Does first trimester serum pregnancy-associated plasma protein A differ in pregnant women with sickle cell disease?

Short running title: Serum PAPP- A levels and pregnancy outcomes in women with sickle cell disease

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What is already known about this topic?

- Pregnancies in women with SCD are at increased risk of stillbirth and birth of small for gestational age neonates.
- Women with HbSS disease have worse perinatal outcome compared to those with HbSC disease.

What does this study add?

- In women with HbSS disease serum PAPP-A at 11-3 weeks' gestation is significantly reduced and this may be an early marker of adverse perinatal outcome in these pregnancies.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT:

Objective: To assess whether levels of first-trimester pregnancy-associated plasma protein A (PAPP-A) differ between women with and without sickle cell disease (SCD).

Methods: Retrospective study of 101 singleton pregnancies in women with SCD (including 55 with genotype HbSS, 37 with genotype HbSC and 9 with other genotypes). Measured levels of PAPP-A were converted to multiple of the median (MoM) values corrected for gestational age and maternal characteristics. Median PAPP-A MoM in the SCD group was compared to that of 1010 controls.

Results: In the SCD group median PAPP-A MoM was lower than in the non-SCD group (0.72, interquartile range [IQR] 0.54-1.14 versus 1.09, IQR 0.74-1.49; $p<0.001$). Within the SCD group median PAPP-A MoM was lower for those with genotype HbSS than HbSC (0.62, IQR 0.44-1.14 versus 0.94, IQR 0.72-1.25; $p=0.006$). In 7.3% (4/55) of the HbSS group there was stillbirth and in these cases PAPP-A was ≤0.5 MoM; in the control group the incidence of stillbirth was lower (1%; $p<0.001$). In HbSS disease the incidence of small for gestational age (SGA) neonates was increased.

Conclusion: Pregnancies with HbSS have lower PAPP-A MoM values and higher incidence of stillbirth and birth of SGA neonates than in non-SCD controls.

INTRODUCTION

Sickle cell disease (SCD) is the commonest single gene defect in the world.[1-3] It is defined as an autosomal recessive hemoglobinopathy that includes sickle cell HbSS disease and various compound heterozygous genotypes, such as sickle cell HbSC disease or sickle cell β-thalassemia (HbSβ-thal) disease, characterized by chronic hemolytic anemia and vaso-occlusive complications. A recent systematic review and meta-analysis has shown a strong association between SCD and adverse perinatal outcomes, including stillbirth and birth of small for gestational age (SGA) neonates.[4] These manifestations could be as a result of abnormal placentation, which may result in reduced levels of first trimester pregnancy associated plasma protein-A (PAPP-A). However, there are no reported studies that examined the association of SCD and first trimester PAPP-A levels.

The objective of this study is to investigate whether serum PAPP-A levels differ in pregnancies of women with SCD compared to those in women without SCD and whether it is associated with adverse pregnancy outcome.

METHODS

Study population

The data for this study were derived retrospectively and included all pregnant women affected by SCD who attended for combined first-trimester screening for aneuploidies (fetal nuchal translucency, serum free ß-HCG and PAPP-A) at St. Thomas' Hospital, London, UK, between 2009 and 2017. Each SCD pregnancy was matched with 10 non-SCD controls, selected from women of the same racial origin who underwent first-trimester screening within 10 days of the date of screening of each SCD pregnancy.

The inclusion criteria were singleton pregnancy with live fetus at 11-13 weeks' gestation, delivery >20 weeks' gestation with livebirth or stillbirth and known pregnancy outcome. Pregnancies with fetal abnormalities and those ending in induced abortion were excluded. Gestational age was determined from the fetal crown-rump length (CRL).[5] The following characteristics were extracted from maternal records: maternal age, weight, height and racial origin (Black, White, Asian), method of conception (spontaneous or assisted), cigarette smoking during pregnancy (yes or no), genotype of SCD (HbSS, HbSC, HbSß-thal, non-specified SCD).

Serum PAPP-A was measured by an automated devise (Kryptor analytical system, Brahms AG, Berlin, Germany) with results being available within 40 min of blood collection. The measured levels were converted into multiple of median (MoM) for gestational age and corrected for maternal weight, smoking status, racial origin and mode of conception.[6] Data on PAPP-A levels recorded in MoM were obtained from the fetal medicine unit recording system (Astraia Software GmbH, Version 1.24.10, Munich, Germany, 2016). Data on pregnancy outcome were obtained from hospital records in UK Maternity Patient Data Management, National Maternity Care Record System for the NHS (BadgerNet Version 2.9.1.0).

Statistical analysis

Data were not normally distributed and therefore non-parametric tests were used. Characteristics were compared across study groups by the Mann-Whitney U test for continuous variables and by the chi-square test for categorical variables. Data are presented as median and interquartile range (IQR) or n (%). PAPP-A MoM values for SCD pregnancies were compared to the reference group (non-SCD pregnancies), and the process repeated for the two main SCD genotypes (HbSS and HbSC). Pregnancy outcome (live birth or stillbirth, birthweight and birthweight percentile according to the

Fetal Medicine Foundation fetal and neonatal population weight charts[7]) were presented by SCD group.

Analysis was conducted using Stata 15. All tests were two-tailed and *P* values <0.05 were taken to be statistically significant.

RESULTS

The genotype of the 101 women with SCD, included 37 cases of HbSC, 55 of HbSS, 3 of HbCC, 5 of HbSß-thal and 1 of non-specified SCD. There were no significant differences in maternal characteristics between the SCD group and controls (Table 1). In patients with SCD, compared to controls, the median birth weight in grams and centiles was significantly lower (2940 versus 3280 grams; p<0.001 and 17.5 versus 34.7 centiles respectively.) Stratifying by SCD genotype, median birth weight in grams and centiles in pregnancies complicated by HbSC was not significantly different from non-SCD pregnancies (median for HbSC 3070; p=0.007 and 23.7 centiles p=0.06). Median birth weight in grams and centiles was significantly lower in those with genotype HbSS compared to non-SCD pregnancies (median for HbSS 2860 grams; p<0.001 and 12 centiles p<0.001).

In the SCD group median PAPP-A MoM was lower than in the non-SCD group (0.72, IQR 0.54-1.14 versus 1.09, IQR 0.74-1.49; p<0.001) (Table 2). Within the SCD group median PAPP-A MoM was lower for those with genotype HbSS than HbSC (0.62, 0.44-1.14 versus 0.94; 0.72-1.25; p=0.006). The incidence of PAPP-A ≤0.5 MoM was significantly higher in the HbSS group than the controls, but there was no significant difference between the HbSC group and controls (Table 3). Similarly, the incidence of

stillbirth and birth of SGA neonates was significantly higher in the HbSS group than the controls. The four stillbirths in the HbSS group were diagnosed at 20,23, 25 and 29 weeks' gestation, respectively; the birthweight was <3rd percentile in three and serum PAPP-A was ≤ 0.5 MoM in all four. In the 10 stillbirths of the control group the birthweight was <3rd percentile in four and serum PAPP-A was ≤ 0.5 MoM in three.

DISCUSSION

Main findings of the study

The findings of this study demonstrate that first, in pregnant women with SCD first-trimester levels of PAPP-A are significantly lower than in non-SCD controls, second, in HbSS disease, but not in HbSC disease, median PAPP-A MoM is lower and incidence of PAPP-A ≤0.5 MoM is higher than in non-SCD pregnancies, third, in HbSS disease, but not in HbSC disease, the incidence of stillbirth and birth of SGA neonates were higher than in non-SCD pregnancies, and fourth, in HbSS disease, but not in HbSC disease, the incidence of PAPP-A ≤0.5 MoM in pregnancies with stillbirths and SGA neonates was higher than in non-SCD pregnancies.

Interpretation and implications of the study

Low first-trimester serum PAPP-A is associated with increased prevalence and severity of placental pathology during pregnancy such as stillbirth and fetal growth restriction.[8,9] A recent systematic review and meta-analysis including > 26 000 pregnancies in women with SCD showed strong association between SCD and adverse perinatal outcome, including 4-fold increased risk of stillbirth and 2.5-fold increased risk of fetal growth restriction; women with HbSS disease had significantly worse perinatal outcomes compared with those with HbSC disease.[4]

In SCD there is abnormal placentation and or sickling in the placenta[4,10] and the low serum PAPP-A may be a reflection of this placental pathology. Pregnancies with HbSS disease and low levels of PAPP-A ≤ 0.5 MoM seem to be at highest risk of adverse outcome. In this respect, serum PAPP-A could potentially act as a marker of increased risk of complications and stimulate the need for closer surveillance and earlier therapeutic interventions in this specific group of women. Additionally, in the calculation of PAPP-A MoMs in screening for fetal trisomies it may be necessary to adjust for maternal SCD, otherwise the associated low PAPP-A would increase the false positive rate for trisomies 21, 18 and 13 and lead to unnecessary invasive testing for fetal karyotyping.

Strengths and limitations

This study was conducted at one of the largest referral centers for SCD in the UK, but the sample of affected pregnancies is small. Despite this small number we were able to demonstrate significant differences between HbSS disease and non-SCD pregnancies in serum PAPP-A levels and adverse pregnancy outcome. The incidence of stillbirth in the controls (1%) was higher than the national average (04%)[11], but this could be explained by first, inclusion in this study of stillbirths at ≥ 20 weeks' gestation, rather than ≥ 24 weeks in national statistics, and second inclusion of a highly vulnerable inner city Black population. A similar reason may also be true for the observed incidence of SGA in the control group (26%), which was higher than the rate of 17% reported in the reference range for Black women.[7]

Conclusion

In HbSS disease first-trimester serum PAPP-A is decreased and these pregnancies are at high risk of stillbirth and birth of SGA neonates.

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Table 1. Maternal and pregnancy characteristics in SCD and non-SCD control group

Characteristic	**Controls (N=1010)**	**Sickle Cell Disease (SCD)**		
		All SCD (N=101)†	**HbSS (N=55)**	**HbSC (N=37)**
Gestation at PAPP-A analysis (weeks)	12.6 (12.1-13.0)	12.4 (12.0-12.9)	12.3 (11.7-12.9)	12.4 (12.0-13.3)
Age (years)	31.0 (26.8-35.3)	30.5 (26.0-35.4)	30.5 (26.0-35.5)	31.5 (28.1-35.9)
Body mass index‡	26 (23-30)	24 (22-27)	23 (21-25)	25 (22-29)
Racial origin				
Black	1000 (99.0)	100 (99.0)	55 (100)	37 (100)
East Asian	10 (1.0)	1 (1.0)	0	0
Spontaneous conception §	795 (97.8)	82 (97.6)	44 (100)	30 (93.8)
Cigarette smoking	46 (4.6)	0	0	0
Nulliparous	430 (42.6)	43 (42.6)	26 (47.3)	13 (35.1)
Birthweight percentile	34.7 (8.9-68.5)	17.5 (1.6-45.5)	12.0 (0-41.4)	23.7 (3.3-48.8)
Birthweight (gm)	3280 (2950-3580)	2940 (2500-3210)	2860 (2400-3140)	3070 (2720-3460)
Gestation at birth (weeks)	39.9 (38.9-40.7)	38.4 (37.9-39.1)	38.3 (37.7-38.9)	38.6 (38.1-39.9)

Values are given as median (interquartile range) or n (%)

† Including nine women with other types of SCD

‡ Available BMI values: all cases in SCD and 821 cases in comparison group

§ Available data on conception method: 85 cases in SCD and 813 cases in comparison group

Table 2. PAPP-A MoM levels in SCD group by genotype and non-SCD control group

Study group	**Median (IQR)**	**p-value***
Comparison group (n=1010)	1.09 (0.74-1.49)	-
All SCD (n=101)†	0.72 (0.54-1.14)	<0.001
HbSS (n=55)	0.62 (0.44-1.14)	<0.001
HbSC (n=37)	0.94 (0.72-1.25)	0.14

IQR = interquartile range

† Including nine women with other types of SCD

** Mann-Whitney U test, using non-SCD group as comparison group*

Table 3. Birth outcomes and proportion of low PAPP-A by study group.

Characteristic	Controls (N=1010)	Sickle Cell Disease (SCD)					
		All SCD (N=101)†		HbSS (N=55)		HbSC (N=37)	
	n (%)	n (%)	p-value*	n (%)	p-value*	n (%)	p-value*
PAPP-A ≤0.5 MoM	95 (5.0)	24 (23.8)	<0.0001	21 (38.2)	<0.001	2 (5.4)	0.44
Stillbirth	10 (1.0)	4 (3.9)	0.01	4 (7.3)	<0.001	0 (0)	0.54
PAPP-A ≤0.5 MoM	3 (30.0)	4 (100)	<0.0001	4 (100)	<0.0001	-	-
Small for gestational age‡	263 (26.0)	43 (42.3)	<0.001	24 (43.6)	<0.001	14 (37.8)	0.12
PAPP-A ≤0.5 MoM	40 (15.2)	14 (32.6)	<0.0001	10 (41.7)	<0.0001	2 (14.3)	0.65

** p values from x2 test, using non-SCD group as comparison group*

† Including nine women with other types of SCD

‡ Birthweight ≤10th percentile for gestational age.[7] In this section we include only live births