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MATERNAL SERUM ALPHA-FETOPROTEIN AND FREE BETA-HUMAN CHORIONIC GONADOTROPIN IN THE SECOND TRIMESTER: IMPLICATION FOR HIGH RISK OF DOWN'S SYNDROME IN THE GHANAIAN POPULATION

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Abstract: Maternal serum screening during pregnancy for adverse changes in levels of alpha-fetoprotein (AFP) and free beta- Human Chorionic Gonadotropin (free β-HCG) in the second trimester to assess risk of Down's syndrome (DS) is a rare obstetric practice in Ghana. The aim of this study was to determine changes in the levels of these markers, and assess for risk of DS and adverse pregnancy outcomes in the Ghanaian population. Levels of AFP and free β-HCG were determined in maternal serum samples from eighty one women with singleton pregnancy collected at recruitment (12-13 gestational weeks), and in the second trimester (16 gestational weeks). AFP and free β-HCG markers were expressed in multiples of the normal median (MoM) for gestation. Delivery outcomes and features of the new born at delivery were also noted. The mean age of subjects at recruitment was 33.9±3.2 years. AFP levels at recruitment and in the second trimester were 18.8 ± 7.5 ng/ml (mean ± SD) and 30.9 ± 9.8 ng/ml (mean ± SD) respectively. Similarly, free β-HCG at recruitment and in the second trimester were 47.2 ± 12.2 ng/ml (mean ± SD) and 29.8 ± 11.3 ng/ml (mean ± SD) respectively. The differences in the serum levels of AFP and free β-HCG between the first and second trimesters were significant ($p<0.0001$: 95% CI -14.9-(-9.3) and $p<0.0001$: 95% CI 13.6-21.2). Whilst the AFP level did not correlate with weight, free β-HCG weakly correlated with maternal weight ($p=0.29$ and $r^2=0.017$; $p=0.08$ and $r^2=0.055$ respectively), but not age in the second trimester. There were no cases of DS observed in the study population. The mean concentrations of free β-HCG in adverse pregnancies were higher than in normal pregnancies (38.4 ± 6.6; 29.3 ± 10.6) but not for AFP ($p=0.059$, and $p=0.477$ respectively). Although no DS was seen for comparison, free β-HCG appears to be a useful marker for risk assessments of poor pregnancy outcomes in the population.

Key Words: Alpha-fetoprotein, free beta-Human Chorionic Gonadotropin, Down's syndrome, prenatal screening, adverse pregnancies, Ghanaian population.

INTRODUCTION

Down's syndrome (DS) or trisomy 21 has been documented in many populations throughout the world. In Africa, awareness of this condition appears to be low, and as a result, little attention has been given to its documentation [1]. DS is caused by chromosome 21 non-disjunction during meiosis [2]. It is characterized by observable, phenotypic characteristics attributed to genotypic variations on chromosome 21 [3]. This condition is more prevalent in females than in males. Females constitute 90% of the cases due to non-disjunction during meiosis I, whilst the remaining 10% of male cases is due to non-disjunction during meiosis I and II [4]. Approximately 50-75% of embryos with a DS genotype abort spontaneously [4, 5, 6]. The rest survive to term, and account for the observed incidence of DS which is approximately 1 in 600 to 1 in 1000 live births [4, 7]. DS is a leading cause of mental retardation and birth defects worldwide. Mental retardation usually varies from mild to moderate [8]. At least six phenotypic characteristics including patterns of dismorphic features (flat facial profile, poor Moro reflex, hypotonia, loose skin on the back of the neck, slanted palpebral fissures, and small rounded ears) are usually present at birth. Other features are short stature, sparse hair, and a tongue protruding through thick lips. Defects in heart and kidney, hearing and vision loss may also be present [4, 8, 9]. Most children with the syndrome often have difficulty with language and communications skills. Adults with the disease have a high prevalence of early Alzheimer's disease [2, 8]. Maternal age is the single most identified factor contributing to risk [7, 10, 11]. For mothers aged 25, 35 and 45 years, the risk is 1/1341, 1/354 and 1/36 respectively [12]. The biological component of aging that increases the risk of non-disjunction remains unexplained [11, 13]. DS occurs in all ethnic groups and among all economic classes [14]. Screening programs have been instituted in developed countries since the early 1980s [15, 16]. Currently, the most commonly used biochemical markers consist of alpha-fetoprotein (AFP), and free beta- human chorionic gonadotropin (free β-HCG) in the second trimester (15-18 weeks of gestation) [17, 18]. AFP is a fetal specific protein produced by the fetal yolk sac and fetal liver. In pregnancies affected by DS, the concentration falls in about 25% of cases than in unaffected pregnancies [19, 20]. HCG is produced by the syncytiotrophoblast cells

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of the placenta. Free β-HCG is the most discriminatory protein for trisomy 21 compared with normal pregnancies. The concentration of free β-HCG in maternal serum is elevated almost two fold in DS fetuses [19, 20]. Up until 1955, it was believed that DS did not occur in African populations [21]. The first reported case was in Nigeria, in 1982, when it was shown that the prevalence was 1.16 per 1000 live births [1]. Unlike European populations, 58.6% of the cases were seen in women aged 26-35, and 20.7% in those aged greater than 35 years [1]. In the middle 1990s, three separate prospective studies in South Africa suggested that DS may occur in Africa with frequencies similar to other populations with higher incidence in women aged 35 or greater [21, 22]. In Ghana there is no data in the literature about its prevalence, as is the case in most of West Africa. All over the world, the aspiration of women to attain higher education is dictating their age at first pregnancy [23]. Ghana may have a similar trend of women aged greater than 30 years before having their first pregnancy. This trend may increase the risk associated with having children with DS. It is important that basic data is accumulated on DS prevalence. In addition, currently identified biochemical markers need to be assessed in different populations for cut-off points to be determined to facilitate screening programs and counseling of mothers within the risk category. The aim of the present study was therefore to determine levels of serum AFP and free β-HCG in blood samples of pregnant women, with the view of assessing differentials if any, in these markers, for high risk DS pregnancies and poor pregnancy outcomes in the Ghanaian population.

MATERIALS AND METHODS

Participants and study area

Pregnant women, who were aged 30 years and above with singleton pregnancies were included in the study. A total of 130 pregnant women attending the antenatal clinic of the Department of Obstetrics and Gynecology, Ridge Regional Hospital, Accra, Ghana were interviewed over a period of 13 months, out of which 81 singleton pregnant women were found eligible, and recruited into the study. Detailed information was given about the study and informed consent obtained for participation in the study. Each subject was randomly and consecutively selected. Gestational age was estimated from the date of last menstrual period (LMP), if available, and confirmed from ultrasound investigation (USI). Gestational age was expressed in completed weeks. Venous blood samples for AFP and free β-HCG assays were taken at recruitment, 12-13 weeks (first trimester), and at 16 weeks (second trimester) of gestation. Each subject was followed up from recruitment till delivery. Information concerning pregnancy outcomes was obtained from medical records, and included the date of birth, weight and length of the newborn baby, and Apgar score. Some features of newborns were taken after delivery or within first 7 days after delivery. Pregnant women who were aged less than 30 years or had multiple pregnancies were excluded from the study.

Ethical consideration

The study was approved by the Research and Ethical Review Committee of the University of Ghana Medical School (UGMS), Korle-Bu.

Serum AFP and free β-HCG assay

Serum AFP and free β-HCG were assayed by quantitative sandwich ELISA technique using commercial test kits obtained from R & D System (Minneapolis, USA) and GenWay Biotech., Inc. (San Diego, USA) respectively. Absorbance was measured spectrophotometrically at 450 nm.

Statistical analyses

Microsoft Excel 2007 software was used for data storage and analyses. Concentration of AFP and free β-HCG was presented in ng/ml as mean with standard deviation, median and range (maximum and minimum), and as the gestation- specific multiples of the median (MoM). MoM values were calculated by dividing the observed marker concentration by the median value for the gestational week at which the sample was obtained. Statistical significance was determined at $P< 0.05$. The VasarStarts online software was used in all calculations (http://faculty.vassar.edu/lowry/VassarStats.html).

RESULTS

Maternal characteristics of the study population at recruitment are presented in Table 1. Complete first (12-13 gestational weeks) and second (16 gestational weeks) trimesters data were obtained on 69 subjects (85%) out of the 81, with 12 subjects (15%) lost to follow up. One subject was lost due to miscarriage. For delivery outcomes however, data on 66 subjects (82%) were obtained. The outcome of the remaining 15 pregnancies (18%) could not be ascertained, because the participants either changed their address or delivered at a different location and so could not be traced. The patients' age at the time of recruitment ranged from 30 to 44 years (mean ± SD: 33.9 ± 3.2 with median of 33 years).

Table 1: Maternal demographics and clinical variables at recruitment

Parameters (n=81)	Mean ± SD	Median	Range
Demographics			
Age, yrs	33.9 ± 3.2	33	30-44
Gravidity	3.0 ± 1.4	3	1-7
Parity	1.1 ± 1.1	1	0-5
Weight, kg	71.7 ± 15.8	70	44-150
Height, m	1.7 ± 0.1	1.7	1.5-1.8
BMI, kg/m^2	26.4 ± 6.3	25.7	18.9-30.7
Clinical variables			
Hb, g/dl	11.4 ± 1.3	11.5	13.4-12.2
SBP, mmHg	111.7 ± 9.9	110	90-160
DBP, mmHg	68.5 ± 8.8	70	50-100

Levels of AFP and free β-HCG in the first and second trimesters are presented in Table 2. There was a significant difference in the serum levels of AFP and free β-HCG between the first and second trimesters [(p<0.0001; 95% CI-14.9–(-9.3)] and p<0.0001; 95% CI 13.6–21.2 respectively)]. The mean values at recruitment and the second trimesters for AFP were 18.8 ± 7.5ng/ml (mean ± SD), and 30.9 ± 9.8ng/ml (mean ± SD). Similarly, free β-HCG at recruitment and the second trimesters were 47.2 ± 12.2ng/ml (mean ± SD) and 29.8 ± 11.3 ng/ml (mean ± SD) respectively.

Table 2: Concentrations of AFP and free β-HCG at recruitment (12-13 gestational weeks) and the second trimester (16 gestational weeks)

Parameters	AFP, ng/ml		Free β-HCG, ng/ml	
	Recruitment (n=81)	Second trimester (n=69)	Recruitment (n=81)	Second trimester (n=69)
Mean ± SD	18.8 ± 7.5	30.9 ± 9.8	47.2 ± 12.2	29.8 ± 11.3
Median	17.6	29.3	46.0	29.5
Min	5.0	13.3	16.5	8.5
Max	43.0	52.6	72.5	54.0
P- value	<0.0001		<0.0001	
95% CI	-14.9-(-9.3)		13.6-21.2	

Maternal weight and age did not correlate with AFP levels at 16 gestational weeks (p=0.29; r=-0.13; r^2= 0.0169 and p=0.39; r=-0.11; r^2=0.011 respectively). There was a very weak association between maternal weight and free β-HCG levels at 16 gestational weeks, but not for age (p=0.08; r=-0.23; r^2= 0.0552 and p=0.64; r=-0.06; r^2=0.0032 respectively). The median values for AFP increased in the second trimester whilst that for free β-HCG decreased. The second trimester median MoM values for AFP and free β-HCG were each 1.00. We did not observe any MoM value more than 2.0 for both analytes. There were 10 cases of MoM values of AFP that were less than 0.75, and all the babies were normal. Free β-HCG levels in the second trimester in normal and adverse pregnancy outcomes were statistically different (p=0.059) although moderately due to the few numbers. This was not the case for AFP (Table 3).

Table 3: Concentrations of AFP and free β-HCG in normal and adverse pregnancy outcomes in the second trimester

Biochemical marker	Normal pregnancies (n=61)	Adverse pregnancies (n=4)	P - value
AFP, ng/ml	30.2 ± 9.0	31.7 ± 3.1	0.477
Free β-HCG, ng/ml	29.3 ± 10.6	38.4 ± 6.3	0.059

There were sixty one (61) live babies comprising twenty seven (27) males and thirty four (34) females. The mean (SD) weight, full length and head circumference for the entire group (male and female) were 3.32 (0.61) kg, 49.46 (2.79) cm and 33.98 (1.89) cm respectively. The corresponding median values were 3.3 kg, 50.0 cm and 34.0 cm. There was no significant difference in weight, length, head circumference and chest circumference between males and females (p=0.43, p=0.96, p=0.25 and p=0.77 respectively). The number of neonates transferred to neonatal intensive care unit (NICU) was 5: one was a case of large size baby, one case of neonatal asphyxia, one case of hypoglycemia (diabetic mother), one case of difficulty in breathing, and one case of noisy breathing. Neonates were discharged from NICU after 5-10 days. After delivery and during the first 7 days, the newborns were observed for the presence or absence of 13 features which are more common in DS. These features are: Moro reflex, hypotonia, flat facial profile, upslanted palpebral fissures, smallish ears, ear positioned lower on the heard, excess, excess skin on the back of neck, single transverse palmar crease, hyperextensible joints, dysplasia of pelvis (in male), slightly protruding tongue, fifth finger clinodactyly and slightly enlarged gap between the big toe and second toe. There was no newborn having at least 6 of the features. Some newborns had one of the indicated features present, such as slightly flat facial profile, clinodactyly, upslanted palpebral fissures. This can be also due to some other genetic factors. The two newborns that had poor Moro reflex and mild hypotonia did not survive due to neonatal asphyxia and SGA respectively.

DISCUSSION

The present study is the first report of DS screening carried out in Ghana. The study shows that only 7.5% of the study participants were aware of the maternal age related effect on DS occurrence. This segment of participants comprised mainly health workers, or mothers with tertiary level education. A previous study carried out in Nigeria also revealed that maternal knowledge about DS was very low [1]. Clearly, the need for maternal education on DS during antenatal care is necessary in Ghana. This will be a first step in the establishment of a DS registry. Currently no program exists for this purpose. Information gathered

from mothers at antenatal visits will serve as part of a passive data collection in addition to review of medical record on DS prevalence. It is cost-effective and without any safety issues. In the present study no DS case was seen. The study showed that in general the mean/ median levels of the double markers (AFP and free β-HCG) of Ghanaian subjects in this study were different from those of other populations. The median concentration of AFP was approximately 19% lower, while that of free β-HCG was approximately 38% higher in our study, than was found in Asian and Caucasian populations [24, 25, 26]. Other reports indicate that the concentrations of these analytes are higher in Africans than in Caucasians and Asians [24, 26, 27]. If our observation is confirmed in a larger cohort, it will mean that whilst the trends in the serum levels of the markers are similar among different populations, cut-off points for detection may differ and have to be established for each population. The double marker test is based on changes in serum concentrations of the two markers between the first and the second trimesters, and has a detection rate of 60-71% [28, 29]. For the purpose of this study, the first trimester sample was taken at 12-13 gestational weeks (first trimester) and the second trimester at 16 gestational weeks which consisted of 16^{0}, 16^{+1} and 16^{+2} gestational weeks. We observed that while AFP levels increase in the second trimester levels of free β-HCG decreased. This is consistent with previous studies in other populations [27, 30]. Most of the cases with adverse pregnancy outcomes had higher free β-HCG levels than normal pregnancies. Notwithstanding the limited sample size, the mean achieved a moderate level of statistical significance. High free β-HCG levels have also been stated in some reports in the absence of chromosomal abnormalities, placental abnormalities and multiple pregnancies [30, 31, 32, 33]. All the studies adopted "2-2.5" MoM cut-off points to define individuals with high unexplained level of free β-HCG, which was also considered as a positive result in the screening program [30]. Other reports have alluded to increases in the free β-HCG levels in preeclampsia [12]. A comparison of the median MoM in Ghanaian pregnant women with unaffected fetuses in this study showed that the AFP and free β-HCG medians MoM compared very well with those from other countries. The findings clearly present a case for a follow up with a larger cohort. As no DS outcomes were seen, a comparison of the median MoM values of the analytes of unaffected pregnancies with those from literature was made. In the absence of DS, the median MoM is 1.0 for AFP but decreases to 0.75 when DS is present [34]. In our study the median MoM was 1.0. Similarly, the median MoM reported for free β-HCG in the presence of DS is between 2.0 and 2.79 [34]. In our study it was 1.0. Compared to other procedures like amniocentesis and chorionic villus sampling which pose 1-2% risk of miscarriage [16], the use of serum biomarkers would be a safer and less expensive option [16, 35].

CONCLUSION

In this preliminary study, we have used the double marker test (AFP and free β-HCG) to assess DS frequency in a subset of the Ghanaian population. While no DS was seen for comparison, the difference in the observed values of normal and adverse pregnancies for free β-HCG in particular may suggest that these markers could be useful. It was also observed that awareness of DS in the maternal population is low. The study provides the basis for further studies to be carried out for collection of additional data on DS prevalence in Ghana.

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