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**The impact of betel quid chewing during pregnancy
on pregnancy outcomes in Bhutan**

Thesis submitted in fulfilment of the requirements for the
degree of Doctor of Philosophy

October 2016

Yuka Karasawa

Department of Clinical Research
Faculty of Infectious and Tropical Diseases
London School of Hygiene & Tropical Medicine
University of London

This work was partially funded by the Joint Japan/World Bank Graduate Scholarship
Programme and LSHTM travelling scholarship.

Declaration

I, Yuka Karasawa, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this this has been indicated in the thesis.

Sign: _____

Yuka Karasawa

October 2016

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Abstract

Background

Betel (areca) nut is the fourth most widely used psychoactive substance globally, accounting for 10-20% of the world's population. Its most basic form is betel 'quid' which consists of betel leaf, betel nut (the main psychoactive ingredient) and slaked lime. Evidence that betel quid and betel nut alone are associated with oral cancer has been established.

While there is a substantial body of evidence on the impact of health-risk behaviours including smoking and drinking alcohol on adverse pregnancy outcomes, studies on the impact of betel quid chewing on pregnancy outcomes are sparse and heterogeneous. Although several studies report the negative impact of betel quid chewing on pregnancy outcomes, the evidence is inconclusive. One of the challenges in understanding the impact of betel quid is to distinguish the impact of betel quid chewing from the impact of smoking. Bhutan, where low prevalence of smoking and high prevalence of betel-quid chewing are reported, provides a natural experimental environment for taking a close look at the impact of betel quid chewing alone.

As a part of the global agenda to address preterm births (PTB) as a public health priority and in order to provide evidence to inform efforts to reduce neonatal morbidity and mortality in Bhutan, this study explores the impact of betel quid chewing on birth outcomes and its importance in relation to other risk factors.

Methods

This study used a multi-centre case-control design. A case was defined as a mother of a singleton live born infant whose gestational age is less than 37 completed weeks and/or an infant whose birth weight is less than 2500 g. A control was defined as a mother of singleton live born term babies whose birth weight was more than 2500g and gestational age was greater than 37 weeks. Information was collected using a semi-structured questionnaire from February 2015 to the beginning of March 2016 at the three referral hospitals in Bhutan. Study participants were recruited by a trained interviewer during their post-delivery stay before discharge from each hospital. A statistical approach and a causal directed acyclic graph (DAG) approach were used for building logistic regression models.

Results

Of the 669 study participants, 55% of the case mothers and 52% of the control mothers chewed betel quid during pregnancy. About 22% of cases and 22% of controls used commercial betel products during pregnancy. In total, 60% of the case mothers and 57% of the control mothers chewed either betel quid or packaged betel products during pregnancy. Neither the statistical approach nor DAG approach provided clear evidence of an association between betel quid use and low birth weight (LBW) or PTB. The adjusted odds ratio (aOR) of term LBW was 1.07 (95% CI:

0.54-2.13, $p=0.845$) in the statistical approach while the aOR of term LBW was 1.30 (95% CI: 0.74-2.27, $p=0.439$) in the DAG approach. Using the DAG approach, the aOR of PTB in association with betel quid chewing during pregnancy was 1.20 (95% CI: 0.72-2.00, $p=0.614$). When the total number of betel nuts consumed during the last three months of pregnancy was used as an exposure variable, the aOR for mothers who consumed more than one nut per day was 1.39 for term LBW (95%:0.52-3.68, $p=0.514$) and the aOR of PTB was 0.66 (95% CI: 0.27-1.66, $p=0.383$) compared to non-chewers. For a secondary outcome, the data suggest betel quid chewing is associated with increased odds of anaemia (aOR 2.09, 95% CI 1.27-3.43, $p=0.004$). Using the DAG approach, tobacco and alcohol use during pregnancy, low gestational weight gain, and urinary tract infection showed a clear association with term LBW and PTB.

Conclusion

In the present study, the results provide no clear evidence of an association between term LBW or PTB and betel quid chewing during pregnancy. For a secondary outcome, the data suggest betel quid chewing is associated with increased odds of anaemia. The present study provides rich baseline data for mothers and established a cohort of cases and controls, which could be followed up to understand the short- and long-term effects of LBW and PTB and may help design effective interventions.

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To end acknowledgement, I would like to share a short script I learned during my first research trip. It was so inspiring that I kept it on the last page of my fieldwork notebook throughout the research.

བསམ་པ་བཟང་ན་ས་དང་ལམ་ཡང་བཟང།།

བསམ་པ་ངན་ན་ས་དང་ལམ་ཡང་ངན།།

(If intentions are positive, both the path and the journey will be in excellence - anonymous)

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Abbreviations

AC	Abdominal circumference
ACOG	American College of Obstetricians and Gynaecologists
AIC	Akaike Information Criterion
AN	Areca catechu nut
ANC	Antenatal care
AOR	Adjusted odds ratio
AUDIT	Alcohol Use Disorders Identification Test
BLSS	Bhutan Living Standard Survey
BMI	Body mass index
BMIS	Bhutan Multiple Indicator Survey
BP	Blood pressure
BPD	Biparietal diameter
BQ	Betel quid chewing
CI	Confidence interval
CRL	Crown-rump length
CRRH	Central Region Referral Hospital
CS	Caesarean section
DAG	Directed acyclic graphs
DHS	Demographic and Health Surveys
EDD	Expected date of delivery
ERRH	Eastern Region Referral Hospital
FFQ	Food frequency questionnaires
F-GF	Fractional graduated-frequency
FL	Femur length
GA	Gestational age
GF	Graduated frequency
GNH	Gross National Happiness
GWG	Gestational weight gain
Hb	Haemoglobin
HC	Head circumference
HIV	Human immunodeficiency virus
INTERGROWTH-21 st	International Fetal and Newborn Growth Consortium for the 21st Century
ITC	International Tobacco Control
IUGR	Interuterine growth restriction
JDWNRH	Jigme Dorji Wangchuck National Referral Hospital

KGUMSB	Khesar Gyalpo Medical University of Bhutan
LBW	Low birth weight
LMIC	Low and middle income countries
LMP	Last menstrual period
LOA	Limits of agreement
Lt	Length
MAR	Missing at random
MCH	Maternal and child health
MOH	Ministry of Health
MNAR	Missing not at random
NCD	Non-communicable disease
OR	Odds ratio
PIH	Pregnancy-induced hypertension
PNG	Papua New Guinea
PTB	Preterm delivery
QF	Quantify-frequency measures
RR	Relative risk
SD	Standard deviation
SES	Socioeconomic status
SFH	Symphysis pubis-fundal height
SGA	Small for gestational age
SLT	Smokeless Tobacco
STEPS	WHO STEPwise approach to Surveillance Survey
STI/RTI	Sexually transmitted and other reproductive tract infections
SVD	Spontaneous vaginal delivery
TLFB	Timeline followback
UK	United Kingdom
US	Ultrasonography
USA	United States of America
UTI	Urinary tract infection
WHO	World Health Organization

Chapter 1

Background

This chapter introduces the thesis and gives background information on the burden of low birth weight (LBW) and pre-term birth (PTB) and Bhutan, where the fieldwork for the thesis was undertaken. This chapter ends with a description of the research aims and an outline of the thesis.

1.1 Introduction

“The state shall strive to promote those conditions that will enable the pursuit of Gross National Happiness” – Article 9, the Constitution of Bhutan

Globally, despite an improvement in under-5 mortality rates, much less progress has been made for neonatal mortality rates (deaths in the first 28 days of life) [1-3]. In 2010, 7.6 million children were estimated to have died before their fifth birthday and two fifths of these occurred in the first 28 days of life [4]. Almost all (99%) neonatal deaths occur in low-income and middle-income countries (LMIC) [3]. In 2010, the number of neonatal deaths was highest in Southeast Asia, accounting for approximately 1.1 million deaths or 52.3% of regional deaths in children younger than 5 years [4]. The crucial importance of reducing neonatal deaths has been widely recognized in the global agenda [2, 4, 5].

Complications of PTB are the leading direct cause of neonatal mortality, accounting for 35% of 3.5 million neonatal deaths every year and are the second most common cause of under-5 deaths after pneumonia [2][5]. Babies born prematurely are at risk due to loss of body heat, inability to take enough nutrition, breathing difficulties and infections [1]. In 2010, 14.9 million babies were estimated to be born preterm, representing 11.1% of all live births worldwide [6]. Preterm babies often have LBW; however, babies can often have a LBW because they are born small for gestational age (SGA), or a combination of both prematurity and SGA [7, 8]. Prematurity and LBW are both strongly associated with neonatal mortality and morbidity and weight at birth and gestational age are both used indicators for the newborn’s chances for survival, growth, long-term health and psychosocial development [9]. In particular, birth weight is most commonly used partly because it is relatively easy to measure with validity and precision [7]. In 2004, it was estimated that more than 20 million LBW babies were born each year, accounting for 15.5% of all live births worldwide [10] and this number is believed to be similar today. More than half of these babies were born in South Asia [3, 10].

Bhutan, which lies between China and India, has made great progress in reducing under-5 child mortality from an estimated 123 per 1,000 live births in 1990, to 69 per 1,000 live births in 2010 [8], an approximately 50% decline. This is in keeping with ultimate goal of the Royal Government of Bhutan which is “to maximize the happiness of all Bhutanese and to enable them to achieve their full and innate potential as human beings” (The Bhutan Vision 2020) [11]. This has been achieved through an emphasis on development in the social and health sectors, including the

provision of essential health care services free of charge [12]. Article 9 of the Bhutan constitution clearly states that the state shall provide free access to basic public health services in both modern and traditional medicines.

Approximately 48% of under-5 mortality is estimated to occur during the first month after birth in Bhutan [1]; therefore in order to reduce under-5 mortality it is essential to address the issues of PTB and LBW in Bhutan through prevention and improved care.

Prevention of PTB and LBW depends on an understanding of the underlying risk factors [9]. There have been many studies on the risk factors for LBW and PTB which show that they are caused by a broad range of socioeconomic, behavioural, biological, and environmental factors. However, there are some features unique to Bhutan and her neighbours, which means that it may not be possible to extrapolate from elsewhere. One particular feature is the habit of chewing of betel quid, locally known as “doma”, - a concoction of betel nut and betel leaf with a dash of lime.

Betel quid chewing is very popular in the general population of Bhutan. According to the 2010 Gross National Happiness Survey, 72% of the respondents aged 15 to 98 had ever chewed doma in their life and 58.5% of men and 61.5% of women were currently chewing doma [13]. It is also one of the major commercial agricultural products, yielding 9,781 tonnes in 2011 and 6,250 tonnes in 2013 [14].

Betel nut, also known as areca nut, is the fruit of the Areca catechu tree. As the terms ‘betel nut’ and ‘betel nut chewing’ are more common than the areca nut, they are used throughout this thesis. It is the fourth most widely used psychoactive substance in the world and is commonly used in Central, Southern, and South-east Asian countries, accounting for 600 million people worldwide or 10-20% of the world’s population [15, 16]. The nut contains alkaloids, including arecoline and arecaidine, which have been reported to stimulate the central nervous system [15]. The effects of betel nuts on the autonomic nervous system include feeling warm, sweating, cardioacceleration, salivation, and heightened alertness [17]. The maturity of the betel nut used and the contents of the quid vary between individuals locally and across countries [18, 19]. The ripe nut has a high concentration of arecoline compared to the unripe nut [18]. The content of the quid, including the maturity of the nut, and spitting or swallowing of the quid may induce different health effects. In some cultures, betel nut is used with tobacco and it may be that this combination is most harmful [20].

With regard to health effects, in 2004 the International Agency for Research on Cancer (IARC) evaluated the carcinogenic risks of betel-quid and betel nut alone and concluded that both betel quid with or without tobacco and betel nut are carcinogenic to humans [19].

While there is a substantial body of evidence on the impact of health-risk behaviours including smoking and alcohol on adverse pregnancy outcomes, studies on the impact of betel quid chewing on pregnancy outcomes are sparse and heterogeneous. Although several studies report the negative impact of betel quid chewing on pregnancy outcomes, the evidence is inconclusive. One of the challenges in understanding the impact of betel quid is to distinguish the impact of betel quid chewing from the impact of smoking. Bhutan, where low prevalence of smoking and high

prevalence of betel-quid chewing are reported, provides a natural experimental environment for taking a close look at the impact of betel quid chewing alone.

As a part of the global agenda to address PTB as a public health priority and in order to provide evidence to inform efforts to reduce neonatal morbidity and mortality in Bhutan, this study will explore risk factors for adverse pregnancy outcomes focusing on betel quid chewing.

1.2 Key concepts

Low birth weight (LBW): is defined by WHO as a birth weight of less than 2500 g[21].

Preterm Birth (PTB): is defined by WHO as all births before 37 completed weeks of gestation or fewer than 259 days since the first day of the mother's LMP [22]. PTBs can be classified according to the gestational age at which they occur [23]. Those with gestational age at delivery of less than 28 weeks (<28 weeks) are extremely preterm, 28 weeks to less than 32 weeks (28 to <32 weeks) are very preterm, and 32 weeks to less than 37 weeks (32 to <37 weeks) are moderate to late preterm births.

Intrauterine growth retardation (IUGR) or small for gestational age (SGA): is defined by WHO as birth weight below the tenth percentile of the recommended gender-specific birth weight for gestational age reference curves [24].

1.3 Burden of LBW and PTB

Birth weight and gestational age are major indicators of neonatal mortality and morbidity, and are also related to health in later years.

In 2004, it was estimated that more than 20 million LBW babies are born each year, accounting for 15.5% of all live births worldwide [10] and this is believed to still be the case in 2016. More than half of these births were in South Asia [3, 10]. In 2010, 14.9 million babies were estimated to be born preterm, representing 11.1% of all live births worldwide [6]. In Bhutan, 72.2% of babies surveyed in the 2010 Bhutan Multiple Indicator survey were weighed at birth and approximately 9.9 % of infants were estimated to weigh less than 2,500g [13]. PTB was estimated to be 10.2% of live births in 2010 [6].

PTB is a major cause of mortality, morbidity and disability worldwide [2, 25, 26]. Complications of PTB account for 35% of 3.5 million neonatal deaths every year and are the second most common cause of under-5 deaths after pneumonia [2][5]. Babies born preterm are more likely to die during the first 28 days and in the first year of life than babies born full term [23]. Gestational age is a strong predictor of outcomes of PTB and costs of care [8, 26, 27]. Katz et al estimated that overall pooled RRs of mortality for PTB across all regions in low- and middle- income countries were 6.82 (95% CI, 3.6-13.07) for neonatal mortality and 2.50 (95% CI, 1.48-4.22) for post-neonatal mortality [8]. In Asia, the estimated RRs of mortality are 2.64 for gestational age 34 to <37 weeks, 5.44 for

32-33 weeks and 16.60 for <32 weeks [8]. Regional statistics on mortality, LBW, and prematurity are summarized in Table 1.1.

Even if preterm babies survive, complications of PTB arise from immature organ systems that are not sufficiently well developed to support life outside the mother [23]. In the short term, survivors are at risk of respiratory distress syndrome, intraventricular haemorrhage, necrotizing enterocolitis, sepsis and retinopathy of prematurity [2, 23, 25]. In the long term, the effect of PTB may increase the risk of cerebral palsy, learning disabilities, visual and hearing impairment, chronic lung disease of prematurity and non-communicable disease [2, 25, 28, 29]. Furthermore, a large body of studies demonstrated that retarded growth in foetal life and infancy increases the risk of chronic diseases such as cardiovascular disease, hypertension, and diabetes or developmental outcomes in adult life [30-34].

Preterm infants have more hospital readmission in the first year after discharge, often due to respiratory illness, than full term babies [35, 36]. Approximately 80% of infants born before 27 weeks of gestation will develop respiratory distress syndrome (RDS) [23]. Chronic lung disease follows RDS in preterm infants as a result of inflammation, injury and scarring of the airways and the alveoli [23]. The primary cause of chronic lung disease is lung immaturity which is associated with growth, health and neurodevelopmental problems during childhood [23].

A growing body of studies shows that birth weight and gestational age at birth may be related to health in adult life. Two main hypotheses in the literature that aim to explain potential long-term effects are the foetal origins hypothesis, known as the “Barker hypothesis” [37], and the rapid catch up hypothesis. A systematic review identified 21 studies that investigated the Barker hypothesis and 18 studies that investigated the rapid catch-up hypothesis before 2013 [38]. The foetal origins hypothesis suggests that alterations in foetal nutrition and endocrine status result in development adaptations that permanently change structure, physiology, and metabolism, which may lead to increased risks of cardiovascular disease, hypertension, and diabetes in adult life [39]. The rapid catch up growth hypothesis suggests that undernutrition during early development is followed by improved nutrition later in development, SGA newborns retain some capacity to compensate, by increasing their growth rate [31, 38]. As a result, SGA newborns who were exposed to rapid postnatal growth may have an increased risk of chronic diseases in adulthood [31].

Increasing mortality and morbidity arising from low birthweight and PTB also imposes a heavy financial burden on families, society and the health system [2, 23, 27, 40]. Examples of direct costs include the value of the resources used to treat the condition, such as medical care and special education [23]. Indirect costs are the value of resources lost to society, such as missed opportunities for labour or a reduced level of household productivity due to morbidity or premature mortality [23]. In a study that reviewed economic consequences of PTB in the USA, Australia, the UK, and Finland, the hospital costs associated with initial hospitalization varied between £584 per full term baby and £317,166 per extremely preterm survivor using 2008 prices [41]. Furthermore, in the USA, societal economic burden associated with PTB was estimated to be \$26.2 billion or

\$51,600 per PTB using 2005 prices [23, 41]. A summary of the key findings on the burden of LBW and PTB is provided in Table A.1 in Appendix A.

Table 1.1. Mortality, LBW and prematurity in Bhutan and neighbouring countries.

Countries	First day mortality (per 1000 live births) ¹	Share of U5 deaths that occur on the first day ²	Neonatal mortality (per 1000 live births) ³ (2011)	Share of U5 deaths that occur during the first month ⁴	Infant Mortality Rate (per 1000 live births) ⁵ (2011)	U5 Mortality (per 1000 live births) ⁶ (2011)	LBW infants (% of live births) ⁷ (2007-2011)	Prematurity estimates (% of live births) (2010) ⁸
Nepal	10	21%	27	58%	39	48	18%	14%
India	11	19%	32	53%	47	61	28%	13%
Sri Lanka	3	22%	8	63%	11	12	17%	10.7%
Bangladesh	9	21%	26	60%	37	46	22%	14%
Bhutan	9	17%	25	48%	42	54	10%	10.2%
World	8	15%	22	43%	37	51	15.5% ⁹	11.1%

¹ Save the Children, State of the World's Mothers 2013

² ibid

³ UNICEF, WHO, World Bank, UN Population Division, *Levels and trends in child mortality*. 2012.

⁴ Save the Children, State of the World's Mothers 2013

⁵ UNICEF, WHO, World Bank, UN Population Division, *Levels and trends in child mortality*. 2012.

⁶ ibid

⁷ UNICEF, *UNICEF Multiple Indicator Cluster Survey*.

⁸ Blencowe, H., et al.(2012)

⁹ UNICEF, *Low Birthweight: Country, Regional and Global Estimates*, 2004.

1.4 Bhutan

1.4.1 General background

The Kingdom of Bhutan is a small land-locked country in the eastern Himalayas, lying between the Tibetan Plateau in the north and the Indian plains in the south [42]. A total population of 0.76 million [14, 22] reside in the total area of 38,394km² [42]. The country is mountainous with an elevation ranging from about 160m above sea level in the south to more than 7500m above sea level in the north [42]. Approximately 70% of the total land is under forest cover [14, 42]. GDP per capita was US\$ 2532.5 in 2015 [43]. Bhutan's absolute monarchy was established in 1907 and moved towards a constitutional monarchy in 1953 [44]. In 2008, Bhutan held its first national democratic elections and the parliament endorsed the country's first constitution [45]. The country aims to maximize Gross National Happiness for people, supported by four pillars – sustainable socio-economic development, preservation and promotion of culture, environmental conservation, and good governance [46]. The royal government of Bhutan recognizes health and education as pre-requisites for economic and spiritual development, poverty reduction and the road to Gross National Happiness. Article 9 of the constitution mandates the state to provide free access to basic public health services in both modern and traditional medicines and to provide free education to all children of school age up to tenth standard and ensure that technical and professional education is made generally available and that higher education is equally accessible to all on basis of merit (Article 9, The Constitution of the Kingdom of Bhutan).

1.4.2 The health system

Bhutanese formal health care originated in Tibetan medicine known as *Gso-ba Rig-Pa* (science of healing), believed to be introduced by the founder of Bhutan, Zhabdrung Ngawang Namgyal, and developed by traditional doctors who underwent training in Tibet [45]. Western biomedicine was introduced to Bhutan mainly through British medical officers who accompanied political missions from India to Bhutan [45]. The first hospital was established in 1956 in the capital, Thimphu [47]. Today the integrated services of both traditional medicine and western biomedicine are offered by the government inside the same facilities across the country, not to compete but to complement each other[45].

The parliament oversees the government, including the health sector [45]. The Gross National Happiness Commission (GNHC) prepares the five-year plan which set targets and goals for the country's socio-economic development, negotiates budgets with the sector ministries and monitors progress towards the stated goals [45]. The Ministry of Health is responsible for formulating policies and guidelines and regulating and monitoring the health sector, while the districts have authority over, and responsibility for the provision and implementation of health services in their respective areas [45].

The National Health Policy, approved in 2011, shapes the development of the health sector along with three acts: the Bhutan Medical and Health Council (BMHC) Act 2002 to provide the

basis for regulation of the medical and health profession; the Medicines Act 2003 to set a framework for medicines and supplies, their import, sales and use in the country; and the Tobacco Act 2010 to ban commercial tobacco import, trade and use [45].

The provision of health services is predominantly through the public sector. Since 2015, healthcare services have been delivered through 31 hospitals (including one indigenous medicine hospital in the capital), 235 basic health care units (BHUs) and sub-posts and 562 outreach clinics in all 20 districts (Table 1.2) [47]. In BMIS 2010, each region was coded into three categories: Western Region (Thimphu, Paro, Ha, Samtse, Chhukha, Punakha, and Gasa), Central Region (Wangduephodrang, Daga, Tsirang, Sarpang, Zhemgang, Trongsa, and Bumthang), and East Region (Lhuntse, Mongar, Pemagatsel, Samdrup Jongkhar, Trashigang, and Trashigang Yangtse). Nationally, there are 251 doctors (including Bachelor of Medicine graduates and specialists), 47 indigenous medicine physicians, 1105 nurses, and 548 health assistants [47]. The national referral hospital is located in the capital, Thimphu. There are two regional referral hospitals; one in Mongar in eastern Bhutan and one in Gelephu in the South. District hospitals have basic diagnostic facilities including x-ray, blood glucose levels and microscopic services for diagnosing tuberculosis and malaria with generally two doctors, four to five nurses, one laboratory technician and health assistants [12]. BHUs are staffed with three health assistants who have completed 2 years of training at the Royal Institute of Health Sciences (RIHS) [12]. These health assistants assist at normal deliveries and organize outreach health clinics once a month to provide maternal and child health services in remote communities [12]. Referral hospitals, district hospitals and BHUs refer patients to a higher level and back to the community for monitoring and rehabilitation [12]. The government sponsors patients who need treatment that is not available in Bhutan to benefit from treatment outside of the country, mainly in India, based on the national referral guidelines.

The free health services are financed through the funding from the Royal Government of Bhutan, International Aid, Royal Bhutan Army, and out-of-pocket expenditure [45]. Government funding accounts for approximately 60% of the total health expenditure, comprising revenues and donor grants [45, 48]. The Government of India has provided the largest external financial support, especially for the construction of the referral hospitals in Thimphu, Mongar and Gelephu [45]. Total expenditure on health per capita in 2012-2013 was Bhutanese Ngultrum (BTN) 5409 or US 81 dollars (at \$1 =BTN 66) and total expenditure on health as a percentage of GDP was 3.6% in 2012 [48, 49]. With prolonged life-expectancy and an increasing number of non-communicable diseases such as diabetes and cancer, the referral cost is sharply increasing, totalling BTN 90 million in 2010 [45]. This imposes a financial challenge on the sustainability of free health services in Bhutan.

1.4.3 General statistics

Life expectancy at birth improved dramatically from 52 years in 1990 to 70 years in 2015 [50]. Use of improved drinking water sources was 100% (in both urban and rural) while access to improved sanitation remained only 50% (urban 78% vs rural 33%) in 2015 [50]. The primary enrolment ratio between 2009 and 2012 was 89.3% [50]. The total literacy for adults was 53% and for youth (15-24 years) the literacy rate was 80% for males and 68% for females between 2009 and 2014 [50]. Of pregnant mothers, 98% received at least one antenatal care (ANC) visit and 85% received at least four visits between 2010 and 2015 [50].

1.4.4 Betel quid chewing

Betel nut is the fourth most common addictive substance in the world after tobacco, alcohol, and caffeine [15, 16] and is an indispensable part of Bhutanese culture and lifestyle. It is offered during celebrations, religious rituals, festivals, and gatherings and is widely consumed by both men and women. In 2010 61.5% of women in Bhutan were reported that they were current betel quid chewers [51].

In Bhutan, typically one betel quid contains one quarter of a ripe nut and slaked lime wrapped in a piper betel leaf (Figure 1.1). Unripe nuts are usually seasonally available in May and June but ripe nuts are available throughout the year and more commonly consumed. A package of betel quid is prepared at retail shops. One quid contains one leaf, a pinch of lime, and a quarter of one betel nut. Three sets of betel quid were sold for Bhutanese Ngultrum (BTN) 10 or US 0.15 dollar (at \$1 =BTN 66) on average in 2014.



Figure 1.1. Preparation of betel quid in Bhutan.

1.4.5 Packaged betel nut products (Pan Masala)

Betel nut products are available under several names, mostly marketed as mouth refresher and imported from India. Wiz and Rajiniganda are the most popular brands for flavoured pan masala: Two packages of Wiz are sold for BTN 5 or US 0.08 dollar in Bhutan. A small Rajiniganda package weighs 4 grams and is sold for BTN 20 or US 0.30 dollar.

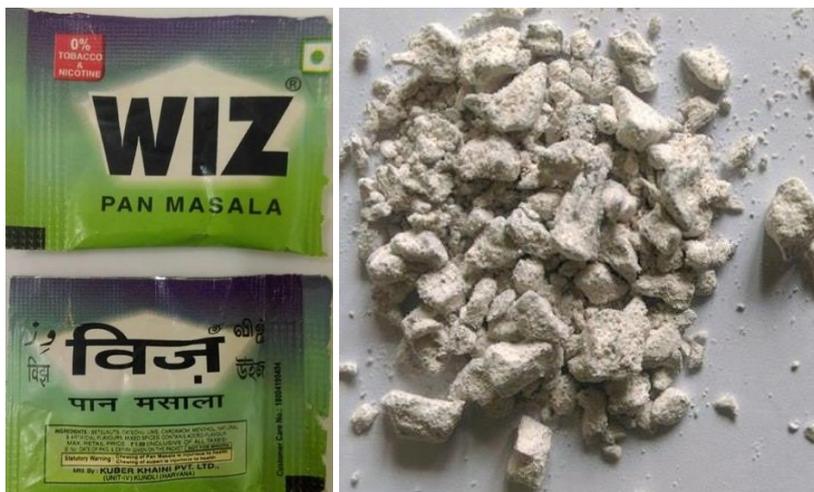


Figure 1.2. Package (1.4 gram) and content of Wiz.



Figure 1.3. Package (4 grams) and contents of Rajiniganda.

1.4.6 Smoking and smokeless tobacco

According to the Tobacco Act 2010, the cultivation, harvest, manufacture, supply, distribution and sale of tobacco products is banned in Bhutan [52]. Though sale of tobacco products is banned, consumption is not prohibited except in areas identified as smoke-free zones by the government. Cigarettes, piped tobacco and other tobacco products can be imported for personal consumption in specific import quantities. Tobacco products cannot be imported for sale. It is “illegally” sold at retail shops. One cigarette cost BTN 15 or US 0.23 dollar in 2014.

In Bhutan, current smokers among females aged 15 and above ranged between 2.1% [53] in 2014 and 2.4% in 2010 [51] in the interviewer-administered population-based surveys after the ban on tobacco in 2010. However, prevalence of current smokers among girls aged between 13 and 15 was higher (6.6%) in the self-administered population-based survey [54]. Smokeless tobacco use was 9.9 % in 2014[53].

Smokeless tobacco was also banned under the Tobacco Act 2010. The brand “Baba”, imported from India, is one of the most commonly used smokeless tobacco products in Bhutan, sold “illegally” at retail shops. The average package contains 10 grams for BTN 15 or US 0.23 dollar in 2014.



Figure 1.4. Package and content of Baba, a smokeless tobacco product available in Bhutan.

1.4.7 Alcohol

Alcohol is widely produced and consumed on cultural, social, and religious occasions [55]. Home-produced alcohol is very common and widely available, although the sale of home-made alcohol is banned [56]. Home-brewed alcohol accounts for more than 40% of alcohol expenditure in the household (32.2% in urban areas and 52.9% in rural areas) [57]. Common home-produced alcohol

products are spirits (Ara) and wines (Changkey, Singchang, and Bangchang), made from maize, rice, wheat, millet and fruits [55, 56]. A study conducted in Tashiyangtse in eastern Bhutan in 2010 found that females consumed more than five times more home-brewed alcohol than industry-made alcohol, while males consumed more than twice as much home-made alcohol as industrial alcohol [56]. Estimates of female current drinkers in the surveys between 2010 and 2014 were 32.8% [51, 53], which is similar to the prevalence in a research study in Tashiyangste (30%) [56]. Out of 81 female current drinkers in the Tashiyangste study, 60.5% drank for medical use while 39.5% were social drinkers [56]. 43% of the total respondents believed that alcohol was necessary for reducing pain and for the initiation of breast feeding during the post-partum recovery period [56]. There is a local belief that “Changkey” will enhance production of breastmilk. This drink is made of rice mixed with yeast and stored in a bucket for more than three months. It is usually prepared hot, sometimes with eggs. Similarly, heated “Ara” with eggs and dry meat is also believed to be nutritious and good for pregnant women. In southern Bhutan, “Tongpa”, a fermented drink made of millet is often consumed for the same reason.

Industrial alcoholic beverages sold in Bhutan are available in 750 ml, 650 ml (beer), 500 ml (canned beer), 335 ml (spirits), 180 ml (spirits) measures [55]. Widely available and consumed local beer (Druk 11000) contains 8% (v/v) and locally produced whiskies contains 42.8% alcohol (v/v) [55]. Among industrial products, beer is most popular [56]. The strength of home-brewed drinks has never been measured using biochemical analyses [55, 56]. Standard size of home-brewed alcoholic beverages are not available as serving size varies depending upon the different cups and vessels used and the amount poured for each serving also differs from home to home. This poses a significant challenge in quantification of alcohol consumption. Only a few studies have attempted to quantify per-capita consumption [55, 56]. One study used the 2010 trade and production data and unrecorded information from a 2007 population-based survey based on assumptions of average alcohol strengths of 25-30% for ara, 15-20% for bangchang, 20-25% for singchang and 15-20% for tongba [55]. The per capita adult pure alcohol consumption was estimated to be 8.47 litres based on the production, imported and domestic sales statistics and 0.97 litres based on data derived from the Bhutan Standard Living Survey 2007 [55]. Another study assumed the strengths of local wine and local spirit (ara) to be 5% and 15% respectively and estimated that annual per capita alcohol consumption was 5442 g for men and 2566 g for women [56].

The societal and healthcare burden of alcohol-related morbidities and mortality is high in Bhutan. In 2014, alcohol liver disease was the leading cause of hospital inpatient mortality and accounts for more than 40% of mortality related to non-communicable diseases in 2013 [14, 58]. There is a huge urgent need for research and interventions in this area.



Figure 1.5. Takin wine, a local wine widely available in retail shops (alcohol concentration, 16% v/v).

Table 1.2. Health facilities and deliveries attended by trained personnel (modified from Annual Health Bulletin 2013).

District	Number of health facilities				Deliveries attended by trained personnel					
	Referral hospital	District Hospital	BHU I	BHU II	Absolute number of deliveries	%	Referral Hospitals	District Hospitals	BHU I	BHU II
Bumthang	0	1	0	5	196	1.8%	-	159	-	37
Chhukha	0	3	1	12	1073	9.9%	-	944	34	95
Dagana	0	1	2	7	175	1.6%	-	94	37	44
Gasa	0	0	1	3	6	0.1%	-	-	1	5
Haa	0	1	1	3	110	1.0%	-	-	93	17
Lhuentse	0	1	0	11	156	1.4%	-	78	-	78
Mongar	1	0	1	23	929	8.6%	640	-	30	259
Paro	0	1	0	3	442	4.1%	-	420	-	22
Pemagatshel	0	1	1	11	230	2.1%	-	115	45	70
Punakha	0	1	0	6	666	6.2%	-	627	-	39
Samdrup Jongkhar	0	2	2	8	450	4.2%	-	310	63	77
Samtse	0	3	0	9	491	4.6%	-	366	-	125
Sarpang	1	1	0	11	951	8.8%	771	68	-	112
Thimphu	1	4	1	9	3418	31.7%	3342	52	14	10
Trashigang	0	3	2	17	567	5.3%	-	275	75	217
Trashiyangtse	0	1	0	7	109	1.0%	-	51	-	58
Trongsa	0	1	0	6	113	1.0%	-	57	-	56
Tsirang	0	1	0	6	217	2.0%	-	174	-	43
Wangdue Phodrang	0	2	0	9	336	3.1%	-	251	-	85
Zhemgang	0	1	2	12	156	1.4%	-	28	32	96
Total	3	29	14	178	10791	100%	4753	4069	424	1545

1.5 Research aim and objectives

The aim of this research is to understand LBW and PTB in Bhutan and the impact of betel quid chewing on birth outcomes and to draw policy implications. The specific objectives are:

1. To explore modifiable risk factors for LBW and PTB in Bhutan
2. To develop methods for assessing betel quid use during pregnancy
3. To describe the pattern of betel quid chewing during pregnancy among Bhutanese women
4. To examine the impact of betel quid chewing on birth outcomes
5. To disseminate the research findings to inform policy makers

The findings of this study will improve our understanding of PTB and LBW in Bhutan by exploring risk factors and the impact of betel quid chewing on pregnancy outcomes. The study will also provide baseline data to inform policy makers to improve modifiable risk factors.

1.6 Outline of the thesis

The thesis is divided into four parts (and eight chapters). Part 1 introduces the thesis (Chapter 1). Part 2 presents a brief overview of the literature (Chapter 2). It also comprises an overview of the methods used and a detailed description of how the questionnaire to assess risk factors and betel quid chewing was developed and how each covariate was modelled and used in the subsequent analyses (Chapter 3). Part 3 presents results of the validation of outcomes (Chapter 4), descriptive analyses of the various risk factors identified in the literature (Chapter 5) and patterns of betel nut chewing during pregnancy in addition to drinking and smoking (Chapter 6), and logistic regression analysis (Chapter 7). Part four concludes with the overall discussion, recommendations for future research, and the implications for policy and practice (Chapter 8).

1.7 Contribution of the author

The author conducted a literature review to design the study and drafted the semi-structured questionnaire. She organized and led a research team comprised of policy makers, medical professionals and research assistants and trained research nurses who collected data and monitored data collection. She obtained all the ethical and administrative approvals. She served as a data supervisor to clean and finalize a double-entered dataset and conduct all descriptive and regression analyses. She also interpreted the results and did all the writing.

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Chapter 2

Literature review

This chapter provides the context of the thesis with a literature review on measurement issues of outcome, risk factors for PTB and LBW in the literature, areca nut and measurement of exposure. The first section describes the general background on issues of measuring birthweight, gestational age, and SGA. The second section presents and discusses a variety of risk factors in the literature. The third section introduces a systematic review of previous studies on the relationship between areca nut chewing and adverse outcomes in order to identify a research gap. The fourth section reviews existing tools for the measurement of exposure. The chapter ends with a summary followed by an introduction of the conceptual framework and causal diagrams.

2.1 Definitions and issues in measuring birth weight, gestational age and small for gestational age

Size at birth is the combined result of foetal growth and gestational duration [1]. LBW may be the result of either shortened gestation (preterm birth) or slowed rate of growth (intrauterine growth restriction). There are various ways to measure the size of a baby including birth weight, length and head circumference. Among these, birth weight is most easily measured. Measuring length and other aspects of growth such as head circumferences requires more skills and training, and therefore is less commonly performed [1].

2.1.1 Birth weight

Birth weight is determined by both duration of gestation and rate of foetal growth [1, 2]. LBW is defined by WHO as a birth weight of less than 2500g. One of the main criticisms of this simple cut-off is that although boys tend to be heavier than girls, the definition of LBW does not take gender into account [1].

2.1.2 Gestational age

Gestational age is defined as the time elapsed between the first day of the last menstrual period and the day of delivery [1]. There are several ways to estimate gestational age. In the last menstrual period method (LMP), the average pregnancy is assumed to last 280 days from the first day of the last menstrual period, mainly the woman's self-reported LMP. Ultrasonography (US) can be used to estimate or confirm gestational age if it is done early in the pregnancy [1]. Various measurements of the foetus, such as biparietal diameter and/or the length of the femur, are taken and compared against age-specific references using standard formulae. LMP measures the length of pregnancy and US measures the size of the foetus [3]. An estimate of gestational age based on the LMP confirmed by an early second trimester US scan is often considered as the most reliable estimate or gold standard [1, 3]. However, in low-income settings, lack of US machines, lack of trained staff,

late presentation to the first antenatal care, and lack of quality control of US measures make reliable US-based gestational age less available [4-7].

Other ways to estimate gestational age include neonatal assessments such as the Ballard Score and the Dubowitz Score, as well as measurements during pregnancy including symphysis pubis-fundal height (SFH) (single or multiple measurements), quickening, and mid- and late pregnancy foetal biometry [6].

The Ballard Score assesses the neuromuscular and physical maturity of new born infants [8]. Neuromuscular maturity is measured using six parameters: posture, square window, arm recoil, popliteal angle, scarf sign, heel to ear, and skin. Physical maturity is measured using seven parameters: skin, lanugo, plantar surface, breast, ear, genitals. The sum points for each parameter are used to estimate gestational age.

The Dubowitz Score assesses an infant for apparent gestational age by considering both neurological and external signs of development [9]. Ten neurological signs include posture, square window (wrist), ankle dorsiflexion, arm recoil, leg recoil, popliteal angle, heel to ear scarf sign, head lag, and ventral suspension. Twelve external signs include edema, skin texture, skin colour, skin opacity, lanugo, plantar creases, nipple formation, breast size, ear form, ear firmness, genitals (female/male). The total points for each parameter are used in a formula to estimate gestational age. White et al. (2011) proposed a formula that incorporates at least three measurements of symphysis-pubis fundal height (SFH), the distance measured from the top of the symphysis pubis to the depression in front of the pad of the middle fingers making the top of the uterine fundus in the middle of the woman's abdomen, to predict gestational age as a low-cost alternative to US [5]. It has been suggested that this may be more reliable than previously published methods such as LMP, the new Ballard score or the Dubowitz given a realistic number (6-7) of repeated SFH measurements, at least two weeks apart with corresponding dates derived from routine ANC.

In comparing different approaches to measurement of gestational age, LMP is usually considered the most low-cost and simple approach. However, the limitation is that the accuracy of the gestational age depends on accurate LMP recall which is often correlated with mothers' literacy rate, regularity of the menstrual cycle, and factors that could influence ovulation timing such as previous oral contraceptive use, a recent pregnancy or breastfeeding[3]. It could also depend on how health workers enquire about LMP [6]. While early second-trimester US is superior to LMP-based dating in predicting the actual date of delivery, accurate estimation also depends on the gestational age at the time of the US examination, the accuracy of the measurement and the quality of the US equipment and on whether the assumption holds, that foetal growth is uniform. A systematic review of 83 publications from 32 countries including low, middle, and high income countries between 1971 and 2008 found substantial heterogeneity of methodology used in US studies of foetal biometry [10]. There were also significant differences in median values and percentile curves and the review suggested standardisation of methodologies. Although gestational age estimates are often clinically used for care, both the Ballard and Dubowitz scores require more technical skills to perform [5, 11].

In order to assess the accuracy of the LMP approach in low-income settings, the literature comparing LMP to US-based gestational weeks in low-income settings was systematically searched on 24 March 2015. Details of the search strategy are given in Appendix B.1. In addition, in order to compare the predictivity of the SFH formula by White et al. [5] to LMP or US-based gestational age, academic papers citing the SFH formula were also searched and included in the review. The key findings of the selected studies are summarized in Table 2.1.

The search identified studies conducted in Gambia [4], Guatemala [12], Papua New Guinea (PNG) [6], Bangladesh [11] and the Thai-Myanmar border [5, 7]. For studies with US-based gestational age used as a gold standard, there were differences in terms of which measurements were taken during US scans according to different gestational weeks and which reference was used (local [4] vs Hadlock growth curve). None of the studies explained how mothers were questioned about their LMP or how certain mothers were about their LMP details. The sample sizes ranged from 80 [4] to 2,437 [5].

Mean LMP underestimated gestational age by 0.6 days in Gambia [4], 1 day in Bangladesh [11], and 5.4 days (0.77 weeks) in Guatemala [12], while overestimating by 3 days in PNG [6]. Ballard overestimated gestational age by 6 days in the PNG study [6] and underestimated by 2.9 days in Bangladesh [11]. Dubowitz overestimated gestational age by 2.57 weeks in the Thai-Myanmar study [7] and by 3.9 days in the Bangladesh study [11]. While estimation based on a single SFH resulted in a huge variation of about 10 weeks, six SFH measurements resulted in a prediction accuracy of ± 14 days using the new formula proposed by White et al. [5]. However, in a later study in the same population, in 2015, the multiple SFH formula overestimated by 3.94 weeks (95% LOA: 2.5 to 5.38) [6]. This could be because the group, on average, only collected a maximum of three fundal height measurements as explained by the authors.

In summary, lack of US machine, lack of trained staff, late presentation for the first ANC, and lack of quality control of ultrasound measures constrain availability of reliable US-based gestational age in low-income settings. Furthermore, estimation of gestational age ultrasound could produce significant errors. LMP and fundal height are more reliable than other clinical measures.

2.1.3 Small for gestational age (SGA)

In order to separate the effects of gender and gestational age, growth reference values are developed for each gender and gestational age separately in terms of percentiles or standardized scores, also known as standard deviation scores. There is no universally accepted definition of SGA. A common working classification is birth weight below the tenth percentile of the recommended gender-specific birth weight for gestational age reference curves [13]. In terms of the reference curves that are used to classify SGA infants, a number of studies generated and validated the reference curves using different methods and populations, and have produced more than 104 published charts since 1990 [14]. The challenges to classifying SGA infants are that it depends on the measurement of gestational age which could be erroneous and also that there are many reference curves to decide the percentiles.

Until the late 1970s, various growth charts were used clinically to assess child growth. In 1977, the National Center for Health Statistics (NCHS) published a new set of growth charts for children aged under 18 years, based on data from the Fels Longitudinal Growth Study and nationally representative surveys [15, 16]. In 1978, the USA Center for Disease Control and Prevention (CDC) extrapolated the published percentiles to compute z scores, allowing for the generation of more extreme cut-offs, including 2 and 3 standard deviations below the median [15]. WHO then recommended that these z scores be used as a global reference for the definition of malnutrition [16]. Although the curves began to be used worldwide, there were numerous methodological limitations to these charts, including a lack of racial diversity in the infant sample, a sample composed of infants who were almost all formula fed, and a disjunction in length and stature measurements when transitioning from the charts for younger children to those for older children [15, 16]. Other charts were still being developed. Hadlock and colleagues used the US measurement, between 10 and 41 weeks of gestation, of 392 pregnant women of the European Continental Ancestry Group living in the USA to create an optimum growth equation [17, 18]. Gardosi and colleagues proposed an individualized approach that took into account ethnic origin, maternal height and weight, parity, and sex of the infant [19]. To respond to the need for upgraded international growth standards to assess the growth and development of infants and young children around the world, WHO initiated the Multicentre Growth Reference Study (MGRS) from 1997 to 2003 in six sites (Pelotas, Brazil; Accra, Ghana; Delhi, India; Oslo, Norway; Muscat, Oman; and Davis, California) to generate new growth curves to assess the growth of infants and young children throughout the world. One of the main challenges in any approach was that the growth curve should show how children should grow rather than describe how they grow and that international sampling should include diverse ethnic groups. In 2006, the WHO Child Growth Standards for children under 5 years was generated based on this study and was supplemented by INTERGROWTH-21st in 2014 [14]. INTERGROWTH-21st is a population-based project that assessed foetal growth and new born size in eight geographically defined urban populations: Pelotas, Brazil; Turin, Italy; Muscat, Oman; Oxford, UK; Seattle WA, USA; Shunyi County in Beijing, China; the central area of Nagpur, India, and the Parklans suburb of Nairobi, Kenya [14]. A cohort of 20,486 pregnant women were selected, according to strict inclusion criteria to identify a population at low risk of impaired foetal growth, and followed up. New born anthropometric measures were obtained within 12 hours of birth by identically trained anthropometric teams using the same equipment at all sites. Sex-specific observed and smoothed centiles for weight, length, and head circumference for gestational, age at birth were calculated and the observed and smoothed curves were almost identical. What differentiates INTERGROWTH-21st from other studies is that it is a prospective multi-centre study that attempted to define an international birth weight standard in the context of optimal maternal health and foetal growth using accurate information on gestational age and other critical measurements to produce the reference curves while previous studies focused on simply describing the birth weights among the general population [20]. While MGRS and INTERGROWTH-21st reference curves assume that children grow uniformly worldwide before the

age of five, another group from WHO provides a global reference curve that can be adjusted to any local population according to the mean birth weight at 40 weeks of gestation and standard deviation of birth weight using data from 24 countries in Africa, Latin America, and Asia that participated in the 2004-08 WHO Global Survey on Maternal and Perinatal Health (237,025 births) [17].

2.1.4 Mother's subjective assessment of the size of the infant

Although birth weight is the most reliable and widely reported measure to assess size at birth, not all the infants are weighed at birth globally, especially in locations where infants are not delivered in health facilities. When infants are not weighed at birth, mother's recall is often used to estimate the prevalence of LBW to estimate the percentage of infants with LBW in Demographic and Health Surveys (DHS) and other surveys. The mothers are often asked, "When (the name of the infant) was born, was he/she very large, larger than average, average, smaller than average or very small?" in the surveys. A study which critically examined the data used to produce estimates of the proportion of infants with LBW showed that birth weights reported by mothers are rounded up or "heaped" in multiples of 500 grams and those who were weighed were more likely to have mothers who live in urban areas and are educated and to be born in a medical facility with medically-trained personnel [21]. It suggested that the current survey-based figures for the prevalence of LBW are underestimated.

2.1.5 Relevance to Bhutan

In Bhutan, according to BMIS 2010, a total of 72.2 % of new borns were weighed at birth. Gestational age is recorded based on mother's recalled LMP, ideally confirmed by US. In terms of US scans, the following are advised to be measured to estimate gestational age: gestational sac diameter (GSD) for less than 8 weeks; crown-rump length (CRL) for 9-12 weeks; biparietal diameter (BPD) and head circumference (HC) for 12-16 weeks; and BPD, HC and abdominal circumference (AC) and femur length (FL) for more than 16 weeks[22]. According to the Ministry of Health Mother and Child Health Handbook [23], if estimates by LMP and US are different by less than one week, and US is done early, the expected date of delivery (EDD) by LMP is recorded in the ANC records. If the difference is more than one week and US is done within 24 weeks of gestation, EDD by US is recorded.

Neonatal estimates are currently not widely practiced even at the referral hospital level as of November 2015. In recent years resident doctors were trained on the new Ballard score and it has been introduced for clinical care only at the NICU at the Jigme Dorji Wangchuck National Referral Hospital (JDWNRH). Length (Lt) and head circumference (HC) were not widely measured at any of the three referral hospitals although in late 2015, the JDWNRH birth centre started to put more effort into measuring HC and Lt (according to personal communication with the nurse in charge). Considering 77.3 % of women go to four ANC visits or more in Bhutan according to BMIS 2010, the multiple SFH formula proposed by White et al.[5] could be also used to predict gestational age. It should be noted that accuracy of prediction depends on the quality of recording and measurement

of SFH and the opportunity for mothers to have a minimum of six measures as using only three measures can result in a huge variation of gestational age [5, 7].

Table 2.1. Validation of measurements to predict gestational age in low-income settings.

Authors (Year)	Sample size and population	Measurements compared	Definition of reliable menstrual history	Ultrasound timing	Dubowitz, Ballard, SFH	Major findings
Moore et al. (2015) [7]	Retrospective analysis of clinical records of 2 cohorts of women who gave births to live singletons on the Thai-Myanmar Border: 1. 250 women who attended ANC between July 2001 and May 2006 with both US CRL and a Dubowitz gestational age assessment. 2. 975 women attending ANC between April 2007 and October 2010 who had US CRL, SFH, US (BPD and HC) between 16 and 50 weeks gestation.	US CRL (reference) Late US (BPD and HC) between 16 and 40 weeks Dubowitz SFH formula (multiple) by White et al (2011)[5].	NA	CRL between 7 and before 14 weeks by trained sonographers	Trained staff, monitored regularly	For preterm babies, Dubowitz overestimated GA by 2.57 weeks (95% Limits of Agreement (LOA): 0.49 to 4.65). SFH overestimated by 3.94 weeks (95% LOA: 2.5 to 5.38) Late US scan of HC underestimated by 0.39 weeks (95% LOA: -2.60 to 1.82) Late US scan of BPD overestimated by 0.83 weeks (95% LOA: -0.93 to 2.58)
Karl et al. (2015) [6]	668 singleton pregnancies from rural Papua New Guinea between November 2009 and December 2012 at 8 health facilities in the Madang municipality.	US (reference) LMP Ballard SFH (single/multiple developed by White et al.(2011)[5] Quickening	Maternal recorded date	British Medical Ultrasound Society guidelines CRL for 6-13 weeks; HC weeks for 13-25 weeks Before 24 weeks by trained clinicians and checked by external expert	Nurses underwent biannual training sessions	LMP overestimated GA by 3 days (LOA: -37 to 44 days) SFH (repeated) overestimated GA by 4 days (LOA: -19 to 26 days) Ballard overestimated GA by 6 days (LOA:-27 to 39 days) Quickening underestimated by 6 days (LOA: -46 to 35 days)

White et al. (2011) [5]	2,437 women with US-dated pregnancies and SFH measurements from 5 clinics on the Thai-Myanmar border between April 2002 and May 2006.	US (reference) A new SFH formula that requires a minimum of three SFH measurements	NA	CRL 8 - < 11 weeks and BPD, femur length and abdominal circumference for 16-<21 weeks	Not mentioned	The multiple measures model using the six SFH measurements resulted in a predication accuracy of \pm 2weeks.
Taylor et al. (2010) [4]	80 singleton babies at the Medical Research Council's (MRC) station in Keneba, Kiang West, an isolated district of Lower River Division in the Gambia. Between May and November 2007.	US (reference) LMP The External Ballard Examination (EBE)	Recalled from mothers at first booking	1st/early 2nd trimester US by a trained clinician CRL for less than 14 weeks and BPD for 14-24 weeks using charts validated in African populations	EBE (modified Ballard score: only 6 external criteria(skin appearance, presence of lanugo hair, plantar creases, breast tissue, ear formation, and external genitalia formation) were scored) by one trained midwife with previous experience	EBE underestimated US-based GA by 15.6 days (LOA:-5.9 to 37.1) EBE underestimated LMP-based GA by 15.4 (LOA:-30 to 61 days) Mean LMP-dated US underestimated GA by 0.6 day
Rosenberg et al. (2009) [11]	355 out-born infants admitted to the Special Care Nursery at the Dhaka Shishu Hospital in Bangladesh who enrolled in a trial of topical emollient therapy from 1998 to 2003.	US (reference) LMP Ballard Dubowitz criteria	Reported by the mother or family	1st/2nd trimester at various centers at Dhaka	Not mentioned	LMP underestimated GA by one day (\pm 11) with concordance coefficient for LMP0.878 Ballard underestimated GA by 2.9 days (\pm 7.8) with concordance coefficient 0.914 Dubowitz overestimated GA by 3.9 days (\pm 7.1) with concordance coefficient 0.886
Neufeld et al. (2006) [12]	171 women aged between 19 and 34 years old from 4 rural villages in eastern Guatemala from August 1996 until June 1999.	US (reference) LMP The Capurro neonatal examination SFH (multiple)	Maternal recalled date	BPD using the regression equation of Hadlock et al conducted between 15 and 24 weeks by one of two trained obstetricians	Five physical items (size of mammary gland, nipple form and areola size, ear-fold development, skin texture and plantar creases) Within 72 hours of birth SFH by trained nurses at each prenatal visit	LMP underestimated GA by 0.77 weeks on average (Range: -22 and 17 weeks). The Capurro estimates underestimated GA by 3.33 weeks (Range: -33 and 25 weeks).

2.2 A summary of published literature on risk factors for LBW and PTB

Adverse pregnancy outcomes could be reduced by diagnosis and timely treatment of pregnancy complications or by eliminating or reducing modifiable risk factors [24]. Babies can be LBW because they are born early (preterm), are born SGA, a proxy for interuterine growth restriction (IUGR), or a combination of the two [25, 26]. PTB and IUGR can differ in potential risk factors. A Lancet paper estimates that 83% of LBW infants were SGA and 33% were PTB using eight datasets from Bangladesh, India, Nepal, Pakistan, Philippines and Thailand [26]. LBW, SGA and PTB may result from a broad range of socioeconomic, behavioral, biological, and environmental factors [24]. A review of the literature on risk factors for LBW and PTB was conducted to bring into focus the modifiable risk factors that are relevant to Bhutan by identifying a broad range of risk factors, categorizing them into modifiable and non-modifiable factors, and prioritising the risk factors according to magnitude of association and prevalence in Bhutan. As the multitude of different risk factors makes it impossible to discuss them all, the selection discussed here focuses on those most relevant to Bhutan. This will help the study identify covariates, control for appropriate confounding variables, and design the content of the questionnaire.

Systematic reviews and other peer reviewed papers in MEDLINE and the Cochrane library were systematically searched with no date restriction on 29 May 2013 and updated on 8 April 2016. No language restriction was applied. There was no study type restriction. For infant outcomes, the following keywords were used: exp infant, LBW/ or exp infant, small for gestational age/ or exp infant, very LBW/ or exp infant, premature/OR LBW* OR PTB* OR small for gestational age OR intrauterine growth retardation. For determinants, exp risk/ or exp logistic models/ or exp risk assessment/ or risk factors/ or exp regression analysis/ or exp epidemiologic research design/OR risk factor* OR determinant* OR associat* were used. In addition to these search terms, the systematic review filter was used. The reference lists of relevant papers were also manually searched and other academic papers were searched in Google scholar and PubMed in April 2016. Abstracts were then screened for possible relevance to developing countries, Southeast or South Asia, or Bhutan. If there were several versions of systematic reviews on a similar subject, the most recently updated article was included in the review if there were no major disparities in the results. The key findings from the selected studies are summarized in Table 2.2.

2.2.1 Modifiable risk factors in the short run

(a) Maternal health-risk behaviours

Certain maternal behaviours such as smoking and drinking alcohol hamper foetal growth. Heavy alcohol consumption during pregnancy increases the risk of PTB by 23% (RR 1.23, 95% CI 1.05-1.44) whereas light to moderate alcohol consumption shows no effect. Tobacco chewing and smoking have been widely reported to be risk factors for LBW and PTB due to carbon monoxide and nicotine [24, 27-30]. A systematic review reported that any maternal smoking increased the risk of preterm delivery by 27% (1.27, 95% CI 1.21-1.33), compared to non-smoking [29]. Caffeine

is the most commonly used psychoactive substance in the US and Europe and a meta-analysis showed that each 100-mg per day increment (around one cup of coffee) in maternal caffeine intake increased the risk of LBW by 13% (RR 1.13, 95%CI 1.06-1.21) [31].

In Bhutan, alcohol consumption is common. A cross-sectional survey in Tashiyangtse in eastern Bhutan reported that 18.5% of women aged 18 years and above consumed more than 40g of alcohol per drinking episode, the level defined as high-intensity drinking in the study [32]. On the other hand, the sales of tobacco was banned in 2004 and this ban was legislated and strengthened by the Tobacco Act of 2010 [33]. The rate of smoking is 2.8% according to the 2011 report [33].

The most popular recreational substance use in Bhutan is chewing betel quid known as “doma”, betel nut and betel leaf with a dash of lime. According to the 2010 Gross National Happiness Survey, 72% of the population had ever chewed betel quid in their life and 58.5% of men and 61.5% of women were currently chewing betel quid [34]. Betel quid is the fourth most widely used psychoactive substance globally and is commonly used in Central, Southern, and South-east Asian countries, accounting for 600 million people worldwide or 10-20% of the world’s population [35, 36]. In its most basic form, betel quid consists of betel leaf, areca nut (the main psychoactive ingredient) and slaked lime (calcium hydroxide). While evidence of the carcinogenic risk of betel quid and areca nut alone is widely established, studies on the impact on adverse pregnancy outcomes are scarce and the quality of the studies is heterogeneous. This will be explored in detail in the next section.

(b) Infectious diseases

Some studies suggest that intrauterine infection may account for 25-40% of PTBs [25, 28, 37-39]. Although the exact magnitude of sexually transmitted infections in Bhutan is not known, syphilis prevalence among pregnant women screened during the antenatal care visits was 2.3% in 2007 [40]. In Bhutan, malaria has significantly declined with an incidence of 6.1 cases per 100,000 population in 2012 [41, 42]. A short cervix may induce intrauterine infection by shortening the distance between microorganisms in the lower genital tract and chorioamniotic membranes [43]. Maternal periodontal disease may be associated with increased risk of PTB and LBW through organisms such as *Fusobacterium nucleatum*, similar to those associated with genital tract infection and through production of inflammatory mediators such as cytokines and prostaglandins, known to be associated with the onset of labour and PTB [44-47]. A number of studies show that betel quid chewers may be predisposed to periodontal disease [48, 49]. In Bhutan, the prevalence of periodontal disease is unknown and screening and treatment of dental health is limited.

(c) Nutritional factors

Normal foetal growth and development depend on maternal nutritional intake and stores [24]. Thus, insufficient caloric intake, inadequate micronutrient intake including folate, iron, zinc, vitamins A, B6, B12, C, E and riboflavin, and low gestational weight gain during pregnancy are associated with LBW, SGA or PTB [24, 50-53]. Gestational weight gain comprises protein, fat, water, and minerals

deposited in the foetus, placenta, amniotic fluid, uterus, mammary glands, blood, and adipose tissue [54] and modifiable behaviours such as dietary intake and physical activity influence the amount of weight gained in pregnancy [55].

Anaemia, particularly iron-deficiency anaemia, may increase the risk for LBW and PTB [56-59]. A meta-analysis showed that maternal anaemia during early pregnancy increased the risk of PTB by 32% [60]. In Bhutan, traditionally, red rice, ema dates (chilli pepper and cheese stew) and suja (salted butter tea) are the national dishes and salt intake is believed to be high among the Bhutanese [61]. Although there are no publicly available data on vitamin A deficiency or anaemia, WHO estimated that 16.6% of pregnant women are deficient in vitamin A and 50% of pregnant women are anaemic [62, 63]. Iodized salt is widely consumed in Bhutan[64].

(d) Antenatal care (ANC)

The relationship between ANC (timing of first visit, frequency, and quality of care) and LBW is still uncertain [24, 65-68]. Routine ANC aims to deliver effective and appropriate screening, preventive, and treatment interventions [67]. Thus, some adverse outcomes could be prevented by early diagnosis and timely treatment of pregnancy complications or by eliminating or reducing modifiable risk factors [24]. Modifiable risk factors include smoking, alcohol consumption, genital tract infection, and caloric consumption. For instance, a recent Cochrane review found that antenatal nutritional advice, which aims to increase energy and protein intake in the general obstetric population, appears to be effective in reducing the risk of PTB, increasing head circumference at birth and increasing protein intake[66].

2.2.2 Modifiable risk factors in the long-run or un-modifiable risk factors

(a) Socioeconomic status

Different measures of socioeconomic disadvantage such as family income, maternal education, occupation, and marital status are reported to be associated with LBW, PTB or SGA [24, 69, 70]. A recent systematic review found that LBW, PTB or SGA were most prevalent among women in the most socioeconomically disadvantaged group [69]. These women may have limited access to basic sanitation, good nutrition, psychosocial support, and health services and may be more exposed to heavy workload during pregnancy, stress, heavy alcohol consumption and smoking. Furthermore, it may be more difficult for them to comply with health messages due to their social circumstances and lack of education.

In terms of socioeconomic variations in Bhutan, in the eastern region, more households are rural (85%) than in the western (80%) and central (70%) regions [71]. In the eastern region, lack of access to roads going to and from markets hampers commercial farming [72]. Moreover, the main agricultural product in the eastern region is maize, accounting for 72% of total maize production [72]. Although farm households consume rice in three to four meals a day, maize substitutes for rice in the eastern region [72]. From the public health perspective, few studies have

been conducted on the eastern region of Bhutan. Thus, this regional variation should be taken into account in the study design and analysis.

(b) Maternal pre-pregnancy weight, short birth spacing, very young maternal age

Considering maternal pre-pregnancy weight as a predictor for nutritional stores potentially available to the growing foetus, maternal underweight may be associated with LBW and PTB [24, 51]. Very young maternal age and short birth spacing may increase the risk of bearing a LBW infant. Very young maternal age may have a negative, biological impact on maternal growth and infant growth due to foeto-maternal competition for nutrients [73]. Closely spaced pregnancies do not allow mothers to fully recover the macro- and micro-nutrients necessary for the next pregnancy and may lead to LBW or PTB for mothers and infants [74].

(c) Maternal and obstetric factors

Potential maternal characteristics associated with PTB and LBW include ethnicity and race [24, 37, 75], and chronic medical conditions [24, 76, 77]. Previous history of adverse pregnancy outcomes such as PTB, intrauterine growth, abortion, neonate loss, and stillbirth are also reported to increase the risk of PTB, LBW, and SGA in a subsequent pregnancy [24, 78-80]. A meta-analysis reported that women with a previous preterm singleton birth at <37 weeks had an increased unadjusted risk for recurrent PTB compared with women with a previous term birth (unadjusted OR 5.43, 95% CI 4.03-7.31) using a random-effects model [80]. Paternal factors including age, height, and paternal LBW may be risk factors [81]. In addition, medically-indicated preterm deliveries are reported to account for 15-20% of all PTBs and the most common medical indications for preterm delivery are pre-eclampsia, foetal distress, SGA, and placental abruption [78, 82].

Nulliparity was associated with a significantly increased unadjusted risk of LBW or SGA birth but not with PTB, although this could be affected by confounders [24, 83]. A meta-analysis shows that unadjusted odds of LBW increased among nulliparous mothers compared to parous (parity 2-4) mothers by 41%. Prior history of PTB may increase the risk of PTB in the next pregnancy [28, 84]. One study reported mothers with a prior spontaneous PTB carried a 2.5-fold increase in the risk of spontaneous preterm delivery in the current gestation compared to mothers with no prior spontaneous preterm delivery [85]. PTB is more common in boys [56, 86] but girls weigh less than boys for the same gestational age [87].

Obesity may increase the risk of PTB and LBW as obese women are more likely to have infants with congenital anomalies, and or, develop pre-eclampsia and diabetes, which is likely to lead to provider-initiated preterm delivery [28, 88-90]. While micronutrient deficiency is still prevalent, Bhutan has experienced a recent increase in adult obesity. A population-based survey conducted in Bhutan between April and June 2014 analysing 1748 women aged 18-69 reported that the prevalence of obesity was 6.5% (95% CI 4.9-8.1) among women aged 18-39 and 12.1% (95% CI 8.7-15.4) among women aged 40-69 respectively [91]. A survey conducted in the capital of

Bhutan in 2008 analysing 1342 women aged 25-74 reported the prevalence of diabetes was 4.5% among women aged 25-34 and 4.5% among women aged 35-44 [85].

(d) Hypertensive disorders

Hypertension in pregnancy is a leading cause of maternal mortality and adverse birth outcomes [92]. Many efforts during antenatal care are made to detect and manage hypertensive disorders during pregnancy [93]. A meta-analysis of USA data showed chronic hypertension increased PTB and LBW 2.7 times [76]. The association between pre-eclampsia or gestational hypertension and poor foetal growth is inconclusive [2, 94-101] (Appendix B.3.).

Pre-eclampsia is defined as hypertension (diastolic blood pressure of ≥ 90 mm Hg) accompanied by proteinuria (≥ 300 mg or more per 24- hour period), and usually occurs during the second half of pregnancy (at or after 20 weeks' gestation) [95, 96]. Pre-eclampsia complicates 2%-8% of pregnancies [95-97, 102]. Women with moderate pre-eclampsia generally have no symptoms [95]. Women with severe pre-eclampsia, or with very high blood pressure, may feel unwell, with symptoms such as headache, upper abdominal pain, or visual disturbances [95]. One hypothesis of the pathogenesis of pre-eclampsia is that reduced placental blood flow hampers foetal growth with an increased risk of IUGR and LBW [98]. Moreover, PTB could be a result of treatment for pre-eclampsia [78].

The 2014 population-based survey reported that 9.1% of women aged 18-39 had been diagnosed with hypertension in last 12 months compared with 25.8% of women aged 40-69[91]. The prevalence of pre-eclampsia in Bhutan is not known.

(e) Psychosocial factors

Poor mental health and intimate partner violence are reported to be associated with LBW and PTB [56]. A meta-analysis suggests women who reported physical, sexual or emotional abuse during pregnancy were 40% more likely than non-abused women to give birth to a LBW baby [103]. Meta-analyses reported increased risk of LBW and prematurity due to maternal anxiety [104] and depression [105]. Association with IUGR is inconclusive [105]. One potential pathway is that maternal psychological stress/distress releases stress hormones such as cortisol and catecholamines, which results in placental hypo-perfusion and the consequent restriction of oxygen and nutrients to the foetus, which may lead to foetal growth impairment and or precipitation of PTB [105-108]. Although the prevalence of intimate partner violence in Bhutan is not known, the National Commission for Women and Children of Bhutan conducted an exploratory study in 2007 and showed that 574 out of 688 married women said that verbal conflicts were common in their marriage and 188 (32.8%) said that these verbal conflicts normally led to physical conflicts[109].

(f) Altitude

In addition to the above socioeconomic, behavioural, and biological factors, research findings suggest the negative impact of a high altitude on birth weight in South America (including Bolivia

and Peru), Tibet, and the USA [110-115] (Appendix B.3.). Altitude is one of the contextual factors that is particular to Bhutan. Bhutan's elevation varies about 160m above sea level in the south to more than 7500m above sea level in the north [116].

Although there is no precise definition of "high altitude", the majority of individuals experience certain clinical, physiological, anatomical, and biochemical changes above 3,000m with a huge individual variation [117]. A commonly used working definition is altitudes of 2,500 meters and above. It is estimated that 83 million people live above 2,500 meters, mostly between 2,500 m and 4,000m [117]. Only Asia and South America have sizable populations residing above 4,000m [117]. Indigenous populations have resided in the Tibetan, Andean and East African plateaus for 10,000 (Andeans) to 20,000 years (Tibetans) [118]. European or Han populations have lived at high altitudes for less than 500 years (less than 400 years in South America, less than 150 years in North America, and less than 50 years in western China) [119].

The unique stress at high altitude is hypobaric hypoxia. Other factors could be cold, aridity, solar radiation, diet, disease ecology, and life style [117]. For example, altitude may constrain agricultural production and increase the cost of transporting fresh food products, which may result in maternal nutritional deficiencies [111]. Hypoxia is a frequent complication of prenatal life. The chronic hypoxia at high altitudes was reported to be associated with a birth weight reduction. Research shows different patterns of birth weight reduction in relation to altitude within a range of less than 500g. Several studies show a linear relation between birth weight reduction and altitude [120]. Most of the evidence suggests that the decline in birth weight is curvilinear with the breakpoint occurring at about 2000m [118]. The entire distribution of birth weights is shifted to lower values [121].

When prolonged, hypoxia is associated with IUGR and increased perinatal mortality and morbidity [118]. Research shows that foetal growth starts to slow in the third trimester after 28-31 weeks' gestation, leading to a reduction in birth weight at high altitude.

One study in South America evaluated the marginal effect of altitude [111] and most of the studies compared the means of birth weight controlling for confounding variables in two communities at extremely low and high altitudes. No studies used multi-level analyses to account for neighbourhood effect. One study showed that birth weight declined an average of 102g per 3300 ft (1000m) elevation, increasing the percentage of LBW by 54% from the lowest to the highest elevations in Colorado, USA, after controlling for gestational age, weight gain, parity, cigarette consumption, number of ANC visits, and hypertensive complications of pregnancy [110]. Several studies suggest that the reduction in birth weight at altitude is largely attributable to IUGR rather than prematurity. A few studies suggest the presence of protective mechanisms against altitude-associated foetal growth retardation in Bolivians and Tibetans from prolonged high-altitude resident ancestry [115, 122, 123]. In past studies, ancestry has been identified using last names [122], place of birth [124], or self-identification confirmed by dress, language, and parental information [123] and was controlled for in the models to estimate the effect of altitudes on birth weight. The results suggest that birth weight reduction may be less severe in populations that have resided longer at

high altitudes. Birth weight declines by an estimated 88 or 89g per 1000m increase in elevation among Tibetan and Andean new borns but by 119 and 153 g per 1,000m for European and Han Chinese new borns respectively [123]. The physiology behind this protection and whether these mechanisms are at the maternal and/or placental and/or foetal level remain unknown.

Several studies shows that the incidence of pre-eclampsia is increased at high altitude and this is likely to contribute to the altitude-associated birth weight decline [110, 114, 120, 122]. Pre-eclampsia and gestational hypertension were 1.7 times (95% CI: 1.3-2.3) more frequent at high altitude in Bolivia comparing 300m and 3600m [114] and 3.6 times more frequent (95% CI: 1.1-11.9) in Colorado comparing 1260m and 310 m [120].

(g) Season

Several systematic reviews reported seasonal patterns of adverse pregnancy outcomes in both high income countries and low/middle income countries [125-127]. The time of birth than the time of conception were more documented as an exposure in the past studies[125]. The seasons that are associated with an increased risk of adverse pregnancy outcomes vary depending on countries due to geographical and climate variations. Seasonal patterns of adverse pregnancy outcomes may be explained by seasonal patterns in food availability and infection, or exposure to cold temperature, humidity, or sunlight[127]. For example, exposure to cold temperatures during mid-gestation could compromise placental blood flow and may lead to lower birth weight[128]. Bhutan has four seasons and seasonal rainfall with monsoon rains occurring from June to September [42]. Seasonal food insecurity and hunger due to shortage of grain from May to July is often observed in the eastern region [129].

(h) Other environmental factors

Other environmental factors include indoor air pollution from solid fuel use. A systematic review suggests that such pollution is associated with increased risk of percentage LBW by 38% and stillbirth and reduced mean birth weight [27]. In Bhutan, 53.6% of the household population in the rural areas use solid fuel [71].

(i) Neighbourhood effect

The neighbourhoods in which people live may influence health through the availability and accessibility of health services, healthy foods, clean water, social support, the prevailing attitudes towards health and health related behaviours, and environmental pollution [130, 131]. One of the early studies which analysed the impact of macro-level factors on LBW suggested that multilevel models should be used in future analyses of maternal and child health outcomes [130]. The study examined both neighbourhood-level and individual-level risk factors for LBW by combining individual data from the city health department's Bureau of Biostatistics and census tract level data including per capita income and information on household wealth, home ownership, number of

housing violations issued and per capita crime data between 1985 and 1989 in Baltimore, USA (n=50,757). The study indicated that there is substantial interaction between macro-level factors and individual-level risk factors. A review of neighbourhood effects on health, which reviewed 25 studies from developed countries before June 1998, found that 23 of the 25 studies reported a statistically significant association between at least one neighbourhood measure of socioeconomic status and health, controlling for at least individual socioeconomic status [131]. Although there is a chance of overestimation of the effect of neighbourhood due to inadequate control of individual socioeconomic status, it suggests the importance of investigating neighbourhood level effects. In Bhutan, there is a cultural diversity across the regions and where mothers live can determine accessibility to health services.

2.2.3 Relevance to Bhutan

The review of the literature suggests that PTB and LBW can be caused by a broad range of socioeconomic, behavioural, biological, and environmental factors. Regarding potentially modifiable risk factors particularly relevant to Bhutan, there is a knowledge gap in the literature on the impact of betel quid chewing on PTB and LBW. Considering the high prevalence rate of this habit in the Bhutanese population, there is a need for further studies to examine its impact on pregnancy outcomes.

Table 2.2. Summary of systematic reviews of determinants of LBW, PTB and SGA.

	Outcomes	Author	Review method	Summary of the key findings
Overall summary	Determinants of LBW	Kramer (1987) [24]	Methodological assessment and meta-analysis (1970-1984)	43 determinants of LBW were analysed from 895 published papers in the English and French literature from 1970-1984. In developed countries, the most important factor was cigarette smoking, followed by nutrition and pre-pregnancy weight. In developing countries the major determinants were racial origin, nutrition, low pre-pregnancy weight, short maternal stature, and malaria. For gestational duration, pre-pregnancy weight, prior premature birth or miscarriage, diethylstilbestrol exposure and smoking were the major determinants, but the majority of PTBs were unexplained in both developed and developing countries.
Modifiable in the short run	Alcohol consumption	Patra et al. (2011) [132]	Systematic review of case-control or cohort studies (1 January 1980-1 August 2009)	36 studies were included. Compared with abstainers, the overall dose-response relationships for LBW and SGA showed no effect up to 10 g pure alcohol/day (an average of about 1 drink/day) and PTB showed no effect up to 18 g pure alcohol/day (an average of 1.5 drinks/day); thereafter, the relationship showed a monotonically increasing risk of LBW and SGA for increasing maternal alcohol consumption. The risk of PTB for mothers who consumed more than three alcoholic drinks per day increased by 23%, compared to nondrinking mothers (RR 1.23, 95% CI 1.05-1.44).
	Smoking	Lumley et al. (2009) [133]	Intervention review of randomised controlled trials (searched in June 2008)	Smoking cessation: The 21 trials to promote smoking cessation in pregnancy with information on perinatal outcomes revealed a reduction in LBW (RR 0.83, 95% CI 0.73 - 0.95), a reduction in PTB (RR 0.86, 95% CI 0.74 -0.98), and an increase in mean birth weight of 39.26 g (95% CI 15.77 g - 62.74 g) in the treatment group.
		Shah and Bracken (2000) [29]	Systematic review and meta-analysis of prospective studies (1966-1997)	Maternal smoking: 20 prospective studies were included. Most studies controlled for maternal age, race, gravidity, parity, income, and other social and demographic factors. The pooled OR of PTB for any maternal smoking during pregnancy versus no smoking is 1.27 (95% CI 1.21-1.33).
	Smokeless tobacco (SLT)	Suliankatchi and Sinha (2016) [134]	Systematic review and meta-analysis (31 July 2015)	2 cohort studies conducted in India were included. One study failed to control for smoking. SLT was statistically associated with LBW (OR 1.88, 95% CI 1.38-2.54); PTB (OR 1.39, 95% CI 1.01-1.91); and still birth (OR 2.85, 95% CI 1.61-5.01).
		Inamdar et al. (2014) [135]	Systematic review (searched in July 2013)	9 studies (7 cohort studies, 1 case-control study and 1 cross-sectional study from Asia (6 studies), Sweden (1), USA (1), and South Africa (1) were included. Significant associations with SLT use were seen in 5/7 studies for LBW, in 3/6 studies for preterm, in all 4 studies for still birth and in 1/2 studies assessing SGA. 3 studies did not report any confounding factors. Studies of oral forms of khat and betel quid without tobacco were excluded.
	Caffeine intake	Chen et al. (2014) [31]	Systematic review (searched on 17 July 2013)	13 prospective studies were included from HICs. Caffeine was statistically significant with LBW (50-149 mg/day: RR 1.13, 95% CI 1.06-2.08; 150-349 mg/day: RR 1.38, 95% CI 1.18-1.62; and ≥350 mg/day: RR 1.60, 95% CI 1.24-2.08 compared to <50 mg). In the dose-response analysis,

			each 100-mg/day increment in maternal caffeine intake was associated with 13% increased risk of LBW (RR 1.13, 95% CI 1.06-1.21).
Maternal infectious disease	Gomez et al. (2013) [37]	Systematic review and meta-analysis (searched in December 2011)	Syphilis: 6 studies were included. Random-effects meta-analyses found that prematurity or LBW are 5.5% more frequent among untreated pregnant women with syphilis than among women without syphilis.
	Corbella et al. (2012) [47]	Systematic review and meta-analysis (searched in January 2011)	Periodontal disease: 17 case-control studies were included (10,148 women). The fixed effect models show that periodontal disease was associated with an increased odds of PTB (OR 1.78, 95% CI 1.58-2.01, 14 studies), LBW (OR 1.82, 95% CI 1.51-2.20, 7 studies), and preterm LBW (OR 3.00, 95% CI 1.93-4.68, 3 studies).
	George et al. (2011) [44]	Systematic review and meta-analysis (searched in 2010)	Periodontal treatment: 10 randomised trials were included (5,645 women, 1 study from India). Using the random-effects model, the periodontal treatment significantly decreased the risk of PTB (OR 0.65, 95% CI 0.45-0.93) and LBW (OR 0.53, 95% CI 0.31-0.92).
	Leitch and Kiss (2007) [38]	Systematic review and meta-analysis (updated on May 2005)	Bacterial vaginosis: 32 studies were included for meta-analysis (30,518 women). Bacterial vaginosis was associated with preterm delivery in asymptomatic patients (OR 2.16, 95% CI 1.56-3.00) and in patients with symptoms of preterm labour (OR 2.38, 95% CI 1.02-5.58).
Micronutrient supplementation	Haider and Bhutta (2015) [136]	Systematic review and meta-analysis (searched on 11 March 2015)	Multiple micronutrients supplementation (MMN): 17 trials (137,791 women: 15 trials from LMIC) were included. MMN resulted in a significant decrease in LBW (RR 0.88, 95% CI 0.85-0.91), SGA (RR 0.90, 95% CI 0.83-0.97) but not with PTB (RR 0.96, 95% CI 0.89 - 1.03) using the fixed effect models.
	Lassi et al. (2013) [137]	Intervention review (searched on 21 December 2012)	Folic acid supplementation: 4 studies were included (3,113 participants) for birth weight. Folic acid supplementation during pregnancy did not show any impact on reducing LBW (RR 0.83, 95% CI 0.66-1.04). 3 studies were included (2,959 participants) for PTB. Administration of folic acid supplementation during pregnancy has no impact on reducing PTB (RR 1.01, 95% CI 0.73 - 1.38).
	Kawai et al. (2011) [52]	Meta-analysis and meta-regression (searched on 1 August 2010)	MN: 15 trials were included. Fixed-effect meta-analyses found that pregnant women who received micronutrient supplements were less likely to deliver LBW infants (RR 0.86, 95% CI 0.79-0.93) or SGA infants (RR 0.85, 95% CI 0.78-0.93) than women who received iron and folic acid supplements. Micronutrient supplementation had no effect on PTB (Pooled RR 0.99, 95% CI 0.95-1.03).
Gestational weight gain	McDonald et al. (2011) [50]	Systematic review and meta-analysis (1950-2 January 2009)	Gestational weight gain (GWG): 28 studies were reviewed (2,124,907 women). High GWG was associated with lower risk of LBW (RR 0.64, 95% CI 0.53-0.78) using 25 to 35 lbs as the reference GWG. Women with high GWG had a decreased risk overall of PTB < 37 weeks (RR 0.75, 95% CI 0.60 - 0.96), PTB 32 to 36 weeks (RR 0.70, 95% CI 0.70 - 0.71), and < 32 weeks (RR 0.87; 95% CI 0.85 - 0.90). However, women with high weekly GWG were at increased risk of PTB. Women with the highest weekly GWG had greater risks of PTB (RR 1.51, 95% CI 1.47 - 1.55) than women with moderately high weekly GWG (RR 1.09, 95% CI 1.05- 1.13). Women with high weekly GWG

			were at increased risk of PTB 32 to 36 weeks (RR 1.14, 95% CI 1.10-1.17 and < 32 weeks (RR 1.81, 95% CI 1.73 - 1.90).
Anaemia	Haider et al. (2013) [59]	Systematic review and meta-analysis (PubMed:1966 -1 May 2012, Embase:1974 - 31 May 2012)	Anaemia: 48 randomised trials (17,793 women: 21 studies from LMICs) and 44 cohort studies (1,851,682 women: 22 studies from LMICs) were included. In analyses of cohort studies, anaemia (defined as Hb<100g/L to <115g/L) was associated with an unadjusted OR of LBW (1.25, 95% CI 1.08-1.45) and adjusted OR of PTB (1.28, 95% CI 1.12-1.47) but not adjusted OR of LBW (1.13, 0.94-1.35), using random effects.
	Kozuki et al. (2011) [57]	Systematic review and meta-analysis (searched in February 2011)	Anaemia: 7 studies were included for meta-analysis. Moderate to severe maternal anaemia (the 90< or <80-g/L) was associated with a 53% increase in risk of the new born being SGA (pooled OR 1.53, 95% CI: 1.24-1.87). Mild anaemia (the <110- and <100-g/L category) showed no significant relationship with SGA.
Work activities	Palmer et al. (2013) [138]	An updated review of meta-analysis (1966-2011)	56 studies were reviewed for birth weight. Any risks from long working hours, shift work, prolonged standing, heaving lifting and high physical workload associated with LBW are at most small.
Antenatal care (ANC)	Carroli et al. (2001) [67]	Systematic review of randomised trials (searched in June 2000 and updated in December 2000)	Number of ANC: 7 studies were included (57,418 women). There was no clinically differential effect of a reduced number of antenatal visits when the results were pooled for LBW (OR 1.04, 95% CI 0.93-1.17).
	Hodnett et al. (2010) [68]	Intervention review randomised controlled trials (searched on January 2010)	Additional social support during ANC: 17 trials (12,264 women) were reviewed. Programs offering additional social support for at-risk pregnant women were not associated with improvements in any perinatal outcomes. 11 trials (10,429 women) were included for PTB (RR 0.92, 95% CI 0.83-1.01). 11 trials (8681) were included for LBW (RR 0.92, 95% CI 0.83-1.03). However, there was a reduction in the likelihood of antenatal hospital admission (three trials; n = 737; RR 0.79, 95% CI 0.68 - 0.92) and caesarean birth (nine trials; n = 4522; RR 0.87, 95% CI 0.78 - 0.97).
	Ota et al. (2012) [66]	Intervention review of randomised controlled trials (search updated on 12 July 2012)	Nutritional advice during ANC: 4 trials (790 women) of nutritional advice were reviewed. Women given nutritional advice had a lower relative risk of PTB (two trials, 449 women) (RR 0.46, 95% CI 0.21 - 0.98), head circumference at birth was increased in one trial (389 women) (mean difference (MD) 0.99 cm, 95% CI 0.43 - 1.55) and protein intake increased (three trials, 632 women) (protein intake: MD +6.99 g/day, 95% CI 3.02 - 10.97). No significant differences were observed in any other outcomes.
Modifiable in the long run or unmodifiable factors	Socioeconomic measures	Ruiz et al. (2015)	Meta-analysis of prospective cohort studies (April 1983- October 2006)
			Mother's education: 12 prospective cohort studies (75296 births between April 1983 and October 2006) in Europe were included. The excess risk of PTB associated with low maternal education was 1.48 (95% CI 1.29-1.69) when using the prevalence ratio of the child outcome between children at the lowest and those at the highest end of the maternal education hierarchy and 1.84

(95% CI 0.99-2.69) when using the prevalence difference of the child outcome between the two ends, adjusting for child sex, maternal age, and ethnicity.

	Shah et al. (2011) [70]	Systematic review and meta-analysis (until April 2010)	Marital status: 21 studies were included. Unadjusted odds of LBW were increased among unmarried (OR 1.46, 95% CI 1.25-1.71), single (OR 1.65, 95% CI 1.44-1.88) and cohabitating (OR 1.29, 95% CI 1.25-1.32) mothers, compared to married mothers. PTB was increased among unmarried (OR 1.22, 95% CI 1.14-1.31), single (OR 1.54, 95% CI 1.39-1.72) and cohabitating (OR 1.15, 95% CI 1.08-1.23) mothers. SGA birth was increased among unmarried (OR 1.45, 95% CI 1.32-1.61), single (OR 1.70, 95% CI 1.47-1.97) and cohabitating (OR 1.36, 95% CI 1.30-1.42) mothers.
	Blumenshine et al. (2010) [69]	Systematic review (1999-2007)	Socioeconomic disadvantage: 106 studies in industrialized countries defined by OECD were included. Socioeconomic disadvantage was consistently associated with increased risk of LBW, PTB or SGA across socioeconomic measures and countries. Many studies observed racial/ethnic differences in the effect of socioeconomic measures.
Parity	Shah et al. (2010) [83]	Systematic review and meta-analysis (until October 2009)	41 studies were included. Unadjusted odds of LBW increased among nulliparous mothers compared to parous (parity 2-4) mothers (OR 1.41, 95% CI 1.26-1.58). Nulliparity was associated with increased unadjusted odds of SGA (OR 1.89, 95% CI 1.82-1.96), but not with PTB (<37 weeks) (OR 1.13, 95% CI 0.96-1.34).
Early age at first childbirth	Gibbs et al. (2012) [73]	Systematic review and meta-analysis (searched on 31 January 2011)	20 studies were reviewed. The evidence suggests the effect on LBW for very young maternal age (<15 years or 2 years post-menarche) is moderate. Meta-analysis of 9 studies indicates moderate association between young maternal age and PTB (OR 1.68, 95% CI 1.34-2.11).
Birth spacing	Kozuki et al. (2013) [139]	Systematic review and meta-analysis (searched in February 2009)	Birth intervals (the time between the previous and index live birth): short (<18 months) and long (> 60 months): 5 cohort studies from LMICs were included. Short IPIs are associated with PTB (aOR 1.58, 95% CI 1.19-2.10) and SGA (aOR 1.51, 95% CI: 1.31-1.75) using the random-effects model. Long IPIs (>60 months) was associated with SGA (aOR 1.14, 95% CI 1.07-1.39) but not with PTB.
	Wendt et al. (2012) [74]	Systematic review and meta-analysis	Short inter-pregnancy intervals (the time between birth and conception) (<12 months): 5 studies were reviewed for the meta-analyses. Short intervals are associated with LBW (<6 m adjusted OR 1.44, 95% CI 1.30-1.61, 6-11 m adjusted OR 1.12, 95% CI 1.08-1.17). The evidence suggests significant impacts of short inter-pregnancy intervals on extreme PTB (<6 m adjusted OR 1.58, CI 1.40-1.78, 6-11 m adjusted OR 1.23, 95% CI 1.03 -1.46) and moderate PTB (<6 m adjusted OR 1.41, 95% CI 1.20-1.65, 6-11 m adjusted OR 1.09, 95% CI 1.01-1.18).
Maternal underweight and obesity	Han et al. (2010) [51]	Systematic review and meta-analysis (1950-2 January 2009)	Maternal underweight: 78 studies were reviewed (1,025,794 women). In both developed and developing countries, underweight women were at increased risk of having an LBW infant (RR 1.48, 95% CI 1.29-1.68, and RR 1.52, 95% CI 1.25-1.85, respectively). The overall risk of PTB was increased in the cohort studies of underweight women (adjusted RR 1.29, 95% CI 1.15-1.46)

			with adjusted RR 1.32 (95% CI 1.10-1.57) for spontaneous PTB and adjusted RR 1.21 (95% CI 1.07-1.36) for induced PTB.
	McDonald et al. (2010) [140]	Systematic review and meta-analysis (1950 – 2 January 2009)	Maternal overweight or obesity: 84 studies were included (1,095,834 women), predominantly from developed countries. The definition of exposure varied across the studies. There was no statistically higher risk of PTB for overweight or obese women with singleton pregnancies (RR 1.06, 95% CI 0.87-1.30, 38 studies) whereas the risk of induced PTB increased (RR 1.30, 95% CI 1.23-1.37, 5 studies). Overweight and obesity was associated with a decreased risk of having an infant of LBW (RR 0.84, 95% CI 0.75-0.95, 28 studies). The decrease was higher in developing countries (RR 0.58, 95% CI 0.47-0.71, 11 studies, 4,710 women) compared to developed countries. After accounting for publication bias, the protective effect of overweight and obesity on LBW disappeared (RR 0.95, 95% CI 0.85-1.07) whereas the risk of PTB appeared to increase in overweight and obese women (RR 1.24, 95% CI 1.13-1.37).
Maternal chronic diseases	Bramham et al. (2014) [76]	Systematic review and meta-analysis (searched in June 2013)	Chronic hypertension: 55 studies (795,221 women) were included (mostly from HICs, 4 studies from Asia: India, China, Taiwan, and Japan). In meta-analyses of US studies (22 studies) using the random-effects model, chronic hypertension increased PTB (2.7, 95% CI 1.9-3.6) and LBW (2.7, 95% CI 1.9-3.8), compared to the USA national population dataset.
Maternal obstetric history	Kazemier et al. (2014) [80]	Systematic review and meta-analysis (searched on 8 January 2013)	History of previous preterm singleton: 6 studies were included (4 USA, 1 Taiwan and 1 Denmark). Women with a previous preterm singleton birth at <37 weeks had an increased risk for recurrent PTB compared with women with a previous term birth (unadjusted OR 5.43, 95% CI 4.03-7.31) using the random-effects model.
Psychosocial stress, anxiety, and abuse	Wosu et al. (2015) [141]	Systematic review (1992-2010)	Maternal history of childhood sexual abuse (CSA): 6 studies from developed countries (4 USA, 1 Norway, 1 Germany) were included. The associations of maternal history of CSA and PTB were inconclusive. 3 studies reported statistically significant associations and 3 studies did not observe statistically significant differences.
	Ding et al. (2014) [142]	Systematic review and meta-analysis (up to June 2013)	Maternal anxiety: 12 (17,304 women from prospective cohort studies in HICs) studies for PTB and 6 studies (4,948 women) for LBW were included. Maternal anxiety during pregnancy was significantly associated with an increased risk of PTB (RR 1.50, 95% CI: 1.33-1.70) and LBW (RR 1.76, 95% CI: 1.32-2.33) using the fixed effects model.
	Grote et al. (2010) [105]	Systematic review and meta-analysis (January 1980-December 2009)	Maternal depression: 29 studies were included (12 non-USA countries including 2 LMICs). Using the random effects models, maternal depression was statistically significantly associated with LBW (RR 1.18, 95% CI 1.07-1.30), PTB (RR 1.13, 95% CI 1.06-1.21), but not with IUGR (RR 1.03, 95% CI 0.99-1.08).
	Murphy et al. (2001) [103]	Systematic review and meta-analysis (1966-1999)	Maternal physical, sexual or emotional abuse during pregnancy: 8 studies were selected for meta-analysis including a few studies that did not adjust for confounders. Fixed-effect meta-analyses

			found that women who reported physical, sexual or emotional abuse during pregnancy were more likely than non-abused women to give birth to a LBW baby (OR 1.4, 95% CI 1.1–1.8).
Paternal factors	Shah et al. (2010) [81]	Systematic review (searched on March 2009)	36 studies were included for a systematic review with qualitative synthesis. Paternal age, height, and birth weight may be associated with LBW.
Indoor air pollution	Pope et al. (2010) [27]	Systematic review (1966-2008)	5 studies were reviewed for LBW. Fixed-effect meta-analyses found that indoor air pollution from solid fuel use was associated with increased risk of percentage LBW (OR 1.38, 95% CI 1.25-1.52)
Agricultural pesticide	Shirangi et al. (2011) [143]	Systematic review (1950-2007)	25 studies were reviewed. The strength of evidence for an association between living near agricultural pesticide applications and adverse reproductive outcomes is generally weak although it may be an important source of ambient environmental exposure. The exposure measurements and outcome measures need to be improved.
Neighbourhood effect	Pickett and Pearl(2001) [131]	Systematic review (before June 1998)	25 studies from developed countries (13 USA, 9 UK, 2 Netherlands, and 1 Finland) which adjusted for at least one individual level socioeconomic status were reviewed. 16 studies used single level linear and logistic regression analysis and Cox proportional hazards models to estimate the impact of neighbourhood factors on health and 9 used multilevel models. 23 out of 25 studies reported a statistically significant association between at least one neighbourhood measure of socioeconomic status and health, controlling for individual socioeconomic status. 3 studies only controlled for one measure of individual level socioeconomic status, which may lead to overestimation of neighbourhood level effect as proxies for unmeasured aspects of individual socioeconomic status.

2.3 The impact of betel quid chewing on pregnancy

In order to understand prior studies on the impact of betel quid chewing on pregnancy and help design the present study, relevant literature was systematically searched in MEDLINE and EMBASE with no date restriction in January 2014 and updated in March 2016. No language restriction was applied. There was no study type restriction. *For areca, the following keywords were used: exp Areca, Areca*, betel*, Supari, Puwak, Mak, Gua, Pinang, Daka, Piper, Catechu, Catechu*, OR doma. For infant outcomes, exp Premature Birth, exp infant, low birth weight/ or exp infant, small for gestational age/ or exp infant, very low birth weight/ or exp infant, premature/ OR low birth weight*, preterm birth*, OR small for gestational age OR intrauterine growth were used.* In total, abstracts of 530 articles were screened for possible relevance to developing countries, Southeast or South Asia, or Bhutan. The reference list was manually searched and other academic papers were searched in Google scholar and PubMed. All papers that referred to the relationship between adverse pregnancy outcomes and betel quid chewing in humans were assessed. As a result, 10 research articles and two case reports were identified and reviewed in depth. Issues on the measurement, potential causal pathways, strengths of an association, and confounders identified in the literature were summarised in narratives. A meta-analysis was conducted to estimate the crude odds ratio from each study and the pooled estimate using the random effect model. Table 2.5 summarises key findings from 10 observational studies and two case reports.

2.3.1 Overall description of the studies

Findings from the 10 observational studies and two case reports show that the impact of betel (areca nut) quid chewing (BQ) during pregnancy is inconclusive. The pregnancy outcomes and indicators of maternal health status that have been described in the literature in association with BQ include LBW, birth weight, PTB, birth length, infant gender ratio, and maternal anaemia and folate deficiency. Studies have been conducted in PNG [144-146], Bangladesh [147], India [148, 149], the Thai-Myanmar border [150, 151], and Taiwan [152-154]. The sample size of studies varied from 186 [154] to 7,685 [151]. All the studies except for the two case reports used interviewer-administered questionnaires to obtain use and patterns of BQ. Only Yang et al. (1999, 2001, 2008) [152-154] in Taiwan validated the questionnaire using test-retest reliability.

One study from India combining BQ with smokeless tobacco [148, 149] and five studies measuring BQ independently from smoking in Taiwan [152-154] and PNG [144, 145] reported an association between BQ and LBW and PTB and reduction in birth weight. On the other hand, two studies with more than 1,000 participants did not report any evidence of association [146, 151].

The observational studies which reported an association between BQ, independently measured from smoking, and LBW and/or PTB and reduction in birth weight [144, 145, 152-154] suggest that the magnitude of this association is modest.

2.3.2 A summary of key findings on the associations between BQ and adverse birth outcomes

In Taiwan, the unripe areca nut, slaked lime, and a piece of unripe fruit from the species *Piper betel* are the most common ingredients in betel quid [153, 155]. Tobacco is not added to BQ [155]. Yang and colleagues [152-154] conducted several studies to assess the risk posed by BQ on adverse birth outcomes among aborigines in Taiwan using a questionnaire validated by test-retest reliability [154]. All the three studies reported a statistically significant association between BQ and pregnancy outcomes. Most recently, a 2008 study collected detailed information about BQ and reported a statistical significant impact of BQ on birth weight loss and LBW among aboriginal women. This was a retrospective study conducted with a total of 1,264 aboriginal women who had just given birth in 10 hospitals in southern and eastern Taiwan between January 2003 and February 2004. Trained nurses administered a questionnaire to collect the data including a detailed history of consumption of betel quid, alcohol, cigarettes, over-the-counter drugs and illicit drugs with regard to frequency and amount consumed both pre-pregnancy and during pregnancy before the mothers were discharged from their respective hospitals. Betel quid chewers were defined as women who had ever used betel nut at any stage during pregnancy including those who subsequently abstained. Non-users were defined as those who had never chewed betel quid during the period of pregnancy. The prevalence rate of betel quid chewing during pregnancy was 36.7% with a daily average of 5.68 quids consumed among the chewers [153]. The chewers were more likely to have a lower educational level, and be unmarried, or unemployed ($p < 0.0001$). Chewers tended to smoke more than non-chewers (non-chewers: 13.4% [107/800] vs chewers: 42.67% [198/464], $p < 0.0001$) and drank more (non-chewers: 12.3% [98/800] vs 53.0% [246/800], $p < 0.0001$). Pre-pregnancy BMI was higher among chewers (non-chewers: 22.3 vs 24.4, $p < 0.0001$) whereas maternal gestational weight gain during pregnancy was lower among chewers (non-chewers: 15.3 kg (SD=6.1) vs 13.6 kg (SD=7.1), $p < 0.0001$). Maternal betel quid use is significantly associated with a loss of 89.54 g in birth weight ($p = 0.0028$) and a reduction of 0.43 cm in birth length ($p = 0.0466$) after adjusting for alcohol, cigarettes, drugs, maternal age, marital status, education level, employment status, BMI, gestational weeks, weight gain during pregnancy and newborn sex. Compared to mothers who did not drink, smoke or chew betel quid, chewers had a higher risk of delivering a LBW newborn (aOR 2.40, 95% CI 1.21–4.80) adjusting for maternal age, marital status, education level, employment status, gestational weeks, parity, weight gain during pregnancy, BMI, maternal drug use and newborn sex. The study did not find a statistically significant association between preterm delivery and BQ in the univariate analysis ($p = 0.0628$).

Senn et al. (2009) investigated the sociological behaviours associated with betel nut use, and its effects on adverse pregnancy outcomes in PNG [144]. In PNG, unripe or uncured ripe areca nut is chewed, sometimes with betel leaf, betel inflorescence or wild ginger and tobacco is never added [155]. A total of 310 of mothers were recruited and interviewed by a trained nurse using a semi-structured questionnaire including the frequency and amount consumed during pregnancy, the reasons for chewing and understanding of the risks and benefits of chewing, during the 3 days following delivery at a health center. Women who ever chewed during pregnancy were included as

users including those who stopped during the pregnancy. The prevalence rate of “users” was 94% with 44% of the users chewing more than 5 nuts a day. There were no demographic differences between chewers and non-chewers. Smoking was 10% among chewers and 0% in non-chewers but not statistically significantly different. Unlike this study, drinking alcohol was less than 1%. After controlling for primigravidity, low BMI of mothers, BQ was associated with an average reduction of birth weight of 238g ($p=0.02$). The study did not find a statistically significant association between BQ and LBW (OR 1.9, 95% CI 0.4-17) or preterm delivery (OR 0.6, 95% CI 0.1-5.4). The small sample size led to the wide confidence intervals and high prevalence of betel quid limits the validity of the findings from this study. Prior to this study, a matched case-control study conducted between April and June 1981, reported that mean birth weight was 2998.5g (SD=492.5) for daily users ($n=400$) and 3079.5g (SD=464.1) among non-users($n=400$) ($0.01 < p < 0.02$) [145].

On the other hand, Ome-Kaius et al. (2015) assessed the impact of BQ on adverse pregnancy outcomes including stillbirth, LBW and anaemia at delivery in a longitudinal cohort of 2700 pregnant women residing in rural lowland PNG between November 2009 and February 2013 [146]. The researchers questioned whether women chewed or not, the frequency of chewing, and the amount of BQ per day (less than one nut per day, 1-2 nut per day, 3-5 nut per day, more than 6 nuts per day) at enrolment. However, the study assumed chewing patterns as reported at baseline would continue during pregnancy and this can lead to underestimating the impact of BQ if mothers quitted chewing BQ after they found out about their pregnancy. The study reported 47.8% chewed more than 3 nuts per day. It did not report evidence of associations between betel quid chewing and LBW in the multivariate logistic regression models adjusting for smoking, malaria, ethnic group (highlander), income, primigravida, mother’s height, mother’s mid-upper arm circumference, frequency of ANC visits and receipt of insecticide treated bed net (aOR 0.94, 95% CI: 0.65-1.38, p -value=0.77).

Prior to this study, a large retrospective cohort study was conducted with a total of 7685 pregnant women attending ANC on the Thai-Myanmar border between July 1997 and November 2006 [151]. This study also measured BQ at the first ANC and reported no statistical association between BQ and adverse pregnancy outcomes. The proportions of heavy betel quid use (defined as daily or more than once a day) was 23.7% (1174/4963) and was significantly higher amongst women who also smoked (31.5%, 782/2479) compared to non-smokers (15.8%, 392/2484). There was no information on maternal education level or socioeconomic status of the study participants. The study also did not provide evidence of associations between BQ and adverse birth outcomes in the multivariate analysis adjusting for primigravida, first ANC visit after the first trimester, malaria, smoking, gestational age at birth (weeks), and maternal weight at enrolment. The adjusted odds ratio was not provided in the original manuscript. The crude odds ratio calculated with the data provided is 0.92 (95% CI: 0.80-1.06, p -value=0.264).

Two studies reported an association between BQ and maternal anaemia [146, 150]. Ome-Kaius et al. (2015) reported pregnant chewers were more likely to be anaemic (haemoglobin $< 11\text{g/dL}$) at delivery than non-chewers (aOR 1.67, 95% CI: 1.27-2.20, $p < 0.001$) [146]. Prior to this

study, two studies in PNG reported no association between anaemia and BQ. Senn et al. (2009) reported that mean haemoglobin level was 94 g/l (95% CI 92-96), slightly lower among chewers and 100 g/l (95% CI 95-106) among non-chewers but not statistically significant[144]. This could be due to the small sample size and high prevalence of betel chewing (94%). De costa (1982) reported that slightly more chewers (48%, 192/400) had a haemoglobin value of less than 10 g/100ml on at least one occasion compared to non-chewers matched on parity and province of origin (46%, 184/400) but the difference was not statistically significant. This could be due to baseline differences of chewers and non-chewers[145]. Chue et al. (2012) reported that chewers were more anaemic (defined as Haematocrit <30%) than non-chewers (non-chewers:17.3% [425/2,459] vs chewers: 19.4% [868/4,422], p=0.031) in the univariate analysis but no association was apparent after controlling for smoking, malaria, multigravida, anaemia at first ANC visit, and first ANC visit after the first trimester[151]. Anaemia was treated at each ANC visit. However, using the same population, Stuetz et al. (2016) reported that daily BQ had a negative effect on haemoglobin level(g/L) after adjusting for smoking, parity and BMI at the time of sampling (Beta -2.90., 95% - 4.62 to -1.16)[150]. Anaemia treatment was not controlled for. In the Chue et al. (2012) study, over-adjustment of covariates in the models may bias the estimate towards the null, especially by controlling the factors in the causal pathway between BQ and anaemia[151].

One study reported an association between BQ and folate deficiency [147]. Using data from 730 pregnant women aged 14-50 in a large randomised control trial of food and multiple micronutrient supplementation in Matlab, Bangladesh, Kader (2016) examined folate deficiency at 14 weeks of gestation. Women were asked about their consumption of BQ during their last pregnancy at the time of their postpartum visit. Almost two-thirds (61% [376/730]) of women consumed BQ during their last pregnancy. Of the 376 chewers, 10.7% women consumed 2-3 times per day, 13.4% consumed once per day, and 5.6% consumed less than once a day. Dry nuts were more commonly consumed than fresh nuts (dry 57.7 % vs fresh 3.6%, denominator unknown). Less than 10% (9.8%, denominator unknown) of women added chewing tobacco to the quid. Adjusting for calculated asset score, women's age, vitamin B-12 status and literacy, the women who consumed betel nut with chewing tobacco were 2.57 times more likely to have folate deficiency compared to the non-chewers (aOR 2.57, 95% CI 1.23-5.36; P=0.012). BQ use two to three times per day (regardless of whether combined with tobacco or not) was significantly associated with folate deficiency among users compared to non-users adjusting for calculated asset score, women's age, vitamin B-12 status, and literacy (aOR 2.51, 95% CI 1.07-5.92, P=0.035). There was no information as to whether this chewing pattern during the last pregnancy changed during the current pregnancy.

Ome-Kaius et al. (2015) reported that chewers more commonly had male babies than non-chewers (non-chewers: 39.8% [123/309] vs chewers: 46.1% [670/1455], p=0.045) while Yang et al. (2008) reported a reduced male new born rate (non-chewers: 56.4% vs chewers: 48.7%, p=0.0084) [153]. Yet, Chue et al. (2012) did not find sufficient evidence of associations between

betel chewing and the sex of new borns (non-chewers: 51.8% [1080/2083] vs chewers: 53.3% [2346/4401], p-value not reported).

Two case reports documented neonatal withdrawal syndrome and the presence of arecoline in the placenta among Asian immigrant mothers in Spain [156, 157].

The majority of the studies did not describe dose-response while one study explored dose-response in relation to folate deficiency [147]. Two main methodological limitations were identified in the studies: measurement of exposure and insufficient control of the confounders. A number of research questions could be formulated for further understanding of the impact. For example, whether timing of exposure during pregnancy (1st trimester vs 3rd trimester) or the amount of exposure would make a difference. If there is a difference between acute or chronic exposure is also unanswered.

2.3.3 Patterns and prevalence of BQ during pregnancy

The maturity of the betel nut used and the contents of the quid vary between individuals locally and across countries (Table 2.4) [151, 155]. Ripe nuts were preferred at the Thai-Myanmar border [151] whereas unripe nuts were more often consumed in PNG [144, 146] and Taiwan [153]. Use of BQ during pregnancy was common although there is a variation in the reported magnitude (83.3% [146]- 94.4% [158] in PNG, 64.6% [151] in the Thai-Myanmar border, 61% [147] in Bangladesh and 36.7% [153] in Taiwan). This variation could be due to true prevalence or differences in the measurement. Daily use was 27.7% in the Thai-Myanmar border [150]. In PNG, 47.8% used more than 3 nuts per day [146]. In Bangladesh, frequency (how many times per day) was reported rather than the number of nuts per occasion [147].

2.3.4 Measurement of BQ

The majority of the studies simply defined “users” as women who reported having ever chewed betel quid during pregnancy and did not separate regular users from light users. This may underestimate the effect of BQ by including a large number of women with very little use of betel nut, for example, even just once in pregnancy in the users group.

In the studies that used a questionnaire to measure BQ, it was mostly measured during the post-delivery stay [144, 153], before entering the labour ward [145], or at ANC visits [148, 150, 151]. The problem of measuring only once at ANC in the Thai-Myanmar border [151] and PNG is that mothers may have stopped BQ after the first ANC. This could lead to biasing the effect of BQ towards the null.

The majority of the papers did not attempt to quantify the “dose” of BQ and to relate this to response in terms of birth weight, or to describe whether the pattern of use changed through pregnancy. Data on how a woman prepares and consumes her betel quid, the type of areca nut (ripe or unripe), estimated number of nuts per day, contents of the quid and whether the woman chews, spits or swallows were non-existent or sparse in the literature. The actual questions used in Taiwan,

the Thai-Myanmar border and PNG are summarized in Table 2.3. These were obtained from the literature [153, 158] or the author [151].

2.3.5 Potential causal pathways

Potential mechanisms as to how BQ could lead to adverse birth outcomes may include indirect pathways through anaemia, hypertension, periodontal diseases, and disrupted blood flow.

There are several potential pathways for BQ to cause maternal anaemia; firstly, by suppressing the appetite and secondly, by blocking vitamin D absorption. Several studies suggest that areca nut chewers have a higher resting metabolic rate due to areca nut metabolites that effect the thermoregulatory pathways, altering the thermogenic effects of the meal and also through centrally mediated effects by decreasing the appetite for food [159]. This may be associated with a reduction in food intake including essential nutrients such as iron, leading to iron-deficiency anaemia. Another study suggests BQ could aggravate the effects of vitamin-D deficiency [160].

A second possible mechanism is through hypertension. The association between hypertension and BQ is not well-studied in the literature. A systematic review by Yamada et al. (2013) identified two studies examining BQ and high blood pressure between 1951 and January 2013 [161]. A study using the data from 44,000 Taiwanese women with type 2 diabetes mellitus (T2DM) interviewed by phone between March 1995 and April 2002 reported that prevalence of BQ (current and ex-chewers compared to non-chewers) was 1.1% (471/44,000) [162]. BQ was associated with an increased risk of hypertension (aOR 1.897, 95% CI 1.534–2.346) adjusting for age, BMI, diabetic duration, and smoking. In Bangladesh, among the 251 women, BQ was associated with 67% increased odds of hypertension (aOR 1.67, 95% CI 1.08–2.59) after adjusting for baseline age, pack-years of tobacco smoking, BMI at baseline, use of hypertensive medications at follow-up, education, land ownership, religion, marital status, and daily intake of meat, vegetables and fruit, baseline blood pressure, change in weight over the time period and diabetes at baseline [163]. There was no study on pregnancy-induced hypertension. Ome-kaius et al. (2015) reported that there was no statistical difference in the mean arterial pressure (mmHg) among chewers and non-chewers ($p=0.41$) [146]. De Costa (1982) reported 39/400 of chewers presented signs of preeclampsia not requiring drug treatment compared to 45/400 among non-chewers matched on parity and province of origin. Preeclampsia requiring drug treatment was excluded from this study [145]. Other studies did not provide any information on hypertensive disorders during pregnancy [147, 151, 152, 154].

The third possible mechanism is through periodontal disease. The red juice from betel quid can stain oral structures and the teeth may become nearly dark brown after chewing habitually for many years [164]. A number of studies show that betel quid chewers may predispose to periodontal disease [48, 49]. Maternal periodontal disease may be associated with increased risk of PTB and LBW [44]. Of the 10 studies that examined the relationship between adverse birth outcomes and BQ, no study reported information on periodontal diseases.

Finally, maternal-foetal blood flow may be disrupted when the central nervous system gets stimulated by BQ, causing acceleration of heart rate, increase of blood flow in carotid arteries and decrease in diastolic blood pressure via a peripheral cholinergic effect [153].

2.3.6 Covariates adjusted in the models in the literature:

The studies reviewed above are heterogeneous in the sample size, study design, measurement, and covariates controlled in the models. Four observational studies examined an association between LBW and BQ during pregnancy. No causal assumptions were explicitly mentioned in these studies and the covariates controlled seemed to be determined by using a statistical approach. As a result, covariates controlled in the model were highly heterogeneous. In the study by Yang et al. (2008), nine variables were included (maternal age, marital status, education level, employment status, gestational weeks, parity, weight gain during pregnancy, BMI, maternal drug use and newborn sex). Chue et al. (2010), included six variables (primigravida, first ANC visit after the first trimester, malaria, smoking, gestational age at birth (weeks), and maternal weight at the first ANC). Ome-Kaius et al. (2015) controlled nine covariates (smoking, malaria, ethnic group (highlander), income, primigravida, mother's height, mother's mid-upper arm circumference, frequency of ANC visits and receipt of insecticide treated bed net). Senn et al. (2009) controlled for primagravidity and low BMI for the birth weight analysis but did not specify the covariates used to estimate the effect on LBW.

Alcohol use and tobacco could confound the association between BQ and pregnancy outcomes. Alcohol use and smoking are known risk factors of adverse pregnancy outcomes and they may be associated with BQ. In Thailand, Myanmar, The Lao People's Republic, Cambodia, and the Philippines, unlike Taiwan and PNG, tobacco is often added to areca nut, slaked lime, catechu (an extract from the acacia tree), and betel leaf [36, 155]. Smokers were slightly higher among BQ users in PNG [146] and significantly higher in Taiwan [152]. Tobacco use was more likely in women who used more than one whole areca nut per day compared with women who use less than one nut per day in the Thai-Myanmar border [151]. However, a few studies in the review fail to measure smoking and alcohol use. Alcohol use was not measured in the Thai-Myanmar border as sale of alcohol is prohibited in the study site [150, 151]. Neither smoking nor alcohol use were measured or reported in the Bangladesh study [147] and in Bhutan, BQ is rarely mixed with tobacco.

In addition, season could confound the association between BQ and pregnancy outcomes. Several systematic reviews seasonal patterns of adverse pregnancy outcomes [125-127]. Due to the effect of increasing body temperature, BQ consumption may increase in winter. BQ is widely consumed during the festivals or occasions in Bhutan. Hence, more people tend to chew BQ during the festival seasons. Season was not explored in any of the studies.

In addition to the measurement issues described in the above, insufficient control of confounders or over-adjustment of covariates also makes it difficult to assess the true association

between BQ and pregnancy. For example, maternal hypertensive disorders were not taken into consideration in any of the models above.

A meta-analysis of crude RR of LBW among BQ users compared to non-users from four observational studies was conducted using the random effects model. The forest plot is shown in Figure 2.1. Analyses were conducted using STATA 14.1 with user-contributed commands for meta-analyses: metan [165]. The number of BQ users, non-users, LBW, and non-LBW were extracted from the original manuscripts. The estimates of effect size and corresponding 95% CIs were 0.94 (95% CI: 0.79-1.27)[146], 0.92 (95% CI: 0.80-1.06)[151], 1.70(95% CI: 0.45-6.40)[144], and 1.95 (95% CI: 1.41-2.71)[153]. The pooled crude RRs for LBW was 1.20 using the random effects model (95% CI: 0.81 - 1.80). The result shows a high heterogeneity across studies ($I^2= 83.2\%$, $p<0.0001$).

2.3.7 Issues of measurement and validation of BQ

A further literature review was conducted in order to understand how betel quid use has been measured and validated in other BQ studies. As the information pertaining to the measurement of BQ was insufficient, the search was further extended to learn from the measurement of other associated health risk behaviours such as drinking and smoking.

Health-risk behaviours including smoking, drinking and other drug use are often measured by administering questionnaires that require retrospective self-reports about engaging in these behaviours. The validity of self-report questionnaires can be influenced by cognitive factors and situational factors. For the former, errors can arise at each stage of cognitive processes for answering questions including comprehension, retrieval, decision-making, and response generation [166]. One example is a recall bias. A systematic review reported recall beyond a 1 year-period tends to be inaccurate when asking adolescents to recall the age at which they initiated tobacco use [166]. Self-reported use might be underreported because of concerns about social desirability and fear of reprisal, especially for behaviours that are illegal, stigmatised, or associated with moral implications [166]. Brener et al. suggested reporting of alcohol and tobacco use is affected by perceptions of privacy and confidentiality and found that the self-administered questionnaire format produced higher reported rates of alcohol and other drug use compared to interviewer-administered questionnaires in the studies on adolescents they included in a review [166].

One way to validate consumption reported by the respondent is to compare the self-reports against biochemical measures of the substance or one of its metabolites [166, 167]. For example, for tobacco exposure, the amount of cotinine, a metabolite of nicotine has been measured in the blood or urine as a biomarker of exposure[168]. There is evidence to suggest that there is strong agreement between self-reported and biochemical measures of tobacco use [166]. However, in terms of alcohol, biomarkers tend to lack sensitivity and specificity [166, 167]. For example, a breath test can only capture alcohol use within the 24 hours preceding the test [166].

Where reliable biochemical measures are not available, other options to validate questionnaires have been explored. These include a “bogus pipeline” approach and corroboration

of self-report data with collateral information. A bogus pipeline is an approach where respondents are led to believe their true behaviour can be detected even though it cannot [166, 167]. Several studies used a saliva test as a bogus pipeline to examine the validity of self-reported drinking [166, 167]. However, these studies reported limited impact of the bogus pipeline on the validation of self-reported alcohol use [166, 167]. Other studies tried to assess the validity of self-report data by examining the concordance of such information with that obtained from collateral sources, such as spouses, children, parents, or close friends who know the person well [169]. A study using a large clinical trial data compared self-reported alcohol use collected periodically for one year, periodically collected collateral interviews, and biochemical tests and concluded biochemical tests and collateral informant reports do not add sufficiently to self-report measurement accuracy [170]. Several other studies and reviews concluded self-reports of alcohol use are considered as reliable and valid approaches to measuring alcohol [166, 167, 170, 171].

Of the three studies that reported dose response between BQ and health outcomes including oral cancer, cardiovascular, and metabolic diseases, the amount of betel quid chewing was most often measured in self-administered [172] or interview-administered questionnaires [173, 174]. In most of the studies, assessment of the validity of the self-administered or interview-administered questionnaire was not reported [172-174].

The amount of exposure could be considered in dimensions of amount of consumption, frequency of consumption, and duration of consumption. For example, Lin et al. (2009) [172] assessed the association between betel quid chewing and obesity, they collected data on the duration (years), frequency (times/day), quantity (numbers/times) of betel quid chewing among 2,359 Taiwanese citizens over 40 years old using self-administered questionnaires. Average betel quid chewing was defined as the average amount of areca nut consumed each time across the interviews (quids/times). The average frequency of betel quid chewing was recorded as times/day. Cumulative quids/day-years of betel quid chewing was calculated as areca nut consumed each time X daily average frequency of areca nut chewing X exposure years.

As for biochemical markers of betel quid chewing, a few recent studies attempted to use arecoline and arecaidine as biomarkers of a chewing habit. One study reported that after administration of areca nuts extracts, the major urinary metabolite was arecaidine with a half-life of 4.3 hours and very low levels of arecoline with a half-life of 0.97 hours [175]. For the quantitative analysis of blood arecoline and arecaidine, serum arecoline and arecaidine was reported to be good measurement of the quantities of betel quid used before the day of drawing blood and arecoline or arecaidine [176]. Therefore, biochemical markers of betel quid chewing seem to be good indicators for recent betel quid exposure but may not be appropriate to measure habitual use quantity in adults. However, acute and chronic foetal exposure might be measured by biomarkers as a case report detected arecoline in the placenta and meconium in 2014 [177].

2.3.8 Relevance to Bhutan

Biochemical analyses was not felt to be technically possible or appropriate for the present study. In terms of measuring BQ, methods from alcohol and tobacco studies were used to inform the development of measurement of BQ. In particular, the questionnaire needed to take into account a possible change of behavior during pregnancy and to attempt to collect detailed information on patterns to improve understanding of BQ during pregnancy. Quantification and dose-response may provide more information on exposure to BQ.

2.3.9 Summary of key findings

In conclusion, a review of the literature revealed that heterogeneous contents of betel quid chewing and population across the studies makes it difficult to assess the effect of betel quid chewing on PTB, LBW and birth weight reduction. Also presence or magnitude of dose response between betel quid chewing and those pregnancy outcomes is not clear. The methods and design of the study so far have several limitations including lack of validation of measurement, insufficient control of confounding variables, simplified categorisation of betel quid chewing, and failure to capture the change of behaviours during pregnancy. There is a need to develop methods to measure BQ to understand the true effect of BQ on pregnancy.

Table 2.3. Questions used to measure betel quid chewing in the literature.

Author (year)	Questions used to measure betel nut consumption during pregnancy	Validation
Yang et al. (2008) [153]	Do you chew betel quid during pregnancy? (yes/ no) If yes: how frequently do you chew betel quid during pregnancy? (ever use but abstained, <1 day/week, 1-3days/week, 4-6 days/week and daily) On average, how many betel quids do you chew per day when you chew betel quid during pregnancy?	Not reported
Senn et al. (2009) [144]	Do you chew betel nut? (yes/no) If yes: how many nuts do you chew per day? (none, less than 5 nuts, 5-10, more than 10) Why do you chew betel nut during your pregnancy? Do you think betel nut chewing will have an effect on your baby?	Not reported
Chue et al. (2012) [151]	Do you eat betel? yes/no (never takes betel) If yes: how often – sometimes (not every day); daily; more than once daily Same questionnaire design used for smoking was applied: Please give an average number of nuts consumed per day. When do you take betel nut? When you take betel do you take it with piper leaf? (yes/no) Do you add tobacco when you take betel? (yes/no) Do you add slaked lime when you take betel? (yes/ no) Do you spit when you are eating betel? (yes/no)	Not reported

Table 2.4. Constituents of betel quid (Gupta 2002 [36]).

Constituent	Preparation
Areca nut	Sliced fresh ripe nut
	Roasted
	Dried/baked
	Fermented
Piper Betel	Fresh leaf
	Inflorescence
Lime	From coral
	From shell fish
	From limestone
Tobacco	Fermented
	Sun dried
	Powdered with molasses with lime
Catechu	Extract of acacia catechu
	Extract of acacia suma
Spices	Cloves
	Cardamom
	Aniseed
Sweeteners	Coconut

Table 2.5. A summary of literature on betel quid chewing during pregnancy.

Outcome of main interest	Authors (Year)	Study design/ Sample size	Measurements of BQ	Timing of measurement of BQ	Validation (Biochemical measures or others) /category of users	Outcome	Confounders	Model	Key findings
LBW, PTB, Anaemia	Stuetz et al. (2016) [150]	Cross-sectional study/1,048 pregnant women who received micronutrient supplements (1 st to 3 rd trimester) in the ANC clinic on the Thai Myanmar border (in Jun 2004 and Nov 2006)	Questionnaire (details not reported)	At enrolment	NA/daily consumption of betel	Anaemia: Hemoglobin (Hb) in the blood sample collected once: <110g/L in 1 st and 3 rd and <105g/L in 2 nd trimester	Smoking, parity, and BMI at the time of sampling (no information on alcohol)	Linear regression	Prevalence of daily use of BQ: 19.7% (2004) and 27.7% (2006) Prevalence of smoking: 28.1%(2004) and 26.8% (2006) Reduction of Hb (g/L): -2.90, 95% CI: -4.62 to -1.16
	Ome-Kaius et al. (2015) [146]	Prospective cohort study/ 2,700 pregnant women residing in rural lowland PNG (Nov 2009 – Feb 2013)	Interview-administered questionnaire	At enrolment (first ANC)	NA/Non-users vs users (occasional users <1 nut/d; mild users 1-2 nuts/d; moderate users 3-5 nuts/d; and heavy users >5 nuts/d)	Primary: (1) BW within 24 h of delivery and LBW (BW<2,500g) (2) PTB: US-Based GA before 37 weeks Secondary: Anaemia: Hb<11g/dL at delivery	Smoking, malaria, gravida, alcohol use, income, education, bed net use, maternal height, number of ANC visits, infant gender, maternal nutritional status, timing of bw/Hb measurement, receipt of intermittent preventive treatment in pregnancy	Multivariate logistic regression or linear regression (no imputation of missing data)	Prevalence of BQ: 83.3% (47.8 % used more than 3 nuts) Prevalence of smoking: 18.9% (21.7% for BQ users) LBW: aOR 0.94, 95% CI: 0.65-1.38 Anaemia: aOR 1.67, 95% CI: 1.27-2.20 PTB: OR not reported
	Chue et al. (2012) [151]	Retrospective cohort study/7,685 refugee pregnant women in the ANC clinic on the	Interview-administered questionnaire	At enrolment (first ANC)	NA/Non-users vs users (heavy users who used daily or more than once daily and light	BW within 72 h of delivery and LBW(BW<2,500g)	LBW: gravida, trimester of first ANC, malaria, infant gender, smoking, GA at	Multivariate logistic regression	Prevalence of BQ: 64.6% (76.8% used occasionally – number of nuts not defined)

	Thai-Myanmar border (July 1997 – Nov 2006)			users who do did not take every day)		birth, and mother's weight at the first ANC		Prevalence of smoking: 11.3% LBW: aOR was reported as not significant (NS) and aOR not reported. Anaemia: aOR NS (OR not reported)
Senn et al. (2009) [144]	Cross-sectional study/310 mothers at Alexishafen health center, Madang Province in PNG (Sep 2007-Jun 2008)	Interview-administered questionnaire	Within 72h of delivery during the post-delivery stay in the health center	NA/ Non-users vs users (including mothers who abstained during pregnancy)	BW, LBW(<2500g), PTB	Primigravidity, and low BMI	Univariate and multivariate analysis	Prevalence of BQ: 94% Hb(g/l) (chewers vs non-chewers): 94 vs 100 (NS) BW: a reduction of 238g (p<0.02) by BQ LBW: crude OR 1.9 (95% CI: 0.4-17) Term LBW: OR 3.0, 95% CI: 0.4-13.0, p=0.48 PTB: OR 0.6, 95% CI 0.1-5.4, p=3.4
Yang et al. (2008) [153]	Retrospective cohort study/1,264 aboriginal women who gave birth in 10 hospital in Southern and Eastern Taiwan	Interview-administered questionnaire	During the post-delivery stay (within 72 h on average) In the hospitals	As described in Yang et al. (1999)/Non-users vs users who had ever used betel nut at any stage during pregnancy	BW, LBW(<2500g), PTB (<37 weeks), birth length	Maternal age, marital status, education level, employment status, BMI, GA, weight gain during pregnancy and infant gender,	Multivariate logistic regression	Prevalence of BQ:36.7 % with a daily average of 5.68 quids

	(Jan 2003 – Feb 2004)			including those who abstained.		maternal drug use, smoking, and alcohol			Smoking:24.1% (42.7% for BQ users) LBW: aOR 2.4, 95% CI: 1.21-4.80 Birth length reduction: 0.43 cm (p=0.0466)
Gupta and Sreevidya (2004) [148, 149]	Prospective cohort/1167 pregnant women who were 3 to 7 months pregnant recruited in 8 primary health post areas in the city of Mumbai, India (Jun – Nov 2002)	Interview-administered questionnaire	At enrolment (3 -7 months of pregnancy, asking about daily use in the past 6 months)	NA/ users are defined as smokeless tobacco product at least once a day for the past six months (light users: 1-4 times/day or heavy: ≥5/ times day)	BW, LBW, PTB (<37 weeks)	Maternal age, education, socioeconomic status, weight, anaemia, ANC, and GA (alcohol and smoking not controlled, smokers excluded from the study).	Multivariate logistic regression (missing data excluded)	Prevalence of daily smokeless tobacco use: 17.10% BW reduction: 105 g (95% CI 30 to 181 g) LBW: aOR 1.6, 95% CI: 1.1 – 2.4 PTB: 1.4, 95% CI: 1.0-2.1	
Yang et al. (2001) [152]	Retrospective unmatched case-control study/229 aboriginal women (32 with adverse pregnancy outcomes and 197 controls) in a regional hospital in Eastern Taiwan (Feb-Sep 1998)	Interview-administered questionnaire	During the post-delivery stay (majority within 72 h) in the hospital	As described in Yang et al. (1999)/non-users and non-users (details not reported)	Adverse pregnancy outcomes: (1) LBW (BW<2500g & GA >37 weeks), (2) PTB (GA<37 weeks) or (3) Any malformation	Maternal age, cigarette smoking, alcohol consumption	Conditional logistic model	Prevalence of BQ (cases vs controls):68.7% vs 48.9% Smoking: 37.5% vs 62.5% Adverse pregnancy outcomes: aOR 5.7, 95% CI: 1.6-20.3	

	Yang et al. (1999) [154]	Retrospective matched case-control study/ 186 aboriginal women (62 with adverse pregnancy outcomes and 124 age-matched controls with normal pregnancy outcomes) in Southern Taiwan (Mar – Oct 1994)	Interview-administered questionnaire	At home (information about previous pregnancies such as timing was not mentioned and could be a source of potential biases if recall was about more than 1 year ago)	Test-retest reliability: 0.78-0.89 for continuous and 0.85-0.91 for categorical variables after 4 weeks of the first interview/non-users vs users during pregnancy (details not reported)	Adverse pregnancy outcomes (not specified)	Maternal illness and number of previous pregnancies (not possible to control for age as samples were matched on age, alcohol and smoking were not controlled in the model as it was not associated with the outcome)	Conditional logistic model	Prevalence of BQ (cases vs controls): 14.5% vs 8.1% Prevalence of smoking: 43.6% vs 38.7% Adverse pregnancy outcomes: aOR 2.8, 95% CI 1.2-6.8
	De Costa, C. (1982) [145]	Case-control study/400 mothers who use BQ daily during pregnancy and 400 mother who have never used at Port Moresby General Hospital in PNG (Apr-Jun 1981)	Self-report at enrolment (details not reported)	At enrolment (booked patients entering the labour ward)	NA/non-users vs daily users	BW	Both cases and controls reported no use of smoking, alcohol, and drugs), matched on province of origin and parity within the 3 groups (1, 2-5, >5)	Chi-square test	Mean BW (cases vs controls): 2,998.5 vs 3,079.5 (p<0.05)
Other aspect of pregnancy	Kader, M. (2016) [147]	Retrospective data from a randomised control trial/ 730 pregnant women in Matlab, Bangladesh (2002)	Interview-administered questionnaire	During the postpartum visit	NA/ (1) Non-users vs users (less than daily, once daily, 2-3 times daily) (2) non-users vs users (with BQ only and BQ with chewing tobacco)	Folate deficiency at 14 weeks of GA (serum folate <6.8 nmol/L)	Asset score, women's age, vitamin B-12 status and literacy (alcohol and smoking were not controlled in the model) (frequency of BQ consumption)	Multivariate logistic regression	Prevalence of BQ: 61% Prevalence of smoking: cigarette smoking not measured Mean serum reduction (users vs non-users): 1.56(nmol/L) (p<0.05) Folate deficiency for BQ 2-3 times a day: aOR 2.51, 95% CI 1.07-5.93

Garcia-Algar et al. (2014) [157]	Prospective clinical observation study/6 Asian mothers during pregnancy and newborn at the time of delivery at the Hospital del Mar in Barcelona Spain	Biological matrices	Clinical examination during the pregnancy	Arecoline concentration in meconium and placenta ($\mu\text{g/g}$)	Clinical signs of birth outcomes (neonatal withdrawal syndrome, LBW (BW<2500g), small for GA, low intrauterine growth, hyporeflexia, hypotonia)	NA	Observation	2 adverse birth outcomes were found in the six exposed newborns.
Lopez-Vilchez (2006) [156]	Case-report/ a healthy 38-year-old mother who was an immigrant from Bangladesh and came to the obstetrics emergency department for childbirth	Biological matrices, observation of dental condition (a brownish-red discoloration of the oral mucosa and tongue)	Clinical examination during treatment of the neonate	Arecoline in the placenta 0.012 $\mu\text{g/g}$ of placental tissue	Clinical signs of neonate withdrawal syndrome	NA	Observation	A diagnosis of neonatal abstinence syndrome resulting from maternal consumption of areca nut was established.

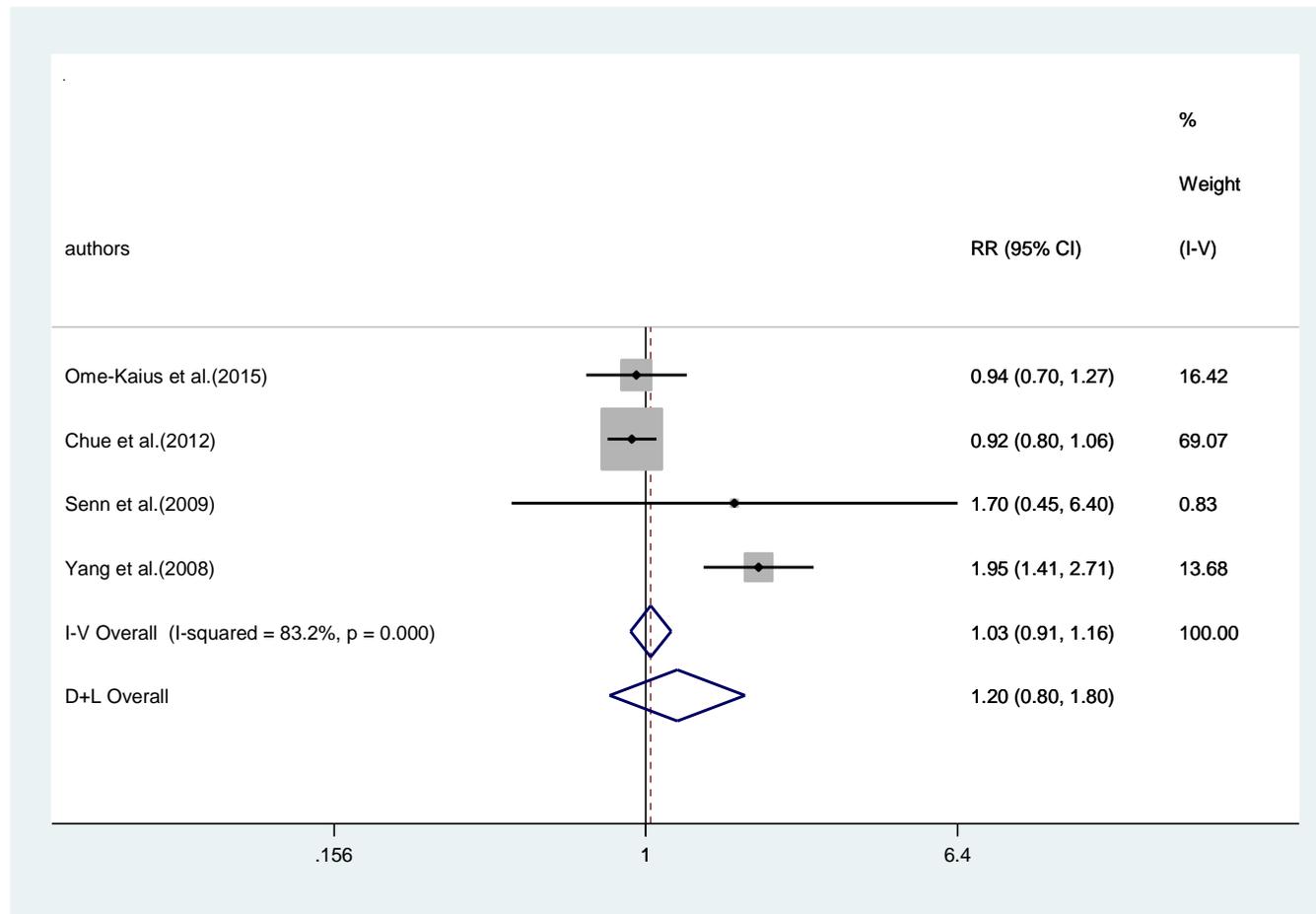


Figure 2.1. Forest plots of betel quid chewing during pregnancy and LBW using the random effects model.¹⁰

¹⁰ I-V Overall = Fixed effect meta-analysis. D+L Overall = Random effect meta-analysis.

2.4 Review of existing tools to measure alcohol, smoking and betel quid chewing

A review of the literature on the impact of betel quid chewing on pregnancy revealed that the methods and design of measuring betel quid in the literature so far have several limitations such as lack of validation of measurement, insufficient control of confounding variables, simplified categorisation of betel quid chewing, and failure to capture the change of behaviours during pregnancy. In particular, accurate measurement of alcohol, which was considered to be highly prevalent in Bhutan and a strong confounder, is critical to separate the effect of betel quid chewing adjusting for alcohol. In section 2.4.1, published and grey literature, as well as government reports from Bhutan, are reviewed to seek a validated questionnaire to measure betel quid chewing, alcohol, and tobacco. Section 2.4.2 provides findings from a review of globally available tools.

2.4.1 A summary of review of existing tools to measure alcohol, smoking, and betel quid-chewing in Bhutan

Seven key publications on betel quid chewing, alcohol, or tobacco use, published before October 2014, were identified (five population-based surveys [34, 91, 178-180], one monograph [181], and one research article [32]). If there were several versions of the population-based surveys, the most recently updated version was reviewed. Betel quid chewing was measured in the 2012 Bhutan Living Standard Survey (BLSS) [178] and the 2010 Gross National Happiness Survey (GNH) [34]. Alcohol was measured in a study conducted in the eastern part of Bhutan by Subady et al. (2012) [32], the 2014 NCD Step Survey (STEP) [91], the International Tobacco Control (ITC) Policy Evaluation Project: The ITC Bhutan Project Report (2011) [180], and the 2010 GNH [34]. In addition to the above article and surveys, Dorji, L. (2012) described the history and context of alcohol, alcohol products, and health care costs relating to alcohol abuse in his monograph. Tobacco was measured in the 2013 Global Youth Tobacco Survey (GYTS) Bhutan Report [179] and the 2014 NCD Step Survey [91]. Supplementary materials used in the STEP survey were obtained from the Royal Government of Bhutan where available. The key findings from the review of existing tools in Bhutan are summarised in Table 2.6.

All five population-based surveys used a variation of Quantify-Frequency measures (QF) which capture quantity within an event and frequency within a reference period [182]. The mode of interview was predominantly interviewer-administered. Only one population-survey for youth [179] used a self-report questionnaire.

Typical questions included:

- **Betel quid chewing:** Did you chew doma (betel quid) in the past 12 months? Do you chew daily or occasionally?
- **Alcohol:** How often do you drink and how many cups or glasses do you usually take when you drink alcohol?; and

- **Smoking:** Do you smoke/ use manufactured cigarettes daily, at least weekly, less than weekly, or not at all?

Major challenges of the QF measures to assess alcohol consumption are that most of the QF measures cannot capture combined drinks and often underestimate the true volume consumed.

As for tools other than QF measures, only one research article conducted in Bhutan, in which only alcohol was measured, was available. The research article aimed to detect harmful alcohol use and used the Alcohol Use Disorders Identification Test (AUDIT), a simple 10-item questionnaire developed by WHO for early detection of hazardous or harmful alcohol use [183] and a tri-level method. AUDIT asks “how many drinks containing alcohol do you have on a typical day when you are drinking” and “how often do you have six or more drinks on one occasion”. While AUDIT is a simple tool for screening of harmful drinking and globally used [184], a problem arises when there is no agreed concept of a standard drink as is the case in Bhutan. A tri-level method asks what type of alcoholic beverage and what quantity per occasion for maximum level, medium level and lower level. Although it captures combined drinks, the concept of high, medium and low could be subjective.

By reviewing existing tools in Bhutan, it was identified that there was no existing tool to sufficiently measure betel quid to understand the pattern and quantity of betel quid chewing during pregnancy and that measuring alcohol imposed a methodological challenge in the present study. All the questionnaires used in Bhutan so far to measure potentially toxic behaviour faced a number of methodological challenges to be adopted in this research. Although a past survey shows much higher prevalence of chewing betel than tobacco[34], it was often included in smokeless tobacco and not explored in detail. Another key finding was that measurement of alcohol imposes a challenge to the present study. First, it is difficult to capture a variety of alcohol beverages, which are often home-brewed. Second, the percentage of alcohol in the home-brewed beverages is unknown [32, 181]. Finally, there is no agreed definition of a standard drink size [181]. As a result, quantifying alcohol consumption is challenging, which makes it difficult to understand the true prevalence and burden of alcohol abuse. No information regarding drinking, smoking, and chewing betel nuts during pregnancy in Bhutan was available. To overcome the challenge of measuring alcohol in Bhutan, globally available tools are explored in the next section.

Table 2.6. A summary of key literature reviewed for development of the questionnaire.

Source	Study objective	Questions	Mode of Interviews	Validated (reference questionnaires)	Sample size	Strengths	Weaknesses	Main Findings:
Subady et al. "Prevalence, patterns and predictors of alcohol consumption in a mountainous district of Bhutan." (2012) [32]	Detection of harmful alcohol use, patterns and quantification of alcohol consumed and attitude and beliefs.	A 10-item questionnaire(AUDIT) Tri-level method to ask the quantity and the type of alcohol beverage in terms of high-, medium, and low-level drinking and the number of days in the past 12 months such as: Starting with a special occasion where you drank maximum level, what type of alcoholic beverages you usually drink and how much? (Type of beverages, Alcohol %, Volume (ml)) How often did you drink like this in the past 12 months? (Every day, 5-6 days/week, 3-4 days/week, 1-2d/week, 2-3d/month, 1d/month, 7-11d / 12month, 4-6d/12month, 2-3 d/12month, or 1 d/12 month) 5 questions as attitude and beliefs.	Interviewer administered	AUDIT, Tri-level method	442 respondents aged ≥18 of 270 household in 17 villages of Tashiyangtse dzongkhag Sampling methods: multi-stage systematic sampling Interview periods: 2012	Quantification of alcohol consumed using validated methods.	Limited sample population. Uncertain assumptions of alcohol % used to quantify alcohol: local wine 5% and local sprit (Ara) 15%.	Female current drinkers: 30% Annual female per capita alcohol consumption: 2566g. Annual per capita home-made alcohol was 2127g.
2014 NCD Step Survey [91]	Prevalence and patterns of drinking and smoking. Frequency and amount of alcohol, tobacco, and smokeless tobacco. Perception of tobacco control policy Identification of heavy episodic drinking.	17-25 questions each for smoking (betel quid is included in smokeless tobacco). and drinking such as: Have you consumed any alcohol within the past 12 months? (Y/N) During the past 7 days, did you consume any home-brewed alcohol, any alcohol brought over the border/from another country, any alcohol not intended for drinking or other untaxed alcohol? (Y/N) On average, how many standard drinks of the following did you consume during the past 7 days? (Homebrewed spirits, e.g. ara, homebrewed beer or wine, e.g. beer, palm or fruit wine, alcohol brought	Interviewer administered	A variation of QF measures	2,822 adults aged 18 to 69 Sampling methods: multiple stage stratified cluster sampling Interview periods: April-June 2014	Wide coverage. Example of pictures of drinks were provided.	No concept of a standard drink: No detailed information about betel quid chewing	Female current drinkers in the past 30 days: 32.8% Female current daily smokers: 2.1% Female current daily users of smokeless tobacco:9.9%

over the border/from another country, alcohol not intended for drinking, e.g. alcohol-based medicines, perfumes, after shaves, other untaxed alcohol in the country)

2013 Global Youth Tobacco Survey (GYTS) Bhutan Report [179]	Prevalence and pattern of consumption of tobacco and smokeless tobacco among youth. Frequency and amount and perceptions.	60 multiple-choice questions in English (43 questions from the GYTS Standard Core Questionnaire, 15 selected optional questions, and two country-specific questions).	Self-administered in the classroom	A variation of QF measure (GYTS Standard Core Questionnaire)	1,378 students aged 13–15 Sampling methods: a national-level representative sample of students in grades 7–9 from 25 sampled schools and random classes selected within the sampled schools Interview periods: March-June 2013)	Prevalence among youth aged 13-15.	No information on drinking	Current smokeless tobacco users among girls aged 13-15: 18.9% Current smokers among girls aged 13-15: 6.6%
Bhutan Living Standard Survey (2012) [178]	Expenditure on betel quid chewing, alcohol, and tobacco.	6 tobacco and betel items: cigarette, bedi, chewing tobacco, doma, pan, doma khamtok 5 alcohol items: ara, bangchang, other wines, beer, liquor. Questions regarding the last 7 days, 30 days and 12 months: What quantity did you consume? (Quantity and Unit) Total value (Nu.) Amount spent (if purchased), or estimated market value (if home produced).	Interviewer administered	NA (The World Bank Living Standards Measurement Study (LSMS) methodology)	8,968 households with a total of 39,825 individuals selected Sampling methods: a stratified two-stage sampling of households Interview periods: March-May 2012; August 2012 in one <i>gewog</i> (Lunana) of Gasa dzongkhag due to accessibility	Wide coverage:	No information on types, amount and frequency of drinking	Tobacco and betel quid chewing: mean monthly household expenditure: 246.84 NU Per capita: 54.33 NU Alcohol: mean monthly household expenditure: 295.36 NU Per capita: 65.01
The International Tobacco Control (ITC) Policy Evaluation	Psychosocial and behavioural effects of Bhutanese tobacco control	ITC global survey questionnaire: Extensive questionnaires on Tobacco products such as:	Interviewer administered	QF measure (ITC global survey questionnaire)	1,806 respondents from Bumthang, Chukha, Thimphu,	Globally comparable. Limited to only 4	No information on betel quid chewing.	Current female having used tobacco products (either smoked or smokeless including snuff)

Project: The ITC Bhutan Project Report(2011) [180]	legislation, including a nationwide ban on the sale of tobacco	Do you smoke/ use manufactured cigarettes daily, at least weekly, less than weekly, or not at all? (Y/N) Ask if respondent's last purchase of tobacco was smokeless tobacco such as Baba, Raja Chap, Surti, Golden Khaini, Chaini, Snuff, other smokeless (specify) How often is smokeless tobacco available in your area? (Always, Most of the time, Sometimes, Rarely, Never, Refused, Don't know)	and Trashigang. (age:≥18)	districts	No explanation of a standard size of cup No information on types of alcohol	at least once within the last 30 days(weighted):4.9% Current drinkers:35% (no information by gender)		
		Alcohol (4 questions): Do you drink alcohol? (Y/N) How often do you drink alcoholic drinks? (Every day or nearly every day, most days, 2 or 3 days a week, about once a week, Less than once a week, refused, don't know) When you drink alcohol, how many cups or glasses would you drink? (1 cup, 2-3 cups, 4-6 cups, 7 cups, more than 7 cups: cups/glasses) How often would you drink more than [4 (females)/ 6 (males)] drinks in one session?						
2010 Gross National Happiness Survey [34]	General history of betel quid chewing, alcohol, tobacco.	Alcohol (4 questions): In your entire life, have you ever consumed any kind of alcohol? How old were you when you first started drinking? (Y/N) Have you consumed any type of alcohol during the past 12 months? (Y/N) How often did you consume alcohol during the past 12 months? Tobacco (6 questions): Does anyone in your household smoke regularly inside the house? (Y/N) Have you ever smoked cigarettes? (Y/N) At what age did you begin to smoke?	Interviewer administered (average: 3 hours)	QF measures	7,142 respondents aged 15 to 98 with the mean of 41 years	Wide coverage of individuals	No information on types, amount and frequency of drinking	Female current smokers in the past 12 months: 2.4% Mean initiation age (years): 19.4 (urban) 20.2(rural) Female current betel chewers in the past 12 months: 61.5% Female current drinkers in the past 12 months: 32.8%
				Sampling methods: multi-stage sampling	Provides current prevalence of smoking, alcohol, betel quid chewing and overview of the pattern.	Extensive information (750 variables)		
				Interview periods: April – December 2010				

Did you smoke during the last 12 months? (Y/N)
Do you smoke daily or occasionally? (Y/N)
How many cigarettes do you smoke each day now?

Tobacco includes Baba, Raja, Surti, Leaf, snuff, others (4 questions):

Have you ever chewed/snuffed tobacco? (Y/N)
At what age did you begin to chew/snuff tobacco?
During the last 12 months, did you chew/snuff tobacco? (Y/N)
If yes, do you chew/snuff tobacco daily or occasionally? (Y/N)

Betel quid chewing (5 questions):

Have you ever chewed doma? (Y/N)
At what age did you begin to chew doma?
Did you chew doma during the past 12 months? (Y/N)
Do you chew daily or occasionally?
How many khamto do you chew each day now?

For smoking, alcohol, and drug use (scale question):

From what you know and heard, are the following issues, a concern in the schools in your community? (A Major concern/Of Some Concern/A Minor Concern/Not a Concern/ Don't know)

2.4.2 A summary of the review of globally available tools to measure alcohol

To solve the challenge of measuring alcohol, globally available tools were sought and reviewed to adapt to the context of Bhutan. Once tools were identified, their applicability to measuring smoking and betel quid chewing was considered.

Sobell et al. (1995) assessed five major drinking measures which are used with adults and adolescents for both males and females and with clinical and normal drinker populations: Lifetime Drinking Measures, Drinking Self-Monitoring Log, Form 90, the Alcohol Timeline Followback (TLFB), and Quantify-Frequency Measures (QF) for their recommended use, advantages and limitations [171]. The Lifetime Drinking Measures is a good estimate for a lifetime overall picture of alcohol consumption rather than a detailed daily account. The Drinking Self-Monitoring Log is used for collecting various aspects of drinking such as amount, frequency, mood, and urges prospectively, especially to assess and monitor treatment. Form 90 and TLFB both use a calendar method and TLFB is recommended to understand drinking patterns of more than the last 90 days [171]. TLFB is a calendar-based measure of self-reported use of alcohol, tobacco, and illicit substances which aims to evaluate specific changes and is the most widely used and validated in high/middle/low income countries [185-187]. It is administered by interviews or self-administered in person or on a computer in research. QF measures estimate average quantity of drinking per occasion and average frequency of occasion and is available in a number of different formats. The Graduated-Frequency (GF) Measure is one of the few QF measures which can account for occasions when different types of beverages were combined [182]. Typical GF questions ask the largest number of drinks an interviewee had on any single day during the past 12 months (24 or more drinks, 12-23, at least 8 but less than 12 drinks, 5-6 drinks, 3-4 drinks or 1 or 2 drinks). However, GF still has a limitation in that there is no standard drink size.

The majority of research on drink size has been conducted in high income countries. In low- and middle- income countries, the lack of commonly agreed standard drinks and a wide range of alcoholic beverages presents serious challenges to the accurate assessment of alcohol consumption [188]. In India, Nayak et al. (2008) explored beverage types, beverage-specific standard drink sizes and pour-size using key informant interviews and participant observation and performed biochemical analyses to measure ethanol content of local beverages in three different study sites (Delhi, Goa and Rajasthan) [188]. In Goa, approximately 2,000 study participants including both men and women (exact sample number not provided) were interviewed while only a small sample of men were studied in Delhi (n=172) and Rajasthan (n=172). The study confirmed a wide range of alcohol drinks with varied ethanol concentrations and differences in drink sizes and pour-sizes, and recommended accounting for the variations in future research. Later in 2010, the same group proposed a new adaptation of the GF measure, the Fractional GF (F-GF) to be used in the context where no standard drink size is available, and validated it against AUDIT and a 28-day diary among 743 male drinkers aged 18 to 49 years in Goa, India [189].

The F-GF first establishes the maximum amount consumed on any day in the previous 12 months by asking, for different beverage types, the numbers of various drink pours associated with the type. Once the maximum is determined, the profile is developed by asking how often the respondent drank about this amount (the maximum); then about how often the respondent drank about three-quarters of that amount ($3/4$ the maximum); followed by identical questions for “about half that amount” and finally “about one quarter of that amount.”

In terms of applicability of these tools to smoking and drinking, TLFB is also widely applied to quantify tobacco [190], marijuana and other drug use [191]. On the other hand, examples of shortcomings include: time required and difficulty in capturing a variety of containers and vessels used for drinking; and the variation in the content of alcohol in Bhutan.

The F-GF is most relevant for the present study to measure alcohol to take into account a wide variety of home-brewed alcohol drinks and absence of standard size. However, F-GF has not been applied to other substances. Additional questions to measure betel quid and tobacco consumption are required for the present study to adopt the F-GF. The development of the questionnaire is further discussed in Chapter 3.

2.5 Summary and introduction of conceptual framework and causal diagrams

Chapter 2 presented the background and context of the present study. Based on the review of the available studies presented in this chapter, Figure 2.2 shows the conceptual framework used for this study, followed by directed acyclic graphs (DAGs) in Figure 2.3 and Figure 2.4 [192]. The framework is specifically intended to describe Bhutan, where data collection was conducted.

The conceptual framework was informed by Dahlgren & Whitehead’s social model [193] of health and Kramer’s epidemiology of LBW [194]. Risk factors were stratified by maternal factors, paternal factors, household factors, and environmental factors and also if risk factors were modifiable in the short term or modifiable in the long term or unmodifiable. The main risk factor of interest is betel quid chewing.

While the conceptual framework presents potential risk factors, it does not show how the present study assumes causal relationships between variables. In order to explicitly present causal assumptions to identify covariates needing to be controlled in the models, causal diagrams were constructed. Directed acyclic graphs (DAGs) help the study to identify whether there is confounding and which variables should be controlled or not controlled in order to achieve conditional exchangeability, conditional on assumptions [192, 195]. The DAGs identify a set of factors that are associated with betel quid chewing during pregnancy and term LBW and PTB. Within these DAGs, the relationships between the factors are assumed rather than proven. The following analyses in Chapter 7 are conducted with covariates informed by the DAGs using the backdoor criterion to identify sufficient sets of confounders. Hence, if the assumptions of underlying relations are wrong, the results of the analyses may be biased.

DAGs were constructed using a six-step approach suggested by Shrier and Platt [196]. In the DAG approach, a child is defined as the direct effect of a particular variable and a parent is a direct cause of a particular variable. A descendant is a direct effect or indirect effect of a particular variable and an ancestor is a direct cause or indirect cause of a particular variable.

Step 1. The covariates chosen to reduce bias should not be descendants of the exposure variable or X (BQ during pregnancy).

Step 2. Delete all variables that satisfy all the following criteria: 1) non-ancestors of X, 2) non-ancestors of the outcome and 3) non-ancestors of the covariates that one is including in the model to reduce bias.

Step 3. Delete all lines emanating from X.

Step 4. Connect any two parents sharing a common child.

Step 5. Strip all arrowheads from lines.

Step 6. Delete all lines between the covariates in the model and any other covariates

If X is dissociated from the outcome after Step 6, then the statistical model chosen minimizes the bias of the estimate of X on the chosen outcome.

These DAGs visualise the relations between variables in the analyses. Single-headed arrows represent causal effects from exposures to outcomes. The absence of an arrow or a variable implies that there is assumed to be no corresponding causal effect. The ways in which different factors may influence betel quid chewing during pregnancy and/or the adverse birth outcomes (term LBW or PTB), either directly or indirectly, are shown. Factors that are important but unobserved in the present study are shown in grey circles. The unobserved factors included stress, anxiety, or abuse during pregnancy, periodontal diseases, paternal factors such as father's height and birthweight, and genetic factors. History of delivering a LBW and/or PTB infant was not included as many causal factors were believed to be overlapping. ANC visit was also not included in the causal diagram as it is considered to be an intervention to reduce the impact of potential risk factors.

This graph can be viewed as a simplified representation of selected aspects of the associations and provides an easily understood depiction of the assumptions about the relationships between betel quid chewing and the adverse birth outcomes (term LBW or PTB). The framework is useful for identifying variables that must be measured and controlled to obtain un-confounded measures of association given the assumptions outlined in the graph. For example, based on Figure 2.3 and Figure 2.4, betel quid chewing before pregnancy is a confounder of the association between betel quid chewing before pregnancy and adverse birth outcomes, whereas micronutrient deficiency, anaemia and periodontal diseases are on the causal pathway between betel quid chewing and adverse birth outcomes.

The selected covariates are explained in details in Chapter 3.

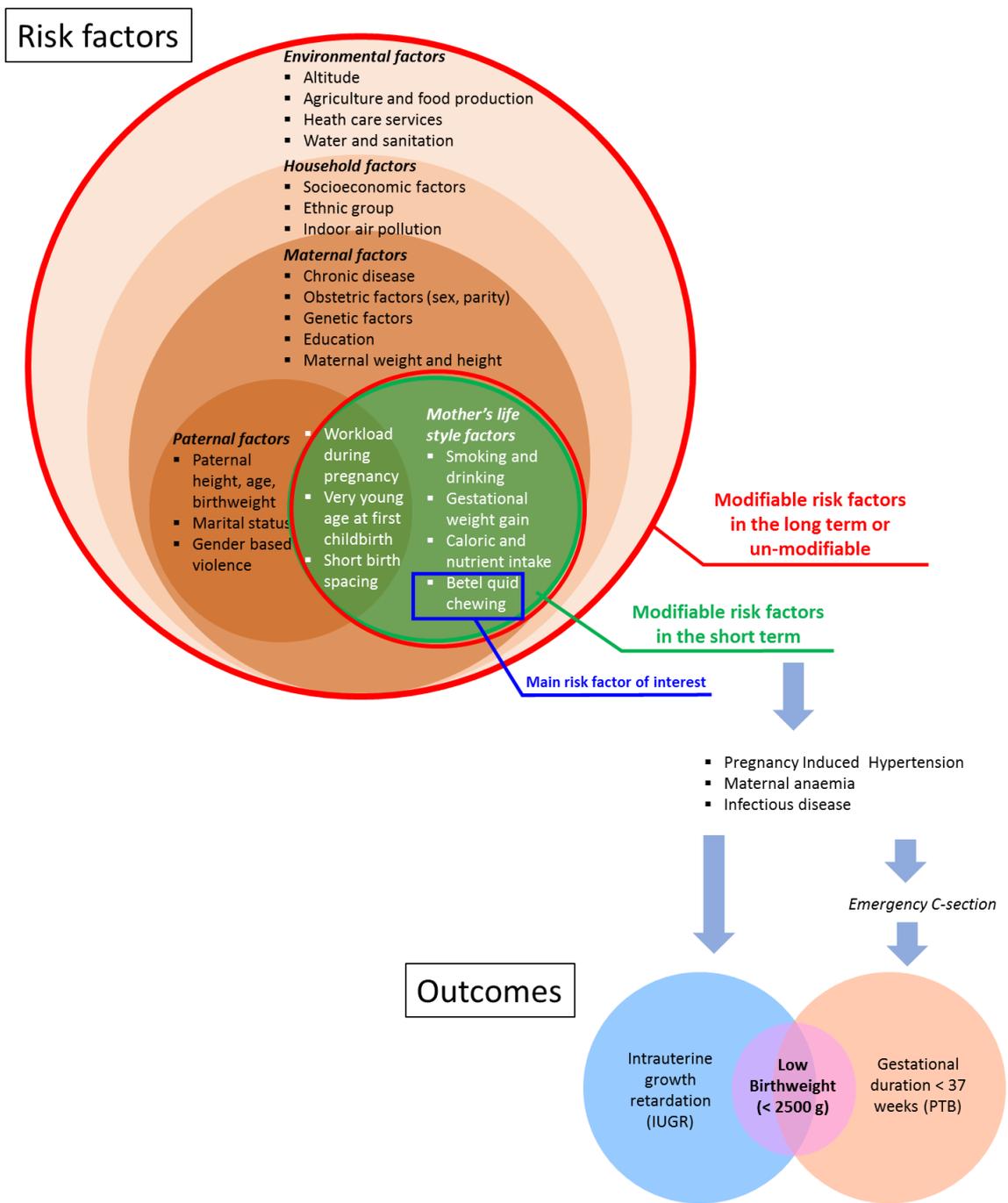


Figure 2.2. Conceptual Framework.

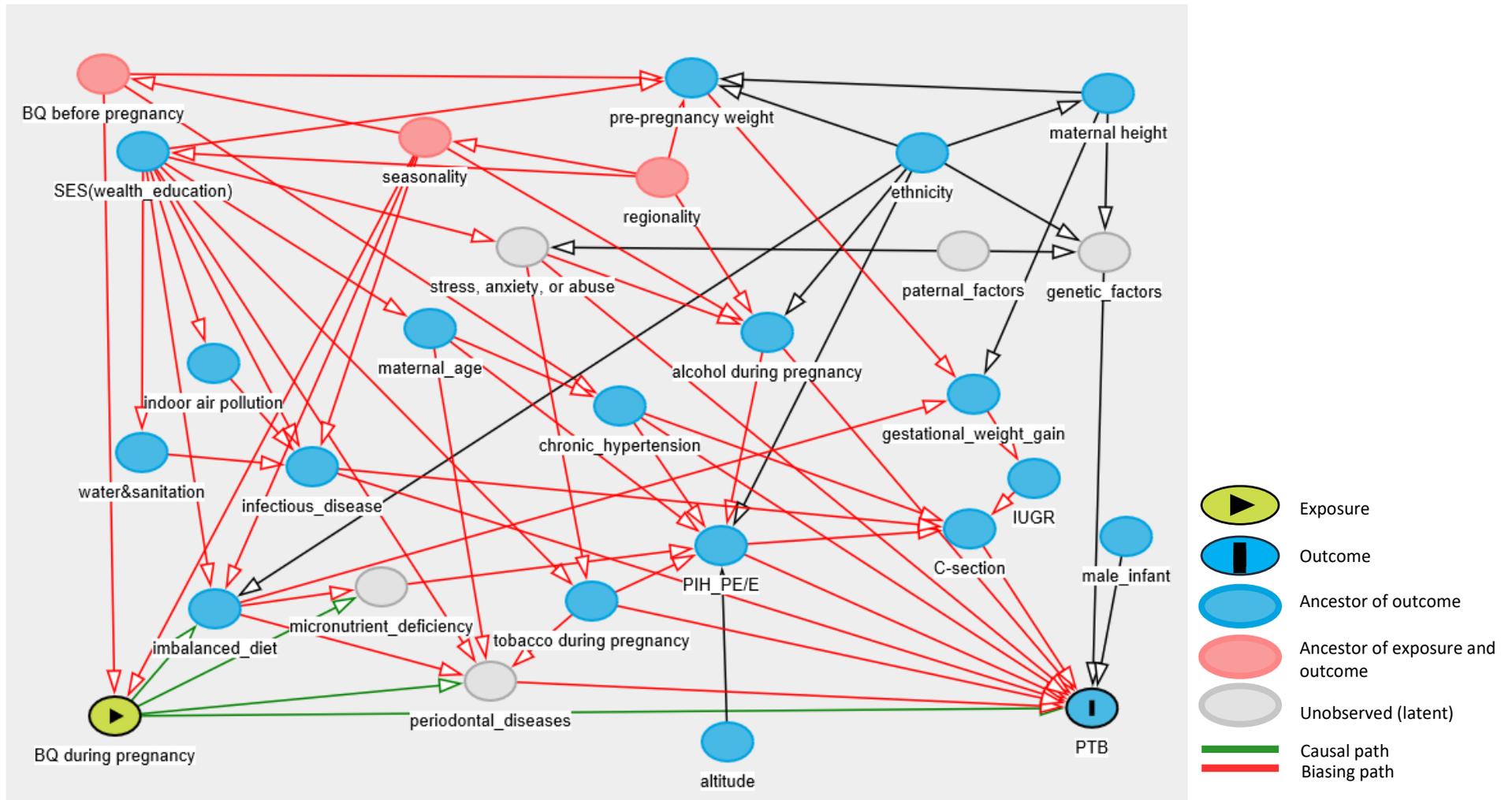


Figure 2.4. DAG for PTB.

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Chapter 3

Methods

This chapter describes methods of the case-control study and consists of four parts. The first part gives details of the study design. The second part describes questionnaire development and preparation for data collection. The third part contains data collection and management. The chapter ends with methodological information on the data analysis.

Part I Study Design

The first methodological section introduces the study design and justification (3.1), testable hypothesis (3.2), and sample size calculation (3.3).

3.1 Justification

In order to achieve the objectives, a hospital-based unmatched case-control study was conducted using a semi-structured questionnaire to collect information on the details of betel quid chewing and potential confounding variables from the mothers of LBW and/or PTB (cases) and normal weight (control) babies.

An observational study rather than experimental study was chosen, as the main aim of this study was to examine a causal factor rather than learning about a prevention or treatment for a disease. Although there is a greater chance of bias due to the retrospective nature of the data collection compared to a cohort study, a case-control study was chosen because the prevalence of LBW and PTB is estimated to be approximately 10%. When the disease is rare, as defined as a frequency of 20% or less, a case-control study is more efficient than a cohort study in terms of the cost and time [1]. Given the multifactorial nature of LBW and PTB, a case-control design also allows multiple risk factors or exposures for one outcome to be assessed [2]. However, it should be noted that the study designs provide evidence of association but not causation [3].

An unmatched case-control study was selected in order to avoid the selection bias introduced by the matching process. If there is a confounder in the source population, the process of matching will superimpose a selection bias over the initial confounder, generally leading to biasing the results toward the null value of effect [4]. If controls are selected to match the cases on a factor that is correlated with the exposure, controls who are more like cases with respect to exposure will be selected compared to when controls are randomly selected [4]. As a result, the crude exposure frequency in controls will become similar to that of cases.

A hospital-based case-control study, particularly based at JDWNRH in Thimphu and the two referral hospitals in Sarpang and Mongar, was chosen over population-based study mainly for two reasons. First, it was reasonably assumed that almost half of all facility births in Bhutan occur at these three referral hospitals. According to BMIS 2010, 63% of births in Bhutan were delivered in public health facilities [5]. In 2012, approximately 47% (4,751), of all health facility births

(10,169) were recorded at the three health facilities [6]. These hospitals are also located in three distinctly different regions and their inclusion of all three ensured that regional variations should be captured. The second reason was for the ease of implementation. A hospital-based case control study would enable interviewers to contact cases and controls soon after delivery and facilitate data monitoring and data quality assurance within a given time and budget. Extensive training for interviewers and close monitoring of data were required. It was believed that this would be done better by organizing an interviewer team consisted of the co-investigators and focal points at each study site rather than sending an individual interviewer to interview mothers at their households.

3.2 Testable hypotheses

Using a case control study design, there were many possible hypotheses that could be tested. The ones that I chose to focus were as follows:

1. Betel quid chewing (yes or no) increases the rate of LBW
2. Betel quid chewing (yes or no) increases the rate of PTB
3. Betel quid chewing (yes or no) increases the rate of maternal anaemia
4. There is a dose-response relationship between betel quid chewing and the odds of LBW and/or PTB deliveries.
5. There is a dose-response relationship between betel quid chewing and the odds of maternal anaemia.

3.3 Sample calculations

The sample size was calculated based on the above-mentioned hypotheses 1 and 2 using Epi Info 7 based on the formula proposed by Fleiss for an unmatched case-control study [7]. As odds ratios and proportion of cases and controls with exposure (betel quid chewing) in the literature are not applicable to the Bhutanese population, a sample size table was created for different scenarios (Table 3.1).

The following assumptions were used: For 80% power, $Z_{\beta}=.84$; For 0.05 significance level, $Z_{\alpha}=1.96$; $r=1$ (equal number of cases and controls); and the proportion exposed in the control group and case group.

Given that 61.5% of women were currently chewing betel quid according to the 2010 Gross National Survey [8], the estimates for exposure in the case group from 40 to 90% were used, while exposure in the control group was estimated to be lower than that in the case group.

The response rate in recent surveys in Bhutan ranged from 82% (7,142 respondents out of 8,700 samples) of the 2010 Gross National Happiness Survey to 97% of the 2012 Bhutan Living Standards Survey. There were no published data on case-controls or other facility-based observational studies in Bhutan at the time of calculating the sample size. To allow for an uncertainty in the response rate, 80% was used for sample size calculation. With the most

conservative scenario of minimum sample size of 388 in both the case and control group, with an 80% response rate, an estimated 485 participants were required in each group. This sample size would be sufficient to show an odds ratio of at least 1.5 with 60% of cases and 50% of controls being exposed.

Table 3.1. Sample size table.

Proportion of exposure (betel quid chewing) in the control group	Proportion of exposure (betel quid chewing) in the case group (power =0.8 & significant level =0.05)					
	40%	50%	60%	70%	80%	90%
10%	32	21	15	11	8	7
20%	83	40	24	16	11	8
30%	356	95	44	25	16	11
40%	-	388	99	44	24	14
50%	-	-	388	95	40	20
60%	-	-	-	356	83	33
70%	-	-	-	-	295	63
80%	-	-	-	-	-	201

Part II Questionnaire development and preparation for data collection

Part II of Chapter 3 covers issues pertaining to preparation for data collection. These include questionnaire development, pilot testing and finalisation, training and assignment of field staff, data monitoring, data entry and data cleaning.

3.4 Development of questionnaire

3.4.1 Introduction

The 22-page semi-structured questionnaire in English was developed and used to collect information on potential confounding variables from the mothers.

The questionnaire was developed according to the following steps [9-11]:

1. Identify domains and items based on literature review of the factors associated with PTB and LBW (Chapter 2.2)
2. Search for relevant validated survey instruments (Chapter 2.4)
3. Review relevant questionnaires used in studies and surveys of betel quid chewing (Chapter 2.4)
4. Draft questionnaire
5. Seek advice from an expert panel including health professionals in Bhutan
6. Pilot the questionnaire with 13 participants from the three hospitals. The participants were asked about clarity, comprehensibility, relevance to the topics and length of the questionnaire. Revision and repeat pre-testing of the questionnaire were done until it was satisfactory (Chapter 3)

The questionnaire consisted of 154 questions on the mother's general information, obstetric and medical information, and behavioural information. The majority of the questions were multiple-choice closed ended questions. Obstetric history, morbidity, and the results of ANC examinations were obtained from the MCH and hospital records. To supplement medical records, mothers were asked if they had been told that they had hypertension or high blood pressure and if they had selected symptoms of infectious diseases. As maternal psychosocial factors are difficult to measure in a quantitative questionnaire, and evidence from the literature is mixed, information regarding these issues was kept in the observational notes. Observations by the interviewers, interviewers' impression, any unexpected events, and other information captured during the interview were also recorded in the open space in the end of the questionnaires. The questionnaire was finalised in December 2015. The final questionnaire is attached in Appendix F.

3.4.2 Availability of routinely collected data

To avoid redundancy of data collection and mitigate the burden on the interviewers, routine collected information was reviewed. Although the MOH and JDWNRH were working on building

an electronic information system to keep patient records, at the time of this study the majority of the records were paper based and carried by the patients themselves. At each site there was also a paper-based birth registry book¹¹ in which nurses record details of the pregnancy and birth with a slight variation of the formats and information. Only at JDWNRH, were the data routinely being put into an electronic (Excel) database.

For the purpose of this study, relevant data were extracted from the birth registry books at each site and input into an Excel-based database for the year for 2015 in order to estimate the prevalence of LBW and PTB. This was undertaken by the author and her research team with agreement from the MOH and the study sites.

3.4.3 Content of the questionnaire

The content of the questionnaire was determined by the findings of the literature review (Chapter 2) according to their relevance to Bhutan. Factors included in the survey ranged from environmental factors (altitude, exposure to agriculture, accessed health care services, and access to improved water and sanitation), household factors (socio-economic factors and ethnic group), maternal factors (chronic diseases, obstetric factors, genetic factors, education, maternal weight and height), mother's life style factors (smoking, drinking, betel quid chewing, gestational weight gain, caloric and nutrient intake, physical activity, workload during pregnancy, and infectious diseases) to paternal factors. Published and grey literature, as well as government reports, were searched to identify a validated tools for measuring key independent variables, especially potentially toxic behaviour such as tobacco, alcohol, and betel nut consumption, applicable to the Bhutanese population (Chapter 2.4). Socio-demographic questions were positioned at the beginning to serve as a warm-up and behavioural and other sensitive questions were positioned in the later part of the questionnaire.

A summary of measurement of selected variables is provided below.

¹¹ Information included in the birth register books: serial number, registered number, admission date & time, delivery date & time, name, age, address and phone number of the mother, name of attendant if any, obstetric histories (gravida, para, abortions, still births, preterm, alive, dead, other medical problems), blood group, VDRL/RPR, mode of delivery (SVD, breech, LSCS and Indication of LSCS, forceps, vacuum, and others) and birth outcomes (single/twins, sex of the infant, birth weight, alive/dead, Apgar score, full-term/premature/post mature, IUGR, deformity, and other information if any). Maternal complications (circle if any) for JDWNRH and ERRH: APH, PPH, retained placenta, prolonged labour, obstructed labour, PIH, eclampsia, ruptured uterus, dead, and others (comments). Maternal complications are recorded in an open space at CRRH. Name of the medical professionals who conducted the delivery, and referral information if any. In addition, JDWNRH collects information on: number and place of ANC visits, maternal height and maternal weight from last ANC record, booking weight (kg), occupation, educational level, and income level of the mother.

(a) Outcome variables

Birth weight (Question 54)

Birthweight was measured within 24 hours of delivery at respective hospitals using the birth scales mentioned in Appendix H.

Gestational age (Question 55)

Date of first day of LMP by mother's recall, estimated date of delivery by first US scan, date of first US scan, and gestational age at first US scan were obtained from the MCH.

Mother's subjective assessment of the size of the infant at birth (Question 62)

When infants have not been weighed at birth, mother's recall has often been used to decide if the new born is LBW in order to estimate the percentage of infants with LBW in Demographic and Health Surveys (DHS) and other surveys. The question was taken from BMIS 2010 [5] to compare mother's subjective assessment of the size of the infant at birth and actual birthweight and gestational age.

(b) Independent variables

In relation to independent variables, ethnicity, altitude of residence, urban/rural residence, gestational weight gain, pre-pregnancy and pregnancy intervals were constructed from the information collected. Calculations, quantification, and modelling of the variables are explained in the following section (Section 3.11.2).

Maternal age (Questions 12 and 13)

Mother's stated age in completed years was recorded. The birth date, month, and year was recorded from the MCH if available.

Mother's name, permanent, current address (Questions 11, 14 and 18)

Mother's stated address was recorded. The MCH was also checked to validate the information.

Maternal anthropometric measures

Maternal height (Question 21)

Mother's height was recorded from the ANC initial general examination from the MCH.

Pre-pregnancy weight (Question 61)

Mothers' recalled pre-pregnancy weight and height were recorded during the interview.

Maternal weights (Questions 21 and 43)

Records of weights at the ANC visits and dates were recorded from the MCH.

Socioeconomic Status (Questions 93-105)

Questions were taken from BMIS 2010[5].

Quantifying the welfare of individuals or household is often difficult [12]. In theory, the best indicator of welfare is the actual consumption of the individuals and ideally this consumption would include both food and other goods as well as consumption of services such as education and health. However, in agricultural/rural economies home production may account for a significant proportion of a household's consumption. The valuation of such production is a major issue for the calculation of both expenditure and income for households that are both producers and consumers [12]. In Bhutan, health and education are provided free of costs. The 2012 Bhutan Living Standard Survey reported that consumption expenditure was 34% higher than income on average, possibly owing to underreporting of income including unreported transactions in agricultural products in the informal market and consumption of home-produced food and food received as gifts [13]. Therefore, income may be an inadequate measure to construct a socioeconomic variable. An asset based-approach is often used in these settings and was chosen for this study. Asset based measures or wealth index reflect long-run household wealth or living standards [14]. However, it should be noted that asset-based measures do not take account of short-run or temporary interruption, or shocks to the household and do not capture the quality of the assets [12].

In the present study, the principal component analysis (PCA), a multivariate statistical technique used to reduce the number of variables in a dataset into a smaller number of dimensions [12, 15], was used. Clumping and truncation are major challenges in producing PCA-based asset indices [16]. Clumping or clustering is defined as households being grouped together in a small number of distinct clusters [15]. Truncation refers to a more even distribution of socio-economic status (SES), but spread over a narrow range, making differentiating between socioeconomic groups difficult [15]. To avoid problems of clumping and truncation, it should be ensured that a broad range of asset variables that capture inequality between households and the stability of household classification into SES groups are included.

Maternal education attainment, marital status, and partner's education attainment (Questions 16, 20, 72)

Information was recorded in multiple-choice questions from the MCH and confirmed with the mothers during the interview.

Maternal occupation and partner's occupation (Question 68, 69, and 73)

Mother's self-reported occupation was recorded using multiple choice questions. Mother's description of work was recorded using open-ended questions. Partner's occupation and description work were also recorded using open-ended questions.

Access to the delivery hospital, means of transportation, hours of travel from home to the delivery hospital and reasons for delivery at the referral hospitals (Questions 64-67)

Information regarding access to health facility such as mother's self-reported mode of transportation and travel time from home to the delivery hospital were recorded. Reasons to deliver at the referral hospitals were recorded using a multiple-choice question.

Gravida, parity, number of abortions, children alive, and children dead (Questions 33-37)

Information was obtained and respective number was recorded from the MCH and confirmed with the mother during the interview.

Month and year of last pregnancy (Question 38)

Month and year the last pregnancy ended were recorded.

Number of ANC visits and the timing of the first ANC visit (Questions 39-42)

Information was recorded from the recall of the mothers and the MCH.

Obstetric records (Questions 22-26)

The following information was obtained from the ANC records where they were recorded as "yes/no" responses: Previous still birth or neonate loss; history of three or more consecutive spontaneous abortions; birth weight of last baby less than 2500 grams; birth weight of last baby more than 4500 grams; admission for hypertension, pre-eclampsia, or eclampsia in the last pregnancy; diastolic BP over 90mm Hg; pelvic mass; suspected STI/RTI, vaginal bleeding; cardiac disease; hypertension; thyroid disease; family history of twins; family history of congenital defects; known "substance " abuse; diabetes; hepatitis; tuberculosis; blood transfusion; renal disease.

Test results for syphilis, hepatitis B, and diabetes were recorded with dates from the ANC records. The MOH guideline advises against writing the test results in the MCH book. If positive, it was indicated in the MCH book and hospital medical records. Hence, absence of test results could imply negative results.

Anaemia (Question 46)

Interviewers ticked "Yes" in the questionnaire if mother's haemoglobin level in the MCH book and medical records was less than 10 g/dL. Detailed information on haemoglobin concentration was not collected.

Diabetes (Question 47)

Interviewers ticked "Yes" in the questionnaire if mothers were diagnosed with diabetes in the ANC or medical records. All pregnant women between 24- 28 weeks' gestation with 75g at the Oral Glucose Tolerance Test are screened at JDWNRH, ERRH and CRRH. Gestational diabetes mellitus is diagnosed if any one of the two readings are abnormal: fasting blood sugar ≥ 92 mg /dL (≥ 5.1

mmol/L) and 2 hours after 75g glucose ≥ 140 mg/dL (≥ 7.8 mmol/L). Detailed information on level of sugar or glucose was not collected in the questionnaire.

Hypertensive disorders (Questions 42, 43 and 57)

Information on gestational hypertensive complications was obtained from medical records and ANC records and recorded as “Yes/No/Unknown” (Q.43). Blood pressure measures from ANC records were recorded with the dates if available (Q.42). Mothers were asked if they had been told that they had high blood pressure or hypertension (Q.57).

Bhutan’s definition was consistent with the widely-used definition of hypertensive disorders[17] as below:

Pre-existing or chronic hypertension

Blood pressure greater than or equal to 140 and/or 90 mmHg occurring on two occasions at least four hours to a week apart before the twentieth week of pregnancy.

Pregnancy-induced hypertension (PIH) or Gestational hypertension

Blood pressure greater than or equal to 140 and/or 90 mmHg occurring on two occasions at least four hours to a week apart after the twentieth week of pregnancy without proteinuria.

Pre-eclampsia

Blood pressure greater than or equal to 140 and/or 90mmHg occurring on two occasions at least four hours to a week apart after the twentieth week of pregnancy.

AND

Proteinuria: ≥ 300 mg in 24 hours urine specimen

Eclampsia

Occurrence of seizures (convulsions) in association with pre-eclampsia.

Urinary Tract infection (UTI) (Question 44)

Information on UTI was obtained from the medical records and recorded as “Yes” or “No”. Information on classification of microbiologically-confirmed/not-microbiologically-confirmed was not collected.

Symptoms of potential infectious diseases (Question 56)

To supplement hospital records, mothers were asked if they had the following symptoms [18] and recorded as “Yes”, “No”, or “Unknown”: [a] Constant feeling of needing to urinate, even after having just urinated; [b] Pain or burning while urinating, or straight afterwards; [c] Pain in the lower belly, behind the front of the pelvis; [d] Cloudy or bloody urine; [e] Fever, feeling very hot and sweating; [f] Feeling very sick or weak; [g] Flank pain (in one or both sides); [h] Repeated

vomiting requiring medical treatment; [i] Chills, rigours or shivering persistently; and [j] Having a rash.

Mode of delivery (Question 52)

Hypertensive disorder during pregnancy may affect both the mother and foetus. Because there is no effective cure for preeclampsia other than delivery, delivery is always the treatment of choice for the mother [17]. Mode of delivery (spontaneous vaginal delivery (SVD), caesarean section (CS)-elective, CS-emergency, vacuum, forceps, breech, unknown) was recorded from medical records.

Working hours /shift during pregnancy (Question 70 and 71)

Information on working hours per week, whether they worked in shifts and if this included night shifts was collected by the interviewer. The questions did not take into account changes or interruption during pregnancy such as maternity leave or sick leave¹².

Physical activity (Questions 76-92)

General lifestyle, physical activity during work and leisure, and sedentary activities were asked about using questions and visual aids from the Bhutan STEP survey 2014 [19]. The STEP survey adopted the Global Physical Activity Questionnaire (GPAQ) developed by WHO for physical activity surveillance in countries. It collects information on physical activity participation in three settings (activity at work, travel to and from places, and recreational activities) in addition to sedentary behaviour, comprising 16 questions. The questionnaire defined vigorous intensity as “physical activities that cause large increases in breathing or heart rate” and moderate intensity as “physical activities that cause a small increase in breathing or heart rate.” The American College of Obstetricians and Gynaecologists (ACOG)’s guidelines recommend at least 150 minutes per week of moderate-intensity aerobic activity (equivalent to brisk walking) for healthy pregnant and postpartum women [20]. The guidelines suggested that the activity should be spread throughout the week and adjusted as medically indicated. Mother’s self-evaluation of physical activities during pregnancy (very active, moderately active, somewhat active, and not active) was also recorded.

Maternal nutrition intake during pregnancy (Questions 140-153)

Assessment of maternal nutritional status can be complicated. There are various approaches to assess individual nutritional status and these can be classified into two main categories: the retrospective reporting of intake from the recent or remote past and the prospective recording of consumption. Weighed dietary records involve recording the weights of all foods prior to their consumption [21]. “Precise

¹² As per the Bhutan Civil Service Rules (BCSR) 2012, civil servants, including teachers and nurses, were entitled to three month maternity leave before March 2016 which was extended to six months in March 2016. Duration of voluntary leave from work before delivery depends on the individual.

weighing” which measures all ingredients of the cooked composite dishes are weighed raw, before cooking and after cooking is considered the most accurate of all available methods and is often used as the gold standard in validation studies [21]. Chemical analyses of diets require the individual to provide a duplicate of all foods consumed, including beverages, over the stipulated number of days and different chemical analyses are conducted according to interest of the research [21]. Typical forms of questionnaire-based methods to measure retrospective intakes are dietary history method, food frequency questionnaires (FFQ), and 24-hour recall method. As these are often based on recall of the individuals, they are subject to recall bias. The 24-hour recall was designed to quantitatively assess current nutrient intake [22]. Food frequency questionnaires are most commonly used in epidemiological studies to estimate habitual energy and nutrient intake, especially in large cohorts [22]. They are inexpensive and easy to administer and demonstrate relatively high validity in validation studies [23, 24]. Challenges are lengthy questionnaires that ask how often a standard portion of each food or beverage was consumed during the previous year. The number of food and beverage items included in the FFQs could vary significantly from less than 22 to 170. The FFQs have been applied to pregnant mothers in epidemiological studies [23, 24]. In the present study, after reviewing several nutritional surveys using the FFQ approaches, questions regarding vegetable and fruit intake and the visual aid of examples of fruits were taken from the 2014 STEP Survey in Bhutan [19]. Frequency of food and appetite questions were adapted from Health and Nutrition Questionnaire from the Vermont Department of Health (USA) [25]. In addition, questions regarding dried meat, local butter tea which contains butter, salt, and hot water, coffee and milk tea with sugar were taken from the Bhutan STEP Survey 2007 [26].

Exposure to betel quid chewing (including commercial betel nut products), smoking (cigarettes and smokeless tobacco), and alcohol (Questions 106-139)

As this research aimed to measure betel quid chewing during the course of pregnancy and sufficiently control confounders such as drinking and smoking, the applicability of timeline followback (TLFB) and the fractional graduated-frequency (F-GF) introduced in the previous chapter of this thesis was assessed to understand the pattern of smoking, drinking and chewing betel nuts during the pilot study. The original questionnaires for TLFB and F-GF were obtained and adapted to Bhutan with the authors’ permission.

TLFB can collect rich information on content, amount, and pattern of daily use, and can yield high reliability. TLFB is also widely applied to quantify tobacco [27], marijuana and other drug use [28]. However, several challenges were identified: time required, the variety of containers and vessels used for drinking; and the variation in the alcohol content in Bhutan. In terms of application to this

research, one of the main concerns was time and risk of exhausting both the mothers, who had recently delivered, and interviewers by collecting daily consumption for all alcohol, tobacco, and betel quid chewing. The main study questionnaire (18-20 page) was expected to take approximately one hour. Adding the TLFB calendar to ask about the amount of drinking, smoking, and betel quid chewing in the past 90 days was expected to add another 10-15 minutes for each substance [29]. In addition, checking medical records was expected to take additional 20 minutes.

The F-GF has the advantage of taking into account a variety of alcoholic drinks as commonly used in Bhutan, although no previous studies have used this tool among the obstetric population. One of the concerns before the pilot study was whether Bhutanese mothers can understand the concept of three quarters, half and a quarter of an amount.

To compare the applicability of TLFB and F-GF to this study, the TLFB 3-month calendar and F-GF questions to measure maximum alcohol, in addition to a set of questions and a 10-month (pregnancy period), monthly calendar were added to the questionnaire. The 10-month calendar asked how much betel quid/how many smokeless tobaccos/how many cigarettes a participating mother used on an average day in each month during the past 10 months and approximately how many days the mother drank in each month during the past 10 months.

During the pilot study between October and December 2015, the 18-page questionnaire including the F-GF questions and the TLFB 3-month calendar including the Bhutanese event days was administered to both cases and controls at the three study sites. An identical plastic cup was provided for each study site and by showing the cup to the mothers, nurses asked how many cups (of this size) of alcoholic drink the mother took. To explain the concept of three-quarters, the half and one quarter to mothers, a visual aid card depicted a beaker with fluid levels at three quarters, half and a quarter of the full beaker (the greatest amount) was provided. This process was standardised across the study sites.

3.4.4 Validation of the questionnaire

Validity refers to whether a questionnaire is measuring what it intends to [10, 30]. The present study validated the questionnaire in respect of content validity and face validity. Content validation ensures the content of the questionnaire has enough items and adequately covers the domain under investigation and is usually undertaken by a group of experts [30]. Face validity indicates where the instrument appears to be assessing the desired qualities [31]. To assess content validity, a group of health professionals from London School of Hygiene and Tropical Medicine and from Bhutan was asked to assess the questionnaire. Each question and definition was reviewed and confirmed by all the co-investigators and interviewers at the meetings prior to the pilot study.

A pilot study was conducted at each site to evaluate feasibility, readability, consistency of style and formatting and the clarity of the language used for face validity [31]. The pilot questionnaire included three questions for mothers and five questions for the interviewers at the end to ask the mothers and the interviewers if questions were understood by respondents; questions were in the right order; all questions were necessary and sufficient; instructions to interviewers were adequate; and space and process of the interview was appropriate (Appendix E). After the pilot study at each site, the answers were reviewed and necessary changes were made based on the feedback.

3.4.5 Training and piloting

Training for interviewers was conducted at the three study hospitals from October to December 2014. The process of randomisation, timing and process of recruitment of cases and controls, how to obtain informed consent and how to build a rapport were demonstrated to research nurses and co-investigators and questionnaires and the process were evaluated and revised during the discussion after the pilot study. In total, 70 nurses and 6 co-investigators participated in the training. Continuing Medical Education (CME) credit was granted to each participant for training to everyone who completed the initial training and certificates will be given to those who participated in the actual data collection at the end of study. During the monitoring visits, trained interviewers were invited to capacity-building lectures. A list of the interviewers is provided in the annex.

Pilot studies were conducted at each study site to try out the process of data collection and questionnaire application. In total, 13 mothers were interviewed (Table 3.2). During the pilot studies, the questionnaire was revised and pre-testing was repeated until satisfactory. Mothers participating in the pilot studies were given a baby towel as a token of appreciation (Figure 3.1).

Table 3.2. Dates and number of participants in the pilot study, by study site.

Hospital	Date	Number of Cases	Number of Controls	Total
JDWRH	3 Nov – 16 Nov 2014	5	5	10
CRRH	22 Nov	1 (training purpose only not qualified for data analysis as home delivery)	1	2
ERRH	5 Dec	1	0	1
Total		7	6	13

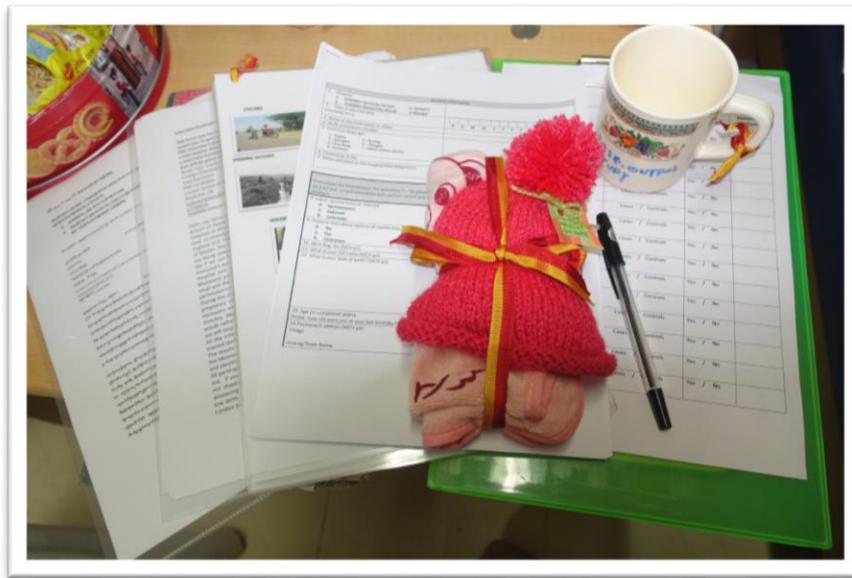


Figure 3.1. A set of the questionnaire and visual aids used in the study and thank you gift for mothers.

3.4.6 Finalisation of the questionnaire

TLFB [32] and F-GF combined with QF questions and responses to the questions were compared to measure patterns of drinking, smoking and betel chewing. During the pilot study, 8 out of 13 mothers reported their history of smoking, drinking, and/or betel nut chewing during pregnancy. Two mothers left the TLFB-calendar blank and three mothers reported having stopped before the last three months of pregnancy. Four mothers answered both TLFB-calendar and relevant questions that measure the same content. Four mothers answered the same frequency and quantity in the TLFB calendar compared to QF questions. One mother reported lower consumption of betel quid in the TLFB calendar than she reported in the question that asked average number of quids per day in each month in the past 10 months (Figure 3.2). Although there were not enough questionnaires to conduct statistical analyses, a few challenges of TLFB were identified. First, daily consumption was not well recalled during the interview and left blank or the same number was entered for every day. The questions which ask about average days or quantity for each month over the past 10 months revealed that the 3-month TLFB which asked about the previous three months does not capture the change of behaviour if mothers changed their behaviour during the pregnancy. In the pilot study, 3 out of 8 mothers stopped drinking after the first or second trimester when they realised that they were pregnant. This led to a decision to keep only the F-GF questions to measure drinking combined with a set of QF questions and 10-month calendar to record the quantity of average consumption of cigarettes, smokeless tobacco, betel quid and number of days of drinking.

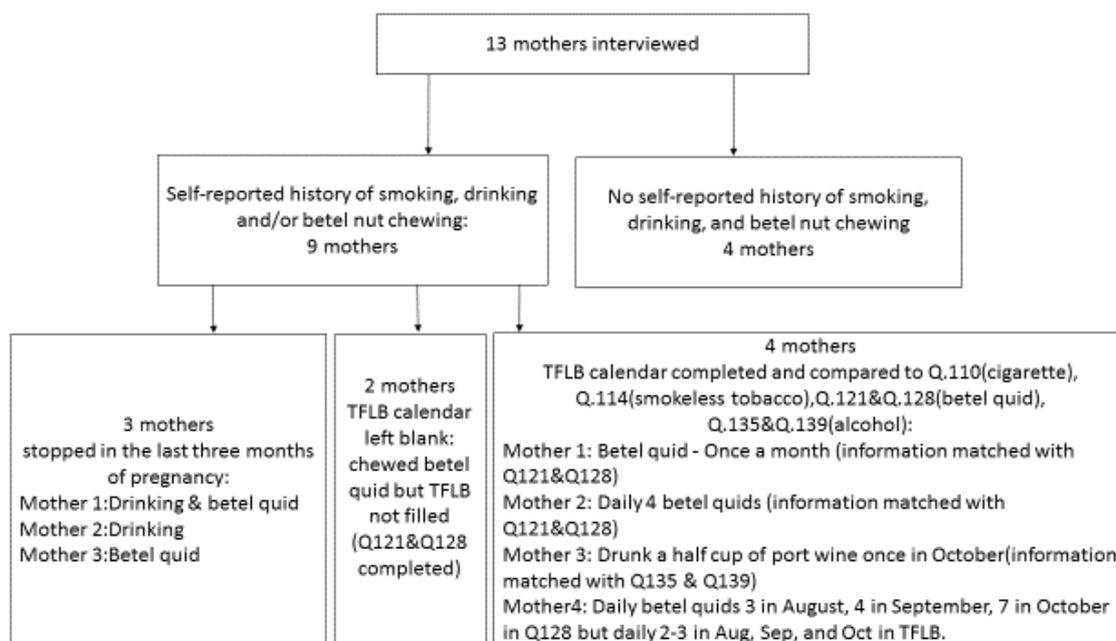


Figure 3.2. Completion of TFLB calendar in the pilot study.

3.5 Mode of interview

Health-risk behaviours including smoking, drinking and other drug use are often measured by administering questionnaires that require retrospective self-reports about engaging in these behaviours. Self-reported use might be underreported because of concerns about social desirability and fear of reprisal, especially for behaviours that are illegal, stigmatised, or associated with moral implications compared to measuring the actual consumption [33]. Brener et al. suggested alcohol and tobacco use is affected by perceptions of privacy and confidentiality and found that the self-administered questionnaire format produced higher reported rates of alcohol and other drug use than interviewer-administered questionnaires in the studies on adolescents they included in their review [33]. In this study, in order to interview the mothers about their tobacco, smokeless tobacco, alcohol, and betel quid chewing during pregnancy, the questions were administered by the interviewers later in the interview after they felt they had built enough rapport with the mothers and again mothers were reminded that these questions were only for research purposes and would not be reported to the government. However, the ban on import and production of tobacco and smoking in public in Bhutan by the Tobacco Act of 2010 could have stigmatised smoking. There is a chance of underreporting of smoking and alcohol due to cultural shame. Interviewers closely observed the mothers. For example, if they observed teeth with betel quid stains in mothers who reported that they do not chew betel nuts, a note was added to the questionnaire after the interview. The quantitative analysis was conducted as per mothers' stated information but the observational notes were reviewed for future research implications.

3.6 Interview language and translation

In Bhutan, English is the language of education. Dzongkha is the national language and the only language with a literacy tradition. Different dialects are spoken depending on the region. The questionnaire was developed in English and training was conducted in English to make sure the definition was clearly communicated. The questionnaire was translated into Dzongkha or other dialects (Sharchoop, Lhotsham (Nepali), Bumpat, Khengkha, and others if any) if necessary during the interviews by the interviewers. Ambiguities regarding translations were discussed and shared during the training and monitoring. The language in which the interview was conducted was recorded in the questionnaire.

Part III Data collection

Part III describes data collection and data management including the study sites and population, ethical consideration, data entry and data cleaning.

The study was conducted at the three referral hospitals between February 2015 and March 2016.

3.7 The study sites and population

The study population was the LBW or PTB neonates born at these three hospitals, and their mothers. A case was defined as a mother of a singleton live born infant whose gestational age is less than 37 completed weeks and/or an infant whose birth weight is less than 2500 g [2].

A control was defined as a mother of singleton live born term babies whose birth weight was more than 2500g and gestational age was greater than 37 weeks.

Mothers were excluded from the study in the case of:

- Multiple pregnancy;
- Still births;
- Maternal age younger than 17 years old at time of delivery;
- Referred to the hospital after home delivery; or
- Refused to participate in the study.

In general, mothers without complications were discharged between 12 and 24 hours after delivery and mothers who delivered by Caesarean section were discharged after 72 hours at the studysites. Figure 3.3 summarises the flow of recruitment process.

All mothers of a singleton live born preterm or/and LBW infant were approached to take part in the study and recruited by a trained interviewer during their post-delivery stay before discharge from each hospital.

Each time a new case was recruited, a list of mothers who were staying in the same maternity ward within +/- 3 days of the birth of the case was checked for a control. Mothers of singleton live born term babies whose birth weight was more than 2500g and gestational age was greater than 37 weeks were recruited during their post-delivery stay. If there was more than one control for one case, a standardised programmed excel file was used to randomly select the control. The purpose of the study was explained to all eligible subjects and their consent obtained by a trained interviewer.

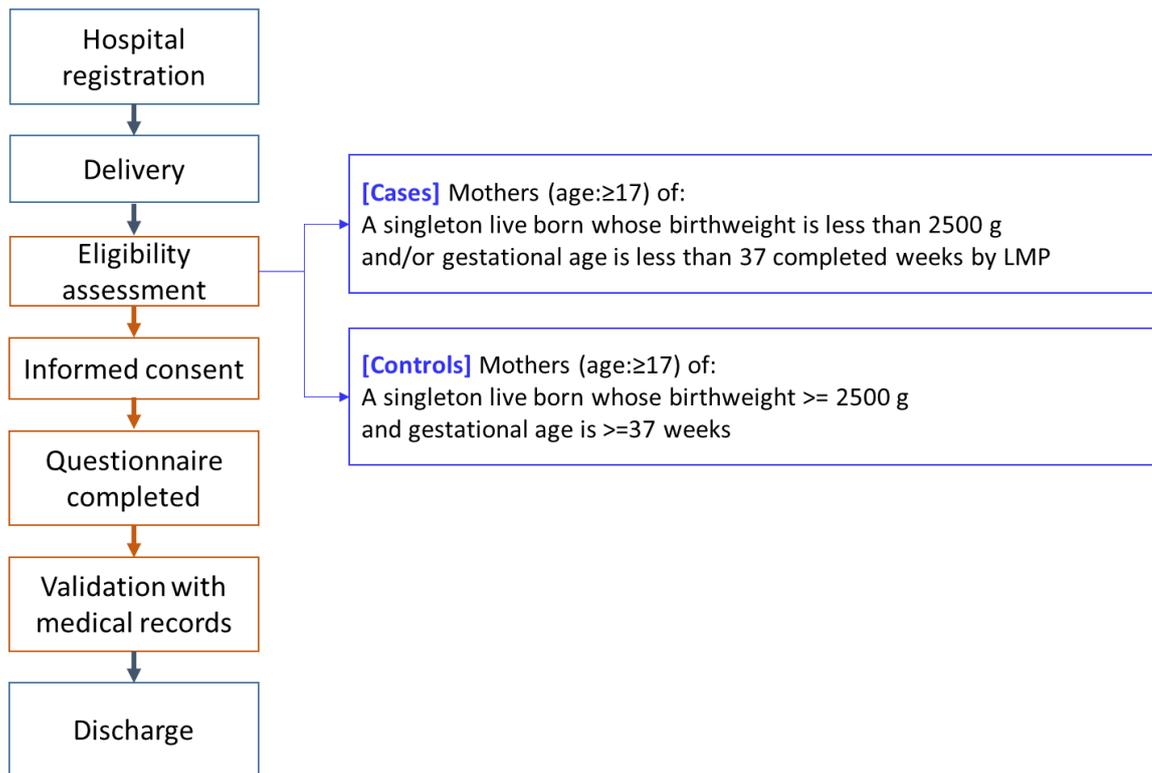


Figure 3.3. Recruitment process.

3.8 Estimates of gestational age used in the recruitment process

In Bhutan, the Maternal and Child Health Handbook (MCH) guideline by the Ministry of Health (MOH) advises that if the difference between the LMP and US estimates was different by less than one week and US is done early, the LMP estimate should be used to estimate gestational age, and if the difference is more than one week, then the US-based estimate should be used [34]. For those without US estimates, LMP is used. This policy was widely practiced across the country at the time of the study. For logistical reasons, recruitment in this study was based on gestational estimates in the MCH or hospital records and the validity of using this estimate of gestational age was explored in the analysis.

3.9 Ethical considerations and the informed consent process

Ethical approval for this research was obtained from the Royal Ethics Board of Health of the Royal Government of Bhutan (REBH/Approval/2014/017 (Amendment)) and the London School of Hygiene and Tropical Medicine (8348-01). Initial ethical approvals were obtained from the London School of Hygiene (8348) and Research Ethics Board of Health, Royal Government of Bhutan (REBH/Approval/2014/017) in July 2014. After the pilot studies, approvals for amendments were obtained in February 2015. Monitoring of one of the study sites, CRRH, was blindly conducted by REBH in May 2015 and checked for compliance. Good compliance was confirmed.

Informed consent forms were prepared in both English and Dzongkha as required by the Royal Government of Bhutan. Confidentiality and anonymity were explained to each participant and informed consent was obtained. As some of the mothers may have lost babies in previous pregnancies, some questions may raise powerful and upsetting issues. The interviewers were trained to be extremely sensitive and to respond appropriately if a participant did become upset. The trained interviewers clearly explained that participation was voluntary and that there were no consequences for refusing to take part in the study or to answer specific questions. A written informed consent form was read by the participants or read out loud by a trained interviewer if the participant could not read. If the mother agreed to participate, she was asked to sign the consent form. If she was unable to sign, her fingerprint and a witness's signature were obtained. The witness was a family member, friend, patient advocate, or someone independent of the research team. The mother was given a copy of the information sheet and consent form to keep (Appendix D).

3.10 Data entry and data management

3.10.1 Assignment of field staff

Questionnaires were administered to mothers by trained interviewers. At each site, at least two trained interviewers were in charge of managing the questionnaires and sending them regularly to the central data entry site (KGUMSB). The assignment of nurses and trainings was conducted with approval from each hospital and the MOH. One research assistant was recruited for entering the pilot study data and four research assistants (one site manager at JDWNRH, two data entry clerks and one data manager) who are fluent in English, Dzongkha, and Lhotsham (Nepali) with basic EXCEL and Microsoft skills and attention to detail were recruited during the data collection period. All the data entry clerks were trained on confidentiality and anonymity of data and EpiInfo7.

3.10.2 Data entry

A data entry form was developed using both EpiData and EpiInfo7 and tested using data from the pilot study by local staff. After assessment, EpiInfo7 was preferred. At the end of data collection cycles (every two months from CRRH and ERRH and every two weeks from JDWNRH), questionnaires from each study site were sent to KGUMSB and data from the questionnaires was centrally entered into an Epi Info 7 database at KGUMSB, including scripts from the open questions and observation notes. After the first data entry clerk scanned and entered data from the questionnaires, the paper questionnaires were handed over to the data entry clerk to enter the same questionnaires. As a result, two datasets were produced at the end of study period. The two datasets and scanned copies of the questionnaire were electronically stored and reported to the author by the data manager. The original questionnaires were kept secured in a locked room at the KGUMSB by the data manager.

3.10.3 Monitoring of recruitment and data quality

During the data collection period from February 2015 to the beginning of March 2016, recruitment of cases and controls and the quality of data was closely monitored by co-investigators and the author. The hospital registration records were checked to see if there were any cases discharged before being contacted. In the early stage of data collection, due to the high burden of routine work at the JDWNRH, missing eligible cases was identified as a challenge. One site manager who was a former nurse in the birthing centre and was studying to upgrade her qualification to Bachelor of Public Health at the KGUMSB, adjacent to the JDWNRH, was recruited to back up data collection. All questionnaires were checked at the start of data collection and randomly selected samples of the questionnaires were manually checked by the author who was based at the KGUMSB throughout the data collection period. Problems were detected and corrected immediately. Data entry clerks also reported potentially erroneous responses to the author. The author frequently visited JDWNRH and maintained communication by email and phone with the focal points at each study site to clarify any questions and to correct problems throughout the study. In addition to the initial training and pilot study, two monitoring visits to regional referral hospitals were conducted (CRRH: March 2015 and September 2015, ERRH: April 2015 and October 2015). Three interim analyses were conducted to monitor data quality and sample size in the field. Any ambiguities or problems were clarified and discussed in person and detailed feedback was shared during the monitoring visits.

3.10.4 Data cleaning

After the study period, the two datasets were compared by the author using STATA version 14 “Cf” command. Any discrepancies between the two datasets were compared to the original questionnaire. If further clarifications were needed, the interviewers were contacted. At the end of data cleaning process, the final dataset was produced for analysis.

Part IV Data Analysis

Part IV describes how variables were modelled for analysis. The methods of descriptive analysis and logistic regression modelling, using a statistical approach and directed approaches, and handling of missing data are explained.

3.11 Modelling of dependent and independent variables:

In this section, construction and categorisation of dependent and independent variables is described.

3.11.1 Modelling of outcome measures

Low birth weight

LBW was defined as a birth weight of less than 2500 g and modelled as a binary variable.

Gestational age at birth in days

As described previously, in Bhutan, the MOH MCH guideline advises that if the difference between the LMP and US estimates is less than a week and US is done early, the LMP estimates should be used and if the difference is more than 1 week, the US estimates should be used [34]. For those without US estimates, LMP or clinical estimates are used. This policy was widely practiced across the country at the time of the study. Recruitment was based on the hospital recorded gestational age. To understand the validity of hospital recorded gestational age, two estimates were calculated: gestational age at birth (in days) from LMP (date of birth - date of LMP) and US estimate calculated by [280-(estimated date of delivery – date of birth)]. The final gestational age reported in the present study was based on the US estimate where available. If US estimates were missing or erroneous, gestational age from hospital records was used.

Preterm birth

The US and LMP estimates were compared to the hospital recorded gestational age used in the recruitment to finalise classification of PTB. When classification of preterm by the three methods did not agree (n=26), the details were examined.

PTB was defined as a gestational age at birth of less than 259 days or before 37 completed weeks by the hospital records or by US estimate and modelled as a binary variable.

Case/control

Case was defined as PTB and/or LBW and modelled as a binary variable.

Small for gestational age (SGA) (Appendix B.2)

SGA was defined as a birth weight below the 10th percentile of a sex-specific birth weight distribution by gestational age.

Although SGA was not included in the main analyses, it was calculated using 2 different distributions: the US 2000 birth weight reference for gestational ages 20-44 weeks

[35] and the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) birth weight standard for gestational ages 24-42 weeks [36]. Two references differ mainly on the point that the INTERGROWTH-21st birth weight standard is a description of birth weight in fetuses in eight countries that experienced optimal growth whereas the US 2000 reference is a description of birth weight in the population, not a description of optimal birth weight. The details are given in Chapter 2.

3.11.2 Modelling of independent measures

Maternal Age

Age was calculated as (the date of delivery – the date of birth of the mother). In Bhutan, it was common to register the date of birth as January 1 of the birth year in the census due to the ambiguity of the actual date of birth. If the birth month and birth year were missing, January 1 was used. If the birth year was missing, self-reported age by the mother was used as age. Age was modelled as a categorical variable with six categories (<20, 20-<25, 25-<30, 30-<35, 35-<40, 40+ years) in the descriptive analysis and three categories (<20, 20-35, and 35+ years) in the logistic regression analysis after checking the distribution in the descriptive analyses.

Socioeconomic status

Using questions taken from BMIS 2010[5], a descriptive analysis was conducted on information on the following: ownership of consumer goods (watch, mobile phone, bike, motorcycle/scooter, car/truck, computer, foreign bow, camera, VCR/VCD/DVD player, and occasional dresses made with silk [sersho gho/kira or silk suits/sari]), dwelling characteristics (persons per sleeping room, type of floor, type of roof, type of wall, and type of cooking fuel), and access to improved water and sanitation.

A binary variable was produced to examine problems of truncation and clumping. Then, tetrachoric or polychoric correlation coefficients for the binary variables were calculated and the resulting correlation matrix was used. Weights (factor scores) were assigned to each household asset. A wealth score based on these weights and the assets owned by that household was assigned to each household; it ranged from 0.01 to 1.46. In the final analysis, 13 variables (ownership of watch, mobile phone, bicycle, motor cycle, car, computer, foreign bow, camera, VCR/VCD, DVD player, occasional dresses made with silk, access to improved sanitation, finished walls, finished floor, number of persons in a sleeping room) were included. The household population in the study was then ranked into five equal quintiles from lowest (poorest) to highest (richest) based on the wealth score. Finally, socio-economic status (SES), called “wealth index”, was created by dividing the sample population into quintiles.

Urban/rural

Residences were classified to urban or rural based on the population census classification using a list of village and thromdes codes obtained from the National Statistics Bureau and reviewed by a local research assistant for consistency.

Obstetric records

The following information was treated as binary variables in the analysis: previous still birth or neonate loss; history of three or more consecutive spontaneous abortions; birth weight of last baby less than 2500 grams; birth weight of last baby more than 4500 grams; admission for hypertension, pre-eclampsia, or eclampsia in the last pregnancy; diastolic BP over 90mm Hg; pelvic mass; suspected STI/RTI; vaginal bleeding; cardiac disease; thyroid disease; family history of twins; family history of congenital defects; known “substance abuse; diabetes; hepatitis; anaemia; tuberculosis; blood transfusion; and renal disease.

Hypertensive disorders

Hypertensive complications were modelled as a categorical variable, classed as: no complications (reference), pre-existing or chronic hypertension, gestational hypertension, pre-eclampsia, and eclampsia in the descriptive analysis. In the process of regression model building, it was modelled as a categorical variable with four categories: 0: no complications (reference), 1: pre-existing or chronic hypertension; 2: gestational hypertension; 3: pre-eclampsia, and 4: eclampsia. In the final model, eclampsia and pre-eclampsia were combined into one category. In the logistic regression models using the conventional approach, hypertension was modelled as a categorical variable with three categories (0: no hypertensive disorder, 1: chronic hypertension, 2: gestational hypertension, and 3: pre-eclampsia or eclampsia). In the logistic regression models using the DAG approach, chronic hypertension and pregnancy-induced hypertension composed of gestational hypertension, pre-eclampsia or eclampsia were modelled as a binary dummy variable respectively.

HIV, syphilis, and hepatitis B

HIV, syphilis, and hepatitis B were modelled as a binary variable if positive in the respective test results. The MOH guideline advises against writing the test results in the MCH book but if the test was positive, it was indicated in the MCH book and hospital medical records. Hence, absence of test results could imply negative results. The percentage of mothers with missing records was reported.

Urinary tract infection

Information on UTI was obtained from the medical records and categorised as a binary variable. Information on classification of microbiological-confirmed and not-microbiologically-confirmed results was not collected. A potential source of bias is that UTI is often undetected and unreported in Bhutan, which leads to misclassification of mothers with UTI as non-UTI. This leads to biasing association towards the null.

Symptoms of potential infectious diseases

The percentage of mothers with selected symptoms of infectious diseases were reported in the descriptive analyses.

Mode of delivery

In the descriptive analysis, mode of delivery was modelled as a categorical variable with five categories (SVD, CS-elective, CS-emergency vacuum, and breech; no forceps delivery in the study participants) and three categories (SVD as reference and CS-elective or CS-emergency) in the logistic regression analyses after checking the distribution in the descriptive analyses.

Pre-pregnancy weight and height

Mothers' recalled pre-pregnancy weight and height were modelled as a continuous variable. The accuracy is unknown and subject to recall bias. Another possible bias could be that mothers who remember or measure their weight could be more health conscious or more literate. Accordingly, more mothers of controls could remember their weight than mothers of cases. Also, overweight women tend underreport their weight [37]. Potential bias could lead to biasing towards the null.

Self-reported pre-pregnancy body mass index (BMI):

Although systematic reviews exploring the relationship between BMI and birth outcomes show heterogeneous cut-off points used in the literature [38-40], this study follows the international classification recommended by WHO [41].

Pre-pregnancy BMI was calculated as weight divided by height in (kg/m^2) and categorised as underweight (< 18.5), average ($18.5\text{--}25.0$), overweight (≥ 25) and obese (≥ 30).

As this is calculated based on mothers' recalled pre-pregnancy weight, it is subject to recall bias.

Pre-pregnancy BMI was used as, while several studies in the literature used first trimester BMI, only 18% of women went to ANC in the first trimester in the present study.

Gestational weight gain (GWG)

Three major options to measure GWG were identified: (1) the difference between the self-reported pre-pregnancy weight and the final pre-delivery weight; (2) linear weekly gain using a linear regression model; and (3) calculating the area under the GWG curve [42]. In this study, the difference between the self-reported pre-pregnancy weight and the final pre-delivery weight was calculated as the most feasible option. The Institute of Medicine recommends the GWG range according to pre-pregnancy BMI (for underweight: 28-40lbs/12.5-18kgs; normal: 25-35 lbs/11.5-16 kgs; overweight: 15-25lbs/7.0-11.5 kgs; obese: 11-20lbs/5-9kgs) [43]. GWG was modelled as a categorical variable with three categories: 0 (as per IOM recommendations); 1 (High GWG); and 2 (Low GWG). When the mothers were missing pre-pregnancy BMI in the complete case analysis, BMI 23-35 was used. As this is calculated based on mothers' recalled pre-pregnancy weight, it is subject to recall bias.

Maternal nutrition intake during pregnancy

All the food intake was modelled as a binary variable in the descriptive analyses. Quantification was not undertaken for most food intake. Hence, a dose response was not examined in the present study.

Parity

Parity was categorised into 4 categories: 0 (nulliparous), 1, 2-3, more than 4 in the descriptive analyses. While controlling for parity was important, having more categories did not materially change the results. As parsimonious models were preferred, the number of categories was reduced to a binary variable (nulliparity or not) in the logistic regression models.

Pregnancy intervals

Inter-pregnancy interval was modelled as a categorical variable, classed as: less than 12 months, 12-<18 months, 18-<24 months, 24 -<60 months, and 60 months or more. Pregnancy intervals could be subject to a recall bias. Although the questionnaire asked the month that the last pregnancy ended, more than 40% of the mothers (42.78%) did not remember the month. Thus, difference between the year of last pregnancy and the year of the most recent delivery was used to calculate pregnancy intervals when mothers were missing the month of last pregnancy.

Number of ANC visits and the timing of the first ANC visit

Number of ANC visits was coded into four categories: 0 (No ANC visits), 1-3, 4-8, more than 8 visits. The cut-off of four was used as WHO recommends at least four ANC visits. The timing of first ANC visit was examined and mean gestational weeks at the first ANC visit was reported. In the logistic regression models, number of ANC visits was modelled as a continuous variable as number of ANC visits per gestational week between the first ANC and time of delivery.

Ethnicity

Ethnicity was classified by the mother's name by a member of the research team and modelled as a binary variable (reference: Northern Bhutanese).

Working hours /shift during pregnancy

In the literature, prolonged working hours is often defined as more than 40 hours per week. In order to make comparison with other studies easy, this definition was used [44].

Working hours were classified into categories: 0 for less than 20 hours, 1 for 20 - 40 hours and 3 for >40 hours. Information on the shift work, whether mothers worked in shifts and if this included night shifts was collected. The questions did not take into account changes or interruption during pregnancy such as maternity leave or sick leave.

Physical activity

The percentage of mothers who reported having engaged in at least 10 minutes of vigorous or moderate physical activity through work or leisure and mean minutes of each activity were reported. The percentage of mothers who did not meet at least 150 minutes of

moderate-intensity activity per week was reported. Mother's self-evaluation of physical activities during pregnancy (very active, moderately active, somewhat active, and not active) was also reported in the descriptive analyses.

Access to health facility

In order to estimate geographical accessibility to health services, mean travel time to each delivery hospital from the mother's current residence was calculated based on the mother's self-reported travel time in hours. Mode of transportation was also asked about in the questionnaire. The place of the mother's first ANC visit, number of participants, and proportion of cases and controls were visualised using ArcGIS Desktop 10.3 (ESRI 2011. Redlands, CA: Environmental Systems Research Institute). ArcGIS is a Geological Information System (GIS) software. GISs are designed to capture, store, manipulate, analyse, manage, and present all types of geographical data. GIS programmes are capable of merging and analysing data on geographical positioning and attributes using a unique identifier. Coordinate systems define how points relate to each other and to the earth's surface, using X, Y, and Z (altitude) coordinates. X and Y can be expressed as a latitude and longitude (in degrees), in minutes and seconds or in units that are specific to a large selection of map projections. The XY codes of health facilities in Bhutan were obtained from the MOH. Other options such as straight line or Euclidean distance or distance along a path, road, train or other transport network were considered. However, as Bhutan does not have an address system for individual houses and identification of individual locations is difficult, measuring straight lines or Euclidean distance using ArcGIS was not feasible in the present study.

Altitude

Mean altitude of permanent and current altitudes in meters at Gewog level (administrative unit below prefecture or dzongkhag level) and difference in altitudes in meters between permanent and current residence Gewogs were calculated to understand the impact of altitude of residence on adverse birth outcomes. As information on altitude at the individual residence level was not available, the altitude of the Gewog centre was used as a proxy for altitude of residence. Information on the altitudes of Gewog centres was obtained from the Royal Government of Bhutan. Altitude was examined as continuous in the descriptive analyses and modelled as a categorical variable with four categories (<0 m (reference), 0-<1000 m, 1000-<2000 m, and 2000 m≤).

Seasonality

Month of delivery in the study participants was examined and explained when there was a difference in the number of participants in the study. A categorical variable of season of month of delivery (0: Fall [September, October, and November]; 1: Winter (reference) [December, January, and February]; 2: Spring [March, April, and May]; 3: Summer [June, July and August]) was analysed in the logistic regression models.

Exposure to betel quid chewing (including commercial betel nut products), tobacco (cigarettes and smokeless tobacco), and alcohol

Betel quid chewing and packaged betel nut products

The number of nuts consumed during the last three months of pregnancy was estimated as explained below in order to examine the dose response. In the logistic regression models, consuming betel quid or packaged betel nut products were combined and modelled as binary variables after checking prevalence and distribution. A categorical variable of betel nuts consumed during the period (0: none; 1: less than or equal to 1 nut per day; 2: more than 1 nut per day) was also analysed in the logistic regression.

Calculating betel nut consumption

Aggregate consumption during the last three month of pregnancy was calculated based on mother's self-reported average number of quids per day and frequency.

The daily consumption was multiplied by the frequency of betel quid chewing to calculate cumulative quids in the last three months of pregnancy. The average frequency of betel quid chewing was recorded as daily, weekly, monthly and others (specified). A few assumptions were made for "daily"- once a day or 30 days per month; for "weekly"- once a week or four times a month; and for "monthly" - once a month unless specified others. "Rarely", "sometimes", and "frequently" were reclassified as "monthly", "weekly" and "daily" respectively.

In the present study, chemical analyses were not conducted and serum arecoline levels were not measured.

Commercial betel nut products (Pan Masala)

The percentage of mothers who used betel nut products during pregnancy and frequency was reported in the descriptive analysis.

Tobacco (cigarettes and smokeless tobacco)

Dose response was examined using the amount of consumption of tobacco during the last three months of pregnancy. In the logistic regression models, consuming cigarettes or smokeless tobacco during pregnancy was combined and modelled as a binary variable after checking prevalence and distribution in the descriptive analyses. A categorical variable of the total grams of smokeless tobacco with two categories (0: none; and 1: less than 5 grams per day) was also analysed.

Cigarettes

The percentage of maternal cigarette use, patterns, and quantity were reported in the descriptive analysis.

Smokeless tobacco

The question asked how many packets of smokeless tobacco the mother used on an average day in each month in the past 10 months. Mean smokeless tobacco consumption in grams was calculated assuming one package of smokeless tobacco contains 10 grams.

Alcohol

The number of days mothers drank as opposed to aggregate ethanol grams was used to examine a dose effect of alcohol. In the logistic regression models, consuming alcohol during pregnancy was modelled as a binary variable and a categorical variable.

The graduated frequency (GF) measure was adapted. First, the maximum amount consumed on any day in the past 10 months was asked for different types of alcohol drinks (beer, wine, local spirits, local wines, liquor). Table 3.3 provides the grid used. Once the maximum is determined, the mother is asked how often she drank about this amount (the maximum), then how often did she drink about three quarters of that amount, followed by about half that amount and finally about one quarter of that amount. The visual aid card depicted a beaker with fluid levels at three quarters, half, and a quarter full and a plastic cup to show the size of the cup (200ml) was used. Three variables were constructed: (1) total maximum volume of consumption per occasion by using the grid (sum of number of cups * amount of ethanol (grams) in 200 ml of each drink) was calculated: (2) total amount of ethanol was calculated by multiplying the amount of ethanol and frequency: and (3) drinking intensity was classified based on maximum volume of ethanol in grams into low (<20 grams), moderate (20-40 grams), and high(>40 grams).

Table 3.3. Assumptions of alcohol concentrations in the commonly-consumed alcoholic drinks in Bhutan.

Type of alcohol	Brand	% (v/v)	Grams of ethanol per 200 ml cup
Local spirit	Ara	25%	39.7 g
Local wine	Changkey, Singchang, Bangchang, Tongpa	15%	23.8 g
Industrial Beer A	Dansberg, Budweiser, Heineken, Singha Beer, Chang beer, Calsberg, Orchim, Haywards, Royal challenge, Golden eagle, San Miguel	5%	7.9 g
Industrial Beer B	Druk 11000, HIT	8%	12.7 g
Industry-made wine drink	SPY	5%	7.9 g
Table Wine	Takin, Santa Barbara, Happiness	16%	25.4 g
Port Wine		18%	28.6 g
Liquor	Rum, Whisky, Brandy	42.8%	68.0 g

*Gram equivalents of pure alcohol = ethanol concentration of beverage consumed X metric volume X 0.794 (relative weight of alcohol). Assumptions from Dorji (2012) [45] were adopted.

3.12 Statistical methods

This section describes methodologies relating to the statistical analyses for outcome validation, descriptive analysis, logistic regression analysis using a statistical approach and a causal directed acyclic graph (DAG) approach, and sensitivity analysis.

3.12.1 Examining validity of classification of preterm

The distribution of gestational age at birth (in days) from LMP (date of birth - date of LMP) and US [280-(estimated date of delivery – date of birth)] were compared. The difference in days between the LMP and US estimates was calculated (LMP estimate – US estimate, hereafter referred to as “gestational age difference”) and analysed as a continuous variable and categorised into five groups (<-14, -14 to -8, -7 to +7, +8 to +14 and >+14 days). Positive values indicate that the LMP-based gestational age estimate exceeds the US estimate and negative values indicate that the LMP-based gestational age estimate is shorter than the US-based estimate.

The LMP estimate of gestational age was compared to the measure of the US gold standard using Lin’s concordance correlation coefficient and Bland-Altman analysis for exact comparison of continuous values for continuous gestational days and Kappa’s coefficient for classification into PTB. The chi-square test for trend was used to analyse the differences in proportion of selected maternal and infant characteristics in five categories of the discrepancy between gestational estimates by LMP and US scan and in the mothers with early scans and late or no scans. Where the count was smaller than five, the Fisher’s exact test was reported. Multivariate logistic regression was used to investigate the association between selected maternal and infant characteristics and the magnitude and direction of the discrepancy and the probability of having early scans compared. Results were presented as adjusted odds ratios with 95% confidence intervals. P-value <0.05 was considered statistically significant.

3.12.2 Descriptive analysis

A descriptive analysis was performed in order to understand the socioeconomic situation, nutritional status, physical activity, health seeking behaviours and intake of alcohol, tobacco and betel nut during pregnancy, as well as key obstetric factors in the study participants.

For categorical variables, the differences in the proportion of controls and cases were tested using the chi-squared test. Where the count was smaller than five, the Fisher’s exact test was reported. The number of study participants in each category, percentages and the corresponding P value were presented.

For continuous variables, the two-sample t test was used. When variance was not equal, Welch's t test was used. The number of study participants in each category, mean, standard deviation, and the corresponding P-value were presented.

Similarly, controls and the mothers of term LBW or preterm were compared and presented in the same tables as cases. P-value <0.05 was considered statistically significant.

3.12.3 Logistic regression modelling

(a) A statistical approach

Selected independent variables were analysed in the univariable analysis. Covariates that were significant at $p < 0.10$ in addition to wealth quintile, education, ethnicity, and number of ANC visits were included in a multivariate logistic regression model. Modelling of each variable was finalised considering its distribution and nature of the relationship with the outcome.

Multivariable analysis models were built using Akaike Information Criterion (AIC) to minimise the AIC (Table J.1 in Appendix J). Multicollinearity was checked to see how the standard error changed as variables were added to the model (Table J.2 in Appendix J). If there is a sudden big increase in a standard error when a variable is added to the model, it indicates a problem.

Two models were used. They only differed in the modelling of betel quid chewing, tobacco, and drinking. Model 1 used a binary variable of betel nuts during pregnancy including betel quid chewing or packaged betel products during pregnancy, tobacco during pregnancy including cigarette smoking and smokeless tobacco, and drinking during pregnancy. Model 2 used a categorical variable of number of betel quids consumed during the last three month of pregnancy with three categories (0: none; 1: less than or equal to 1 nut per day; 2: more than 1 nut per day), a categorical variable of the total grams of smokeless tobacco with 2 categories (0: none; and 1: less than 5 grams per day), and a categorical variable of number of days of drinking during the last three months of pregnancy with 3 categories (0: none; 1: less than or equal to once a week; and more than once a week).

Model 1

$$\text{Logit (P)} = \alpha + \beta_1 \text{CRRH} + \beta_2 \text{ERRH} + \beta_3 \text{Season(fall)} + \beta_4 \text{Season(spring)} + \beta_5 \text{Season(summer)} + \beta_6 \text{Female Infant} + \beta_7 \text{age}(<20) + \beta_8 \text{age}(35<) + \beta_9 \text{education(NFE)} + \beta_{10} \text{education(primary school)} + \beta_{11} \text{education(secondary school)} + \beta_{12} \text{education(diploma)} + \beta_{13} \text{wealth(poorest)} + \beta_{14} \text{wealth(second)} + \beta_{15} \text{wealth(fourth)} + \beta_{16} \text{wealth(richest)} + \beta_{17} \text{number of ANC} + \beta_{18} \text{ethnicity} + \beta_{19} \text{GWG(high)} + \beta_{20} \text{GWG(low)} + \beta_{21} \text{number of meals per day} + \beta_{22} \text{urinary tract infection} + \beta_{23} \text{nulliparity} + \beta_{24} \text{previous history of preterm} + \beta_{25} \text{hypertensive disorders(chronic/pre-existing hypertension)} + \beta_{26} \text{hypertensive disorders(gestational hypertension)} + \beta_{27} \text{hypertensive disorders(pre-eclampsia/eclampsia)} + \beta_{28} \text{mode of delivery(cs-elective)} + \beta_{29} \text{mode of delivery(cs-emergency)} + \beta_{30} \text{betel quid/betel products during pregnancy} + \beta_{31} \text{cigarette/smokeless tobacco during pregnancy} + \beta_{32} \text{alcohol during pregnancy}$$

Model 2

$$\text{Logit (P)} = \alpha + \beta_1 \text{ CRRH} + \beta_2 \text{ ERRH} + \beta_3 \text{ Season(fall)} + \beta_4 \text{ Season(spring)} + \beta_5 \text{ Season(summer)} + \beta_6 \text{ Female Infant} + \beta_7 \text{ age(<20)} + \beta_8 \text{ age(35<)} + \beta_9 \text{ education(NFE)} + \beta_{10} \text{ education(primary school)} + \beta_{11} \text{ education(secondary school)} + \beta_{12} \text{ education(diploma)} + \beta_{13} \text{ wealth(poorest)} + \beta_{14} \text{ wealth(second)} + \beta_{15} \text{ wealth(fourth)} + \beta_{16} \text{ wealth(richest)} + \beta_{17} \text{ number of ANC} + \beta_{18} \text{ ethnicity} + \beta_{19} \text{ GWG(high)} + \beta_{20} \text{ GWG(low)} + \beta_{21} \text{ number of meals per day} + \beta_{22} \text{ urinary tract infection} + \beta_{23} \text{ nulliparity} + \beta_{24} \text{ previous history of preterm} + \beta_{25} \text{ hypertensive disorders(chronic/pre-existing hypertension)} + \beta_{26} \text{ hypertensive disorders(gestational hypertension)} + \beta_{27} \text{ hypertensive disorders(pre-eclampsia/eclampsia)} + \beta_{28} \text{ mode of delivery(cs-elective)} + \beta_{29} \text{ mode of delivery(cs-emergency)} + \beta_{30} \text{ number of betel quid}(\leq 1 \text{ nut per day}) \text{ during the last three months of pregnancy} + \beta_{31} \text{ number of betel quid}(> 1 \text{ nut per day}) \text{ during the last three months of pregnancy} + \beta_{32} \text{ less than 5 grams of smokeless tobacco during the last three months of pregnancy} + \beta_{33} \text{ frequency of drinking during the last three months of pregnancy}(\leq \text{ once a week}) + \beta_{34} \text{ frequency of drinking during the last three months of pregnancy}(> \text{ once a week})$$

First, the LBW and/or PTB (case) was used as an outcome and the results are provided in Appendix Table.J.3. Next, the sub-analyses of term LBW and PTB were conducted separately to compare with the DAG approach. Adjusted odd ratios are presented with their 95% CI.

(b) A causal directed acyclic graph (DAGs) approach

The aforementioned statistical models embody many parametric assumptions that are not known to be correct and may well be incorrect [46]. Without taking direct and indirect causal assumptions into account, covariates informed in a statistical approach may lead to biased estimates by controlling for an intermediate variable or due to an introduction of associations which are otherwise not related [47]. For example, if two covariates both cause a third covariate (it is called a “collider” in the DAG as two directed arrows collide at the covariate), then adjustment for the third covariate creates a conditional association between the first two covariates and could introduce bias [46-48]. Causal diagrams are graphical models for causal relations that can complement conventional models. Minimum sets of covariates controlled for in the models were identified based on the two criteria: (1) they should not be on the causal pathway between the exposure and outcome and should not be a collider; and (2) controlling them blocks every path between the exposure and the outcome [49]. DAGs used in the present study are provided in Chapter 2. Preterm and term LBW were considered separately in the DAGs as it was assumed that there were slightly different causal pathways from betel quid chewing leading to preterm and term LBW deliveries (Figure 2.3. **DAG for term LBW.** and Figure 2.4. **DAG for PTB.**). Sufficient sets from the DAGs were determined using DAGGITY software [50]. Separate logistic analyses were conducted using the minimum sets of covariates for different exposure variables.

Table 3.4 and Table 3.5 summarise covariates controlled in the model informed by the DAG. In the logistic models, wealth quintile and education variables were used to represent SES. Delivery

hospital was used as a proxy for regionality. Number of meals per day was used as a proxy for inadequate or imbalanced diet.

As for alcohol and tobacco, it was originally assumed that low SES has an indirect relationship with alcohol and tobacco consumption during pregnancy through psychosocial factors such as stress, anxiety, and abuse. Also it was assumed that there is a direct causal relationship between the psychosocial factors on preterm or term LBW deliveries. With these assumptions, if psychosocial factors were observed, a set of covariates that should be controlled for to estimate the total effect of alcohol on PTB or LBW would have been ethnicity, psychosocial factors, regionality, and seasonality. A set of covariates that should be controlled for to estimate the total effect of tobacco use during pregnancy on PTB or LBW would have been SES and psychosocial factors. However, as the present model did not aim to measure psychosocial factors, it was not possible to estimate the total effect of alcohol or tobacco on PTB or LBW.

In order to measure the total effect of alcohol and tobacco on adverse birth outcomes, assumptions on SES and psychosocial factors were slightly modified and the modified DAG is presented as DAG3. DAG3 assumes that there is no direct or indirect effect of psychosocial factors and that there is a direct effect of SES on alcohol or tobacco consumption, leading to term LBW (Figure 3.4) or PTB (Figure 3.5). In this model, the total effect of alcohol on PTB can be estimated by controlling for several different sets of covariates such as (ethnicity, SES, regionality, and seasonality) or (betel quid chewing during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, and seasonality). Similarly, the total effect of alcohol on term LBW can be estimated by adjusting for betel quid chewing during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, and seasonality. Using the same DAG, the total effect of tobacco on term or preterm LBW can be estimated adjusting for SES.

For the secondary outcome, anaemia, seasonality was adjusted based on the assumed causal framework provided in DAG 4 (Figure 3.6) and seasonality and SES were adjusted based on the assumed casual framework in DAG 5 (Figure 3.7). Although there seemed to be no causal effect between SES and betel quid chewing in Bhutan, DAG 5 was compared to DAG 4 to see the plausibility of the causal assumptions in DAG 4.

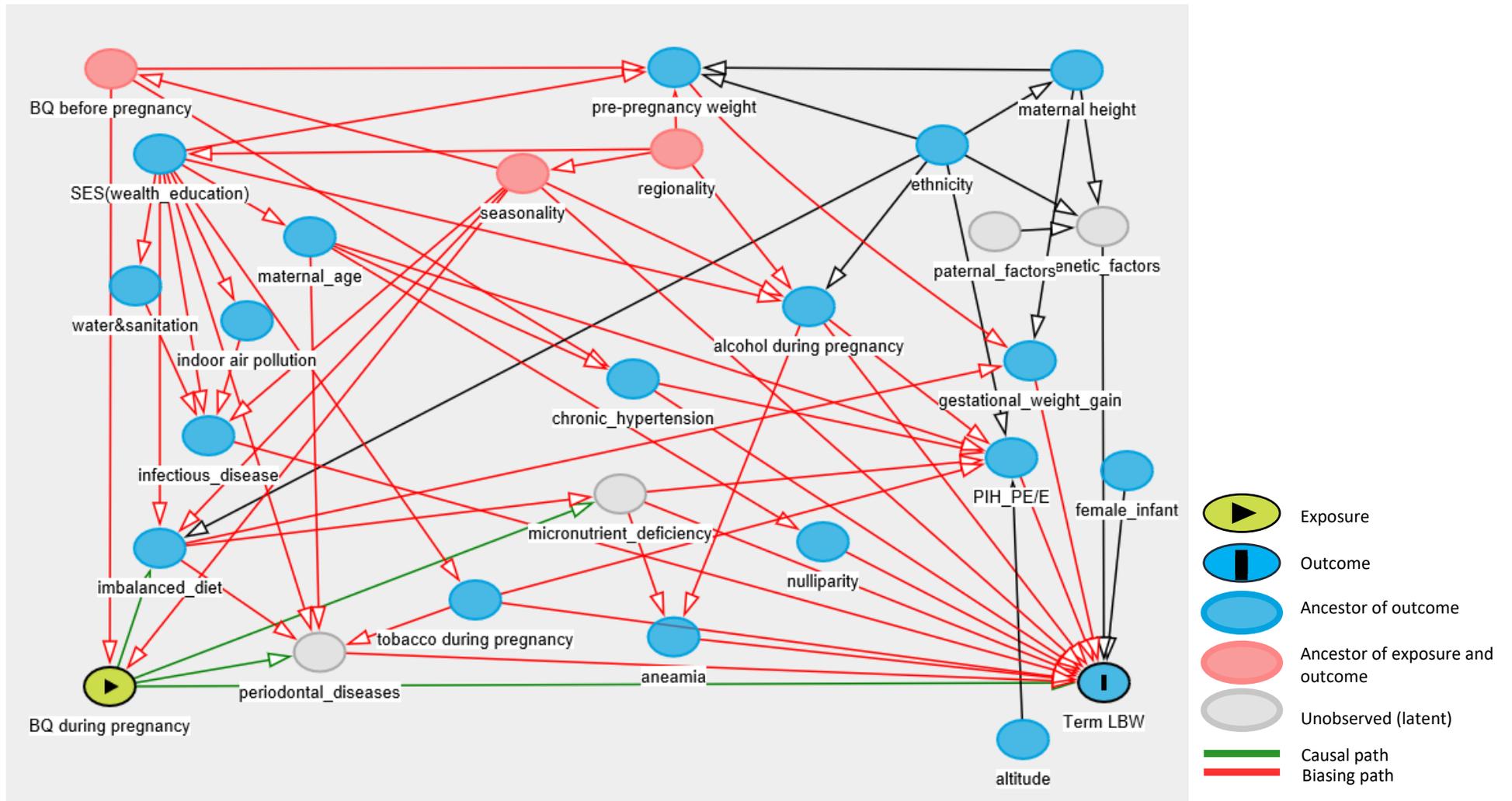


Figure 3.4. DAG3 (LBW) assuming there is no indirect or direct causal relationship between psychosocial factors and term LBW.

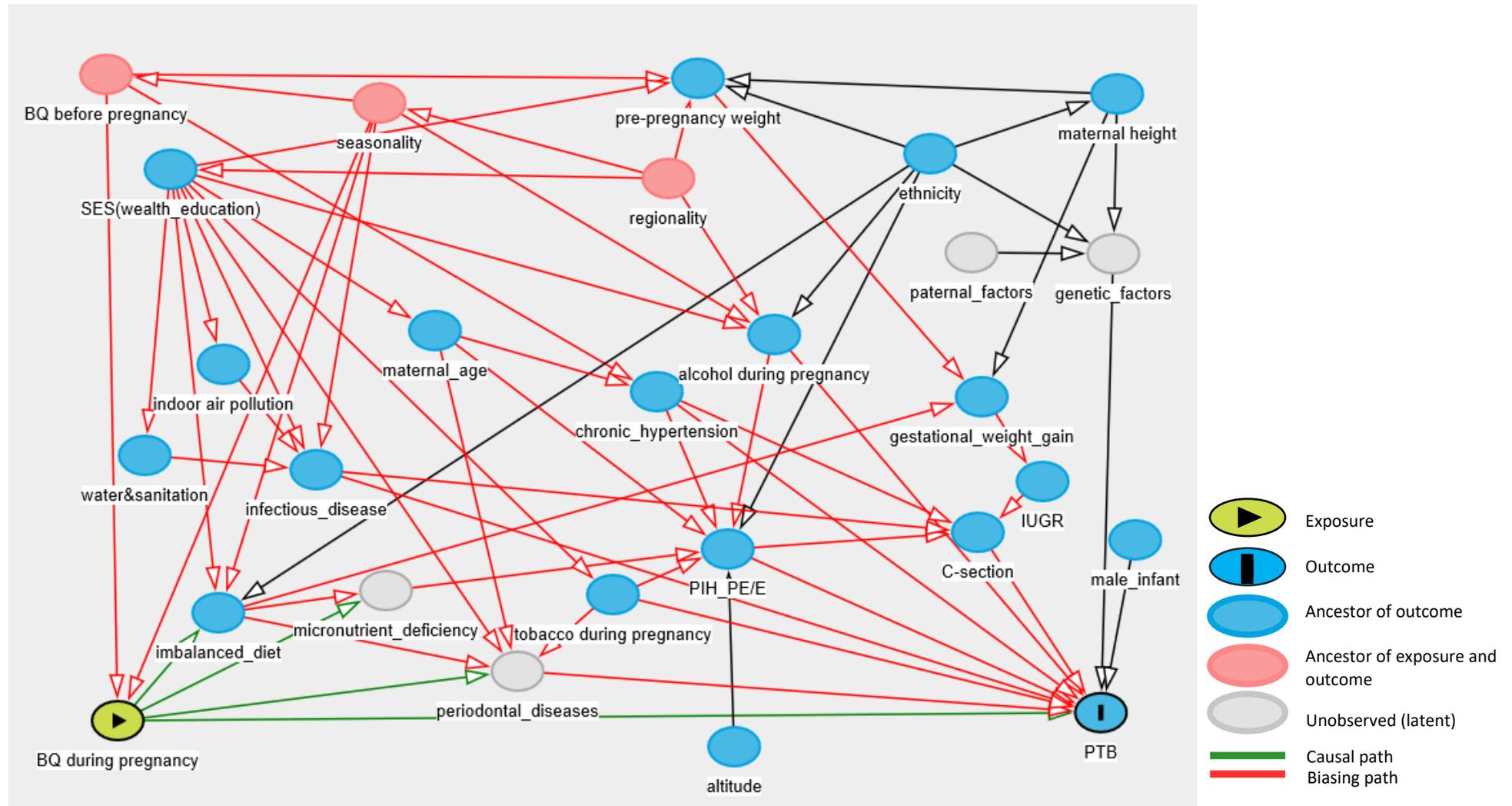


Figure 3.5. DAG3 (PTB) assuming there is no indirect or direct causal relationship between psychosocial factors and PTB.

Table 3.4. Sets of covariates to estimate the total effects on preterm delivery for different exposure variables identified in the DAG approach.

Exposure	Factors adjusted for
Betel quid chewing during pregnancy	<i>Covariates sets 1:</i> Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality <i>Covariates sets 2:</i> BQ chewing before pregnancy, seasonality
Gestational weight gain	<i>Covariates sets 1:</i> BQ during pregnancy, ethnicity, SES, chronic hypertension, imbalanced diet, maternal height, maternal age, regionality, seasonality <i>Covariates sets 2:</i> Imbalanced diet, maternal height, pre-pregnancy weight
UTI	SES, seasonality
Chronic hypertension	BQ during pregnancy, ethnicity, SES, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality
PIH, Pre-eclampsia or Eclampsia (PIH, PE/Eclampsia)	Alcohol during pregnancy, BQ during pregnancy, ethnicity, chronic hypertension, imbalanced diet, maternal age, tobacco during pregnancy
C-section	PIH, PE/Eclampsia, chronic hypertension, gestational weight gain, UTI
Wealth quintile	Regionality
Education	Regionality
Maternal age	SES
Male infant	No adjustment is necessary
Ethnicity	No adjustment is necessary
Alcohol	DAG 3 : BQ during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, seasonality DAG 3: Ethnicity, SES, regionality, seasonality
Tobacco	DAG 3: SES

Table 3.5. Sets of covariates to estimate the total effects on term LBW for different exposure variables identified in the DAG approach.

Exposure	Factors adjusted for
Betel quid chewing during pregnancy	<i>Covariates set 1:</i> Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality <i>Covariates set 2:</i> BQ chewing before pregnancy, seasonality
Gestational weight gain	<i>Covariates set 1:</i> BQ during pregnancy, ethnicity, SES, chronic hypertension, imbalanced diet, maternal height, maternal age, regionality, seasonality <i>Covariates set 2:</i> Imbalanced diet, maternal height, pre-pregnancy weight, seasonality
Chronic hypertension	BQ during pregnancy, ethnicity, SES, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality
UTI	SES, seasonality
Nulliparity	Maternal age
Wealth quintile	Regionality
Education	Regionality
Maternal age	SES
Sex of the infant	No adjustment is necessary
Ethnicity	No adjustment is necessary
Alcohol	<i>DAG3:</i> BQ during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, seasonality
Tobacco	<i>DAG 3:</i> SES

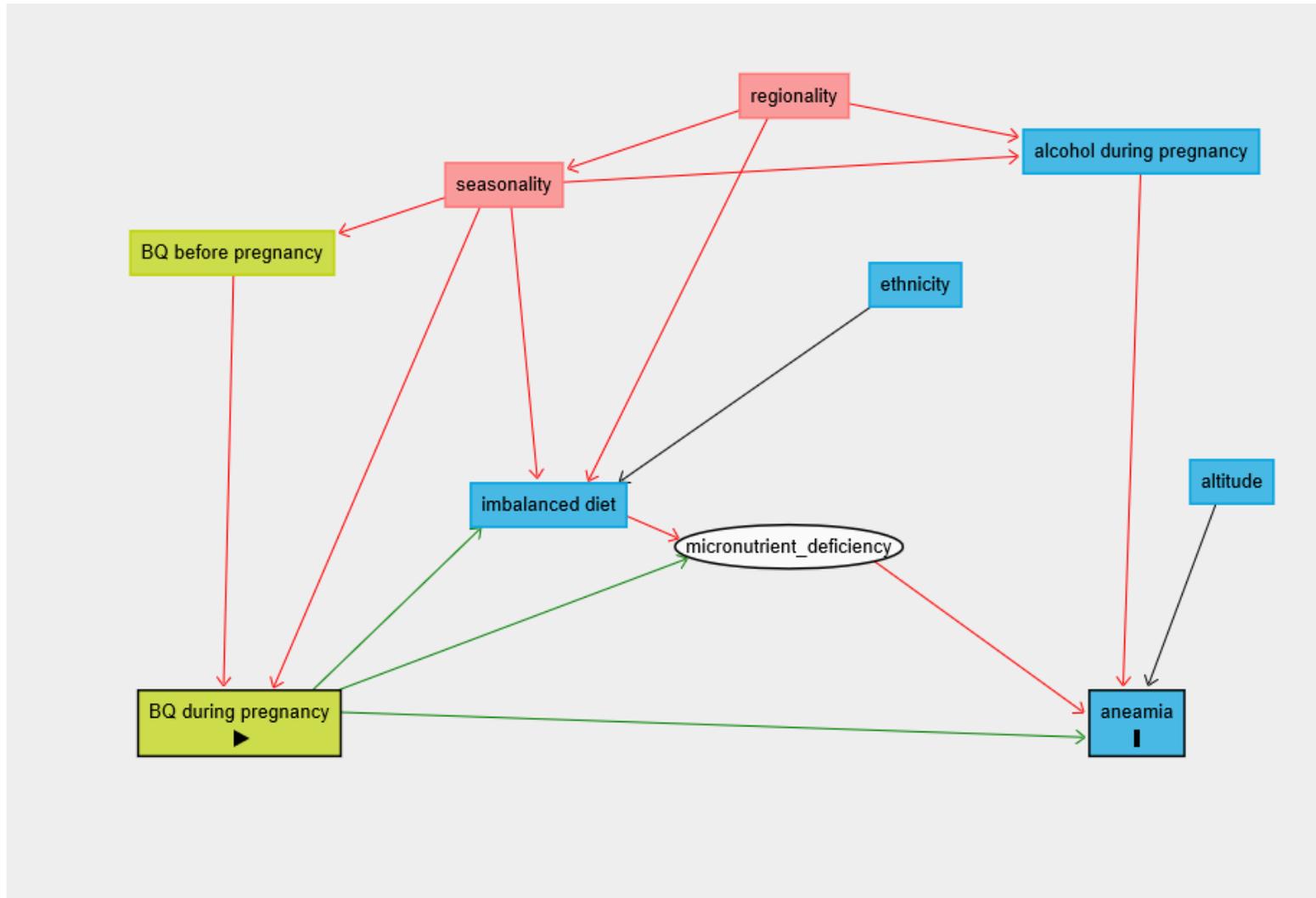


Figure 3.6. DAG 4 to show causal assumptions between betel quid chewing during pregnancy and anaemia

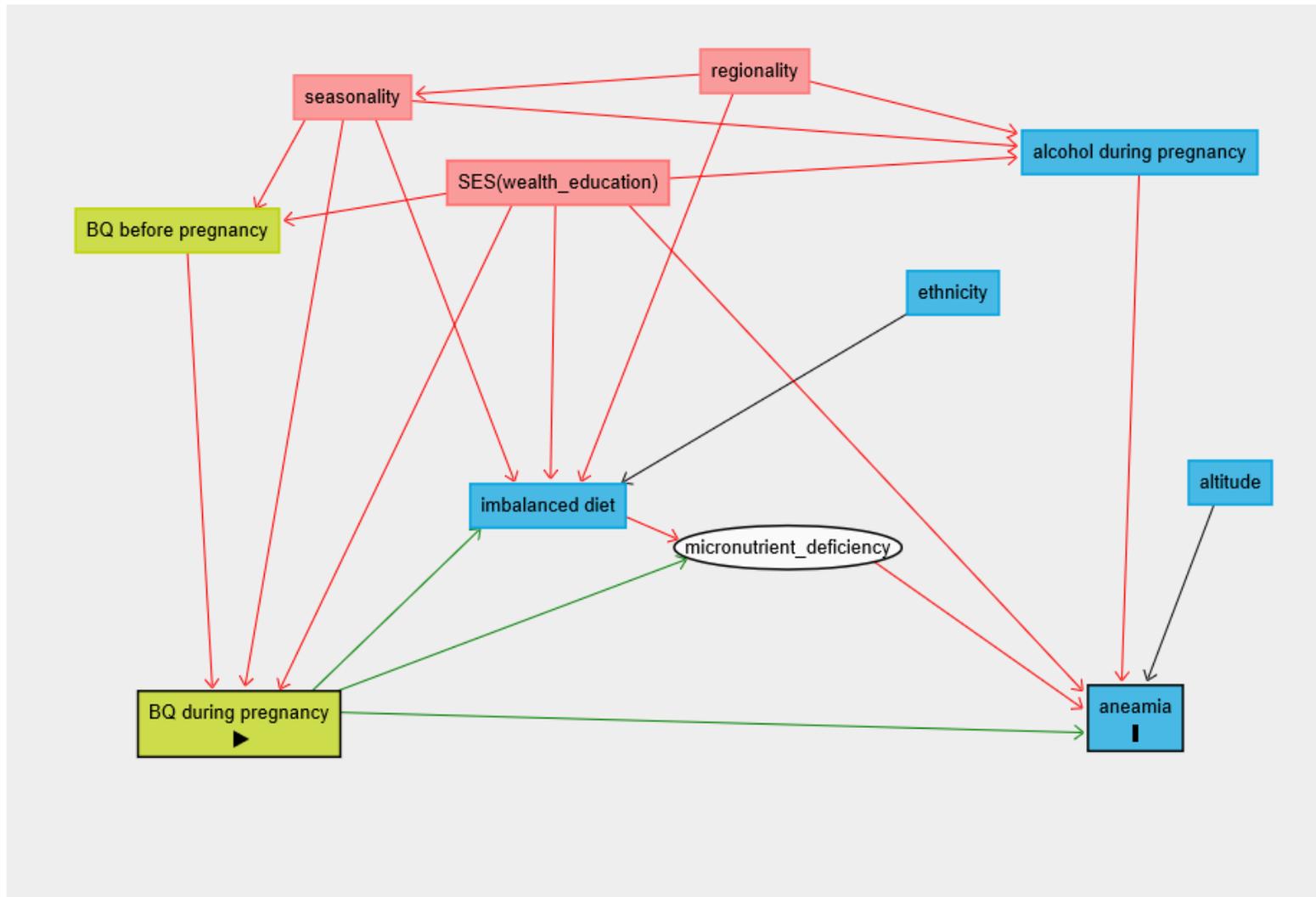


Figure 3.7. DAG 5 to show causal assumptions between betel quid chewing during pregnancy and anaemia assuming there is a direct causal relationship between SES and betel quid chewing

Sensitivity analyses for the DAG approach:

For the DAG approach, three sensitivity analyses were conducted: (1) limiting data to mothers with early scans (first scan before 24 weeks) and certain LMP dates; (2) multiple imputation based on Missing at random (MAR) assumption; and (3) sensitivity analysis using missing not at random (MNAR) assumption. Handling of missing data is described in detail in the next section. Finally, as the second outcome variable, the relationship between anaemia and betel quid chewing during pregnancy was examined using the DAG approach.

3.12.4 Handling of missing data

Estimating the causal effect of interest from these data requires consideration of the measurement error in the exposure, and the possibility of unmeasured confounders, as well as missing data.

Missing data may lead to biased and inefficient parameter estimates if inadequately handled during the analysis [51, 52]. There are several ways to handle missing data and the missing data mechanism plays a central role in informing the analysis.

Rubin (1976) classified the process that governs the probability of being missing into three categories [53].

- (1) Missing completely at random (MCAR) : the missingness mechanism is unrelated to any inference we wish to draw;
- (2) Missing at random (MAR): the missingness mechanism does not depend on the unobserved data; and
- (3) Missing not at random (MNAR): the missingness mechanism depends on the unobserved data, even after taking into account all the information in the observed data.

Different missing mechanisms can result in different implications for the analysis. Although several tests are proposed to test MCAR versus MAR, they are not widely used and it is not possible to test MAR versus MNAR since the information that is needed for such a test is missing [53]. In practice, it is advised to postulate a missingness mechanism; identify its class, and perform a valid analysis for that class of missingness mechanism. In addition, sensitivity analyses should explore the robustness of conclusions to the suggestion of alternative plausibility when considering missing data [52].

There are different ways of handling missing data. In much research, multiple imputation, developed by Donal B. Rubin in the 1970s is widely used to deal with incomplete data. Multiple imputation is a general approach which aims to allow for the uncertainty about the missing data by creating several different plausible imputed datasets and appropriately combining results obtained from each of them [54].

The analysis starts with observed, incomplete data. Multiple imputation creates several complete versions of the data by replacing the missing values with plausible data values drawn from a distribution specifically modelled for each missing entry. The imputed datasets are identical for

the observed data entries, but differ in the imputed values. The magnitude of these differences reflects our uncertainty about what value to impute. The second step is to estimate the parameters of interest from each imputed dataset and then pool those parameter estimates into one estimate and estimate variance. The variance combines the conventional sampling variance (within imputation variance) and the extra variance caused by the missing data (between-imputation variance) [53].

The STATA “mi impute chained” command was used. In the imputation, binary variables were imputed in the logistic regression models. Nominal variables were imputed in the multinomial logistic regression models. Continuous variables were imputed in the regression models. The “Augment” option was used to deal with perfect prediction. The “mi passive” command was used to transform variables after imputation.

To check the multiple imputation model, the Monte Carlo error was checked to see if it was reasonably small (Table J.4 and Table J.5 in Appendix J) and convergence was checked by plotting the mean estimate against the cycle (iteration) number (in this study, 100) for each variable with a high proportion of missing data (Figure J.1 - J.6 in Appendix J). If the imputation model is working well, the mean values from the imputations at successive iterations should move around randomly in the trace plots.

The Monte-Carlo error represents how much variability there is due to the fact we have used 100 imputations. With an infinite number of imputations, the Monte-Carlo error would be zero.

White et al. (2010) propose the following guideline to decide the number of iterations [55].

1. The Monte Carlo error of a coefficient should be less than or equal to 10% of its standard error;
2. The Monte Carlo error of a coefficient's T-statistic should be less than or equal to 0.1; and
3. The Monte Carlo error of a coefficient's P-value should be less than or equal to 0.01 if the true P-value is 0.05, or 0.02 if the true P-value is 0.1.

In the present research, both the multiple imputation and complete record analysis were conducted and if they differed, the assumptions and mechanisms causing the distinct results were explained.

Finally, as the multiple imputation model assumes MAR, a sensitivity analysis was conducted using two MNAR assumptions to check if the variables included in the imputation model make MAR plausible. The multiply imputed datasets were produced with 100 times iterations in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using a fully conditional specification (FCS) statement, MNAR, and ADJUST option with an adjustment parameter (SHIFT). The MNAR statement imputes missing values by using the pattern-mixture model approach, assuming the missing data are MNAR [56]. The rest of the analysis was conducted using the aforementioned methods in STATA 14.1. Inferential results for these values under MNAR and results for imputed under MAR were compared.

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Chapter 4

Results of validation of outcome measurements

Chapters 4 to 7 presents the results. Chapter 4 provides validation of outcome measurements. Chapter 5 provides a descriptive analysis of covariates, followed by a descriptive analysis of betel quid chewing, packaged betel products, smoking and drinking in Chapter 6. Chapter 7 provides the results of the multivariate analysis and sensitivity analysis.

4.1 Methods

When analysing potential risk factors for PTB, accurate classification of preterm is a very important factor to understand the validity of the analysis. Hence, before going to the detailed analysis of potential risk factors, the validity of outcome measurements, especially if there was misclassification of preterm, and potential impact of misclassification on the results of the studies, if any, was examined and discussed.

Uncertainties around measuring gestational age (GA) in Bhutan can arise mainly from the availability of early US scans and recording and accuracy of LMP. To examine these, first, the percentage of mothers with information about LMP and early scans was described. Then, mothers with early scans were compared to mothers with late scans on selected maternal and infant characteristics. Subsequently, GA estimates using the first day of LMP by mother's recall were compared to the estimates by US scan. Finally, factors contributing to the differences in the estimates were examined.

The details of statistical methods were described in Ch3 (see Section 3.13.1).

4.2 Description of mothers with early scans and mothers with late scans

A total of 672 mothers were interviewed (351 cases and 321 controls) during the study period. Three mothers with LBW and/or PTB babies were excluded due to the exclusion criteria (age \leq 16 years). A total of 669 mother-baby pairs were included in the analysis.

4.2.1 Proportion of mothers without information on LMP and US scans and timing of US scans (n=669)

Out of 669 mothers who participated in the study, 26% or 176 mothers of the mothers were missing records of the first day of LMP and 3% mothers were missing US estimates. Eighteen percent or 117 mothers had US scans after 24 weeks or no scans. Eight percent or 51 mothers had neither recalled LMP nor early scans before 24 weeks' gestation. The mean GA at the first US scan was 16.4 (95% CI 15.9-17.0) weeks for the total study participants. The mean GA at birth by LMP estimate was 267 days (95% CI: 265-270) while the mean GA at birth by US was 266 days (95% CI: 264-268). The average difference between LMP and US estimates was 1 day (\pm 17 days). GA difference between LMP and US was within \pm 7 days for 54% of the mothers, \pm 14 days for

73.0% and +/- 30 days for 90% among the 480 mothers with both LMP and US estimates. Among 669 mothers, 26 % or 171 were classified as PTB by US estimate of GA. On the other hand, due to the large number of the mothers who were missing LMP, fewer mothers were classified as PTB by LMP (22.7%). The agreement was 87%. The Kappa coefficient for classification into two categories was 0.67, which indicates good agreement [1]. In total, 193 mothers were classified as PTB in the study. Among 193 mothers, 80% had a GA based on early scans before 24 weeks and 20% were based on late scans or clinical estimates.

4.2.2 Comparison of maternal and neonate characteristics between mothers with early scans and mothers with late scans

There were no statistical differences between mothers with early scans and mothers with late scans in terms of proportion of cases, LBW and PTB (Table 4.1). However, more mothers with late scans were missing LMP estimate than mothers with early scans.

In terms of socio-economic background, more mothers with late scans were under 20 years old or over 40 years old and single, divorced, or widowed, and students, unemployed, or self-employed. More mothers with late scans were classified in the third wealth quintile or below, had a parity greater than one and smoked during pregnancy (Table 4.2).

Controlling for any other variables in the model in Table 4.3, mothers who were younger than 25 years old compared to age 25-<30, who smoked during pregnancy, whose parity was greater than two, who were single, divorced, or widowed had higher odds of having late scans or no scan. Interestingly, mothers who drank during pregnancy tended to have a higher odds of having early scans. This could be due to insufficient control of confounding factors. Another explanation could be that drinking alcohol may not indicate a lack of concern about their health in the cultural context of Bhutan where many women believe that alcohol has medical benefits [2].

Table 4.1. Comparison between early scans and late scans in relation to outcome categories.

Outcome categories	Early scan (n=552)		Late or no scan (n=117)		P value	
	N	%	N	%		
Case	280	50.7	68	58.1	0.146	
Low birthweight	247	44.8	59	50.4	0.263	
Preterm	Preterm	154	27.9	39	33.3	0.176
	Missing	0	0.0	3	2.6	
Missing LMP		125	22.6	51	43.6	<0.0001
Missing US		5	0.9	18	15.4	<0.0001
Difference between LMP and US estimates	<-14	41	7.4	18	15.4	<0.0001
	-14 to -8	26	4.7	4	3.4	
	-7 to 7	240	43.5	19	16.2	
	8 to 14	55	10.0	7	6.0	
	>14 days	60	10.9	10	8.6	
	Missing	130	23.6	59	50.4	

Table 4.2. Comparison between early scans and late scans in relation to selected maternal and neonate characteristics.

Maternal and neonatal characteristics		Early scan (n=552)		Late or no scan (n=117)		P value
		N	%	N	%	
Delivery hospital	JDWNRH	382	69.2	73	62.4	0.187
	Gelephu	81	14.7	17	14.5	
	Mongar	89	16.1	27	23.1	
Sex of infant	Male	257	46.6	65	55.6	0.244
Age	<20	27	4.9	14	12.0	<0.0001
	20-<25	144	26.1	40	34.2	
	25-<30	211	38.2	18	15.4	
	30-<35	101	18.3	23	19.7	
	35-<40	50	9.1	13	11.1	
	40+	19	3.4	9	7.7	
Marital status	Single, divorced, widow	10	1.8	7	6.0	0.009
	Married or living with a partner	539	97.6	109	93.2	
	Missing	3	0.5	1	0.9	
Education	Never attended school	134	24.3	33	28.2	0.221
	Non-formal education (NFE)	37	6.7	12	10.3	
	Primary	84	15.2	18	15.4	
	Middle Secondary or Secondary	252	45.7	49	41.9	
	Diploma, College, and postgraduate	45	8.2	4	3.4	
	Missing	0	0.0	1	0.9	
Occupation	Housewife	331	60.0	73	62.4	<0.0001
	Unemployed	3	0.5	4	3.4	
	Student	2	0.4	5	4.3	

	Self-employed	74	13.4	20	17.1	
	Employee	140	25.4	15	12.8	
	Missing	2	0.4	0	0.0	
Wealth quintile	Poorest	102	18.5	32	27.4	0.003
	Second	104	18.8	28	23.9	
	Third	105	19.0	28	23.9	
	Fourth	118	21.4	15	12.8	
	Richest	119	21.6	13	11.1	
	Missing	4	0.7	1	0.9	
Ethnicity	Southern Bhutanese	170	30.8	35	29.9	0.851
Parity	0	249	45.1	47	40.2	0.001
	1	162	29.4	21	18.0	
	2 or 3	122	22.1	35	29.9	
	4>=	18	3.3	11	9.4	
	Missing	1	0.2	3	2.6	
Maternal height categorical	<145	42	7.6	10	8.6	0.823
	145 - <150	108	19.6	17	14.5	
	150-<155	191	34.6	34	29.1	
	155<=	180	32.6	32	27.4	
	Missing	31	5.6	24	20.5	
Pre-pregnancy BMI	Underweight (< 18.5)	32	5.8	3	2.6	0.473
	Average (18.5–25.0)	245	44.4	51	43.6	
	Overweight (≥25)	83	15.0	12	10.3	
	Obese (≥30)	18	3.3	2	1.7	
	Missing	174	31.5	49	41.9	
Betel nut chewing during pregnancy	Yes	303	54.9	56	47.9	0.149

	Missing	3	0.5	0	0.0	
Smoking during pregnancy	Yes	11	2.0	8	6.8	0.004
Smokeless tobacco during pregnancy	Yes	37	6.7	13	11.1	0.095
	Missing	6	1.1	2	1.7	
Pan masala during pregnancy	Yes	128	22.3	27	23.1	0.884
	Missing	9	1.6	1	0.9	
Alcoholic drinks during pregnancy	Yes	151	27.4	28	23.9	0.447
Hypertensive disorder	No hypertensive complications	435	78.8	80	68.4	0.478
	Pre-existing or chronic hypertension	16	2.9	3	2.6	
	Gestational hypertension	44	8.0	11	9.4	
	Pre-eclampsia	25	4.5	8	6.8	
	Eclampsia	7	1.3	0	0.0	
	Missing	25	4.5	15	12.8	
UTI	Yes	49	8.9	11	9.4	0.696
	Missing	53	9.6	18	15.4	
Mode of Delivery	SVD	336	60.9	77	65.8	0.908
	CS-Selective	62	11.2	13	11.1	
	CS-Emerg.	144	26.1	26	22.2	
	Vacuum	7	1.3	1	0.9	
	Breech	3	0.5	0	0.0	

Table 4.3. Crude and adjusted odds of having late scans or no US scans compared to the mothers with early scans by selected maternal characteristics.

Maternal characteristics		Late scans vs. early scans (reference)					
		Crude Odds Ratio	95% CI	P value	Adjusted Odds Ratio (n=644)	95% CI	P value
Delivery hospital (ref: JDWNRH)	Gelephu	1.10	0.62-1.96	0.751	1.04	0.53-2.04	0.905
	Mongar	1.59	0.96-2.61	0.069	1.39	0.73-2.65	0.313
Age (reference: 25-<30)	<20	6.08	2.72-13.60	<0.0001	5.39	1.95-14.90	0.001
	20-<25	3.26	1.80-5.91	<0.0001	3.21	1.61-6.39	0.001
	30-<35	2.67	1.38-5.17	0.004	2.18	1.02-4.60	0.048
	35-<40	3.05	1.40-6.63	0.005	1.72	0.77-4.73	0.244
	40+	5.55	2.20-14.04	<0.0001	2.81	0.82-8.40	0.082
Marital status (ref: Married or living with a partner)	Single, divorced, widow	3.46	1.29-9.29	0.014	1.66	0.42-6.58	0.471
Education (ref: Never attended school)	Non-formal education (NFE)	1.32	0.62-2.80	0.474	1.53	0.66-3.57	0.324
	Primary	0.87	0.46-1.64	0.668	0.96	0.47-2.00	0.921
	Middle Secondary or Secondary	0.79	0.48-1.29	0.343	1.70	0.87-3.33	0.122
	Diploma, College, and postgraduate	0.36	0.12-1.07	0.067	1.46	0.35-6.05	0.605
Occupation (ref: Housewife)	Unemployed	6.05	1.32-27.59	0.020	8.9	1.54-51.49	0.015
	Student	11.34	2.16-59.58	0.004	14.9	1.96-113.57	0.009
	Self-employed	1.23	0.70-2.14	0.473	1.9	1.02-3.63	0.045
	Employee	0.49	0.27-0.88	0.016	0.8	0.38-1.66	0.539
Wealth quintile (ref: Third)	Poorest	1.18	0.66-2.09	0.580	0.85	0.43-1.66	0.626
	Second	1.01	0.56-1.82	0.975	0.89	0.46-1.71	0.726
	Fourth	0.48	0.24-0.94	0.033	0.49	0.23-1.03	0.060

	Richest	0.41	0.20-0.83	0.014	0.37	0.16-0.86	0.020
Ethnicity (ref: Northern Bhutanese)	Southern Bhutanese	0.96	0.62-1.48	0.851	1.01	0.59-1.74	0.959
Parity (ref: Nulliparity)	1	0.69	0.40-1.19	0.181	1.28	0.66-2.50	0.469
	2 or 3	1.52	0.93-2.48	0.093	2.89	1.33-6.31	0.007
	4>=	3.24	1.44-7.29	0.005	5.30	1.51- 18.62	0.009
	Chewing betel or pan masala during pregnancy	0.77	0.52-1.16	0.215	0.80	0.49-1.31	0.377
	Chewing smokeless tobacco or smoking during pregnancy	2.18	1.24-3.85	0.007	2.87	1.46-5.65	0.002
	Drinking during pregnancy	0.84	0.53-1.33	0.448	0.56	0.32-1.00	0.049

4.3 Understanding the difference in estimated gestational age (GA) between LMP estimates and US estimates

4.3.1. Testing for normality of distribution of the GA difference between LMP estimates and US estimates

The Shapiro-Wilk W test for normal data (<0.001) and the Skewness/Kurtosis tests for Normality (<0.001) suggest the difference in estimated GA by the two methods is not normally distributed (Figure 4.1 and Figure 4.2). The skewness is -0.62 indicating that the difference in the estimated GA is skewed to the left. The Kurtosis is 6.3 .

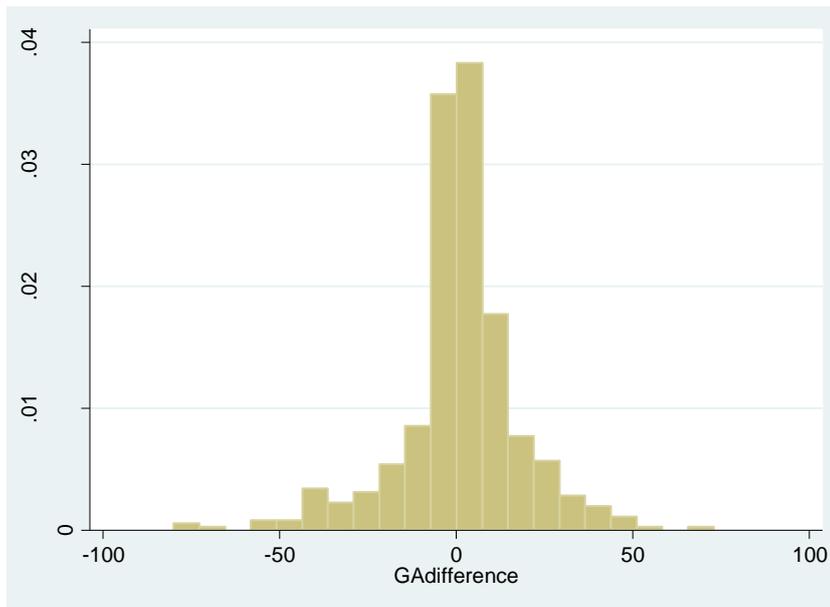


Figure 4.1. Distribution of the difference in estimated GA for the study participants.

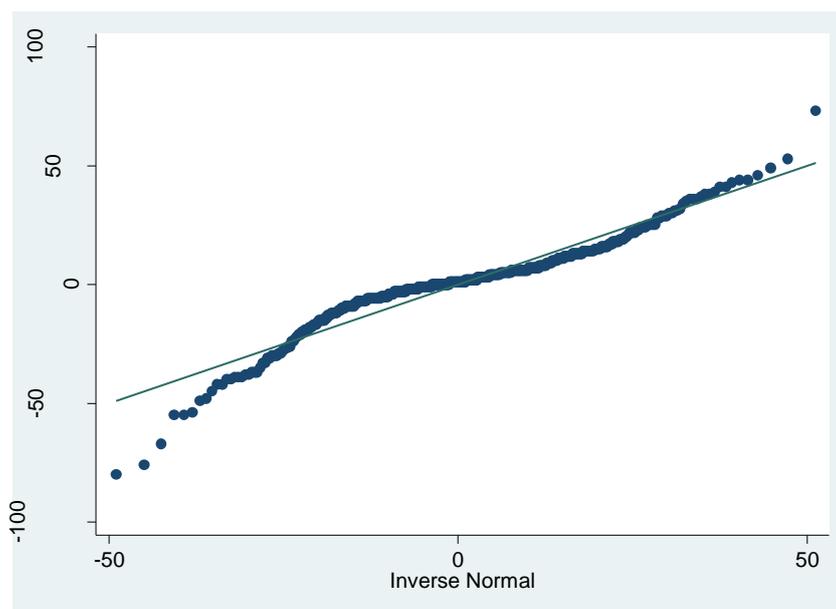


Figure 4.2. Q-Q plot of the difference in estimated GA difference.

4.3.2 Examining the relationship between the two estimates

The Bland-Altman limits of agreement test analyses the differences of paired variables against the average of the two values in a pair (Figure 4.3) [3]. The mean difference is 1.1 (95% CI: -0.4 to 2.7) and the limits of agreement (Reference Range for difference) were -35.4 to 33.1. The overall Lin's concordance correlation coefficient was 75.6% (95% CI: 0.720 -0.792) (Figure 4.4).

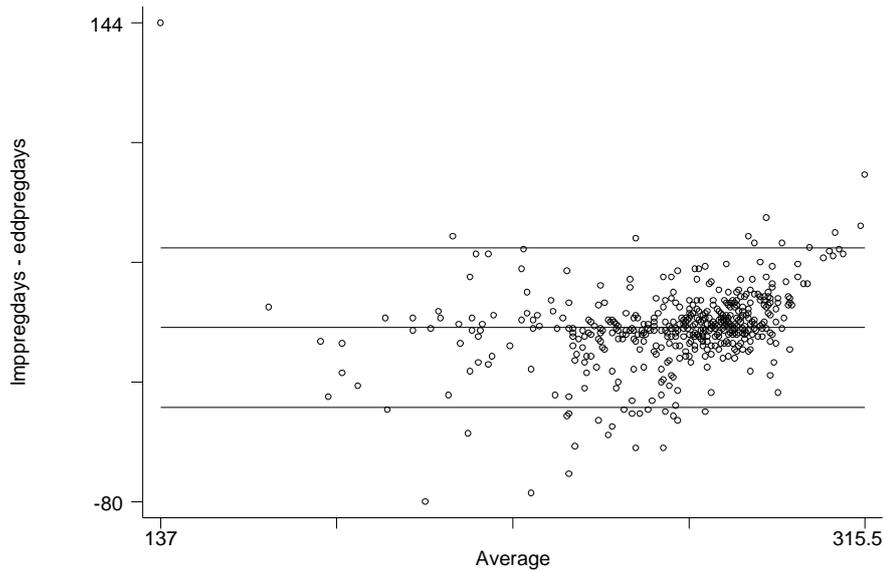


Figure 4.3. Bland-Altman plots of the estimated GA by prenatal ultrasound (eddpregdays) with LMP in days. The B&A graph plot represents every difference between two estimates against the average. The mean difference is 1.135 (95% CI: -0.432 to 2.703) and the limits of agreement (Reference Range for difference) are -35.392 to 33.121.

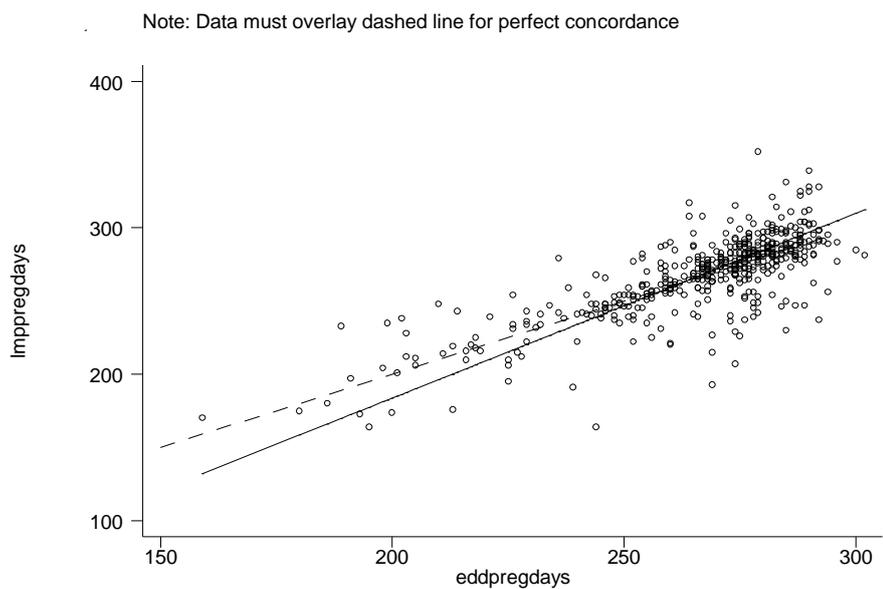


Figure 4.4. Concordance correlation coefficient ultrasound with LMP.

4.3.3 Understanding the factors contributing to the difference in estimated GA

In Bhutan, the MCH book advises that if the difference between the LMP and US estimates are different by less than one week and US is done early, then the GA should be based on the LMP estimates and if the difference is more than one week, then it should be based on the US estimates.

The difference in estimated GA between LMP and US was within +/-7 days for 39% of the mothers (Table 4.5). Twenty percent (132/669) had a GA difference of greater than +7 days (LMP estimate > US estimate), while 13% (89/669) had a GA difference of less than -7 days (LMP estimate < US estimate).

Table 4.4 and Table 4.5 describe magnitude of difference in estimated GA in days, between LMP and US estimates by adverse birth outcomes and selected maternal and infant characteristics.

Mothers with late scans had 3.5 times higher odds of the GA estimated by LMP being shorter than the one by US, by more than 7 days, controlling for any other variables in the model (Figure 4.5 and Table 4.6), thus, classifying fewer babies as PTB by US based estimate. This could indicate that there is a chance of underestimating the burden of PTB if the LMP based estimate was more accurate than late US scans.

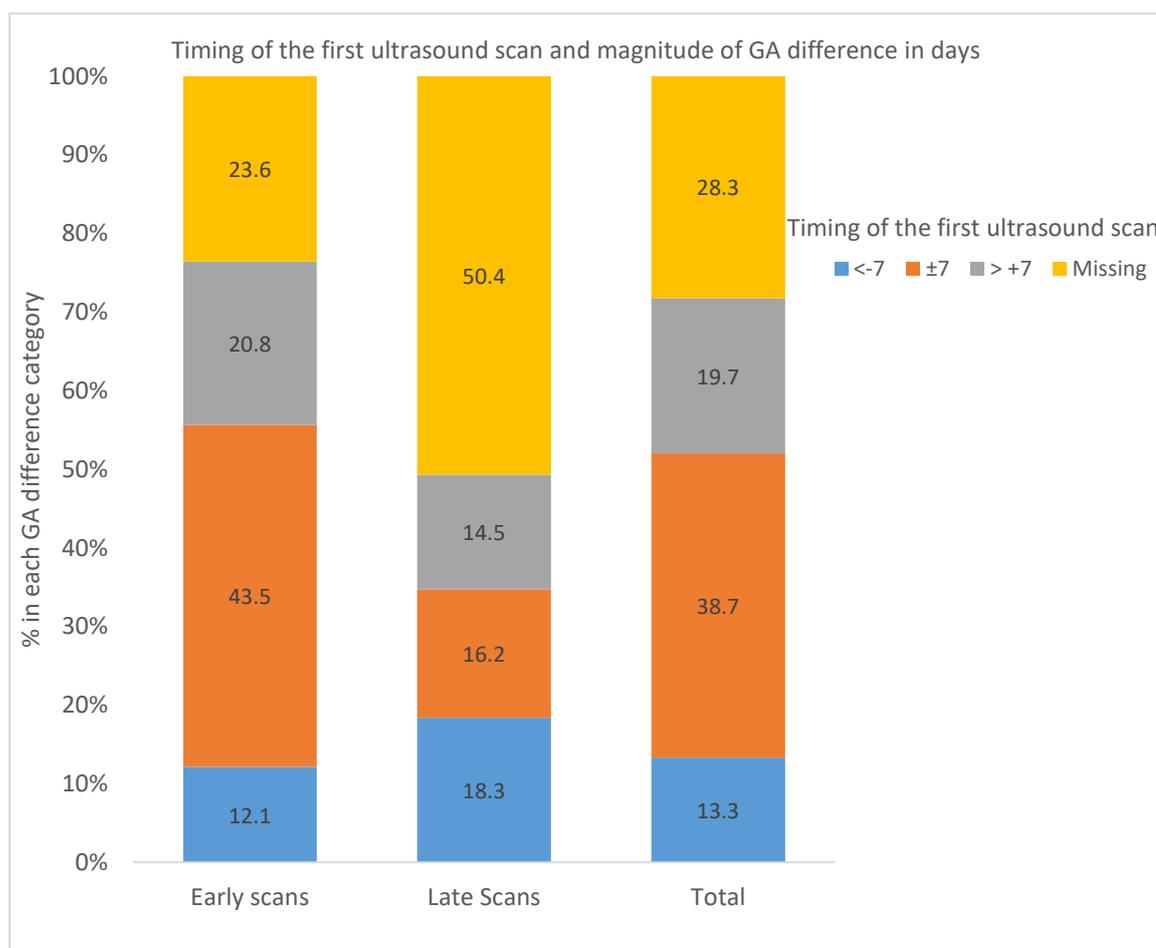


Figure 4.5. Magnitude of difference in estimated GA in days, between LMP and US estimates and timing of the first US scan.

Table 4.4. Magnitude of difference in estimated GA in days, between LMP and US estimates by adverse birth outcomes.

Adverse birth outcomes		GA difference (in days): LMP-US estimate						Total	P value
		<-14	-14 to -8	±7	8 to 14	>+14	Missing		
Case	Case	10%	3%	39%	7%	10%	31%	100%	0.213
	Control	8%	6%	38%	11%	11%	26%	100%	
Preterm	GA<259	9%	5%	38%	5%	10%	33%	100%	0.375
	GA >=259	9%	4%	39%	11%	11%	26%	100%	
	Missing	0%	0%	0%	0%	0%	100%	100%	
Low birthweight	Birthweight <2500	10%	2%	39%	8%	9%	31%	100%	0.046
	Birthweight >=2500	7%	6%	38%	11%	11%	26%	100%	

Table 4.5. Magnitude of estimated difference in GA, in days between LMP and US estimates by selected maternal and infant characteristics.

Maternal and infant characteristics		GA difference (in days): LMP-US estimate						Total
		<-14	-14 to -8	±7	8 to 14	>+14	Missing	
Delivery hospital	JDWNRH	10%	4%	44%	10%	10%	22%	100%
	Gelephu	9%	9%	34%	7%	12%	29%	100%
	Mongar	5%	1%	25%	8%	12%	53%	100%
Timing of scans	<=24	7%	5%	43%	10%	11%	24%	100%
	>24	15%	3%	16%	6%	9%	50%	100%
Sex of infant	Male	9%	5%	36%	9%	12%	28%	100%
	Female	9%	4%	41%	10%	9%	28%	100%
Age	<20	20%	2%	24%	10%	5%	39%	100%
	20-<25	10%	6%	34%	10%	11%	29%	100%
	25-<30	7%	6%	44%	12%	12%	20%	100%
	30-<35	8%	2%	43%	6%	10%	32%	100%
	35-<40	6%	5%	37%	5%	11%	37%	100%
	40+	11%	0%	36%	7%	7%	39%	100%
Marital status	Single, divorced, widow	6%	0%	35%	6%	0%	53%	100%
	Married or living with a partner	9%	5%	39%	9%	11%	28%	100%
	Missing	0%	0%	25%	25%	25%	25%	100%
Education	Never attended school	10%	2%	32%	4%	10%	42%	100%
	Non-formal education (NFE)	8%	6%	27%	14%	8%	37%	100%
	Primary	7%	8%	37%	5%	9%	34%	100%
	Middle Secondary or Secondary	10%	5%	44%	12%	10%	19%	100%
	Diploma, College, and postgraduate	2%	2%	49%	12%	18%	16%	100%
	Missing	0%	0%	0%	0%	0%	100%	100%

Occupation	Housewife	9%	5%	37%	9%	11%	29%	100%
	Unemployed	0%	0%	14%	0%	0%	86%	100%
	Student	14%	0%	29%	0%	0%	57%	100%
	Self-employed	10%	3%	34%	7%	9%	37%	100%
	Employee	8%	3%	47%	13%	12%	17%	100%
	Missing	0%	0%	50%	0%	0%	50%	100%
Wealth Quintile	Poorest	10%	1%	30%	7%	12%	40%	100%
	Second	14%	7%	33%	7%	8%	31%	100%
	Third	8%	9%	34%	8%	8%	32%	100%
	Fourth	5%	3%	50%	14%	11%	18%	100%
	Richest	7%	2%	47%	11%	14%	20%	100%
	Missing	0%	0%	60%	0%	0%	40%	100%
Ethnicity	Southern Bhutanese	10%	5%	47%	9%	9%	19%	100%
	Northern Bhutanese	8%	4%	35%	9%	11%	32%	100%
Parity	0	9%	4%	44%	9%	13%	21%	100%
	1	10%	7%	37%	11%	10%	26%	100%
	2 or 3	8%	4%	35%	8%	9%	36%	100%
	4>=	7%	0%	21%	3%	3%	66%	100%
	Missing	0%	0%	0%	0%	0%	4%	100%
Maternal height categorical	<145	19%	4%	25%	12%	17%	23%	100%
	145 - <150	10%	4%	38%	10%	11%	26%	100%
	150-<155	8%	5%	39%	11%	9%	27%	100%
	155<=	6%	4%	47%	7%	11%	25%	100%
	Missing	7%	5%	22%	5%	5%	55%	100%
Pre-pregnancy BMI	Underweight (< 18.5)	9%	6%	46%	9%	9%	23%	100%
	Average (18.5–25.0)	9%	4%	44%	11%	10%	21%	100%

	Overweight (≥ 25)	5%	2%	44%	12%	12%	25%	100%
	Obese (≥ 30)	5%	5%	35%	15%	25%	15%	100%
	Missing	10%	6%	28%	6%	9%	41%	100%
Betel nut chewing during pregnancy	Yes	9%	4%	37%	10%	11%	29%	100%
	Missing	0%	0%	1%	0%	0%	2%	100%
Smoking during pregnancy	Yes	5%	0%	42%	5%	16%	32%	100%
Snuff and chewing tobacco during pregnancy	Yes	8%	4%	28%	6%	16%	38%	100%
	Missing	13%	0%	50%	0%	13%	25%	100%
Pan Masala during pregnancy	Yes	9%	8%	42%	11%	7%	23%	100%
	Missing	10%	0%	80%	0%	0%	10%	100%
Alcoholic drinks during pregnancy	Yes	9%	6%	38%	8%	11%	28%	100%
Hypertensive disorder	No hypertensive complications	9%	5%	40%	10%	11%	25%	100%
	Pre-existing or chronic hypertension	11%	0%	37%	5%	11%	37%	100%
	Gestational hypertension	5%	5%	35%	7%	9%	38%	100%
	Pre-eclampsia	15%	0%	30%	3%	18%	33%	100%
	Eclampsia	14%	0%	29%	0%	14%	43%	100%
	Missing	8%	5%	35%	5%	3%	45%	100%
Mode of Delivery	SVD	9%	5%	38%	10%	10%	28%	100%
	CS-Elective	8%	5%	33%	13%	15%	25%	100%
	CS-Emerg.	8%	4%	42%	6%	11%	29%	100%
	Vacuum	13%	0%	38%	13%	0%	38%	100%
	Breech	33%	0%	33%	0%	0%	33%	100%

Table 4.6. Results of logistic regression analysis for the odds of having a difference in estimated GA of more than ± 7 days.

GA difference		>7 vs. -7 \geq to +7			<-7 vs. -7 \geq to +7		
		Adjusted Odds Ratio (n=377)	95% CI	P value	Adjusted Odds Ratio (n=377)	95% CI	P value
Timing of US (ref: early scans)	Late scans	1.61	0.74-3.49	0.231	3.45	1.58-7.55	0.002
Delivery hospital (ref: JDWNRH)	Gelephu	1.46	0.74-2.90	0.277	1.63	0.75-3.53	0.214
	Mongar	1.49	0.74-3.01	0.265	0.65	0.23-1.80	0.404
Age (ref: 25-<30)	<20	1.36	0.40-4.65	0.620	2.57	0.78-8.51	0.122
	20-<25	1.23	0.69-2.22	0.480	1.42	0.70-2.86	0.328
	30-<35	0.54	0.27-1.08	0.082	0.54	0.23-1.26	0.153
	35-<40	0.63	0.26-1.55	0.318	0.78	0.26-2.33	0.658
	40+	0.47	0.12-1.77	0.263	0.46	0.10-2.08	0.311
Marital status (ref: Married or living with a partner)	Single, divorced, widow	0.32	0.03-2.99	0.318	0.27	0.03-2.86	0.277
Education (ref: Never attended school)	Non-formal education (NFE)	1.52	0.55-4.16	0.417	1.71	0.53-5.56	0.370
	Primary	0.78	0.34-1.80	0.558	1.04	0.42-2.54	0.934
	Middle Secondary or Secondary	1.15	0.58-2.30	0.685	1.15	0.52-2.52	0.733
	Diploma, College, and post graduate	1.66	0.58-4.76	0.342	0.56	0.10-3.24	0.515
Occupation (ref: Housewife)	Student	-	-	-	1.78	0.10-30.60	0.691
	Self-employed	0.92	0.44-1.94	0.828	1.44	0.60-3.49	0.415
	Employee	0.99	0.54-1.82	0.978	0.91	0.42-1.94	0.803
Wealth quintile (ref: Third)	Poorest	1.37	0.63-2.97	0.433	0.87	0.37-2.06	0.755
	Second	1.04	0.47-2.30	0.914	1.30	0.60-2.82	0.509
	Fourth	1.05	0.52-2.10	0.898	0.32	0.13-0.79	0.014

	Richest	1.21	0.57-2.55	0.626	0.43	0.17-1.08	0.073
Ethnicity (ref: Northern Bhutanese)	Southern Bhutanese	0.64	0.38-1.07	0.089	0.64	0.35-1.19	0.163
Parity (ref: Multiparty)	Nulliparity	0.72	0.42-1.22	0.217	0.57	0.30-1.11	0.096
Chewing betel or pan masala during pregnancy		1.13	0.70-1.84	0.618	1.08	0.60- 1.94	0.794
Chewing smokeless tobacco or smoking during pregnancy		1.56	0.70-3.47	0.274	0.66	0.22-1.97	0.454
Drinking during pregnancy		1.03	0.61-1.74	0.899	0.73	0.37-1.44	0.363

4.4. Conclusions

In the sample population, for mothers with both LMP and US information, the LMP and US based estimate demonstrated a good agreement. The average difference between LMP and US based estimates was 1 day (± 17 days). Uncertainty of classification of PTB could be mostly introduced by estimating GA from late US scans.

There were no statistical differences between mothers with early scans and mothers with late scans in terms of proportion of cases, LBW and PTB. However, more mothers with late scans were missing LMP estimates than mothers with early scans. In terms of socio-economic background, more mothers with late scans were younger than 25 years old, divorced, or widowed, students, self-employed and unemployed. More mothers with late scans had a parity greater than one and smoked during pregnancy according to a logistic regression model controlling for any other variables in the model. Wealthier mothers had a more than 50% reduced odds of having late scans or no scans compared to the middle quintile.

With regard to the magnitude of the difference in estimated GA between LMP and US based estimates, mothers with late scans had higher odds of having a difference between LMP and US estimates of GA of greater than 7 days.

In order to examine the impact of this uncertainty, in the multivariate analyses examining the potential risk factors of adverse birth outcomes, an analysis limited to mothers with early scans before 24 weeks of gestation will be conducted and compared to the analyses using the whole dataset.

References

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3. Giavarina, D., *Understanding Bland Altman analysis*. *Biochem Med (Zagreb)*, 2015. **25**(2): p. 141-51.

Chapter 5

Results of descriptive analysis of the study population and selected maternal and infant characteristics

In the previous chapter, the validity of the outcome measures was explored and it was concluded that the classification of PTB was relatively reliable with slight uncertainties in estimating GA due to late US scans or no scans. Using these outcome measures, a descriptive analysis was performed in order to understand the socioeconomic situation, nutritional status, physical activity and key obstetric factors of the study participants.

For categorical variables, the differences in the proportion of controls and cases were tested using the chi-squared test. If any expected values were equal to or smaller than five, Fisher's exact test was used. The number of study participants in each category, percentages and the corresponding P value were presented.

For continuous variables, the two-sample t test was used. When variance was not equal, Welch's t test was used. The number of study participants in each category, mean, standard deviation, and the corresponding P value were presented.

Similarly, controls and mothers of LBW or PTB babies were compared and presented in the same tables as cases.

These analyses will inform the logistic regression models using a statistical approach and missing data and subsequent sensitivity analysis using the directed acyclic graph approach.

5.1 Recruitment of cases and controls at the three referral hospitals

According to the national surveillance data, there were 5601 deliveries at these hospitals, of which 64 were stillbirths, in the year 2015 [1].

During the study period from February 2015 to the beginning of March 2016, 5,472 mothers of singleton live births were eligible (468 mothers for cases and 5,000 for controls) based on the birth register from the three study sites (Figure 5.1). There were 464 mothers of LBW babies and 302 mothers of PTB babies (204 babies were both LBW and PTB). In the source population (singleton live births), prevalence of LBW was 8.5% [464/5468]. Of the eligible cases, 76% [357/468] were asked for consent and 74% [348/468] participated in the study (Figure 5.2). The main reasons for not approaching the cases for consent was that the mothers had already been discharged. Approximately 2% of the mothers did not consent. The main reasons given by the mothers were that they were feeling tired or feeling shy.

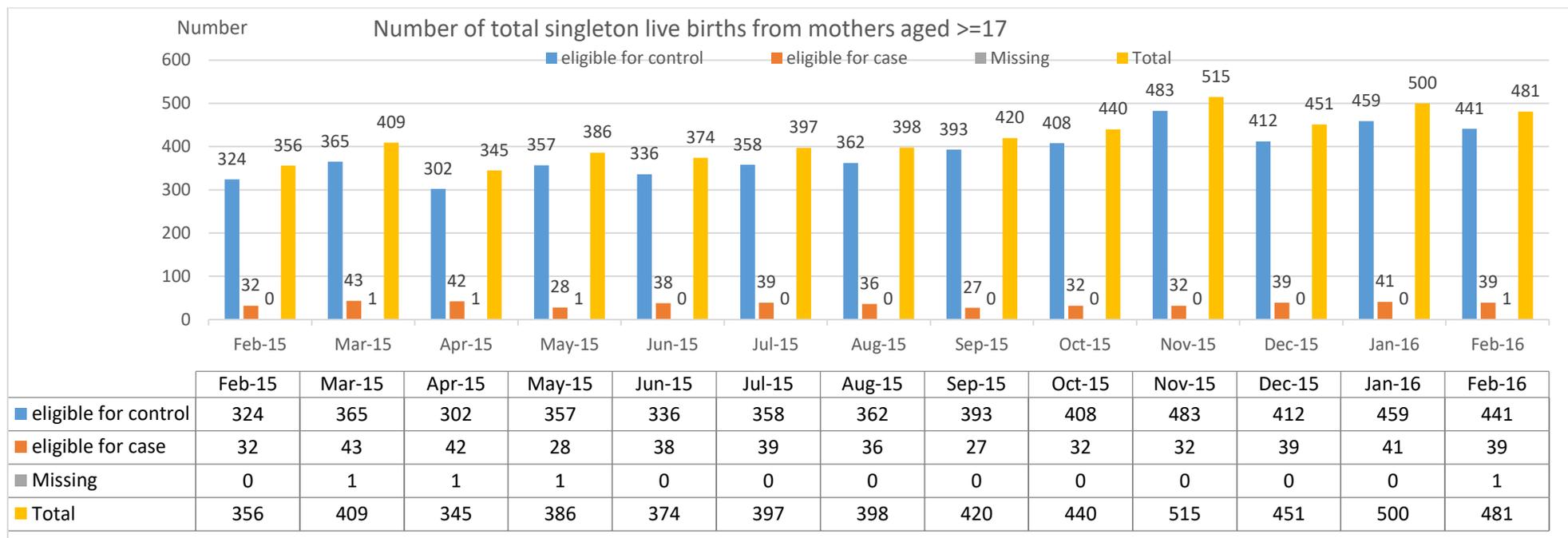


Figure 5.1. Number of total singleton live births from mothers aged ≥ 17 at the three referral hospitals in Bhutan between February 2015 and February 2016.

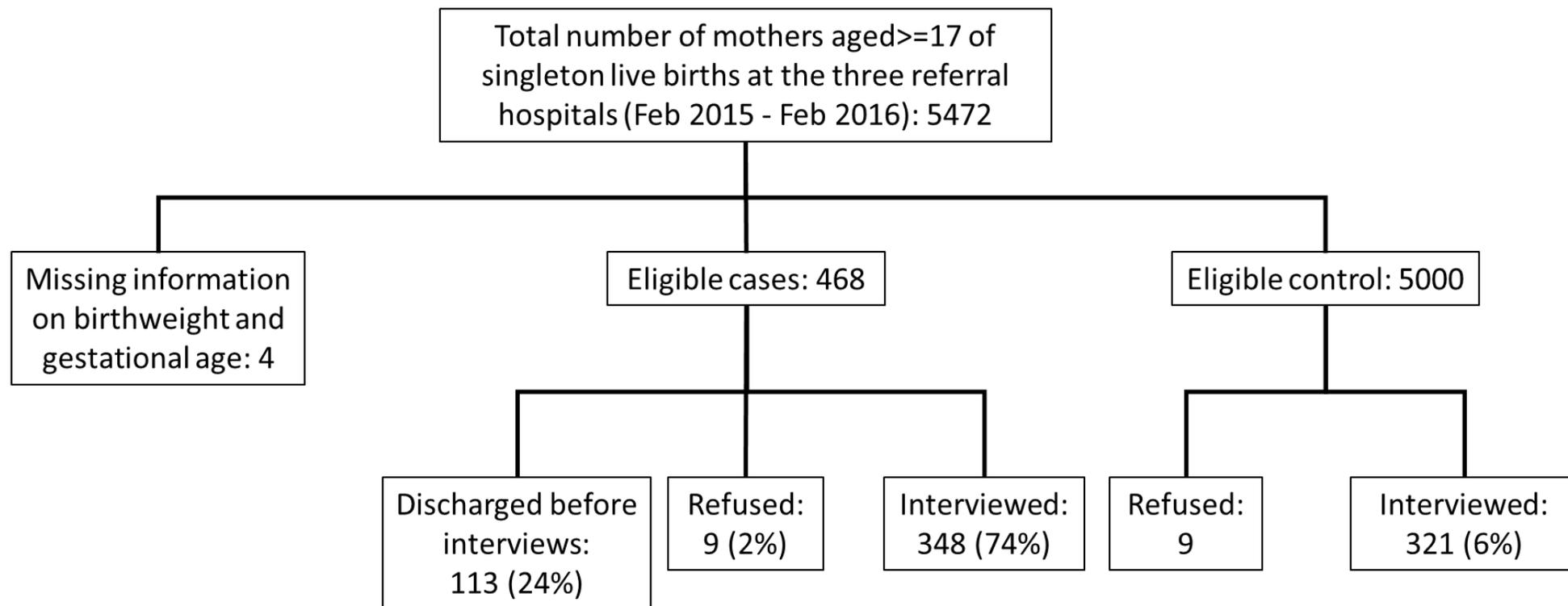


Figure 5.2. Recruitment of cases and controls (the number of refusals in the control group at ERRH is not reflected in the chart as the information was not documented).

5.2 Delivery hospital

In total, 672 mothers were interviewed. Three mothers with low LBW and/or PTB babies were excluded due to the exclusion criteria (age <17 years). A total of 669 mother-baby pairs were included in the analysis (Figure 5.4). Case mothers comprised 193 mothers of PTB babies, 152 mothers of term LBW babies, and three mothers of babies whose birthweight was less than 2500 grams but whose GA was uncertain. About 68% of the total study participants were recruited at JDWNRH, followed by ERRH in Mongar (17%) and CRRH in Gelephu (15%) (Figure 5.3, Table 5.2, and Table 5.3).

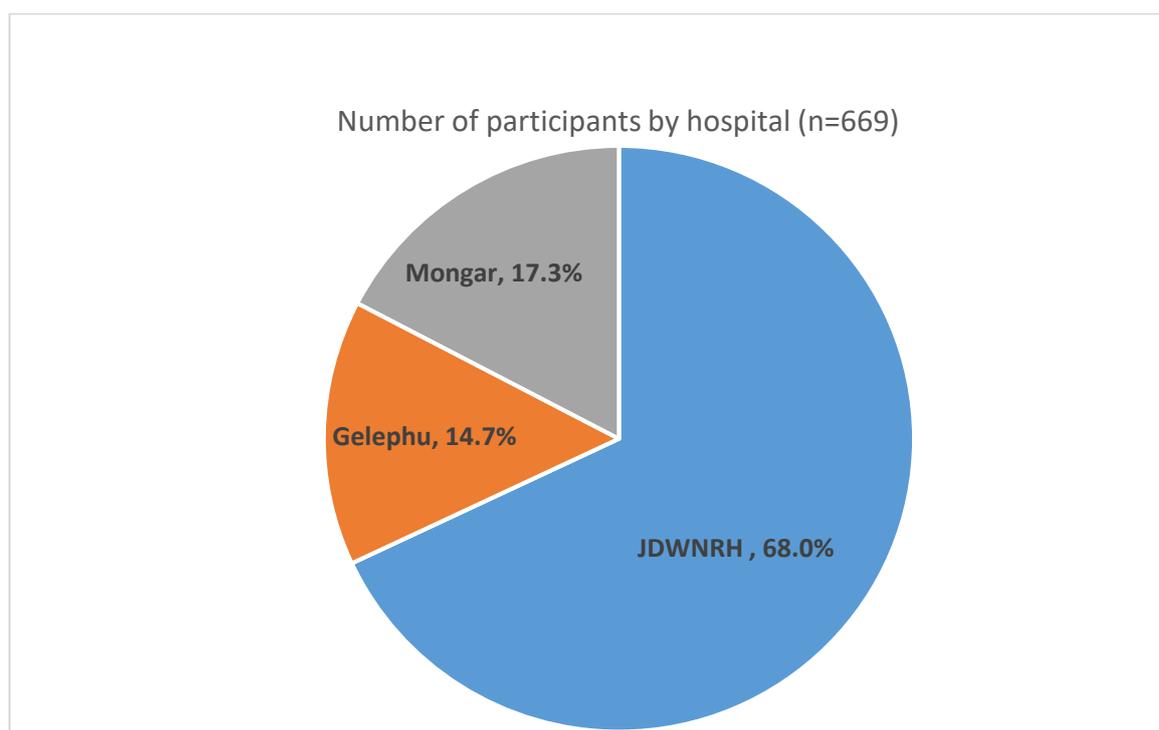


Figure 5.3. Number of participants by hospital.

Table 5.1. Number of recruited cases and controls by hospital

Hospital	Total (n=669)		Control (n=321)		Case (n=348)		P value
	N	%	N	%	N	%	
JDWNRH	455	68%	215	67%	240	69%	0.853
Gelephu	98	15%	49	15%	49	14%	
Mongar	116	17%	57	18%	57	17%	

Table 5.2. Number of term LBW and controls by hospital

Hospital	Control (n=321)		Term LBW (n=152)		P value
	N	%	N	%	
JDWNRH	215	67%	102	67%	0.999
Gelephu	49	15%	23	15%	
Mongar	57	18%	27	18%	

Table 5.3. Number of PTB and controls by hospital

Hospital	Control (n=321)		PTB (n=193)		P value
	N	%	N	%	
JDWNRH	215	67%	138	70%	0.708
Gelephu	49	15%	26	13%	
Mongar	57	18%	32	16%	

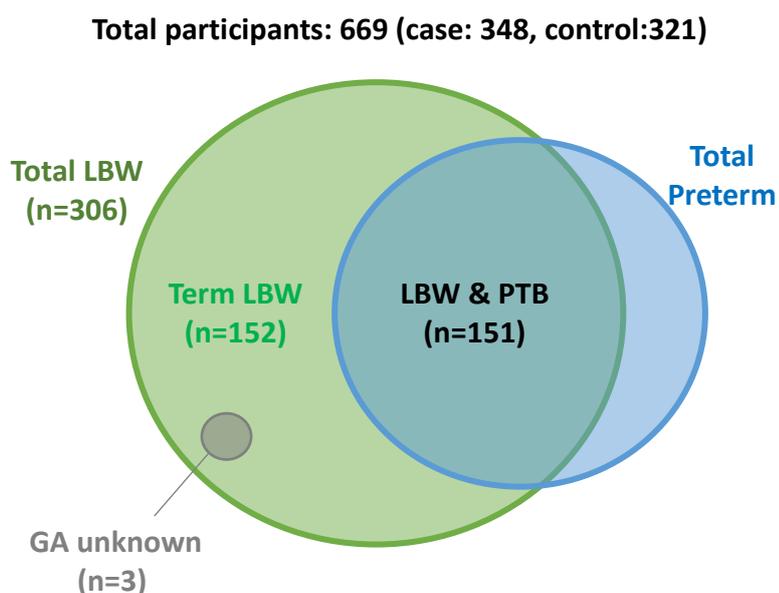


Figure 5.4. Description of the study participants

5.3. Distribution by delivery month

The number of recruited cases and controls varied by month ($p < 0.05$) (Figure 5.5 and Figure 5.6). In February 2015, as the study was just launched, the research team focused on interviewing more cases to become familiar with the process. The focus was on not missing cases thereafter. While the actual study period was from February 2015 to the beginning of March 2016, it was originally planned to end in December 2015 and was extended due to insufficient sample size. There was

some confusion at JDWNRH in January 2016 and data collection was suspended for two weeks, which could explain the lower number of participants in January 2016. In the last month of the study period, the team focused on interviewing more controls to match the number of cases to avoid overloading staff during the royal birth¹³.

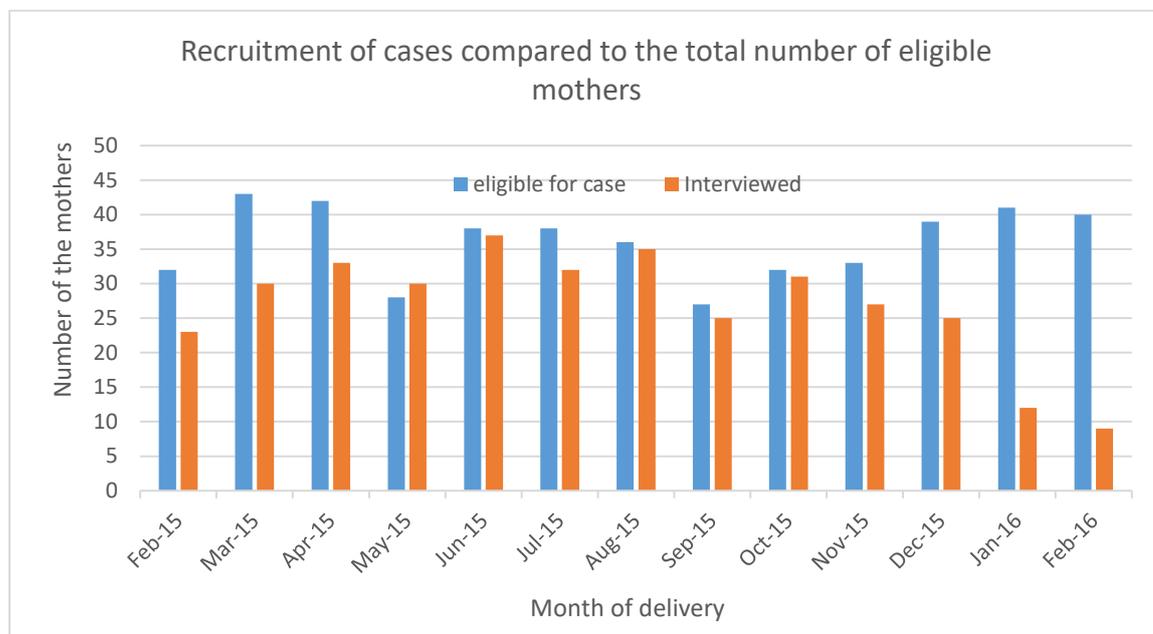


Figure 5.5. Recruitment of cases compared to the total number of eligible mothers.

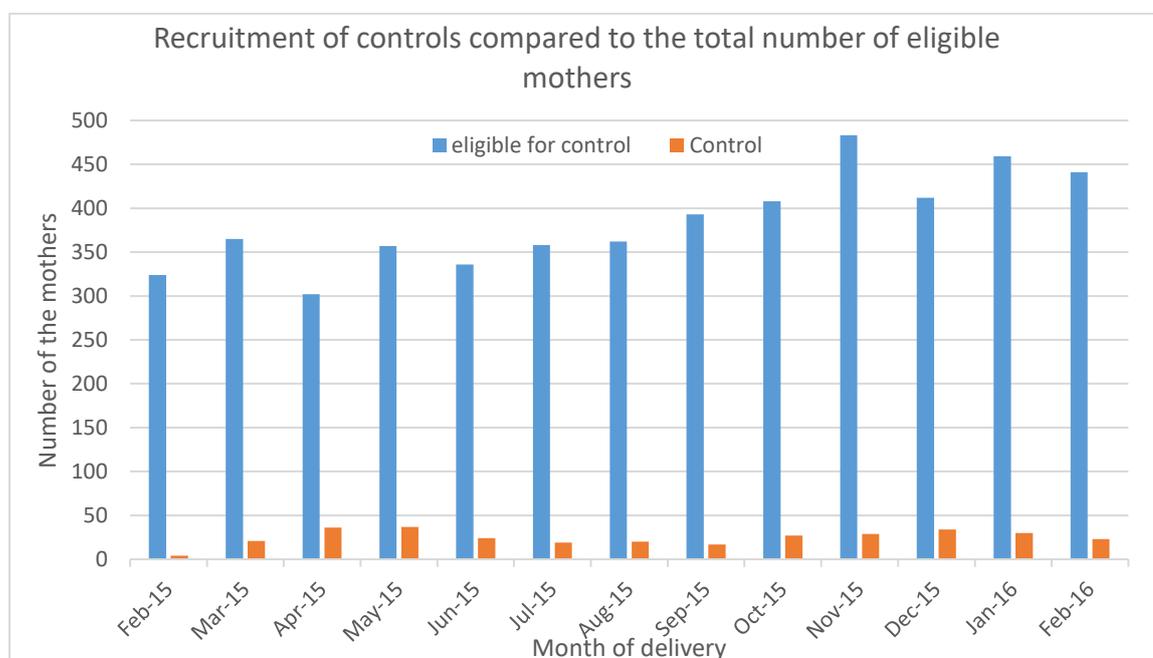


Figure 5.6. Recruitment of controls compared to the total number of eligible mothers.

¹³ Bhutan’s Queen gave birth to the Crown Prince in February 2016.

5.4 Descriptive analysis of maternal and infant characteristics (general)

5.4.1 Socioeconomic status

(a) Age, marital status, and education

Mean maternal age at the time of delivery was 27.5 years old. There were more mothers aged under 20 years and over 40 years in the cases compared to the controls (<20 years :control 4.4% [14/321] vs case 7.8% [27/348]; and 40+years : control 1.6% [5/321] vs case 6.6% [23/348]). Data suggested an association between age categories and adverse outcomes in the descriptive analysis (p=0.003) (Table 5.4).

Of the sample, 98% of the women were married. More than 20% of the mothers had not had any form of education, while 60% attended middle secondary education or more. The percentage of mothers who completed professional diploma, college or postgraduate education was 9%. Similarly, 17% of the husbands or partners never had any form of education. Two percent had non-formal education and another 2% had monastic education. More than 50% had middle secondary education or more.

Other national surveys reported notably lower levels of highest education attainment. For example, a national non-communicable disease survey conducted in 2014 reported that out of 2,819 respondents aged between 18 and 69 years old, 63% had no formal schooling, 14% had less than primary school, 9% had completed primary school, 7% had completed secondary school, 5% had completed high school, 2% had completed college/university, and 1% had completed postgraduate degree studies [2].

Table 5.4. Age, marital status, education and adverse birth outcomes.

Age, marital status, education		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
		N	%	N	%	P value	N	%	P value	N	%	P value
Age (years)	<20	14	4.4%	27	7.8%	0.003	9	5.9%	0.049	18	9.3%	0.002
	20-<25	85	26.5%	99	28.5%		40	26.3%		57	29.5%	
	25-<30	123	38.3%	106	30.5%		51	33.6%		54	28.0%	
	30-<35	66	20.6%	58	16.7%		24	15.8%		34	17.6%	
	35-<40	28	8.7%	36	10.1%		18	11.8%		17	8.8%	
	40+	5	1.6%	23	6.6%		10	6.6%		13	6.7%	
Marital Status	Married or living with a partner	321	97.2%	336	96.6%	0.295	149	98.0%	>1.000	185	95.9%	0.129
	Missing	3	0.9%	1	0.3%		1	0.3%		0	0.0%	
Mother's Highest level of education attained	Never attended school	72	22.4%	95	27.3%	0.180	48	31.6%	0.094	47	24.4%	0.472
	Non-formal education (NFE)	28	8.7%	21	6.0%		11	7.2%		10	5.2%	
	Primary	43	13.4%	59	17.0%		26	17.1%		33	17.1%	
	Middle Secondary or Secondary	154	48.0%	147	42.2%		55	36.2%		89	46.1%	
	Diploma, College, and postgraduate	24	7.5%	25	7.2%		12	7.9%		13	6.7%	
	Missing	0	0.0%	1	0.3%		0	0.0%		1	0.5%	
Husband education's highest level of education attained	Never attended school	44	13.7%	72	20.7%	0.203	38	25.0%	0.122	34	17.6%	0.601
	NFE	8	2.5%	8	2.3%		3	2.0%		5	2.6%	
	Primary	71	22.1%	72	20.7%		32	21.1%		39	20.2%	
	Middle Secondary or Secondary	132	41.1%	127	36.5%		52	34.2%		74	38.3%	
	Diploma, College, and postgraduate	53	16.5%	47	13.5%		19	12.5%		28	14.5%	
	Monastic Education	8	2.5%	6	1.7%		3	2.0%		3	1.6%	
	Divorced	5	1.6%	10	2.9%		2	1.3%		7	3.6%	
	Missing	0	0.0%	6	1.7%		3	2.0%		3	1.6%	

(b) Wealth Index

After examining 17 asset variables, 13 (ownership of watch, mobile phone, bicycle, motor cycle, car, computer, foreign bow, camera, VCR/VCD, DVD player, silk dress (gho, kira, sari or suits), access to improved sanitation, finished walls, finished floor, number of persons in a sleeping room) were included to create a wealth index which was used to group the study population into quintiles. Access to improved water supply, using solid fuel for cooking, having a kitchen as a separate room, and a finished roof were not included after checking the correlation matrix (Table 5.6). Data shows weak or no association between adverse birth outcomes and wealth quintile ($p < 0.198$) (Table 5.7).

Compared to the 2010 BMIS, the percentage of asset ownership was higher in general in the present study (Table 5.5). This could be the result of capturing more urban dwellers in the sample population or rapid urbanisation and modernisation especially after democratisation in 2008.

Table 5.5. Proportion of asset ownership and comparison with the BMIS 2010 survey.

Asset variables	The present study	BMIS (2010)
	% or Means (SD)	% or Means (SD)
Use of solid fuel for cooking	4.8%	39.5%
Motorcycle	5.4%	3.6%
Foreign bow	9.4%	5.8%
Bicycle	10.8%	5.2%
Computer	30.2%	8.0%
Car	30.5%	14.2%
Camera	35.1%	13.2%
Silk dress	43.1%	12.7%
VCR/VCD/DVD Player	45.1%	25.4%
Finished Floor	56.5%	26.5%
Watch	67.6%	58.9%
Finished walls	76.4%	46.3%
Improved sanitation	80.9%	63.3%
Finished roof	95.4%	85.4%
Not cooking in the bedroom	96.4%	74.3%
Mobile phone	96.7%	84.7%
Improved water	98.4%	96.1%
Number of persons per bed room	0.6 (0.4)	3.2 (1.7)

Table 5.6. Factor scores for each variable included in principal component analysis.

Asset variables	Factor1	Factor2	Factor3
Camera	0.22862	-0.02727	-0.28036
Computer	0.18760	0.00484	0.12733
Car	0.17632	-0.04161	-0.07731
Bicycle	0.13905	-0.19183	0.23474
Silk dress	0.10328	-0.00387	-0.14417
Improved sanitation	0.09590	0.04286	0.07146
Finished walls	0.09560	0.11267	0.22284
Foreign bow	0.08458	-0.27825	-0.22346
VCR/VCD/DVD Player	0.08097	0.03214	-0.05779
Mobile phone	0.06870	0.49561	-0.19115
Watch	0.06844	0.15903	-0.06327
Finished Floor	0.05733	0.10405	0.41615
Motor cycle	0.04665	-0.12558	0.12586
Number of persons per bed room	0.02734	0.03598	0.02199

Table 5.7. Wealth quintile and adverse birth outcomes.

Wealth quintile	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Poorest	55	17.2%	78	22.4%	0.198	37	24.3%	0.372	40	20.7%	0.402
Second	67	20.9%	65	18.7%		29	19.1%		36	18.7%	
Middle	59	18.4%	74	21.3%		29	29.1%		44	22.8%	
Fourth	63	19.7%	70	20.1%		31	20.4%		38	19.7%	
Richest	73	22.8%	59	17.0%		26	17.1%		33	17.1%	
Missing	3	0.9%	2	0.6%		0	0.0%		2	1.0%	

(c) Ethnicity

A previous study reported that there was a statistical significant difference in birth weight by ethnicity [3]. In the study, which used the information from the national referral hospitals on 13,647 singleton neonates of GA between 37 completed weeks and 41 weeks 6 days between January 2011 and December 2014, the mean birthweight was $3,177 \pm 435$ g (the mean birth weight of northern Bhutanese was: $3,260 \pm 436$ g and the mean of southern Bhutanese was: $3,060 \pm 411$ g). Ethnicity was classified by the mother's name and language by a member of the research team. About 31% (205/669) were identified as southern Bhutanese. There were no statistically significant differences in proportion of mothers of southern Bhutanese origins between cases and controls ($p=0.572$) (Table 5.8).

Table 5.8. Ethnicity and adverse birth outcomes.

Ethnicity	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Southern Bhutanese	95	29.6%	110	31.6%	0.572	58	38.2%	0.063	193	26.42%	0.440

(d) Urban and rural residence

Residential status was classified as urban or rural based on the population census classification using a list of villages and thromdes codes obtained from the National Statistics Bureau and reviewed by a local research assistant for consistency. Permanent address is where the mothers registered for the census whereas current residence is where they were staying at the point of delivery. Only 9% (60/669) of the study population were originally from urban areas whereas 55% (372/669) were staying in urban areas at the point of delivery, indicating the mothers moved from their original rural residence to an urban residence at some point before delivery. Of those who stayed in urban areas at the point of delivery, more were in the richest category compared to the rural areas ($p=0.003$) (Table 5.9), suggesting urban and rural disparities of wealth. However, the data show weak or no association between adverse birth outcomes and urban residence ($p=0.932$) (Table 5.10).

Table 5.9. Wealth quintile and urban residence.

Ethnicity	Urban (n=372)		Rural (n=296)		P-value
	N	%	N	%	
Poorest	64	17.3%	69	23.3%	0.003
Second	65	17.5%	67	22.6%	
Middle	73	19.7%	60	20.3%	
Fourth	74	20.0%	58	19.6%	
Richest	92	24.8%	40	13.5%	

Table 5.10. Urban residence and adverse birth outcomes.

Urban residence	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Urban current residence (n=372)	178	55.5%	193	55.5%	0.932	82	54.0%	0.759	108	56.0%	0.810
Missing	0	0.0%	2	0.6%		0	0.0%		2	1.0%	

(e) Altitude

Bhutan's elevation varies from 160 to 7,500 meters above sea-level. Thimphu (JDWNRH), the capital, is located 2,320 meters above sea-level; Mongar town (ERRH) is located 1,541 meters above sea-level and Gelephu town (CRRH) is situated 224 meters above sea-level.

The altitude of gewog centre (administrative unit below prefecture or dzongkhag level) was used as a proxy for altitude of residence. Mean altitude of current gewog was 1,823 meters above sea-level (Figure 5.8). On average, mothers moved to a gewog 557 meters higher than their original gewog at some point before delivery (Figure 5.7 and Figure 5.9). Data show weak or no association between adverse birth outcomes and difference in altitudes ($p=0.2305$) (Table 5.11).

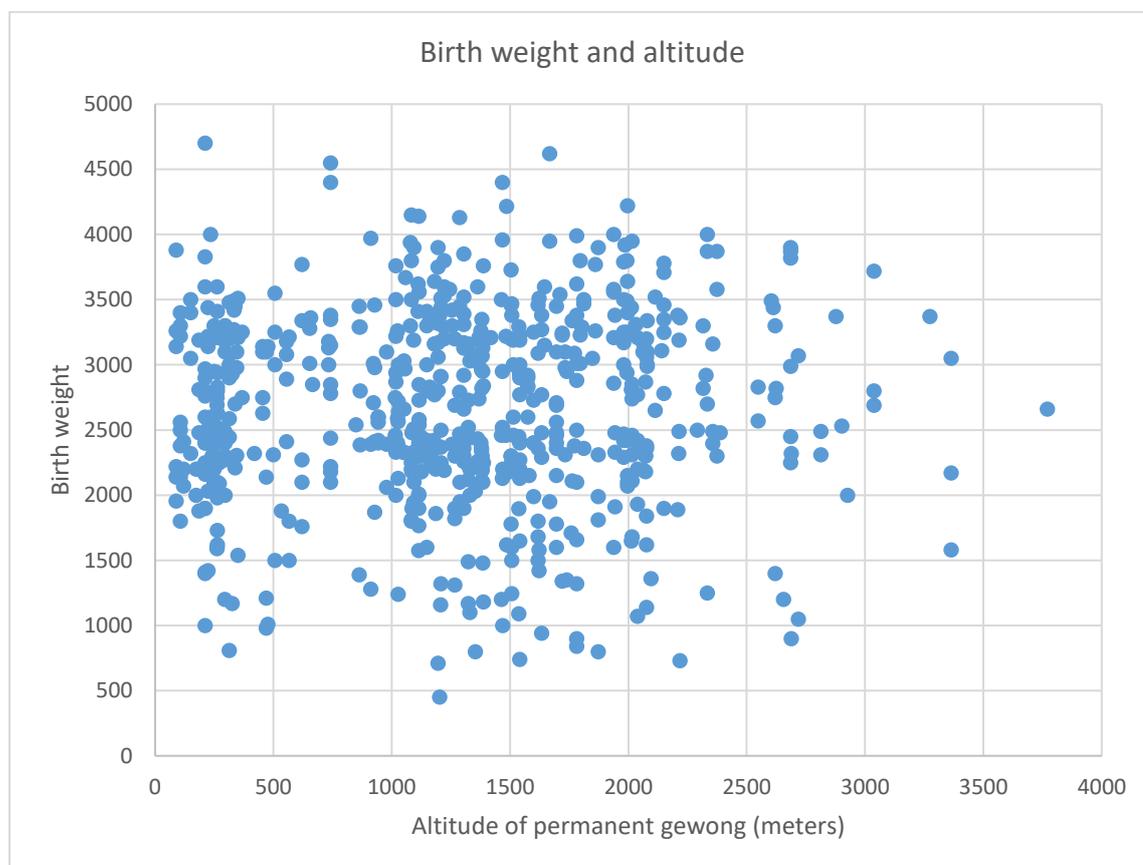


Figure 5.7. Birth weight and permanent gewogs (in meters).

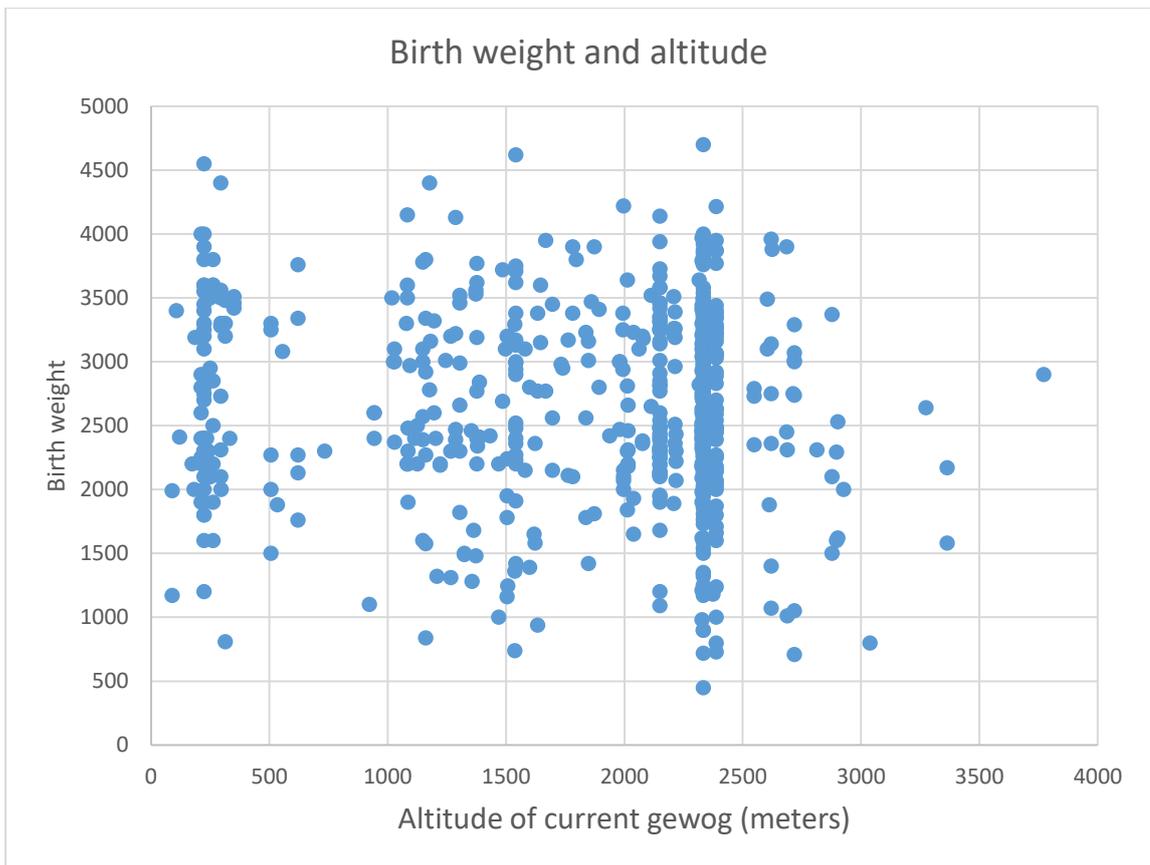


Figure 5.8. Birth weight and altitude of current gewog (in meters).

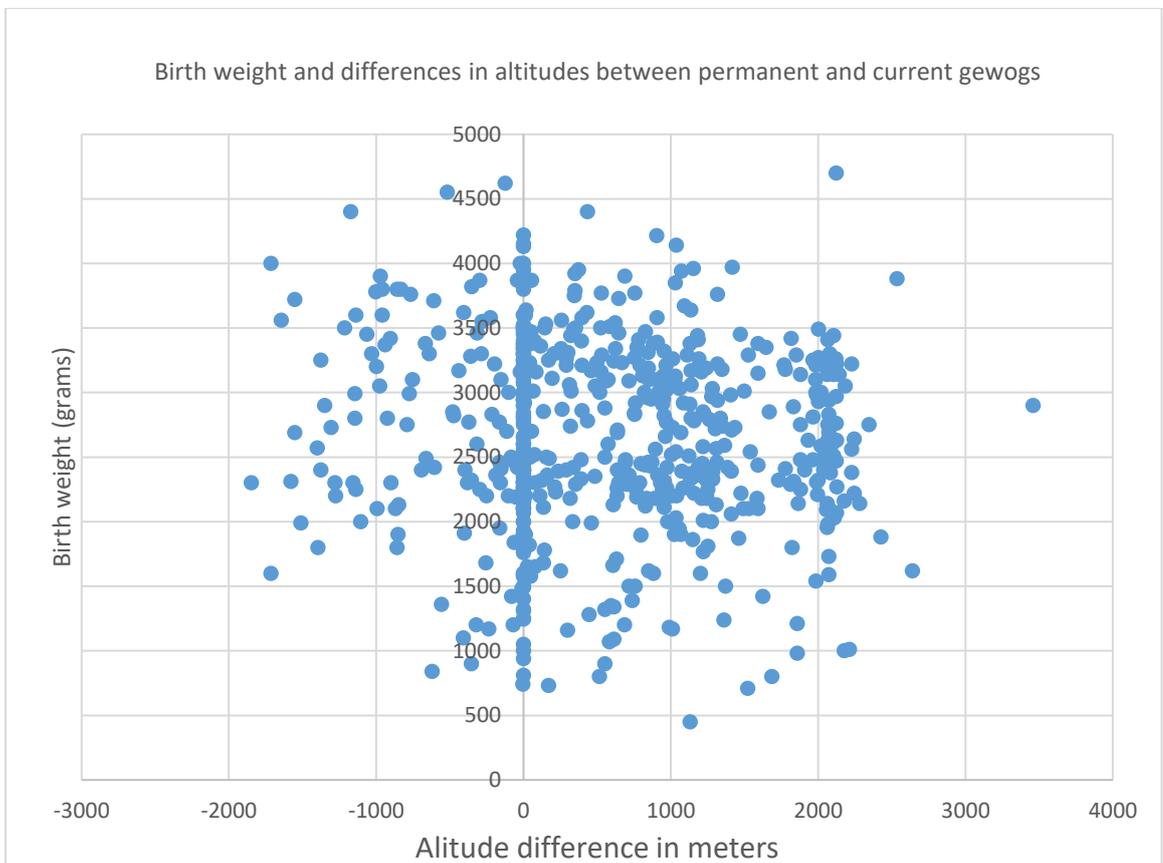


Figure 5.9. Birth weight and differences in altitudes (meters) between permanent and current gewogs.

Table 5.11. Altitude (in meters) and adverse birth outcomes

Altitude (in meters)		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
		N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Altitude of current residence (meters)		307	1791 (781)	338	1853 (753)	0.3067	149	1796 (763)	0.9483	186	1890 (748)	0.1611
Difference between current and permanent altitudes (meters)		301	512 (918)	333	596 (888)	0.2305	147	593 (880)	0.3680	183	591 (895)	0.3475
<hr/>												
By ethnicity: Southern Bhutanese (n=205)	Altitude of current residence (meters)	91	1662 (883)	108	1731 (844)	0.5707	56	1755 (844)	0.5251	51	1693 (854)	0.834
	Difference between current and permanent altitudes (meters)	88	898 (1043)	106	1008 (953)	0.4486	56	1028 (966)	0.4450	49	961 (135)	0.7169

(f) Employment situations of study participants and partner

Among the case mothers, 61% were housewives including those who were engaged in farming (16%) or weaving (7%) at home. Among 245 (36%) employed mothers, 36% were self-employed and 28% were employed in the public sector including education and health. The majority of the self-employed mothers were engaged in retail shop-keeping (42%) or farming (35%). Employment activities of the rest included weaving, embroiling, herding, managing a travel agency and other businesses. In total, 15% of the case mothers were engaged in farming either as self-employed farmers or assisting with family farming. Partner's occupation is described in Figure I.1 in Appendix I.

Sixty one percent of the employed mothers and 47% of the self-employed mothers worked more than 40 hours per week (Figure I.2 in Appendix I). Although information regarding timing was not included in the survey, a few mothers mentioned that they resigned or went on leave in their third trimester.

Among mothers who worked in weaving, farming, shop-keeping and office work, in addition to house chores, most of the pregnant women (91%) worked during the day time while 5% worked in rotating shift work with night or fixed night work, or stayed from morning to night in their own shops (Figure I.3 in Appendix I). Occupations of mothers who worked in rotating shift work included police officers, security-related workers, service-related workers including hotel managers, housekeepers and bar or karaoke staff.

There were no statistically significant different associations between pregnancy outcomes and maternal occupation, working hours or working shifts in the descriptive analyses (maternal occupation $p=0.362$; working hours: $p=0.990$; and working shift: $p=0.753$, table not presented).

(g) A summary of key findings from the analysis of socio-economic factors

The study was able to capture more than 70% of the total PTB and/or LBW mothers and the refusal rate was low. However, there is a possibility of interview bias in that those mothers who cannot speak or write may not be willing to participate in research. The descriptive analysis of socio-economic factors suggested an association between age and adverse outcomes but showed little variation by education, wealth quintile, ethnicity, occupation, marital status, urban residence and altitude. The analysis also revealed that the study participants had a higher level of educational attainment and more often lived in urban areas compared to other national level surveys such as a recent STEPS survey in 2014. This could imply that the study participants represent those who had complications or were referred by primary health care facilities, also those who preferred to deliver at the referral hospitals who have the means and knowledge to come to the referral hospitals and may have a higher education, better access to the health facilities and higher wealth level compared to the general population. Selected socioeconomic factors are further examined in the multivariable analysis in Chapter 7 and discussed in comparison with other studies in Chapter 8.

5.4.2 Health seeking behaviour

(a) Number of ANC visits and timing of ANC

Of the sample mothers, only 2% (13/669) had no ANC visits. 83% (558/669) of the mothers had at least four ANC visits as per WHO recommendation. Three mothers were missing information on the number of ANC visits.

Mean number of ANC visits was 5.6 (SD=6.2, 95% CI 5.4-5.7) (Table 5.12). Mothers of LBW and PTBs had fewer ANC visits (term LBW vs no-LBW: 5.7 vs 6.7; PTB vs Non-PTB: 3.8 vs 6.7) and the difference was statistically significant ($p<0.0001$). Number of ANC visits was coded into four categories: 0 (no ANC visits), 1-3, 4-8, more than 8 times (Table 5.12). The cut-off of four was used as WHO recommended at least four ANC visits. The data suggest a strong association between number of ANC visits and adverse birth outcomes.

Mean gestational weeks at the first ANC visit was 15 weeks (mean 15.2, SD=6, 95% CI 14.7-15.7). There were no statistically significant differences in the mean gestational weeks between cases and controls (Table 5.13).

This indicates that the timing of the first ANC was not particularly late for the mothers of adverse delivery outcomes (Figure 5.10). However, the number of ANC visits was lower. This could be due to shorter pregnancy duration or could indicate insufficient management of pregnancy or screening of potential high risk pregnancy.

Table 5.12. Mean number of ANC and standard deviation by adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Number of ANC visits	319	6.7 (1.7)	346	4.6 (2.0)	<0.0001	151	5.7 (1.8)	<0.0001	193	3.8 (1.7)	<0.0001

Table 5.13. Number of ANC (categorical) and adverse birth outcomes.

Number of ANC	GA weeks at the 1 st visit (n=652)		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	Mean (SD)	P-value	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Number of ANC visits	-	<0.0001	0	0.0%	12	3.5%	<0.0001	2	1.3%	<0.0001	9	4.6%	<0.0001
1 to 3 times	21 (7)		7	2.2%	88	25.3%		14	9.2%		73	37.6%	
4 to 7 times	15 (6)		204	63.6%	222	63.8%		112	73.7%		110	56.7%	
8 times or more	12 (4)		108	33.6%	24	6.9%		23	15.1%		1	1.0%	
Missing	-		2	0.6%	2	0.6%		1	0.7%		0	0.0%	

Table 5.14. Gestational weeks at the 1st ANC visit by adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
GA weeks at 1st ANC	319	14.9 (6.2)	333	15.6 (6.5)	0.1705	294	15.7 (6.2)	0.2052	184	15.5 (6.8)	0.3222

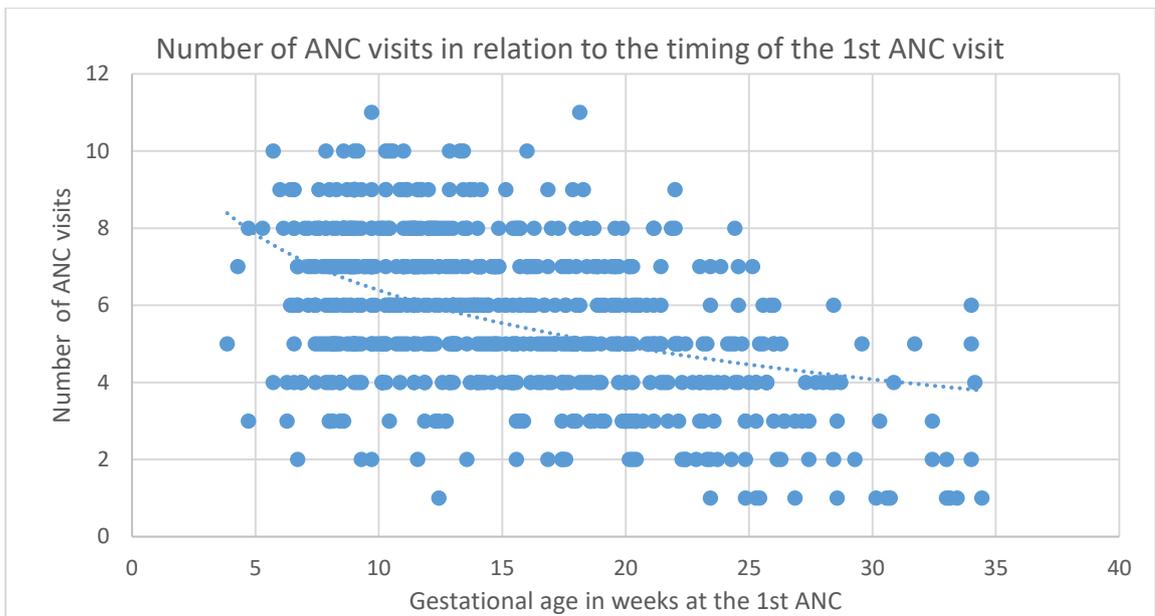


Figure 5.10. Number of ANC visits in relation to the timing of the 1st ANC visit.

(b) Location of ANC visits

The following map (Figure 5.11) summarises the cases and controls by location of ANC visits.

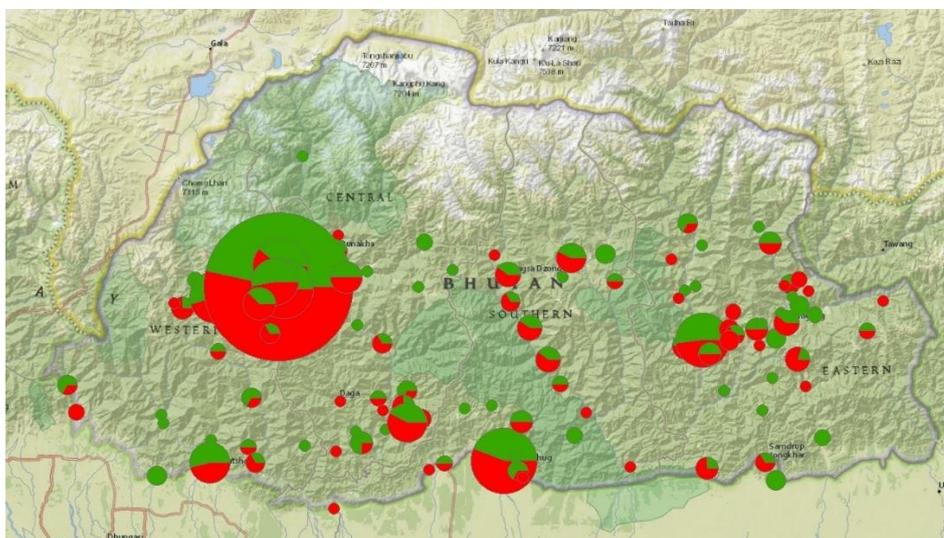


Figure 5.11. Number of participants with the proportion of cases by ANC facility (n=610). The size of the graph represents the size of the total number of participants who registered at ANC clinics. Red represents the proportion of cases out of the total while green represents the proportion of controls. Note: the following ANC centres were not included in the map due to lack of geographic information. Chuhukha (Baikunza Sub-Post and Chapchha BHU), Dagana (Lunana BHU), Haa (Haa IMTRAT Hospital), Lhuentse (Dangling BHU, Ganglakhema BHU, and Thimyul BHU), Pema Gatshel (Borangma Sub-Post, Khangma Sub-Post, and Naskhar Sub-Post), Punakha (Goenshari BHU), Samdrup Jongkhar (Maenjiwoong BHU), Samtse (Ganthong BHU, Sangang Choeling BHU, Sengteng BHU, and Ugyentse BHU), Sarpang (Chokorling BHU, Jangchubling BHU, and Lhayul BHU), Thimphu (Kuzugchen BHU), Trashigang (Jeonkhar BHU), Tashiyangtse (Jangphutse sub-post), Trongsa (Kella Sub-post), Tsirang (Barshong BHU and Pungtenschuu BHU), Wandue Phodrang (Esa BHU I, Khotokha sub-post, Manas BHU, and Namregang BHU).

(c) Reasons for delivery at this hospital

The most common reason was for better health care and services (Table 5.15). Second, 30% (201/669) was referral from other hospitals. In addition to those referred, 3% (19/669) had a medical condition such as pain or bleeding or had been advised by doctors due to high blood pressure, bleeding, or past obstetric records and came voluntarily to the referral hospitals. Among those who were referred, advised, or had a medical condition (pain or bleeding), 57% delivered by caesarean section and approximately half of the mothers delivered LBW (54%) or PTB babies (40%).

More control mothers delivered at the referral hospital for better health care and services than cases (control 51.1% [164/321] vs case 43.1% [150/348]). On the other hand, more case mothers were referred to the delivery hospital (control 25.6% [82/321] vs case 34.2% [119/348]) or had some medical condition that motivated them to deliver at the referral hospitals voluntarily (control 0.6% [2/321] vs case 4.3%[15/348]) ($p=0.003$) than controls.

Table 5.15. Reasons of delivery and adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Because I or my relatives live or work nearby	57	17.8%	45	12.9%	0.003	22	14.5%	0.251	23	11.9%	<0.0001
For better health care and services	164	51.1%	150	43.1%		77	50.7%		71	36.8%	
Better equipped	3	0.9%	4	1.2%		3	2.0%		1	0.5%	
Has more qualified staff	9	2.8%	10	2.9%		3	2.0%		6	3.1%	
Referral from other hospitals or BHUs	82	25.6%	119	34.2%		42	27.6%		77	39.9%	
Medical condition or advised by a doctor	2	0.6%	15	4.3%		5	3.3%		10	5.2%	
Other (please specify)	3	0.9%	5	1.4%		0	0.0%		5	2.6%	
Missing	1	0.3%	0	0.0%		0	0.0%		0	0.0%	

(d) Transportation and travel time

In Bhutan, the road is fairly rough and most of the case mothers travel by land. About half of the mothers (367/669) travelled on rough roads during pregnancy. Mean travel time from home to the delivery hospital was 1.9 hours (95% CI 1.5-2.3) (Table 5.16). The travel time was not statistically significantly different between delivery outcomes (Table 5.17). However, more mothers of LBW or PTB babies travelled by ambulance ($p=0.011$) (Table 5.19).

Table 5.16. Mean travel time to each delivery hospital from place of residence.

	JDWNRH (n=455)		Gelephu (n=98)		Mongar (n=116)		P-value
	N	Mean (SD)	N	Mean (SD)	N	Mean (SD)	
Travel time (hours)	448	1.4 (4.1)	97	2.8 (9.8)	114	3.1 (2.7)	0.0011

Table 5.17. Mean travel time in hours by different adverse outcome.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Travel time (hours)	319	2.0 (7.0)	343	1.7 (2.5)	0.5069	150	1.7 (2.5)	0.5111	190	1.8 (2.5)	0.5767

Table 5.18. Mean travel time in hours by reason.

Reason	N	Mean (SD)
Because I or my relatives live or work nearby	101	1.5 (9.6)
For better health care and services	312	0.8(1.7)
Better equipped	7	1.0 (1.8)
Has more qualified staff	19	0.7(1.0)
Referral from other hospitals or BHUs	195	4.0(5.7)
Medical condition or advised by a doctor	17	0.8(1.4)
Other	8	0.4(0.2)
Total	659	1.9(5.2)

Table 5.19. Mode of transport and adverse outcomes

Mode of transport	Mean travel time (hours)		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	Mean (SD)	P-value	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
By car	1.4 (6.2)	<0.0001	143	44.6	119	34.2	0.011	54	35.5	0.149	65	33.5	0.001
By bus	7.0 (12.4)		13	4.1	19	5.5		13	8.6		6	3.1	
By taxi	0.7 (1.3)		97	30.2	101	29.0		51	33.6		47	24.2	
By ambulance	3.4 (2.9)		54	16.8	86	24.7		25	16.5		61	32.0	
On foot	0.8 (1.5)		12	3.7	23	6.6		9	5.9		14	7.2	
Others	-		1	0.3	0	0.0		0	0.0		0	0.0	
Missing	-		1	0.3	0	0.0		0	0.0		0	0.0	

(e) A summary of key findings from analysis of health seeking behaviours

The descriptive analysis suggested an association between number of ANC visits and adverse birth outcomes. The timing of the ANC was not particularly late for the mothers with adverse birth outcomes. More mothers with adverse outcomes travelled by ambulance and had been referred. The referred mothers travelled longest (4 hours on average) on the journey from home. More than half of those mothers referred or those who had a medical condition and came voluntarily to the referral hospitals delivered a baby by caesarean section and approximately half of these mothers delivered LBW and/or PTB babies.

5.4.3 General health, infectious or chronic diseases

(a) Mean BMI, weight gains

Mean height was 152.5 cm (SD=6.0, 95% CI 152.0-153.0). 8% (55/669) of the mothers were missing height information. This is similar to the results from the Bhutan 2014 STEPS survey, which reported that the mean height was 153.2 cm (152.6-153.8) for females aged between 18 and 69 [2]. There were no statistical significant differences in means for mothers of LBW and/or PTB babies compared to the control mothers (Table 5.20).

Mean pre-pregnancy weight was 53.7 kg (SD=9.5, 95% CI 52.9 – 54.6). Of the study participants, 187/669 (28%) of mothers were missing pre-pregnancy weight information. Compared to the control mothers, mothers of term LBW babies were slightly lower in weight (term LBW 52.2 [SD=8.7] vs non-LBW 54.6 [SD=9.1], $p=0.0209$). There was no statically significant difference for PTB ($p=0.2423$).

Mean maternal weight at first antenatal care was 55.8 kg (SD=9.3, 95% CI 55.0-56.5). 5% (36/669) of mothers were missing maternal weight information.

Mean pre-pregnancy BMI was 23 (SD=3.75, 95% CI 22.7-23.4). There were no statistically significant differences in the mean BMI for different birth outcomes (control vs case, $p=0.0870$; control vs term LBW, $p=0.1824$; and control vs PTB, $p=0.1637$). While 33.3% of the mothers were missing information on BMI due mainly to lack of pre-pregnancy weight information, 44.3% of (296/669) mothers had a BMI within an average range between 18.5 and 25.0; 5.2% of mothers were underweight (BMI< 18.5); 14.2% were overweight (BMI \geq 25); and 3.0% were obese (BMI \geq 30).

Mean gestational weight gain (defined as the difference between the self-reported pre-pregnancy weight and pre-delivery weight measured at the last ANC visit) was 9.4 kg (SD= 5.9, 95% CI 8.8-9.9) (Figure 5.12). Approximately one third (29.9% [200/669]) of the women were missing information on gestational weight gain. Gestational weight gain was statistically significantly smaller for the case mothers than the controls (control 10.6 kg [SD=5.6] vs case 8.1[SD=6.0, <0.0001]). The Institute of Medicine recommends the gestational weight gain range according to pre-pregnancy BMI (for underweight: 28-40 lbs/ 12.5-18 kg; normal BMI: 25-35 lbs/11.5-16 kg; for overweight:15-25lbs/7.0-11.5 kg; obese:11-20 lbs/5-9 kg) [4]. By this

classification, 17.32% had the recommended gestational weight gain whereas 41% had a lower weight gain than recommended (Figure 5.13 and Figure 5.14). On the other hand, 12% had a higher weight gain than recommended.

Table 5.20. Mean height, pre-pregnancy weight, weight at 1st ANC visit, pre-pregnancy BMI, and gestational weight gain.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Height	300	152.7 (5.5)	314	152.3 (6.6)	0.4450	146	151.5 (7.6)	0.0962	168	153.0 (5.4)	0.5356
Pre-pregnancy weight	241	54.6 (9.1)	241	52.9 (9.9)	0.0403	102	52.2 (8.7)	0.0209	138	53.4 (10.7)	0.2423
Weight at 1st ANC	309	56.2 (8.8)	324	55.3 (9.8)	0.2245	149	53.6 (8.3)	0.0019	174	56.9 (10.7)	0.5009
Pre-pregnancy BMI	227	23.3 (3.5)	219	22.7 (3.8)	0.0870	97	22.7 (3.8)	0.1824	122	22.7 (4.1)	0.1637
Gestational weight gain	237	10.6 (5.6)	232	8.1 (6.0)	<0.0001	99	7.9 (6.0)	0.0002	133	8.3 (5.9)	0.0003

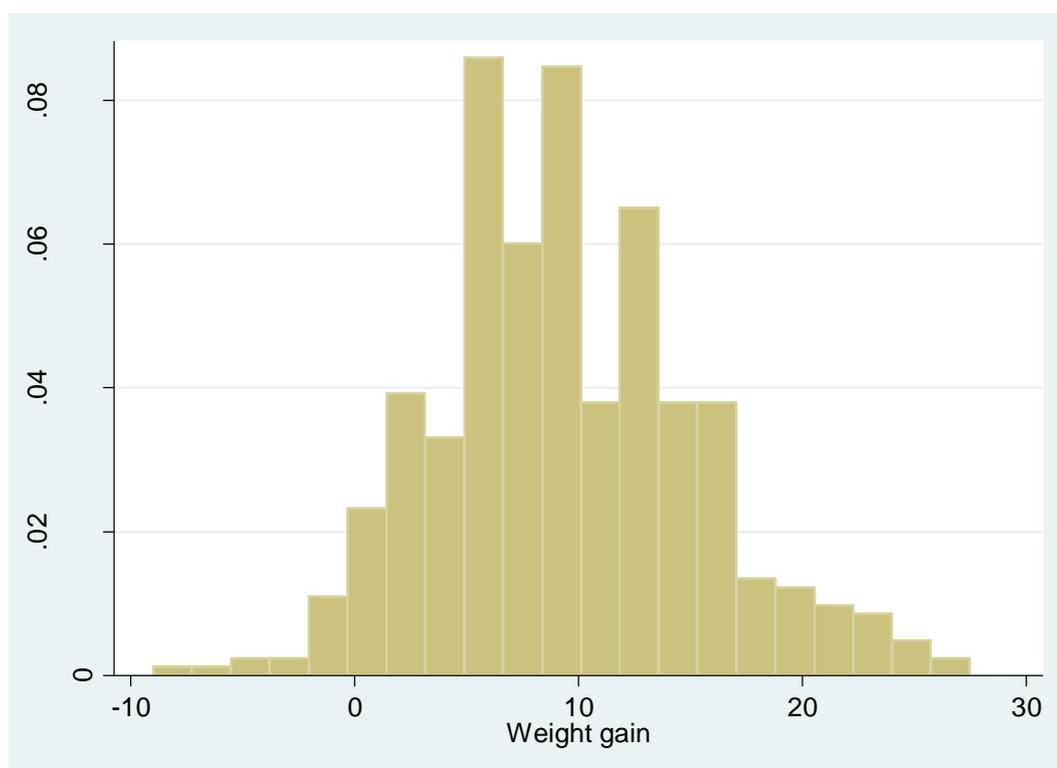


Figure 5.12. Distribution of gestational weight gain (kg) defined as the difference between the self-reported pre-pregnancy weight and pre-delivery weight measured at last ANC visit.

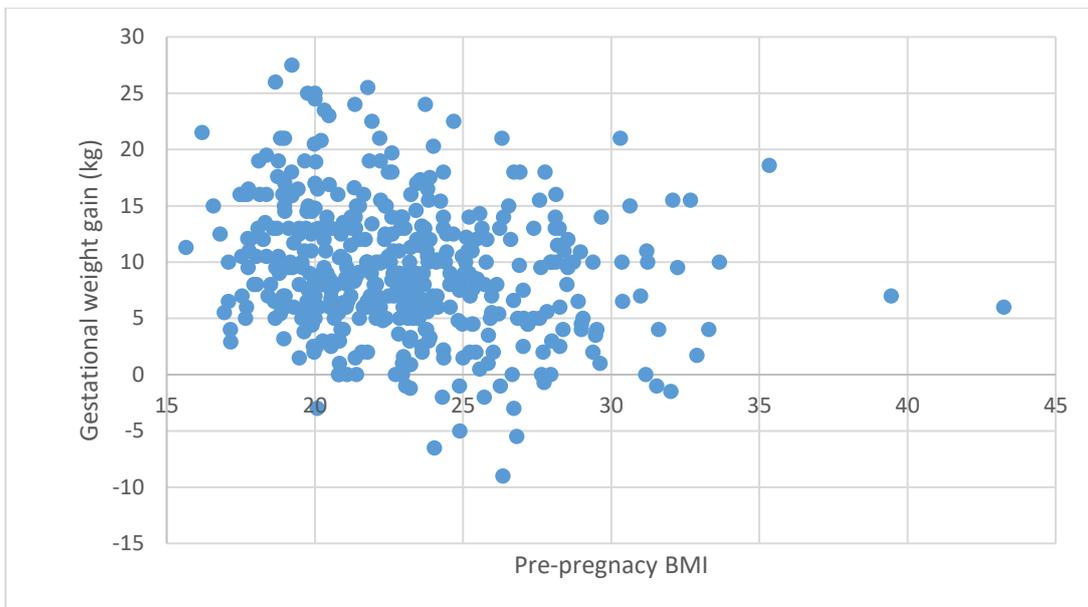


Figure 5.13. Scatterplot of gestational weight gain (kg) and pre-pregnancy BMI

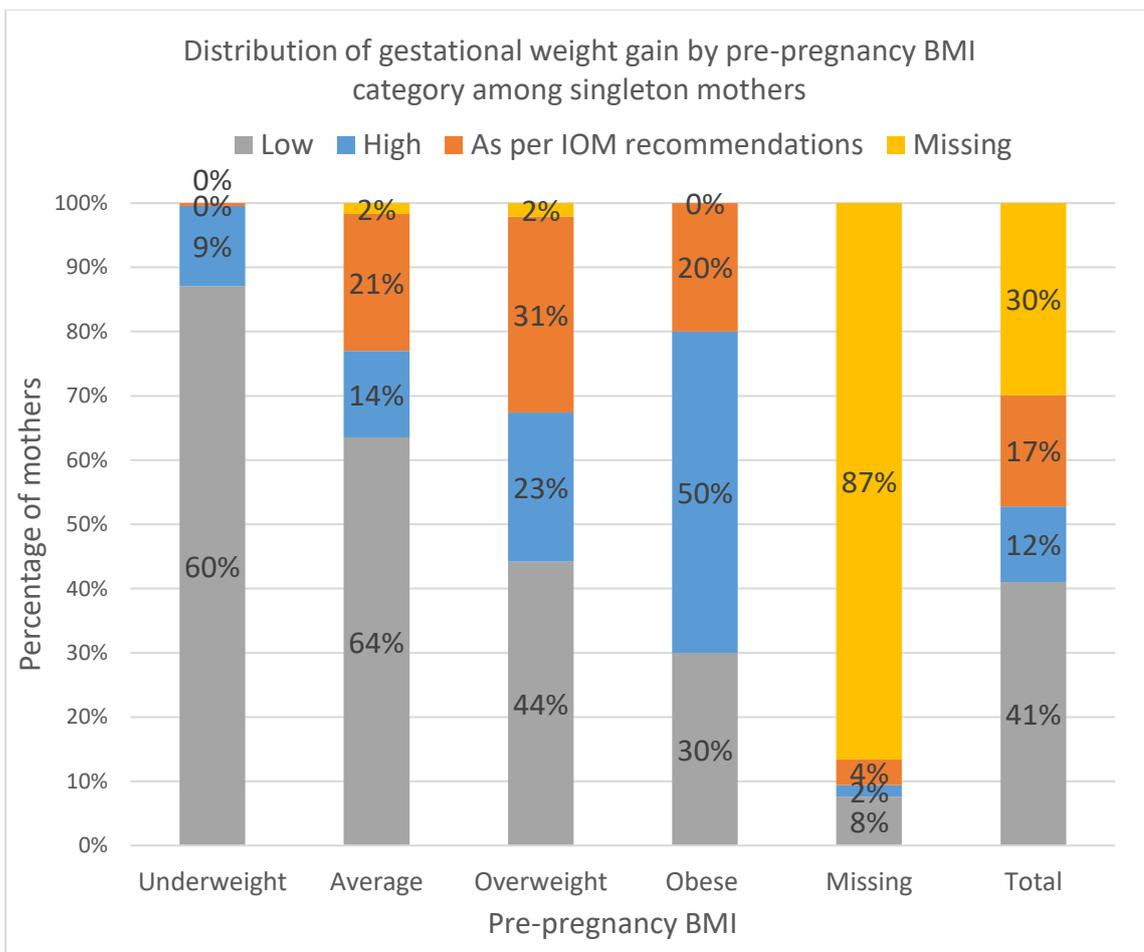


Figure 5.14. Distribution of gestational weight gain by pre-pregnancy BMI category among singleton mothers (n=669). Note: BMI categories were used (underweight, < 18.5 kg/m²; normal, 18.5 to 26.0 kg/m²; overweight, 26.1 to 29.0 kg/m²; obese, > 29.0 kg/m²).

(b) Infectious diseases

i) HIV, syphilis and hepatitis B

More than 30% of mothers were missing information on the test results in the MCH book (mothers missing results of HIV 259/669; mothers missing results of syphilis 267/669; and hepatitis B 286/669). This could be due to the MOH guideline which advised against writing the test results in the MCH book. Absence of test results could imply negative results. If positive, it was indicated in the MCH book and hospital medical records. According to the positive records, assuming the absence of data indicates negative results, the prevalence of HIV, syphilis, and hepatitis B was less than 1% (HIV 0.2%, syphilis 0.3%, and hepatitis B 1.5%). This is comparable with the government report and other studies where HIV prevalence was reported as below 0.2% [5]. In a study conducted among 4885 people in the most-at-risk populations (MARPS), sero positivity was 0.7% for HIV, 1.2% for syphilis and 1.3% for hepatitis B [6].

ii) Urinary tract infection (UTI)

Less than 10% (60/669) of mothers had a recorded UTI. More mothers in the case group experienced UTI compared to the control group (control 6.9% [22/321] vs case 10.9% [38/348], $p=0.036$). More mothers in the case group were missing information on UTI compared to the control group (control 7.8% [25/321] vs case 13.2% [46/348]) (Table 5.21).

Table 5.21. UTI and adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Urinary tract infection	22	6.9%	38	10.9%	0.036	19	12.5%	0.040	19	9.8%	0.118
Missing	25	7.8%	46	13.2%		12	7.9%		32	16.6%	

iii) Symptoms of potential infectious diseases

Mothers were asked if they had had selected symptoms of infectious diseases at some point during pregnancy. Even though only about 11% of the mothers with adverse birth outcomes had a recorded UTI, about 16% reported possible symptoms such as fever, feeling very hot and sweating and 28% of the mothers reported feeling very sick or weak at some point during pregnancy, which could indicate a possible unscreened or untreated infectious diseases. More mothers in the cases group reported pain in the lower belly, behind the front of the pelvis (control 22.7% [73/321] vs 31.3% [109/348], $p=0.012$) and flank pains (control 24.9% [80/321] vs case 35.1% [122/348], $p=0.005$) compared to the controls (Table 5.22).

Table 5.22. Symptoms of potential infectious diseases and adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Constant feeling of needing to urinate, even after having just urinated	117	36.5%	152	43.7%	0.053	63	41.5%	0.282	89	46.1%	0.029
Missing	1	0.3%	2	0.6%		1	0.7%		1	0.5%	
Pain or burning while urinating, or straight afterwards	43	13.4%	62	17.8%	0.121	25	16.5%	0.376	37	19.2%	0.086
Missing	2	0.6%	1	0.3%		1	0.3%		0	0.0%	
Pain in the lower belly, behind the front of the pelvis	73	22.7%	109	31.3%	0.012	45	29.6%	0.105	64	33.2%	0.009
Missing	4	1.3%	5	1.4%		2	1.3%		3	1.6%	
Cloudy or bloody urine	29	9.0%	37	10.6%	0.507	9	5.9%	0.231	28	14.5%	0.057
Missing	4	1.3%	2	0.6%		0	0.0%		2	1.0%	
Fever, feeling very hot and sweating	31	9.7%	57	16.4%	0.012	24	15.8%	0.060	33	17.1%	0.015
Missing	5	1.6%	1	0.3%		0	0.0%		1	0.5%	
Feeling very sick or weak	51	15.9%	96	27.6%	<0.0001	36	23.7%	0.039	60	31.1%	<0.0001
Missing	3	0.9%	3	0.9%		2	1.3%		1	0.5%	
Flank pain (in one or both sides)	80	24.9%	122	35.1%	0.005	58	38.2%	0.003	63	32.6%	0.065
Missing	4	1.3%	3	0.9%		2	1.3%		1	0.5%	
Repeated vomiting requiring medical treatment	31	9.7%	45	12.9%	0.184	21	13.8%	0.168	24	12.4%	0.338
Missing	4	1.3%	5	1.2%		3	2.0%		1	0.5%	
Chills, rigours, or shivering persistently	10	3.1%	24	6.9%	0.028	12	7.9%	0.022	12	6.2%	0.099
Missing	4	1.3%	1	0.3%		1	0.7%		0	0.0%	
Having a rash	16	5.0%	20	5.8%	0.671	8	5.3%	0.908	12	6.2%	0.555
Missing	4	1.3%	3	0.9%		1	0.7%		2	1.0%	

(c) Chronic diseases

The prevalence of anaemia defined as Hb<100g/L based on antenatal records and hospital records was 12.4%. There was no statistical difference in the proportion of anaemic mothers in each adverse pregnancy outcome (control 11.2% [36/321] vs case 13.5% [47/348], p=0,279) (Table 5.23). More mothers in adverse outcome groups were missing information on Hb level (control 0.6% [2/321] vs case 4.3% [15/348]).

The prevalence of diabetes based on medical records was 1.1%. There was no statistical difference in the proportion of diabetic mothers in each adverse pregnancy outcome (control 1.6% [5/321] vs case 0.6% [2/348], p=0.275) (Table 5.23).

Table 5.23. Selected chronic diseases (anaemia and diabetes) and adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Anaemia	36	11.2%	47	13.5%	0.279	20	13.2%	0.474	27	14.0%	0.270
Missing	2	0.6%	15	4.3%		5	3.3%		9	4.7%	
Diabetes	5	1.6%	2	0.6%	0.275	0	0.0%	0.183	2	1.0%	>0.999
Missing	6	1.9%	16	4.6%		5	3.3%		11	5.7%	

(d) Obstetric history (previous preterm, previous history, pregnancy interval, parity)

In Bhutan, termination of pregnancy at the mother's request is illegal. The prevalence of previous history of a LBW infant was statistically significantly higher in the adverse birth outcome groups (p<0.0001) (Table 5.24). Similarly, previous admission for gestational hypertension, pre-eclampsia, eclampsia was significantly higher among mothers with PTB babies (p=0.013) but not among mothers with term LBW babies.

Pregnancy intervals could be subject to a recall bias. Although the questionnaire asked the month that the last pregnancy ended, more than 40% of the mothers (42.78%) did not remember the month. Thus, difference between the year of last pregnancy and the year of the most recent delivery was used to calculate pregnancy intervals when mothers were missing the month of last pregnancy. Mean pregnancy interval was 4 years (mean=4.4, SD=3.5, 95% CI: 4.0-4.8).

Table 5.24. Obstetric history and adverse birth outcomes.

Selected obstetric history		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
		N	%	N	%	P-value	N	%	P-value	N	%	P-value
Parity	0	131	40.8%	165	47.4%	0.032	71	46.7%	0.166	93	48.2%	0.058
	1	100	31.2%	83	23.9%		38	25.0%		44	22.8%	
	2 to 3	80	24.9%	78	22.1%		34	22.4%		43	22.3%	
	4 or more	9	2.8%	20	5.8%		9	5.9%		11	5.7%	
	Missing	1	0.3%	3	0.9%		0	0.0%		2	1.0%	
Pregnancy intervals for the mothers	less than 12 months	10	5.2%	12	6.2%	0.445	4	4.6%	0.097	8	7.7%	0.818
	12 to <18 months	10	5.2%	9	4.6%		4	4.6%		5	4.8%	
	18 to <24 months	4	2.1%	3	1.6%		0	0.0%		3	2.9%	
	24 to <60	93	47.9%	76	39.2%		32	36.4%		44	42.3%	
	60 months or more	54	27.8%	68	35.1%		38	43.2%		30	28.9%	
	Missing	23	11.9%	26	13.4%		10	11.4%		14	13.5%	
Abortions	0	291	90.7%	305	87.6%	0.226	132	86.8%	0.155	172	89.1%	0.532
	1	26	8.1%	33	9.5%		15	9.9%		17	8.8%	
	2 or more	3	0.9%	9	2.6%		5	3.3%		4	2.1%	
	Missing	1	0.3%	1	0.3%		0	0.0%		0	0.0%	
	Previous stillbirth or neonate loss	Yes	17	5.3%	25		7.2%	0.310		9	5.9%	
Missing	1	0.3%	2	0.6%	0	0.0%	1		0.5%			
Previous history of PTB	Yes	14	4.4%	38	10.9%	0.002	13	8.6%	0.106	25	13.0%	<0.0001
	Missing	6	1.9%	7	2.0%		3	2.0%		2	1.0%	
Previous history of LBW	Yes	11	3.4%	41	11.8%	<0.0001	36	11.8%	0.017	27	14.0%	<0.0001
	Missing	6	1.9%	6	1.7%		6	2.0%		2	1.0%	
Last pregnancy: admission for hypertension, pre-eclampsia, or eclampsia	Yes	1	0.3%	6	1.7%	0.125	0	0.0%	>0.999	6	3.1%	0.013
	Missing	7	2.2%	8	2.3%		2	1.3%		5	2.6%	

(e) Pregnancy induced hypertension (PIH)

More mothers in the case group had hypertensive disorders (pre-existing or chronic hypertension, gestational hypertension, preeclampsia and eclampsia) compared to the control group (pre-existing or chronic hypertension: control 2.2% [7/321] vs case 7.8% [27/348]; gestational hypertension: control 3.4% [11/321] vs case 13.2% [46/348]; pre-eclampsia control 1.3% [4/321] vs case 8.3% [29/348]; and eclampsia: control 0.0% vs 2.0% [7/348], $p < 0.0001$) (Table 5.25).

Strength of associations of different hypertensive disorders and adverse birth outcomes are further examined in the multivariable analysis.

Table 5.25. Hypertensive disorders and adverse birth outcomes.

Hypertensive disorders	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
No hypertensive complications	285	88.8%	218	62.6%	<0.0001	106	69.7%	<0.0001	111	57.5%	<0.0001
Pre-existing or chronic hypertension	7	2.2%	27	7.8%		11	7.2%		16	8.3%	
Gestational hypertension	11	3.4%	46	13.2%		23	15.1%		23	11.9%	
Pre-eclampsia	4	1.3%	29	8.3%		6	4.0%		23	11.9%	
Eclampsia	0	0.0%	7	2.0%		0	0.0%		7	3.6%	
Missing	14	4.4%	21	6.0%		6	4.0%		13	6.7%	

(f) A summary of key findings from analysis of general health, infectious or chronic diseases

Data suggested an association between gestational weight gain and adverse outcomes. Recorded positives for HIV, syphilis, and hepatitis B in the study participants were fairly low and comparable to other studies in Bhutan. Data suggested an association between UTI and other symptoms of potential infectious diseases and adverse birth outcomes. Nulliparity, previous history of preterm, and LBW were statistically significantly associated with adverse birth outcomes. Chronic and pregnancy-induced hypertension was strongly associated with adverse birth outcomes. These factors are further examined in the multivariable analysis in the statistical approach.

5.4.4 Mode of delivery

More mothers in the case group delivered by caesarean section (CS) (elective and emergency) than in the control group (control 31.5% [101/321] vs case 41.4% [144/348], $p=0.001$) (Table 5.26). Nearly half (45%) of the PTB babies and 37% of the term LBW babies were delivered by CS.

About 62% of the mothers of premature babies with gestational hypertension or preeclampsia and/or eclampsia delivered their babies by CS (Figure 5.15).

Table 5.26. Mode of delivery and adverse birth outcomes.

Hypertensive disorders	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
SVD	215	67.0%	198	56.9%	0.001	93	61.2%	0.344	102	52.9%	<0.0001
CS-Elective	41	12.8%	34	9.8%		17	11.2%		17	8.8%	
CS-Emergency	60	18.7%	110	31.6%		39	25.7%		71	36.8%	
Vacuum	5	1.6%	3	0.9%		3	2.0%		0	0.0%	
Breech	0	0.0%	3	0.9%		0	0.0%		3	1.6%	

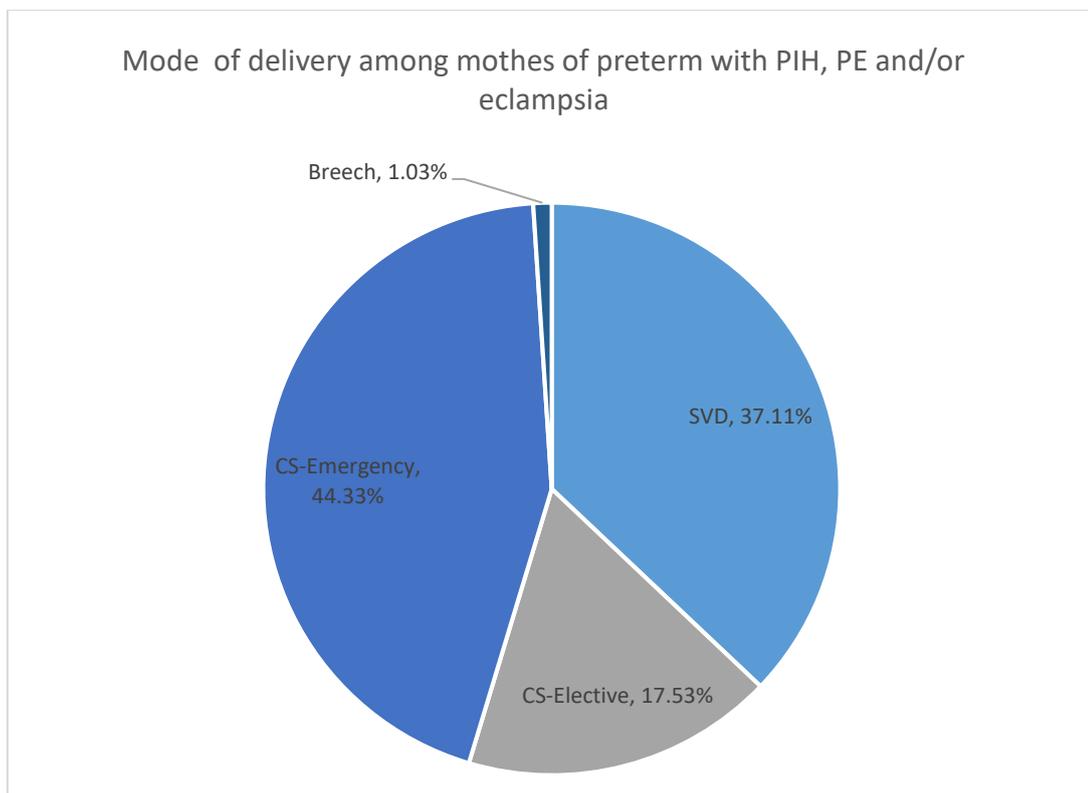


Figure 5.15 Mode of delivery among mothers of preterm babies with PIH, PE and/or eclampsia.

5.4.5 Sex of the infants

More female babies were born in the case group than in the control group (control 47.4% [152/321] vs case 55.8% [194/348], $p=0.030$) (Table 5.27). Among mothers of term LBW babies, there were more female babies than in the control group and the difference was significant (control vs term LBW 63.8% [97/152], $p=0.001$). There was no statistically significant difference between the control group and the preterm group ($p=0.323$).

Table 5.27. Sex of the infants and adverse birth outcomes.

Sex of the infants	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Male	169	52.7%	153	44.0%	0.030	55	36.2%	0.001	95	49.2%	0.323
Female	152	47.4%	194	55.8%		97	63.8%		97	50.3%	
Ambiguous	0	0.0%	1	0.3%		0	0.0%		1	0.5%	

5.4.6 Physical activity during pregnancy

In the study sample, the majority of the mothers reported having engaged in at least 10 minutes of vigorous (18% [119/669]) or moderate physical activity (81% [544/669]) through work including domestic chores and travel between places (66% [439/669]) at least once during pregnancy. In a typical day, mean hours of work-related vigorous intensity activities and moderate intensity activities were 4.6 hours and 3.4 hours respectively (Table I.2 in Appendix I). Among the mothers who engaged in vigorous activities, 34% were involved in farming.

On the other hand, engaging in recreational activities during pregnancy was not very common (Table I.1 in Appendix I). Only 4% (29/669) engaged in vigorous intensity sport, fitness, or recreational activities such as running, swimming, football, badminton, basketball or martial arts and 17% (113/669) engaged in moderate-intensity recreational activity such as brisk walking or dancing for leisure at least once during pregnancy.

Less than 10% of the mothers (8%, [51/669]) mothers reported they did not engage in any moderate or vigorous intensity activities during pregnancy and 17% of the mothers did not meet the ACOG recommendation (150 minutes physical activity per week) (Table I.3 in Appendix I).

The data did not suggest an association between level and hours of physical activity and adverse outcomes except for work-related moderate physical activity. More mothers of preterm babies reported that they engaged in work-related moderate physical activity (control 78.2 % [251/321] vs case 86.5 [167/193], $p=0.03$). Almost 25% of the mothers reported being very active while 6% reported being not active. In the case group, more mothers reported being not active compared to the control group (control 1.3% [4/321] vs case 5.2% [18/348], $p=0.03$) (Table 5.28).

Table 5.28. Self-evaluation of physical activities during pregnancy.

Self-evaluation of physical activity level	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Very active	79	24.6%	85	24.4%	0.03	34	22.4%	0.158	51	26.4%	0.02
Moderately active	171	53.3%	179	51.4%		76	50.0%		100	51.8%	
Somewhat active	62	19.3%	57	16.4%		31	20.4%		26	13.5%	
Not active	4	1.3%	18	5.2%		7	4.6%		11	5.7%	
Missing	5	1.6%	9	2.6%		4	2.6%		5	2.6%	

5.4.7 Dietary habits, fruits and vegetable consumption

WHO recommends eating at least 400 g, or five portions, of fruit and vegetables per day to reduce the risk of non-communicable diseases, and help ensure an adequate daily intake of dietary fibre. In addition, WHO recommends limiting salt, oil and sugar [2].

Traditionally, Bhutanese food often contains green or red chilli; salt, butter and oil and are taken with one or two plates of rice while small portions of green and yellow vegetables and fruits are also served.

The number of cups of daily green and yellow vegetables and fruits, and intake and frequency of common sources of salt, oil, sugar were described.

On average, the study sample mothers consumed two servings of green vegetables (mean 1.95, SD=2.2, 95% CI 1.78 - 2.12) every day. Similarly, the mean number of fruit servings per day was 1.8 (mean 1.77, SD=1.7, 95% CI 1.63-1.90). There was no difference in the means of vegetable or fruit intake across mothers with different pregnancy outcomes (vegetable $p=0.82$; and fruits $p=0.58$, table not presented). Vegetable oil was the most commonly used oil for cooking (99%). On average, the mothers consumed roughly 40 ml of oil in total for cooking per day including vegetable oil and butter (mean 39.18 g, SD=23, 95% CI 37.41 - 40.95). There was no difference in the means of total oil intake for cooking across mothers of different pregnancy outcomes ($p=0.90$, table not included).

Seventy seven percent of mothers did not have the recommended five servings of fruits and/or vegetables a day. This is comparable to the results from the 2014 STEPS survey which reported that 70% of the women aged between 18 and 69 years old ate less than five servings of fruits and/or vegetables, thus not meeting the WHO recommendation [2].

Bhutanese traditional “Ezay” (chilli pickles with cheese and salt) and “Suja” (butter tea with salt) are common sources of salt (Figure.I.4 in Appendix I). In total, 74% of the mothers consumed ezay at least once a week during pregnancy while 16% consumed it daily. Also, 24% of the mothers consumed suja at least once a week and 4% consumed it daily. About 23% consumed dried meat at least once a week. There were no differences in the frequency of ezay, suja, or dried meat consumption across mothers of different pregnancy outcomes (ezay $p=0.134$; suja $p=0.157$; and dry meat $p=0.260$, table not presented).

The majority (74%) of the pregnant women consumed milk tea with sugar at least once a week and 30% consumed it daily. Coffee was consumed less than milk tea during pregnancy; less than 26% drank coffee during pregnancy and 6% of the mothers consumed coffee at least once a week. There were no differences in the frequency of milk tea and coffee consumption across the mothers of different pregnancy outcomes (milk tea $p=0.594$; and coffee $p=0.613$). In terms of caffeine from tea and coffee, the daily recommended maximum is 200 mg of caffeine, equivalent to two mugs or four cups of coffee [7]. Unfortunately, this study did not include information on daily caffeine intake. Therefore, examining the amount of caffeine and adverse pregnancy outcomes is beyond the scope of this study.

On average, pregnant women had meals three times a day (mean 3.1, SD=0.6, 95% CI 3.1-3.2) and snacks twice a day (mean 1.7, SD=1.0, 95% CI 1.7-1.8). The mothers of LBW, PTB or SGA neonates reported slightly fewer meals per day and mean differences were statistically significant at 5% level of significance (control 3.2 times [SD=0.6] vs case 3.0 times [SD=0.5], $p=0.0001$) (Table 5.29).

Likewise, the proportion of mothers who reported having poor appetite during pregnancy was slightly higher among the mothers of LBW, PTB or SGA babies (control 2% [5/321] vs 7% [22/348], $p<0.0001$) (Table 5.30).

Table 5.29. Mean frequency of meals and snacks during pregnancy.

Frequency of meals and snacks during pregnancy	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
# of meals	319	3.2 (0.6)	347	3.0 (0.5)	0.0001	151	3.1 (0.5)	0.0061	193	3.0 (0.5)	0.0001
# of snacks	309	1.8 (1.0)	332	1.7 (1.1)	0.277	140	1.7 (1.1)	0.4319	189	1.7 (1.0)	0.395

Table 5.30. Self-evaluation of appetite during pregnancy.

Self-evaluation of appetite during pregnancy	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Good	260	81%	185	60%	<0.0001	93	61%	<0.0001	118	61%	<0.0001
Fair	53	17%	90	29%		47	31%		55	29%	
Poor	5	2%	22	7%		12	8%		11	6%	
Missing	3	1%	9	5%		0	0%		9	5%	

5.5 Mother's subjective assessment of the baby's size

Although birth weight is the most reliable and widely-reported measure to assess size at birth, not all infants are weighed at birth globally, especially when they are not delivered in health facilities. When infants are not weighed at birth, mother's recall is often used to assess LBW in order to estimate the percentage of LBW infants in Demographic and Health Surveys (DHS) and other surveys. In the present study, mother's subjective concept of the baby's size was explored in relation to birth weight and gestational age (Figure 5.16 and Table 5.31). When both cases and controls were averaged, the categories seem to match up well with the mean birthweight if the extreme large category was excluded. However, among mothers of LBW neonates, more than 38% assessed their babies as average or larger (Table 5.32). Ten mothers of preterm babies did not answer this question as the baby had been taken to the NICU directly and mothers had not seen the baby at the time of the interview. The mean birth weight was 1450 g (95% CI: 1109 g -1791 g) and mean gestational age for these babies was 221 days (95% CI: 207- 234) (Table 5.31). The results suggest that the current survey-based estimates of the prevalence of LBW could be underestimated.

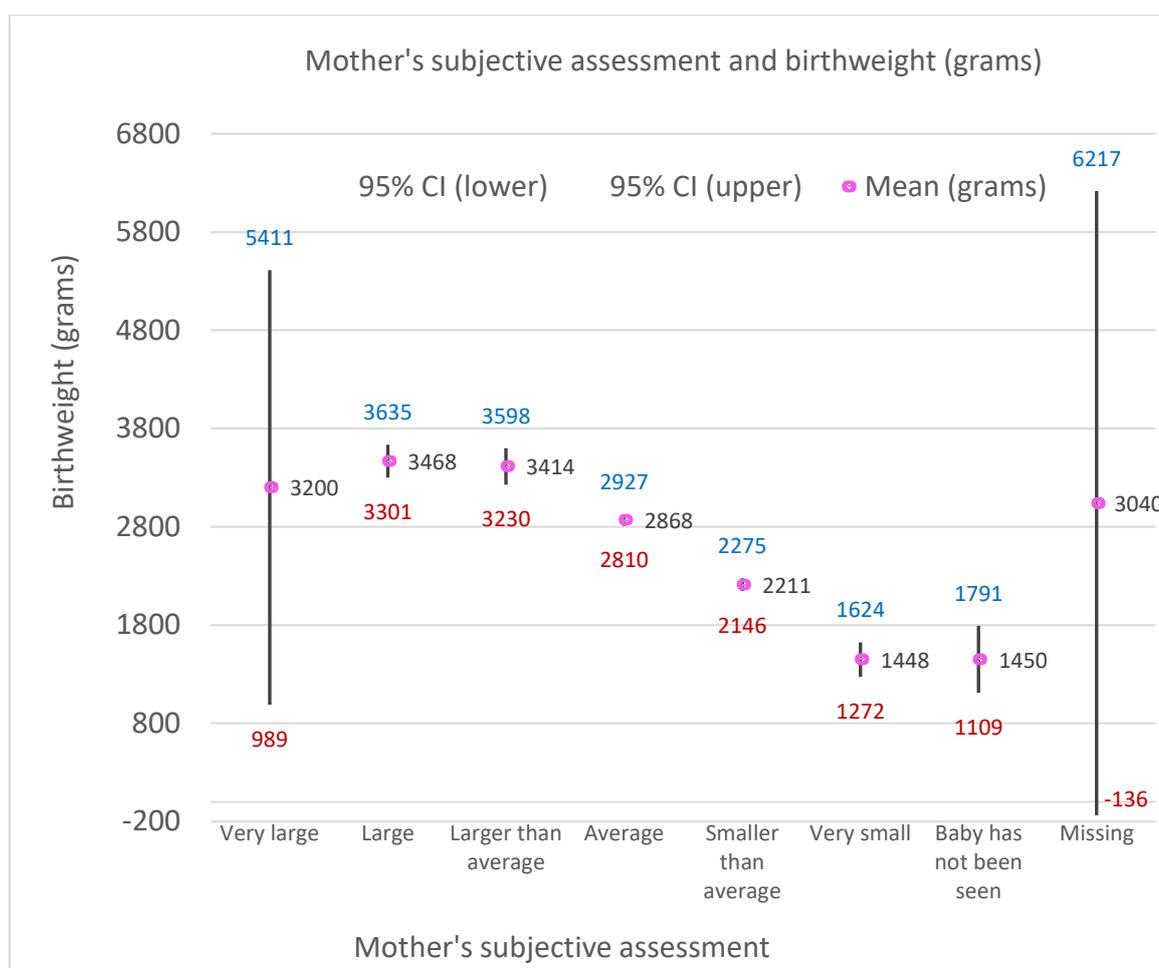


Figure 5.16. Mother's subjective assessment and birth weight (grams).

Table 5.31. Distribution of mother’s subjective assessment.

Mother’s subjective assessment	Total (n=669)		Gestational age (days)	Birth weight (grams)
	N	%	Mean (SD, 95% CI)	Mean (SD, 95% CI)
Very large	3	0.45%	279 (17, 236-321)	3200 (890, 989-5411)
Large	47	7.03%	278 (9, 276-281)	3468 (570, 3301-3635)
Larger than average	32	4.78%	279 (10, 275-282)	3414 (511, 3230-3598)
Average	354	52.91%	271 (18, 269-273)	2868 (559, 2810-2927)
Smaller than average	179	26.91%	260 (20, 257-263)	2211 (440, 2146-2275)
Very small	39	6.13%	228 (31, 218-238)	1448 (559, 1272-1624)
Baby has not been seen	10	1.49%	221 (19, 207-234)	1450 (477, 1109-1791)
Missing	2	0.3%	287 (4, 255-318)	3040 (356, -136-6217)

Table 5.32. Mother’s subjective assessment and adverse birth outcomes.

Mother’s subjective assessment	Control (n=321)		Term LBW (n=152)		Preterm (n=193)	
	N	%	N	%	N	%
Very large	2	0.6%	1	0.7%	0	0.0%
Large	41	12.8%	4	2.6%	2	1.0%
Larger than average	30	9.4%	1	0.7%	1	0.5%
Average	225	70.1%	53	34.9%	76	39.4%
Smaller than average	20	6.2%	86	56.6%	73	37.8%
Very small	1	0.3%	7	4.6%	31	16.1%
Baby has not been seen	0	0.0%	0	0.0%	10	5.2%
Missing	2	0.6%	0	0.0%	0	0.0%

5.6 Summary

Descriptive analyses suggest an association between adverse birth outcomes and the following variables: age of the mother, nulliparity, sex of the infant, urinary tract infection, hypertensive disorders, previous history of PTB, previous history of LBW, gestational weight gain, number of meals per day, number of ANC visits, mode of transportation to the hospital, and mode of delivery. Data suggest weak or no evidence of association between adverse birth outcomes and socio-economic factors such as education, ethnicity, wealth quintile, and urban residence. Data did not suggest sufficient evidence for an association between adverse birth outcomes and difference in altitudes between permanent and current gewogs. These findings will inform the multivariable regression analysis. Patterns and prevalence of betel quid chewing, tobacco and alcohol are described in Chapter 6.

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Chapter 6

Results of descriptive analysis of maternal betel quid chewing, tobacco, and alcohol

In the previous chapter, descriptive analyses of the study population and selected maternal and infant characteristics were provided. In this chapter, betel quid and packaged betel products (6.1), cigarette and smokeless tobacco (6.2), and alcohol consumption (6.3) are examined in detail to identify patterns of use during pregnancy and investigate the effects on birth weight and gestational age. Descriptive analyses were conducted to understand the patterns of consumption, followed by quantification of consumption. The chapter ends with a summary.

6.1 Betel quid chewing and packaged betel products

6.1.1 Patterns of betel nut chewing during pregnancy among study participants

(i) Prevalence

Among 669 mothers who participated in the study, 471/669 (70.40%) reported chewing betel quid at least once in their lifetime and 359 mothers (53.7%) chewed betel quid during pregnancy. The observed difference of prevalence between the cases and controls was small and not statistically significant (control 52.3% [168/321] vs case 54.9% [191/348], $p=0.485$) (Table 6.2).

Most commonly, mothers preferred to chew after meals (69.9%) (Figure 6.1) and when they felt cold (54.5%) (Figure 6.2). The mean starting age was 18 years old (95% CI: 17.6-18.6) and the mean duration of betel chewing is 9.5 years (95% CI: 8.8-10.2) (Table 6.1). Comparing the mothers of LBW and/or PTB babies to the control mothers, the mean duration of betel chewing was slightly longer (10.2 vs 8.7, $p=0.04$). Betel quid chewing during pregnancy was more common among the mothers who delivered at JDWNRH (59.6%) than CRRH (32.7%) and ERRH (48.3%) ($p<0.001$) (Table 6.4). The number of betel quids consumed during the last three months of pregnancy was higher at JDWNRH (mean 380, SD=677) than CRRH (mean 102, SD=151) and ERRH (mean 122, SD=234) (Figure 6.3).

(ii) Pattern of betel quid chewing and adverse birth outcomes

The majority of the mothers chewed a combination of piper leaf (always: 87.5% vs never: 4.5%), lime (always: 87.5% vs never: 7.0%), and one quarter of a ripe nut, and spit after chewing (always spit: 47.1% vs never-spit: 18.9%) (Table 6.3). Of the mothers who chewed during pregnancy ($n=359$), 40.7% (146/359) were daily users. The majority (76.3%) used weekly or more than weekly. Only 10.9% of the total chewers during pregnancy added tobacco to betel nut chewing. More mothers in the case group added tobacco compared to the mothers in the control group (always add tobacco, control 6.0% [10/168] vs case 13.1% [25/168], $p=0.045$; control vs term LBW 10.5% [9/86], $p=0.111$; and PTB 15.4% [16/104], $p=0.027$).

(iii) Quantity

On average, mothers consumed 5.5 quids (95% CI: 4.7-6.3) or approximately 1.4 nuts per day and cumulative consumption during the last three months of pregnancy was 324 quids (95% CI: 252 – 396) or approximately 81 nuts among the mothers who chewed betel quid during pregnancy (Table 6.3). The majority of the mothers continued chewing throughout pregnancy (Figure 6.6).

(iv) Comparison of chewers and non-chewers

In the descriptive analyses comparing chewers and non-chewers, there was no statistically significant difference in education or wealth index by status of betel quid chewing (Table 6.4). On the other hand, more mothers who chewed betel quid used other substance such as alcohol (Non-chewers: 17.6% [54/307] vs chewers: 34.8% [125/359], $p<0.0001$), pan masala (Non-chewers: 11.1% [34/307] vs chewers: 32.3% [116/359], $p<0.0001$), cigarettes (Non-chewers: 1.3% [4/307] vs chewers: 4.2% [15/359], $p=0.034$), and smokeless tobacco (Non-chewers: 3.3% [10/307] vs chewers: 10.9% [39/359], $p<0.0001$) compared to the mothers who did not chew during pregnancy. More mothers who chewed betel quid were anaemic compared to non-chewers (non-chewers: 8.1% [25/168] vs chewers 16.2% [58/168], $p=0.004$). The daily vegetable intake among betel quid chewers was lower compared to non-chewers (non-chewers: 1.7[SD=1.8] vs chewers: 2.2[SD=2.5], $p=0.004$).

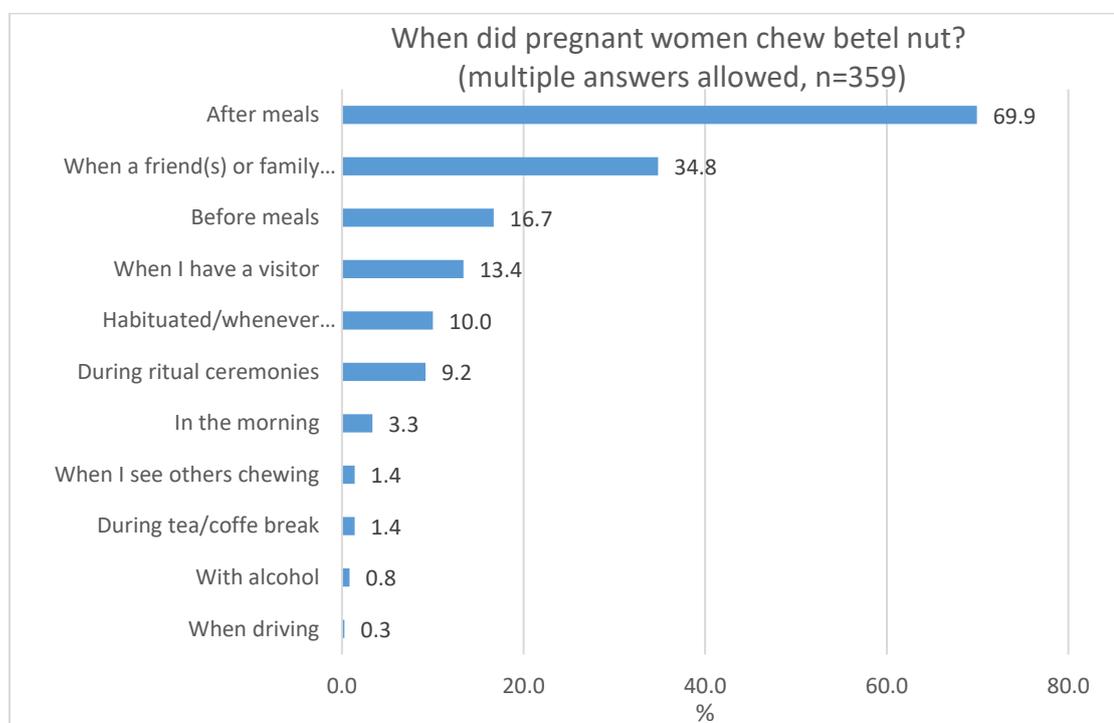


Figure 6.1. The timing of chewing betel quid during pregnancy.

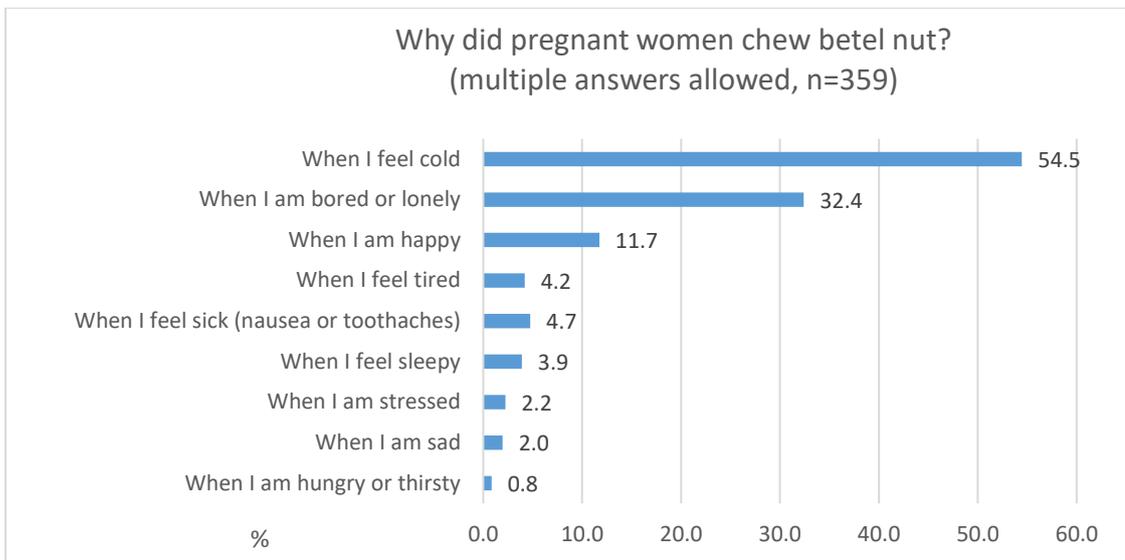


Figure 6.2. The reasons for chewing betel quid during pregnancy.

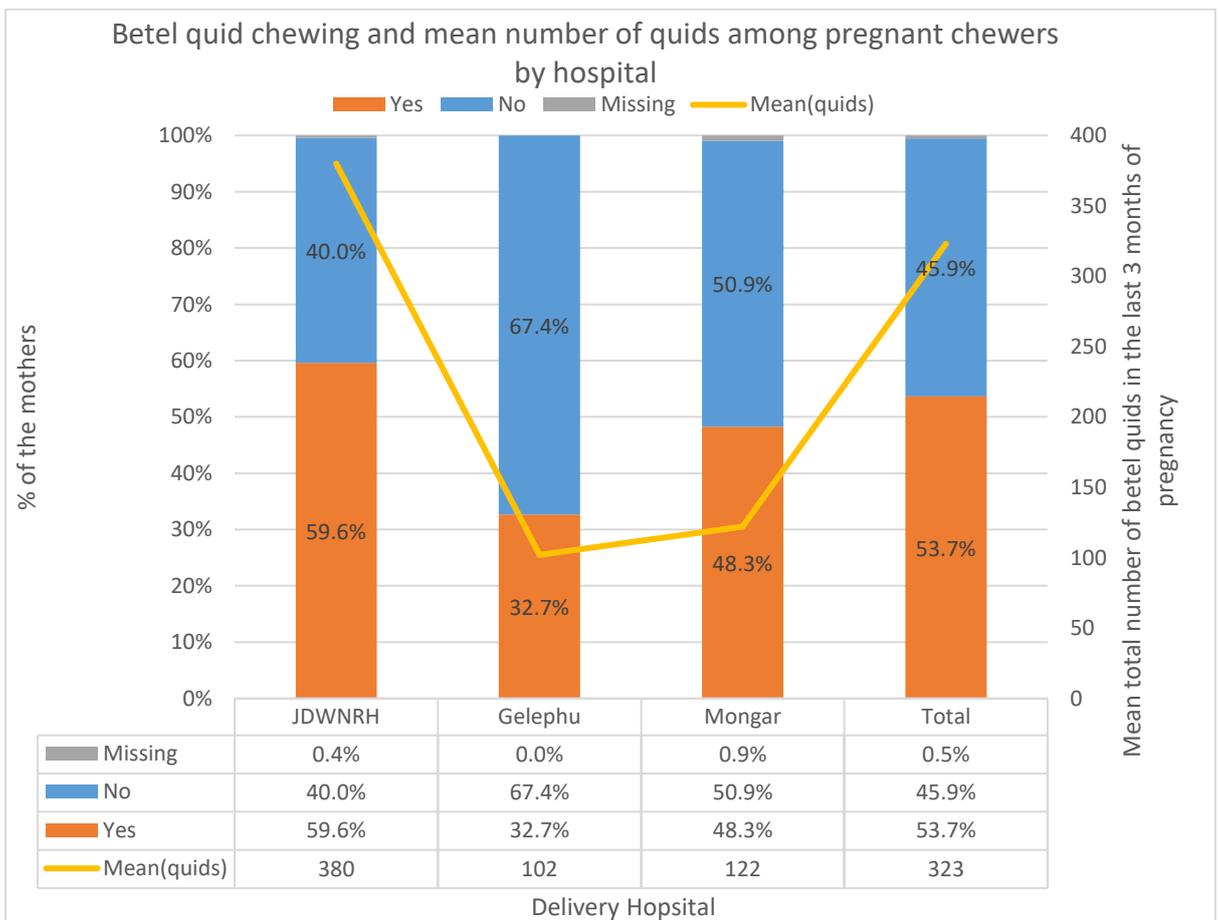


Figure 6.3. Betel quid chewing during pregnancy and mean total number of betel quids among pregnant chewers by delivery hospital.

Table 6.1. Mean age of starting betel quid chewing, duration of consumption and amount of consumption (per day and cumulative for the last three month of pregnancy).

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Age of starting BQ	212	18.4 (5.5)	244	17.9 (5.6)	0.311	107	18.2 (5.5)	0.8314	135	17.5 (5.6)	0.1644
Years of chewing	160	8.7 (5.7)	184	10.2 (7.2)	0.0352	84	10.0 (6.9)	0.1505	99	10.4 (7.46)	0.0564
Daily consumption	144	5.4 (6.3)	160	5.6 (7.9)	0.7587	75	6.6 (9.8)	0.3356	84	4.8 (5.6)	0.5032
Cumulative consumption	134	317 (553)	134	317 (553)	0.8746	69	388 (845)	0.5297	81	282 (478)	0.6236

Table 6.2. Prevalence of BQ chewing during pregnancy.

Prevalence of BQ chewing and patterns of consumption		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
		N	%	N	%	P-value	N	%	P-value	N	%	P-value
Ever chewed BQ (n=669)	Yes	220	68.5%	251	53.3%	0.339	109	71.7%	0.513	140	72.5%	0.364
	Missing	1	0.3%	0	0.0%		0	0.0%		0	0.0%	
Age of starting (n=471)	<10 yrs old	9	4.1%	11	4.4%	0.866	5	4.6%	0.747	6	4.3%	0.362
	10-<18 yrs old	83	37.7%	101	40.2%		37	33.9%		63	45.0%	
	>=18 yrs old	120	54.6%	132	52.6%		65	59.6%		66	47.1%	
	Missing	8	3.6%	7	2.8%		2	1.8%		5	3.6%	
Chewed during pregnancy (n=669)	Yes	168	52.3%	191	54.9%	0.485	86	56.6%	0.406	104	53.9%	0.669
	Missing	1	0.3%	2	0.6%		0	0.0%		2	1.0%	

Table 6.3. Prevalence of BQ chewing and patterns of consumption during pregnancy.

Prevalence of BQ chewing and patterns of consumption		Control (n=168)		Case (n=191)			Term LBW (n=86)			Preterm (n=104)		
		N	%	N	%	P-value	N	%	P-value	N	%	P-value
Frequency of chewing during pregnancy (n=359)	Daily	70	41.7%	76	39.8%	0.726	32	37.2%	0.773	44	42.3%	0.693
	Weekly or more	60	35.7%	68	35.6%		29	33.7%		39	37.5%	
	Monthly or more	31	18.5%	36	18.9%		19	22.1%		16	15.4%	
	Rarely	4	2.4%	8	4.2%		4	4.7%		4	3.9%	
	Other (not specified)	3	1.8%	1	0.5%		1	1.2%		0	0.0%	
	Missing	0	0.0%	2	1.1%		1	1.2%		1	1.0%	
Add tobacco (n=359)	Always	10	6.0%	25	13.1%	0.045	9	10.5%	0.111	16	15.4%	0.027
	Usually	0	0.0%	1	0.5%		1	1.2%		0	0.0%	
	Sometimes	1	0.6%	2	1.1%		2	2.3%		0	0.0%	
	Never	156	92.9%	162	84.8%		74	86.1%		87	83.7%	
	Missing	1	0.6%	1	0.5%		0	0.0%		1	1.0%	
Add slaked lime (n=359)	Always	144	85.7%	170	89.0%	0.015	78	90.7%	0.382	91	87.5%	0.005
	Usually	6	3.6%	1	0.5%		1	1.2%		0	0.0%	
	Sometimes	1	0.6%	7	3.7%		1	1.2%		6	5.8%	
	Rarely	0	0.0%	2	1.1%		1	1.2%		1	1.0%	
	Never	15	8.9%	10	5.2%		5	5.8%		5	4.8%	
	Missing	2	1.2%	1	0.5%		0	0.0%		1	1.0%	
Add piper leaf (n=359)	Always	143	85.1%	171	89.5%	0.044	79	91.9%	0.372	91	87.5%	0.180
	Usually	7	4.2%	0	0.0%		0	0.0%		0	0.0%	
	Sometimes	5	3.0%	8	4.2%		2	2.3%		6	5.8%	
	Rarely	4	2.4%	4	2.1%		2	2.3%		2	1.9%	
	Never	9	5.4%	7	3.7%		3	3.5%		4	3.9%	
	Missing	0	0.0%	1	0.5%		0	0.0%		1	1.0%	

Spit after chewing (n=359)	Always	76	45.2%	93	48.7%	0.172	43	50.0%	0.119	50	48.1%	0.788
	Usually	13	7.7%	5	2.6%		1	1.2%		4	3.9%	
	Sometimes	39	23.2%	53	27.8%		26	30.2%		26	25.0%	
	Rarely	6	3.6%	4	2.1%		1	1.2%		3	2.9%	
	Never	33	19.6%	35	18.3%		15	17.4%		20	19.2%	
	Missing	1	0.6%	1	0.5%		0	0.0%		1	1.0%	
Duration of BQ chewing (n=359)	0 to 5 years	57	33.9%	57.0	29.8%	0.103	28	32.6%	0.270	29	27.9%	0.125
	5< to 10 years	52	31.0%	48.0	25.1%		21	24.4%		26	25.0%	
	>10 yrs	51	30.4%	79.0	41.4%		35	40.7%		44	42.3%	
	Missing	8	4.8%	7.0	3.7%		2	2.3%		5	4.8%	
Daily number of quids of chewing (n=359)	>20	7	4.2%	9	4.7%	0.892	6	7.0%	0.632	3	2.9%	0.738
	11 to 20	11	6.6%	14	7.3%		5	5.8%		9	8.7%	
	1 to 10	126	75.0%	137	71.7%		64	74.4%		72	69.2%	
	Missing	24	14.3%	31	16.2%		11	12.8%		20	19.2%	
Cumulative consumption during the last three months of pregnancy (n=359)	Stopped	10	6.0%	10	5.2%	0.745	4	4.7%	0.723	6	5.8%	0.640
	<=total 280 quid or <=1 nut per day	85	50.6%	105	55.0%		47	54.7%		57	54.8%	
	280 < to <=1800 quids or 1-5 nuts per day	34	20.2%	31	16.2%		14	16.3%		17	16.4%	
	1800 quids+ or more than 5 nuts per day	5	3.0%	5	2.6%		4	4.7%		1	1.0%	
	Missing	34	20.2%	40	20.9%		17	19.8%		23	22.12%	

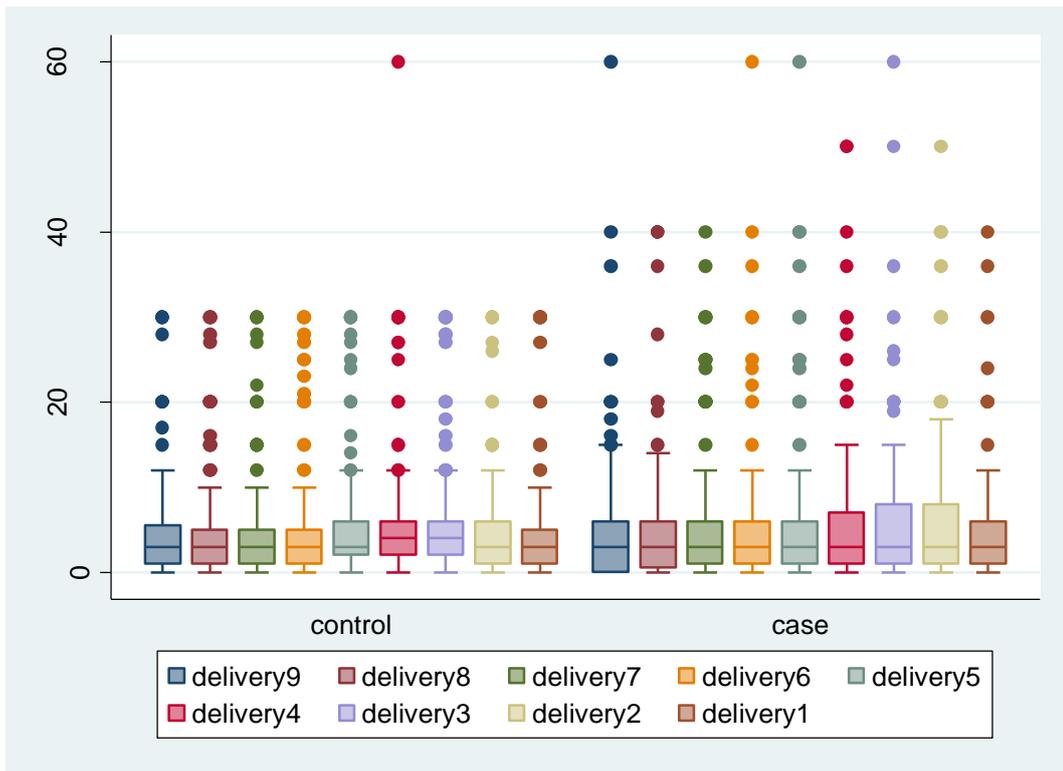


Figure 6.4. The daily consumption during pregnancy for controls and cases. Delivery # represents # of month from the month of delivery (n=359, 53 missing details of daily consumption).

Table 6.4. Baseline characteristics by status of betel quid chewing during pregnancy (n=666).

Selected characteristics		Betel quid non-users (n=307)	Betel quid users (n=359)	P-value
Delivery hospital (%)	JDWRH	182 (59.3%)	271 (75.5%)	<0.0001
	Gelephu (CRRH)	66 (21.5%)	32 (8.9%)	
	Mongar (ERRH)	59 (19.2%)	56 (15.6%)	
Season of delivery (%)	Spring	75 (24.43%)	112 (31.2%)	0.076
	Summer	85 (27.7%)	79 (22.0%)	
	Fall	79 (25.7%)	77 (21.5%)	
	Winter	68 (22.2%)	91 (25.4%)	
Maternal age, mean (S.D.)		27.5 (6.1)	27.5 (5.6)	0.890
Maternal education level (%)	"No school or NFE"	106 (34.5%)	108 (30.1%)	0.424
	Primary or middle secondary	44 (14.3%)	60 (16.7%)	
	High school/Masters/college/diploma	157 (51.1%)	190 (52.9%)	
	Missing	0 (0.0%)	1 (0.3%)	
Wealth Quintile (%)	Poorest	62 (20.2%)	72 (20.1%)	0.518
	Second	68 (22.2%)	62 (17.3%)	
	Middle	61 (19.9%)	72 (20.1%)	
	Fourth	58 (18.9%)	75 (20.9%)	
	Richest	55 (17.9%)	76 (21.2%)	
	Missing	3 (1.0%)	2 (0.6%)	
Maternal pre-pregnancy BMI, mean (S.D.)		22.8 (3.6)	23.2 (3.9)	0.270
Gestational weight gain as per IOM recommendation, number (%)	Within recommendations	59 (19.2%)	57 (15.9%)	0.309
	High GWG	31 (10.1%)	47 (13.1%)	
	Low GWG	129 (42.0%)	144 (40.1%)	

	Missing	88 (28.7%)	111 (30.9%)	
Alcohol drinking during pregnancy, number (%)	Yes	54 (17.6%)	125 (34.8%)	<0.0001
	Total number of days of drinking in the last 3 months of pregnancy (n=661), mean(S.D.)	3 (12.9)	5 (13.6)	
Cigarette smoking during pregnancy, number (%)	Yes	4 (1.3%)	15 (4.2%)	0.034
	Total number of cigarettes in the last 3 months(n=665), mean(S.D.)	2 (26)	1 (14)	
Smokeless Tobacco during pregnancy, number (%)	Yes	10 (3.3%)	39 (10.9%)	<0.0001
	Missing	5 (1.6%)	3 (0.8%)	
	Total ST in grams in the last 3 months of pregnancy (n=652), mean(S.D.)	4.2 (33.3)	19.1 (73.0)	
Pan masala during pregnancy, number (%)	Yes	34 (11.1%)	116 (32.3%)	<0.0001
	Missing	5 (1.6%)	5 (1.4%)	
Anaemia (recorded), number (%)	Yes	25 (8.1%)	58 (16.2%)	0.004
	Unknown	13 (4.2%)	2 (0.6%)	
	Missing	1 (0.3%)	1 (0.3%)	
Hypertensive disorders, number (%)	Chronic or existing hypertension	18 (5.9%)	16 (4.5%)	0.518
	Gestational hypertension	23 (7.5%)	34 (9.5%)	
	Pre-eclampsia	11 (3.6%)	21 (5.9%)	
	Eclampsia	3 (1.0%)	4 (1.1%)	
	Missing	18 (5.9%)	17 (4.7%)	
Daily cup of vegetables (n=613), mean (S.D.)		2.2 (2.5)	1.7 (1.8)	0.004
Daily cup of fruits (n=595), mean (S.D.)		1.8 (1.7)	1.7 (1.7)	0.596
Number of meals per day (n=663), mean (S.D.)		3.1(0.5)	3.1(0.6)	0.307
Sex of the infants, male (%)		154 (50.2%)	168 (46.8%)	0.482

6.1.2 Packaged betel nut products - Pan masala (PM)

Pan masala (PM) refers to packaged betel nut products which are available under several names, mostly marketed as a mouth refresher. About 22% or 150/669 of the mothers used pan masala during pregnancy (Figure 6.6). Mothers who used packaged betel products also chewed betel quid (77% [116/150]). Use of packaged betel products during pregnancy was more common among the mothers who delivered at JDWNRH (26.1%) compared to CRRH (19.2%) and ERRH (12.4%) ($p=0.005$) (Figure 6.5).

There was no statistical difference in the proportion in different adverse outcome groups (Table 6.5).

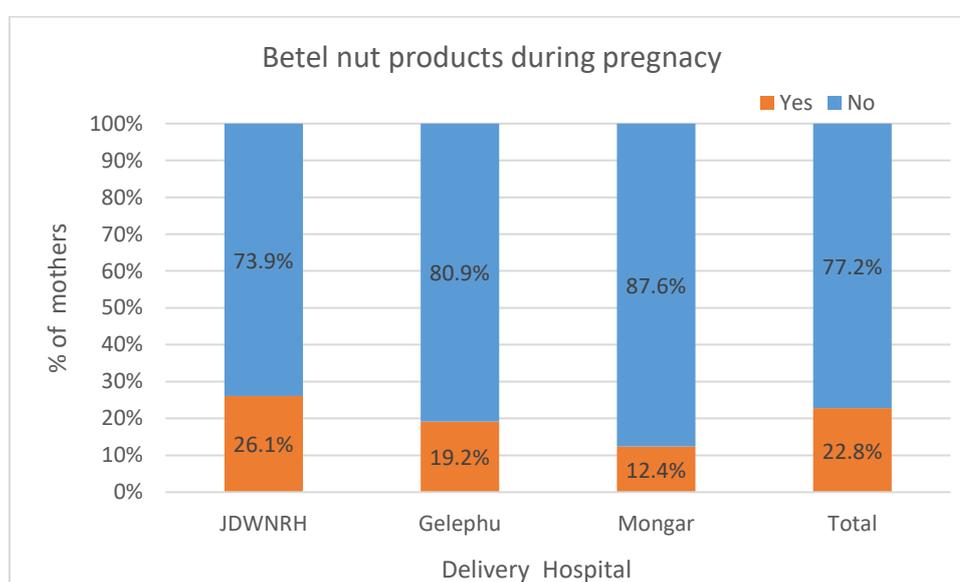


Figure 6.5. BQ products by delivery hospital.

Table 6.5. Use of BQ products and adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	%	N	%	P-value	N	%	P-value	N	%	P-value
Pan masala during pregnancy	72	22.4%	78	22.4%	0.989	31	20.4%	0.631	47	24.4%	0.639
Missing	5	1.44%	5	1.44%		3	2.0%		2	1.0%	

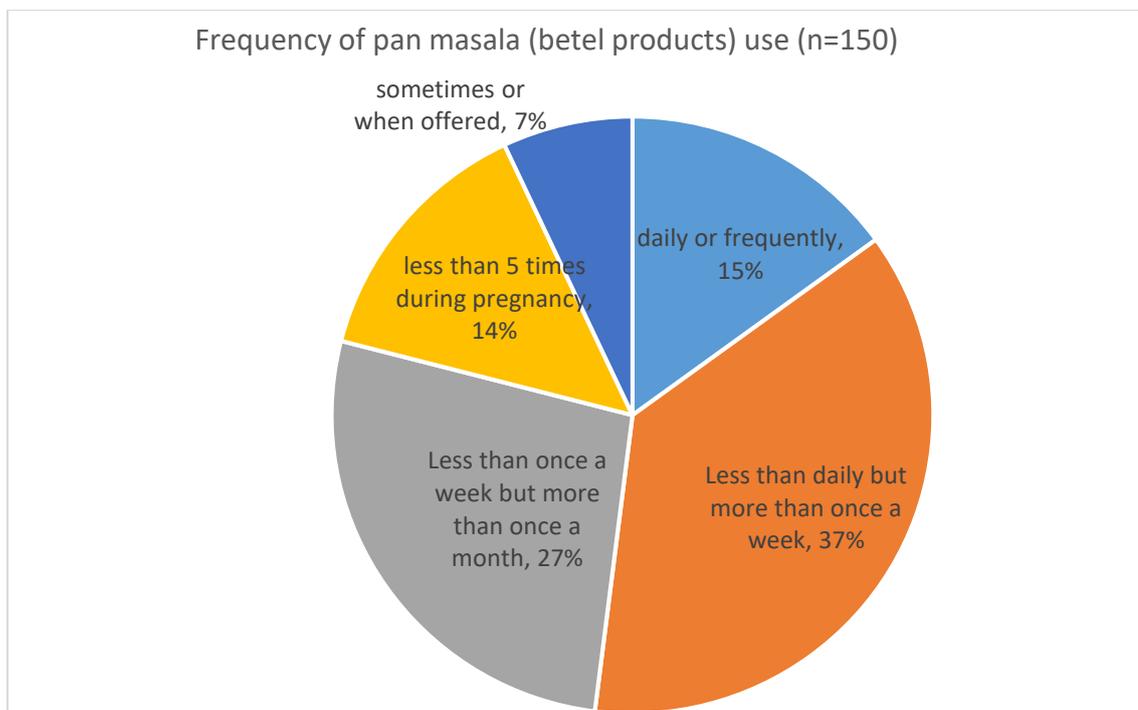


Figure 6.6. Frequency of BQ product use among study participants.

6.1.3 A summary of key findings from analysis of betel nut and betel product use

Betel quid chewing or use of betel products such as pan masala during pregnancy was highly prevalent among study participants, accounting for 59% of the total study participants. The majority (77%) of the mothers who used packaged betel products chewed betel quid. It was more common among the mothers who delivered at JDWNRH, located in the western part of the country compared to the Eastern or Southern Region Referral Hospitals. However, the amount of consumption remained low. The mothers who had chewed in the last three months of pregnancy consumed 5 quids or 1.4 nuts per day on average. Mixing tobacco with the quid was not common practice among the study population (less than 10%). The data did not suggest sufficient evidence of association between betel nut and betel nut products and adverse birth outcomes in the descriptive analysis. However, the data does suggest an association between betel quid chewing with added tobacco and adverse outcomes. This may imply that previous studies that reported an association between birth weight reductions or PTB could not sufficiently control for consumption of tobacco added to betel quid. Chewers were more anaemic compared to non-chewers. This will be further examined in the logistic regression analysis.

6.2 Cigarette smoking and smokeless tobacco

6.2.1 Cigarette smoking

Among 669 mothers who participated in the study, 15.3% (102/669) of mothers had smoked at least once in their lifetime and less than 3% (19/669, 3.9% of the control group and 1.9% of the case group) smoked during pregnancy (Table 6.6). There was no statistically significant difference in proportion of mothers who smoked during pregnancy by delivery hospital (3.5% at JDWNRH, 3.1% at CRRH, and 0% at ERRH, $p=0.125$).

Mean starting age of smoking was 19 years old (mean =19.5, SD=3.6, 95% CI 18.8 - 20.2 years). Mean duration of smoking for mothers who smoked during pregnancy was 5.5 years (mean=5.5, SD=3.6, 95% CI 3.6-7.3). More than 50% of them were daily smokers. Mean number of cigarettes per day was 3 (SD=4.5, 95% CI 1.0- 5.5). Sixty seven percent of the mothers stopped smoking during the third trimester. On average, mothers smoked 2 cigarettes per day during pregnancy among mothers who smoked during pregnancy (mean= 2.2, SD=1.7, 95% CI: 1.3- 3.0) (Figure 6.10). The total consumption in the last three months of pregnancy was 51 cigarettes (mean 50.6, SD=116, 95% CI: -7.2 -108.3) (Table 6.7). The observed difference of prevalence of smoking during pregnancy in the cases and controls was small and not statistically significant (controls 1.6% [5/321] vs cases 4.0% [14/348], $p=0.055$). This could be due to the low prevalence (2.8%) and low level of consumption (2.2 cigarettes per day).

6.2.2 Smokeless tobacco

On the other hand, 7.5% of the mothers (50/669) consumed smokeless tobacco during pregnancy (Figure 6.6). The majority (98%) of the mothers who used smokeless products did not smoke cigarettes. Smokeless tobacco use during pregnancy was more common among the mothers who delivered at JDWNRH (9.5%) compared to CRRH (5.1%) and ERRH (1.7%) ($p=0.007$) (Figure 6.7). Mean total consumption during the last three months of pregnancy was 190 grams (SD=141) among mothers delivered at JDWNRH, 194 grams (SD=151) at CRRH and 123 grams (SD=144) at ERRH.

The majority of the mothers (76%) were daily users and chewing tobacco was the most popular type of product (78%) (Figure 6.8 and Figure 6.9). Interestingly, the majority of the mothers kept using smokeless tobacco while many of the mothers who smoked cigarettes quit smoking during pregnancy. More mothers of LBW and/or PTB babies used smokeless tobacco compared to the control mothers (control 4.1% [13/321] vs case 10.6% [37/348], $p=0.001$).

On average, mothers who used smokeless tobacco during pregnancy consumed 2.4 grams per day during pregnancy (SD= 1.4, 95% CI: 1.9-2.8) (Table 6.7). The total consumption in the last three months of pregnancy was 187 grams (SD= 142, 95% CI: 144-230) (Figure 6.11). At this level of consumption, the data did not suggest a statistical difference in the mean of daily consumption during pregnancy or total consumption during the last three months of pregnancy between the case and control groups.

Table 6.6. Proportion of mothers who ever smoked, smoked during pregnancy and used smokeless tobacco during pregnancy by different adverse outcomes.

		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
		N	%	N	%	P-value	N	%	P-value	N	%	P-value
Cigarette	Ever smokers	52	16.2%	50	14.4%	0.488	27	17.8%	0.505	22	11.4%	0.126
	Smoked during pregnancy	5	1.6%	14	4.0%	0.055	6	4.0%	0.187	7	3.6%	0.132
Smokeless tobacco	Used during pregnancy	13	4.1%	37	10.6%	0.001	19	12.5%	0.001	18	9.3%	0.016

Table 6.7. Mean total consumption of cigarettes and smokeless tobacco (grams) during the last three months of pregnancy among users.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Total number of cigarettes during the last three months of pregnancy (n=19)	5	0 (0)	13	70 (133)	0.0818	6	52 (95)	0.2410	6	25 (40)	0.1852
Total ST consumption in grams during the last three months of pregnancy (n=50)	11	173 (178)	33	192 (130)	0.7421	18	204 (147)	0.6347	15	179 (110)	0.9227

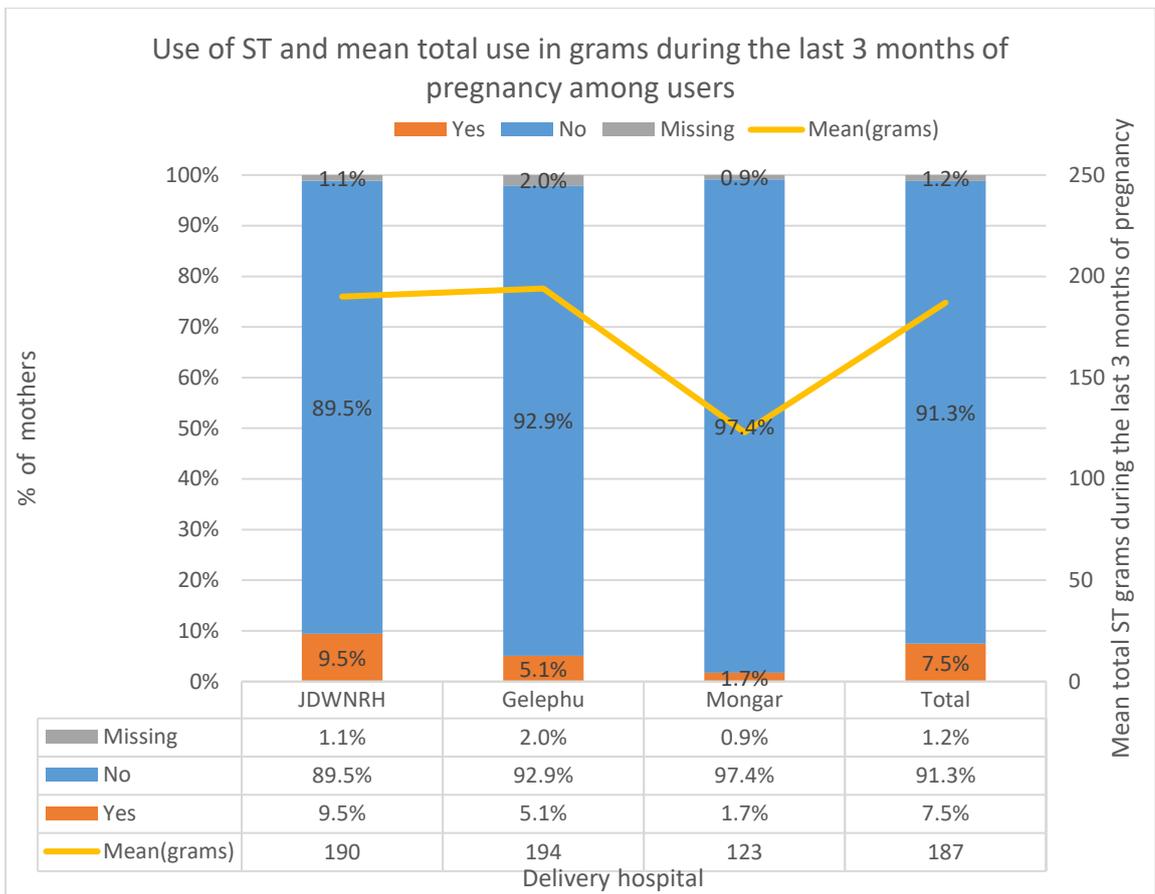


Figure 6.7. Use of smokeless tobacco (ST) and mean total use in grams during the last 3 months of pregnancy among users by delivery hospital.

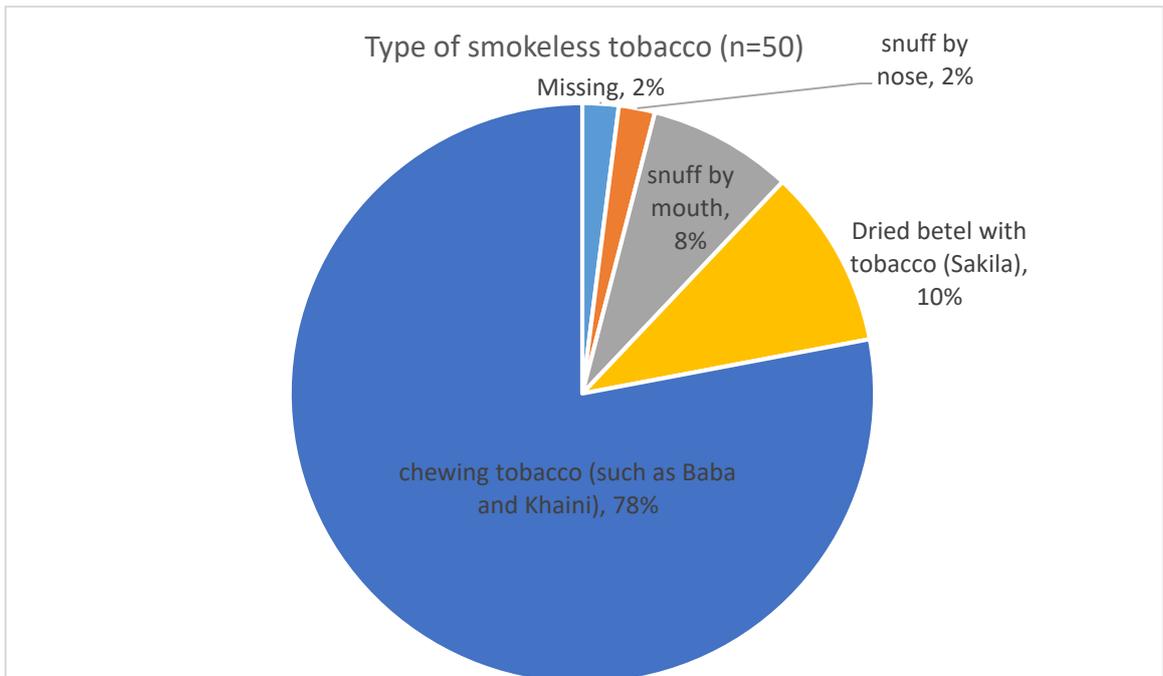


Figure 6.8. Type of smokeless tobacco used during pregnancy (n=50).

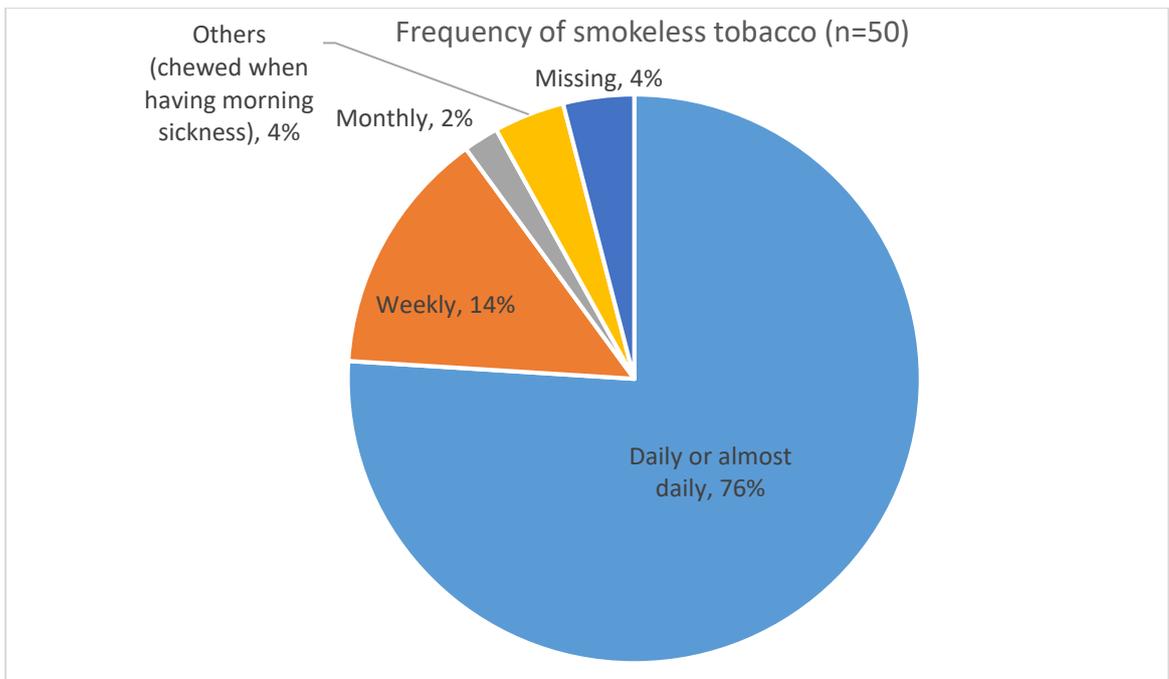


Figure 6.9. Frequency of smokeless tobacco use during pregnancy.

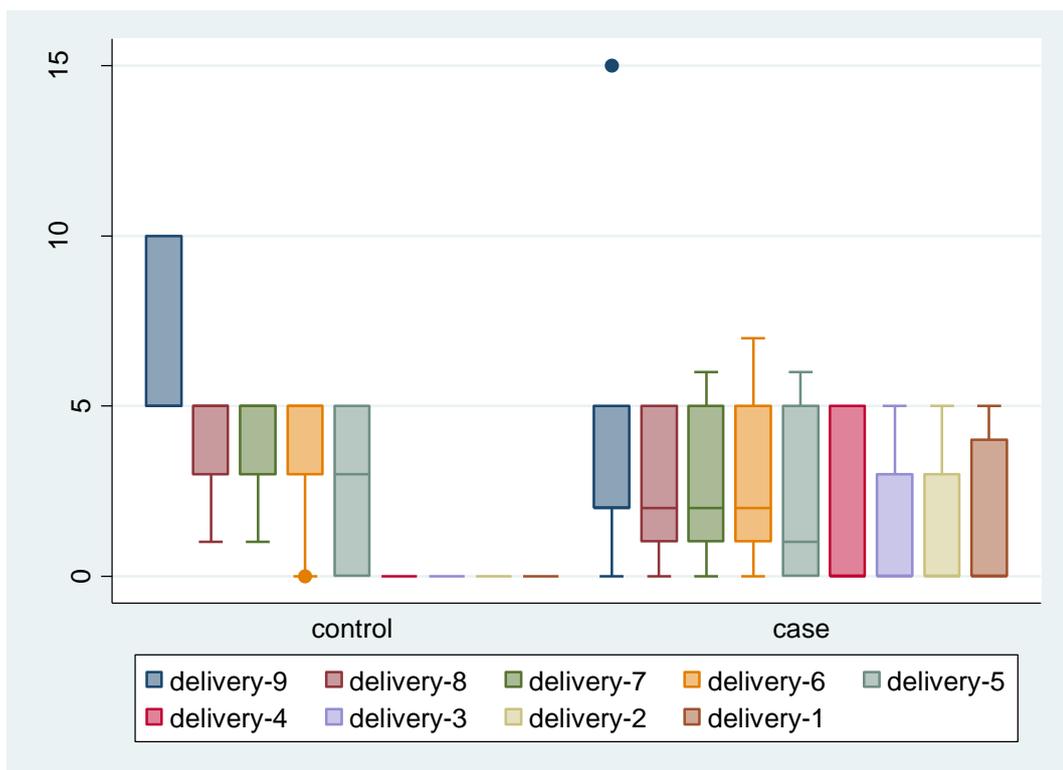


Figure 6.10. Boxplots of daily cigarette consumption during pregnancy for controls and cases. Delivery # represents # of month from the month of delivery (n=18).

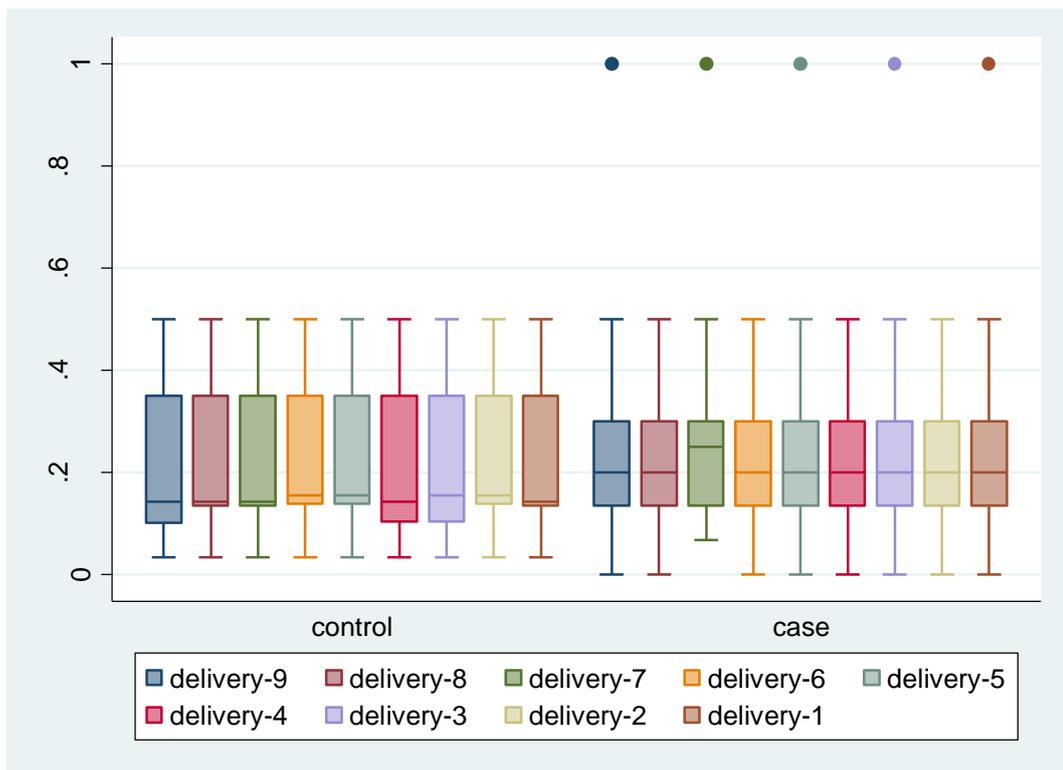


Figure 6.11. Boxplots of daily smokeless tobacco package consumption during pregnancy for controls and cases. Delivery # represents # of month from the month of delivery (n=44).

6.2.3 A summary of key findings of analysis of cigarette and smokeless tobacco use

Among the study participants, smoking during pregnancy or use of smokeless tobacco was low, accounting for 10.2% of the total. Cigarette smoking was less than 3% (2.8%), whereas smokeless tobacco use was 7.5%. The majority (98%) of the users of smokeless tobacco did not smoke cigarettes. The low cigarette smoking was comparable to the results in the 2011 report [1]. Most of the mothers quit before the last three months of pregnancy. The observed difference of prevalence of smoking during pregnancy between the cases and controls was small and not statistically significant (controls 1.6% [5/321] vs cases 4.0% [14/348], $p=0.055$). This could be due to the low level of prevalence and consumption. For example, other studies which reported a strong association used exposure criteria of smoking less than 20 cigarettes per day versus non-smokers [2]. More mothers of LBW and/or PTB babies used smokeless tobacco compared to the control mothers (control 4.1% [13/321] vs case 10.6% [37/348], $p=0.001$). In the logistic regression analysis, a binary variable of cigarette smoking or use of smokeless tobacco will be examined.

6.3 Alcohol

6.3.1 Prevalence and adverse birth outcomes

About half (49.6%) of the mothers had consumed alcohol at least once in their lifetime (controls 45.5% [146/321] vs cases 53.5% [186/348], $p=0.048$) (Table 6.8). Mean age of starting drinking was 19 years old ($SD=5.3$) with 5% starting before reaching 10 years old (Table 6.9). Twenty seven percent of the mothers drank during pregnancy. More mothers of LBW and/or PTB babies drank during pregnancy compared to the controls (controls 21.5% [69/321] vs cases 31.6% [110/348], $p=0.003$; controls vs term LBW 35.5% [54/152], $p=0.001$; and PTB 28.5% [55/193], $p=0.072$). Mean duration of drinking was 9.1 years ($SD=7.5$) among the mothers who drank during pregnancy (controls 7.6 years [$SD=6.8$] vs cases 10.0 years [$SD=8.0$], $p=0.03$). Pregnancy drinking was more common among the mothers who delivered at the Eastern Region Referral Hospital (ERRH) (37%) compared to JDWNRH in the capital (27%) and Central Region Referral Hospital (CRRH) in South (13%) ($p<0.0001$) (Figure 6.12). Mean maximum level of ethanol grams per occasion was higher at the Eastern Referral hospital (mean 79.8 grams, SD 99.6) compared to JDWNRH (mean 46.6 grams, SD 47) and CRRH (39.4 grams, SD 27.9).

6.3.2 Pattern and adverse birth outcomes

The most commonly consumed alcoholic drink among the mothers during their pregnancy was “Changkey” (home-made rice wine, 32%), followed by Ara (home-made spirit, 31%) and wine (15%) (Figure 6.13). On average, mean days of drinking during the last three months of pregnancy was 14 days ($SD=22.9$, 95%CI: 11-18 days). Mothers drank on average 3 days per month for controls and 6 days per month for cases ($p=0.0219$) (Figure 6.17). This suggests that control mothers drank less than once a week whereas case mothers drank once or twice a week. About 40% of the mothers had a heavy episodic drinking (>40grams) at least once during pregnancy.

6.3.3 Quantity

The maximum amount of alcohol consumed during pregnancy reported by the study participants on the F-GF measure ($n=179$) had a mean value of 54.1 g ($SD=64.2$) of ethanol in any day with a skewed distribution extending to 476g of ethanol on any day in the past 10 months (Table 6.10 and Table 6.11).

The data suggested that the higher the maximum ethanol grams per occasion, the more the mother drank during the last three months of pregnancy (Figure 6.15).

Mean total ethanol consumption in the past 10 months among the study participants who consumed alcohol was 7753 grams ($SD= 15764$, 95% CI: 5395-10112 grams). The results confirm a study from Tashiyangtse, which reported the annual consumption was 8554 grams ($SD=12501$) among drinking women and the main motivation for drinking was medical reasons [3]. This is equivalent to approximately 33 bottles (700ml) of whisky in the past 10 months or 3 bottles of whisky per month during pregnancy.

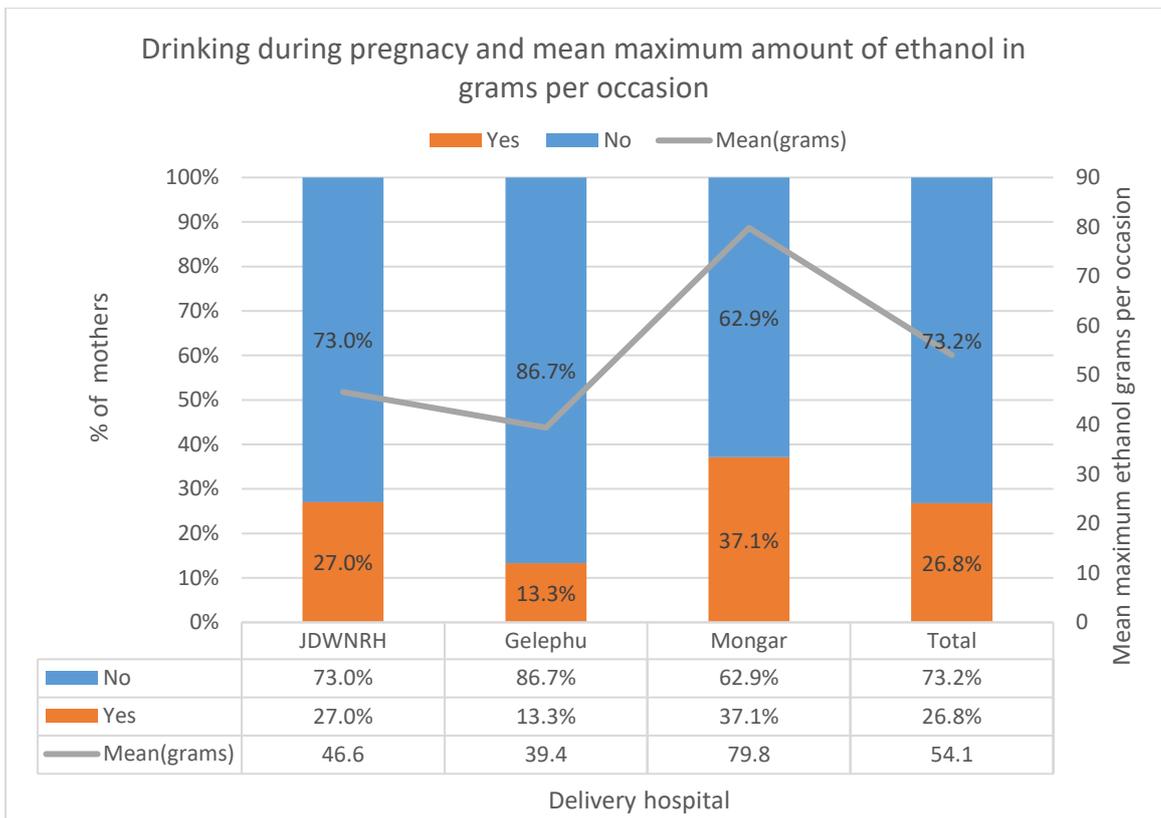


Figure 6.12. Pregnancy drinking by delivery hospital and mean maximum ethanol grams per occasion among pregnant drinkers.

Table 6.8. Descriptive statistics of drinking among study participants.

		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
		N	%	N	%	P-value	N	%	P-value	N	%	P-value
Ever drank	Yes	146	45.5%	186	53.5%	0.048	84	55.3%	0.054	101	52.3%	0.15
	Missing	2	0.6%	0	0.0%		0	0.0%		0	0.0%	
Age of starting drinking (n=332)	<10 yrs old	12	8.2%	5	2.7%	0.048	2	2.4%	0.099	3	3.0%	0.212
	10-<19 yrs old	43	29.5%	68	36.6%		33	39.3%		34	33.7%	
	>=19 yrs old	87	59.6%	111	59.7%		47	56.0%		64	63.4%	
	missing	4	2.7%	2	1.1%		2	2.4%		0	0.0%	
Drank during pregnancy (n=669)	Yes	69	21.5%	110	31.6%	0.003	54	35.5%	0.001	55	28.5%	0.072
Duration of drinking among pregnant drinkers (n=179)	0 to 5 years	28	40.6%	41	37.3%	0.295	18	33.3%	0.185	23	41.8%	0.738
	5< to 10 years	17	24.6%	20	18.2%		9	16.7%		11	20.0%	
	>10 yrs	21	30.4%	46	41.8%		25	46.3%		20	36.4%	
	missing	3	4.4%	3	2.7%		2	3.7%		1	1.8%	
Drinking intensity among pregnant drinkers (n=179)	Low (<20g)	19	27.5%	27	24.6%	0.308	10	18.5%	0.151	17	30.9%	0.533
	Moderate (20-40g)	28	40.6%	35	31.8%		17	31.5%		17	30.9%	
	High(40g)	22	31.9%	47	42.7%		26	48.2%		21	38.2%	
	Missing	0	0.0%	1	0.9%		1	1.9%		0	0.0%	

Table 6.9. Age of starting drinking and years of drinking among mothers with adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Age (years old) of starting drinking (n=332)	142	19.0 (5.5)	183	19.4 (5.2)	0.521	82	19.1 (5.2)	0.9667	100	19.8 (5.0)	0.262
Years of drinking (n=179)	66	7.6 (6.3)	107	10.0 (8.0)	0.030	52	10.9 (8.4)	0.0225	54	9.1 (7.6)	0.255

Table 6.10. Mean maximum ethanol grams per occasion, total ethanol grams in the past 10 months, number of days of drinking in the last 3 months of pregnancy among drinking study participants (n=179).

	Control (n=69)		Case (n=110)			Term LBW (n=54)			Preterm (n=55)		
	N	Mean (SD)	N	Mean (SD)	P-value	N	Mean (SD)	P-value	N	Mean (SD)	P-value
Maximum ethanol grams per occasion	69	53 (71)	108	55 (61)	0.8093	52	69 (78)	0.2393	55	42 (34)	0.2934
Total ethanol grams in the past 10 months	69	6443 (14046)	105	8614 (16808)	0.3583	50	12541 (22507)	0.0948	54	5122 (7607)	0.5063
Number of days of drinking in the last 3 months of pregnancy	65	9 (18)	109	18 (25)	0.0108	54	24 (29)	0.0022	54	12 (18)	0.4139

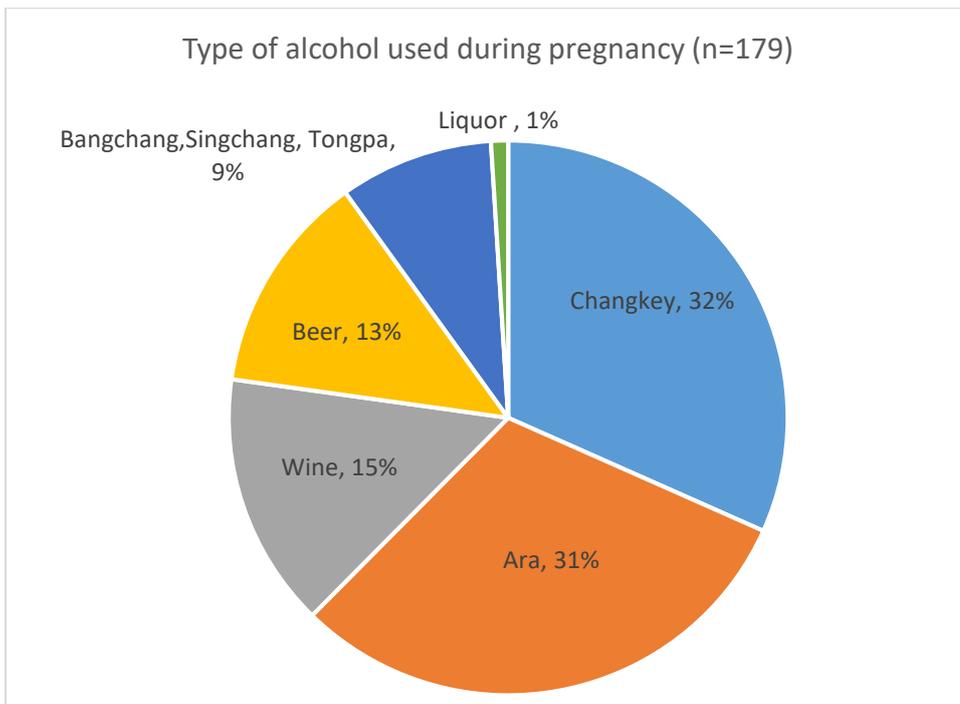


Figure 6.13. Type of alcohol used during pregnancy.

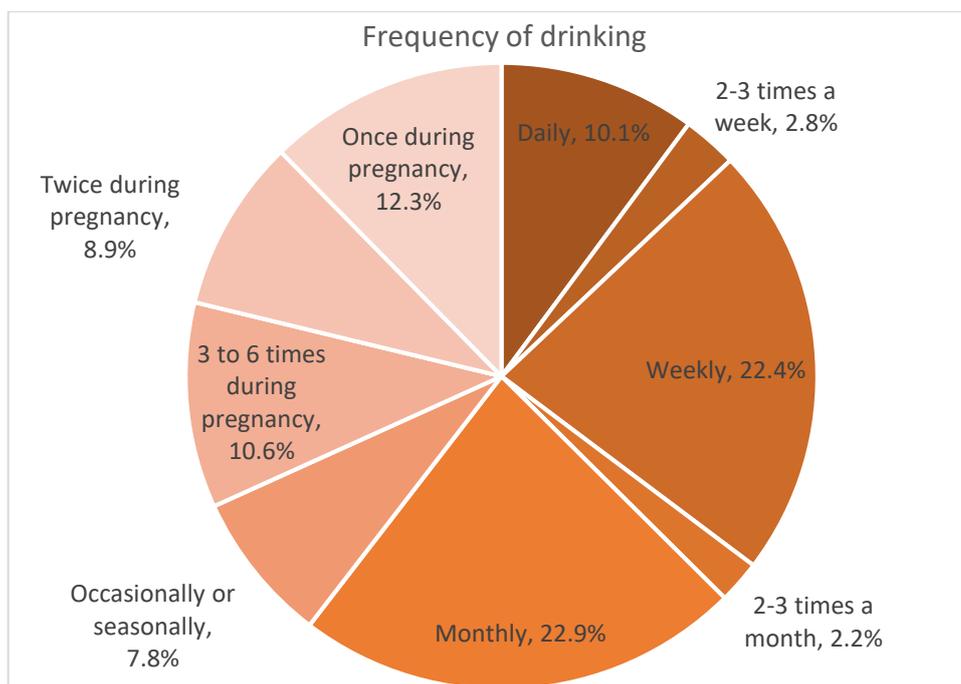


Figure 6.14. Frequency of drinking among study participants (n=179).

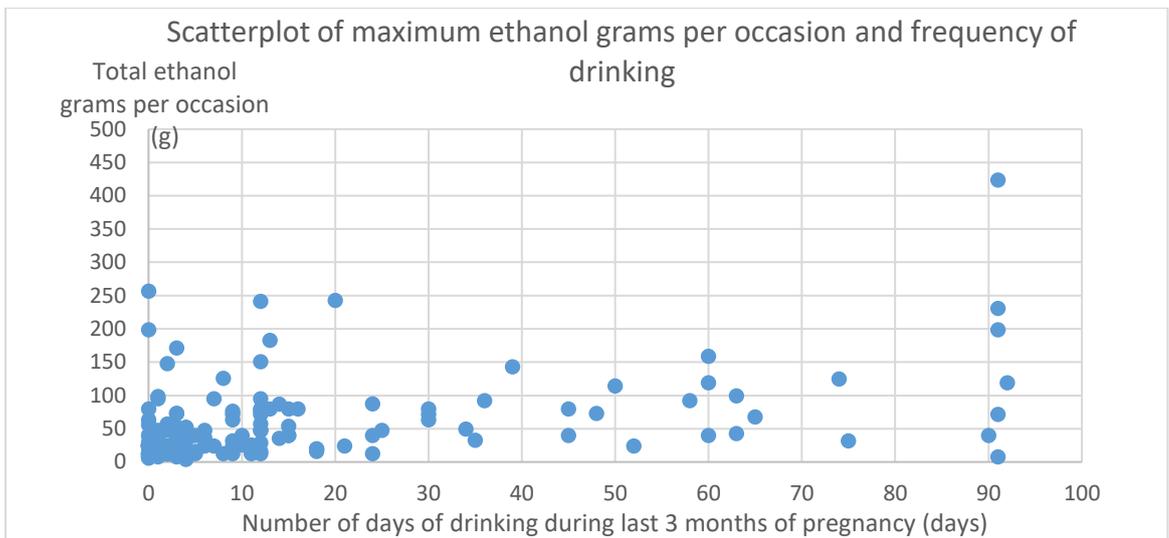


Figure 6.15. The fractional graduated frequencies (F-GF measure): The maximum amount of ethanol during pregnancy on one occasion and number of days of drinking during last 3 months of pregnancy.

Table 6.11. The fractional graduated frequencies (F-GF) measure: mean quantities and percentage of each level of drinking out of total ethanol grams at four levels of F-GF measure.

	Mean (SD) quantity grams ETOH	Percent of total volume
The maximum	54.1 (64.2)	40.7%
3/4 maximum	40.6 (48.2)	29.8%
1/2 maximum	27.0 (32.1)	20.2%
1/4 maximum	13.5 (16.1)	9.3%

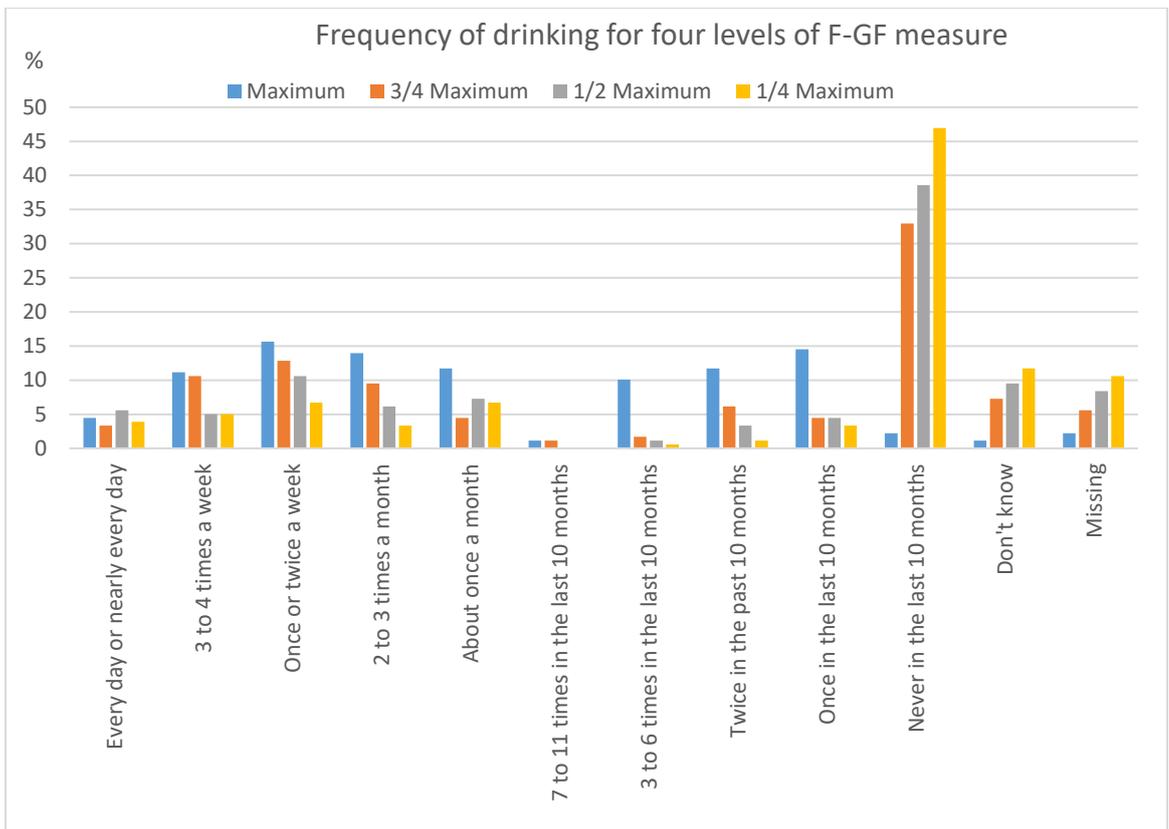


Figure 6.16. Frequency of drinking for four levels of F-GF measure.

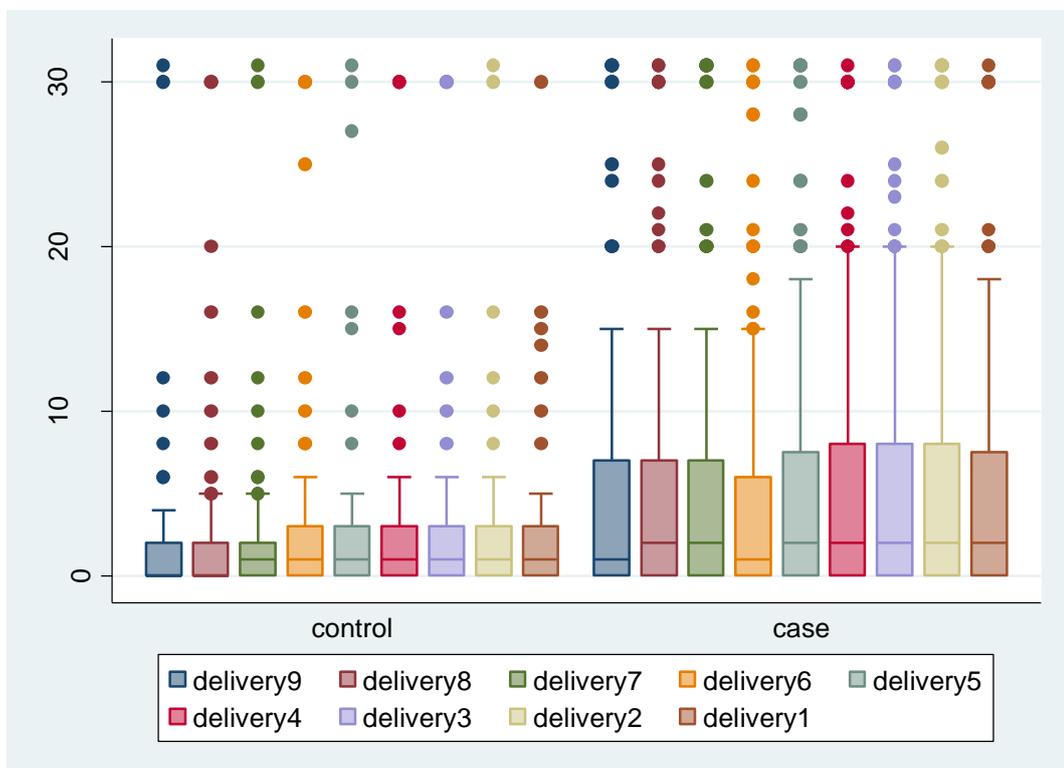


Figure 6.17. Boxplots of number of drinking days per month among pregnant drinkers by controls (0) and cases (1). Delivery # represents # of months from the month of delivery (n=176).

6.3.4 A summary of the key findings from analysis of pregnancy drinking

In Bhutan, 27% of the study mothers drank during pregnancy. Although chemical analyses of actual alcohol content were not conducted, estimates based on the assumptions produced a similar result to past studies and suggested a high volume of drinking during pregnancy. More mothers of LBW and/or PTB babies drank during pregnancy compared to the controls (controls 21.5% [69/321] vs cases 31.6% [110/348], $p=0.003$; controls vs term LBW 35.5% [54/152], $p=0.001$; and PTB 28.5% [55/193], $p=0.072$). Mothers drank on average 3 days per month for controls and 6 days per month for cases ($p=0.0219$). In the logistic regression models, this will be further examined. The number of days of drinking during the last three months of pregnancy will be classified into 3: No-drinking, less than or equal to once a week, more than once a week.

6.4 Summary

Bhutanese pregnant women are exposed to a wide variety of substances, most commonly betel quid and alcohol (Figure 6.18). Substance use seems to continue throughout pregnancy except for cigarette smoking where the majority of the mothers stopped smoking before the last three months of pregnancy. The most common substance use during pregnancy was betel quid chewing (53%, 359/669) followed by alcohol (27%, 179/669) and pan masala (23%, 153/669). Prevalence of cigarettes and smokeless tobacco was 3% and 5% respectively. Mean age of starting betel quid chewing was 18. Drinking was more common in the eastern part of Bhutan whereas betel quid chewing, use of other betel nut products and smokeless tobacco were more common among the mothers who delivered at the national referral hospital located in the western part of the country. The descriptive analyses suggest associations between drinking and smokeless tobacco and adverse birth outcomes. While the data did not provide sufficient evidence of association between adverse outcomes and betel quid chewing, betel quid seems to be associated with anaemia. The high prevalence of drinking and high intensity of drinking during pregnancy raises a public health concern in Bhutan. A recent national survey on risk factors for non-communicable diseases (NCD) in 2014 reported that NCD risk factors such as use of tobacco and alcohol, unhealthy diet including high dietary salt consumption, and high blood pressure were highly prevalent among Bhutanese adults [4]. Descriptive analyses in Chapters 5 and 6 confirmed that this pattern was also observed among the Bhutanese pregnant women in our study and it could be leading to pregnancy complications. This may have resulted in delivering LBW and/or PTB babies directly and indirectly. The potential risk factors identified in the descriptive analyses will be further examined in the regression analysis.

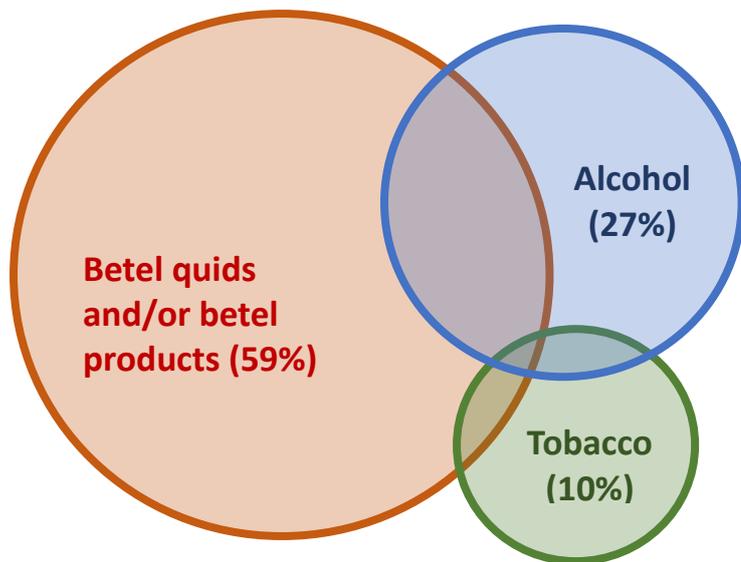


Figure 6.18. Common potentially toxic behaviour among study participants

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2. Shah, N.R. and M.B. Bracken, *A systematic review and meta-analysis of prospective studies on the association between maternal cigarette smoking and preterm delivery*. American Journal of Obstetrics & Gynecology, 2000. **182**(2): p. 465-72.
3. Subady, B.N., S. Assanangkornchai, and V. Chongsuvivatwong, *Prevalence, patterns and predictors of alcohol consumption in a mountainous district of Bhutan*. Drug Alcohol Rev, 2013. **32**(4): p. 435-42.
4. Ministry of Health (Royal Government of Bhutan) and World Health Organization, *National NCD STEPS Survey Instrument Bhutan 2014*. 2014: Thimphu.

Chapter 7

Results of the logistic regression analyses and sensitivity analyses

As proposed in Chapter 3, a statistical approach and a causal directed acyclic graph (DAG) approach were used for building logistic regression models. In this chapter, firstly, results from the logistic regression models using a statistical approach are provided, followed by the results from the logistic regression analyses using the DAG approach. Univariable analysis and multivariable analysis were conducted for both approaches. Using the DAG approach, sensitivity analyses were conducted. As a secondary outcome, association between betel quid use during pregnancy and maternal anaemia was examined using the DAG approach. The chapter ends with a summary of the overall analysis.

7.1 A statistical approach

7.1.1 Methods

Multivariable analysis models were built using Akaike Information Criterion (AIC) to minimise the AIC (Table J.1 in Appendix J). Multicollinearity was checked to see how the standard error changed as variables were added to the model (Table J.2 in Appendix J). If there was a sudden large increase in a standard error when a variable was added to the model, it was considered to indicate a problem.

Two models were used. They only differed in the modelling of betel quid chewing, tobacco, and drinking. Model 1 used a binary variable of betel quid chewing during pregnancy, tobacco use during pregnancy, and drinking during pregnancy. Model 2 used a categorical variable of number of betel quids consumed during the last three months of pregnancy with three categories (0: none; 1: less than or equal to 1 nut per day; 2: more than 1 nut per day), a categorical variable of the total grams of smokeless tobacco with 2 categories (0: none; and 1: less than 5 grams per day), and a categorical variable of number of days of drinking during the last three months of pregnancy with 3 categories (0: none; 1: less than or equal to once a week; and more than once a week)

Delivery hospital, season of delivery, sex of the infant, highest level of education attainment, mother's age at the time of delivery, ethnicity, GWG, hypertensive disorders, UTI, mode of delivery, nulliparity, previous history of preterm birth, number of ANC visits per gestational week after the first visit, number of meals per day, drinking during pregnancy, and smoking or using smokeless tobacco during pregnancy were adjusted based on the findings from the univariable analysis.

Modelling of each variable was finalised considering its distribution and nature of the relationship with the outcome. After examining the dataset, the regression analysis was restricted to mothers who delivered by spontaneous vaginal delivery or caesarean section, either elective or emergency. The mothers who delivered by breech or vacuum were omitted due to the empty cells, which resulted in 658 mothers in the final models. Eclampsia and pre-eclampsia were combined into one category. As a result, hypertension was modelled as a categorical variable with four

categories (0: None hypertensive disorder; 1: chronic hypertension; 2: gestational hypertension; and 3: Pre-eclampsia or eclampsia). The following tables present the results from the logistic regression analyses.

Analyses of term LBW and PTB were conducted separately. The results are provided in Table 7. 1. The results of the LBW and/or PTB (cases) are provided in Table J.3 in Appendix J.

7.1.2 Results

Multivariable analyses using a statistical approach did not provide clear evidence of the impact of betel quid use, including packaged betel products or pan masala, at the level of consumption of 1.4 nuts per day on LBW and/or PTB in the present study. Adjusting for delivery hospital, season of delivery, maternal age, number of ANC visits, wealth quintile, mother's education level, nulliparity, history of previous PTB, smoking or using smokeless tobacco during pregnancy, drinking during pregnancy, hypertensive disorders, UTIs, number of meals per day, and mode of delivery, the data did not provide sufficient evidence of association between betel quid chewing during pregnancy and adverse birth outcomes compared to those who did not chew during pregnancy in Model 1 (term LBW: the adjusted odds [aOR] 1.07, 95% CI: 0.54-2.13, $p=0.845$; and PTB: aOR 1.23, 95% CI: 0.65-2.34, $p=0.529$) (Table 7.1). In Model 2, compared to the mothers who did not chew during the last three months of pregnancy, the aOR for mothers who chewed less than or equal to one nut per day in the last three months of pregnancy was 1.36 for term LBW (95% CI: 0.61-3.00, $p=0.452$) and 0.62 for PTB (95% CI: 0.28-1.37, $p=0.236$) (Table 7.2). The aOR for mothers who chewed more than one nut per day was 1.40 for term LBW (95% CI: 0.37-5.24, $p=0.621$) and 0.62 for PTB (95% CI: 0.16-2.36, $p=0.481$) compared to non-chewers.

As discussed in the Methods section, the aforementioned statistical models embody many parametric assumptions that are not known to be correct and may well be incorrect [1]. Without taking direct and indirect causal assumptions into account, covariates informed in a statistical approach may lead to biased estimates by controlling for an intermediate variable such as previous history of PTB which may share many causal factors with the present preterm birth. In fact, history of previous PTB was positively associated with term LBW (aOR 4.26, 95% CI: 1.18-15.32, $p=0.027$) and PTB (aOR 3.45, 95% CI: 1.01-10.09, $p=0.036$) adjusting for all other variables in Model 1. In the next section, results from logistic regression models using the DAG approach are provided.

Table 7.1. Results of logistic regression models using a statistical approach for Model 1.

Model 1		Term LBW(n=289) Adjusted Odds ratio			PTB (n=310) Adjusted Odds Ratio		
Independent variables		Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
Hospital (ref: JDWNRH)	CRRH	0.53	0.18-1.52	0.236	0.79	0.30-2.13	0.646
	MRRH	0.29	0.10-0.81	0.019	0.32	0.13-0.81	0.016
Season (ref: Winter)	Fall	1.00	0.37-2.66	0.995	0.82	0.32-2.13	0.689
	Spring	0.96	0.38-2.43	0.926	1.07	0.44-2.64	0.875
	Summer	3.67	1.41-9.54	0.008	3.57	1.44-8.82	0.006
Sex of the infant (ref: Male)	Female	3.76	1.88-7.56	<0.0001	0.95	0.52-1.75	0.872
Age (ref: 20-35)	<20	0.79	0.12-5.42	0.813	2.24	0.64-7.87	0.208
	35<	0.96	0.31-2.97	0.938	0.65	0.23-1.80	0.404
Education (ref: Never attended school)	Non-formal education (NFE)	0.20	0.03-1.20	0.079	0.42	0.11-1.65	0.168
	Primary	1.34	0.43-4.16	0.616	1.03	0.35-3.04	0.984
	Middle Secondary or Secondary School	0.31	0.11-0.91	0.033	0.51	0.21-1.28	0.152
	Diploma, college, and postgraduate	0.53	0.12-2.35	0.402	0.29	0.06-1.27	0.166
Wealth Quintile (ref: Middle)	Poorest	0.76	0.23-2.46	0.646	1.29	0.46-3.60	0.622
	Second	0.81	0.28-2.32	0.695	1.03	0.40-2.65	0.959
	Fourth	2.05	0.75-5.61	0.161	1.35	0.51-3.57	0.539
	Richest	1.37	0.49-3.81	0.543	1.46	0.56-3.79	0.435
Number of ANC visits per gestational week after the first ANC visit		0.07	0.00-17.89	0.352	0.32	0.06-1.66	0.177
Ethnicity (ref: northern Bhutanese)	Southern Bhutanese	1.92	0.93-3.96	0.076	0.74	0.36-1.54	0.422

GWG (ref: IOM recommendations)	High GWG	0.57	0.19-1.77	0.334	0.47	0.16-1.37	0.169
	Low GWG	3.12	1.42-6.84	0.004	2.19	1.06-4.52	0.034
Number of meals per day		0.65	0.37-1.14	0.134	0.53	0.31-0.92	0.023
UTI		2.13	0.70-6.50	0.183	2.49	0.92-6.78	0.073
Nulliparity		2.14	1.00-4.59	0.050	1.58	0.80-3.11	0.189
Previous history of PTB		4.26	1.18-15.32	0.027	3.45	1.01-10.09	0.036
Hypertension (ref: Non-hypertensive disorder)	Chronic or pre-existing	2.97	0.61-14.47	0.177	6.19	1.60-23.88	0.008
	Gestational hypertension	4.68	1.39-15.73	0.013	3.90	1.18-12.86	0.026
	Pre-eclampsia or eclampsia	0.76	0.12-4.88	0.771	9.39	2.44-36.11	0.001
Mode of delivery (ref: SVD)	CS-elective	1.08	0.35-3.38	0.891	1.05	0.37-3.03	0.923
	CS-emergency	1.35	0.60-3.03	0.469	3.74	1.80-7.76	<0.0001
Chewing betel quid or pan masala during pregnancy		1.07	0.54-2.13	0.845	1.23	0.65-2.34	0.529
Chewing smokeless tobacco or smoking during pregnancy		3.74	1.25-11.14	0.018	1.13	0.32-4.03	0.854
Drinking during pregnancy		1.78	0.84-3.81	0.134	1.26	0.61-2.58	0.529

Table 7.2. Results of logistic regression models using a statistical approach for Model 2.

Model 2	Independent variables	Term LBW (n=261) Adjusted Odds ratio			PTB (n=280) Adjusted Odds ratio		
		Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
Hospital (ref: JDWNRH)	CRRH	0.53	0.16-1.81	0.315	0.66	0.21-2.10	0.486
	MRRH	0.24	0.07-0.83	0.025	0.25	0.09-0.70	0.008
Season (ref: Winter)	Fall	0.98	0.30-3.19	0.967	0.67	0.22-2.01	0.473
	Spring	1.32	0.44-3.97	0.617	1.26	0.45-3.51	0.657
	Summer	4.94	1.51-16.18	0.008	3.79	1.35-10.65	0.012
Sex of the infant (ref: Male)	Female	4.08	1.89-8.84	<0.0001	1.09	0.54-2.19	0.818
Age (ref: 20-35)	<20	1.28	0.15-10.75	0.818	2.75	0.67-11.19	0.159
	35<	0.86	0.23-3.31	0.831	0.67	0.20-2.19	0.509
Education (ref: Never attended school)	Non-formal education (NFE)	0.26	0.04-1.79	0.171	0.54	0.12-2.50	0.431
	Primary	1.34	0.38-4.73	0.652	1.27	0.38-4.33	0.697
	Middle Secondary or Secondary School	0.28	0.08-0.93	0.038	0.61	0.21-1.75	0.356
	Diploma, college, and postgraduate	0.78	0.15-4.11	0.771	0.48	0.09-2.52	0.385
Wealth Quintile (ref: Middle)	Poorest	0.84	0.23-3.02	0.788	1.60	0.48-5.30	0.44
	Second	0.57	0.17-1.89	0.356	1.27	0.43-3.75	0.665
	Fourth	3.21	1.02-10.09	0.046	2.91	0.95-8.88	0.061
	Richest	1.05	0.33-3.42	0.929	2.03	0.69-5.95	0.197
Number of ANC visits per gestational week after the first ANC visit		0.32	0.00-97.70	0.694	0.32	0.05-2.22	0.248
Ethnicity (ref: northern Bhutanese)	Southern Bhutanese	3.06	1.34-7.02	0.008	0.63	0.27-1.47	0.285

GWG (ref: IOM recommendations)	High GWG	0.68	0.18-2.52	0.567	0.58	0.18-1.84	0.358
	Low GWG	4.18	1.66-10.52	0.002	2.26	1.03-4.97	0.043
Number of meals per day		0.69	0.38-1.28	0.241	0.51	0.27-0.95	0.033
UTI		2.56	0.68-9.57	0.163	2.70	0.89-8.23	0.08
Nulliparity		1.77	0.76-4.15	0.188	1.95	0.92-4.15	0.083
Previous history of PTB		6.71	1.55-29.05	0.011	4.81	1.29-17.88	0.019
Hypertension (ref: Non-hypertensive disorder)	Chronic or pre-existing	2.85	0.49-16.39	0.241	8.69	2.18-34.59	0.002
	Gestational hypertension	2.76	0.69-11.09	0.153	2.69	0.70-10.42	0.151
	Pre-eclampsia or eclampsia	0.74	0.08-7.10	0.791	11.07	2.30-53.24	0.003
Mode of delivery (ref: SVD)	CS-elective	1.54	0.41-5.79	0.519	1.43	0.41-5.02	0.573
	CS-emergency	1.57	0.63-3.91	0.337	4.26	1.91-9.49	<0.0001
Total number of betel nuts during the last 3 months of pregnancy (ref: None)	<=90 nuts during the 3 months or 1 nut per day	1.36	0.61-3.00	0.452	0.62	0.28-1.37	0.236
	More than 1 nut per day	1.40	0.37-5.24	0.621	0.62	0.16-2.36	0.481
Chewing smokeless tobacco or smoking during pregnancy (ref: None)	Less than 5 grams per day	1.59	0.36-7.11	0.541	0.22	0.02-2.19	0.196
Total number of days of drinking during the last three months of pregnancy (ref: no drinking)	Less or equal to once a week	1.55	0.57-4.24	0.392	2.14	0.88-5.19	0.093
	More than once a week	9.74	1.59-59.60	0.014	13.23	1.68-104.18	0.014

7.2 A causal directed acyclic graph approach

7.2.1 Methods

Separate logistic regression analyses were conducted to estimate the total effect of different exposure variables on adverse birth outcomes using the minimum sets of covariates for different exposure variables as described in Chapter 3. In the logistic models, wealth quintile and education variables were used to represent socioeconomic status (SES). Delivery hospital was used as a proxy for regionality. Number of meals per day was used as a proxy of imbalanced diet.

Term LBW and PTB were used as separate outcomes. Three sensitivity analyses were conducted: (1) Limiting data to mothers with early scans and certain LMP dates for PTB; (2) multiple imputation based on MAR assumption; and (3) sensitivity analysis using a pattern mixture approach under MNAR assumptions. In the pattern mixture sensitivity analysis, two assumptions under MNAR were made for UTI. UTI was selected as it was one of the variables with more than 10% missing observations and as the literature provides a substantial amount of evidence of an association between UTI and adverse birth outcomes.

(a) Multiple imputation

As the total number of observations included in the multivariable analyses decreased by roughly 50% due to missing data, the multiple imputation model was used to compare the results to see the validity of the analyses assuming MAR.

The percentage of missing values across the 19 variables varied between 0 and 30%. Many mothers were missing pre-pregnancy weight.

The minimum number of cases with complete data for the analyses of interest was 282/473 for term LBW and 247/516 for PTB models. The multiple imputation model (Rubin, 1987a) [2] was used to create 100 multiply imputed datasets. Model parameters were estimated with multiple regression applied to each imputed dataset separately. These estimates and their standard errors were combined using Rubin's rules.

The STATA "mi impute chained" command was used to create 100 imputed datasets. The variables included in the model and model specifications are provided in Table 7.3 and Table 7.4.

The Monte-Carlo error represents how much variability there is due to the fact we have used 100 imputations. With an infinite number of imputations, the Monte-Carlo error would be zero. From Table J.4 in Appendix J, it was confirmed that the conditions proposed by White et al. (2010) were met [3] as described in Chapter 3. Convergence was checked by plotting the mean estimate against the cycle (iteration) number (in this study, 100) for each variable with a high proportion of missing data. In the trace plots, it was confirmed that the values varied randomly (Figures J.1 – J.6 in Appendix).

MNAR Model 1 assumes that the odds of having UTI during pregnancy for mothers who were missing information on UTI was two times more than that of mothers who have information on UTI (OR=2). The MNAR Model 2 odds of having UTI during pregnancy for mothers who were

missing information on UTI was 50% less than that of mothers who have information on UTI (OR=0.5). The imputation datasets were produced with 100 times of iteration in SAS 9.4 (SAS Institute Inc., Cary, NC, USA) using a fully conditional specification (FCS) statement, MNAR, and ADJUST option with an adjustment parameter (SHIFT). The MNAR statement imputes missing values by using the pattern-mixture model approach, assuming the missing data are MNAR [4]. The rest of the analyses were conducted using the aforementioned methods in STATA 14.1.

Table 7.3. Completeness of variables included in the model and model specification for multiple imputation (all numbers are counts of available and missing data).

		Complete	Missing	Model specification in the imputation Model
Outcome variable	LBW	473	0	logistic
Independent variable	GWG (kg)	336	137	regress
	Maternal pre-pregnancy weight (kg)	343	130	regress
	UTI	436	37	logistic
	Maternal height (cm)	446	27	regress
	Hypertension	453	20	mlogit
	Smoking or smokeless tobacco during pregnancy	467	6	logistic
	Betel quid during pregnancy	468	5	logistic
	Wealth quintile	470	3	mlogit
	Number of meals per day	470	3	regress
	Betel quid chewing before pregnancy	472	1	logistic
	Nulliparity	472	1	logistic
	Mother's age at delivery	473	0	regress
	Ancestry by name	473	0	logistic
	C-section	473	0	logistic
	Education	473	0	mlogit
	Delivery hospital	473	0	mlogit
	Season	473	0	mlogit
	Sex of infant	473	0	logistic
	Drinking during pregnancy	473	0	logistic

Table 7.4. Completeness of variables included in the model and model specification for multiple imputation (all numbers are counts of available and missing data).

		Complete	Missing	Model specification in the imputation Model
Outcome variables	PTB	513	3	logistic
Independent variables	GWB (kg)	369	147	regress
	Maternal pre-pregnancy weight in (kg)	379	137	regress
	UTI	457	59	logistic
	Maternal height (cm)	467	49	regress
	Hypertension	487	29	mlogit
	Smoking or smokeless tobacco during pregnancy	509	7	logistic
	Betel quid during pregnancy	509	7	logistic
	Wealth quintile	511	5	mlogit
	Number of meals per day	514	2	regress
	Betel quid chewing before pregnancy	515	1	logistic
	Education	515	1	mlogit
	Drinking during pregnancy	516	0	logistic
	Sex of the infant	516	0	logistic
	Delivery hospital	516	0	mlogit
	Season	516	0	mlogit
	C-section	516	0	logistic
	Ancestry by name	516	0	logistic
	Mother's age at delivery	516	0	regress

7.2.2 Results

The results from the complete case analysis and sensitivity analyses varied little. Thus, the results were integrated and presented. The following section mainly summarises the results from the complete case multivariable analyses unless the results of multiple imputation substantially changed. If the results from the complete case analysis and results from multiple imputation models were different, the discrepancies were addressed. Inferential results for these values under MNAR and results for imputed values that were obtained under MAR were virtually identical (Table 7.7 and Table 7.8). Therefore, it may indicate that the MAR assumption is plausible.

The differences in the statistical approach and DAG approach were addressed where the results substantially changed. Possible reasons for discrepancies are discussed.

(a) Modifiable risk factors in the short run

I. Maternal potential health-risk behaviours

(1) Betel quid chewing or pan masala use during pregnancy

The aOR of delivering a term LBW baby among the mothers who chewed betel quid or used pan masala during pregnancy compared to those who did not chew betel quid or betel products was 1.30 (95% CI: 0.74-2.27, p-value=0.361) adjusting for ethnicity, wealth quintile, education, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, delivery hospital and season of delivery (Table 7.5). The aOR of PTB was 1.20 (95% CI: 0.72-2.00, p-value=0.481) adjusting for ethnicity, wealth quintile, education, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, delivery hospital, and season of delivery (Table 7.6). When a categorical variable of the total number of betel nuts chewed during the last three months of pregnancy was used in the model instead of the binary variable, the aOR of term LBW among the mothers who chewed betel quid less than or equal to one nut per day was 1.32 (95% CI: 0.69-2.51, p-value=0.402) adjusting for ethnicity, wealth quintile, education, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, delivery hospital, and season of delivery in the model compared to the mothers who did not chew during pregnancy. The aOR of PTB among the mothers who chewed betel quid less than or equal to one nut per day was 0.76 (95% CI: 0.39-1.47, p-value=0.411) compared to the non-chewers during pregnancy.

(2) Tobacco

The level of consumption was low compared to past studies (on average 3 cigarettes per day or 2.4 grams of smokeless tobacco per day). In the present study, the maximum amount of smokeless tobacco was 5 grams per day on average during the last three months of pregnancy and the majority of women quit smoking cigarettes during the last three month of pregnancy. The aOR of term LBW among mothers who used smokeless tobacco and/or smoked cigarettes during pregnancy was 3.12 (95% CI: 1.62-5.99, p=0.001) adjusting for wealth quintile and maternal education compared to mothers who did not use any tobacco. The aOR of PTB was 2.14 (95% CI: 1.12-4.11, p=0.022).

Tobacco was one of the variables that had significantly different estimates in the statistical and DAG approaches. In the model using a statistical approach, the aOR of PTB was 1.13 (95% CI: 0.32-4.03, p=0.854). This estimate may be biased due to the over-adjustment of the variables that could be in the causal pathway such as hypertensive disorders. Hypertensive disorders, assumed to be on the causal pathway between tobacco and adverse pregnancy outcomes in the DAGs was adjusted for in the statistical approach, allowing only direct effect to be estimated. This may explain why the odds ratio in the statistical approach was 50% smaller than the DAG analysis.

(3) Alcohol during pregnancy

Mothers who drank during pregnancy had 1.96 times higher odds of term LBW compared to non-drinkers after adjusting for betel quid chewing during pregnancy, wealth quintile, education, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, and season of delivery (aOR 1.96, 95% CI: 1.10-3.49, p=0.022). Mothers who drank less than or equal to once a week had 2.16 higher odds of PTB (95% CI: 1.11-4.19, p=0.023) and mothers who drank more than once a week had 3.43 times higher odds of PTB (95% CI: 0.88-13.37, p=0.075) compared to non-drinkers. Association between alcohol during pregnancy and pregnancy outcomes was not clear in the statistical approach (Term LBW: aOR 1.78, 95% CI: 0.84-3.81, p=0.134; and PTB: aOR 1.26, 95% CI: 0.61-2.58, p=0.529). This estimate may be biased due to the over-adjustment of the variables that could be on the causal pathway such as hypertensive disorders. Hypertensive disorders, assumed to be on the causal pathway between alcohol and adverse pregnancy outcomes in the DAGs, was adjusted for in the statistical approach, allowing only direct effect to be estimated. In addition, the number of observations included in the statistical approach was smaller than the DAG approach due to the different portions of missing data as they include different covariates. As a result, loss of information in the statistical model decreased the power.

II. Nutritional factors (GWG)

Having lower GWG than recommended by the Institute of Medicine (IOM) according to pre-pregnancy BMI, had a 2.62 times higher odds ratio (aOR 2.62, 95% CI: 1.33-5.15, p=0.005) of term LBW and a 2.15 times higher odds ratio (aOR 2.15, 95% CI: 1.14-4.04, p=0.018) of PTB compared to those with GWG as per IOM recommendations (For underweight: 28-40 lbs/ 12.5-18 kgs ; normal BMI: 25-35 lbs/11.5-16 kgs; For overweight: 15-25lbs/7.0-11.5 kgs; obese: 11-20 lbs/5-9 kgs), adjusting for betel quid chewing during pregnancy, ethnicity, wealth quintile, education, chronic hypertension, imbalanced diet, maternal height, maternal age, delivery hospital, and season of delivery.

III. Infectious disease (UTI)

The aOR of term LBW association with UTI was 2.17 (95% CI: 1.11-4.26, p=0.023) and the aOR of PTB was 1.98 (95% CI 1.00-3.90, p=0.049), adjusting for wealth, education, and season of delivery.

(b) Modifiable risk factors in the long-run or non-modifiable risk factors

I. Socio-economic status

(1) Wealth quintile

The data did not provide sufficient evidence of the impact of wealth quintile on term LBW after adjusting for delivery hospital (middle quintile [reference] vs poorest quintile [aOR: 1.37, 95% CI 0.73-2.54]; middle vs second [aOR: 0.88, 95% CI 0.47-1.65]; middle vs

fourth [aOR: 1.01, 95% CI 0.54-1.89]; and middle vs richest [aOR: 0.73, 95% CI: 0.39-1.37]). The results were similar for PTB.

(2) Education

The data suggested evidence of an association between middle secondary or secondary school compared to no education, with reduced odds of term LBW after adjusting for delivery hospital (None [reference] vs non-formal education [aOR: 0.59, 95% CI 0.27-1.29]; none vs primary[aOR: 0.91, 95% CI 0.49-1.67]; none vs middle secondary or secondary school [aOR: 0.54, 95% CI 0.33-0.86]; and none vs diploma, college, or postgraduate [aOR: 0.75, 95% CI: 0.34-1.64]).

The aOR of PTB for education varied a little by each category of highest education attainment (none [reference] vs non-formal education [aOR: 0.56, 95% CI 0.25-1.27]; none vs primary [aOR: 1.20, 95% CI 0.66-2.15]; none vs middle secondary or secondary school [aOR: 0.89, 95% CI 0.57-1.39]; and none vs diploma, college, or post graduate [aOR: 0.82, 95% CI: 0.38-1.78]).

II. Maternal age

The aOR for maternal age substantially changed in the DAG approach compared to the statistical approach. In the DAG approach, the aOR among mothers who were under 20 years old compared to mothers aged between 20 and 35 at time of delivery was 1.65 (95%CI: 0.68-4.01, $p=0.272$) for term LBW and 2.37 (95%CI: 1.13-4.98, $p=0.023$) for PTB adjusting for wealth and education.

In the statistical approach, the aOR among mothers who were under 20 years old compared to mothers aged between 20 and 35 at time of delivery was 0.79 (95% CI: 0.12-5.42, $p=0.813$) for term LBW and 2.24 (95% CI: 0.64-7.87, $p=0.208$) for PTB.

The aOR of term LBW in the statistical approach is less than half of the aOR in the DAG approach. This could be due to underestimation by adjusting for some of the variables on the causal pathway such as hypertensive disorders and previous history of PTB. This only allowed the model to estimate the direct causal effect of age.

The evidence of association between maternal age older than 35 years and adverse birth outcomes was clearer in the multiple imputation models than complete case analysis although the point estimates were virtually identical. In the complete case analysis, the aOR among mothers over 35 years old was 1.77 (95%CI: 0.996-3.13, $p=0.052$) for term LBW and 1.72 (95%:1.01-2.93, $p\text{-value}=0.047$) for PTB compared to mothers aged between 20 and 35. In the multiple imputation model under MAR, the corresponding aOR of term LBW was 1.76 (95%CI: 1.02-3.06, $p=0.044$) and the aOR of PTB was 1.80 (95%:1.01-3.18, $p\text{-value}=0.045$). Loss of information in the complete case analysis due to missing data may account for decreased power.

III. Hypertensive disorders

(1) Chronic hypertension

In the complete case analysis, the aOR of term LBW among mothers with chronic hypertension was 2.68 (95% CI: 0.70-10.31, p-value=0.151) and the aOR of PTB was 3.23 (95% CI: 1.01-10.30, p-value=0.047) after controlling for betel quid chewing during pregnancy, ethnicity, wealth quintile, education, maternal height, maternal age, pre-pregnancy weight, delivery hospital, and season of delivery. The association between chronic hypertension and adverse pregnancy outcomes was clearer in the multiple imputation models. Under MAR, the aOR of term LBW was 4.24 (95% CI: 1.44-12.42, p=0.009) and the aOR of PTB was 4.73 (95% CI: 1.83-12.19, p=0.001). The results under MNAR were almost identical to those under MAR. The large number of missing data in maternal height and pre-pregnancy weight may have decreased the power for the complete case analysis, resulting in an unclear association between chronic hypertension and term LBW.

(2) Pregnancy-induced hypertension, pre-eclampsia and eclampsia:

The mothers with pregnancy-induced hypertension (PIH), pre-eclampsia, or eclampsia had a 7.08 times higher odds ratio (95% CI: 3.58-14.00, p-value<0.0001) of PTB compared to mothers without hypertensive disorders adjusting for alcohol during pregnancy, betel quid chewing during pregnancy, ethnicity, chronic hypertension, number of meals per day, maternal age, tobacco use during pregnancy. The total effect of PIH, pre-eclampsia, or eclampsia on term LBW cannot be estimated by adjusting for covariates in the proposed DAG.

IV. Maternal and obstetric factors

(1) Nulliparity

The aOR of nulliparous mothers compared to non-nulliparous mothers was 1.50 for term LBW (aOR 1.50, 95% CI 0.98-2.30, p=0.061) after adjusting for maternal age.

(2) Sex of the infant

Female infants had a 1.96 times higher odds ratio of being LBW (crude OR 1.96, 95% CI: 1.32-2.92, p=0.001). The association between male infants and PTB was not clear (crude OR 0.88, 95 % CI: 0.62-1.26, p=0.487).

V. Ethnicity

The crude OR of term LBW among southern Bhutanese compared to northern Bhutanese was 1.47 (95% CI: 0.98-2.20, p=0.064). The crude odds ratio of PTB among southern Bhutanese compared to northern Bhutanese was 0.85 (95% CI: 0.57-1.27, p=0.440).

VI. Altitude difference between the current residence and the permanent residence

The OR of term LBW differed little by altitude difference between the current residence and the permanent residence in the univariable analysis (0-1000 m (reference) vs < 0 m [term LBW: OR 0.81, 95% CI: 0.46-1.42; and PTB: OR 0.93, 95% CI: 0.56-1.55]; 1000-<2000 m [term LBW: OR 1.18, 95% CI: 0.72-1.95; and PTB: 1.27, 95% CI: 0.79-2.02]; and 2000 m and above [OR 1.03, 95% CI: 0.51-2.06; and PTB: OR 1.18, 95% CI: 0.62-2.23]).

Table 7.5. Results from the logistic regression models based on DAGs for term LBW.

Exposure		Category	Factors adjusted for	n	Crude	95%CI	P-value	n	AOR	95%CI	P-value
Maternal Potential Risk Factors	Betel quid chewing during pregnancy		<i>Covariate set 1: Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality</i>	468	1.10	0.74-1.63	0.648	317	1.30	0.74-2.27	0.361
			<i>Covariate set 2: BQ chewing before pregnancy, seasonality</i>					468	1.04	0.61-1.78	0.876
	Total number of betel nuts during the last 3 months of pregnancy (ref: No betel quid chewing)	<=90 nuts during the last 3 months or <1 nut per day	<i>Covariate set 1: Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality</i>	421	1.28	0.81-2.01	0.286	282	1.32	0.69-2.51	0.402
		More than 90 nuts during the last 3 months or >1 nut per day				1.07	0.57-2.00	0.836		1.39	0.52-3.68
	Tobacco use during pregnancy		<i>DAG 3: SES</i>	467	3.28	1.73-6.23	<0.0001	465	3.12	1.62-5.99	0.001
	Grams of ST in the last 3 months of pregnancy (ref: did not use during pregnancy)	Less than 5 grams per day	<i>DAG 3: SES</i>	463	3.55	1.62-7.78	0.002	461	3.04	1.36-6.80	0.007
		Alcohol use during pregnancy		<i>DAG 3: BQ during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, seasonality</i>	473	2.01	1.31-3.08	0.001	320	1.96	1.10-3.49
	Number of drinking days	Less than or equal to once a week	<i>DAG 3: BQ during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, seasonality</i>	469	1.43	0.81-2.52	0.221	317	1.92	0.94-3.90	0.069
		More than once a week				6.07	2.81-13.12	<0.0001		6.30	1.66-23.91

Nutritional Factors	GWG (ref: as per IOM recommendation)	High GWG	Covariate set 1: BQ during pregnancy, ethnicity, SES, chronic hypertension, imbalanced diet, maternal height, maternal age, regionality, seasonality	336	0.69	0.29-1.64	0.398	310	0.72	0.27-1.87	0.494
		Low GWG			2.47	1.38-4.43	0.002		2.62	1.33-5.15	0.005
		High GWG	Covariate set 2: Imbalanced diet, maternal height, pre-pregnancy weight					316	0.92	0.37-2.29	0.859
		Low GWG							2.69	1.43-5.04	0.002
Infectious Diseases	UTI		SES, seasonality	436	1.96	1.02-3.75	0.043	433	2.17	1.11-4.26	0.023
Hypertensive disorders	Chronic hypertension		BQ during pregnancy, ethnicity, SES, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality	468	3.55	1.35-9.36	0.010	317	2.68	0.70-10.31	0.151
	PIH, PE/Eclampsia		The total effect cannot be estimated by covariate adjustment	452	4.79	2.48-9.26	<0.0001				
Socio-economic Status	Wealth quintile (ref: Middle)	Poorest	Regionality	470	1.34	0.73-2.47	0.341	470	1.37	0.73-2.54	0.330
		Second			0.88	0.47-1.64	0.689		0.88	0.47-1.65	0.700
		Fourth			1.00	0.54-1.86	0.997		1.01	0.54-1.89	0.975
		Richest			0.72	0.39-1.36	0.317		0.73	0.39-1.37	0.320
		Education (ref: No education)	Non-formal education	Regionality	473	0.59	0.27-1.29	0.188	473	0.59	0.27-1.31
		Primary			0.91	0.49-1.67	0.753		0.91	0.49-1.67	0.753
		Middle secondary or secondary			0.54	0.33-0.86	0.010		0.53	0.33-0.86	0.010

		Diploma, college, and post-graduate		0.75	0.34-1.64	0.472		0.75	0.34-1.64	0.469	
	Maternal age (ref: 20-35)	<20	SES	473	1.53	0.64-3.64	0.334	470	1.65	0.68-4.01	0.272
		35<			2.02	1.16-3.50	0.012		1.77	0.996-3.13	0.052
Hypertensive disorders	Chronic hypertension		BQ during pregnancy, ethnicity, SES, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality	468	3.55	1.35-9.36	0.010	317	2.68	0.70-10.31	0.151
	PIH, PE/Eclampsia		The total effect cannot be estimated by covariate adjustment	452	4.79	2.48-9.26	<0.0001				
Obstetric Factors	Sex of the infant (ref: Male)		No adjustment is necessary	473	1.96	1.32-2.92	0.001				
	Nulliparity		Maternal age	472	1.26	0.86-1.87	0.237	472	1.50	0.98-2.30	0.061
Ethnicity	Ethnicity	Southern Bhutanese	No adjustment is necessary	473	1.47	0.98-2.20	0.064				
Altitude	Altitude between the current residence and the permanent residence (ref: 0-1000)	<0	No adjustment is necessary	448	0.81	0.46-1.42	0.453				
		1000-2000			1.18	0.72-1.95	0.513				
		>=2000			1.03	0.51-2.06	0.943				

Table 7.6. Results from the logistic regression models based on DAGs for PTB.

Exposure	category	Factors adjusted for	Complete case analysis							Data restricted to mothers with early scans only				
			n	Crude	95%CI	P-value	n	AOR	95%CI	P-value	n	AO R	95%CI	P-value
Maternal Potential Health Risk Behaviour	Betel quid chewing during pregnancy	<i>Covariates set 1: Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality</i>	507	1.16	0.80-1.67	0.433	333	1.20	0.72-2.00	0.481	279	1.17	0.66-2.05	0.594
		<i>Covariate set 2: BQ chewing before pregnancy, seasonality</i>	-	-	-	-	507	1.10	0.68-1.79	0.702	414	1.03	0.60-1.76	0.924
	Total number of betel nuts during the last 3 months of pregnancy (ref: No betel quid chewing)	<i>Covariate set 1: Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality</i>	455	1.19	0.78-1.81	0.420	299	0.85	0.47-1.54	0.586	247	0.76	0.39-1.47	0.411
		More than 90 nuts during the last 3 months or >1 nut per day	-	0.80	0.44-1.49	0.486	-	0.66	0.27-1.66	0.383	-	0.77	0.28-2.14	0.623
	Tobacco use during pregnancy	DAG 3: SES	507	2.38	1.25-4.51	0.008	502	2.14	1.12-4.11	0.022	411	1.64	0.73-3.68	0.233
	Grams of ST in the last 3 months of pregnancy (ref: did not use during pregnancy)	Less than 5 grams per day DAG 3: SES	502	2.39	1.07-5.32	0.033	497	2.11	0.93-4.81	0.076	409	1.71	0.64-4.61	0.287
	Alcohol use during pregnancy	DAG 3: BQ during pregnancy, Ethnicity, SES, chronic hypertension, maternal height,	514	1.46	0.97-2.19	0.073	337	1.53	0.89-2.65	0.126	282	1.61	0.89-2.90	0.117

			maternal age, pre-pregnancy weight, seasonality												
			DAG 3: Ethnicity, SES, regionality, seasonality	-	-	-	-	508	1.46	0.95-2.25	0.082	416	1.46	0.91-2.36	0.118
	Number of drinking days (ref: no drinking)	Less than or equal to once a week	DAG 3: BQ during pregnancy, Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, seasonality	509	1.50	0.90-2.49	0.121	333	2.16	1.11-4.19	0.023	279	2.44	1.22-4.89	0.011
		More than once a week		-	2.37	1.01-5.54	0.046		3.43	0.88-13.37	0.075	-	2.54	0.48-13.44	0.272
		Less than or equal to once a week	DAG 3: Ethnicity, SES, regionality, seasonality	-	-	-	-	503	1.54	0.90-2.64	0.116	412	1.62	0.91-2.89	0.174
		More than once a week		-	-	-	-	-	2.37	0.96-5.85	0.061	-	1.55	0.50-4.82	0.451
Nutritional Factors	GWG (ref: as per IOM recommendation)	High GWG	Covariate set 1: BQ during pregnancy, ethnicity, SES, chronic hypertension, imbalanced diet, maternal height, maternal age, regionality, seasonality	370	1.20	0.59-2.42	0.614	328	1.17	0.52-2.63	0.697	275	1.17	0.49-2.77	0.724
		Low GWG		-	2.61	1.52-4.50	0.001	-	2.15	1.14-4.04	0.018	-	2.59	1.28-5.25	0.008
		High GWG	Covariate set 2: Imbalanced diet, maternal height, pre-pregnancy weight	-	-	-	-	343	1.34	0.64-2.81	0.441	284	1.50	0.67-3.36	0.329
		Low GWG		-	-	-	-	-	2.23	1.26-3.93	0.006	-	2.77	1.45-5.29	0.002
Infectious Diseases	UTI		SES, seasonality	457	1.67	0.87-3.18	0.122	451	1.98	1.00-3.90	0.049	372	1.61	0.74-3.49	0.231

Hypertensive disorders	Chronic hypertension		BQ during pregnancy, ethnicity, SES, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality	499	4.35	1.75-10.78	0.002	333	3.23	1.01-10.30	0.047	279	3.48	0.99-12.14	0.051
	PIH, Pre-eclampsia/Eclampsia		Alcohol during pregnancy, BQ during pregnancy, ethnicity, chronic hypertension, imbalanced diet, maternal age, tobacco during pregnancy	482	7.83	4.24-14.44	<0.0001	460	7.08	3.58-14.00	<0.0001	383	8.97	4.05-19.87	<0.0001
Mode of Delivery	C-section		PIH, pre-eclampsia or eclampsia, chronic hypertension, gestational weight gain, infectious disease	514	1.83	1.26-2.64	0.001	329	2.16	1.26-3.70	0.005	268	1.81	0.99-3.34	0.056
Socio-economic status	Wealth quintile (Ref: Middle)	Poorest	Regionality	509	0.96	0.55-1.68	0.881	509	0.94	0.53-1.66	0.833	416	1.16	0.60-2.26	0.660
		Second		-	0.72	0.41-1.26	0.253	-	0.72	0.41-1.27	0.255	-	0.86	0.45-1.66	0.663
		Fourth		-	0.81	0.46-1.42	0.458	-	0.81	0.46-1.42	0.460	-	1.10	0.58-2.07	0.772
		Richest		-	0.61	0.34-1.07	0.084	-	0.60	0.34-1.06	0.078	-	0.79	0.42-1.50	0.471
		Education (Ref: No education)	Non-formal education	Regionality	513	0.55	0.24-1.23	0.145	513	0.56	0.25-1.27	0.165	420	0.61	0.23-1.58
	Primary		-	1.18	0.66-2.11	0.587	-	1.20	0.66-2.15	0.551	-	1.35	0.69-2.66	0.380	
	Middle secondary or secondary		-	0.89	0.56-1.39	0.596	-	0.89	0.57-1.39	0.604	-	0.99	0.59-1.65	0.955	
	Diploma, college, and postgraduate		-	0.83	0.38-1.79	0.634	-	0.82	0.38-1.78	0.623	-	0.98	0.43-2.22	0.953	

Maternal age	Maternal age (ref: 20-35)	<20	SES	514	2.43	1.17-5.03	0.017	508	2.37	1.13-4.98	0.023	416	2.09	0.86-5.07	0.102
		35<		-	1.72	1.01-2.93	0.047	-	1.70	0.97-2.95	0.062	-	1.63	0.85-3.14	0.144
Sex of the infant	Male infant		No adjustment is necessary	513	0.88	0.62-1.26	0.487	-	-	-	-	419	1.03	0.69-1.54	0.894
Ethnicity	Ethnicity	Southern Bhutanese	No adjustment is necessary	514	0.85	0.57-1.27	0.440	-	-	-	-	420	0.88	0.56-1.38	0.573
Altitude	Altitude between the current residence and the permanent residence (ref: 0-1000)	<0	No adjustment is necessary	484	0.93	0.56-1.55	0.782	-	-	-	-	396	1.02	0.58-1.78	0.958
		1000-2000		-	1.27	0.79-2.02	0.326	-	-	-	-	-	1.46	0.87-2.46	0.152
		>=2000		-	1.18	0.62-2.23	0.614	-	-	-	-	-	1.20	0.57-2.53	0.633

Table 7.7. Missing data imputation for term LBW under MAR and MNAR in comparison with complete case analysis.

Exposure	Category	Factors adjusted for	Complete case				MAR (n=473)			MNAR (ODDS ratio=0.5) (n=473)			MNAR (ODDS ratio=2) (n=473)		
			n	OR	95%CI	P-value	OR	95%CI	P-value	OR	95%CI	P-value	OR	95%CI	P-value
Betel quid chewing during pregnancy		<i>Covariate set 1: Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality</i>	317	1.30	0.74-2.27	0.361	1.40	0.90-2.19	0.140	1.38	0.89-2.16	0.151	1.39	0.89-2.17	0.145
		<i>Covariate set 2: BQ chewing before pregnancy, seasonality</i>	468	1.04	0.61-1.78	0.876	1.06	0.62-1.80	0.873	1.06	0.62-1.80	0.837	1.06	0.62-1.81	0.834
Tobacco use during pregnancy		<i>DAG 3: SES</i>	465	3.12	1.62-5.99	0.001	3.31	1.74-6.28	<0.0001	3.22	1.68-6.19	<0.0001	3.19	1.66-6.14	0.001
Alcohol use during pregnancy		<i>DAG3: BQ during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, seasonality</i>	320	1.96	1.10-3.49	0.022	2.08	1.30-3.32	0.002	2.08	1.31-3.31	0.002	2.07	1.30-3.30	0.002
GWG (ref: as per IOM recommendation)	High GWG	<i>Covariate set 1: BQ during pregnancy, ethnicity, SES, chronic hypertension, imbalanced diet, maternal height, maternal age, regionality, seasonality</i>	310	0.72	0.27-1.87	0.494	0.61	0.25-1.47	0.266	0.61	0.25-1.49	0.280	0.63	0.26-1.52	0.304
	Low GWG			2.62	1.33-5.15	0.005	2.18	1.20-3.96	0.011	2.16	1.18-3.97	0.013	2.19	1.28-4.17	0.016
	High GWG	<i>Covariate set 2: imbalanced diet, maternal height, pre-pregnancy weight</i>	316	0.92	0.37-2.29	0.859	0.65	0.28-1.53	0.327	0.65	0.28-1.54	0.332	0.68	0.29-1.57	0.364
	Low GWG			2.69	1.43-5.04	0.002	2.13	1.22-3.73	0.008	2.11	1.19-3.72	0.010	2.15	1.19-3.88	0.011

Chronic hypertension		BQ during pregnancy, ethnicity, SES, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality	317	2.68	0.70-10.31	0.151	4.24	1.44-12.42	0.009	4.12	1.39-12.17	0.010	4.05	1.39-11.81	0.010
UTI		SES, seasonality	433	2.17	1.11-4.26	0.023	2.23	1.15-4.33	0.018	2.20	1.14-4.28	0.019	2.23	1.15-4.31	0.018
Nulliparity		Maternal age	472	1.50	0.98-2.30	0.061	1.50	0.98-2.30	0.061	1.50	0.98-2.30	0.060	1.50	0.98-2.30	0.061
Wealth quintile (ref: Middle)	Poorest	Regionality	470	1.37	0.73-2.54	0.33	1.35	0.73-2.52	0.339	1.36	0.73-2.54	0.328	1.36	0.73-2.53	0.333
	Second			0.88	0.47-1.65	0.700	0.88	0.47-1.64	0.693	0.89	0.48-1.65	0.705	0.88	0.47-1.65	0.698
	Fourth			1.01	0.54-1.89	0.975	1.01	0.54-1.89	0.971	1.01	0.54-1.89	0.970	1.01	0.54-1.89	0.974
	Richest			0.73	0.39-1.37	0.320	0.73	0.39-1.37	0.323	0.73	0.39-1.38	0.332	0.73	0.39-1.37	0.330
Education (ref: No education)	Non-formal education	Regionality	473	0.59	0.27-1.29	0.188	0.59	0.27-1.31	0.196	0.59	0.27-1.31	0.196	0.59	0.27-1.31	0.196
	Primary			0.91	0.49-1.67	0.753	0.91	0.49-1.67	0.753	0.91	0.49-1.67	0.753	0.91	0.49-1.67	0.753
	Middle secondary or secondary			0.54	0.33-0.86	0.010	0.53	0.33-0.86	0.010	0.53	0.33-0.86	0.010	0.53	0.33-0.86	0.010
	Diploma, college, and post-graduate			0.75	0.34-1.64	0.472	0.75	0.34-1.64	0.469	0.75	0.34-1.64	0.469	0.75	0.34-1.64	0.469
Maternal age (ref: 20-35)	<20	SES	470	1.65	0.68-4.01	0.272	1.66	0.68-4.03	0.267	1.66	0.68-4.03	0.268	1.66	0.68-4.03	0.267

	35<			1.77	0.996-3.13	0.052	1.80	1.01-3.18	0.045	1.80	1.01-3.18	0.045	1.80	1.01-3.18	0.045
Sex of infant (ref: Male)		No adjustment is necessary	473	1.96	1.32-2.92	0.001	1.96	1.32-2.92	0.001	1.96	1.32-2.92	0.001	1.96	1.32-2.92	0.001
Ethnicity	Southern Bhutanese	No adjustment is necessary	473	1.47	0.98-2.20	0.064	1.47	0.98-2.20	0.064	1.47	0.98-2.20	0.064	1.47	0.98-2.20	0.064

*MNAR Model 1 assumes that the odds ratio of having UTI during pregnancy for mothers who were missing information on UTI was two times more than that of mothers who have information on UTI (OR=2). MNAR Model 2 assumes the odds ratio of having UTI during pregnancy for mothers who were missing information on UTI was 50% less than that of mothers who have information on UTI (OR=0.5). The imputation datasets were produced in SAS9.4 using MNAR and shift option and analysed in STATA 14.1.

Table 7.8. Missing data imputation for PTB under MAR and MNAR assumptions in comparison with complete case analysis.

Exposure	Category	Factors adjusted for	Complete case analysis				MAR (n=516)			MNAR (OR=0.5) (n=516)			MNAR (OR=2) (n=516)		
			n	AOR	95%CI	P-value	AOR	95%CI	P-value	AOR	95%CI	P-value	AOR	95%CI	P-value
Betel quid chewing during pregnancy		<i>Covariate set 1: Ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality</i>	333	1.20	0.72-2.00	0.481	1.21	0.81-1.81	0.343	1.21	0.81-1.81	0.343	1.21	0.82-1.81	0.461
		<i>Covariate set 2: BQ chewing before pregnancy, seasonality</i>	507	1.10	0.68-1.79	0.702	1.07	0.66-1.74	0.776	1.07	0.66-1.74	0.776	1.08	0.67-1.92	0.627
Tobacco during pregnancy		<i>DAG 3: SES</i>	502	2.14	1.12-4.11	0.022	2.23	1.17-4.27	0.015	2.23	1.17-4.26	0.015	2.23	1.17-4.27	0.015
Alcohol during pregnancy		<i>DAG 3 : BQ during pregnancy, ethnicity, SES, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, seasonality</i>	337	1.53	0.89-2.65	0.126	1.47	0.94-2.28	0.083	1.47	0.95-2.28	0.083	1.47	0.95-2.27	0.083
		<i>DAG 3: Ethnicity, SES, regionality, seasonality</i>	508	1.46	0.95-2.25	0.082	1.44	0.90-2.20	0.097	1.44	0.94-2.20	0.097	1.44	0.94-2.20	0.096
GWG (ref: as per IOM recommendation)	High GWG	<i>Covariate set 1: BQ during pregnancy, ethnicity, SES, chronic hypertension, imbalanced diet, maternal height, maternal age, regionality, seasonality</i>	328	1.17	0.52-2.63	0.697	1.03	0.51-2.09	0.936	1.03	0.51-2.09	0.936	1.02	0.49-2.11	0.958
	Low GWG		-	2.15	1.14-4.04	0.018	2.08	1.19-3.62	0.010	2.08	1.19-3.62	0.010	2.05	1.14-3.68	0.016
	High GWG	<i>Covariate set 2: Imbalanced diet, maternal</i>	343	1.34	0.64-2.81	0.441	1.06	0.54-2.10	0.857	1.06	0.54-2.10	0.857	1.07	0.53-2.14	0.857

		height, pre-pregnancy weight													
	Low GWG		-	2.23	1.26-3.93	0.006	2.17	1.29-3.66	0.004	2.17	1.29-3.66	0.004	2.15	1.24-3.73	0.006
UTI		SES, seasonality	451	1.98	1.00-3.90	0.049	1.81	0.93-3.53	0.081	1.81	0.93-3.53	0.081	1.79	0.90-3.55	0.097
Chronic hypertension		BQ during pregnancy, ethnicity, SES, maternal height, maternal age, pre-pregnancy weight, regionality, seasonality	333	3.23	1.01-10.30	0.047	4.73	1.83-12.19	0.001	4.73	1.83-12.19	0.001	4.18	1.60-10.87	0.003
PIH, PE/Eclampsia		Alcohol during pregnancy, BQ during pregnancy, Ethnicity, chronic hypertension, imbalanced diet, maternal age, tobacco during pregnancy	460	7.08	3.58-14.00	<0.0001	8.00	4.27-15.00	<0.0001	8.00	3.27-15.00	<0.0001	7.08	3.78-13.26	<0.0001
C-section		PIH_PE/E, chronic hypertension, gestational weight gain, UTI	329	2.16	1.26-3.70	0.005	1.41	0.91-2.19	0.122	1.36	0.87-2.11	0.175	1.46	0.94-2.27	0.090
Wealth quintile (ref: Middle)	Poorest	Regionality	509	0.94	0.53-1.66	0.833	0.96	0.54-1.70	0.894	0.96	0.54-1.70	0.894	0.97	0.55-1.71	0.906
	Second		-	0.72	0.41-1.27	0.255	0.74	0.42-1.29	0.286	0.74	0.42-1.29	0.286	0.74	0.42-1.30	0.300
	Fourth		-	0.81	0.46-1.42	0.460	0.83	0.47-1.45	0.508	0.83	0.47-1.45	0.508	0.82	0.47-1.44	0.494
	Richest		-	0.60	0.34-1.06	0.078	0.61	0.35-1.08	0.090	0.61	0.35-1.08	0.090	0.61	0.35-1.09	0.094
Education (ref: No education)	Non-formal education	Regionality	513	0.56	0.25-1.27	0.165	0.51	0.22-1.18	0.116	0.51	0.22-1.18	0.116	0.51	0.22-1.18	0.116

	Primary		-	1.20	0.66-2.15	0.551	1.20	0.66-2.15	0.540	1.20	0.67-2.16	0.540	1.20	0.66-2.15	0.550
	Middle secondary or secondary		-	0.89	0.57-1.39	0.604	0.89	0.56-1.39	0.613	0.89	0.57-1.40	0.613	0.89	0.57-1.40	0.616
	Diploma, college, and postgraduate		-	0.82	0.38-1.78	0.623	0.82	0.38-1.77	0.615	0.82	0.38-1.77	0.615	0.82	0.38-1.77	0.619
Maternal age (ref: 20-35)	<20	SES	508	2.37	1.13-4.98	0.023	2.34	1.11-4.91	0.025	2.34	1.11-4.91	0.025	2.34	1.11-4.90	0.025
	35<		-	1.70	0.97-2.95	0.062	1.76	1.02-3.06	0.044	1.76	1.02-3.06	0.044	1.77	1.02-3.06	0.043
Male infant		No adjustment is necessary	513	0.88	0.62-1.26	0.487	0.88	0.62-1.26	0.498	0.88	0.62-1.26	0.498	0.88	0.62-1.26	0.492
Ethnicity	Southern Bhutanese	No adjustment is necessary	514	0.85	0.57-1.27	0.440	0.87	0.58-1.29	0.478	0.87	0.58-1.29	0.478	0.86	0.58-1.29	0.477

*MNAR Model 1 assumes that the odds ratio of having UTI during pregnancy for mothers who were missing information on UTI was two times more than that of mothers who have information on UTI (OR=2). MNAR Model 2 assumes the odds ratio of having UTI during pregnancy for mothers who were missing information on UTI was 50% less than that of mothers who have information on UTI (OR=0.5). The imputation datasets were produced in SAS9.4 using MNAR and shift option and analysed in STATA 14.1.

7.3 Anaemia and betel quid chewing

In this section, anaemia was analysed as a secondary outcome using the DAG approach.

7.3.1 Background

The evidence of anaemia and betel quid is mixed in the literature. As a secondary outcome, the relationship between anaemia and betel quid chewing was examined. First, two conditions suggested by Cousens et al. [5] when using data collected during a case-control study for another outcome, were checked.

The first condition is that the effect of betel-quid chewing on the mother's propensity to report for LBW or PTB is independent of any effect of anaemia status. As this was a case-control study and almost all the preterm weight and/or LBW babies were approached in the referral hospitals, the anaemia status is less likely to influence the mother's propensity to report LBW or PTB babies. The Mantel-Haenszel test was conducted to examine the strata specific odds ratios after being stratified by anaemia status. The test suggests that the strata specific ORs are similar ($p=0.4317$).

The second condition is that the effects on PTB or LBW of the betel quid chewing and anaemia do not interact. The interaction term was examined ($p=0.432$). The data suggest that there is no evidence of an interaction between the effect of betel quid and anaemia.

As described in Chapter 3, season of delivery was adjusted based on the assumed causal framework provided in DAG 4 and season of delivery and SES were adjusted based on the assumed casual framework in DAG 5. Although there seemed to be no causal effect between SES and betel quid chewing in Bhutan, DAG 5 was compared to DAG 4 to see the plausibility of causal assumptions in DAG 4. As was suggested in the literature, smoking and altitude were included in the DAGs. Physiological haemoglobin needs are greater at high altitude due to the lower concentration of oxygen in the atmosphere and smoking increases haemoglobin concentrations [6].

7.3.2 Results

The results based on the causal assumptions in DAG 4 and DAG 5 were virtually identical (Table 7.9). Betel quid chewing during pregnancy was associated with increased odds of anaemia during pregnancy. Compared to those who did not chew betel nuts, mothers who chewed during pregnancy had a 2.2 times higher odds ratio of being anaemic (AOR 2.16, 95% CI 1.31-3.58, $p=0.003$) adjusting for season of delivery and (aOR 2.2, 95% CI 1.31-3.64, $p=0.003$) adjusting for season of delivery, education, and wealth quintile. Compared to non-chewers, mothers who chewed more than 1 nut or 4 quids had a 3 times higher odds ratio of being anaemic (aOR 3.41, 95% CI: 1.81-6.42, $p<0.0001$) adjusting for season of delivery and (aOR 3.46, 95% CI: 1.82 – 6.58, $p<0.0001$) adjusting for season of delivery, education, and wealth quintile in Model 2.

Table 7.9. Anaemia and betel quid based on DAG 4 and DAG 5.

Exposure	Category	Factors adjusted for	N	Crude OR	95% CI	P-value	N	AOR	95% CI	P-value
Model 1: Chewing betel quid during pregnancy		DAG 4: Seasonality	649	2.09	1.27-3.43	0.004	649	2.16	1.31-3.58	0.003
		DAG 5: Seasonality, SES		-	-	-	643	2.19	1.31-3.64	0.003
Model 2: Average number of betel nuts per day during pregnancy (ref: None)	<= 1nut	DAG 4: Seasonality	594	1.69	0.94-3.02	0.078	594	1.75	0.97-3.14	0.063
	more than 1 nut per day		-	2.99	1.62-5.51	<0.0001		3.41	1.81-6.42	<0.0001
	<= 1nut	DAG 5: Seasonality, SES		-	-	-	588	1.78	0.98-3.24	0.058
	more than 1 nut per day			-	-	-		3.46	1.82-6.58	<0.0001

7.4 Summary

In this chapter, both the statistical approach and DAG approach were used to build logistic regression models in order to estimate the impact of potential risk factors on adverse birth outcomes. Comparing the results in the two approaches suggests that results in the statistical approach may be biased due to the over-adjustment or unnecessary adjustment of covariates. Using the DAG approach, three sensitivity analyses were conducted: limiting data to mothers with early US scans, missing data imputation under MAR and MNAR. Results in the complete case and sensitivity analyses were virtually identical. In the logistic regression models, there was no clear evidence of an association between betel quid chewing and adverse birth outcomes. For the secondary outcome, the data suggest an association between anaemia and betel quid chewing during pregnancy.

Using the DAG approach, tobacco and alcohol use during pregnancy, low GWG, and UTI showed a clear association with term LBW and PTB. The factors that show clear evidence of association with term LBW were female infant and middle secondary education compared to no education. On the other hand, being under 20 years old compared to 20-35 years old and having PIH, pre-eclampsia, or eclampsia was clearly associated with PTB. Chronic hypertension shows a clear association with PTB. The association between chronic hypertension with term LBW was clearer in the multiple imputation models, suggesting loss of information in the complete case analysis due to a large amount of missing data. The association between term LBW and PTB and mothers over 35 years old compared to 20-35 years old was clearer in the multiple imputation models than complete case analysis. The results will be discussed in detail in the next chapter.

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Chapter 8

Conclusions and Discussions

In the following sections (Section 8.1-8.3), the key results are highlighted and compared with previous studies and the implications for policy and practice are discussed. In Chapter 7, both the statistical approach and DAG approach were used to build logistic regression models in order to estimate the impact of potential risk factors on adverse birth outcomes. Neither approach provided clear evidence of an association between betel quid use and term LBW or PTB, at the level of consumption of 5.5 quids or 1.4 nuts per day. Comparing the results between the two approaches suggests that results in the statistical approach may be biased due to the over-adjustment or unnecessary adjustment of covariates. Using the DAG approach, three sensitivity analyses were conducted; limiting data to mothers with early US scans, missing data imputation under MAR and under MNAR. Results in the complete case and sensitivity analyses were almost identical.

For the secondary outcome, the data suggest an association between anaemia and betel quid chewing during pregnancy. Using the DAG approach, tobacco and alcohol use during pregnancy, low GWG, and UTI showed a clear association with term LBW and PTB. The factors that show clear evidence of association with term LBW were female infant and middle secondary education compared to no education. On the other hand, being younger than 20 years old compared to 20-35 years old and having PIH, pre-eclampsia, or eclampsia was clearly associated with PTB. Chronic hypertension shows a clear association with PTB. The association between chronic hypertension and term LBW was clearer in the multiple imputation models, suggesting loss of information in the complete case analysis due to a large amount of missing data. The association between term LBW and PTB and mothers older than 35 years old compared to 20-35 years old was clearer in the multiple imputation models than in the complete case analysis. The limitations of the research findings are discussed in Section 8.4. The chapter ends with recommendations for future research in Section 8.5. and conclusion in Section 8.6.

8.1 Primary findings from analyses of potential modifiable risk factors

8.1.1 Betel quid chewing during pregnancy

(a) A summary of key findings

Neither the statistical approach nor DAG approach provided clear evidence of an association between betel quid use and LBW or PTB, at the level of consumption of 5.5 quids or 1.4 nuts per day.

In the DAG, it was hypothesised that betel quid chewing during pregnancy has both a direct and indirect impact on term LBW and PTB. It was assumed that seasonality causes betel quid chewing before and during pregnancy and betel quid chewing during pregnancy is associated with the adverse birth outcomes indirectly through reduced appetite, increased periodontal disease, and increased micronutrient deficiency, whereas betel quid chewing before pregnancy has an impact on

chronic hypertension. The detailed causal assumptions were graphically presented in the DAGs in Chapters 2 and 3. Covariates adjusted in the logistic regression to estimate the total effect of betel quid chewing during pregnancy, informed by the DAG, were ethnicity, SES (education and wealth quintile), chronic hypertension, maternal height, maternal age, pre-pregnancy weight, regionality (delivery hospital), and season of delivery.

The estimated odds ratio of term LBW was slightly smaller and 95% CIs were slightly wider in the statistical approach compared to the DAG approach. This could be due to over-adjustment of factors that could stand on the casual pathway between betel quid chewing and adverse birth outcomes such as GWG. For example, using the statistical approach, the adjusted odds ratio (aOR) of term LBW was 1.07 (95% CI: 0.54-2.13, $p=0.845$) while the aOR of term LBW was 1.30 (95% CI: 0.74-2.27, $p=0.439$) in the DAG approach.

Using the DAG approach, three sensitivity analyses were conducted; limiting data to mothers with early US scans, missing data imputation under MAR and under MNAR. Results in the complete case and sensitivity analyses were virtually identical. Hence, the results of the complete case analysis of the DAG approach are reported below.

The aOR of PTB in association with betel quid chewing during pregnancy was 1.20 (95% CI: 0.72-2.00, $p=0.614$). When the total number of betel nuts consumed during the last three months of pregnancy was used as an exposure variable, the aOR among mothers who consumed less than or equal to one nut per day compared to the mothers who did not chew during pregnancy was 1.32 for term LBW (95% CI: 0.69-2.51) and 0.85 for PTB (95% CI: 0.47-1.54). The aOR for mothers who consumed more than one nut per day was 1.39 for term LBW (95%:0.52-3.68) and the aOR of PTB was 0.66 (95% CI: 0.27-1.66).

The observed difference in betel quid chewing between cases and controls was small and not statistically significant. Of the 669 study participants, 55% of the case mothers and 52% of the control mothers chewed betel quid during pregnancy. About 22% of cases and controls used commercial betel products during pregnancy. In total, 60% of the case mothers and 57% of the control mothers chewed either betel quid or packaged betel products during pregnancy

Other surveys and reports conducted in Bhutan reported similar prevalence. According to the 2010 Gross National Happiness Survey, 72% of respondents aged 15 to 98 had ever chewed betel quid in their lives and 59% of men and 62% of women were currently chewing [1]. The National Nutrition Survey in 2015 reported that 43% of 148 pregnant women in the survey reported consuming betel nuts regularly during the week before the survey and 50% reported that had they chewed betel in the first trimester [2].

In this study, there was no statistically significant difference between users and non-users in terms of demographic factors such as maternal age, highest level of education attained, wealth quintile, pre-pregnancy BMI, and GWG. Of the mothers who chewed betel during pregnancy ($n=359$), the majority (76%) chewed weekly or more than weekly with 41% reporting daily use, and 70% of the mothers who chewed during pregnancy chewed after meals. This confirms past literature which suggests that the users of betel nut believe it is helpful for the digestion [3]. On

average, mothers consumed 5.5 quids (95% CI: 4.7-6.3) or approximately 1.4 nuts per day. Almost half (47 %) always spit after chewing versus 19% never-spitting.

Adding tobacco to betel quid was not common in Bhutan. The majority used one quarter of a ripe nut and chewed it combined with piper leaf and lime. On the other hand, more mothers who chewed betel quid used other substances such as alcohol, pan masala, cigarettes, and smokeless tobacco compared to the mothers who did not chew during pregnancy. In Bhutan, the sale of tobacco was banned in 2004 and this ban was legislated for and strengthened by the Tobacco Act of 2010 [4]. This policy may account for the low prevalence of cigarette smoking during pregnancy in Bhutan.

It should be noted that the study sample size was designed to be able to show an odds ratio of at least 1.5 based on the assumption that 60% of cases and 50% of controls had been exposed. However this assumption of a 10% difference in betel quid chewing between cases and controls did not hold. The actual observed difference in the study was very small (52% in the controls and 55% in the cases), resulting in a large p-value for the association between betel chewing and term LBW or PTB. Therefore, there was no evidence for an association between betel chewing and adverse birth outcomes. However, this is not evidence of no association. The 95% C.I. around the OR provides an indication of how large any association might be. In the present study, the 95% CIs were 0.74-2.27 for term LBW and 0.72-2.00 for PTB.

Among the singleton live born babies delivered at the three referral hospitals during the study period, the proportion of babies born LBW and PTB could be estimated as 8.5% (464/5472) and 5.5% (302/5472) respectively. Given that the proportions of LBW and PTB are relatively small, the "rare" disease assumption is met and the odds ratios provide an estimate of the relative risk. To examine the odds ratio in the context of the relative and absolute risk, if the risk of LBW in the general singleton live born infants is 8.5%, the 1.3 times increased risk with betel quid chewing during pregnancy would result in an incidence in 11.1%, a 2.6 % increase. Similarly, if the risk of PTB in the general singleton live born infants is 5.5%, the 1.2 times increased risk with betel quid chewing during pregnancy would result in an incidence of 6.6%, a 1.1% increase.

(b) Comparison with other observational studies

Association with birth outcomes

As described in Chapter 2, the pooled crude RRs from the meta-analysis using the random effect model for LBW suggests slightly increased odds of LBW. However, this was not statistically significant (RR: 1.20, 95% CI: 0.81 - 1.80). This is similar to the crude odds ratio for term LBW obtained in the present study (1.10, 95% CI: 0.74 -1.63).

There was a high heterogeneity across studies ($I^2 = 83.2\%$, $p < 0.0001$) (Figure 2.1). The estimates of effect size in the meta-analysis and corresponding 95% CIs were 0.94 (95%CI: 0.79-1.27) (Ome-Kaius et al. (2015)) [5], 0.92 (95% CI: 0.80-1.06) (Chue et al. (2012)) [6], 1.70 (95% CI: 0.45-6.40) (Senn et al. (2009))[7], and 1.95 (95% CI: 1.41-2.71) (Yang et al. (2008)) [8].

To understand why there might be differences in the results, the details of similarities and differences between this study and previous studies are discussed below. Studies were compared in terms of characteristics of the study populations, patterns and level of consumption of betel quid chewing, timing of measurement, and covariates adjusted in the model. Association between betel quid chewing and anaemia is also discussed.

(i) Characteristics of the study populations

Differences in sample size and study population may account for the differences in the results. In the present study, the total number of women included in the logistic analysis was 473 for term LBW and 513 for PTB. The largest study was by Chue et al. (2012) [6] and included 7,685 refugee pregnant women, followed by 2,700 in the Ome-Kaius et al. study (2015) [5], 1,264 in the Yang et al. study (2008) [8] and 310 in the Senn et al. study (2009).

In the present study, there was no statistically significant difference between chewers and non-chewers by socio-economic characteristics in the descriptive analyses. This was similar to the Senn et al. (2009) study [7]. In the Ome-Kaius et al.(2015) study, more areca (betel) nut chewers tended to reside in rural areas and pursue an income-generating activity but numbers were similar for literacy rate, maternal age, and primigravida [5]. However, in the Yang et al. study, the chewers were more likely to have a lower educational level, be unmarried, or be unemployed $p<0.0001$ [8]. In the Chue et al. (2012) study, chewers were older and more chewers were multigravida compared to non-chewers ($p<0.001$)[6]. They did not provide information on maternal education level or SES of the study participants. In the present study, like the Chue et al. (2012) study [6], there was no statistically significant difference of BMI. In Taiwan, the pre-pregnancy BMI was higher among chewers (non-chewers: 22.3 vs 24.4, $p<0.0001$) whereas maternal GWG during pregnancy was lower among chewers (non-chewers: 15.3 kg (SD=6.1) vs 13.6 kg (SD=7.1), $p<0.0001$) [8].

(ii) Patterns and level of consumption of betel quid chewing

The differences in the patterns and level of consumption do not provide a clear explanation for the differences in the results. In Bhutan ripe nuts are preferred as on the Thai-Myanmar border [6], unlike PNG [5, 7] and Taiwan [8] where unripe nuts are more often consumed. The level of consumption in Bhutan was lower (on average 5.5 quids or 1.4 nuts per day) than PNG (44% chewed more than 5 nuts per day in the Senn et al.(2009) study [7] and 47.8% chewed more than 3 nuts per day in the Ome-Kaius et al. (2015) study[5]). It was, however, similar to Taiwan (the prevalence rate of betel quid chewing during pregnancy was 36.7% with a daily average of 5.68 quids consumed among the chewers in the Yang et al. (2008) study [8]) and the patterns of Karen and Burmese pregnant women at the Thai-Myanmar border [6]. At the Thai-Myanmar border [6] , more than 50% (53.9%) of the women used less than one whole nut per day and 31.6% used one to four whole nuts per day [6]).

Adding tobacco to betel quid chewing was rare in Bhutan like the rest of the studies and thus would not account for the differences.

Tobacco use (cigarette smoking and smokeless tobacco) and alcohol use were more common among chewers than non-chewers in Bhutan. This was similar to PNG [5, 7] and Taiwan. There was not enough information to assess if alcohol may account for discrepancies. Information on alcohol was not collected in Thailand as alcohol was banned in the refugee camp where the study was conducted [6].

(iii) Timing of assessing betel nut use

The timing of assessing betel nut use may account for the discrepancies. The large studies in Thailand [6] and in PNG [5] did not report any evidence of association. As discussed in Chapter 2, these two studies enquired about mother's betel nut use at the first ANC clinic and assumed they did not stop during pregnancy. This could lead to biasing the effect of betel quid chewing towards the null. The present study aimed to overcome this methodological challenge by collecting information on consumption over the past 10 months at delivery. As a result, the crude OR in the present study suggested slightly increased odds of term LBW whereas crude ORs in Thailand and in PNG suggest reduced odds. The 95% CI was slightly wider in the present study. This could be due to the small sample size of the present study compared to the other two studies.

(iv) Covariates adjusted in the models

Differences in covariates may explain the differences in the results. As described in Chapter 2, most of the previous studies seemed to use a statistical approach such as the stepwise approach, and covariates controlled in the model were highly heterogeneous.

For example, all studies controlled for parity and some maternal anthropometric characteristics such as weight, height, or BMI. Smoking was controlled for in the Chue et al. study (2012) and the Ome-Kaius et al. study (2015) but not in the Yang et al. (2008) and Senn et al. (2009) studies. In PNG, malaria was controlled for in the Ome-Kaius study but not in the Senn et al. (2009) study. Yang et al. (2008) and Ome-Kaius et al. controlled for some socioeconomic characteristics whereas Senn et al. (2009) and Chue et al. (2012) did not control. Gestational weeks was controlled for in Yang et al (2008) and Chue et al. (2012) not in the PNG studies. Maternal age and GWG were only adjusted in the Yang et al. (2008) study. No studies controlled for hypertensive disorders or season of delivery.

In the study by Yang et al. (2008), nine variables were included (parity, pre-pregnancy BMI, maternal age, marital status, education level, employment status, gestational weeks, weight gain during pregnancy, maternal drug use, and newborn sex). In Chue et al. (2012), six variables were included (primigravida, maternal weight at the first ANC visit, timing of the first ANC visit after the first trimester, malaria infection, smoking, gestational age at birth (weeks)). Ome-Kaius et al. (2015) controlled for nine covariates (primigravida, mother's height, mid-

upper arm circumference of the mothers, smoking, ethnic group (highlander), income, frequency of antenatal visits, malaria, and receipt of insecticide treated bed net). Senn et al. (2009) controlled for primigravida and low BMI for birth weight analysis but did not specify the covariates used to estimate the effect on LBW.

In the present study, maternal age, ethnicity, SES (education and wealth quintile), chronic hypertension, maternal height, pre-pregnancy weight, delivery hospital, and season of delivery were adjusted in the logistic regression model based on the causal assumptions in the DAGs. Primigravida was controlled for in all studies but not in the present study. Instead, maternal age was controlled for as it was assumed to have a direct causal relationship on primigravida, which is associated with increased odds of term LBW in the literature but not with PTB. GWG was not controlled for as it was assumed to stand on the causal pathway through reduced appetite due to betel quid chewing. Gestational weeks was not included as the outcome was restricted to term LBW. Malaria prevalence was low in Bhutan. Chronic hypertension was not adjusted for in any of the studies. Association between hypertension and betel quid chewing is not well-studied in the literature. A systematic review by Yamada et al. (2013) identified two studies that reported an association between betel quid chewing and high blood pressure between 1951 and January 2013 [9]. There were no studies on pregnancy-induced hypertension. Ome-kaius et al. (2015) reported that there was no statistical difference in the mean arterial pressure (mmHg) among chewers and non-chewers ($p=0.41$)[5]. De Costa (1982) reported 39/400 of chewers presented signs of preeclampsia not requiring drug treatment compared to 45/400 among non-chewers matched on parity and province of origin. Preeclampsia requiring drug treatment was excluded from this study [10]. Other studies did not provide any information on hypertensive disorders during pregnancy [6, 11-13].

Several studies suggest that betel quid chewing is associated with increased odds of periodontal diseases as described in Chapter 2. Periodontal diseases were included in the DAG as an unmeasured factor in the present study and it is worth examining the impact of betel quid on periodontal diseases and association with adverse birth outcomes.

(v) Association between anaemia and betel quid chewing

Although the multivariable analyses did not provide clear evidence of the impact of betel quid on term LBW or PTB, the data suggest betel quid chewing is associated with increased odds of anaemia. Compared to those who did not chew betel nuts, mothers who chewed during pregnancy had 2.1 times higher odds of being anaemic (aOR 2.09, 95% CI 1.27-3.43, $p=0.004$). Compared to non-chewers, mothers who chewed more than 1 nut or 4 quids had 3 times higher odds of being anaemic (aOR 2.99, 95% CI: 1.62-5.51, $p < 0.0001$).

Two previous studies have reported an association between betel quid chewing and maternal anemia [5, 14]. Ome-Kaius et al. (2015) reported pregnant chewers were more likely to be anaemic (haemoglobin < 11 g/dL) at delivery than non-chewers (aOR 1.67, 95% CI: 1.27-2.20, $p < 0.001$) [5]. Prior to this study, two studies in PNG reported no association between

anaemia and betel quid chewing. Senn et al. (2009) reported that mean haemoglobin level was 94 g/l (95% CI 92-96) slightly lower among chewers and 100 g/l (95% CI 95-106) among non-chewers but not statistically significant. This could be due to the small sample size and high prevalence of betel chewing (94%). De costa (1982) reported that slightly more chewers (48%, 192/400) had a haemoglobin value of less than 10 g/100ml on at least one occasion compared to non-chewers matched on parity and province of origin (46%, 184/400) but the difference was not statistically significant. This could be due to baseline differences between chewers and non-chewers. Chue et al. (2012) reported that chewers were more anaemic (defined as Haematocrit <30%) than non-chewers (non-chewers:17.3% [425/2,459] vs chewers: 19.4% [868/4,422], $p=0.031$) in the univariate analysis but no association was apparent after controlling for smoking, malaria, multigravida, anaemia at first ANC visit, and first ANC visit after the first trimester. Anaemia was treated at each ANC visit. However, using the same population, Stuetz et al. (2016) reported that daily betel quid chewing had a negative effect on haemoglobin level (g/L) after adjusting for smoking, parity and BMI at the time of sampling (Beta -2.90., 95% -4.62 to -1.16). Anaemia treatment was not controlled for. Differences in classification of betel users (users/nonusers vs daily use) and covariates in the models might account for these discrepancies. Chue et al. could be adjusting for factors on the causal pathway such as anaemia at first ANC visit after the first trimester.

There are several potential pathways for betel quid causing maternal anaemia. One of the pathways is through suppressed appetite. Several studies suggest that betel nut chewers had a higher resting metabolic rate due to betel nut metabolites that effect the thermoregulatory pathways, altering the thermogenic effects of the meal and also through centrally mediated effects by decreasing the appetite for food [3]. Another study suggests betel nut chewing could aggravate the effects of vitamin-D deficiency [15]. Although causation between vitamin-D deficiency and anaemia is not established, a meta-analysis of observational studies suggests vitamin-D deficiency is associated with anaemia [16, 17].

In the present study, 12.4% of the mothers were identified to be anaemic from medical records during pregnancy. Anaemia was defined as haemoglobin level less than 10g/dL by the interviewers from antenatal records and medical records; altitude was not controlled for as details of haemoglobin were not collected in the present study. The 2015 National Nutrition Survey (NNS), which measured haemoglobin level using a Hemocue 301 and recorded to the nearest 0.1 g/dL, reported much higher prevalence of anaemia among pregnant women (27.3%) after adjusting for the altitudes of each chiwog (a basic electoral precinct) surveyed [2]. The lower anaemia prevalence in the present study could be because altitude was not adjusted for, which led to underestimated anaemia prevalence.

(c) Summary

In the present study, the results suggest that there is no evidence of an association between term LBW or PTB and betel quid chewing during pregnancy. This could be due to the small observed

difference in betel quid consumption between cases and controls. For a secondary outcome, the data suggest betel quid chewing is associated with increased odds of anaemia.

8.1.2 Alcohol

There was clear evidence of an association between drinking and term LBW or PTB. Mothers who drank during pregnancy had 1.96 times higher odds of term LBW compared to non-drinkers after adjusting for betel quid chewing during pregnancy, wealth quintile, education, chronic hypertension, maternal height, maternal age, pre-pregnancy weight, and seasonality (95% CI: 1.10-3.49, $p=0.022$). Mothers who drank more than once a week had 3.43 times higher odds of PTB (95% CI: 0.88-13.37, $p=0.075$) compared to non-drinkers. Mothers who drank less than or equal to once a week had 2.16 higher odds of PTB (95% CI: 1.11-4.19, $p=0.023$).

Of the study participants, 27% of the mothers reported drinking alcohol during pregnancy. About 38% of the pregnancy drinkers had a heavy drinking episode (defined as more than 40 grams of ethanol per one drinking occasion) at least once during pregnancy. Pregnancy drinking was more common among the mothers who delivered at the Eastern Region Referral Hospital (ERRH) (37%) compared to JDWNRH in the capital (27%) and the Central Region Referral Hospital (CRRH) in the south (13%) ($p<0.0001$). Mean maximum level of ethanol grams per occasion was higher at the Eastern Referral Hospital (ERRH) (mean 79.8 grams, SD 99.6) than JDWNRH (mean 46.6 grams, SD 47.x) and CRRH (39.4 grams, SD 27.9). This suggests that prevalence of drinking and intensity of drinking are higher in the eastern region.

These results are comparable with other studies such as the NNS 2015 [2], a study in eastern Bhutan[18], and a population survey on risk factors for non-communicable disease using the WHO Stepwise Non-communicable Disease Risk Factor Survey (STEPS) method conducted in 2014[19].

The NNS 2015 showed that 16% of 148 pregnant women reported consuming alcohol regularly during the week before the survey and 23.9% reported they drank once or twice a week in the second trimester [2].

The research article showed that prevalence of alcohol consumption among women aged between 26 and 40 years old was 30% and prevalence of high intensity drinking (>40 grams) was 18.5% [18]. The research article used a tri-level method where an interviewee was asked about up to three different kinds of types of beverage and amount in millilitres for maximum, medium, and low level.

In the STEPS survey, data from 1,748 female respondents aged between 18 and 69 years showed that 32.8% were current drinkers (past 30 days) and 10.3% of the current drinkers reported a high level of alcohol intake (≥ 40 grams per occasion) [19]. The STEPS survey reported the lowest percentage of mothers of high intensity drinking. This could be due to a methodological difference as the survey asked the number of “standard drinks” participants consumed and quantified the response without taking different kinds of alcoholic drinks into account. The present study asked about the maximum number of alcoholic drinks for each different kind of drink per one occasion

and quantified grams of ethanol. The higher prevalence of heavy episodic drinking in the present study could be because more than half of the sample comprised mothers with adverse birth outcomes.

In the present study, a Fractional Graduated Frequencies (F-GF) measure proposed by Greenfield et al.[20] was adapted to overcome the difficulty in measuring the volume of home-brewed alcohol and unavailability of a standard drink. Compared to a population survey conducted in northern Goa [20], number of ethanol grams at each level of drinking was slightly lower (Table 8.1). The population difference (general male drinkers vs pregnant female drinkers) may account for some of the observed differences. Overall, the results were comparable and it may suggest that the F-GF is a practical tool to describe prevalence and intensity of alcohol where there is no standard drink size.

Table 8.1. Comparison of mean ethanol (ETOH) in grams using F-GF measure for the maximum amount, ¾, ½ and ¼ amount.

Study population	Present study		Greenfield et al. (2010) [20]	
	Mean (SD) quantity grams ETOH	Percent of total volume	Mean (SD) quantity grams ETOH	Percent of total volume
Among 177 women who drank during pregnancy interviewed at delivery in the three referral hospitals			Among 743 male drinkers in urban and rural cities in northern Goa	
The maximum	54.1(64.2)	40.7%	65.8(56.9)	56.10%
¾ Maximum	40.6(48.2)	29.8%	49.4(42.6)	12.10%
½ Maximum	27.0(32.1)	20.2%	32.9(28.4)	24.40%
¼ Maximum	13.5(16.1)	9.3%	16.5(14.2)	7.40%

In addition to increased risk of LBW and PTB, maternal exposure to heavy drinking can lead to foetal alcohol syndrome (FAS). The royal government of Bhutan initiated birth defect surveillance on 1 January 2015 to collect information on number of stillbirths, birth defects including Down syndrome, congenital heart defects, and FAS [21]. According to the report, prevalence of FAS was 1.47 per 1000 births, and was more prevalent in births reported from ERRH (4/812). This coincides with the findings of the present study that show higher prevalence of pregnancy drinking and higher intensity of drinking among the mothers who delivered at ERRH.

8.1.3 Tobacco

Although the prevalence of tobacco consumption was low, there was clear evidence of an association between tobacco use and term LBW and PTB.

Mothers who used smokeless tobacco and/or smoked cigarettes during pregnancy had 3.04 times higher odds of term LBW (95% CI: 1.36-6.00, p=0.007) after adjusting for wealth quintile

and maternal education, compared to mothers who did not use tobacco. The aOR of PTB was 2.14 (95% CI: 1.12-4.11, $p=0.022$). The confidence intervals were wider and the ORs were larger, compared to the systematic reviews which reported an association between PTB and any maternal smoking (OR 1.39, 95%CI 1.01-1.91) [22] and smokeless tobacco (OR 1.39, 95% CI:1.21-1.33) [23]. This could be due to the small sample size or due to some recall biases such as underreporting in the controls compared to the cases introduced in the case-control study design, which may lead to the overestimated point estimates.

The prevalence of tobacco consumption was low. Smoking during pregnancy or use of smokeless tobacco was only 10.2% across all study participants. Cigarette smoking was less than 3% (2.8%), while smokeless tobacco use was 7.5%. The majority (98%) of the users of smokeless tobacco did not smoke cigarettes. On average, mothers who used tobacco consumed three cigarettes per day or on average 2.4 grams of smokeless tobacco per day during pregnancy. The maximum amount of smokeless tobacco used was 5 grams per day on average during the last three months of pregnancy.

The low cigarette smoking was comparable to the results in the 2011 report [4]. Most of the mothers quit cigarette smoking before the last three months of pregnancy. More mothers of LBW and/or PTB neonates used smokeless tobacco compared to the control mothers (control 4.1% [13/321] vs case 10.6% [37/348], $p=0.001$). The observed difference of prevalence of cigarette smoking during pregnancy in the cases and controls was small and not statistically significant (controls 1.6% [5/321] vs cases 4.0% [14/348], $p=0.055$).

8.1.4 Low gestational weight gain and imbalanced diet

This study showed clear evidence of an association between GWG and adverse pregnancy outcomes.

Mothers who had a lower GWG than recommended by the IOM, according to pre-pregnancy BMI, had 2.67 times higher odds (aOR 2.67, 95% CI: 1.38-5.19) of delivering a term LBW and 2.31 times higher odds (aOR 2.31, 95% CI:1.25-4.27) of PTB compared to those with GWG as per IOM recommendations, after adjusting for betel quid chewing during pregnancy, ethnicity, wealth quintile, education, chronic hypertension, imbalanced diet, maternal height, maternal age, delivery hospital, and season of delivery.

Although the IOM recommendation was the most accepted guidance in the literature [24], it was derived from studies in high-income countries where there is a greater problem of excessive GWG and therefore the results may need to be re-examined using a chart more relevant to low-and middle-income countries.

On average, mothers consumed two servings of green vegetables per day (mean 1.96, SD=2.2, 95% CI 1.79 - 2.13). Similarly, the mean number of servings of fruit per day was 1.8 (mean 1.77, SD=1.7, 95% CI 1.63-1.90). This is comparable with the findings from the 2014 STEPS survey which reported that the mean number of vegetable servings per day was 3.5 servings and mean number of fruit servings per day was 0.7 days.

Seventy seven percent of the mothers did not have the recommended five servings of fruits and/or vegetables in a day. This is similar to the results from the 2014 STEPS survey which reported that 70% of the women aged between 18 and 69 years old ate less than five servings of fruits and/or vegetables, thus not meeting the WHO recommendation[19].

Maternal malnutrition and dietary imbalance may lead not only to maternal and child mortality and morbidity but also have intermediate effects as demonstrated in the associations between LBW and raised blood pressure in childhood and adult life in the literature [25].

8.1.5 Urinary tract infection (UTI)

This study showed clear evidence of an association between UTI and adverse pregnancy outcomes.

The aOR of term LBW in association with UTI was 2.17 (95% CI: 1.11-4.26, p=0.023) and the aOR of PTB was 1.98 (95% CI 1.00-3.90, p=0.049), after adjusting for wealth, education, and season of delivery.

Infection is thought to be one of the main biological pathways leading to PTB and UTI is recognized as one of the most important and potentially modifiable risk factors for early PTB [26]. Although there are other kind of infection that affect pregnant women, UTI was used as it was the best documented.

In the descriptive analysis, less than 10% of mothers had a recorded UTI. More mothers in the case group experienced UTI compared to the control group. More mothers in the case group were missing information on UTI compared to the control group. In terms of symptoms of potential UTIs, more mothers among the cases reported pain in the lower belly, behind the front of pelvis and flank pains compared to the controls.

8.2 Modifiable risk factors in the long-run or non-modifiable risk factors

8.2.1 Hypertensive disorders

Having hypertensive disorders was associated with increased odds of term LBW and PTB compared to the mothers without hypertensive disorders.

The mothers with pregnancy-induced hypertension (PIH), pre-eclampsia, or eclampsia had 7.08 times higher odds (95% CI: 3.58-14.00, p <0.0001) of PTB compared to mothers without hypertensive disorders after adjusting for alcohol during pregnancy, betel quid chewing during pregnancy, ethnicity, chronic hypertension, number of meals per day, maternal age and tobacco use during pregnancy. The total effect of PIH, pre-eclampsia, or eclampsia on term LBW cannot be estimated by adjusting for covariates in the proposed DAG using the backdoor criterion due to an unobserved confounder that directly effects the exposure and outcome variables. Micronutrient deficiency was assumed to have a direct causal relationship with term LBW and PIH, pre-eclampsia, or eclampsia in the proposed DAG but was unobserved in the present study.

The evidence of association between chronic hypertension and adverse birth outcomes was clearer in the multiple imputation models than the complete case analysis. In the complete case

analysis, the aOR of term LBW among mothers with chronic hypertension was 2.68 (95% CI: 0.70-10.31, p-value=0.151) and the aOR of PTB was 3.23 (95% CI: 1.01-10.30, p-value=0.047) after controlling for betel quid chewing during pregnancy, ethnicity, wealth quintile, education, maternal height, maternal age, pre-pregnancy weight, delivery hospital, and season of delivery. The association between chronic hypertension and adverse pregnancy outcomes was clearer in the multiple imputation models. Under MAR, the aOR of term LBW was 4.24 (95% CI: 1.44-12.42, p=0.009) and the aOR of PTB was 4.73 (95% CI: 1.83-12.19, p=0.001). The results under MNAR were almost identical to those under MAR. The large amount of missing data on maternal height and pre-pregnancy weight may have decreased the power for complete case analysis, resulting in an unclear association between chronic hypertension and term LBW.

The direction and strength of association was similar to previous studies but in the present study, the confidence interval was wider. This could be due to the small sample size in the present study. Morisaki et al. (2014) reported prevalence of chronic hypertension was 0.4% and preeclampsia or eclampsia was 2.4% using data from 299,878 singleton deliveries collected in 359 health facilities from 29 countries in Africa, Asia, Latin America and the Middle East [27]. In the multi-nominal, multilevel, multivariate logistic regression models, the study reported that chronic hypertension and preeclampsia/eclampsia was associated with increased odds of PTB (chronic hypertension: aOR 2.28, 95% CI: 1.94-2.68 and preeclampsia/eclampsia: aOR 5.03, 95% CI: 4.72-5.37) after adjusting for maternal age, marital status, education, parity and previous caesarean section. Gestational hypertension was not reported in the study.

8.2.2 Wealth index

The data did not show a clear association between social gradient and term LBW and PTB. The odds of term LBW varied a little by different wealth quintiles after adjusting for delivery hospital (middle quintile [reference] vs poorest quintile [aOR: 1.37, 95% CI 0.73-2.54]; middle vs second [aOR: 0.88, 95% CI 0.47-1.65]; middle vs fourth [aOR: 1.01, 95% CI 0.54-1.89]; and middle vs richest [aOR: 1.43, 95% CI: 0.68-3.05]). The results were similar for PTB.

There are two main possible explanations for this. Firstly, most of the mothers were sufficiently well-off to go to the referral hospitals for delivery. Secondly, free education and free access to health care is mitigating the effect of socio economic factors on adverse health outcomes.

8.2.3 Education

The data suggested middle secondary or secondary school compared to no education was associated with reduced odds of term LBW after adjusting for delivery hospital. On the other hand, the adjusted odds of PTB for education varied a little by each category of highest educational attainment. The impact of education may be underestimated. The population in the present study is relatively more educated than in other national surveys. For example, the STEPS 2014 survey using a nationally representative sample aged between 18 and 69 years old (total 2822 respondents) reported that more than 60% of participants had no formal schooling [19]. This could imply that the study participants

included more mothers who preferred to deliver at the referral hospitals, had the means and knowledge to access the referral hospitals and may have a higher education level, access to health facilities and higher wealth level compared to the mothers who deliver at primary health care level or at home.

8.3 Primary findings from validation of outcome measurements

In the present study, for mothers with both LMP and US information, the LMP and US estimates demonstrated good agreement. The mean LMP overestimated US estimate was 1 day (± 17 days) and was similar to previous studies. In the literature, mean LMP underestimated gestational age by 0.6 day in Gambia [28], 0.77 weeks in Guatemala [29], and 1 day in Bangladesh [30] while overestimating by 3 days in PNG [31].

There was no statistical difference between mothers with early scans and mothers with late scans in terms of proportion of cases that were LBW and PTB in the sample. Hence, it could be assumed that classification of LBW and PTB in the present study was reasonably reliable. However, more mothers with late scans were missing LMP estimates and US estimates, compared to the mothers with early scans. In terms of demographic and socio-economic background, more mothers with late scans were under 25 years old, divorced, or widowed, students, self-employed and unemployed. More mothers with late scans had a parity greater than one and smoked during pregnancy according to a logistic regression model controlling for any other variables in the model. Wealthier mothers compared to the middle quintile had more than 50% reduced odds of having late or no scan (adjusted odds ratio 0.37, 95% CI: 0.16-0.86 vs reference group (middle quintile)).

The findings may inform identification of the mothers who do not receive adequate ANC. Interventions targeted at mothers who are under 25 years old, divorced or widowed, students, self-employed, unemployed, smokers, have a parity greater than one and are in the lower wealth quintile may help improve coverage of ANC.

Although birth weight is the most reliable and widely-reported measure to assess size at birth, not all the infants are weighed at birth globally, especially when they are not delivered in health facilities. When infants are not weighed at birth, mother's recall is often used to determine if the infant is LBW in order to estimate the percentage of LBW infants in Demographic and Health Surveys (DHS) and other surveys. In the present study, mother's subjective concept of the baby's size was explored in relation to birth weight and gestational age. Among mothers of LBW infants, more than 33% assessed their babies as average or larger. The results suggest that the current survey-based estimates of the prevalence of LBW could be underestimated.

8.4 Limitations

In this section, limitations are discussed in terms of selection bias, recall bias, measurement errors, confounding and sample size. There are three main biases that can arise in observational studies: biases related to the selection of subjects into the study, biases arising from the way in which the

data are collected such as recall bias, truncation bias, measurement error and finally bias due to confounding [32]. The retrospective case-control study is more susceptible to selection bias than other epidemiological studies [32].

8.4.1 Selection bias

Selection bias is introduced when the association between exposure and outcome within the study population is different from that in the target population or cases and controls are not drawn from the same source population. In the study design, in order to capture regional variations, a multi-centre case-control study was conducted rather than a single site study design. Given that the present study recruited cases in a hospital setting, controls were recruited from among mothers who would have attended the hospital if they had experienced the outcome of interest i.e. LBW or PTB in the present study. By recruiting controls from the same hospital, it could reasonably be assumed that they would have been recruited as cases if they had been delivered PTB or LBW babies. The usual concern with recruiting hospital-based controls is that they tend to be more similar to cases with respect to exposure than the general population, which would lead to an underestimate of the effect of the exposure of interest. This happens when the exposure leads to conditions other than the outcome of interest which led the control to attend the facility. For example, if controls included some mothers who had been referred for conditions which are linked to betel quid chewing, this could bias the odds ratio towards the null.

The hospital population could differ from deliveries at primary health care facilities and home deliveries on a number of points. According to a systematic review in Africa, facility deliveries in general are associated with first births or those lower in the birth order, maternal higher level of education, higher household wealth, from urban residence, shorter distance to the nearest facility, higher number of ANC visits [33]. In the present study, cases and controls were identical in terms of socio-economic factors such as highest level of educational attainment, wealth quintile, marital status and baseline characteristics such as ethnicity and pre-pregnancy BMI. Thus, by capturing relatively well-educated or wealthier population compared to the general population, the effect of education or wealth could be underestimated compared to the community-based case control study in the present study.

8.4.2 Recall bias

Case-control studies can be subject to a number of sources of recall bias. Chemical analyses or laboratory tests were not conducted in the present study to measure exposure to and quantification of betel quid chewing, smoking, and drinking. Betel quid was widely consumed and had no stigma attached. As a result, mothers were generally open about answering. However, it is still possible that betel quid chewing, smoking, and alcohol use were underreported if mothers felt hesitant about sharing the actual consumption due to some attached stigma, which may bias the estimates towards the null. Generally, it is said that controls underreport exposure more than cases in case-control studies due to differential reporting of exposure information between cases and controls based on

their disease status or interviewer's knowledge of an individual's disease status (interviewer/observer bias). In such case, the odds may be overestimated. In the present study, betel quid chewing had a similar prevalence among cases and controls but alcohol and tobacco were more common in the cases than controls. The point estimates and 95% CIs were comparable to others studies and systematic reviews for alcohol but larger for tobacco. Therefore, the results should be interpreted with these limitations in mind.

8.4.3 Measurement errors

The ambiguity on the accuracy of gestational age used to classify PTB is one of the biggest limitations that could impact the results of the study. To examine this ambiguity, validity of outcome measure was examined in detail and sensitivity analyses limiting to mothers with early scans were conducted.

The second methodological challenge was missing data in the independent variables. The present study collected information such as mother's self-reported symptoms or subjective assessment to supplement hospital records to deal with this challenge. The multiple imputation method was used to deal with missing information.

In terms of measurement of exposure, a tool was developed after careful consideration of the culture and context. The present study used a modified calendar method to assess the betel quid consumption during pregnancy in Bhutan. Compared to other studies, the practice of mixing betel quid with tobacco among pregnant women was less common, allowing the researchers to look at the effect of betel quid separately. For alcohol use, the graduate frequency method was used to overcome the challenge of not having a standard drink size. Amount of ethanol was calculated based on the conservative assumptions of alcohol concentration of home-brewed alcohol. It might have been overestimated if the assumptions of alcohol concentration were higher. In the logistic regression, whether mothers drink alcohol or not and the number of drinking days were used as an exposure. Thus, this limitation does not materially influence the results.

The questionnaire was not validated in terms of the concurrent validity and construct validity. Concurrent validity can be demonstrated by correlating the measure with related and or dissimilar measures [34]. Construct validity refers to the degree of how well the items in the questionnaire represent the underlying conceptual structure [34, 35]. In addition to validity, it is also important that a measure can demonstrate reliability as defined as repeatability, stability or internal consistency [34, 35]. This could have been explored by asking the mothers about their consumption of betel quid, alcohol, and smoking at every ANC visit using the same questionnaire to check stability using test-retest reliability [34] or by keeping track of expenditure or asking mothers to keep diaries of the amount consumed.

8.4.4 Confounders

Pregnancy and birth outcomes may be influenced by a multitude of biological, behavioural, and socio-economic factors. For the present study this complexity was addressed by using both

statistical and directed acyclic graphs approaches. The causal assumptions were explicitly presented in the DAGs and covariates controlled for in the logistic regression models were determined based on the causal assumptions. However, it should be noted that several factors such as periodontal disease were not measured and not controlled for in the model. The causal assumptions made in the DAGs substantially impact the results of the logistic regressions. In relation to this point, a statistical approach was also conducted and compared to the results from the DAG approach. Any substantial discrepancies were addressed in [Chapter 7](#).

8.4.5 Sample size

The study did not recruit long enough to get the planned 485 sample size for cases ([Chapter 3.3](#)). In addition, given the very large number of eligible controls, many opportunities were missed to recruit controls ([Chapter 5.1](#)). As the research nurses were trained for the first time to conduct a case control study, it was difficult to institutionalise the study in the beginning and it was often the case that mothers were discharged before nurses had a chance to contact them. The study period was extended from the original time period and additional research assistant was recruited to assist the data collection. As a result, the resources were running short. To avoid pressuring the health system and compromising the quality of data collection, the study ended before reaching the planned sample size.

The sample size was based on the assumption of 10% difference in prevalence of betel chewing. The observed small difference in the prevalence led to a bigger P-value. This limited the ability to produce clear evidence of association or no association between betel quid chewing and adverse birth outcomes.

8.4.6 Translation Bias

Although the research nurses were fluent in English and Dzongka, verbal translations were agreed during the training and monitoring visits ([Chapter 3.6](#)), the lack of a formal written translation (back-translation, validation) process could have led to some bias if different interviewers translated differently and conveyed different meanings of some questions to respondents. About 20% of the interviews were done in English, combined with other languages such as Dzongkha (40% of the total interviews), Sharchop (33%), and Lhotsham (29%) (Figure I.5).

8.4.7 Statistical approach

The statistical model have too many variables for the number of outcome events and as a result, may be lacking power (Table 7.1 and Table 7.2). There are 32 variables and the number of outcome events were less than 200. This may partially explain considerable uncertainty around the estimates [36]. In this point, the DAG analyses were used to inform the statistical model to reduce the number of variables that should be controlled for in the models.

8.4.8 Imputation of missing data

MNAR analysis was conducted using only UTI ([Chapter 7.2.2](#)) in the present study. However, there could be a number of other MNAR scenarios. For example, multiple variables can have MNAR missingness mechanism at the same time. More MNAR analysis with more variables may be needed in order to determine whether or not the missing data is MAR or not more robustly.

8.5 Recommendations

Globally, the Sustainable Development Goals set targets for major reductions in maternal, neonatal, and child mortality and call for universal access to sexual and reproductive health services by 2030 [37].

In Bhutan, several initiatives are being taken to increase awareness of the importance of the first 1000 days between the beginning of a woman's pregnancy and her child's second birthday in the child's health, ultimately to reduce maternal and child mortality through improved health and nutritional status of adolescents, pregnant and lactating women as well as young children. The present study provides rich baseline data for mothers and established a cohort of cases and controls, which could be followed up to understand the short- and long-term effects of LBW and PTB.

The following recommendations are made to inform policy makers on modifiable risk factors.

8.5.1 Recommendations for further research

In order to reduce the burden of LBW and PTB, research into both prevention and care are necessary [38]. The present study focused on prevention by exploring potential risk factors for LBW and PTB. The present study also identifies possible areas for future studies as follows:

- **Further research on association between betel quid chewing during pregnancy and adverse birth outcomes**

The studies so far could not provide sufficient evidence that betel is not associated with adverse outcomes due to sample size, confounders, and measurement issues. The present study aimed to develop methods for assessing betel quid use during pregnancy and described the pattern of betel quid chewing during pregnancy among Bhutanese women. In addition, the present study tried to quantify the amount of betel quid chewing during pregnancy. However, the observed difference of betel chewing in cases and controls were smaller than the assumption used to calculate the sample size, which limited the ability to provide clear evidence of association. An association between the amount, timing, and type of betel and adverse birth outcomes may be further examined. Although further research with larger sample size may be warranted to build on the findings, given the very small differences in exposure to betel quid between cases and controls, an effect may be very

small. As described in [Section 8.1.1.](#), in relation to a possible relative and absolute risk according to the results, if the risk of LBW in the general singleton live born infants is 8.5%, the 1.3 times increased risk with betel quid chewing during pregnancy would result in an incidence in 11.1%, a 2.6 % increase. Similarly, if the risk of PTB in the general singleton live born infants is 5.5%, the 1.2 times increased risk with betel quid chewing during pregnancy would result in an incidence of 6.6%, a 1.1% increase. Following up the cohort of babies may be useful to examine the short term and long term impact of betel quid chewing on growth, morbidity, and morbidity of babies if any.

- **Further research on validation of measurement of betel quid chewing during pregnancy**

A prospective cohort study is required to establish the utility of the questionnaire developed in this study. Test-retest reliability can be established by asking the mothers how much betel quid they chewed, alcohol they drank and tobacco they smoked at every ANC visit using the same questionnaire [34] or by keeping track of expenditure or asking mothers to keep diaries of the amount consumed.

- **Research on mechanisms or causal association between betel quid chewing and anaemia**

While the present study shows clear evidence of association between anaemia and betel quid chewing during pregnancy, causality is not established and the mechanism is not clearly understood. Further research on potential mechanisms may be warranted. Such research may include systematic research on chemical constituents of betel nuts and packaged betel products.

- **Research on association between betel quid chewing and periodontal disease and adverse birth outcomes**

Several studies suggest that betel quid chewing is associated with increased odds of periodontal disease as described in Chapter 2. Periodontal disease was included in the DAG as an unmeasured factor in the present study. To estimate the precise effect of betel quid chewing, it is worth examining the impact of betel quid on periodontal disease and association with adverse birth outcomes.

- **Qualitative study on practice of alcohol and betel quid chewing**

Considering the high prevalence of alcohol and betel quid chewing during pregnancy, qualitative research is necessary to understand the cultural and social norms that encourage use of alcohol and betel quid chewing during pregnancy. There might be cultural beliefs about health benefits associated with alcohol and betel quid chewing during pregnancy. This would help design effective prevention and intervention programmes and policies.

- **Research and development of more accurate measurement of home-brewed alcohol**

Considering the large amount of home-brewed alcohol and high burden of alcohol-related disease, a greater understanding of alcohol consumption needs to be built. There is no accurate information on the ethanol concentration of home-brewed alcohol. Validation research of F-GF with the obstetric population using chemical analysis is recommended.

- **Research and development of alcohol screening tools for obstetric population**

Studies indicate that even the low levels of prenatal alcohol exposure can negatively affect the birth outcomes [39]. The present study showed mothers who drank less than or equal to once a week at 2.16 higher odds of PTB compared to non-drinkers. Screening pregnant women for alcohol use at the primary care level is necessary. Although the Maternal and Child Health Handbook and National Standards of Midwifery Practice for Safe Motherhood state that alcohol use during pregnancy should be discouraged, no concrete screening process exists and no screening questions are asked.

There are a number of validated screening questions on harmful drinking recommended at the primary care level such as the 10-item Alcohol Use Disorders Identification Test (AUDIT) [40] and the 4-item CAGE [41]. Of these screening tools, the 4-item T-ACE [42] is developed and validated for routine use in obstetric-gynecological practice. Based on the CAGE questions, the T-ACE consists of four questions:

- (1) How many drinks does it take to make you feel high? (Tolerance);
- (2) Have people Annoyed you by criticizing your drinking?;
- (3) Have you ever felt you ought to Cut down on your drinking?;
- (4) Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover? (Eye opener).

However, these screenings tools seem to be developed and validated in societies where alcohol drinking during pregnancy is associated with some negative value judgement. Considering the high prevalence of drinking during pregnancy in Bhutan, research to develop the screening tools applicable to the Bhutanese population is required to initiate screening.

- **Research and development of culturally appropriate interventions on alcohol during pregnancy**

To reduce consumption of alcohol, there are pharmacological, psychological and educational interventions described in the literature [43]. However, Cochrane reviews of

pharmacological [44], psychological and educational interventions [43] showed that the effect of such interventions is inconclusive. There is an urgent need for research and development of effective interventions among pregnant mothers. In order to introduce interventions for Bhutanese population, such interventions should be culturally appropriate and validated for the Bhutanese obstetric population.

- **Research on management of hypertensive disorders during pregnancy**

The association between hypertensive disorders and adverse outcomes is well established. Additionally, hypertension is the second most common direct cause of maternal deaths after haemorrhage, accounting for 14% of all deaths (11.1-17.4%) [45]. Although not the focus of the present study, a relatively high prevalence of hypertensive disorders was found and was associated with an increased odds of PTB and LBW. Further research focusing on the detection, management and outcome of hypertensive disorders of pregnancy in Bhutan may be helpful in order to identify whether prevention and treatment can be optimised.

- **Follow-up study of the cohort established in this case control study**

Having established a cohort of mother –baby pairs, by enrolled and obtained baseline data on these cases and control, it would be very interesting to do a follow-up study on this cohort in order to describe the short- and long-term impacts of term LBW and PTB in Bhutan. The short-term outcomes may include hospital admission rate, neonatal mortality and morbidity. The long-term outcomes may include health, growth and development in childhood and even into adulthood. Qualitative and quantitative research on care of LBW and PTB by following up the cohort, in combination with the baseline data the present study provides, may contribute to building a comprehensive understanding of prevention and care in Bhutan and help design and scale up low-cost interventions.

- **Qualitative study on psychosocial factors such as stress, anxiety and partner violence**

Recent studies show an association between psychosocial factors and adverse birth outcomes as described in Chapter 2. Although not the focus of the present study, unstructured memos by the interviewers identified some of the repeated keywords such as divorce, unbooking (no ANC), problem with partner or family, alcohol, and PIH. In the present study, psychosocial problem was included as an unobserved factor that has a causal relationship with alcohol and tobacco use during pregnancy and was not investigated in depth. It may be worth conducting qualitative research focusing on the psychosocial factors that Bhutanese women are facing in the rapidly modernising society.

8.5.2 Implications for policy and practice

Antenatal Care (ANC) is a key strategy to improve maternal and infant health. Of the sample mothers, 83% had at least four ANC visits as per WHO recommendation. Mean number of ANC visits was 5.6 and mean gestational weeks at the first ANC visit was 15 weeks. This indicates that

ANC is an effective intervention to reach the obstetric population. Improving the coverage and quality of ANC, focusing on the factors that have clear evidence of an association with adverse pregnancy outcomes, may contribute to improving health outcomes of mothers and babies in Bhutan.

The research shows that more mothers in the case group were missing information on UTI compared to the control group. In terms of symptoms of potential UTIs, more mothers in the cases reported pain in the lower belly, behind the front of the pelvis and flank pains compared to the controls. This may suggest that efforts to screen for and treat UTI during pregnancy at antenatal care could be strengthened.

The present research also reveals imbalanced and inadequate diet during pregnancy in the Bhutanese population. Nutritional interventions that involve correction of micronutrient and macronutrient imbalances in mothers before conception or at critical periods of early development is required.

The present research suggests that prevalence of drinking and intensity of drinking are higher in the eastern region. Alcohol interventions targeting especially in the eastern region should be prioritised.

The present research can help identify the population that comes to ANC late or does not come at all. Policies or campaigns targeted at mothers who are under 25 years old, divorced or widowed, students, self-employed, unemployed, smokers, have a parity greater than one and are in the lower wealth quintile may benefit from improved ANC coverage.

In this study, the main potentially modifiable risk factors in the short-term, associated with PTB and LBW were alcohol, tobacco, low GWG, and UTI. As described above, it should be a priority to design, implement and evaluate interventions aimed at reducing these risks as much as possible and to improve the understanding of other modifiable risks such as other infectious diseases and hypertensive disorders. However, these interventions are likely to take time to put into place, and even with optimal efforts at prevention, a substantial proportion of adverse outcomes may not be avoidable. It is therefore very important that the care of pre-term and low birth weight babies is optimised. As has been highlighted recently, there are a number of effective inexpensive solutions that have not been implemented or scaled up in low income countries that could save the lives of many LBW and PTB babies [1]. Among cost-effective interventions, Kangaroo Mother Care (KMC) has been named as one of the highest impact facility-based interventions that are scalable in low-resource settings and act as entry points for strengthening health systems [2]. KMC includes thermal care through continuous skin-to-skin contact between the mother and baby, support for exclusive breastfeeding or other appropriate feeding, and early recognition of, and response to, complications [46]. A meta-analysis of KMC using three randomized controlled trials showed that KMC in the first week of life reduced the risk of mortality by 51% (relative risk 0.49, 95% confidence interval 0.29-0.82) compared with standard care [46]. In Bhutan, KMC was implemented as a pilot project at JDWNRH in September 2013 and expanded to ERRH and CRRH during the study period.

8.6 Conclusion

In the present study, the results did not provide clear evidence that there are increased odds of an association between term LBW or PTB and betel quid chewing during pregnancy. For a secondary outcome, the data suggest betel quid chewing is associated with increased odds of anaemia. The present study provides rich baseline data for mothers and established a cohort of cases and controls, which could be followed up to understand the short- and long-term effects of LBW and PTB and may help design effective interventions.

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Appendix A

Table A.1. Burden of preterm birth and low birth weight.

Outcome		Author	Review method	Summary of findings	
Mortality	Mortality	Mortality risk in preterm and SGA infants in low and middle income countries	Katz et al. (2013) [1]	Systematic review to identify datasets from published studies and cohorts which may have collected the information and a pooled country analysis (1982 – 2010)	20 studies from Asia, Africa and Latin America were included (2,015,019 live births). Pooled overall RRs for preterm were 6.82 (95% CI 3.6-13.07) for neonatal mortality and 2.50 (95% CI 1.48-4.22) for post-neonatal mortality. Pooled overall RRs for SGA infants were 1.83(95% CI 1.34-2.50) for neonatal mortality and 1.90 (95% CI 1.32-2.73) for post-neonatal mortality. Neonatal mortality rates and relative risks increased as gestational age decreased across studies and regions.
		Adult mortality from all causes, CVD or cancer	Risnes et al.(2011) [2]	Meta-analysis of observational studies (before October 2010)	22 prospective or longitudinal cohort studies from the UK, Denmark, Sweden, Finland, Norway, the Netherland, Australia, and Israel were included (36,834 deaths). Fixed-effect meta-analysis showed that a 6% lower risk (adjusted HR 0.94, 95% CI: 0.92-0.97) per kg higher birthweight for men and women combined. Inverse association with cardiovascular mortality (HR=0.88, 95% CI 0.85-0.91) was reported. Cancer mortality was HR 1.13 95% CI 1.07-1.10 for men and HR 1.04, 95% CI 0.98-1.10) for women.
Long-term morbidity	Neuro-development /behavioral effects	Long-tern neurodevelopmental outcome in high-risk newborns in resource-limited settings	Milner et al. (2015) [3]	Systematic review to identify literature (January 1996 to April 2012), narrative review and median prevalence of key neurodevelopmental outcomes where data quality allowed	33 studies of LBW/PTB populations from resource-limited settings were included. The median sample size was 122 (IGR 84-171). The medial overall prevalence of moderate-to-severe neurodevelopmental impairment was 21.4% (11.6-30.8).
		Language difficulties	Van Noort-van der Spek et al. (2013) [4]	Meta-analysis (January 1995-March 2011)	13 studies were included for simple language function tests and 7 studies were included for complex language function tests. Random effects meta-analysis revealed statistically significant differences between preterm-born children and term-born children both for simple (d= -0.45, 95% CI -0.59 to -0.30) and complex language tests (d=-0.62, 95% CI -0.82 to -0.43) in the absence of major disabilities and independent of SES.
		Brain Development	de Kieviet et al. (2012) [5]	Meta-analysis (before 1 August 2012)	15 studies were included (419 very preterm or very low birth weight (VLBW) and 307 term-born children). Very preterm (<32 weeks) or very

low birth weight (<1500g) was associated with a significantly smaller total brain volume (d= -0.58, 95% CI -0.43 to -0.73).

	Cognitive and behavioral outcome	Bhutta et al (2002) [6, 7]	Meta-analysis (1980-November 2001)	15 studies were included for cognitive data (1556 school-aged children who were born preterm and 1720 school-aged children who were term-born). Term born children had significantly higher cognitive scores than preterm children (weighted mean difference, 10.9, 95% CI 9.2-12.5).
	Motor Impairment	de Kieviet et al. (2009) [8]	Meta-analysis (January 1992-August 2009)	41 studies were included. Very preterm and VLBW children obtained significantly lower scores on all 3 established and widely used motor tests. BSID-II: d = -0.88 (95% CI -0.96 to -0.80), MABC: d = -0.65, 95% CI, -0.70 to -0.60, and BOTMP: d = -0.57, 95% CI, -0.68 to -0.46).
Specific Physical effects	Visual impairment	Fetus and Newborn Committee, Canadian Paediatric Society (1998) [9]	Systematic review (January 1966 – December 1997)	8 population-based studies of retinopathy of prematurity were included. Studies from Canada, New Zealand, Great Britain, Sweden, Norway, and Denmark suggested that infants at greatest risk of ROP were 1500g or less at birth, or 30 weeks gestational age or younger.
	Chronic lung disease	Colin et al. (2010) [10]	Structured review (2000-2009)	24 studies were included. These studies consistently revealed that infants born at 32 to 36 weeks' gestational age, including infants of 34 to 36 weeks' gestational age, have substantial respiratory morbidity compared with term infants.
	Hypertension	Mu et al.(2012) [11]	Systematic review (1995-2011)	20 studies from Japan, Spain, Finland, England, Sweden, USA, China, Brazil were included. Using 9 studies, the fixed effect models revealed that LBW was associated with and increased risk of hypertension (OR 1.21, 95% CI 1.13-1.30) and BW more than 4000g had a negative association with hypertension (OR 0.78, 95% CI, 0.71-0.86).
	Long-term cardiovascular ill-health and non-communicable disease	Parkinson et al. (2013) [12]	Systematic review and meta-analysis (before October 1, 2011)	40 studies were included (17030 preterm and 295261 term-born adults) In adults, preterm birth was associated with significantly higher systolic blood pressure (mean difference: 4.2 mm HG, 95% CI 2.8-5.7, p <0.001).
Economic Costs	Long-term economic costs	Petrou et Khan (2012) [13]	Non-systematic review (January 1980-2011)	20 studies from developed countries defined by OECD were included. Data on economic consequences of moderate and late preterm birth consistently suggest that service provision for infants born between 33 and 36 weeks' gestation is associated with substantial incremental costs during the initial hospital stay and throughout childhood.

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Appendix B

B.1 Search Strategy in Medline (2.1)

Gestational age

Gestational age.mp. or exp Gestational Age/ OR last menstrual period.mp. OR_pregnancy duration.mp. OR pregnancy length.mp. OR_pregnancy dating.mp.

LMP

LMP.mp. OR menstruation.mp. or exp Menstruation/ OR menstrual cycle.mp. or exp Menstrual Cycle/ OR menstru*.mp. OR last menstrual period.mp. OR LMP.mp.

US

exp Ultrasonography, Prenatal/ or Ultrasonography/ or ultrasonography.mp OR ultrasound.mp.

Embase ALL LMIC countries List from World Bank, 2014 was added.

Abstracts were then screened for possible relevance (n=491). Other academic papers were searched in PubMed and the Cochrane library. Table 2.1 summarizes the six studies.

B.2 Comparison of ultrasound birth weight references and the International Fetal and Newborn Growth Consortium for the 21st century standard using the data from case-control study (3.11.1)

Small for gestational age (SGA) was defined as a birth weight below the 10th percentile of a sex-specific birth weight distribution by gestational age. SGA of cases and controls was calculated using 2 different distributions: the US 2000 birth weight reference for gestational ages 20-44 weeks and the International Fetal and Newborn Growth Consortium for the 21st Century (INTERGROWTH-21st) birth weight standard for gestational ages 24-42 weeks. INTERGROWTH-21st is a description of optimal birth weight and the US 2000 birth weight is a description of measured birth weight in the population.

In total, by the US 2000 birth weight reference, 269 infants (41.5%) were classified as SGA while 201(31.0%) infants were classified as were classified as SGA using the US 2000 reference. The agreement was 89.6%. The Kappa coefficient for classification was 0.78, which indicates good agreement. INTERGROWTH-21 classified less study participants as SGA compared to the US 2000. This confirms the previous study by Kozuki et al [1], which compared the two references using data from 10 low- and middle-income countries and reported that a greater than one quarter differences in SGA classifications by the two different distributions.

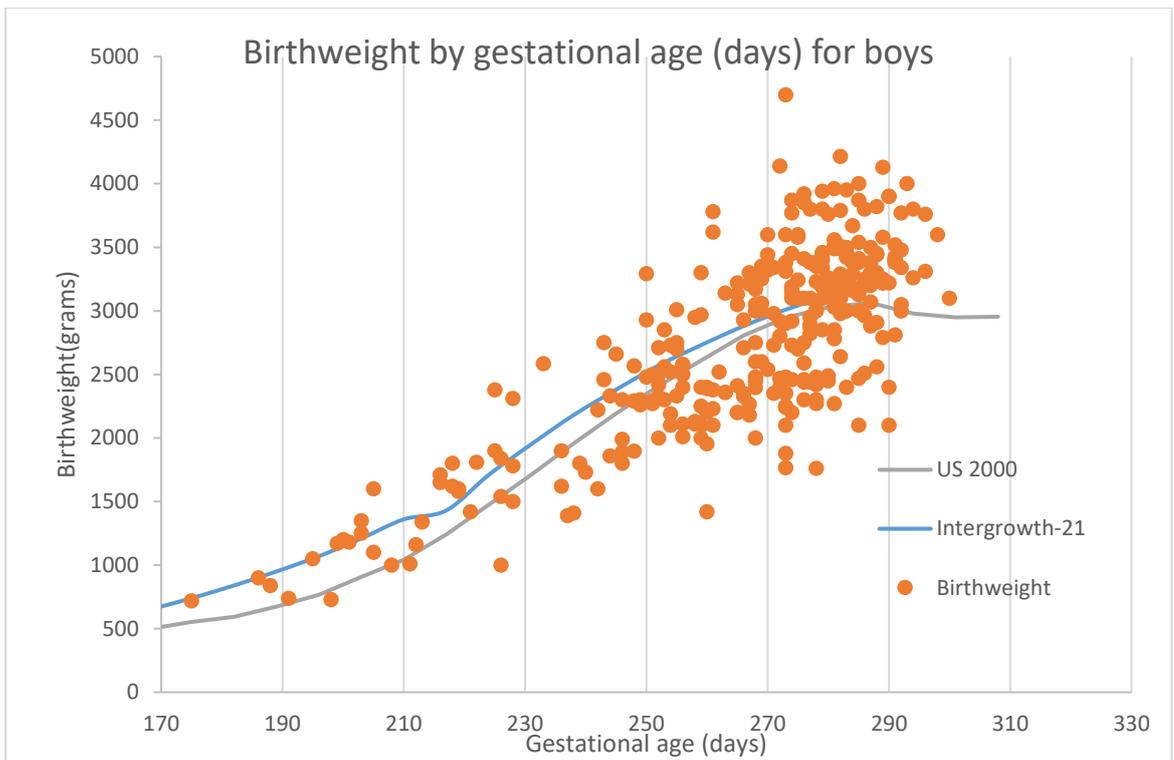


Figure B.1. Birthweight by gestational age for boys.

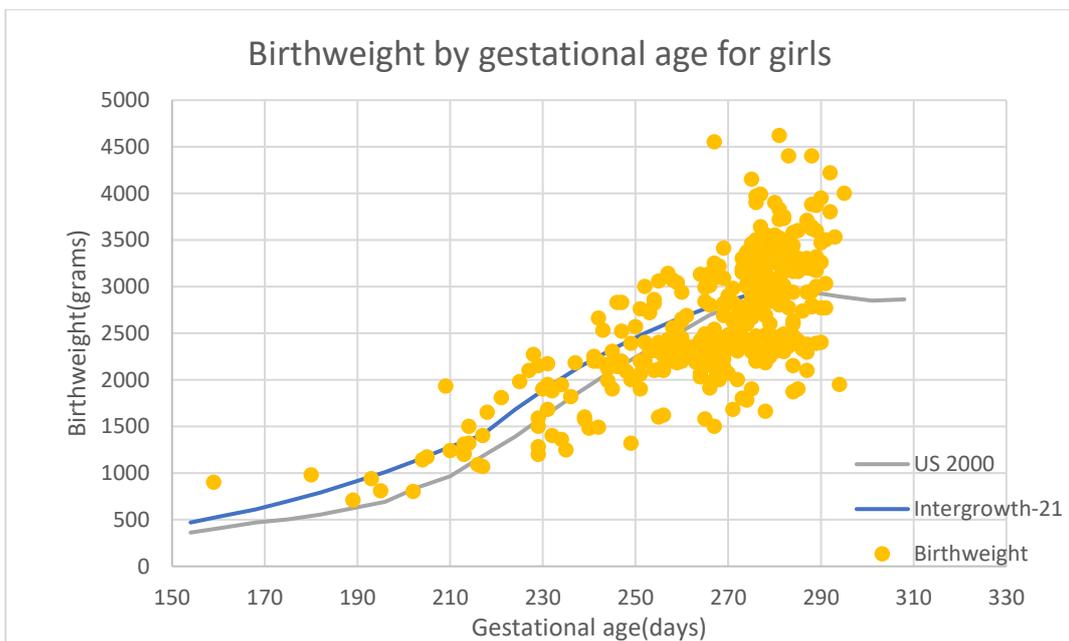


Figure B.2. Birthweight by gestational age for girls.

Table B.1. The 10th percentile birth weight values by gestational age and sex comparing the US2000 reference to the INTERGROWTH-21 Standard in this study participants

Gestational age (weeks)	Boys							Girls						
	Total	US 2000 10th percentile	No and % of SGA by the US 2000		Intergrowth-21st	SGA by Intergrowth 21		Total	US 2000 10th percentile	SGA by US 2000		Intergrowth-21st	SGA by Intergrowth-21	
	N	Weight, g	N	%	Weight, g	N	%	N	Weight, g	n	%	Weight, g	n	%
22	0	393	0	0	-	0	0	1	362	0	0	-	0	0
23	0	453	0	0	-	0	0	0	416	0	0	-	0	0
24	0	498	0	0	500	0	0	0	470	0	0	470	0	0
25	0	554	0	0	570	0	0	1	504	0	0	540	0	0
26	2	594	0	0	650	0	0	0	556	0	0	610	0	0
27	1	674	0	0	740	0	0	3	622	0	0	700	0	0
28	4	766	1	25	840	1	25.00	1	693	0	0	790	0	0
29	5	906	0	0	950	0	0	3	845	0	0	900	0	0
30	5	1044	1	20	1070	1	20.00	7	965	0	0	1010	0	0
31	6	1241	0	0	1210	0	0	3	1180	1	33.33	1140	1	50.00
32	8	1475	1	12.5	1360	1	12.50	9	1390	2	22.22	1280	1	12.50
33	3	1712	2	50	1430	1	33.33	10	1638	3	30.00	1410	3	33.33
34	9	1957	5	55.56	1710	2	22.22	12	1872	4	33.33	1680	4	36.36
35	16	2192	4	25.53	1950	3	18.75	17	2099	6	35.29	1920	3	18.75
36	24	2410	12	46.15	2180	6	25	25	2299	10	40.00	2140	5	20.83
37	27	2609	19	70.37	2380	13	48.15	34	2495	25	73.53	2330	18	52.94
38	36	2807	18	48.57	2570	12	34.29	52	2694	38	73.08	2500	33	64.46
39	69	2947	30	43.48	2730	22	31.88	70	2834	32	45.71	2650	22	31.88
40	54	3029	14	25.45	2880	10	18.52	61	2919	23	37.70	2780	20	33.33
41	36	3063	9	25	3010	8	22.22	35	2949	10	28.57	2890	9	25.71
42	6	2979	0	0	3120	1	16.67	2	2893	1	50.00	2980	1	50.00
43	1	2949	0	0	-	0	0	0	2849	0	0	-	0	0
44	0	2954	0	0	-	0	0	0	2863	0	0	-	0	0
Total	311	-	116	36.36	-	81	26.05	346	-	155	44.80	-	120	35.61

B.3 Hypertension during pregnancy (2.2.2)

Hypertension in pregnancy is a leading cause of maternal mortality and adverse birth outcomes [2]. Many efforts during antenatal care are made to detect and manage hypertensive disorders during pregnancy [3]. A meta-analysis concluded that chronic hypertension increased preterm delivery and low birth weight by 2.7 times[4]. The association between preeclampsia or gestational hypertension and poor foetal growth is inconclusive [5-13].

Pre-eclampsia is defined as hypertension (diastolic blood pressure of ≥ 90 mm Hg) accompanied by proteinuria (≥ 300 mg or more per 24- hour period) , usually occurs during the second half of pregnancy (at or after 20 weeks' gestation)[6, 7]. Pre-eclampsia complicates 2%-8% of pregnancies [6-8, 14]. Women with moderate pre-eclampsia generally have no symptoms[6]. Women with severe pre-eclampsia, or with very high blood pressure, may feel unwell, with symptoms such as headache, upper abdominal pain, or visual disturbances[6].

Pre-eclampsia can affect blood supply to the placenta, leading to poor intrauterine growth, and can precipitate preterm birth related either to the spontaneous onset of preterm labor or to early deliver to protect the mother or the fetus[6]. In order to examine the strength of association between pre-eclampsia/eclampsia and adverse birth outcomes, relevant literature was systematically searched in MEDLINE, EMBASE, and Cochrane Library from the inception of each database in March 2016. For pre-eclampsia/eclampsia, “exp Pre-eclampsia/”OR “exp Eclampsia/”OR “hypertensive disorder*”, OR “Pregnancy Complications/”, OR ”exp Hypertension, Pregnancy-induced/” were used. The reference list was manually searched and other academic papers were searched in Google scholar and PubMed. No language restriction was applied. There was no study type restriction. All papers that referred to the relationship between adverse pregnancy outcomes and pre-eclampsia/eclampsia in humans were assessed. Research shows mixed-evidence, observing inconsistent associations between preeclampsia and the risk of birth complications.

Naeye (1989) presented an evidence that preeclampsia and low uteroplacental blood flow are an important cause of spontaneous preterm delivery using the data from an US-based cohort study of 55,908 pregnancy women in 12 medical school-affiliated hospitals in different regions of the US between 1959 and 1966[15].

Xion et al. conducted several research to explore relationship between pregnancy-induced hypertension and fetal growth in China and Canada [9-11]. In 2001, a study which examined 97,270 pregnancies between 1991 and 1996 at 35 hospitals in northern and central Alberta, Canada reported that the birthweight were statistically significant lower among mothers with pre-eclampsia who delivered at or before 37 weeks of gestation after adjusting for maternal smoking, maternal age, maternal pre-pregnancy weight of ≥ 91 kg or ≤ 45 kg, prior spontaneous and induced abortions, prior SGA new-born, prior large-for-gestational-age newborn, anemia, and premature rupture of membranes (PROM)[9]. However, the birth weights were not lower among mothers with pre-eclampsia who delivered after 37 weeks of gestation. In 1999, Xion et al. reported that preeclampsia and severe preeclampsia increased the risk of intrauterine growth restriction and low birth weight

but not associated with preterm birth using a population-based perinatal database of 16,939 pregnancies from 10 hospitals between 1989 and 1990 in Suzhou, China [11]. Yet, when the group stratified the population by type of hypertension and gestational age in 2004 and adjusted for maternal age, education, parity, BMI at the first antenatal visit, prior induced abortion, PROM, and severe anaemia (< 8g/dL) during pregnancy, the authors concluded that there were no differences in mean birthweight between women with gestational hypertension and women with normal blood pressure [10].

Villar et al. (2005) analysed 39,615 pregnancies collected in the WHO Antenatal Care Trial between August 1990 and December 1998 in Rosario, Argentina, Havana, Cuba, Jeddah, Saudi Arabia, and Khon Kaen, Thailand and reported women with pre-eclampsia had a lower mean gestational age(37.5 weeks) and mean birth weight(2845g) compared to women without any hypertensive conditions and/or IUGR infants and 74% of the preterm deliveries were medically indicated[8].

Bakker et al. (2011) used a multi-linear model with repeated measure to analyse 8,623 women who participated in a population-based prospective cohort study between 2001 and 2005 in Rotterdam, the Netherlands [5]. The authors restricted their analyses to spontaneous deliveries and reported an increase in blood pressure from the second trimester to the third trimester was associated with an increased risk of adverse birth outcomes after adjusting for gestational age at birth (only in birth weight analyses), maternal age, educational level, ethnicity, parity, folic acid supplement use, smoking, alcohol, caffeine intake, weight, height, stress, and fetal sex (SGA OR 5.03, 95% CI: 3.31-7.62; PTB OR 5.89, 95% CI: 2.63-13.14; and LBW OR 8.94, 95% CI: 6.19-12.90)[5].

Ota et al. (2014) analysed 295,829 singleton infants from the WHO Multi-Country Survey on Maternal and Newborn Health between May 2010 and December 2011, collected in 359 health facilities from 29 countries in Africa, Asia, Latin America and the Middle East, and reported that the risk of preterm SGA infants was significantly higher among nulliparous mothers and mothers with chronic hypertension (aOR 1.68, 95% CI 1.22-2.30) and preeclampsia/eclampsia (aOR 2.89, 95% CI: 2.55-3.28) compared to preterm appropriate-for-gestational age (AGA), controlling for maternal age, marital status, education in years, anaemia, malaria/dengue, HIV/AIDs, other heart, lungs, liver and kidneys conditions, and the human development index[13]. The study did not adjust for other potential risk factors such as smoking, alcohol and caffeine intake, maternal BMI, malnutrition, gestational weight gain, maternal stature, psychosocial, stress, interpregnancy interval, and previous history of miscarriage. The study also did not mention regarding mode of delivery of the babies included for the analyses.

Using 299,878 singleton from the same data as Ota et al. (2014) (the WHO Multicounty Survey on Maternal and Newborn Health), Morisaki et al. (2014) reported prevalence of chronic hypertension was 0.4% and preeclampsia/eclampsia was 2.4% [12]. In the multi-nominal, multilevel, multivariate logistic regression models, the study reported that chronic hypertension and preeclampsia/eclampsia was associated with increased risk of preterm (chronic hypertension: aOR 2.28, 95% CI: 1.94-2.68 and preeclampsia/eclampsia: aOR 5.03, 95% CI: 4.72-5.37) adjusting for

maternal age, marital status, education, parity and previous caesarean section. Gestational hypertension was not reported in the study.

Although there are differences in the study design (prospective[5, 8] vs retrospective[9-11, 15], treatment of missing data (simply omitting missing data[8-11] vs multiple imputations[5]), confounders included in the models, and definitions of hypertension ($\geq 130/90$ mmHg in China[10, 11]), the origins of the heterogeneous observed associations are still not clear.

Moreover, preterm could be a result of treatment for pre-eclampsia[16]. Steegers et al.(2010) did not suggest any specific gestational weeks for delivery for mothers with pre-eclampsia but suggested that timing of delivery should be designed to keep perinatal outcomes at an optimum while avoiding maternal risks[7]. Sibai (2003 & 2006) recommended delivery for women who develop preeclampsia at 37 weeks or more and for all women with severe preeclampsia at ≥ 34 weeks' gestation, and at 35 to 37 weeks in the presence of any of the following factors: severe pre-eclampsia, preterm labor or PROM, suspected IUGR-oligohydramnios, vaginal bleeding, or abnormal fetal testing (variable or late decelerations, absent or reverse umbilical artery diastolic flow, or biophysical profile ≤ 6)[17, 18].

Furthermore, a systematic review and meta-analysis of randomized controlled trials of antihypertensive therapy for mild-to moderate pregnancy hypertension found that there was a statistical trend towards an increase in small-for-gestational age infants among women taking antihypertensive therapy, compared with those who took placebo or no therapy combining 15 trials with 1782 women (OR 1.31, 95% CI: 0.988-1.75)[19]. This systematic review was updated in 2004 and reported that 176g birth weight difference or 19% attributable to differential blood pressure control when they analysed 34 RCTs [20].

Measurement of blood pressure and assessment for presence of proteinuria are the cornerstones of screening for pre-eclampsia, and part of routine antenatal care throughout the world [6, 7]. Both are subject to error due to inadequate training in how to make reliable measurements and to poor equipment. Although dipstick testing for screening of proteinuria has issues of intraobserver and interobserver variability and limited sensitivity and specificity, it is widely used and might be the only test available in low-income and middle-income countries[7].

Table B.2. A summary of key findings from studies on high altitude and pregnancy/birth outcomes.

Authors (years)	Altitude (m)	Lowland comparison	Data	Outcome	Ancestry	Confounders	Model	Key findings
Jensen et al.(1997)[21]	USA, Colorado: 3,000 m-3,500m (n=3,836)		Retrospective cohort study: Birth certificates in Colorado(1989-1991)	Birthweight(BW)	-	Gestational weeks, weight gain, parity, smoking, prenatal care visits, Hypertensive complications of pregnancy	ANOVA, χ^2 , Stepwise regression	BW declined 102 g for every 1000-m gain.
Palmer et al.(1999)[22]	USA, Colorado: 3,100 m(Leadville, n=93)	1260m (Burlington, Colorado, n=116)	Retrospective cohort study: Hospital records	BW, Pre-eclampsia(PE), blood pressure	-	Population matched on population size, age, economic characteristics) Confounders: Maternal weight, diabetes, age, gravidity, previous pregnancy complications, smoking, parity	ANOVA, χ^2 , Stepwise regression	BW: 285g lower at 3100m PE: 16% (3100 m) vs 3% (1260m) (OR 3.6, 95% CI:1.1-1.9)
Giussani et al. (2001)[23]	Bolivia: 3,649 m (La Paz, Bolivia, n=200 :100 each from low/high income area)	437 m (Santa Cruz) n=200:100 each from low/high income area)	Retrospective cohort study: Hospital records between 1997 and 1998	BW, body length(Lt), head circumference(HC)	-	Stratified by low and high income regions	ANOVA, χ^2 ,	The cumulative frequency curve across all compiled BW was shifted to the left in babies from a high altitude compared with those in a low altitude, regardless of economic status.
Moore et al. (2001)[24]	The Tibetan Autonomous Region, China 2,700 m- 4,700 m n=452		8 hospitals for 18 months between 1992- 1993.	BW, Preterm(PTB), prenatal/post-natal mortality	Tibetan Han(Chinese) By parents information, language, and dress	Maternal age, parity, and/or gravity	ANOVA, Linear regression	At 2,700 -3,000m: Tibetan babies weighed more than Hans, averaging 310g 95% CI: 126-494) heavier At 3,000-3,800: 530g (95% CI: 210-750g).
Keyes et al. (2002)[25]	Bolivia: 3,649 m (La Paz, Bolivia, n=1,607)	437 m (Santa Cruz, n=813)	Retrospective cohort study: Data from the national health fund (Jan 1998- April 1998) and	BW, IUGR,PE and gestational hypertension(GH)	-	Parity, maternal age, weight at delivery for PE and GH analysis	ANOVA, and stepwise logistic regression to estimate the effect of altitude on PE or GH.	BW: 3084±12g vs 3366 ±18 g (p<0.01) IUGR: 16.8%; 95% CI: 14.9-18.6 vs 5.9%; 95% CI: 4.2-7.5 (p<0.01)

			private clinics(Jan 1996-April 1998)					PE and GH: 1.7(95% CI: 1.3-2.3)
Hartinger et al. (2006)[26]	Peru: 3,800m (Juliaca, n=5,603) & 3,400m(Cuzco, n=3068) & 3,280m (Huancayo, n=12,321))	150 m (Lima, n=63,181)	Retrospective cohort study: Peruvian Perinatal Information System (1995-2002)	BW	Aymara (Juliaca), Quechua (Cuzco), Huancayo By place of residence	Mother's age, marital status, education, parity, maternal BMI, smoking, PE, hemorrhage during pregnancy, gestational age, infant's sex	ANOVA, χ^2 , Multivariate regression	BW reduction relative to Lima Huancayo: 208g Cuzco:170.5g Juliaca: 110.1g BW reduction may be less severe in populations that have resided longer at high altitudes
Julian et al. (2007)[27]	Bolivia: 3,200-4,100 (La Paz or Oruro) & 2,500m (Cochabamba)	437 m (Santa Cruz)	Retrospective cohort study: Hospital records in public hospitals(Jan 1996 - May 1998) and public clinics(Jan 1996-May 1999)	BW, preterm, and SGA	Andean (Aymara/ Quechua), Mestizo European, By last names	For BW: gestational age, maternal weight at term, parity, prenatal visits, PE/GH, infant's sex,	ANOVA, χ^2 , Logistic regression for SGA, and linear regression for BW	BW: Low altitude Andean:3328 (29)g, Mestizo: 3315 (18)g, European: 3215(40)g Intermediate: High altitude Andean:3329 (33)g, Mestizo: 3325 (22)g, European: 3200(45)g High: Andean:3122(20) g, Mestizo: 3119 (13)g, European: 2999(37)g SGA: Andean SGA: European relative to Andean ancestry increased frequency of SGA at high altitude (OR:4.94, 95% CI 2.35-10.38)
Wehby et al. (2010)[28]	1,854-3,600m (Bolivia, Colombia, Ecuador, n=5803) 5 -1,280 m (Argentina, Brazil, Chile and Colombia, n=58,143) High altitude:1,854-3,600 m Low altitude: 5-1,280m		117 primary hospital affiliated with the Latin American Collaborative Study of Congenital Abnormalities between 1982 and 2008	BW, GA	Native, African, European Latin, European non-Latin, Other	Maternal age, infant's sex, ethnic ancestry, characteristics of healthcare institution of birth , time effects, country fixed effects,	Quintile regression	BW reduction: 270-280g decrease in BW mean with moving from 5m up to 3,600m.

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Appendix C
Consent form in Dzongkha

Appendix D
Consent form in English



Interview Participant Consent Form 2014-2015

This form may be read by the participant or read out loud to the participant by the researcher to gain written consent.

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the research with you)**
- **Interview Participant's Agreement (for signatures if you agree to take part)**

You will be given a copy of the full Informed Consent Form.

Study Title: Exploring modifiable risk factors of low birth weight and preterm birth in Bhutan

Foreign Principal Investigator:

Yuka Karasawa

PhD Candidate

London School of Hygiene and Tropical
Medicine, UK

Yuka.karasawa@lshtm.ac.uk

[Tel: XXXXXXXXX](tel:XXXXXXXX)

Lead co-investigator at JDWNRH:

Dr. Phurb Dorji

Head of Department of Obs & Gyne

JDW National Referral Hospital

Hello, my name isI am a part of a research team that is carrying out a study by London School of Hygiene and Tropical Medicine and Ministry of Health, JDWNRH, CRRH, ERRH, and University of Medical Sciences of Bhutan. The principal investigators are Ms. Yuka Karasawa and Dr. Phurb Dorji is the lead co-investigator at JDWNRH. Ms. Yuka Karasawa is a student at London School of Hygiene and Tropical Medicine, UK and research fellow with University of Medical Sciences of Bhutan. She is conducting interviews for her PhD with members of the research team. We are doing research to understand why some babies are born too early or very small and to see if we can find ways of preventing low birth weight and preterm birth in the future for everyone's benefit. This study will be conducted at JigmeDorji National Wangchuck Referral Hospital and the two referral hospitals in Mongar and Gelephu, in collaboration with Bhutanese partners. Both the mothers of new born babies who are born too early or very small and the mothers of normal weight babies will be interviewed. I would like to ask your permission for you to participate in a research study.

During this study, you will be asked to answer some questions about your general health and pregnancy, including your habits and diet. We would like to access your medical records if necessary with your permission. The interview was designed to be between 30 and 60 minutes, depending on the number of questions we ask you. If there are questions you would rather not answer or that you do not feel comfortable answering, please say so and we will stop the interview or move on to the next question, whichever you prefer.

All the information will be kept confidential. The data will be stored securely. Only a few trained staff who are closely concerned with the research will have access to the information. The results of the study will be written up in a PhD dissertation and in a report submitted to the Ministry of Health. Your quotes may be used in the final reports. In such case your name and identity will be kept out of these reports, and your participation will be anonymous.

All participation in this study is voluntary. You are free to decide if you want to take part or not. If you do agree you can change your mind at any time. Choosing not to take part will not disadvantage you in any way. There are no individual benefits to taking part, but in answering our questions you will help us improve our understanding of preterm birth and low birth weight for the benefit of all Bhutanese in the future. The study is approved by London School of Hygiene and Tropical Medicine and Royal Government of Bhutan.



Interview Participant's Agreement:

Study Title: Exploring modifiable risk factors of low birth weight and preterm birth in Bhutan

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have been asked have been answered to my satisfaction. I give permission for responsible individuals from the London School of Hygiene & Tropical Medicine, from regulatory authorities or from this hospital, to access my medical records, where it is relevant to my taking part in this research. I agree for my quote to be used in the publication or report released on the study. I consent voluntarily to be a participant in this study.

Print Name of Participant	Signature of Participant	Date (Day/month/year)
---------------------------	--------------------------	--------------------------

If the participant is unable to sign, a literate witness must sign (if possible, this person should be selected by the participant and should have no connection to the research team). Participants who are illiterate should include their thumb print as well.

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print Name of Witness	Signature of Witness	Date (Day/month/year)
-----------------------	----------------------	--------------------------

And

Thumb print of participant

Statement by the researcher/person taking consent

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands that the following will be done:

1. The data will be used in a Final Report and a PhD dissertation.
2. Sections of the medical notes and data collected during the study may be looked at by responsible individuals from the London School of Hygiene & Tropical Medicine, from regulatory authorities or from this hospital.
3. The participant's quote may be used in the publication or report released on the study. I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily. A copy of this ICF has been provided to the participant.

Print Name of Researcher/person taking the consent	Signature of Researcher /person taking the consent	Date (Day/month/year)
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Appendix E

Check-list during the pilot study

For Pilot Study Only

Please ask the mothers the following questions.

(1) How was the length of the questionnaire? 0 – Too long 1 – OK 2 – Too short	
(2) Are any of the words ambiguous or difficult to understand? 0 – No 1 – Yes (please specify the question numbers)	
(3) Were there any questions where the options you want were not available? 0 – No 1 – Yes (please specify the question numbers)	

For the research nurses:

	Hours	Minutes
(1) How long did the interview take?		
(2) Are any of the questions particularly difficult or sensitive? 0 – No 1 – Yes (please specify the question numbers)		
(3) Are the questions misinterpreted by the mothers? Are any of the words ambiguous or difficult to understand? 0 – No 1 – Yes (please specify the question numbers)		
(4) Does the questionnaire flow smoothly? Can the interviewers follow the instructions easily? Do the interviewers misinterpret the questions? 0 – No 1 – Yes (please specify the question numbers)		
(5) Is there adequate space on the form and the answers clearly coded? 0 – No 1 – Yes (please specify the question numbers)		

Appendix F

Questionnaire

General Information																	
Instructions for interviewers: Please write the number in the right column unless otherwise specified.																	
1. Hospital 1- JDWNRH (Birthing Center) 3- Gelephu 2- JDWNRH (Maternity Ward) 4- Mongar																	
2. Start of the interview (Time & DD/MM/YYYY)	Time: : (am / pm) <table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 20px;"> </td> </tr> <tr> <td>D</td> <td>D</td> <td>M</td> <td>M</td> <td>Y</td> <td>Y</td> <td>Y</td> <td>Y</td> </tr> </table>									D	D	M	M	Y	Y	Y	Y
D	D	M	M	Y	Y	Y	Y										
3. Name of the interviewer																	
4. Study participant number:																	
5. Interview language: 1 – English 5 – Bumtap 2 – Dzongkha 6 –Khengkha 3 – Sharchhop 7 – Others (please specify) 4 - Lhotsham																	
6. Citizenship ID No.																	
7. When admitted to the hospital?(DD/MM/YYYY)	<table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 20px;"> </td> </tr> <tr> <td>D</td> <td>D</td> <td>M</td> <td>M</td> <td>Y</td> <td>Y</td> <td>Y</td> <td>Y</td> </tr> </table>									D	D	M	M	Y	Y	Y	Y
D	D	M	M	Y	Y	Y	Y										

Medical records									
Instructions for interviewers: For questions 7 – 55, please transcribe from the medical records. Please refer to the MCH handbook p2, 3, 4, 5, 6, 8, 12 and Immediate post partum record and maternity history sheet. If anything is not clear, please confirm with the mothers.									
8. Labor: spontaneous or induced 0- Spontaneous 1- Induced 2- Others (Specify) 9- Unknown									
9. Preterm Premature rupture of membranes (PPROM): 0- No 1- Yes 9- Unknown									
10. MCH Reg. No (MCH p2)									
11. Full name of the mother (MCH p2):									
12. Date of birth of the mother (MCH p2)	Date <table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 50px;"> </td> <td style="width: 50px;"> </td> </tr> </table> (If not known, write 98) Month <table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 50px;"> </td> <td style="width: 50px;"> </td> </tr> </table> (If not know, write 9998) Year <table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 20px;"> </td> <td style="width: 20px;"> </td> <td style="width: 20px;"> </td> <td style="width: 20px;"> </td> </tr> </table> (If not known, write 9998)								

<p>13. Age (in completed years): Probe: How old were you at your last birthday?</p>																					
<p>14. Permanent address (MCH p3): Village Gewog/Town Name</p>	<p>Village <input type="text"/> Gewog/Town Name <input type="text"/></p>																				
<p>15. Permanent address (MCH p3): Dzongkhag</p> <table border="0"> <tr> <td>11- Bumthang</td> <td>21 – SamdrupJongkhar</td> </tr> <tr> <td>12 - Chukha</td> <td>22 – Samtse</td> </tr> <tr> <td>13 – Dagana</td> <td>23 – Sarpang</td> </tr> <tr> <td>14 - Gasa</td> <td>24 – Thimphu</td> </tr> <tr> <td>15 – Haa</td> <td>25 – Trashigang</td> </tr> <tr> <td>16 – Lhuntse</td> <td>26 - Trashiyangtse</td> </tr> <tr> <td>17 – Monggar</td> <td>27 – Trongsa</td> </tr> <tr> <td>18 – Paro</td> <td>28 – Tsirang</td> </tr> <tr> <td>19 – Pemagatshel</td> <td>29 – Wangdue</td> </tr> <tr> <td>20 – Punakha</td> <td>30 - Zhemgang</td> </tr> </table>	11- Bumthang	21 – SamdrupJongkhar	12 - Chukha	22 – Samtse	13 – Dagana	23 – Sarpang	14 - Gasa	24 – Thimphu	15 – Haa	25 – Trashigang	16 – Lhuntse	26 - Trashiyangtse	17 – Monggar	27 – Trongsa	18 – Paro	28 – Tsirang	19 – Pemagatshel	29 – Wangdue	20 – Punakha	30 - Zhemgang	
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<p>16. What is the highest education level you finished? (MCH p3)</p> <p>0 - Never attended school 1 - Primary 2 - Secondary 3 – Graduate 4 – NFE 5 –Other(specify) 6 – DON’T KNOW</p>																					
<p>17. Mobile or home phone numbers if available (MCH p3)</p>																					
<p>18. Current address(MCH p3): Village Gewog/Town Name</p>	<p>Village <input type="text"/> Gewog/Town Name <input type="text"/></p>																				
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19 – Pemagatshel	29 – Wangdue																				
20 – Punakha	30 - Zhemgang																				

<p>20. If currently/formerly married/living with a partner: What was the highest level of school your husband/partner attended: primary, secondary, or higher (MCH p4)?</p> <p>0 - Never attended school 1 - Primary 2 - Secondary 3 - Graduate 4 - NFE 5 - Other (specify) 6 - Don't know</p>				
<p>21. ANC Initial General Examination (MCH p4)</p> <p>Record if available. If unknown, please write 9999.</p>	<p>BP</p>			
	<p>Height (cm)</p>			
	<p>Weight (kg)</p>			
<p>22. Medical History (MCH p4): (Please tick "yes" or "no" for each line.)</p>	<p>No Yes</p> <p><input type="checkbox"/> <input type="checkbox"/> Diastolic BP over 90</p> <p><input type="checkbox"/> <input type="checkbox"/> Pelvic mass</p> <p><input type="checkbox"/> <input type="checkbox"/> Suspected STI/RTI</p> <p><input type="checkbox"/> <input type="checkbox"/> Vaginal bleeding</p> <p><input type="checkbox"/> <input type="checkbox"/> Cardiac disease</p> <p><input type="checkbox"/> <input type="checkbox"/> Hypertension</p> <p><input type="checkbox"/> <input type="checkbox"/> Thyroid Disease</p> <p><input type="checkbox"/> <input type="checkbox"/> Family history of twins</p> <p><input type="checkbox"/> <input type="checkbox"/> Family history of congenital defects</p> <p><input type="checkbox"/> <input type="checkbox"/> Known "substance " abuse</p> <p><input type="checkbox"/> <input type="checkbox"/> Diabetes</p> <p><input type="checkbox"/> <input type="checkbox"/> Hepatitis</p> <p><input type="checkbox"/> <input type="checkbox"/> Tuberculosis</p> <p><input type="checkbox"/> <input type="checkbox"/> Blood Transfusion</p> <p><input type="checkbox"/> <input type="checkbox"/> Renal Disease</p> <p><input type="checkbox"/> <input type="checkbox"/> Other severe medical disease(list)</p>			
<p>23. Past Obstetric History(MCH p4) (Please tick "yes" or "no" for each line.)</p>	<p>No Yes</p> <p><input type="checkbox"/> <input type="checkbox"/> Previous still birth or neonate loss</p> <p><input type="checkbox"/> <input type="checkbox"/> History of 3 or more consecutive spontaneous abortions</p> <p><input type="checkbox"/> <input type="checkbox"/> Birth weight of last baby less than 2500 grams</p> <p><input type="checkbox"/> <input type="checkbox"/> Birth weight of last baby more than 4500 grams</p> <p><input type="checkbox"/> <input type="checkbox"/> Last pregnancy: admission for hypertension, pre-eclampsia, or eclampsia</p>			
<p>24. List any medicine(MCH p4)</p>	<p><input type="checkbox"/> Tick if NONE</p> <p><input type="checkbox"/> Tick if not available</p> <p>Regular medications(eg. for asthma) ()</p> <p>Medication started during pregnancy (eg. for high blood pressure or antibiotics for infections) ()</p>			

25. List any allergies (MCH p4)	<input type="checkbox"/> Tick if NONE <input type="checkbox"/> Tick if not available																										
26. Investigations(MCH p5) ⁱ	<table border="1"> <thead> <tr> <th>Primary Test</th> <th>Date(DD/MM/YY)</th> <th>Results</th> </tr> </thead> <tbody> <tr><td>VDRL or RPR</td><td></td><td></td></tr> <tr><td>HBsAG</td><td></td><td></td></tr> <tr><td>HIV</td><td></td><td></td></tr> <tr><td>TPHA</td><td></td><td></td></tr> <tr><td>FBS</td><td></td><td></td></tr> <tr><td>OGTT</td><td></td><td></td></tr> <tr><td>PPBS</td><td></td><td></td></tr> </tbody> </table>	Primary Test	Date(DD/MM/YY)	Results	VDRL or RPR			HBsAG			HIV			TPHA			FBS			OGTT			PPBS				
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27. Was gestational age estimated based on clinical assessment in the late stage of pregnancy due to lack of the first date of last menstrual period and USG scans? 0- No 1- Yes 9- Unknown																											
28. First date of Last Menstrual Period(MCH p6)	<input type="checkbox"/> Tick if not available (comments if any: _____) <table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 25px;"> </td><td style="width: 25px;"> </td> </tr> <tr> <td>D</td><td>D</td><td>M</td><td>M</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td> </tr> </table>											D	D	M	M	Y	Y	Y	Y								
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29. Estimated Date of Delivery (EDD) (MCH p6)	<table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 25px;"> </td><td style="width: 25px;"> </td> </tr> <tr> <td>D</td><td>D</td><td>M</td><td>M</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td> </tr> </table>											D	D	M	M	Y	Y	Y	Y								
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30. EDD by 1 st USG Scan(MCH p6)	<table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 25px;"> </td><td style="width: 25px;"> </td> </tr> <tr> <td>D</td><td>D</td><td>M</td><td>M</td><td>Y</td><td>Y</td><td>Y</td><td>Y</td> </tr> </table>											D	D	M	M	Y	Y	Y	Y								
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32. POG at 1 st scan (wks and days) (MCH p6)	<table style="width: 100%;"> <tr> <td style="width: 50%;"></td> <td style="width: 25%; text-align: center;">Wks</td> <td style="width: 25%; text-align: center;">Days</td> </tr> </table>				Wks	Days																					
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33. Gravida (The woman's total number of pregnancies, including the present pregnancy) (MCH p6)																											
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35. Abortion (MCH p6)																											
36. Living (number of children alive) (MCH p6)																											
37. Dead (number of children dead) (MCH p6)																											

<p>38. When was last time you became pregnant before this pregnancy?</p>	<p><input type="checkbox"/> Tick if not available</p> <p>Year <table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td style="width: 20px; height: 20px;"></td> </tr> <tr> <td style="text-align: center;">Y</td> <td style="text-align: center;">Y</td> <td style="text-align: center;">Y</td> <td style="text-align: center;">Y</td> </tr> </table></p> <p>Month <table border="1" style="display: inline-table; vertical-align: middle;"> <tr> <td style="width: 20px; height: 20px;"></td> <td style="width: 20px; height: 20px;"></td> </tr> <tr> <td style="text-align: center;">M</td> <td style="text-align: center;">M</td> </tr> </table></p>					Y	Y	Y	Y			M	M																																																
Y	Y	Y	Y																																																										
M	M																																																												
<p>39. Did you attend antenatal care at least once during this pregnancy? 0 – No 1 –Yes 9999 - Unknown</p>																																																													
<p>40. How many times did you receive antenatal care during this pregnancy? (If unknown, please write 9999.)</p>																																																													
<p>41. During your pregnancy, was your blood pressure measured at least once? 0 – No 1 –Yes 9999 - Unknown</p>																																																													
<p>42. Please check page 8 of MCH book and record BP and Urine (ALB) for Pregnancy Induced Hypertension.</p>	<table border="1" style="width: 100%; border-collapse: collapse;"> <thead> <tr> <th style="width: 5%;"></th> <th style="width: 25%;">Date (DD/MM/YYYY)</th> <th style="width: 20%;">Urine (Alb)</th> <th style="width: 15%;">Wt</th> <th style="width: 35%;">BP</th> </tr> </thead> <tbody> <tr><td>1</td><td></td><td></td><td></td><td></td></tr> <tr><td>2</td><td></td><td></td><td></td><td></td></tr> <tr><td>3</td><td></td><td></td><td></td><td></td></tr> <tr><td>4</td><td></td><td></td><td></td><td></td></tr> <tr><td>5</td><td></td><td></td><td></td><td></td></tr> <tr><td>6</td><td></td><td></td><td></td><td></td></tr> <tr><td>7</td><td></td><td></td><td></td><td></td></tr> <tr><td>8</td><td></td><td></td><td></td><td></td></tr> <tr><td>9</td><td></td><td></td><td></td><td></td></tr> <tr><td>10</td><td></td><td></td><td></td><td></td></tr> <tr><td>11</td><td></td><td></td><td></td><td></td></tr> </tbody> </table>		Date (DD/MM/YYYY)	Urine (Alb)	Wt	BP	1					2					3					4					5					6					7					8					9					10					11				
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<p>43. Any records for Pre-eclampsia, Gestational hypertension, and eclampsia?</p>	<p>No Yes Unknown</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Pre-ecplamsia?</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Gestational Hypertension?</p> <p><input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/> Eclampsia?</p>																																																												
<p>44. Any records for urinary tract infection? 0 – No 1 – Yes 9999 – None recorded</p>																																																													
<p>45. Please check White Blood Cell count (WBCs) in urine from the urine test. Is WBCs >5-10? 0 – No 1 – Yes 9999 – None recorded</p>																																																													

<p>46. Any records for diabetes?[Interviewers tick “Yes” if mothers were diagnosed with diabetes in the ANC or medical records] 0 – No 1 – Yes 9999 - None recorded</p>																									
<p>47. Any records for anemia?[Interviewers tick “Yes” if mother’s haemoglobin level in the MCH book and medical records was less than 10 g/dL] 0 – No 1 – Yes 9999 - None recorded</p>																									
<p>48. Has the mother taken antenatal corticosteroids? 0 – No 1 – Yes 9999 - None recorded</p>																									
<p>49. Has the mother taken tocolysis? 0 – No 1 – Yes 9999 - None recorded</p>																									
<p>50. Has the mother taken any antibiotics? 0 – No 1 – Yes 9999 - None recorded</p>																									
<p>51. Delivery date and time? (MCH p12)</p>	<table border="1" style="width: 100%; text-align: center;"> <tr> <td style="width: 25px;"> </td> </tr> <tr> <td>D</td> <td>D</td> <td>M</td> <td>M</td> <td>Y</td> <td>Y</td> <td>Y</td> <td>Y</td> </tr> <tr> <td colspan="6">Time</td> <td>:</td> <td>am/pm</td> </tr> </table>									D	D	M	M	Y	Y	Y	Y	Time						:	am/pm
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<p>52. Mode of delivery(MCH p12) 1- SVD 2- CS-Elective 3- CS-Emerg. 4- Vacuum 5- Forceps 6- Breech 9999 - Unknown</p>																									
<p>53. Sex of an infant (MCH p12) 0 – Male 1-Female 9- Ambiguous</p>																									
<p>54. Birth weight of an infant (grams) (MCH p12)</p>	grams																								
<p>55. Period of gestational age (POG) (wks) (MCH p12)</p>	Wks Days																								

Questions to the mothers

<p>63. Have you had any preterm births before this delivery? 0 – No 1 – Yes 9 - Don't Know</p>	
<p>64. How long was your travel time to the hospital on this occasion to give birth? (total travel time to the hospital)</p>	<p style="text-align: center;">Hours Minutes</p> <p style="text-align: center;">_____</p>
<p>65. How did you travel to this hospital? (multiple responses allowed)</p> <p>1 – By car 2 – By motorbike 3 – By bus 4 – By taxi 5 – By ambulance 6 – By stretcher 7 – On foot 8 – Others (please specify)</p>	
<p>66. Where do you usually go for health care services?</p> <p>1 - Referral hospital 2 - District hospital 3 - Military hospital 4 - Basic Health Unit I 5 - Basic Health Unit 6 - Sub-post 7 - ANC satellite (Thimphu only) 8 –Others(please specify) 9 - Don't Know</p>	
<p>67. Why did you come to this hospital to deliver your baby? (please select the best answer from the choices provided)</p> <p>1 - Because I or my relatives live near the hospital 2 - For better health care and services 3 - More equipped 4 - Has more qualified staff 5 - Referral from other hospitals or BHUs 6 - Others (please specify)</p>	
<p>68. What is your occupation?</p> <p>1 – Housewife [skip to Q. 72] 2 - Unemployed [skip to Q. 72] 3 – Student [skip to Q. 72] 4 - Self-employed 5 – Employee</p>	
<p>69. What kind of work do you mainly do? Please describe. (eg. farmers, weavers, civil servants, shop keepers, teachers, daily wage labors, members of parliament(MP) etc)</p>	

<p>70. In the past 10 months, did you work:</p> <ul style="list-style-type: none"> 1 – Only during daytime 2 – Fixed evening work 3 – Fixed night work 4 – Rotating shift work without night 5 – Rotating shift work with night 6 – Others (specify) 																									
<p>71. How many hours did you work per week on average in the past 10 months?</p> <ul style="list-style-type: none"> 1- Less than 20 hours 2 - Between 20 and 40 hours 3 - More than 40 hours 																									
<p>72. What is your current marital status?</p> <ul style="list-style-type: none"> 1 – Single [Skip to Q. 74] 2 – Married 3 – Divorced 4 – Separated 5 – Living with a partner 6 – Widow 7 – Others (specify) 																									
<p>73. If currently/formerly married/living with a partner: What is (was) the occupation of your husband/partner? That is, what kind of work does (did) he mainly do? Please describe. (eg. guides, drivers, farmers, carpenters, civil servants, teachers, daily wage labors, monks, members of parliament(MP) etc)</p>																									
<p>74. During your pregnancy, did you ever travel on a rough or bumpy road in a vehicle such as car, bus, truck?</p> <p>0 – No [Skip to Q. 76] 1 – Yes 9 - Don't Know</p>																									
<p>75. If yes, how often did you travel?</p> <table border="1" data-bbox="180 1266 789 1667"> <tr><td>1</td><td>Every day or nearly every day</td></tr> <tr><td>2</td><td>Three to four times a week</td></tr> <tr><td>3</td><td>Once or twice a week</td></tr> <tr><td>4</td><td>Two to three times a month</td></tr> <tr><td>5</td><td>About once a month</td></tr> <tr><td>6</td><td>Seven to eleven times during pregnancy</td></tr> <tr><td>7</td><td>Three to six times during pregnancy</td></tr> <tr><td>8</td><td>Twice during pregnancy</td></tr> <tr><td>9</td><td>Once time during pregnancy</td></tr> <tr><td>10</td><td>Never during pregnancy</td></tr> <tr><td>11</td><td>Don't know</td></tr> <tr><td>12</td><td>Others (please specify)</td></tr> </table>	1	Every day or nearly every day	2	Three to four times a week	3	Once or twice a week	4	Two to three times a month	5	About once a month	6	Seven to eleven times during pregnancy	7	Three to six times during pregnancy	8	Twice during pregnancy	9	Once time during pregnancy	10	Never during pregnancy	11	Don't know	12	Others (please specify)	
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Life style

Next I am going to ask you about the time you spent doing different types of physical activity in a typical week during your pregnancy. Please answer these questions even if you do not consider yourself to be a physically active person. Think first about the time you spent doing work. Think of work as the things that you had to do such as paid or unpaid work, studying/training, household chores, harvesting food/crops, fishing or hunting for food, seeking employment. In answering the following questions 'vigorous-intensity activities' are activities that require hard physical effort and cause large increases in breathing or heart rate,

'moderate-intensity activities' are activities that require moderate physical effort and cause small increases in breathing or heart rate.

76. During your pregnancy, did your work involve vigorous-intensity activity that causes large increases in breathing or heart rate like carrying or lifting heavy loads, digging or construction work for at least 10 minutes continuously?
0 – No [Skip to Q. 79] 1 – Yes

77. During your pregnancy, how often did you do vigorous-intensity activities as part of your work?

1	Every day or nearly every day
2	Three to four times a week
3	Once or twice a week
4	Two to three times a month
5	About once a month
6	Seven to eleven times during pregnancy
7	Three to six times during pregnancy
8	Twice during pregnancy
9	Once time during pregnancy
10	Never during pregnancy
11	Don't know
12	Others (please specify)

78. During your pregnancy, how much time did you spend doing vigorous-intensity activities at work in total on atypical day?

Hours Minutes

79. During your pregnancy, did your work involve moderate-intensity activity that causes small increases in breathing or heart rate such as housework and domestic chores, brisk walking or carrying light loads for at least 10 minutes continuously?
0 - No [Skip to Q. 82] 1- Yes

80. During your pregnancy, how often did you do moderate-intensity activities as part of your work?

1	Every day or nearly every day
2	Three to four times a week
3	Once or twice a week
4	Two to three times a month
5	About once a month
6	Seven to eleven times during pregnancy
7	Three to six times during pregnancy
8	Twice during pregnancy
9	Once time during pregnancy
10	Never during pregnancy
11	Don't know
12	Others (please specify)

81. During your pregnancy, how much time did you spend doing moderate-intensity activities at work in total on atypical day?

Hours Minutes

Travel to and from places:

The next questions exclude the physical activities at work that you have already mentioned. Now I would like to ask you about the usual way you travel to and from places. For example to work, for shopping, to market, to place of worship and health facility.

82. During your pregnancy, did you walk or use a bicycle (*pedal cycle*) for at least 10 minutes continuously to get to and from places on a typical day?

0 – No [Skip to Q. 85] 1– Yes

83. During your pregnancy, how often did you walk or bicycle for at least 10 minutes continuously to get to and from places?

1	Every day or nearly every day
2	Three to four times a week
3	Once or twice a week
4	Two to three times a month
5	About once a month
6	Seven to eleven times during pregnancy
7	Three to six times during pregnancy
8	Twice during pregnancy
9	Once time during pregnancy
10	Never during pregnancy
11	Don't know
12	Others (please specify)

84. During your pregnancy, how much time did you spend walking or bicycling for travel in total on a typical day?

_____ **Hours** _____ **Minutes**

Recreational activities:

The next questions exclude the work and transport activities that you have already mentioned. Now I would like to ask you about sports, fitness and recreational activities (*leisure*) during your pregnancy.

85. During your pregnancy, did you do any vigorous-intensity sports, fitness or recreational (*leisure*) activities that cause large increases in breathing or heart rate like running or football, martial arts, swimming, badminton, basketball for at least 10minutes continuously?

0 – No [Skip to Q. 88] 1 – Yes

86. During your pregnancy how often did you do vigorous-intensity sports, fitness or recreational (*leisure*) activities?

1	Every day or nearly every day
2	Three to four times a week
3	Once or twice a week
4	Two to three times a month
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6	Seven to eleven times during pregnancy
7	Three to six times during pregnancy
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9	Once time during pregnancy
10	Never during pregnancy
11	Don't know
12	Others (please specify)

87. How much time did you spend doing vigorous intensity sports, fitness or recreational activities in total on a typical day?

_____ **Hours** _____ **Minutes**

<p>88. Did you do any moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities that cause a small increase in breathing or heart rate such as brisk walking, dancing, archery, khuru or degor, volleyball for at least 10 minutes continuously?</p> <p>0 – No [Skip to Q. 91] 1 – Yes</p>																									
<p>89. During your pregnancy, how often did you do moderate-intensity sports, fitness or recreational (<i>leisure</i>) activities?</p> <table border="1" data-bbox="180 436 784 842"> <tr><td>1</td><td>Every day or nearly every day</td></tr> <tr><td>2</td><td>Three to four times a week</td></tr> <tr><td>3</td><td>Once or twice a week</td></tr> <tr><td>4</td><td>Two to three times a month</td></tr> <tr><td>5</td><td>About once a month</td></tr> <tr><td>6</td><td>Seven to eleven times during pregnancy</td></tr> <tr><td>7</td><td>Three to six times during pregnancy</td></tr> <tr><td>8</td><td>Twice during pregnancy</td></tr> <tr><td>9</td><td>Once time during pregnancy</td></tr> <tr><td>10</td><td>Never during pregnancy</td></tr> <tr><td>11</td><td>Don't know</td></tr> <tr><td>12</td><td>Others (please specify)</td></tr> </table>	1	Every day or nearly every day	2	Three to four times a week	3	Once or twice a week	4	Two to three times a month	5	About once a month	6	Seven to eleven times during pregnancy	7	Three to six times during pregnancy	8	Twice during pregnancy	9	Once time during pregnancy	10	Never during pregnancy	11	Don't know	12	Others (please specify)	
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<p>90. How much time did you spend doing moderate intensity sports, fitness or recreational (<i>leisure</i>) activities on a typical day?</p>	<p>_____ Hours _____ Minutes</p>																								
<p>91. How would you describe your daily activity?</p> <p>1 - Very active 2 - Moderately active 3 - Somewhat active 4 - Not active</p>																									

<p>Sedentary behavior:</p> <p>The following question is about sitting or reclining at work, at home, getting to and from places, or with friends including time spent [sitting at a desk, sitting with friends, travelling in car, bus, train, reading, playing cards or watching television], but do not include time spent sleeping.</p>	
<p>92. During your pregnancy, how much time did you usually spend sitting or reclining in total on a typical day? (both at home and at work place)</p>	<p>_____ Hours _____ Minutes</p>

<p>Household Characteristics</p>	
<p>93. How many people live in your household with you, including you but not including this baby. (one household is defined as sharing a single kitchen)?</p>	
<p>94. Number of Children under age 18 (not including this baby):</p>	
<p>95. Number of Adults over age 18 years (including you):</p>	
<p>96. What type of dwelling do you live in?</p> <p>1 - Apartment/flats 2 - Detached house 3 - Village housing 4 - Other (please specify)</p>	
<p>97. How many rooms in your household are used for sleeping?</p>	

98. What is the main material of the dwelling floor?			
Natural floor	Rudimentary floor	Finished floor	
11 - Earthen / clay floor	21- Planks / shingles 22- Bamboo	31- Polished wood 33 - Tiles / marble 34 - Cement / concrete / terrazzo	
96 – Others (specify)			
99. What is the main material of the roof?			
Natural roofing	Rudimentary Roofing	Finished roofing	
11 - No Roof 12 - Thatch	22- Bamboo 23 - Planks / shingles 24 - Cardboard 25 - Tarpaulin	31 -Metal sheets 32 -Tiles / slates 34- Concrete / cement	
96 – Others(specify)			
100. What is the main material of the exterior walls?			
Natural walls	Rudimentary walls	Finished walls	
11 - No walls 12 - Cane / Palm / Trunks/ Bamboo	21 - Bamboo with mud 22 - Stone with mud 24 - Plywood 25 - Cardboard	31 - Cement / RCC wall 32 - Stone with lime / cement 33 - Bricks 34 - Cement blocks 36 - Wood planks 37 - Rammed earth 38 - Mud blocks	
96 – Others (specify)			
101. What is the main source of drinking water for members of your household?			
Piped water	Dug well	Water from spring	
11 - Piped into dwelling 12 - Piped into compound 13 - Piped to neighbour 14 - Public tap	31 - Protected well 32 - Unprotected well	41 - Protected spring 42 - Unprotected spring	
51 - Rainwater collection 61 - Tanker-truck 71 - Cart with small tank / drum 81 - Surface water (river, stream, dam, lake, pond, canal, irrigation channel) 91 - Bottled water 96 - Other (specify)			

<p>102. What kind of toilet facility do members of your household usually use? IF "FLUSH" OR "POUR FLUSH", PROBE: WHERE DOES IT FLUSH TO?</p> <table border="1"> <thead> <tr> <th>Flush / Pour flush</th> <th>Pit latrine</th> </tr> </thead> <tbody> <tr> <td>11 - Flush to piped sewer system</td> <td>21 - Ventilated Improved Pit latrine (VIP)</td> </tr> <tr> <td>12 - Flush to septic tank (without soak pit)</td> <td>22 - Pit latrine with slab</td> </tr> <tr> <td>13 - Flush to septic tank (with soak pit)</td> <td>23 - Pit latrine without slab / Open pit</td> </tr> <tr> <td>14 - Flush to pit (latrine)</td> <td>24 - Long drop latrine</td> </tr> <tr> <td>15 - Flush to somewhere else</td> <td></td> </tr> <tr> <td>16 - Flush to unknown place / Not sure /do not where</td> <td></td> </tr> </tbody> </table> <p>31 - Composting toilet 41 - Bucket 95 - No facility, Bush, Field 96 - Other (specify)</p>	Flush / Pour flush	Pit latrine	11 - Flush to piped sewer system	21 - Ventilated Improved Pit latrine (VIP)	12 - Flush to septic tank (without soak pit)	22 - Pit latrine with slab	13 - Flush to septic tank (with soak pit)	23 - Pit latrine without slab / Open pit	14 - Flush to pit (latrine)	24 - Long drop latrine	15 - Flush to somewhere else		16 - Flush to unknown place / Not sure /do not where										
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<p>103. What type of fuel does your household mainly use for cooking curry?</p> <p>1 - Electricity 2 - Liquefied Petroleum Gas (LPG) 3 - Kerosene 4 - Coal 5 - Wood 6 - Straw/Shrubs/Grass 7 - Dung Cake 8 - No food cooked in household 9 - Other (Specify)</p>																							
<p>104. Is the cooking usually done in the house, in a separate building, or outdoors? If 'In the house', probe: IS IT DONE IN A SEPARATE ROOM USED AS A KITCHEN?</p> <p>1 - In a separate room used as kitchen in the house 2 - Elsewhere in the house 3 - In a separate building 4 - Outdoors 5 - Other (specify)</p>																							
<p>105. Does any member of your household own:</p> <p>[A] A WRIST WATCH? [B] A MOBILE PHONE? [C] A BICYCLE? [D] A MOTORCYCLE OR SCOOTER? [E] A CAR OR TRUCK? [F] A COMPUTER? [G] A FOREIGN BOW? [H] A CAMERA? [I] A VCR/VCD/DVD PLAYER? [J] A SERSHO GHO/KIRA/SILK SARI/SILK SUITS?</p>	<table> <thead> <tr> <th>No</th> <th>Yes</th> </tr> </thead> <tbody> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [A] A WRIST WATCH?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [B] A MOBILE PHONE?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [C] A BICYCLE?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [D] A MOTORCYCLE OR SCOOTER?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [E] A CAR OR TRUCK?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [F] A COMPUTER?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [G] A FOREIGN BOW?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [H] A CAMERA?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [I] A VCR/VCD/DVD PLAYER?</td> </tr> <tr> <td><input type="checkbox"/></td> <td><input type="checkbox"/> [J] A SERSHO GHO/KIRA/ A SILK SARI/SILK SUITS</td> </tr> </tbody> </table>	No	Yes	<input type="checkbox"/>	<input type="checkbox"/> [A] A WRIST WATCH?	<input type="checkbox"/>	<input type="checkbox"/> [B] A MOBILE PHONE?	<input type="checkbox"/>	<input type="checkbox"/> [C] A BICYCLE?	<input type="checkbox"/>	<input type="checkbox"/> [D] A MOTORCYCLE OR SCOOTER?	<input type="checkbox"/>	<input type="checkbox"/> [E] A CAR OR TRUCK?	<input type="checkbox"/>	<input type="checkbox"/> [F] A COMPUTER?	<input type="checkbox"/>	<input type="checkbox"/> [G] A FOREIGN BOW?	<input type="checkbox"/>	<input type="checkbox"/> [H] A CAMERA?	<input type="checkbox"/>	<input type="checkbox"/> [I] A VCR/VCD/DVD PLAYER?	<input type="checkbox"/>	<input type="checkbox"/> [J] A SERSHO GHO/KIRA/ A SILK SARI/SILK SUITS
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Behavioral factors

Smoking: Now we are about to ask the smoking before, during pregnancy and currently.

<p>106. Have you ever smoked any cigarettes?</p> <p>0 - No [Skip to Q. 111] 1 - Yes</p>	
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107. If yes, at what age did you begin to smoke cigarettes?									
108. <u>In the past 10 months</u> , have you smoked any cigarettes even once? 0 - No [Skip to Q. 111] 1 - Yes									
109. If yes, how often did you smoke cigarettes? 1 - Daily or almost daily 2 - Weekly 3 - Monthly 4 - Other (Specify)									
110. <u>In the past 10 months</u> , could you tell us <u>how many cigarettes</u> did you smoke on <u>an average day</u> in each month during your pregnancy? (A pack has 20 cigarettes.) For example, did you smoke more in winter compared to the summer? How about during the festival and holidays?	May 2014	June 2014	July 2014	Aug 2014	Sep 2014	Oct 2014	Nov 2014	Dec 2014	Jan 2015
	Feb 2015	Mar 2015	April 2015	May 2015	June 2015	July 2015	Aug 2015	Sep 2015	Oct 2015
111. Have you used any smokeless tobacco such as snuff and chewing tobacco (not including doma) even once in <u>the past 10 months</u> ? 0 - No [Skip to Q.115] 1 - Yes									
112. If yes, how often did you smokeless tobacco? 1 - Daily or almost daily 2 - Weekly 3 - Monthly 4 - Other (Specify)									
113. What kind of smokeless tobacco did you use? (multiple responses allowed) 1 - Snuff, by mouth 2 - Snuff, by nose 3 - Chewing tobacco (such as baba and khaini) 4 - Others (specify)									
114. <u>In the past 10 months</u> , could you tell us <u>how many packets of smokeless tobaccos</u> did you use on an average day in each month? For example, did you use more in winter compared to the summer? How about during the festival and holidays?	May 2014	June 2014	July 2014	Aug 2014	Sep 2014	Oct 2014	Nov 2014	Dec 2014	Jan 2015
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115. Did you take pan masala such as "Rajnigandha" or "WIZ" in the past 10 months? 0 - No [Skip to Q.118]									

1 – Yes	
116. How often did you take Pan masala such as “Rajnigandha” or “WIZ” in the past 10 months? 1 - Daily or almost daily 2 – Weekly 3 – Monthly 4 – Other (Specify)	
117. How long does it take you to finish 20 g package of pan masala such as “Rajnigandha” or “WIZ”? 1- One day 2- One week 3- One month 4- Two to three month 5- Other(specify)	

Doma: Now we are about to ask doma before, during pregnancy and currently.	
118. Have you ever chewed doma? 0 - No [Skip to Q.129] 1 – Yes	
119. If yes, at what age did you begin to chew doma?	
120. Did you chew doma even once <u>in the past 10 months</u> ? 0 - No [Skip to Q.129] 1 – Yes	
121. If yes, how often did you chew doma? 1 - Daily or almost daily 2 – Weekly 3 – Monthly 4 – Other (Specify)	
122. When do you take doma? Before meals, after meals, with alcohol etc...(multiple responses allowed) 1 - Before meals 2 - After meals 3 - With alcohol 4 - During tea/coffee break 5 - In the morning 6 - When driving 7 - When I have a visitor 8 - When a friend(s) or family offers 9 - During rituals or ceremonies 10 - Others (please specify)	
123. When do you feel like chewing doma?(multiple responses allowed) 1 - When I am bored 2 - When I am happy 3 - When I am stressed 4 - When I am sad 5 - When I feel sick 6 - When I feel sleepy 7 - When I feel tired 8 - When I feel cold 9 - Others (please specify)	

<p>124. When you take doma nut do you take it with piper leaf?</p> <p>1 – Always 2 - Usually 3 – Sometimes 4 – Rarely 5 – Never</p>									
<p>125. Do you add tobacco when you take doma nut?</p> <p>1 – Always 2 - Usually 3 – Sometimes 4 – Rarely 5 – Never</p>									
<p>126. Do you add slaked lime when you take doma nut?</p> <p>1 – Always 2 - Usually 3 – Sometimes 4 – Rarely 5 – Never</p>									
<p>127. Do you spit when you are eating doma?</p> <p>1 – Always 2 - Usually 3 – Sometimes 4 – Rarely 5 – Never</p>									
<p>128. Could you tell us <u>how many doma</u> you chewed on an <u>average day</u> in each month during the past 10 months? For example, did you chew more doma in winter compared to the summer? How about during the festival and holidays?</p>	May 2014	June 2014	July 2014	Aug 2014	Sep 2014	Oct 2014	Nov 2014	Dec 2014	Jan 2015
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Alcohol: Now we are about to ask alcohol before, during pregnancy and currently.

<p>129. Have you consumed any type of alcohol?</p> <p>0 - No [Skip to Q. 140] 1 - Yes</p>									
<p>130. If yes, at what age did you begin to drink alcohol?</p>									
<p>131. Have you consumed any type of alcohol <u>in the past 10 months</u>?</p> <p>0 - No [Skip to Q. 140] 1 - Yes</p>									
<p>132. If yes, how often did you drink alcohol?</p> <p>1 - Daily or almost daily 2 – Weekly 3 – Monthly 4 – Other (Specify)</p>									
<p>133. What beverage do you drink most?</p> <p>1 – Ara 2 – BANGCHANG/ 'SINGCHANG/' TONGPA</p>									

<p>3 –Changkey 4 –Beer 5 – Wine 6 –LIQUOR (rum, whisky, brandy) 7 –Others (please specify)</p>	
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The next few questions are about how much of any type of alcohol you may have had in any one day during the last 10 months. Think of all kinds of alcohol beverages combined that is any combination of beer, wine, liquor, ara, bangchang, singchang, and tongpa.

<p>134. During the last 10 months, what is the largest amount of each type of alcohol you drank on that day when you had the most to drink? (show the sample cup)</p> <p>Ara _____ cups BANGCHANG' _____ cups SINGCHANG _____ cups TONGPA _____ cups Changkey _____ cups Table Wine _____ cups Port Wine _____ cups Fortified Wine _____ cups Spy _____ cups Beer A(Dansberg, Budweiser, Heineken, Singha beer, Chang beer, Carlsberg, Orchim, Haywards, Royal challenge, Golden eagle, Sang Miguel, Tiger) _____ cups Beer B(Druk 11,000, HIT Beer) _____ cups Liquor (run, whisky, brandy) _____ cups Others (specify) _____ cups</p>	<table border="1"> <thead> <tr> <th>Drinks</th> <th>Number of cups</th> </tr> </thead> <tbody> <tr><td>Ara</td><td></td></tr> <tr><td>BANGCHANG'</td><td></td></tr> <tr><td>SINGCHANG</td><td></td></tr> <tr><td>TONGPA</td><td></td></tr> <tr><td>Changkey</td><td></td></tr> <tr><td>Table Wine</td><td></td></tr> <tr><td>Port Wine</td><td></td></tr> <tr><td>Fortified Wine</td><td></td></tr> <tr><td>SPY</td><td></td></tr> <tr><td>Beer A (Dansberg, Budweiser, Heineken, Singha beer, Chang beer, Carlsberg, Orchim, Haywards, Royal challenge, Golden eagle, Sang Miguel, Tiger)</td><td></td></tr> <tr><td>Beer B (Druk 11,000, HIT Beer)</td><td></td></tr> <tr><td>Liquor (run, whisky, brandy)</td><td></td></tr> <tr><td>Others (specify)</td><td></td></tr> </tbody> </table>	Drinks	Number of cups	Ara		BANGCHANG'		SINGCHANG		TONGPA		Changkey		Table Wine		Port Wine		Fortified Wine		SPY		Beer A (Dansberg, Budweiser, Heineken, Singha beer, Chang beer, Carlsberg, Orchim, Haywards, Royal challenge, Golden eagle, Sang Miguel, Tiger)		Beer B (Druk 11,000, HIT Beer)		Liquor (run, whisky, brandy)		Others (specify)	
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<p>135. How many times did you drink as much as the maximum amount you mentioned above in the past 10 months?</p> <table border="1"> <tr><td>1</td><td>Every day or nearly every day</td></tr> <tr><td>2</td><td>Three to four times a week</td></tr> <tr><td>3</td><td>Once or twice a week</td></tr> <tr><td>4</td><td>Two to three times a month</td></tr> <tr><td>5</td><td>About once a month</td></tr> <tr><td>6</td><td>Seven to eleven times in the last 10 months</td></tr> <tr><td>7</td><td>Three to six times in the last 10 months</td></tr> <tr><td>8</td><td>Twice in the past 10 months</td></tr> <tr><td>9</td><td>Once time in the past 10 months</td></tr> <tr><td>10</td><td>Never in the past 10 months</td></tr> <tr><td>11</td><td>Don't know</td></tr> </table>	1	Every day or nearly every day	2	Three to four times a week	3	Once or twice a week	4	Two to three times a month	5	About once a month	6	Seven to eleven times in the last 10 months	7	Three to six times in the last 10 months	8	Twice in the past 10 months	9	Once time in the past 10 months	10	Never in the past 10 months	11	Don't know							
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<p>136. How many times did you drink three-fourth of this amount in the past 10 months?</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr><td style="width: 5%; text-align: center;">1</td><td>Every day or nearly every day</td></tr> <tr><td style="text-align: center;">2</td><td>Three to four times a week</td></tr> <tr><td style="text-align: center;">3</td><td>Once or twice a week</td></tr> <tr><td style="text-align: center;">4</td><td>Two to three times a month</td></tr> <tr><td style="text-align: center;">5</td><td>About once a month</td></tr> <tr><td style="text-align: center;">6</td><td>Seven to eleven times in the last 10 months</td></tr> <tr><td style="text-align: center;">7</td><td>Three to six times in the last 10 months</td></tr> <tr><td style="text-align: center;">8</td><td>Twice in the past 10 months</td></tr> <tr><td style="text-align: center;">9</td><td>Once time in the past 10 months</td></tr> <tr><td style="text-align: center;">10</td><td>Never in the past 10 months</td></tr> <tr><td style="text-align: center;">11</td><td>Don't know</td></tr> </table>	1	Every day or nearly every day	2	Three to four times a week	3	Once or twice a week	4	Two to three times a month	5	About once a month	6	Seven to eleven times in the last 10 months	7	Three to six times in the last 10 months	8	Twice in the past 10 months	9	Once time in the past 10 months	10	Never in the past 10 months	11	Don't know															
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Nutrition:

140. How many times a day do you usually eat including meals and snacks? (___ #meals per day ___# snacks per day)	<u> </u> meals per day	<u> </u> snacks per day
141. How would you describe your appetite in the past 10 months? 1 - Good 2 – Fair 3 - Poor		

Fruits and vegetables: The next questions ask about the fruits and vegetables that you usually eat. I have a nutrition card here that shows you some examples of local fruits and vegetables. Each picture represents the size of a serving. As you answer these questions please think of a typical week in the past 10 months.

142. In a typical week, on how many days did you eat fruit in the past 10 months? If 0 days, skip to 144. If don't know, please write "77".	Days
	Others (Please specify):
143. How many servings of fruit do you eat on one of those days? (<i>one fruit serving is (a) ½ cup of chopped, cooked or canned fruit; (b) 1 medium-sized piece of fruit such as banana , apple, orange; (c) ½ cup of fruit juice (not artificially flavored) (USE SHOWCARD)</i>)	Servings
144. In a typical week, on how many days did you eat vegetables (excluding chilies and tubers such as potatoes) in the past 1- months? If 0 days, skip to 146. If don't know, please write "77".	Days
	Others (Please specify):
145. How many servings of vegetables did you eat on one of those days? (one vegetable serving is (a) 1 cup of raw green leafy vegetable such as spinach, salad greens, etc. (b) ½cup of other vegetables, cooked or chopped, such as carrots, pumpkin, corn, beans, onion, etc. but excluding chilies and tubers such as potatoes .)(<i>USE SHOWCARD and sample cup</i>)	Servings
146. What type of oil or fats most often used for meal preparation in your household? 1 – Vegetable oil 7- Lard or suet(animal fat/tselol) 8- Butter or ghee 9- Margarine 10- Other (please specify) 11- None in particular 12- None used 13- Don't know	
147. Total amount of fat/oil used in a month (all forms together)	<u> </u> Liters <u> </u> ml
148. How many members of the family eat from the same pot?	
149. How frequently did you eat "Ezay" in the past 10 months? 1 – Daily 2- 5-6 days per week 3- 1-4 days per week 4 - 1-3 days per month 5 - Less than once a month 6 – Never 7 – Stopped during pregnancy	

<p>150. How frequently do you eat dry meat in the past 10 months? 1 – Daily 2- 5-6 days per week 3- 1-4 days per week 4 - 1-3 days per month 5 - Less than once a month 6 – Never 7 – Stopped during pregnancy</p>																	
<p>151. How frequently do you drink Suja in the past 10 months? 1 – Daily 2- 5-6 days per week 3- 1-4 days per week 4 - 1-3 days per month 5 - Less than once a month 6 – Never 7 – Stopped during pregnancy</p>																	
<p>152. How often did you drink milk tea with sugar in the past 10 months? 1 – Daily 2- 5-6 days per week 3- 1-4 days per week 4 - 1-3 days per month 5 - Less than once a month 6 – Never 7 – Stopped during pregnancy</p>																	
<p>153. How often did you drink coffee in the past 10 months? 1 – Daily 2- 5-6 days per week 3- 1-4 days per week 4 - 1-3 days per month 5 - Less than once a month 6 – Never 7 – Stopped during pregnancy</p>																	
<p>154. End of the interview (Time & DD/MM/YYYY)</p>	<table border="1" style="width: 100%;"> <tr> <td style="width: 15%;">Time:</td> <td style="width: 10%; text-align: center;">:</td> <td style="width: 50%;"></td> <td style="width: 10%; text-align: right;">(am / pm)</td> </tr> <tr> <td style="width: 12.5%;"></td> <td style="width: 12.5%;"></td> <td style="width: 12.5%;"></td> <td style="width: 12.5%;"></td> </tr> <tr> <td style="text-align: center;">D</td> <td style="text-align: center;">D</td> <td style="text-align: center;">M</td> <td style="text-align: center;">M</td> </tr> <tr> <td style="text-align: center;">Y</td> <td style="text-align: center;">Y</td> <td style="text-align: center;">Y</td> <td style="text-align: center;">Y</td> </tr> </table>	Time:	:		(am / pm)					D	D	M	M	Y	Y	Y	Y
Time:	:		(am / pm)														
D	D	M	M														
Y	Y	Y	Y														

End of questionnaire. Thank you!

ⁱ The following test results were recorded: Venereal Disease Research Laboratory (VDRL) or Rapid Plasma Reagin (RPR), *Treponema pallidum* hemagglutination assay (TPHA) for STI/RTI; HBsAG for hepatitis B; HIV; and Fasting Blood Sugar (FBS), Oral Glucose Tolerance Test (OGTT), and *Post-prandial Blood-Sugar (PPBS)*.

Appendix G

Research Team

Field Research Team

Co-investigators:

Dr. Shunmay Yeung (LSHTM)
Dr. Hannah Blencowe (LSHTM)
Dr. Kinzang Tshering (KhesarGyalpo University of Medical Science of Bhutan)
Dr. Yoriko Nishizawa (KGUMSB)
Dr. Phurb Dorji, (Jigme Dorji Wangchuck National Referral Hospital)
Dr. Tulsi Ram Sharma (Eastern Region Referral Hospital)
Dr. Purushotam Bhandari (Central Region Referral Hospital)
Dr. Nidup Gyeltshen (CRRH)
Mr. Sonam Wandhi (Ministry of Health, MoH)
Ms. Tashi Tshomo (MoH)

Research assistants:

Ms. Asha Rai
Mr. Tshering Samdrup
Ms. Deo Maya Subba
Mr. Dilli Ram Mongar
Mr. Ugyen Tshering (Pilot study)

Translator:

Yeshe Lhendup

Focal Points:

Ms. Yangden Drukpa (JDWNRH)
Ms. Geeta Giri (JDWNRH)
Ms. Kinley Chhimi (CRRH)
Mr. Dorji Gyeltshen (CRRH)
Ms. Jigme Zangmo (CRRH)
Mr. Karma (ERRH)
Ms. Kezang Wangmo (ERRH)

Interviewers:

JDWNRH

Birth Centre:

Ms. Anju Thapa
Ms. Bishnu Maya Rai
Ms. Damanti Bhujel
Ms. Dechen Zangmo
Ms. Dipsika Rai
Ms. Jamyang Dema
Ms. Kinga Om
Ms. Lali Maya Karki
Ms. Puntsho Yangzom
Ms. Rinchen Yangzom
Ms. Sangay Zangmo
Ms. Sonam Choden
Ms. Sonam P Dorji
Ms. Tshewang Gyem
Ms. Ugyen Choden

Maternity Ward:

Ms. Choki Om
Ms. Kezang Lhamo
Ms. Nidup Chenzom
Ms. Passang Kyipa
Ms. Pem Choki
Ms. Rinchen Zangmo
Ms. Sherab Zangmo
Ms. Sonam Pelzom
Ms. Tshering Zangmo
Ms. Tenzin Dema
Ms. Yangchen Lham

NICU:

Ms. Tandin Om

CRRH

Ms. Arun Gautam
Mr. Choki Dorji
Ms. Deepak Gaga
Ms. Dhan Maya
Ms. Karma Yangchen
Ms. Dawa Zangmo
Ms. Hem Raj Rai
Ms. Namgay Pemo
Ms. Pema Zangmo
Ms. Leki Nima
Ms. Lhakpa Choki Sherpa
Ms. Lhamo,
Ms. Mindhu
Ms. Rajesh Sapkota
Ms. Sonam Wangmo
Ms. Thinley Zangmo
Ms. TsheringChoden
Ms. TsheringTashi
Ms. Ugyen Delma
Ms. Ugyen Lhamo
Ms. Zangpo
Ms. Sadhna Gurung
Ms. Tenzin Dema
Ms. Dawa Dema
Ms. Sangay Zangmo
Mr. Khara Nanda
Ghimery
Ms. Laiba lepcha
Ms. Khendu Pem

ERRH

Ms. Chonining Zangmo
Ms. Karma Tshomo
Mr. Khara Nanda Ghimray
Mr. Chencho Dorji
Ms. Rinzin Wangmo
Mr. Ugyen Tshering
Mr. Sherub Dorji
Ms. Pelki Zangmo
Ms. Choten Tshering
Ms. Sonam Wangmo
Mr. Sadha Gurung
Ms. Sonam Dyakar
Ms. Tashi Deki
Ms. Yezer Tshomo
Ms. Tshering Om
Ms. Phuntsho Choden
Ms. Sasha Hiring
Ms. Khara Nanda

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Mothers who participated in this study and their babies

Dr. Tashi Tobgay (KGUMSB)
Dr. Pakila Drukpa (KGUMSB)
Dr. Sonam Gyamtsho (ERRH)
Dr. Gosar Pemba (JDWNRH)
Dr. Tapas Gurung (CRRH)
Mr. Phub Sangay (NSB)
Mr. Cheku Dorji (NSB)

Appendix H

Validation of the baby scales in the study settings

Birthweights were measured within 24 hours of delivery. During the monitoring visits, one identical bag which contains 2 kilo grams of rice were weighed twice at each hospital using their baby scales. The scales were accurate ± 100 grams. At the eastern referral hospital in Mongar, for babies who were taken to NICU, babies' weights are crosschecked using Prestige.

Table H.1. Validation of baby scales at the study sites.

Hospitals	Baby Scales	During the study			After the study		
		Weights (1 st time)	Weights (2 nd time)	Dates	Weights (1 st time)	Weights (2 nd time)	Dates
JDWNRH (Birthing Center)	Tanita Digital Baby Scale (BD-585-WH)	1.99 kg	1.99 kg	19-Sep-15	2.00 kg	2.00 kg	12-Jun-16
JDWNRH (Maternity Ward)	SECA	1.98 kg	1.98 kg	19-Sep-15	-	-	-
Mongar Hospital	SECA	1.90 kg	1.90 kg	20-Oct-15	1.99 kg	1.99 kg	21-Jun-16
Mongar (NICU)	Prestige	1.99 kg	1.99 kg	20-Oct-15	2.01 kg	2.01 kg	21-Jun-16
Gelephu	SK-20	2.05 kg	2.10 kg	21-Sep-15	1.90 kg	1.90 kg	09-May-16

Appendix I

Additional tables for descriptive analysis

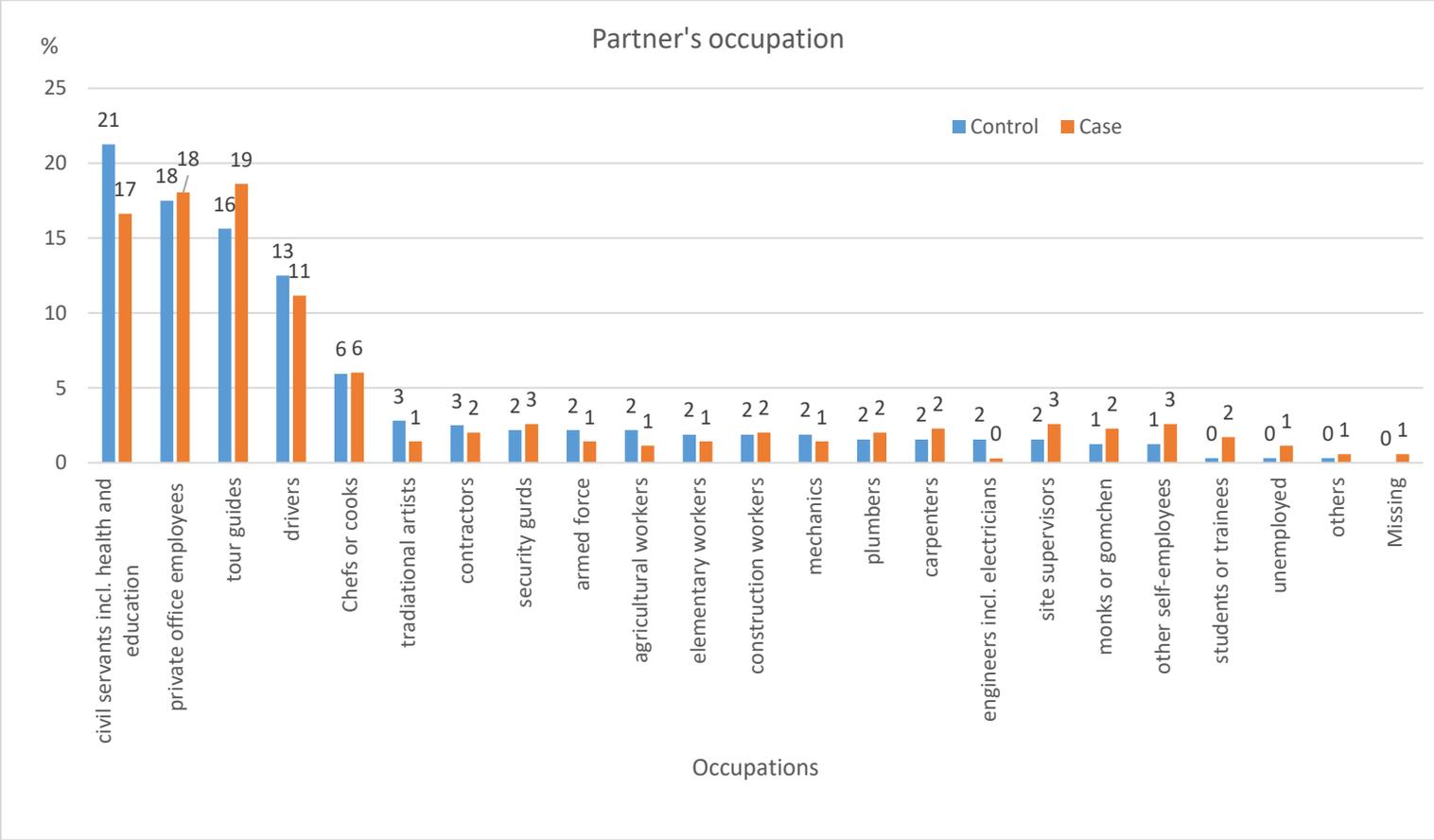


Figure I.1. Partner's occupation.

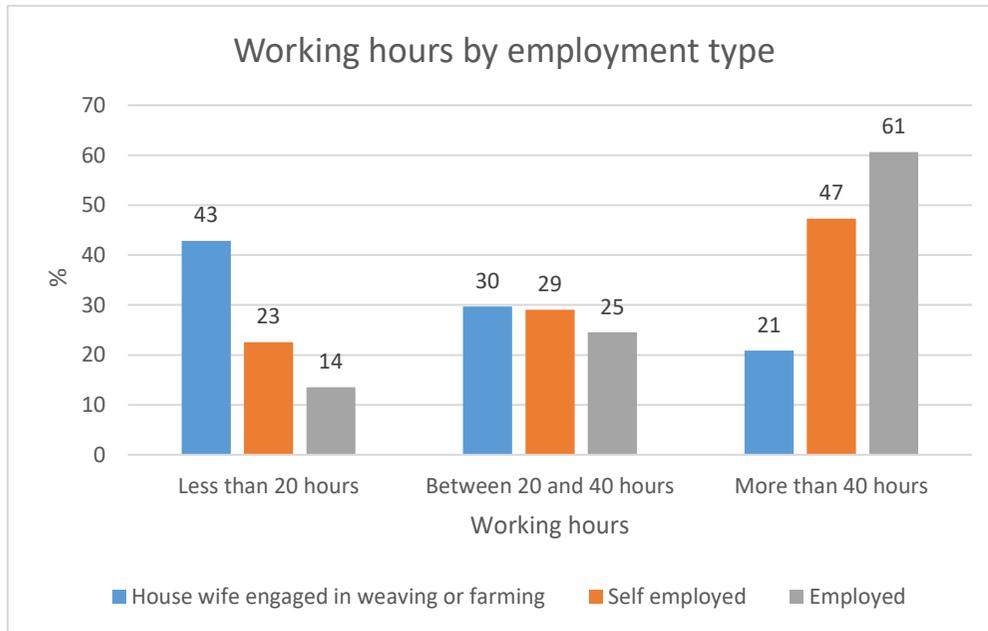


Figure I.2. Weekly working hours by employment type (before maternity leave for employees).

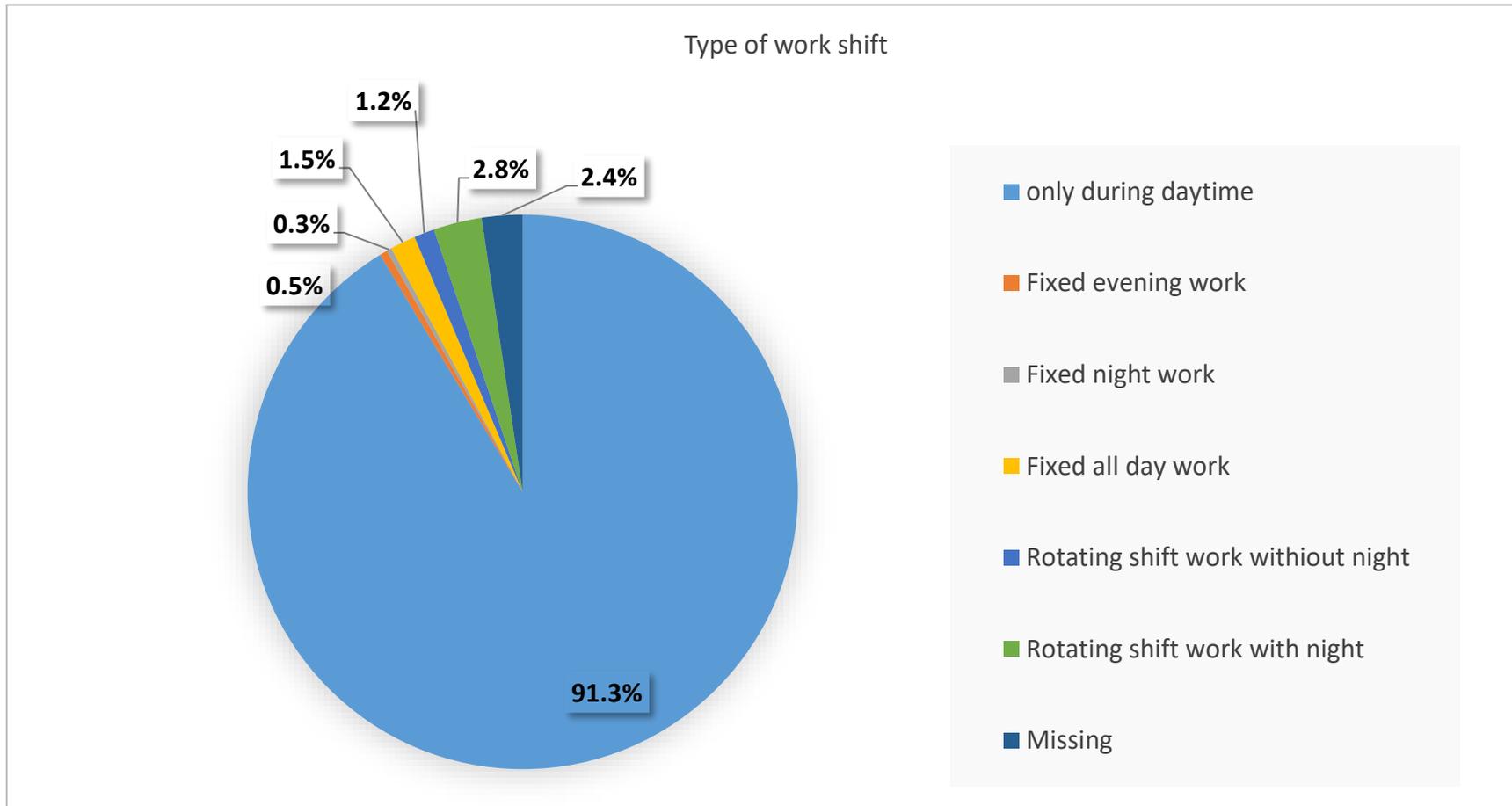


Figure I.3. Type of work shift.

Table I.1. The percentage of the mothers who engaged in vigorous to moderate physical activities (work, leisure, transportation) and adverse birth outcomes.

		Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
		N	%	N	%	P-value	N	%	P-value	N	%	P-value
Work	Vigorous activity (yes)	54	16.8%	65	18.7%	0.54	32	21.1%	0.255	33	17.1%	0.956
	Missing	5	1.6%	5	1.4%		3	2.0%		2	1.0%	
	Moderate activity (yes)	251	78.2%	291	83.6%	0.10	121	79.6%	0.804	167	86.5%	0.03
	Missing	6	1.9%	4	1.2%		2	1.3%		2	1.0%	
Leisure	Vigorous activity (yes)	13	4.1%	15	4.3%	0.86	8	5.3%	0.549	7	3.6%	0.82
	Missing	2	0.6%	3	0.9%		1	0.7%		2	1.0%	
	Moderate activity (yes)	49	15.3%	64	18.4%	0.29	32	21.1%	0.114	32	16.6%	0.73
	Missing	5	0.8%	2	0.7%		2	1.3%		0	0.0%	
Travel	Walk or bike for at least 10 minutes	211	65.7%	228	65.5%	0.90	102	67.1%	0.832	126	65.28%	0.89
	Missing	4	1.3%	3	0.9%		1	0.7%		2	1.04%	

Table I.2. Mean minutes of physical activities in total on a typical day.

Physical activities in total on a typical day	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	Mean minutes (SD)	N	Mean minutes (SD)	P-value	N	Mean minutes (SD)	P-value	N	Mean minutes (SD)	P-value
Work related vigorous activities (n=119)	50	295 (173)	65	269 (199)	0.47	32	295 (173)	0.93	33	249 (167)	0.23
Work related moderate activities(n=542)	244	198 (136)	285	204 (156)	0.64	120	213 (174)	0.42	162	196 (138)	0.89
Leisure related-vigorous activities(n=28)	13	47 (37)	14	81 (121)	0.33	7	101 (169)	0.43	7	61 (45)	0.50
Leisure related moderate activities (n=113)	48	68 (69)	64	58 (64)	0.42	32	63 (83)	0.80	32	52 (37)	0.18
Travel related moderate activities (n=439)	210	61 (62)	224	67 (94)	0.45	99	65 (98)	0.71	125	68 (91)	0.44
Sedentary Activities (n=661)	317	212 (150)	344	193 (135)	0.09	150	193 (133)	0.18	191	191 (135)	0.10

Table I.3. Proportions of the mothers who did not meet the ACOG guideline and adverse birth outcomes.

	Control (n=321)		Case (n=348)			Term LBW (n=152)			Preterm (n=193)		
	N	% or mean (SD)	N	% or mean (SD)	P-value	N	%	P-value	N	%	P-value
Less than 150 minutes of any vigorous/moderate activities per week	57	17.8	56	16.1	0.553	26	17.1%	0.850	30	15.5%	0.507
Missing	1	0.3	0	0.0		0	0.0%		0	0.0%	

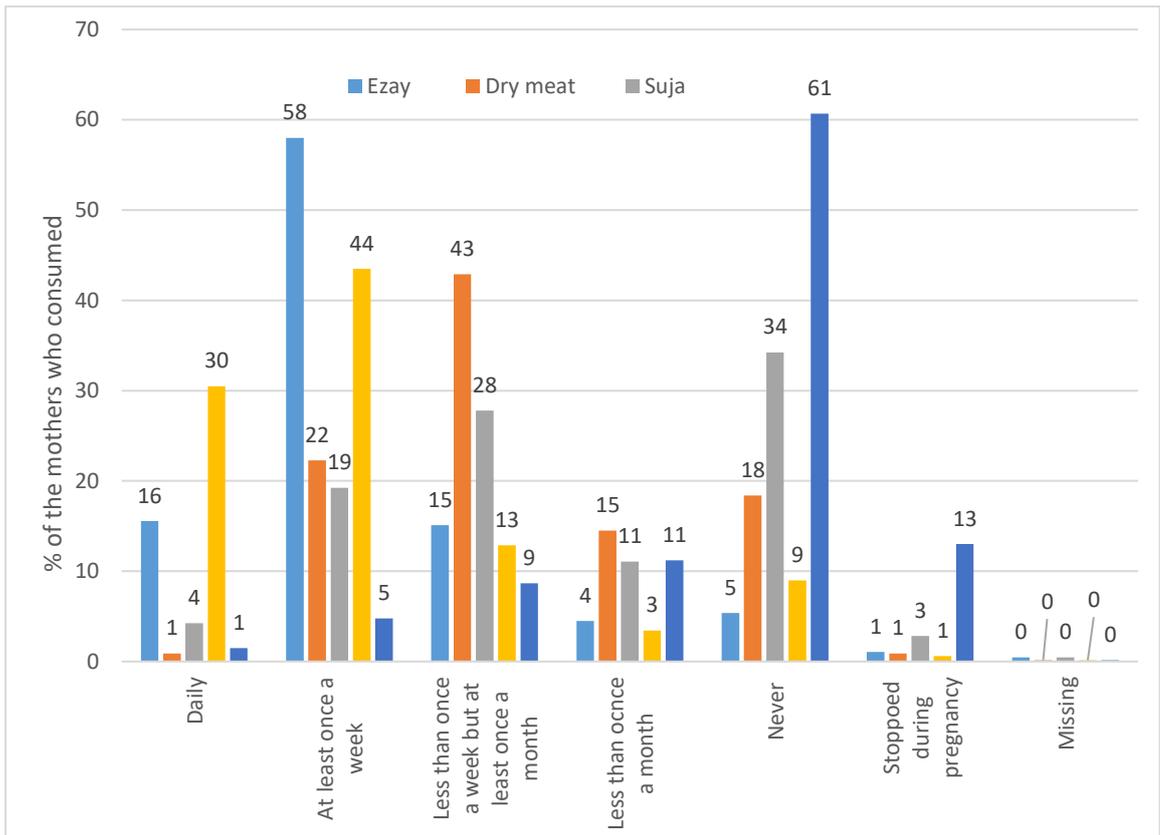


Figure I.4. Patterns and frequency of common sources of sugar and salt among study participants (n=669).

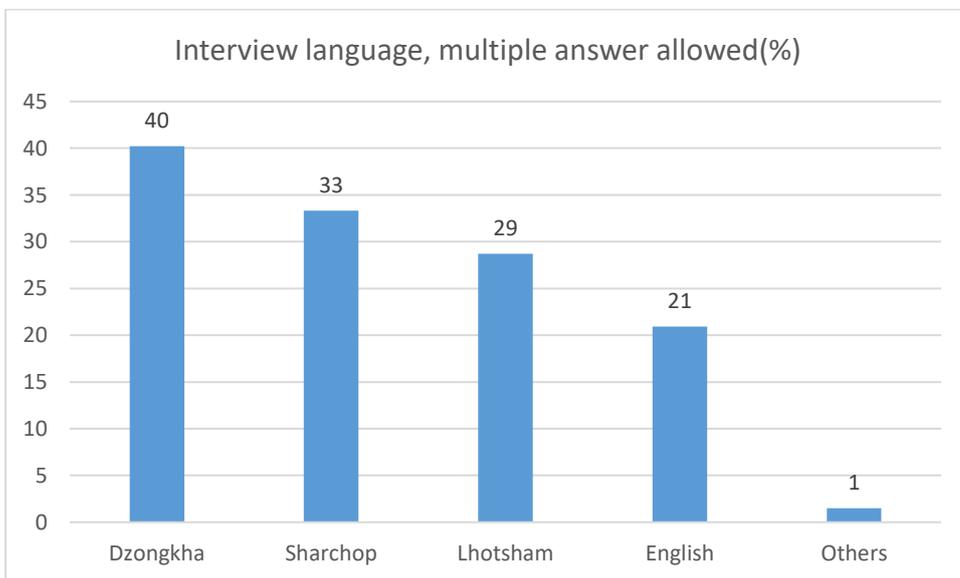


Figure I.5. Interview language.

Appendix J

Table J.1. AIC for the statistical approach.

Added variable	AIC	
	Model 1	Model 2
Hospital	916.8524	916.8524
Age	909.5562	908.0641
No. of ANC	882.8316	881.2978
WQ	881.1862	879.7886
Education	884.1807	882.3977
Ethnicity	886.0866	884.3093
Sex	881.553	881.553
GWG	626.8252	626.8252
Nulliparity	622.0839	622.0839
history of PTB	604.6755	604.6755
AN/PM	603.72	
Amount of AN		542.8519
ST/Smoke	595.3904	
Amount of ST		538.1317
Drinking	595.1822	
No. of drinking days		529.5396
Hypertension	528.043	478.5081
Mode of delivery	527.5638	477.6931
UTI	499.8113	450.3702
No. of meals	491.7174	442.4257
Season	488.3853	431.8197

Table J.2. Regression SE for tatistical approach.

Factors		Standard Error																	
		+hospital	+age	+No. of ANC	+WQ	+education	+ethnicity	+sex	+ GWG	+nulliparity	+History of PTB	+PN/BQ	+ST/smoking	+drinking	+hypertensive	+mode of delivery	+UTI	+No. Of meals	+season
Hospital (ref: JDWNRH)	Gelephu	0.198	0.189	0.205	0.208	0.218	0.218	0.218	0.235	0.238	0.244	0.257	0.256	0.272	0.269	0.294	0.292	0.264	0.279
	Mongar	0.207	0.198	0.211	0.203	0.212	0.221	0.215	0.201	0.189	0.189	0.192	0.205	0.201	0.210	0.206	0.147	0.138	0.139
Maternal age (ref: 20-35)	<20		0.766	0.798	0.761	0.776	0.773	0.805	0.682	0.506	0.597	0.596	0.565	0.535	0.578	0.578	0.685	0.585	0.661
	35<		0.448	0.483	0.472	0.47	0.471	0.48	0.368	0.465	0.506	0.489	0.446	0.441	0.359	0.343	0.351	0.332	0.339
Number of antenatal care per gestational week after the first ANC visit				0.216	0.237	0.236	0.237	0.231	0.422	0.393	0.461	0.46	0.441	0.436	0.264	0.224	0.260	0.268	0.231
Wealth Quintile (WQ) (ref: Middle)	Poorest				0.26	0.256	0.255	0.271	0.329	0.336	0.368	0.372	0.364	0.377	0.394	0.402	0.416	0.430	0.490
	Second				0.194	0.195	0.194	0.21	0.217	0.224	0.227	0.233	0.248	0.256	0.248	0.260	0.329	0.347	0.402
	Fourth				0.222	0.232	0.232	0.24	0.313	0.313	0.33	0.328	0.331	0.334	0.381	0.380	0.454	0.576	0.669
	Richest				0.169	0.181	0.181	0.185	0.261	0.291	0.302	0.299	0.309	0.312	0.385	0.397	0.468	0.503	0.553
Education (ref: No education)	NFE				0.208	0.21	0.203	0.164	0.162	0.164	0.16	0.154	0.155	0.147	0.152	0.161	0.163	0.184	
	Primary				0.267	0.267	0.271	0.421	0.42	0.446	0.426	0.435	0.434	0.467	0.464	0.512	0.531	0.525	
	High school or less				0.185	0.185	0.192	0.214	0.197	0.211	0.208	0.205	0.21	0.182	0.183	0.169	0.178	0.181	
	Masters/college/diploma				0.403	0.401	0.442	0.422	0.366	0.404	0.382	0.359	0.377	0.339	0.325	0.316	0.337	0.297	
Ethnicity (ref: Northern Bhutanese)	Southern Bhutanese				0.194	0.198	0.271	0.273	0.284	0.282	0.276	0.289	0.323	0.337	0.320	0.323	0.360		
Sex of the infant (ref: Male)	Female				0.235	0.269	0.272	0.293	0.289	0.303	0.311	0.370	0.395	0.401	0.408	0.433			

Gestational weight gain (GWG) (ref: IOM recommendations)	High	0.279	0.26	0.258	0.268	0.278	0.287	0.281	0.263	0.232	0.246	0.240	
	Low	0.578	0.582	0.587	0.578	0.567	0.557	0.732	0.753	0.742	0.764	0.717	
nulliparity			0.366	0.43	0.425	0.418	0.422	0.472	0.462	0.464	0.473	0.470	
History of previous preterm birth (PTB)				1.365	1.358	1.401	1.447	1.752	1.755	1.605	1.654	1.77	
PM or BQ during pregnancy					0.232	0.211	0.201	0.238	0.235	0.260	0.265	0.299	
Smokeless tobacco (ST) or smoking during pregnancy						0.913	0.887	0.734	0.791	0.737	0.779	0.893	
Drinking during pregnancy							0.36	0.426	0.434	0.434	0.446	0.503	
Hypertensive disorders (ref: None)	chronic hypertension								3.476	2.898	2.696	2.907	3.209
	Gestational hypertension								2.641	2.750	2.313	2.117	2.205
	PE or eclampsia								5.179	3.984	2.988	3.095	3.373
Mode of delivery (ref: SVD)	CS-Elective								0.429	0.446	0.434	0.414	
	CS-Emerg.								0.532	0.704	0.666	0.728	
UTI										0.769	0.830	0.926	
Number of meals per day											0.128	0.125	
Season (ref. Winter)	Fall											0.336	
	Spring											0.372	
	Summer											1.161	

Table J.3. Results of the uni-variable and multivariable models for LBW and/or PTB (case) using the statistical approach.

Independent variables		Crude Odd Ratio			Model 1 (n=397) Adjusted Odds ratio			Model 2 (n=357) Adjusted Odds Ratio		
		Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
Hospital (ref: JDWNRH)	CRRH	0.90	0.58-1.39	0.621	0.67	0.30-1.52	0.338	0.66	0.26-1.66	0.372
	MRRH	0.93	0.62-1.40	0.717	0.37	0.18-0.77	0.008	0.35	0.15-0.79	0.012
Season (ref: Winter)	Fall	1.48	0.95-2.31	0.082	0.90	0.43-1.87	0.777	1.18	0.51-2.73	0.692
	Spring	1.28	0.84-1.95	0.259	1.04	0.52-2.10	0.902	1.44	0.69-3.01	0.331
	Summer	2.13	1.37-3.32	0.001	3.16	1.54-6.50	0.007	3.94	1.88-8.24	<0.001
Sex of the infant (ref: Male)	Female	1.41	1.04-1.91	0.027	1.73	1.06-2.83	0.028	1.85	1.08-3.18	0.026
Age (ref: 20-35)	<20	2.01	1.03-3.92	0.04	1.10	0.34-3.57	0.872	1.30	0.36-4.66	0.894
	35<	1.83	1.16-2.90	0.01	0.80	0.35-1.84	0.605	0.80	0.31-2.03	0.496
Education (ref: Never attended School)	Non-formal education (NFE)	0.57	0.30-1.08	0.085	0.30	0.09-0.996	0.049	0.39	0.10-1.45	0.160
	Primary	1.04	0.63-1.71	0.878	1.17	0.48-2.82	0.730	1.28	0.49-3.31	0.618
	Middle Secondary or Secondary School	0.72	0.49-1.06	0.096	0.46	0.23-0.997	0.049	0.49	0.21-1.15	0.101
	Diploma, college, and post graduate	0.79	0.42-1.49	0.468	0.51	0.17-1.59	0.249	0.74	0.21-2.52	0.625
Wealth Quintile (ref: middle)	Poorest	1.11	0.68-1.80	0.672	1.12	0.48-2.64	0.793	1.32	0.51-3.38	0.567
	Second	0.77	0.48-1.25	0.297	1.00	0.45-2.20	0.730	0.96	0.40-2.30	0.924
	Fourth	0.89	0.55-1.44	0.623	1.69	0.78-3.67	0.184	2.66	1.12-6.33	0.027
	Richest	0.64	0.40-1.05	0.075	1.40	0.65-3.04	0.393	1.52	0.65-3.58	0.335
Number of antenatal care per gestational week after the first ANC visit		0.31	0.07-1.31	0.11	0.28	0.06-1.40	0.122	0.32	0.04-2.30	0.255
Ethnicity (ref: northern Bhutanese)	Southern Bhutanese	1.10	0.79-1.53	0.572	1.25	0.71-2.20	0.430	1.55	0.84-2.89	0.164

Gestational weight gain (ref: IOM recommendations)	High GWG	0.97	0.53-1.76	0.913	0.57	0.25-1.30	0.181	0.65	0.27-1.60	0.349
	Low GWG	2.55	1.63- 3.99	<0.0001	2.42	1.35-4.32	0.003	2.68	1.42- 5.07	0.002
Number of meals per day		0.58	0.44-0.76	<0.0001	0.56	0.36-0.87	0.010	0.57	0.35- 0.92	0.022
Urinary Tract Infection		1.79	1.03-3.11	0.038	2.16	0.93- 5.01	0.071	2.12	0.83-5.38	0.114
Nulliparity		1.32	0.97-1.80	0.074	1.69	0.98- 2.91	0.061	1.75	0.95- 3.23	0.072
Previous History of preterm birth		2.70	1.43-5.08	0.002	3.57	1.35-9.44	0.010	4.93	1.61-15.11	0.005
Hypertension (ref: Non-hypertensive disorders)	chronic or pre-existing	5.04	2.12-11.80	<0.0001	5.03	1.44-17.57	0.011	5.67	1.58-20.36	0.008
	gestational hypertension	5.47	2.77-10.80	<0.0001	4.40	1.65- 11.75	0.003	3.06	1.03-9.08	0.044
	Pre-eclampsia or eclampsia	11.77	4.13-33.55	<0.0001	5.24	1.48-18.50	0.010	5.19	1.20-22.48	0.028
Mode of delivery (ref: SVD)	CS-Elective	0.90	0.55-1.48	0.677	0.97	0.42-2.24	0.947	1.38	0.53- 3.62	0.507
	CS-Emergency	1.99	1.38-2.88	<0.0001	2.35	1.28-4.31	0.006	2.67	1.38-5.15	0.003
Chewing betel quid or pan masala during pregnancy		1.09	0.80-1.49	0.478	1.13	0.67-1.90	0.646			
Chewing smokeless tobacco or smoking during pregnancy		2.81	1.60-4.92	<0.0001	1.92	0.77-4.78	0.161			
Drinking during pregnancy		1.69	1.19-2.40	0.003	1.69	0.94-3.03	0.077			
Total number of betel nuts during the last 3 months of pregnancy (ref: No betel quid chewing)	<=90 nuts during the 3 months or =<1 nut per day	1.22	0.86-1.75	0.268				0.90	0.49- 1.65	0.730
	more than 90 nuts during the 3 months or >1 nut per day	0.90	0.53-1.53	0.684				0.84	0.31- 2.28	0.735
Total number of smokeless tobacco (grams) during the last three months of pregnancy		1.00	1.00-1.01	0.012				0.76	0.20-2.84	0.679

Total number of days of drinking during the last three months of pregnancy (ref: No drinking)	less or equal to once a week	1.48	0.95-2.31	0.083	1.99	0.97-4.07	0.061
	more than once a week	3.89	1.89-7.98	<0.0001	12.48	2.53-61.54	0.002
Urban/rural residence	Urban current residence	1.01	0.75 -1.38	0.932			
Difference between permanent and current altitude (ref: 0-<1000)	<0 m	0.87	0.56 -1.34	0.524			
	1000-<2000 m	1.24	0.83 -1.85	0.301			
	2000 m and above	1.14	0.66 -1.96	0.648			
Pre-pregnancy BMI (ref: average BMI)	Underweight	1.92	0.92-3.99	0.082			
	Overweight	0.64	0.40-1.02	0.061			
	Obese	1.22	0.49-3.04	0.666			

Table J.4. Monte-Carlo Error (MCE) for term LBW model

Term LBW	MCE of Coef.	MCE of Std. Err.	10% of S.E.	MCE of t	MCE of P>t
PM or BQ during pregnancy	0.003878	0.0004389	0.02281643	0.02	0.004
Ethnicity(southern Bhutanese)	0.0028411	0.0002682	0.02348585	0.01	0.004
Poorest	0.0050061	0.0005986	0.03552561	0.01	0.011
Second	0.0042988	0.0004699	0.03448597	0.01	0.009
Fourth	0.0044232	0.0004865	0.03437589	0.01	0.009
Richest	0.0038387	0.000381	0.03572441	0.01	0.007
NFE	0.0059927	0.0007771	0.04326635	0.01	0.008
Primary	0.0048746	0.0007085	0.03360915	0.01	0.012
High school or less	0.0057427	0.0009843	0.03085304	0.02	0.003
Masters/college/diploma	0.0061996	0.0008012	0.04770796	0.01	0.01
Chronic hypertension	0.0159643	0.0029736	0.05488909	0.03	0.001
Maternal height	0.0006316	0.0001957	0.00192902	0.03	0.026
<20	0.0061081	0.0007207	0.04733843	0.01	0.007
35>	0.0048844	0.000625	0.03207688	0.01	0.001
Pre-pregnacy weight	0.0009855	0.0004658	0.0016614	0.1	0.004
Gelephu	0.0042834	0.0006236	0.03191694	0.01	0.009
Mongar	0.0033428	0.0003473	0.03041451	0.01	0.008
Fall	0.0035577	0.0004115	0.03106183	0.01	0.008
Spring	0.0032335	0.0003349	0.02987462	0.01	0.008
Summer	0.0039265	0.0003931	0.02988117	0.01	0

Table J.5. MCE for Term PTB model

PTB	MCE of Coef.	MCE of Std. Err.	10% of S.E.	MCE of t	MCE of P>t
PM or BQ during pregnancy	0.0036866	0.0004651	0.0204277	0.02	0.009
Ethnicity(southern Bhutanese)	0.0030846	0.0003692	0.0229994	0.01	0.007
Poorest	0.0058309	0.0006629	0.0316441	0.02	0.015
Second	0.0047401	0.0005619	0.0308557	0.02	0.01
Fourth	0.00517	0.0005576	0.0308698	0.02	0.013
Richest	0.004643	0.0004611	0.0325238	0.01	0.004
NFE	0.0051825	0.0005131	0.0456588	0.01	0.003
Primary	0.003932	0.0003748	0.031796	0.01	0.007
High school or less	0.0044583	0.0004781	0.0273764	0.02	0.013
Masters/college/diploma	0.0043376	0.0004316	0.0461999	0.01	0.007
Chronic hypertension	0.0173955	0.0042416	0.0483283	0.04	0
Maternal height	0.000889	0.0002645	0.0013722	0.04	0.027
<20	0.004702	0.0005637	0.0395791	0.01	0.002
35>	0.0051432	0.0007427	0.0306124	0.02	0.001
Pre-pregnacy weight	0.0007578	0.0003172	0.0013722	0.06	0.019
Gelephu	0.00312	0.0003475	0.0290976	0.01	0.008
Mongar	0.0028068	0.0002867	0.027822	0.01	0.006
Fall	0.0019558	0.0001475	0.0287363	0.01	0.001
Spring	0.0037732	0.0003598	0.0282172	0.01	0.006
Summer	0.0020512	0.000155	0.3085231	0.01	0

Figure J.1. Trace plot of mean UTI for term LBW

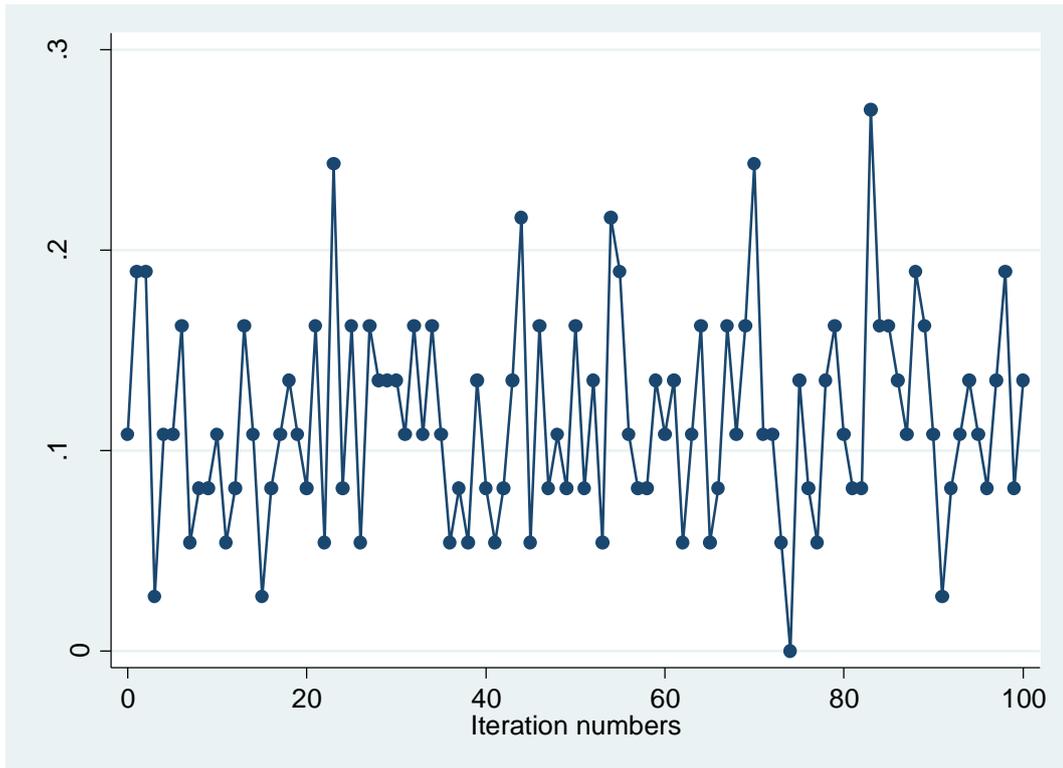


Figure J.2. Trace plot of mean pre-pregnancy weight for term LBW

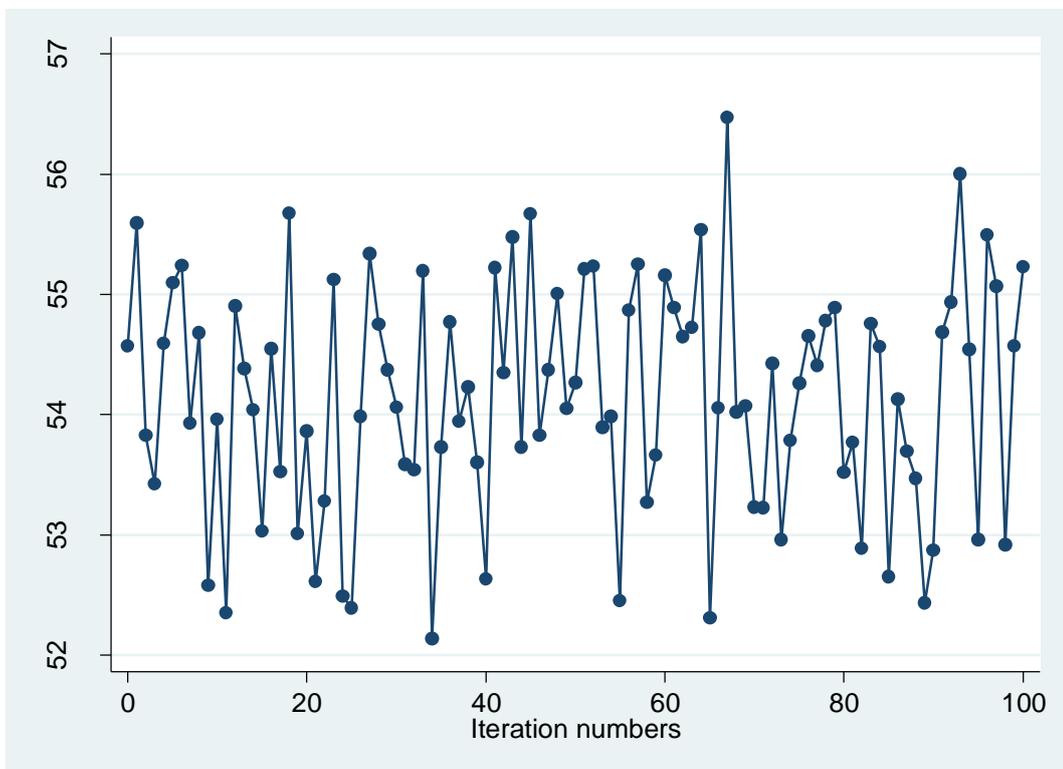


Figure J.3. Trace plot of mean maternal height for term PTB

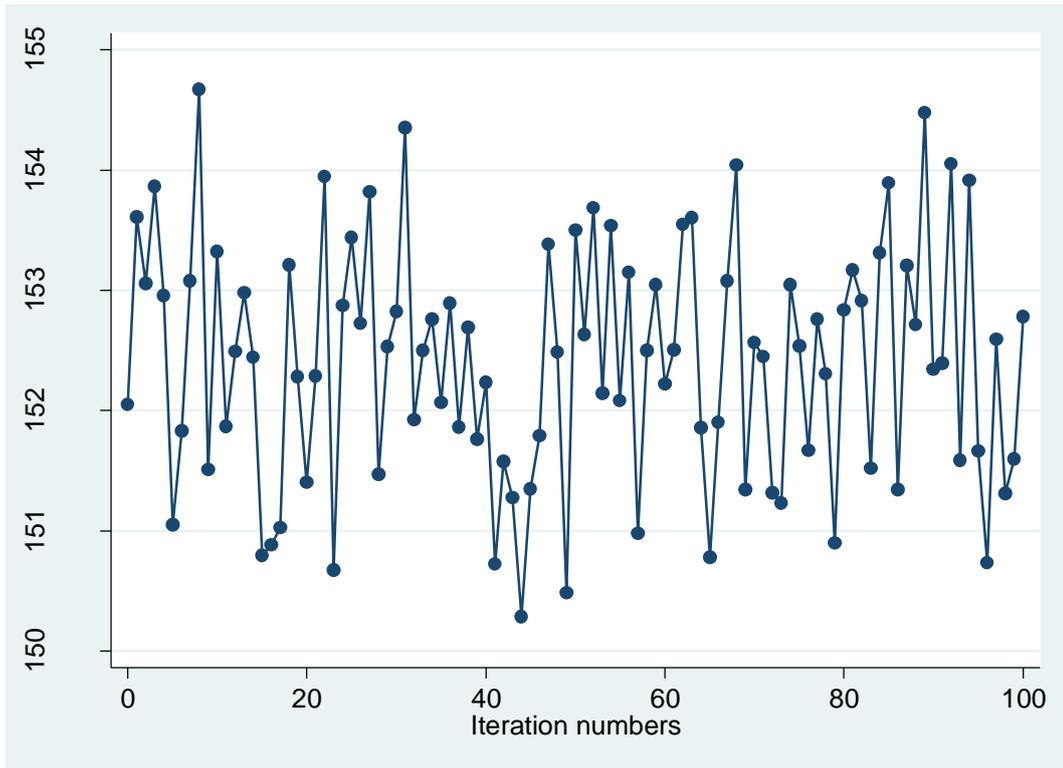


Figure J.4. Trace plots of mean UTI for PTB

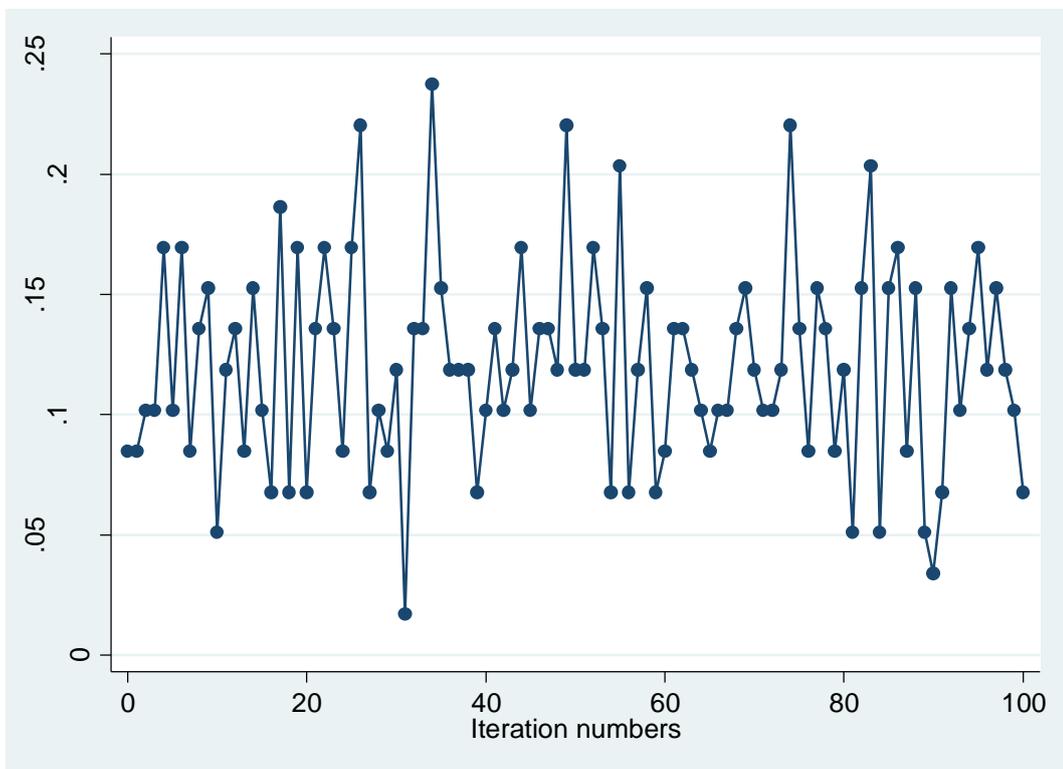


Figure J.5. Trace plots of mean pre-pregnancy weight for PTB

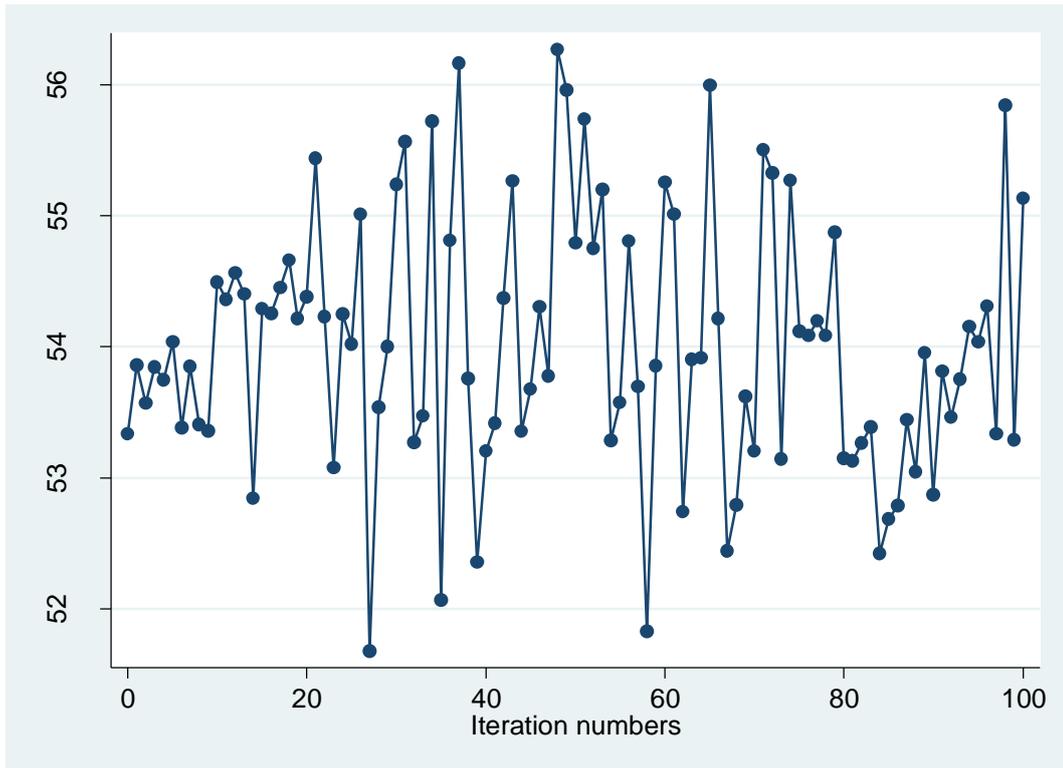


Figure J.6. Trace plots of mean maternal height for PTB

