Journal of Perinatology (2018) 38:797–803
https://doi.org/10.1038/s41372-018-0159-y

ARTICLE

Differences in prenatal aneuploidy screening among African–American women with hemoglobin S variants

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Received: 17 October 2017 / Revised: 30 April 2018 / Accepted: 21 May 2018 / Published online: 2 July 2018

Abstract

Objective It has been shown that hemoglobinopathies increase the risk of pregnancy complications and placental dysfunction. This could alter the placental analytes examined during prenatal aneuploidy screening. Our objective was to determine whether there is a difference in maternal serum screening results for women with hemoglobin S variants (AS, SS, SC, S/beta thalassemia) compared with women with normal hemoglobin (AA).

Study design This is a retrospective cohort study in African–American women receiving aneuploidy screening at MedStar Washington Hospital Center from 2008 to 2015. We evaluated 79 women with hemoglobin S variants (69 AS and 10 sickle cell disease (SCD)) and 79 controls. Descriptive statistics (means, medians, and frequencies) were calculated for each group. For the continuous variables, differences in the averages between the two groups were tested using the *t* test or Wilcoxon rank sum test. Differences in the averages between three or more groups were tested using the analysis of variance test or the Kruskal–Wallis test.

Results Demographics were similar between cases and controls. The overall screen positive rate for Down syndrome among patients with sickle cell trait (AS) was 3% (2/69). For patients with SCD, the overall screen positive rate was 10% (1/10). None of the women in the control population (AA) has a positive Down syndrome screening result (0/79).

Conclusion As expected, the screen positive rate in patients with hemoglobin S variants was higher than controls, however, patients with sickle cell trait do not appear to be at an increased risk for false-positive results with serum aneuploidy screening compared with the general population. We did, however, find an increased risk of false-positive quad screen results in patients with sickle cell disease.

Introduction

Sickle cell disease refers to a group of autosomal recessive disorders involving distorted sickle-shaped hemoglobin (Hb S) [1]. Asymptomatic individuals are carriers and referred to as having sickle cell trait (AS). The most severe form of sickle cell disease is sickle cell anemia, caused by homozygous Hb S (SS), and characterized by chronic hemolytic anemia and vaso-occlusive complications [2, 3]. Sickle cell disorders can also be found in those who co-inherit hemoglobin S and an abnormality of beta-globin structure or production, for example, sickle-hemoglobin C disease (SC) or sickle beta thalassemia (S/beta thalassemia) [1].

The vaso-occlusive nature of sickle cell disease likely leads to placental dysfunction, resulting in poor pregnancy outcomes and may alter placental analytes used in aneuploidy screening [3, 4]. Studies are conflicting, yet limited, regarding whether sickle cell carrier status is associated with an increased risk of adverse maternal or perinatal outcomes [4, 5].

Screening for aneuploidy in pregnancy is predominately performed through maternal serum testing of fetal and placentally derived analytes in the first and/or second trimester. In the first trimester, serum free beta-human chorionic gonadotropin (hCG) or total hCG, and placentally derived pregnancy-associated plasma protein-A (PAPP-A) are measured and in the second trimester alpha-fetoprotein (AFP), unconjugated estriol (uE3), inhibin-A (DIA), and

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hCG are measured. The reported detection rates for Down syndrome with first and second trimester screening are 82–87% and 81%, respectively, with a 5% screen positive rate, however, this varies with maternal age at delivery [6].

In the absence of a chromosome abnormality or neural tube defect, abnormal analyte levels have also been associated with adverse pregnancy outcomes, including early pregnancy loss, stillbirth, placental abruption, abnormal placental adherence, gestational hypertension, preeclampsia, fetal growth restriction, and preterm birth [7, 8]. These abnormalities are thought to be secondary to abnormal trophoblast invasion and placental development, which is similar to what is seen in the placentas of patients with sickle cell disease [9, 10].

Understanding the potential for false-positive results in women with sickle cell variants, and more specifically sickle cell trait, may assist in counseling these patients regarding appropriate prenatal testing for aneuploidy. Therefore, our objective was to compare screen positive rates for aneuploidy screening in patients with hemoglobin S variants versus patients with hemoglobin AA.

Methods

MedStar Washington Hospital Center (MWHC) is a high volume, regional obstetric referral center that cares for a population in which sickle cell variants occur frequently. We performed a retrospective cohort study using an ultrasound database of African–American women receiving care at the perinatal center for MedStar Washington Hospital Center from 2008–2015. Cases were singleton pregnancies in African–American women with hemoglobin S confirmed by hemoglobin electrophoresis and who had undergone first or second trimester aneuploidy screening. Cases were further divided into sickle cell trait (AS) and sickle cell disease (SCD-SS, SC, S/beta thalassemia). Women with multifetal gestations or with no hemoglobin electrophoresis results were excluded. Controls were African–American women with a normal hemoglobin electrophoresis (AA). The controls were selected in a 1:1 ratio from women receiving care at the Perinatal Center during the same period as the cases. This study was approved by the institutional review board at MedStar Health Research Institute.

For each case and control, we collected the following demographic information from the electronic medical record: age, parity, gestational age at testing, gestational age at delivery, and maternal weight (Table 1). We also collected information regarding Down syndrome risk and values (multiple of the median) of each serum analyte in the first or second trimester (Tables 2 and 3). The labs performing the testing adjusted the multiples of the median (MoM) values for maternal race, prior positive screen, diabetic status, smoking status, and weight. A positive Down Syndrome screen was defined by each laboratory's cutoff value. Three different labs were used for first trimester screening due to insurance coverage and cutoffs were as follows: NTD–1 in 313, LabCorp–1 in 250, Genecare–1 in 295. For second trimester screening (quad screening), all testing was performed at LabCorp and the cutoff value was

Table 1 Demographic characteristics

	Controls $n = 79$	Sickle cell trait $n = 69$	Sickle cell disease variant* $n = 10$	*P* value*
Mean maternal age at delivery (years)	25.0 (21.8–29.5)	25.0 (21.2–31.2)	23.1 (20.4–32.3)	0.79
Mean gestational age at delivery (weeks)	38.9 (36.6–39.9)	39.1 (37.9–40.2)	38.4 (36.3–39.4)	0.08
Birthweight (grams)	2862.9 (723.1)	3157.0 (611.9)	2720.9 (381.4)	0.02
Mean maternal weight, *k this should be lbs*	181.0 (145.8–210.8)	168.0 (135.0–193.8)	141.5 (133.0–160.0)	0.02
Pre-gestational diabetes *N* (%)	0 (0)	2 (3)	0 (0)	0.12
Parity *N* (%)				< 0.001
Nulliparous	25 (32)	45 (65)	6 (60)	
Multiparous	54 (68)	24 (35)	4 (40)	

Maternal age, weight, and gestational age are median and IQR. Birthweight is mean and SD

*SS = 5, SC = 4, S/beta thalassemia = 1

*$p < 0.05$ is statistically significant

Table 2 Comparison of screen positive rate and serum analyte value in controls vs sickle cell trait

	Controls $n = 79$	Sickle cell trait $n = 69$	P value*
Mean gestational age at screening (weeks)			
First trimester	12.9 (11.6–13.1)	12.6 (12.0–13.0)	0.49
Second trimester (Quad screen)	17.3(15.9–19.4)	17.1(16.1–18.3)	0.89
Screening type N (%)			0.56
First trimester	37 (47)	29 (42)	
Second trimester (Quad screen)	42 (53)	40 (58)	
Screen positive rate N (%)			
Overall	0 (0)	2 (3)	0.13
First trimester	0 (0)	0 (0)	0.32
Second trimester (Quad screen)	0 (0)	2 (5)	0.14
Mean serum analyte values (MoM)			
PAPPA-A	1.1 (0.62–1.5)	0.93 (0.69–1.2)	< 0.0001
free hCG	1.0 (0.62–1.4)	1.2 (0.67–1.7)	0.38
AFP	1.0 (0.80–1.2)	0.95 (0.71–1.2)	0.43
Total hCG	0.92 (0.71–1.3)	1.2 (0.75–1.6)	0.25
uE3	0.93 (0.71–1.1)	1.0 (0.86–1.2)	0.23
DIA	0.81 (0.66–1.1)	0.73 (0.59–1.1)	0.24

Data are median and IQR unless otherwise specified

*$p < 0.05$ is statistically significant

1 in 270. For all patients with a positive screen, a review of the mother's and infant's medical record was conducted to determine whether the infant had a confirmed diagnosis of Down syndrome or any other chromosome abnormality.

Means, medians, standard deviations, and ranges for continuous variables and frequencies and percentages for categorical variables were calculated. For the continuous variables, differences in the averages between the two groups were tested using the two-tailed t test when the normality assumption of the data was satisfied, and the non-parametric Wilcoxon rank sum test was used when the normality assumption was not satisfied. Differences in the averages between three or more groups were tested using the analysis of variance test when the normality assumption of the data were satisfied, and the non-parametric Kruskal–Wallis test was used when the normality assumption was not satisfied. A p value of $< .05$ was considered to indicate a statistically significant difference. Statistical analysis was performed using SAS 9.4 software.

Results

Between 2008 and 2015, there were 247 patients with reported sickle cell variants receiving care at the MWHC Perinatal Center. Of those patients, 126 had a confirmed diagnosis by hemoglobin electrophoresis. Another 47 cases were excluded for the following reasons: 12 cases with diagnostic testing only, 31 missing demographic information, 3 multiple gestation, and 1 with testing at the wrong gestational age, leaving a final study population of 79 patients (69 AS and 10 SCD).

Descriptive characteristics of the study population are presented in Table 1. Median maternal age at delivery ranged from 23 to 25 years (IQR 20.4 to 32.3 years) and the difference was not statistically significant among groups ($p = 0.79$). This was also true for median gestational age at delivery, range 38.4–38.1 weeks ($p = 0.08$). Birthweight was the highest among patients with sickle cell trait when compared to both controls and patients with sickle cell disease (AS 3157 g vs AA 2862 g vs SCD 2720 g, $p = 0.02$). Maternal weight at testing was higher in controls compared with patients with sickle cell trait or sickle cell disease (AA 181.0 lbs vs AS 168.0 lbs vs SCD 141.5 lbs, $p = 0.02$). Regarding parity, controls were more likely to be multiparous (68%) compared with patients with sickle cell trait (35%) and sickle cell disease (40%), $p < 0.001$.

There was no difference in the percentage of patients receiving first versus second trimester screening (Tables 2 and 3). The overall screen positive rate for Down syndrome among patients with sickle cell trait was 3% (2/69) and when evaluating first trimester and second trimester screening separately, the rates were 0% (0/29) and 5% (2/40), respectively (Table 2). For patients with sickle cell disease, the overall screen positive rate was 10% (1/10) when evaluating first trimester and second trimester screening separately, the rates were 0% (0/4) and 17% (1/6), respectively (Table 3). We identified 79 women for our control population. Of these women, none had a positive Down syndrome screening result (Tables 2 and 3).

When comparing serum analyte levels, patients with sickle cell trait (Table 2) had a significantly lower PAPP-A levels compared with controls. (AS 0.93 MoM vs. AA 1.1 MoM, $p < 0.0001$). However, the levels of AFP, DIA, uE3, free, and total hCG did not differ between cases and controls.

For patients with sickle cell disease (Table 3), they had lower uE3 levels (SCD0.87 MoM vs. AA 0.93 MoM, $p = 0.03$). The other analyte levels of PAPP-A, AFP, free and total hCG levels were similar between women with sickle cell disease variants and controls.

Of cases with a positive screen, none underwent amniocentesis, but two had normal cell-free fetal DNA-screening results (Table 4). Chart review revealed that none of these three infants had clinical manifestations of Down syndrome or any other chromosome abnormality on physical exam at delivery. The hemoglobin diagnoses associated with the positive screen cases were AS (2 patients)

Table 3 Comparison of screen positive rate and serum analyte value in controls vs sickle cell variants

	Controls $n = 79$	Sickle cell disease variant* $n = 10$	P value*
Mean gestational age at screening (weeks)			
First trimester	12.9 (11.6–13.1)	12.8 (12.2–13.2)	0.49
Second trimester (Quad screen)	17.3 (15.9–19.4)	17.2 (15.9–19.4)	0.89
Screening type *N* (%)			0.68
First trimester	37 (47)	4(40)	
Second trimester (Quad screen)	42 (53)	6 (60)	
Screen positive rate *N* (%)			
Overall	0 (0)	1 (10)	0.005
First trimester	0 (0)	0 (0)	
Second trimester (Quad screen)	0 (0)	1 (17)	0.008
Mean serum analyte values (MoM)			
PAPPA-A	1.1 (0.62–1.5)	0.86 (0.67–1.1)	0.06
Free hCG	1.0 (0.62–1.4)	1.1 (0.99–1.1)	0.64
AFP	1.0 (0.80–1.2)	0.87 (6.8–1.3)	0.82
Total hCG	0.92 (0.71–1.3)	1.4 (0.88–2.5)	0.17
uE3	0.93 (0.71–1.1)	0.87 (0.61–1.2)	0.03
DIA	0.81 (0.66–1.1)	1.0 (0.82–2.6)	0.83

Data are median and *IQR* unless otherwise specified

*SS = 5, SC = 4, S/beta thalassemia = 1

*$p < 0.05$ is statistically significant

and SC. Case 1 and 3 underwent second trimester screening and had elevated hCG and DIA levels. Case 2 underwent second trimester screening and had a low uE3 level. Case 1 had a delivery and postpartum course complicated by pre-eclampsia. Cases 2 and 3 had uneventful pregnancy, delivery and postpartum courses (Table 4).

Discussion

For serum aneuploidy screening, a positive test result indicates that a patient's risk for having a baby with Down syndrome is greater than the specified cutoff level based on the performance characteristics of the test. The positive screen rate for these tests is between 2.3% and 5.1% for first trimester screening and 3.6 and 9.4% for second trimester screening [11, 12]. Our study found that there is an increased screen positive rate for the quad screen among patients with sickle cell disease, but no increased screen positive rate among patients with sickle cell trait. However, the false-positive cases only occurred with second trimester screening, which is not surprising given that the false-positive rate is generally lower with first trimester compared with second trimester screening.

The results of this study demonstrate that patients with sickle cell disease have an increased chance of a positive serum screen result for Down syndrome in the second trimester. Our findings are consistent with the study by Kneitel et al., however, their screen positive rate was significantly higher (38.5%) than ours (17%) [13]. This could be secondary to the fact that our population included patients with various types of sickle cell disease, which may have a weaker impact on placental development and in turn, a smaller impact on serum analytes. In addition, we have shown that patients with sickle cell trait have a screen positive rate of 5% in the second trimester, which is not significantly increased over the expected rate. The finding may be explained by the fact that carrier status often confers no symptoms or very mild anemia, which may not alter placental development [4].

General serum analyte trends seen in pregnancies affected with Down syndrome show significantly decreased PAPP-A, mild decrease in AFP and uE3, and increased DIA and hCG. With the exception of AFP, this same trend is associated with preeclampsia, fetal growth restriction, and preterm birth [7, 8, 14]. Theses outcomes and serum analyte trends are also seen in pregnancies affected by sickle cell disease [10]. The exact mechanism underlying the placental dysfunction seen in sickle cell disease is unknown, however, it is hypothesized to be secondary to hypoxia from a chronic inflammatory state [3, 10, 15, 16]. In the placenta, the syncytiotrophoblast is formed by fusion of cytotrophoblasts and this process can be induced by hypoxia [10, 17]. Given that hCG, DIA, and PAPP-A are produced by the syncytiotrophoblasts, it is possible that the hypoxic environment in sickle cell disease could lead to dysregulation of these analytes. uE3 is produced by the placenta using metabolites secreted by the fetal adrenal glands. Therefore, low uE3 levels may result from placental dysfunction or fetal conditions that impair the estriol pathway. Lastly, elevated AFP levels have been associated with placental pathology, but given that it is produced by the fetus and not the placenta it is unclear whether differences in AFP would be expected in patients with sickle cell disease [7].

In our study, serum analytes between cases and controls did not show significant differences overall, but there were some notable trends. Among both patients with sickle cell trait and sickle cell disease, there was a decrease in PAPP-A, which reached statistical significance in patients with sickle cell trait and had a trend towards significance in patients with sickle cell disease. This finding is not

Table 4 False-positive cases

Case number	Diagnosis	First trimester analytes (MoM)		Second trimester analytes (MoM)				T-21 risk by age	T-21 risk after screening	Maternal age at delivery (years)	Gestational age at testing (weeks)	Gestational age at delivery (weeks)	Comments
		PAPPA-A	**Free beta hCG**	**AFP**	**hCG**	**uE3**	**DIA**						
1	AS	0	0	0.88	3.28	1.29	2.63	1 in 1111	1 in 259	22	16.7	41.1	–Negative cell-free fetal DNA screening. –Normal anatomy US. –Uneventful pregnancy course. –No evidence of aneuploidy at delivery. –Delivery/postpartum course complicated by preeclampsia.
2	AS	0	0	0.98	1.91	0.59	1.41	1 in 486	1 in 217	32	16.1	39.1	–No further testing. –Normal anatomy US. –Uneventful pregnancy course. –No evidence of aneuploidy at delivery. –Uncomplicated delivery/ postpartum course.
3	SC	0	0	1.03	3.88	0.82	3.27	1 in 1178	1 in 136	18	18.6	39.0	–Negative cell-free fetal DNA screening. –Normal anatomy US. –Uneventful pregnancy course. –No evidence of aneuploidy at delivery. –Uncomplicated delivery/ postpartum course.

surprising in the patients with sickle cell disease given the chronic inflammatory state previously discussed. In patients with sickle cell trait, the mechanism is unclear but may indicate that there is a spectrum of disease within patients who have sickle cell trait. It is notable, however, that the median levels of 0.86–0.93 MoM found in our study did not reach levels reported to be associated with adverse obstetric outcomes (< 0.52 MoM) in previous studies [7]. As was also seen in the study by Kneitel et al., both patients with sickle cell trait and disease variants had higher hCG levels than controls, but this did not show a trend toward significance[13]. Again, at a median level of 1.1–1.4 MoM, they were also lower than levels reported to be associated with adverse pregnancy outcomes. Lastly, uE3 levels were significantly lower in patients with sickle cell disease variants compared with controls and as with other analyte patterns in our study, the median level was 0.83 MoM, which is still significantly higher than levels reported to be associated with adverse obstetric outcomes (0.5 MoM) in previous studies, therefore the clinical significance of these differences in analyte levels is unclear.

Our population was primarily comprised of patients with hemoglobin AS (87%) and a very small number of patients with sickle cell disease variants (13%), which is reflective of the national prevalence of hemoglobinopathies. In the United States, ~8% of African Americans have sickle cell trait, and 3.3% have some form of sickle cell disease [18]. Although the number with a false-positive screen was low in our cohort, the abnormal analyte patterns were also consistent with those reported in previous studies [7–9]. We also had one patient who developed preeclampsia, which is consistent with previous studies documenting the association between elevated hCG levels and adverse pregnancy outcomes [2, 11].

In addition to this being a retrospective study with a small sample size, another limitation is that our hospital is a referral center and therefore, many patients had to be excluded from this study as their hemoglobin diagnosis could not be confirmed. This could result in selection bias given that we were only able to evaluate charts with complete documentation. Further, the results of our patients with sickle cell disease must be interpreted with caution given that Hb SC and Sβ+-thalassemia typically have a milder clinical course than Hb SS and Sβ°-thalassemia [19].

At present, there is limited data on the effect of hemoglobin variants on maternal serum screening results. This study is unique because it provides information about individuals with both sickle cell disease variants and sickle cell trait. We were also able to obtain specific information regarding both combined first trimester screen and the quad screen owing to the equal distribution in our population. Another strength of this study is that both our cases and controls were selected from the same clinical center and only differed by their beta-globin variant status, providing a representative sample of the population at large.

Conclusion

In conclusion, we found that there was a significantly increased screen positive rate for Down syndrome on the quad screen among patients with sickle cell disease variants, but no increased screen positive rate among patients with sickle cell trait. Women with sickle cell trait can be counseled that the performance of standard first and second trimester screening tests are comparable to women without such variants, but women with sickle cell disease variants are at increased risk for false-positive results on the quad screen. We also found that the serum analyte values, although different from the control group, did not reach levels associated with adverse pregnancy outcomes. This study provides guidance on pre- and post test counseling for women with sickle cell disease and sickle cell trait, which will be significant in clinics providing care to predominantly African–American patients. More studies are needed to evaluate the impact of sickle cell variants on placental growth and function.

Acknowledgements We thank Eshetu Tefera, M.S, and the Medstar Health Research Institute for statistical support.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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