



STUDY PROTOCOL

REVISED **Ambient and Indoor Air Pollution in Pregnancy and the risk of Low birth weight and Ensuing Effects in Infants (APPLE): A cohort study in Bangalore, South India [version 2; peer review: 1 approved with reservations, 2 not approved]**

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Abstract

Background: Exposure to air pollution (IAP) from the combustion of solid fuels is a significant cause of morbidity and mortality in developing countries. Pregnant women exposed to higher pollutant levels are at higher risk of delivering a low-birth-weight (LBW) baby. There is a lack of standardized data regarding the levels and types of specific pollutants and how they impact LBW. We aim to prospectively assess the association between ambient and indoor air pollution levels in pregnancy and low birth weight and understand the subsequent risk of adiposity in these infants.

Methods: We will conduct a prospective cohort study of 516 pregnant women recruited before 18 weeks of gestation in the urban slums of Bangalore, who have voluntarily consented to participate. We will estimate the level of air pollutants including coarse particulate matter 10 ug/m³ (PM₁₀), fine particulate matter 2.5 ug/m³(PM_{2.5}) and carbon monoxide (CO) parts per million (ppm) levels in both indoor and ambient environment. The follow-up of the delivered children will be done at delivery until the infant is two years old. The association between pollutants and LBW will be evaluated using logistic regression adjusting for potential confounders. Further, we will explore the mediation role of LBW in the hypothesized causal chain of air pollution and adiposity. Nested within a larger Maternal Antecedents of Adiposity and Studying the Transgenerational role of Hyperglycemia and Insulin (MAASTHI) cohort, we can estimate the absolute risk of having low birth weight caused by air pollution and other variables.

Discussion: Understanding the association between exposures to ambient and indoor air pollution and low birth weight is essential in India. LBW

Open Peer Review

Reviewer Status

	Invited Reviewers		
	1	2	3
version 2 (revision) 30 Oct 2019		 report	 report
		↑	↑
version 1 19 Oct 2018	 report	 report	 report

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Any reports and responses or comments on the article can be found at the end of the article.

babies have a higher risk of developing obesity and Non-Communicable Diseases (NCDs) during adulthood. The results from this study can inform the efforts for controlling the air pollution-related chronic diseases in India.

Keywords

Ambient air pollution, indoor air pollution, low birth weight, adiposity, Non-communicable disease



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REVISED Amendments from Version 1

The current version incorporates the changes as per the suggestions of the reviewers. The objectives are rewritten along with conceptualization of the hypothesis and methodological approach. Instead of measuring and estimating the independent effects of indoor and ambient air pollution, the current proposal considers the net effect of the air pollutants from all the sources. We are measuring the sources of air pollution from several sources inside the houses and in the ambient air. The total impact of air pollutants is measured through the monitoring devices the women are wearing, is the exposure of interest. There is no change in the assessment of intermediate (low birth weight) and outcome (adiposity). The revised manuscript also contains several grammatical corrections to improve readability. [Figure 1](#) has been modified. [Figure 2](#), [Figure 3](#), and [Figure 4](#) have been deleted; while the next figures - [Figure 5](#), [Figure 6](#), [Figure 7](#) are now [Figure 2](#), [Figure 3](#), [Figure 4](#).

Any further responses from the reviewers can be found at the end of the article

List of Abbreviation

AQI: Air Quality Index
 ATP: Adenosine TriPhosphate
 BMI: Body Mass Index
 CO: Carbon Monoxide
 CSV : comma separated values
 DNA: Deoxyribose Nucleic Acid
 HBsAg: Hepatitis B Surface Antigen
 HIV: Human Immunodeficiency Virus
 IAP: Indoor Air Pollution
 LBW: Low Birth Weight
 NCD: Non-Communicable Disease
 PM10: Particulate matter 10 micrometer
 PM2.5: Particulate matter 2.5micrometer
 PPM: parts per million
 RH: Relative Humidity
 SES: Socio-Economic Status
 SO2: Sulphur Dioxide
 SD: Secure Digital
 T2DM: Type 2 Diabetes Mellitus
 US EPA: The United States Environmental Protection Agency
 USB: Universal Serial Bus
 VDRL: Venereal Disease Research Diagnostic
 XLSX: Excel Microsoft Office Open XML Format Spreadsheet file

Introduction

The developmental origins of health and disease (DOHaD) hypothesis deals with exploring the causal role of intrauterine circumstances to the origins of diseases in adults. The initial studies have revealed that undernutrition during pregnancy is an

important determinant of adult cardiac and metabolic disorders due to fetal programming. This was hypothesized to be mediated via altering the fetus' structure, function, and metabolism. Since then, the development of fetal origins of adult disorders has remained an essential focus of researchers in the exploration of causal mechanisms for hypertension, coronary heart disease, and non-insulin-dependent diabetes. The collation of the prospective evidence using the DOHaD approach helps in assessing and modifying the impact of determinants of health from the life-course perspective. While the evidence regarding nutritional pathways of low birth weight (LBW) is available, other antecedents such as air pollution and psychosocial stress are less investigated¹.

Globally, nearly three billion people use traditional biomass fuels as their primary source of energy comprising of wood, charcoal, and agricultural wastes^{2,3}. In India, nearly 67% of the population uses biomass as a primary source of fuel for cooking. As a result, exposure to indoor air pollution (IAP) from the combustion of these fuels has emerged as an important cause of morbidity and mortality⁴. Air pollution is contributing to the second-highest associated risk factor for mortality and morbidity⁵. Even low-dose exposure to pollutants *in utero* can result in disease, disability, and death in childhood³. Air pollution is associated with several diseases such as respiratory infections, chronic obstructive pulmonary disease, cataracts, cardiovascular events, low birth weight and all-cause mortality. Further, a meta-analysis by Stieb *et al.* indicates that the decrease in birth weight is proportional to higher pollutant concentration⁶.

The putative role of exposure to air pollution during pregnancy resulting in LBW has been assessed in several studies^{1,6-8}. The suggested mechanisms mediating this path include oxidative stress resulting in placental and endothelial dysfunction⁹, and damage in the DNA productivity due to an imbalance between reactive oxygen species¹⁰. Specifically, exposure to particulate matter during pregnancy induces changes in multiple placental compartments, including the maternal vascular space, fetal capillaries, and surface exchange areas¹¹. These alterations in placental function were associated with a higher incidence of LBW among exposed fetuses^{11,12}. The poor nutrition accentuates the propensity of a baby to be LBW and subsequent inadequate development of pancreatic beta-cell mass, resulting in a higher risk of development of type 2 diabetes in the future. These include higher insulin resistance and storage of fat as compared to children with a healthy weight. Intrauterine malnutrition and other fetal constraints induce insulin deficiency (lack of growth-promoting activities of insulin). The resulting postnatal state of regulatory insulin resistance leads to a rapid postnatal increase of adipose tissue that remains stable throughout life¹³.

Available evidence suggests that several environmental factors induce intrauterine growth retardation (IUGR) and subsequent LBW in newborns. These include diet¹⁴, diabetes¹⁵, hormone exposure¹⁶, air pollution¹⁷, psychosocial stressors¹⁸ and hypoxia^{19,20}. Our ongoing, MAASTHI birth cohort study characterises the impact of these factors, including exposure to higher glucose levels during pregnancy, in the intrauterine milieu on the fetus,

barring the effect of air pollution. A cohort study is an efficient study design to assess the association between prenatal exposure to air pollutants and infant health outcomes.

Objectives: We aim to evaluate the association between prenatal exposure to air pollutants and low birth weight in newborns (Figure 1). We will also be exploring the mediation role played by LBW in the causal path between air pollution in pregnancy and adiposity in infancy (Figure 1).

1. To explore the association of prenatal exposure to the total level of air pollutants in pregnancy and low birth weight at birth.

Hypothesis 1: Prenatal exposure to higher levels of air pollution increases the risk of low birth weight.

2. To evaluate the association between prenatal exposure to the total level of air pollutants in pregnancy and adiposity in infants, mediated through low birth weight.

Hypothesis 2: Low birth weight at birth mediates the effect of air pollution in pregnancy on adiposity in children.

Methods

Study design

A prospective cohort study is planned in the urban slums of Bangalore. The study duration is three years. In this regard, a pilot study was carried out in September 2017. Subsequently, the recruitment and follow-up visits are scheduled between August 2018 and December 2020. The study population comprises households with pregnant women. All the pregnant women in the study population will be followed up until delivery, and their infants will be followed up further irrespective of the exposure status. The follow-ups will be performed at birth, at six months, and eighteen months of age. Infant anthropometry, morbidity, feeding practices, and child developmental milestones will be assessed during each follow-up visit

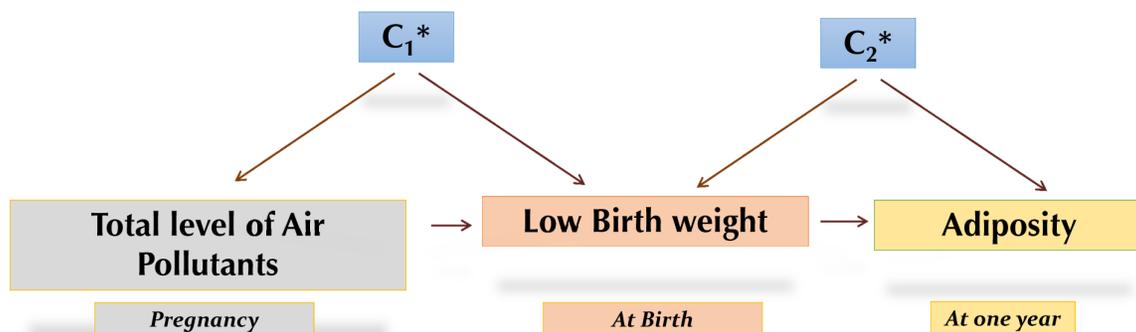
Setting: The study area is located in the slums of the east and west zones of urban Bangalore. The selected areas are:- Srirampura, Kodandarampura, Shirdi Saibaba Nagar, Subasnagar from the West Zone, and DJ Halli, Bagalur Layout and Pulikeshinagar from the East Zone (Figure 2). A slum is defined as an area comprising of at least 60–70 households living in poorly built congested tenements along with the neighbouring well-built houses (see data from the Karnataka Slum Development Board).

Participants: The study participants will be selected from the Bruhath Bangalore Mahanagara Palike (BBMP) health centres. Permissions have been obtained from the Chief Health Officer, Bangalore to conduct the study in the selected BBMP urban health centres. The selected centers are, Ramachandrapura Urban family welfare center (UFWC), Subash Nagar urban health center (UHC), Shirdi Saibaba UHC, Kodandarampura UHC from the west zone and DJ Halli, Bagalur Layout, Robertsonpet and KG Halli UHCs from the east zones of BBMP. The research staff will screen the eligible respondents in the health center and the community. Only eligible respondents will be enrolled in the study after obtaining their informed consent.

Eligibility criteria: Pregnant women aged between 18–45 years with a gestational age of under 18 weeks who reside in the slums and plan to deliver at the study locations are eligible for recruitment in the study. Women with severe co-existing illness, and those who plan to move out of the study location during the study period will be excluded. Women who are mentally or physically not capable of voluntarily consenting or participating in the study will be excluded (Figure 3).

Ethical considerations

Institutional Ethics Committee: Ethical clearance for the proposed study has been obtained from the institutional review board (IEC) at Bangalore, IIPH-H (Approval Number IIPHHB/TRCIEC/121/2017 Dated 27 July 2017).



***Potential confounders**

- C1: Household/indoor temperature, Season, Maternal smoking(active and passive), Socio-economic status, Antenatal stress, Maternal Age, Occupational H/o exposure, Ambient temperature, Heavy traffic roadways
- C2: SES, Gender, Feeding habits, Physical activity, Exact age at measurement, Feeding habits

Figure 1. Hypothesis displaying exposure, intermediate and outcome of interest, APPLE study, 2019.

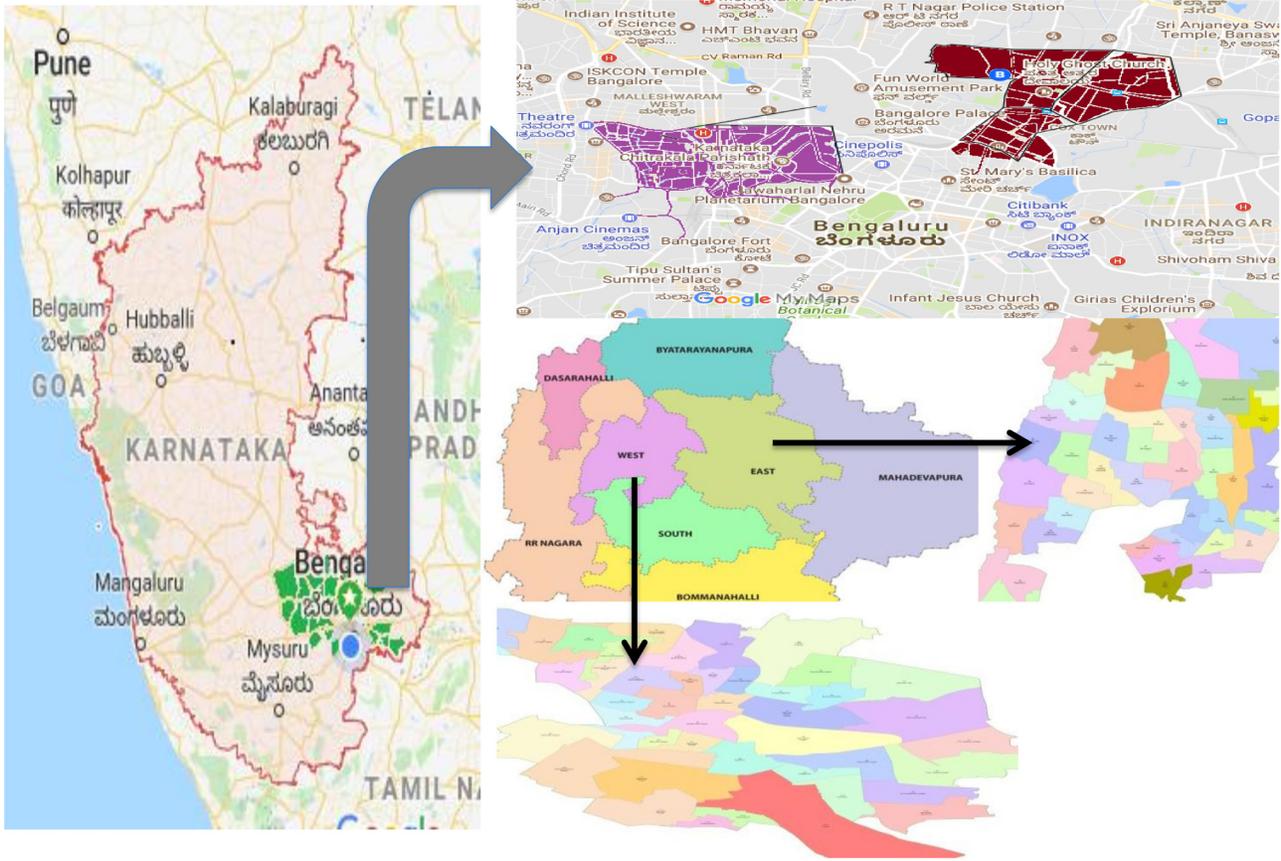


Figure 2. Study Areas for the research in Urban Bangalore. West zone is colored in purple and East zone in brown.

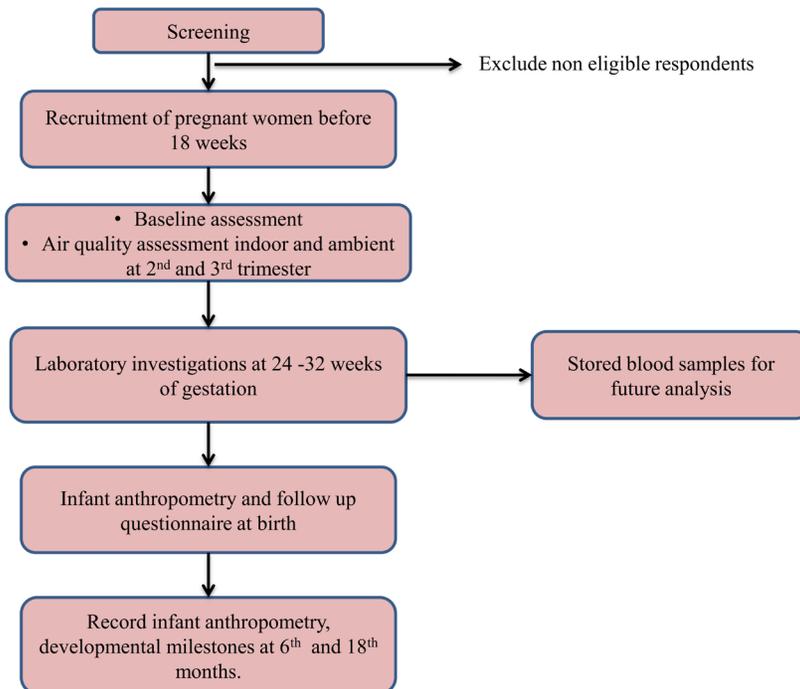


Figure 3. Flow diagram depicting the steps in the APPLE cohort study.

Written informed consent will be obtained from participants before the start of the study. They will be informed in detail about the study, and their voluntary agreement to participate in the research will be obtained.

Exposure assessment. We will assess the level of air pollutants, namely, fine particulate matter (PM_{2.5}) and coarse particulate matter (PM₁₀) and carbon monoxide (CO). All these pollutants will be measured both indoor and in the ambient environment using air quality monitors. Personal sampling monitors will be used to measure the individual exposure level of PM_{2.5}, PM₁₀, and CO at the household level. The eligible participants will be required to wear a personal sampling device for one entire day during each trimester. The samplers will be kept inside a small adequately ventilated, appealing sling bag that they can wear while carrying out the routine household chores. The samplers record the data continuously for 24-hours, and are connected to a portable outlet power source or power bank. The data will be stored in the memory card in the sampler, which is then transferred and stored safely in a hard disk later by the research staff. The exposure data will be measured twice during pregnancy, during the second and the third trimester of pregnancy. A tentative schedule for the distribution of the monitors is provided in Appendix 1 ([Supplementary Table 1](#), [Supplementary File 1](#)). The ambient air quality monitor will be kept outside the house in a protected environment, within 5 km radius from the respective participant's house. This monitor will assess ambient data on all three pollutants (PM_{2.5}, PM₁₀, and CO) for 24 hours. The device will be about five feet above the ground level provided with a shelter to secure the device from direct sunlight and rain. We will also obtain the readings of the ambient air pollutant levels from the nearby ambient air quality monitoring devices and stations installed by the pollution control board (monitored via [Eprolytics](#))

Together, we will assess the ambient exposure level of that particular area. This data will be considered as a proxy for ambient data for each household in that area.

Covariates. We will assess socioeconomic and demographic variables, current and previous obstetric history, fuel used for cooking and heating, and the location of the kitchen, using pre-tested questionnaires ([Supplementary File 1](#)). The information on other sources of pollution such as burning of agarbatti/dhoop (Incense sticks); and the use of mosquito repellents and candles will be collected. The duration of exposure to indoor as well as outdoor air pollution will be collected. (Detailed in [Supplementary Table 1](#), [Supplementary File 1](#)). Trained research staff will record the height and weight of the participants by using SECA 213 (seca Precision for health) stadiometer and digital Omron HN-283 (Omron Healthcare Co., Ltd.) weighing scale. After placing the scale on level ground, the research staff will check for a 'zero' reading. After ensuring that the respondent has removed heavy outer clothing and shoes, two readings to the nearest 10 grams will be recorded. A portable stadiometer will be used for measuring height to the nearest 0.1 cm; measured with the participant standing straight with her feet together, the head plate of the stadiometer

will then be pulled down to ensure that it rests on the crown of the head. Blood pressure will be measured using OMRON HEM-7203 (Omron Healthcare Co., Ltd.) automated blood pressure monitor. We will also measure the fasting and 2-hour postprandial blood glucose levels (Oral glucose tolerance test)²¹, and haemoglobin as markers of hyperglycaemia and anaemia in pregnant women ([Table 1](#)). Medall Healthcare Pvt will conduct the tests. Ltd. using a fully automated analyzer. Non-cyanide methods will be used for hemoglobin estimation, and Glucose oxidase (GOD)-peroxidase (POD) colorimetric method is used to assess glucose levels. The procedure has been detailed further in the laboratory analysis and sample storage section. The field staff will assess obstetric morbidity and hospitalization during the monitoring visits.

Household temperature and the status of anaemia will be considered as a common factor between indoor and ambient air pollution and its association with low birth weight^{22,23}. A list of known effect modifiers will be made and collected using a structured questionnaire ([Supplementary File 1](#)) and later considered during analysis. (Eg; the age of the respondent, occupational exposure²⁴)

Season, socioeconomic status, smoking history, and exposure to second-hand smoke, traffic roadways, maternal stress, and social support have been considered as confounders^{25–30}. As we cannot identify the relative contribution of tobacco smoke to the indoor or ambient air pollution, we will obtain data using the structured questionnaire. Psychosocial stress data will be collected using the standard Edinburgh Postnatal Depression Scale (EPDS), a widely used self-reporting questionnaire explicitly developed to screen women for perinatal depression³¹. EPDS has been validated by Fernandes *et al.* for prenatal depression in South India at a cut-off of ≥ 13 (sensitivity = 100%, specificity = 84.90%, and area under the curve = 0.95)³². Social support will be measured using a questionnaire that has been used and developed by St. John's Research Institute to evaluate a broad range of social support (i.e., emotional, instrumental, informational, and appraisal)³³. This questionnaire has a total of 12 items, and each item is scored between 0 (definitely not enough) to 3 (definitely enough). The highest score being 36 means excellent social support and 0 meaning very low social support.

Outcome variables. Birth weight, length, and skinfold thickness of the baby at birth, 6 and 18 months are the outcomes of interest. Feeding practices, morbidity, and child development milestone will be assessed during the follow-up visits. ([Table 1](#)). The Trivandrum Developmental Scale will be used to measure the developmental milestones³⁴. The 51-items of Trivandrum Development Screening Chart for children of 0–6 y [TDSC (0–6 y)] is a simple, reliable and valid screening tool in the community to identify children between 0–6 years with developmental delay.

For assessment of weight, the baby will be placed naked on the digital SECA 354 weighing scale and readings will be taken to the nearest 0.5g. For measuring infant length, the baby's head

Table 1. Proposed measurements at baseline and follow up in the APPLE study.

Participants	Measurement/tests	Frequency	N	Timepoints
Pregnant women	PM _{2.5} , PM ₁₀ and CO concentration of both indoor and ambient levels.	Two times	516	Once in 2 nd and 3 rd trimester
Pregnant women	1. Blood glucose estimation 2. Haemoglobin 3. Height and weight, Blood Pressure 4. Socio-demographic characteristics, current obstetric history, psychosocial stress, social support	Once	516	During 24–32 weeks of gestation
Newborn	Anthropometry of the child: skinfold thickness (Biceps, triceps and subscapular) and circumferences (Head, chest, waist, hip and mid-arm circumference)	Once	516	At birth
Infants	Skinfold thickness (Biceps, triceps and subscapular) and circumferences (Mid-upper arm, Head, chest, waist and hip circumference), morbidity and feeding practices, child developmental milestones	Two times	516	6 th month 18 th month

PM – particulate matter

will be held against the end of the head plate of the SECA 417 infantometer, and the footplate will be bought up to the heels ensuring that the feet and knees are flat.

Chasmors body circumference tape will be used to measure the circumferences. Mid-upper-arm (MUAC) will be recorded with the arm bent, allowing the measurement to be taken with the baby in its natural position. An ink mark will be made on the anterior and posterior sides of the arm to locate the point for biceps and triceps estimation. Head circumference will be measured with the baby's head on the side. The tape will be placed on the forehead. Waist circumference will be taken by placing the tape around the abdomen immediately above the umbilicus at the end of expiration. Chest circumference will be measured by placing the tape around the chest at the end of expiration.

Skinfold thickness will be measured on the left side of the body using the Holtain Calipers. Three readings to the nearest 0.2mm will be taken. For triceps skinfold thickness, the tape will be placed around the upper arm at the level of the mark done at the posterior side while measuring mid-upper arm circumference (MUAC). With the tape in position, a horizontal line will be drawn on the skin at the level of the mark. The point at which the fold is to be measured will then be marked; the skin will be lifted over the posterior surface of the triceps muscle, above the marked point and the calipers will be applied below the fingers. For subscapular skinfold thickness, the inferior angle of the scapula will be identified, and the skin will be marked immediately below the angle. The skinfold will be picked up above the mark, and the caliper jaws will be applied below the fingers, at the apex of the fold³⁵.

Adiposity: We define adiposity as the sum of the skinfold thickness, namely biceps, triceps, and subscapular measuring above the 70th percentiles are classified as at-risk, and more than 85th percentiles are classified as obese³⁶.

The skinfold thickness equation for body fat composition measurement by Holtain Caliper is correlated and validated against dual-energy x-ray absorptiometry (DEXA), the standard gold method for body fat estimation and reported a reasonable validation with DEXA.

Laboratory analysis and sample storage: Medall Healthcare Pvt. Ltd (MEDALL), a centralized and nationally accredited laboratory will be engaged to carry out all the tests, including haemoglobin and oral glucose tolerance test (OGTT). The pregnant women will be advised by the research staff to visit the hospital after an overnight fasting state for at least 7–8 hours. A trained phlebotomist will draw the fasting and postprandial sample 2 hours following a 75 g oral load of glucose³⁷. The blood from the fasting sample will be centrifuged within 30 minutes of the collection at 3500rpm for about 5 minutes in a REMI Medico centrifuge C-854/6 portable centrifuge. The sample will be transferred into a cold box at 2–8°C and will be transferred to the central laboratory, where assays will be carried out. An aliquot of plasma will be made by centrifuging the fasting Sodium Fluoride tube and haemoglobin processed EDTA samples for 3500rpm for 5 min. The remaining sediment of packed red cells will be centrifuged at high speed at 4500rpm for 5–10 min to extract the buffy coat samples. The plain tube containing 6ml of blood will be made to stand for 40 minutes to clot then centrifuged at a speed of 4500rpm for 5–10 minutes. Totally, six aliquots of serum will be separated from 6ml of a plain blood sample each holding 0.5ml of serum. 4 aliquots of plasma of 0.5ml each, 3 aliquots of buffy coats (0.1 – 0.5ml) will be stored for future analysis. The aliquots will be stored in a bio-repository at the research centre (Indian Institute of Public Health, Public Health Foundation of India-Bangalore), wherein the samples will be stored in a stepwise manner (2 – 8 °C, -20 °C deep freezer and -80 °C deep freezer) for future analysis. The samples are planned to be stored long term and will be moved to a -80 °C ultra-low deep freezer.

Sample size: A research study by Kalpana Balakrishna *et al.* showed that a population-weighted mean of annual $PM_{2.5}$ exposure in India has increased from 59.8 $\mu\text{g}/\text{m}^3$ to 79.9 $\mu\text{g}/\text{m}^3$ (1990–2016). The study also reported that more than 80% of the Indian population were exposed to $PM_{2.5}$ of more than 40 $\mu\text{g}/\text{m}^3$. Delhi has the highest populated weighted mean of $PM_{2.5}$ (>150 $\mu\text{g}/\text{m}^3$) followed by Uttar Pradesh, Bihar, and Haryana. We plan to recruit 516 pregnant women over a period of one year with a hypothesized (based on the evidence in India) 20% of LBW in this population³⁸, accounting for the design effect (DEFF) of (for cluster surveys- 1.5, for 95% confidence interval, the sample size required is 369. Further, due to the transient nature of pregnant women from the slum population, the sample size required is 516 accounting for a 40% loss to follow-up³⁹.

The sample size has been estimated using a formula, $n = Z^2 P(1-P)/d^2$; where n is the sample size, Z is the statistic corresponding to the confidence interval, P is the expected prevalence and d is the precision. The sample size of 246 has been estimated using 20% of the expected prevalence of LBW at a 95% confidence interval and 5% precision. The two zones of the study area considered as a cluster and accounting for the design effect of 1.5. The sample size came out to be 369. Further, due to the transient nature of pregnant women from the slum population, the sample size required is 516, accounting for a 40% loss to follow-up.

Data sources and measurements

The study instruments used in this study have been developed by the research team using previous research findings. The questionnaire has been piloted and validated before administration in the field (Supplementary File 1). The research staff will administer the questionnaire, conduct blood investigation, and record the anthropometric measurements at the health centre. The air monitoring device with power bank will be distributed to

pregnant women by the field staff of the respective area and taken back after the 24 hours estimation is done. The post-monitoring assessment will be performed using a structured questionnaire assessing the cooking practices and activity of the participants during the monitoring period. The enrolled pregnant women due for delivery, will be tracked through periodic phone calls by tracking respondents based on their estimated due date (EDD) and scheduling follow-up visits.

Ambient and indoor air quality monitor: We will use the VAYUSESNE (later renamed as VAYUCARE) device for assessing the outdoor and indoor air pollutants. This is designed by Ambience Monitoring India private limited based in Delhi marketed through their selling partner “I love Clean Air”. It is a portable device that can operate for 24 hours with 10,000mAh external power bank (Figure 4). The devices provide real-time monitoring of particulate pollution $PM_{2.5}$, PM_{10} and CO. It has air sensors to produce details on air quality. The data will be saved in an SD card. The downloaded data can be viewed in comma-separated values (CSV) and Excel Microsoft Office Open XML Format Spreadsheet file (XLSX) format. The monitors are calibrated, tested, and then installed⁴⁰. Upon installation, the monitor will assess the indoor air quality data automatically. The particulate matter is measured in $\mu\text{g}/\text{m}^3$, and CO is measured in ppm. The temperature is measured in degree Celsius, and relative humidity (RH) is expressed as a percentage.

Bias

Specific attention will be paid to limit bias by controlling for confounding, minimizing selection bias, and measurement errors. The data from laboratory investigations and air pollutant estimation is accessed only by the key research staff who are not involved in data collection to prevent any differential misclassification of the outcome. The field team will be trained and certified on anthropometric measurements by St. John’s



Figure 4. Personal air quality monitoring device with holding power bank and ventilated sling bag.

Research Institute and any possible measurement error will be minimized. Accuracy and inter-observer reliability of their measurements will be assessed at the outset and subsequently for every six months. To control for confounding, information about the potential confounders will be obtained and monitored during the analysis stage.

Statistical methods: Descriptive statistical estimates of individual exposure will be reported. Frequencies and percentages will be reported for all the variables. The exposure data distribution will be checked for normality. The mean and standard deviation will be reported for quantitative variables. The minimum and maximum levels of exposure will be reported. The prevalence of low birth weight will be calculated. The fasting and postprandial glucose will be categorized based on the WHO recommended cut off levels (fasting glucose of ≥ 92 mg/dl and 2-hr values of ≥ 153 mg/dl) are considered as hyperglycemia⁴¹. The anemia status will be categorized as normal when the Hemoglobin (Hb) is >11 gm/dl, mild anaemia when Haemoglobin 10 – 10.9gm/dl, moderate anaemia when Hemoglobin 7– 9.9gm/dl and severe when the Hemoglobin level is less than 7gm/dl^{42,43}. The outcomes (birth weight and adiposity) will be modeled both as a continuous and binary indicator variable. The exposure of interest ($PM_{2.5}$, PM_{10} , and CO) will be taken as a continuous scale measure for analysis. The exposure value will then be categorized in terms of quartiles, and the lower quartile of the exposure will be taken as the reference category for each pollutant for further analysis. The odds ratio with confidence interval will be reported. We will also report the dose-response relationship between maternal exposure to indoor and ambient air pollutants ($PM_{2.5}$, PM_{10} and CO). Birth weight will be assessed through sensitivity analysis^{44,45}. Mediation analysis will be used to test the hypothesized causal chain involving low birth weight as an intermediate in the association between air pollution and adiposity.

Dissemination of information: The findings of the study will assist the efforts of the Government to counter climate change. The data from the study will pave the way for future research and policy-making agendas of the government. We have engaged key stakeholders like health officers and Slum board officials to increase their sense of awareness towards the impact of air pollution on health. Through our membership in the

Ms. National Advisory Group and periodic inputs, we will ensure the wider dissemination of the study findings. The findings will be disseminated to fellow researchers through conference presentations, publications in the peer-reviewed journals, and reports to health authorities.

Study status: The baseline assessments have been started at two zones of the study area. The study tool has been piloted in the field and made necessary changes. We are currently calibrating the air quality devices before we use them in the field. The calibration process includes passing a known concentration of air pollutants (CO) in a controlled environment to obtain measurements from the devices within the acceptable range of ± 10 ppm.

Discussion

There is a lack of standardized data for the confluence of risk factors, including the levels and roles of specific pollutants and how they are associated with low birth weight and adiposity in India. By collecting high-quality prospective data on exposures in pregnant women, this study can provide insights into the environmental causes of low birth weight and obesity in childhood. The results from our study may provide evidence regarding the adverse effects of air pollution in pregnancy, and thereby can help in improving neonatal and child health outcomes. The results can inform policy regarding limiting air pollution and designing interventions for use in future studies.

Data availability

No data are associated with this article

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We sincerely thank Dr. Jasper V. Been³, Consultant Neonatologist, University Medical Centre Rotterdam, Rotterdam, Zuid Holland, the Netherlands for a thorough review and helpful inputs to the protocol. We thank Dr. Megha Thakur for proofreading the manuscript. Our sincere thanks to Dr. Suresh Shapeti and Mr. Ramesh for facilitating the administrative support and coordination. We thank Mr. Gurulingaiah, Mr. Kiran, Ms. Keerthi Deshpande, Ms. Maithili Karthik, Ms. Sindhu Gowda, and Ms. Meena KV for their support in carrying out research activities in the field.

Supplementary material

Supplementary File 1: File contain the study questionnaire along with the following Supplementary Tables: Supplementary Table 1: Tentative schedule for using the air pollution monitoring devices for exposure assessment

Supplementary Table 2: Description on variables

[Click here to access the data](#)

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Ryan W. Allen

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I appreciate the opportunity to review and comment on version 2 of this paper. I have read the updated paper, the authors' responses to Dr. Kalpana Balakrishnan's comments on version 1, and Dr. Julian Marshall's comments on version 2.

As I indicated in my review of version 1, this is an important research area. I remain very enthusiastic about the idea of a birth cohort focused on prenatal air pollution exposure and these outcomes. However, the authors have not addressed several of my previous concerns:

- I previously commented that "The investigators plan to use the VAYUCARE device for measuring air pollutants, but the paper does not provide sufficient detail on this instrument or a rationale for choosing it. Has the device been validated against other, more established monitors?"

I remain concerned about the lack of validation data for the VAYUCARE. I agree with Dr. Marshall's recent comments that the VAYUCARE needs to be compared to a "gold standard" device and that six hours is insufficient for such a comparison.

- In my previous comments, I also wrote that "The authors should describe their plans for deriving exposure estimates from the measurements. For example, personal monitoring is planned during two 24-hour periods in pregnancy. These will need to be temporally adjusted and combined in some way to estimate long-term exposure during pregnancy."

The authors do not appear to have addressed this concern. It remains unclear how two 24-hour measurements will be used to estimate exposure over the full pregnancy or during specific trimesters. These short-term measurements will be heavily influenced by temporal variations in concentrations, and that variation will need to be accounted for in some way. Dr. Balakrishnan expressed a related concern in a comment about model specifications, but after reading the authors' responses it is still unclear how this issue will be dealt with.

- I am also still concerned about the power calculations. In response to previous comments, the authors updated their calculations based on a systematic review of solid fuel use (Tielsch *et al.*, 2009). This is a surprising choice because, according to Dr. Balakrishnan, "the prevalence of solid fuel use is not likely to be higher than 5%." Moreover, a key driver of statistical power is the exposure gradient, but the power calculation still does not specify what gradient is being assumed.

Other comments:

- The paper indicates that "the recruitment and follow-up visits are scheduled between August 2018 and December 2020." Has data collection already started, or have the timelines not been updated?
- Will the authors have access to any data on gestational age at birth? For the primary outcome, LBW, gestational age is not needed. But it may also be useful to distinguish between children who were growth restricted vs. those who were born premature (for example, these may pose different risks for adiposity in childhood). For the former, an outcome like small for gestational age might be useful.
- Given emerging evidence that air pollution contributes to pregnancy loss, I encourage the authors to think through the potential role of "live birth bias" in this study (see citations Liew *et al.*, 2015¹ and Nobles and Hamoudi, 2019² for more details).

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Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Air pollution exposure assessment and epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 12 December 2019

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Julian D. Marshall

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I have reviewed the revisions to:

Ambient and Indoor Air Pollution in Pregnancy and the risk of Low birth weight and Ensuing Effects in Infants (APPLE): A cohort study in Bangalore, South India

Shriyan P, Babu GR, Ravi D, Ana Y, van Schayck OC, Thankachan P and Murthy G.

To review these revisions, I read the following:

- The authors' 1-paragraph summary of changes.
- The authors' reply to comments by Dr. Balakrishnan.
- The parts of the new document that are relevant to the issue I focused on in my initial review: the low-cost sensors.
- I also have reviewed a relevant citation from the new document; see below.

As a reminder, the two main concerns I raised in my initial review are:

1. "I am concerned that the proposal's current plan for air pollution measurement is insufficient; the level of accuracy and precision in those measurements may be too low, and as a result, exposure misclassification may overwhelm the statistical signal the authors seek to quantify."
2. "I agree with the review comments from Professors Balakrishnan and Allen regarding air pollution measurement."

I remain concerned about those two points.

I see these new aspects in the report:

- The authors' reply to Dr. Balakrishnan states: "Field calibration: the State Pollution Control Board of Karnataka (KSPCB) possesses several outdoor samplers, with some located in the city of Bangalore, to measure ambient CO and PM2.5 levels. For our calibration, we used the outdoor sampler located at the Indira Gandhi Institute of Child Health Hospital, Bangalore (device name: Ecotech Monitoring Solutions, NIMHANS). The board very frequently calibrates this device. During 6 hours, we placed our outdoor sampler and three of our indoor samplers directly next to the board's sampler. After that, we calibrated our devices against the board's sampler based on the outdoor readings. Correction factors were developed for all our devices."

Six hours is entirely insufficient to test a sensor, develop a calibration curve, and understand how robust the sensor is. The low-cost sensor also needs to be well calibrated against a gold-standard reference device, such as a BAM. This would involve, before commencing the study, many days or weeks of co-location with multiple low-cost sensors and at least one reference sampler. It would also involve, during the study, constantly testing the devices to see how their performance might be changing. See below - I could potentially help facilitate this calibration, if the authors are interested.

- The authors' report states: "The monitors are calibrated, tested, and then installed⁴⁰." That sentence cites this report: http://www.janhensen.nl/team/past/master/Vervoort_2018.pdf. However, the report itself states that the devices were, in that study, not calibrated (see top of page 10); they were not compared against a gold-standard measure. The report also states "The test showed that PM2.5 is measured quite similarly by all monitors, see Figure 2.3" (page 10). However, Figure 2.3 instead shows that the devices do not internally agree with each other. (Also, the test duration was under 2 days, so even if the devices did agree, the test is not long enough to provide robust evidence.)

In conclusion, my two concerns above are unchanged by the revisions I saw.

As a minor note, my prior-round of review comments pointed out a minor typo in the abstract. In the revised version I read, the typo remains uncorrected. This should be edited.

Lastly, I am aware of two BAMs currently operating in Bangalore. If the authors would like to calibrate their low-cost sensors against a BAM (a reference device), and to see how the devices compare, they can reach out to me to discuss logistics; I can potentially help facilitate access. On a related note: I will be in Bangalore early next month (January); if it is of interest, I would be happy to meet to talk further in person about testing and calibrating the low-cost sensors.

Competing Interests: No competing interests were disclosed.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Version 1

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Julian D. Marshall

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Thank you for this opportunity to review the study protocol, “Ambient and Indoor Air Pollution in Pregnancy and the risk of Low birth weight and Ensuing Effects in Infants (APPLE): A cohort study in Bangalore, South India [version 1]”. In addition, I have read two peer review reports included with the manuscript: one by Kalpana Balakrishnan (Balakrishnan, 2018¹) and one by Ryan Allen (Allen, 2019²).

The proposed study focuses on the impact of air pollution on adverse birth outcomes. Air pollution concentrations for PM_{2.5}, PM₁₀, and CO will be measured indoors and in nearby (within 5km) ambient air, using the VAYUCARE low-cost sensor; measurements as 24-hour averages will be conducted twice per subject: once each during the second and the third trimesters. Health parameters will be investigated for the mother and the infant, focusing on two outcomes: adiposity and birth weight.

The research topics investigated here are very important. A successful investigation would meaningfully add to the literature.

To my knowledge, the health measurements and the plans for epidemiological analyses are robust. (Those aspects are not my area of expertise.) Comments below focus on the air pollution measurements.

I am concerned that the proposal's current plan for air pollution measurement is insufficient; the level of accuracy and precision in those measurements may be too low, and as a result, exposure misclassification may overwhelm the statistical signal the authors seek to quantify.

I agree with the review comments from Professors Balakrishnan and Allen regarding air pollution measurement. I would not want to see the important work of this project move forward, and then later the authors find out that the study is unsuccessful or inconclusive solely because of uncertainties in air pollution measurement.

To help shore up this aspect of the investigation, I suggest the authors conduct two exercises: first, a series of pilot studies (e.g., first without people, then on 5 people, then on 20 people, and then on 100 people, before expanding to the full ~500-person cohort). During this pilot study, the researchers should investigate the robustness of the air pollution measurements for use in their study. Strong attention is needed for issues such as error, bias, device reliability/failure rate, reproducibility, within- and between-instrument variability, within- and between-participant variability, interference (e.g., dependency on relative humidity), general spatial and temporal variability, and the range of concentrations observed. This information will usefully inform the study design (e.g., which device to use, how many measurements to make). Running multiple versions of the same device side-by-side will inform reproducibility; running them next to a reference monitor will inform accuracy. Second, a review on devices used to measure air pollution exposures for epidemiology, with special attention to issues of using low-cost, unproven sensors and to approaches that have been used in India.

The research would benefit from greater knowledge of expected concentrations and concentration gradients; such information would inform the sample-size/power calculations. Earlier measurements in Bangalore reported relatively high concentrations (e.g., Both *et al.*, 2011³); I believe current measurements in Bangalore by ILK and CSTEP, using a reference-grade monitor (Beta Attenuation Monitor [BAM]), suggest cleaner concentrations.

Minor typo:

The abstract contains the text "We will estimate the level of air pollutants including coarse particulate matter 10 ug/m³ (PM₁₀), fine particulate matter 2.5 ug/m³(PM_{2.5}) and carbon monoxide (CO) parts per million (ppm) levels in both indoor and ambient environment." Both uses of the phrase "ug/m³" should be "µm" (micrometers), reflecting the size of the particles (<10 µm, <2.5 µm) rather than a concentration measurement (mass per volume: µg/m³).

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2. Allen RW: Referee Report For: Ambient and Indoor Air Pollution in Pregnancy and the risk of Low birth weight and Ensuing Effects in Infants (APPLE): A cohort study in Bangalore, South India [version 1]. *Wellcome Open Research*. 2019; **3** (133). [Reference Source](#)
3. Both AF, Balakrishnan A, Joseph B, Marshall JD: Spatiotemporal aspects of real-time PM(2.5): low- and middle-income neighborhoods in Bangalore, India. *Environ Sci Technol*. 2011; **45** (13): 5629-36 [PubMed Abstract](#) | [Publisher Full Text](#)

Is the rationale for, and objectives of, the study clearly described?

Yes

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Air pollution monitoring; exposure assessment

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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Ryan W. Allen

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This paper describes a birth cohort study to evaluate associations between air pollution exposure and low birth weight (LBW) in areas near Bangalore. The investigators also intend to evaluate the mediating role of LBW in associations between prenatal air pollution exposure and adiposity in early childhood. This is an ambitious study focused on important areas of inquiry. The scientific evidence linking air pollution with fetal growth is expanding rapidly, while the research on prenatal exposure and adiposity/overweight/obesity is relatively limited and results are mixed.

I have several concerns with the study as described:

1. Exposure assessment:

- The measurement plan is difficult to understand, due in part to inconsistent use of terminology. Under “exposure assessment”, the authors describe plans for outdoor, indoor, and personal sampling. Then under “Data sources and measurements” the authors describe only ambient and indoor monitoring, with no mention of personal monitoring. The paper states that “personal sampling monitors will be used to measure...individual exposure...at the household level.” Are the terms “personal” and “indoor” being used interchangeably? Or will each participant undergo both personal and indoor sampling? If so, will these be conducted at the same time?
- The investigators plan to use the VAYUCARE device for measuring air pollutants, but the paper does not provide sufficient detail on this instrument or a rationale for choosing it. Has the device been validated against other, more established monitors? I recommend that the authors carefully

consider their choice and provide a clear rationale for choosing this particular instrument. As interest in relatively low-cost monitors has increased, there have been more evaluations of various monitors and their strengths and weaknesses, and the authors might consider reviewing some of these recent evaluations such as:

- <http://www.aqmd.gov/aq-spec/evaluations>
 - <https://www.epa.gov/air-sensor-toolbox/evaluation-emerging-air-pollution-sensor-performance>
 - Morawska *et al.*, 2018¹.
- The authors should describe their plans for deriving exposure estimates from the measurements. For example, personal monitoring is planned during two 24-hour periods in pregnancy. These will need to be temporally adjusted and combined in some way to estimate long-term exposure during pregnancy. In addition, it is not clear if the investigators have plans to estimate exposure prior to enrolment in the study (the period from conception to enrolment).
 - The investigators plan to place outdoor monitors within 5 km of participants' residences. This may be too far to assess concentrations at the residence location. I suggest that the team consider models for interpolating outdoor measurements.

2. Power calculations:

- The study's power to detect associations will depend on the exposure gradient, but it is not clear what gradient the investigators assumed for their power calculations. Moreover, the ambiguity in the exposure assessment makes the power calculation difficult to interpret; it is unclear whether the exposure of interest is the pregnancy averaged concentration, trimester-specific concentrations, or concentrations over some other averaging time.
- The investigators note the transient nature of this population in assuming a loss of 40% of the cohort. It is not clear if the investigators also accounted for pregnancy losses in their anticipated loss to follow-up. One might expect that $\geq 10\%$ of these pregnancies will result in spontaneous abortion or stillbirth (depending on gestational age at enrolment).

3. Analysis plan:

- Hypotheses 3 and 4 assume an association between air pollution during pregnancy and adiposity in childhood. But the literature on this question is mixed, so it is possible that air pollution will not be associated with adiposity in this cohort. I recommend that the authors revise their hypotheses to formally test the air pollution – adiposity association before moving to the mediation analysis.
- For continuous outcome variables in childhood, will each time point be modelled separately or are there plans to take advantage of the study's longitudinal design to evaluate growth trajectories?
- The literature suggests that rapid "catch-up" growth after birth is strongly tied to poor cardiometabolic health later in life. Thus, in addition to the mediation analysis of LBW on the exposure-adiposity pathway, I suggest that the authors consider also explicitly evaluating catch-up growth as an outcome.
- The paper does not describe how the biomarkers (haemoglobin, oral glucose) will be used. These variables are not listed as potential confounders or possible effect modifiers (in Figure 3 or Table 1), nor are they outcome variables. Collection of these samples will pose a burden to participants, so the use of these samples needs to be justified.

In addition, I have the following minor comments/suggestions:

- The summary of previous studies on air pollution and fetal growth could be updated and expanded to include, for example, some of the papers listed at the end of this review.
- Figure 6 would be more informative as a timeline showing key activities at different stages of pregnancy/childhood.

- The questionnaire (supplementary file 1) needs refinement. Some questions are vague/subjective and may not provide useful information (e.g. “Is that a dusty occupation?”, “Please describe the weather”).

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Is the rationale for, and objectives of, the study clearly described?

Partly

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

No

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Air pollution exposure assessment and epidemiology.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Reviewer Report 13 May 2019

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Kalpana Balakrishnan 

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The proposal focuses on a theme that is being increasingly recognized as an important health outcome associated with air pollution exposures. This is especially pertinent in low-middle income countries where the prevalence of health damaging exposures and low birth weight are both high. The investigators have previously been engaged with cohort studies and thus have experience in launching such studies. The health outcomes section of the proposal is quite strong.

The proposal unfortunately does not provide a correct framing for addressing air pollution exposures and uses terminologies rather loosely making it difficult to interpret the rationale, objectives and the study methods proposed. A detailed listing of the issues and suggestions are provided below for possible consideration by the investigators and the funders prior to a final decision:

1. The term "indoor air pollution" is not defined correctly. The investigators seem to want to address air pollution exposures from solid fuel use and term it as indoor air pollution. Air pollution resulting from solid fuel combustion is defined by WHO as household air pollution and contributes to both indoor and ambient air pollution. However in the study population chosen (urban slums near Bangalore) the contributions from solid fuel use to indoor and ambient air pollution is likely to be negligible. In the event it is not so, the investigators should provide supporting information to make a case for prevalence of solid fuel use in the study population. The all India average of 67% solid fuel use is dated and currently stands at 59%. In large metropolitan cities such as Bangalore, even in slums the prevalence of solid fuel use is not likely to be greater than 5%. Indoor air pollution in this setting therefore will not represent contributions from solid fuel use.

Ambient air pollution in the setting proposed (based on recent source apportionment studies) is primarily from transport-related emissions and is likely to be responsible for bulk of the indoor exposures, especially in slums. In the absence of other prominent indoor sources (as is likely in urban slums) ambient/indoor ratios for most pollutants will be close to 1.

The study design and methods thus cannot examine the independent association of indoor vs ambient air pollution with birth weight in this setting, nor can it address household air pollution from

solid fuel use.

2. The investigators have not used the most recent or pertinent literature for the proposal. See several citations that have been added that provide a more comprehensive background on the topic. In particular recent studies in Chennai have documented the impacts on birthweight in a rural-urban cohort that address the dual risk of household and ambient air pollution through longitudinal household area measurements. A similar such study (DAPHNE) is underway in Delhi that focuses on personal exposures for urban pregnant women.
3. The sample size is not well informed by the expected exposure variability in the setting. The average exposure used in the cited reference (Balakrishnan *et al.*, 2018¹) is a national average for ambient air pollution. This does not address expected variability in personal exposures in an urban slum. It is also not clear if the effects estimate they have used is informed by the recent Chennai cohort study or meta-analytical estimates from other LMIC studies. Further performing two measurements may not adequately capture temporal variability (assuming that indeed women are able to consistently participate in a third trimester personal measurement). A 10000mAh power bank can be quite cumbersome to carry and may pose some safety/personal concerns for pregnant women.
4. The investigators do not provide any validation/citations/specifications for the instrument to be used for personal exposure monitoring. It is not clear if this is a new generation low cost sensor and if so, appropriate validations against reference monitors would be needed to develop quantitative exposure-response relationships. CO exposure is unlikely to be of concern in an urban population (given the expected low prevalence of solid fuel use). CO in the ambient environment is unlikely to be detected at good enough resolution in low cost sensors.
5. Numerous infant outcomes are being measured including growth and development milestones for which there exists a stronger pool of evidence for air pollution-related impacts but yet only mediations through pre-natal exposures on adiposity is being examined. It would be immensely useful to measure post-birth so that each of the outcomes could be independently studied in addition to mediation effects. The sample size requirement will however likely be much higher.
6. Finally, the model specifications need to be elaborated further. Given the spatio-temporal variability and the inability to characterize this fully with just two measurements during pregnancy, there will presumably be a need for mixed effects models to estimate long-term (pregnancy period) exposures. Planned sensitivity analyses should be described in greater detail.

The exposure assessment components of the proposal need to be totally re-structured to focus only on ambient air pollution, using alternative exposure measurement approaches, in a larger cohort and using well validated instrumentation. A wealth of high quality health information is being collected but without a similar rigour in exposure measurements, the ability to develop quantitative exposure-response would not be realized.

Much has advanced in terms of using alternative exposure assessment methodologies in large cohorts. These should find a place in this cohort as well.

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Is the rationale for, and objectives of, the study clearly described?

No

Is the study design appropriate for the research question?

Partly

Are sufficient details of the methods provided to allow replication by others?

Yes

Are the datasets clearly presented in a useable and accessible format?

Not applicable

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Exposure assessment, air pollution and health effects, environmental epidemiology, bio-monitoring, environmental policy

I confirm that I have read this submission and believe that I have an appropriate level of expertise to state that I do not consider it to be of an acceptable scientific standard, for reasons outlined above.

Author Response 01 Oct 2019

Giridhara R Babu, Public Health Foundation of India, Bangalore, India

The proposal focuses on a theme that is being increasingly recognized as an important health outcome associated with air pollution exposures. This is especially pertinent in low-middle income countries where the prevalence of health damaging exposures and low birth weight are both high. The investigators have previously been engaged with cohort studies and thus have experience in launching such studies. The health outcomes section of the proposal is quite strong.

Many thanks for recognizing the strengths of the proposal.

The proposal, unfortunately, does not provide a correct framing for addressing air pollution exposures and uses terminologies rather loosely making it difficult to interpret the rationale, objectives and the study methods proposed. A detailed listing of the issues and suggestions are provided below for possible consideration by the investigators and the funders prior to a final decision:

Our sincere thanks to your time and effort in providing detailed comments. We respect each of the inputs that you have given as a well-established senior mentor and researcher in this field. We have answered your queries in multiple parts to provide valid reasons, rationale, and details on methods. However, we earnestly submit that an appeal to the funders in a platform for peer review of the protocol is unwarranted.

1. The term "indoor air pollution" is not defined correctly. The investigators seem to want to address air pollution exposures from solid fuel use and term it as indoor air pollution. Air pollution resulting from solid fuel combustion is defined by WHO as household air pollution and contributes to both indoor and ambient air pollution. However, in the study population chosen (urban slums near Bangalore) the contributions from solid fuel use to indoor and ambient air pollution is likely to be negligible. In the event it is not so, the investigators should provide supporting information to make a case for prevalence of solid fuel use in the study population. The all India average of 67% solid fuel use is dated and currently stands at 59%. In large metropolitan cities such as Bangalore, even in slums, the prevalence of solid fuel use is not likely to be higher than 5%. Indoor air pollution in this setting, therefore, will not represent contributions from solid fuel use.

Ambient air pollution in the setting proposed (based on recent source apportionment studies) is primarily from transport-related emissions and is likely to be responsible for bulk of the indoor exposures, especially in slums. In the absence of other prominent indoor sources (as is likely in urban slums) ambient/indoor ratios for most pollutants will be close to 1.

The study design and methods thus cannot examine the independent association of indoor vs ambient air pollution with birth weight in this setting, nor can it address household air pollution from solid fuel use.

Thank you for your valuable inputs on this. We have not defined the solid fuel as indoor air pollution in our study. Our objective is to see the total effect of individual's exposure to air pollutants mainly PM2.5, PM10, and CO inside the house as well as their levels in the ambient air during the during the second and third trimester of pregnancy. The exposure of interest in our study is the levels of PM2.5, PM10, and CO, not solid fuel use. Solid fuel use is a contributing factor for these pollutants. Not only cooking fuel but several other contributing factors like incense sticks, frankincense, mosquito repellents (solid/liquid)

are also leading to a higher amount of exposure. Your study has provided a detailed note of all these sources. We have obtained information regarding all these sources and not just solid fuels. However, we are treating only the pollutants (as a result of all these sources) as exposure. Pregnant women are vulnerable to inhalation of a higher level of pollutants, due to the poor housing structure and inadequate ventilation in the urban slums. Our focus is not only on solid fuel use, there are several other activities done at the household level which contribute to the emission of higher level of PM2.5, PM10 and CO. We are recording each of these activities at the household occurring at regular basis. Prospective assessment at early pregnancy is the best design to prove the independent association between exposure to pollution and birth outcomes. We are recording the level of personal exposure to air pollutants during pregnancy, prospectively following them up until delivery to see the effect on birth weight of the baby. We are not here to address the air pollution from solid fuel use. Our findings may provide the contributing factors for higher level of PM2.5, PM10, and CO at the individual level. It is unfortunate that most of your comments are based on the incorrect assumption that we are assessing only solid fuel use. We request you to please see the questionnaire and be assured that this is not what we are doing.

We agree that the study design and methods cannot examine the independent association of indoor vs. ambient air pollution, but it is designed to examine the total effect of all pollutants (irrespective of indoor v/s outdoor) with birth weight in this setting. Accordingly, we have made changes in the manuscript. We did not intend to address estimate household air pollution from solid fuel use.

1. The investigators have not used the most recent or pertinent literature for the proposal. See several citations that have been added that provide a more comprehensive background on the topic. In particular recent studies in Chennai have documented the impacts on birth weight in a rural-urban cohort that address the dual risk of household and ambient air pollution through longitudinal household area measurements. A similar such study (DAPHNE) is underway in Delhi that focuses on personal exposures for urban pregnant women.

Thank you for the comments. The recent literature has been updated in the Background section. The recent articles have been quoted in page no. 3, line no 17 and 24.

1. The sample size is not well informed by the expected exposure variability in the setting. The average exposure used in the cited reference (Balakrishnan *et al.*, 2018¹) is a national average for ambient air pollution. This does not address expected variability in personal exposures in an urban slum. It is also not clear if the effects estimate they have used is informed by the recent Chennai cohort study or meta-analytical estimates from other LMIC studies. Further performing two measurements may not adequately capture temporal variability (assuming that indeed women are able to participate in a third trimester personal measurement consistently). A 10000mAh power bank can be quite cumbersome to carry and may pose some safety/personal concerns for pregnant women.

Thank you for the comment. We have now re-calculated the sample size using a published systematic review report/ risk estimates reported by Tielsch et al, 2009. Risk of Low Birth Weight and Stillbirth Associated With Indoor Air Pollution From Solid Fuel Use in Developing Countries-2010: Increased risk of LBW of 38% (OR ¼ 1.38, 95% CI: 1.25, 1.52)

from exposure to IAP and an associated reduction in birth weight of 96.6 g[1] Tielsch et al, 2009:Adjusted analysis: Risk of Low birth weight by exposure to solid biomass fuel [RR (term) $\frac{1}{4}$ 1.49, 95% CI: 1.25, 1.77; RR (preterm) $\frac{1}{4}$ 1.70, 95% CI: 0.93, 3.10] A cohort study][2]

We agree with you and have planned our measurements trimester-wise, but through field experiences, we have noticed that the majority of the study population contact the health center after completion of the first trimester. This is a practical limitation. We are assuming that one measurement for 24 hours is a proxy for the exposure through that trimester. The funding available does not permit us to assess throughout the trimester for now.

The device weighs approximately 110gms, and the power bank is approximately 200gm at the most. We have designed a sling bag to hold the device along with a power bank and asked specific questions to the study subjects on any discomfort they felt wearing devices. In our initial assessment of around 100 women, except for two women, rest all did not report any discomfort while wearing the bag. The reason for discomfort by these two women was that it is a disturbance in carrying the bag while doing household chores.

1. The investigators do not provide any validation/citations/specifications for the instrument to be used for personal exposure monitoring. It is not clear if this is a new generation low-cost sensor and if so, appropriate validations against reference monitors would be needed to develop quantitative exposure-response relationships. CO exposure is unlikely to be of concern in an urban population (given the expected low prevalence of solid fuel use). CO in the ambient environment is unlikely to be detected at good enough resolution in low cost sensors.

Thanks for the concerns. Please note that a manuscript describing validation is under the process of publication elsewhere. The device we are using is a low cost monitoring device which measures parameters PM2.5, PM10, and CO. The manufacturer is Ambience Monitoring India, New Delhi. It is a custom-built device. The scattered light is transformed into signals, that are amplified and processed. The specifications are as follows:- Range of measuring parameter, Particulate Matter 2.5 (PM2.5) Fine particulate Range: 0.0 – 999.9 $\mu\text{g}/\text{m}^3$, Particulate Matter 10 (PM10) Course particulate Range: 0.0 -1999.9 $\mu\text{g}/\text{m}^3$ Minimum resolution of $< 0.3 \mu\text{m}$. Relative error Maximum of 15% and $\pm 10 \mu\text{g}/\text{m}^3$ Operating Temperature Range -10 degrees C to +50 degrees C Operating Humidity Maximum Limit 70% RH . Carbon Monoxide sensor: Type: Electrochemical Measurement Range: 0 - 1000 ppm Detection Limit: 0.5 ppm Resolution: $< 100 \text{ ppb}$ (instrumentation dependent) Precision: $< \pm 2 \%$ of reading Response: between 15 - 30 seconds Sensitivity: 4.75 \pm 2.75 nA/ppm Expected operating life: > 5 years (up to 10 years if average temperature is 23C \pm 3 degrees) Operating temperature range: -30 to 55 C (-20 to +40 C recommended) . Temperature: Range: -40 – 123 °C Accuracy: ± 0.4 °C Power: 5uW standby, 3mw active Response: 8s (up to 20 minutes to balance if moved out of a location of excessive heat 35C+). Relative Humidity: Range: 0 – 100% humidity Accuracy: $\pm 3\%$; $\pm 4\%$ up to 80% RH $\pm 5\%$ up to 90% RH Power: 5uW standby, 3mw active. Certification CE/FCC/RoHS.

The device has been validated before use. Initially, Zero PM2.5 and CO for both indoor and outdoor samplers performed using a sealed glass container 45cmx45cmx35cm with an inlet and outlet. The glass chamber contains two valves, one at the inlet and outlet that were closed during the zero-calibration measurements. All sides of the glass container were glued together to make it airtight, except for the lid. For the zero-calibration, we had

placed our fully charged indoor and outdoor devices in the glass chamber after switching them on. After that, the lid was closed and sealed with double-sided tape. There was no gas inflow or outflow possible during the experiment. The devices were kept in the closed chamber until all their battery lives have ended (min 24h). The devices were kept in an airtight container without any exposure to CO or PM2.5.

Apart from the zero-calibration, also a one-point CO calibration has been performed in the same glass chamber. For this experiment, we have used calibrated gas cylinders of CO with a volume capacity of 10 litres with 50 ppm of CO concentration. After switching the devices on, they were kept in ambient air for a period of minimum one hour so that the readings of the device stabilized in ambient air. After that, the device was placed in the glass container after which the lid was closed and sealed. At this point, the valve at the outlet was left open and at the same time, the CO gas from the cylinder was released through the inlet to force the present air out of the container. To ensure there was no mixture of gases remaining in the chamber to avoid contamination in the device readings, we connected the outlet pipe to a water container having a measuring cylinder and flushed CO double the volume of the glass chamber. The outlet valve was then closed, and the chamber at this point was filled with 50 ppm. The regulator was closed and the device was left inside the sealed container for an hour. The device was removed after that and left for another hour in ambient air to stabilize the readings post-exposure to CO.

Field calibration: the State Pollution Control Board of Karnataka (KSPCB) possesses several outdoor samplers, with some located in the city of Bangalore, to measure ambient CO and PM2.5 levels. For our calibration, we used the outdoor sampler located at the Indira Gandhi Institute of Child Health Hospital, Bangalore (device name: Ecotech Monitoring Solutions, NIMHANS). The board very frequently calibrates this device. During 6 hours, we placed our outdoor sampler and three of our indoor samplers directly next to the board's sampler. After that, we calibrated our devices against the board's sampler based on the outdoor readings. Correction factors were developed for all our devices.

1. Numerous infant outcomes are being measured including growth and development milestones for which there exists a stronger pool of evidence for air pollution-related impacts but yet only mediations through pre-natal exposures on adiposity is being examined. It would be immensely useful to measure post-birth so that each of the outcomes could be independently studied in addition to mediation effects. The sample size requirement will however likely be much higher.

We agree with you. The current study is a subset of the larger cohort ongoing in Bangalore Government Health facilities with a targeted sample size of 5000 pregnant women with an aim to understand the transgenerational role of glucose and other nutrients, psychosocial environment on the poor infant outcomes and risk of childhood obesity, as an early marker of chronic diseases. Through the recent evidences it has been also proved that air pollution is also a contributing factor for poor infant outcomes. Hence we built our research question on evaluating independent association between indoor and ambient level of PM2.5, PM10 and CO on birth outcomes. We are in constant search for funding. In order to measure several post-birth outcomes independently studied in addition to mediation effects, we are seeking further funding from other sources as you have rightly pointed out that the sample size requirement will be much higher. We got funding for only four air quality monitoring devices and therefore, it has

been difficult to measure the pollution parameters after birth. We will try to cover the post-birth measurements, through additional sources of funding.

1. Finally, the model specifications need to be elaborated further. Given the spatio-temporal variability and the inability to characterize this fully with just two measurements during pregnancy, there will presumably be a need for mixed effects models to estimate long-term (pregnancy period) exposures. Planned sensitivity analyses should be described in greater detail.

The exposure assessment components of the proposal need to be restructured to focus only on ambient air pollution, using alternative exposure measurement approaches, in a larger cohort and using well-validated instrumentation. A wealth of high quality health information is being collected but without a similar rigour in exposure measurements, the ability to develop quantitative exposure-response would not be realized.

Much has advanced in terms of using alternative exposure assessment methodologies in large cohorts. These should find a place in this cohort as well.

We are using a multivariate linear regression model to examine the association between birth weight and pollution parameters (in continuous variables) separately adjusting for listed potential confounders. From the apriori, information covariates will be selected. Forming the causal path from exposure to outcome potential confounders will be listed. The model will be developed by adjusting to various potential confounders. A model with larger adjusted R2 and predicted R2 value would be considered and also with all confounders, we will run step-wise regression analysis. We will also categorize the exposure variables trimester wise as per the percentiles and run multivariate model adjusting for known important potential confounder variables. As we progress, we are keen to included advanced techniques using alternative exposure assessment methodologies in large cohorts. Your advice in this regard is of high value.

Again, we sincerely thank you for all the comments.

Competing Interests: No competing interests were disclosed.