First trimester screening using ultrasound and serum markers in Panamanians: Factors associated with adverse pregnancy outcomes

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Background: There is no published data on the association between serum biochemical and ultrasonographic markers and adverse pregnancy outcomes. Therefore, the aim of this study was to determine the factors associated with perinatal outcomes in singleton pregnancies using ultrasound and maternal serum markers during the first trimester in Panamanians. **Materials and Methods:** This was a prospective observational study of 468 first trimester singleton pregnancies conducted over a 7-year period. All women attending a prenatal screening clinic during the study period were informed of the study and the option to participate. Two maternal serum markers, free β-human chorionic gonadotropin (β-hCG) and pregnancy associated plasma protein-A (PAPP-A), and four fetal ultrasound markers, nuchal translucency thickness, nasal bone, flow across the tricuspid valve, and flow in the ductus venosus (DV), were measured by certified maternal fetal medicine specialists. Adverse outcomes included miscarriage, major structural defects, genetic disorders, and major fetal cardiac defects. **Results:** A total of 454 (97%) pregnancies were unaffected. Median maternal age was 31.5 years (range: 18-50). Maternal age was significantly greater in cases of adverse outcome ($P = 0.007$). The number of adverse outcomes associated with an absent or hypoplastic nasal bone, tricuspid valve regurgitation, and abnormal flow in the DV were significantly greater relative to unaffected pregnancies (Ps < 0.001). No differences were found in fetal crown-rump length or maternal serum levels of β-hCG or PAPP-A. **Conclusion:** Abnormal ultrasound markers are associated with adverse outcomes. Women with normal ultrasound and serum markers should be reassured of low risk of adverse pregnancy outcomes.

Key words: Fetal ultrasonography, nuchal translucency, panama, pregnancy outcome, prenatal screening

INTRODUCTION

The aim of the first trimester screening is to identify fetuses with major aneuploidies using a combination of maternal age, fetal nuchal translucency (NT) thickness and maternal free β-human chorionic gonadotrophin (free β-hCG) and pregnancy associated plasma protein-A (PAPP-A).[1-3] First trimester findings may give insight into other adverse pregnancy outcomes in addition to aneuploidies, such as structural abnormalities, fetal loss, congenital heart defects, and genetic syndromes in euploid fetuses.[1,4]

A large variety of structural anomalies such as skeletal dysplasias, diaphragmatic hernia, cleft lip and palate and renal anomalies have been described in the setting of an enlarged NT.[5] NT is the sonographic appearance of a collection of fluid under the skin behind the fetal neck during the first trimester of pregnancy [Figure 1].[1] An increased NT is not only a marker for chromosomal

anomalies, but also a nonspecific indicator of abnormal development, common to several pathologic pathways, including an increased risk of miscarriage or fetal death, from 1.6% in those with NT between the 95th and 99th percentiles to approximately 20% for values above the 99th percentile,[5] and a 15-fold increased likelihood of lethal or serious malformation.[6] In the study with the largest sample size to date[7] fetuses with even a minimal increase in NT thickness (greater than the 95th percentile) had associated adverse perinatal outcomes.

Congenital heart disease is the most common of all major birth defects and is responsible for 20% of stillbirths and 30% of neonatal deaths associated with congenital defects.[8,9] Several studies have shown that an enlarged NT is associated with an increased risk of congenital heart disease. Accordingly, NT measurement significantly improves the detection of major cardiac malformations, including duct dependent congenital heart disease.[8-11] The combined data from eight studies on euploid fetuses

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Figure 1: Enlarged nuchal translucency in a fetus with an atrioventricular septal defect. The dotted vertical line indicates the extent of collection of fluid under the skin behind the fetal neck

with increased NT demonstrated abnormal ductus venosus (DV) blood flow in 87% of fetuses with cardiac defects, compared with 19% of fetuses without congenital heart defects.[11] Similarly, in a meta-analysis examining the diagnostic performance of the first trimester screening of DV for congenital heart disease, the positive likelihood ratio was 4.97 (95% confidence interval [CI] 2.3-10.8) in chromosomally normal fetuses with increased NT.[12]

Biochemical markers, particularly PAPP-A are of potential value in identifying pregnancies that may result in an adverse outcome. One such adverse outcome is fetal loss, either due to miscarriage (fetal death prior to 24 weeks gestation) or stillbirth (death at or after 24 weeks gestation) prior to labor.[13-16] Increased risk of fetal loss is associated with advanced maternal age, smoking, obesity, parity, and poor obstetric history.[14-19] Some studies show weak to moderate predictive relations between first trimester serum markers such as low PAPP-A and low free β-hCG and various adverse pregnancy outcomes,[20,21] while others find a strong association between these markers and adverse outcome.[22,23]

The Central American region has no published data on the association between serum biochemical and ultrasonographic markers and adverse pregnancy outcomes. Consequently, the purpose of this study was to examine the factors associated to perinatal outcomes in singleton pregnancies that were screened during the first trimester using ultrasound and maternal serum markers.

MATERIALS AND METHODS

This prospective observational study took place between November 2005 and March 2013 in a clinic that provides prenatal screening to both low- and high-risk women. All women attending the clinic during the study period were informed of the study and offered the option to participate. A total of 748 pregnant women were screened during the first trimester. Women received prenatal counseling and informed consent was obtained in each case. Pregnancies with one viable fetus at the time of the scan and with crown-rump length (CRL) between 45 and 84 mm were included in the study. Gestational age was derived from CRL measurement, irrespective of the last menstrual period. All procedures were conducted in accordance with the Helsinki Declaration of the World Medical Association.

Serum free β-human chorionic gonadotropin and pregnancy associated plasma protein-A measurements

A clotted blood sample was obtained from each patient and free β-hCG and PAPP-A were measured using solid-phase, enzyme-labeled chemiluminescent immunometric assay (Siemens Healthcare Diagnostics, Inc., Llanberis, UK). Regressed medians were used to generate multiples of the median (MoM) for each case. MoM values of markers were corrected for maternal weight, smoking status parity, ethnicity, and modes of conception.

Ultrasonographic measurements

Transabdominal and transvaginal ultrasound examination took place at 11-13 + 6 weeks and was performed by Fetal Medicine Foundation (FMF) certified operators. The ultrasound scan included a full structural survey, and NT was measured according to established guidelines.[24-26] Assessment of the nasal bone status, tricuspid flow and DV flow were performed by certified maternal fetal medicine specialists. Ultrasound examinations were performed with high-resolution equipment (Voluson 730 Expert 2008, General Electric, Austria or Siemens G50 Ultrasound, Siemens Medical Solutions USA, Inc.), at 3.5-7.0 MHz. Doppler ultrasound assessments were performed in accordance with the as low as reasonable achievable principle, and in all cases thermal and mechanical indices were displayed on the monitor and were kept below 1.0 and 0.5, respectively.[27-29] Risks for chromosomal abnormalities were calculated using the software provided by the FMF (Astraia, Munich, Germany) which gives individual risks for trisomy 21.18 and 13 according to ultrasound findings and maternal age. A calculated risk ≥1:100 was defined as high-risk. Video clips and images of the studies were digitized for further analysis. Outcome information was obtained from the cytogenetics laboratory and by telephone calls to the mothers or from the maternity units where delivery took place. Adverse perinatal outcome was reported in cases of spontaneous fetal losses, major fetal structural defects, genetic syndromes, or major fetal heart defects.

Statistical analysis

Statistical analysis was performed with IBM SPSS for Windows version 20.0 (IBM Corp., Armonk, NY). Results

are presented as mean ± standard deviation, median (range) or number and percentage of observations. Statistically significant differences between unaffected and adverse outcome groups were examined using analysis of variance for continuous variables and Chi-square or Fisher's exact test for categorical variables. $P < 0.05$ were considered significant.

RESULTS

Over a 7-year period, screening was carried out in 748 singleton pregnancies. Of these, 280 (37.4%) cases were excluded, including 78 women with ongoing pregnancies, 112 cases of missing data regarding pregnancy outcome and 90 cases where two or more biomarkers were not assessed. The maternal and pregnancy characteristics of the 468 cases included in statistical analysis are summarized in Table 1. Of these, 454 (97.0%) resulted in an unaffected pregnancy. Adverse outcomes included four spontaneous fetal losses, five major fetal cardiac defects, three limb transverse defects and two renal fetal defects. Median maternal age was 31.0 years (range: 18-50 years), and 140 (29.9%) of women were aged 35 years or older at the time of assessment. The median gestational age at screening was 89 days (range: 78-98 days).

Comparisons between unaffected and adverse outcome groups are summarized in Table 2. There was a statistically significant difference in maternal age between unaffected and adverse pregnancy outcomes; average age was greater ($P < 0.01$) and more women were aged 35 years or older ($P < 0.01$) in cases of adverse outcome. No differences were found between groups in fetal CRL or maternal serum levels of β-hCG or PAPP-A. Fetal NT was significantly higher ($P < 0.01$), and more cases were above the 95th percentile ($P < 0.01$) in adverse pregnancy outcomes. Lastly, the number of adverse pregnancy outcomes associated with fetal nasal bone absence ($P < 0.001$) and

atypical DV ($P < 0.001$) and tricuspid ($P < 0.001$) flow velocity waveforms was significantly greater relative to unaffected pregnancies.

DISCUSSION

The results of this study demonstrate that an enlarged NT (above the 95th percentile) and advanced maternal age (>35 years) are associated with adverse perinatal outcomes in a mixed population (low- and high-risk), which is consistent with the findings reported in other studies.[4-7] Abnormal nasal bone, tricuspid valve regurgitation and abnormal flow in the DV were significantly greater in cases of adverse outcome, a finding that is consistent with

Table 1: Demographic and biochemical/ultrasound sample characteristics

Characteristic	Value	Total subjects*
Maternal age (years)	31.6±5.1	468
≥35 years (%)	140 (29.9)	
Maternal weight (kg)	62.9±11.3	439
Nonsmoker (%)	461 (98.5)	468
Nulliparous (%)	197 (51.8)	380
Spontaneous conception (%)	467 (99.8)	468
Singleton pregnancy (%)	468 (100)	468
Gestational age at screening (days)	89.1±4.5	468
Crown rump length (mm)	65.0±8.8	468
PAPP-A (MoM)	1.02 (0.16–9.22)	468
Free β-hCG (MoM)	0.79 (0.18–6.15)	468
Nuchal translucency (mm)	1.50 (0.90–9.80)	468
≥P_{95} (%)	18 (3.8)	
Abnormal nasal bone (%)	6 (1.3)	468
Abnormal ductus venosus (%)	6 (1.3)	465
Abnormal tricuspid flow (%)	4 (0.9)	455
Adverse outcomes (%)	14 (3.0)	468

*Data were not available for the entire sample on four measures; Values represent the mean ± SD, median (range) or *n* (%); Free β-hCG = Free beta human chorionic gonadotrophin; MoM = Multiples of the median; NS = Not statistically significant; PAPP-A = Pregnancy-associated plasma protein-A; SD = Standard deviation

Table 2: Comparison of factors between unaffected and adverse outcome groups

Characteristic	Unaffected (*n* = 454)	Adverse outcome (*n* = 14)	*F*	χ^2	*P*
Maternal age (years)	31.5±5.0	35.2±6.6	7.35		0.007
≥35 years (%)	131 (28.9)	9 (64.3)		8.13	0.004
Maternal weight (kg)	63.0±11.3	62.1±11.7	0.07		NS
Crown-rump length (mm)	65.1±8.7	60.5±12.1	3.82		NS
PAPP-A (MoM)	1.02 (0.16–9.22)	1.34 (0.32–2.45)	0.05[a,b]		NS
Free β-hCG (MoM)	0.79 (0.18–6.15)	1.07 (0.20–3.80)	0.70[a,b]		NS
Nuchal translucency (mm)	1.5 (0.9–6.6)	4.7 (1.2–9.8)	17.03[a,b]		0.001
≥P_{95} (%)	10 (2.2)	8 (57.1)			<0.001[c]
Abnormal nasal bone (%)	1 (0.2)	5 (35.7)			<0.001[c]
Abnormal ductus venosus (%)	3 (0.7)	3 (23.1)			<0.001[c]
Abnormal tricuspid flow (%)	1 (0.2)	3 (25.0)			<0.001[c]

Values represent the mean ± SD, median (range) or *n* (%); [a]Analysis conducted using $\log_{10}$ transformation of values; [b]Brown-Forsythe *F* ratio reported; [c]Fisher's exact test; Free β-hCG = Free beta human chorionic gonadotrophin; MoM = Multiples of the median; NS = Not statistically significant; PAPP-A = Pregnancy-associated plasma protein-A; SD = Standard deviation

similar reports.[9-12] A wide range of conditions have been reported in fetuses with abnormal ultrasound markers due to different mechanisms, including systolic and diastolic cardiac dysfunction, fetal infections, superior mediastinal compression found in diaphragmatic hernia, narrow chest in skeletal dysplasia, failure of lymphatic drainage due to abnormal, or delayed development of the lymphatic system, among others.[5,8,30] The uncertainty surrounding pregnancy outcome is a significant cause of anxiety and should not be underestimated. Consequently, counseling parents in the setting of enlarged NT and abnormal ultrasound and maternal markers should include information of an increased risk of miscarriage, fetal loss and diagnosis of major structural and cardiac defects.

In this study, PAPP-A values and β-hCG values and maternal weight were not associated with adverse perinatal outcomes in our population. This result is inconsistent with previous studies that demonstrated an association between these three variables and spontaneous abortion[14-17] and fetal death (stillbirth).[22,23] Our study likely did not have sufficient power to detect these differences. PAPP-A and hCG are peptides synthesized by the syncytiotrophoblast. PAPP-A is a protease for insulin-like growth factor (IGF) binding proteins 4 and 5. Low levels of this marker are expected to result in low free IGF, which is an important determinant of fetal growth and trophoblast invasion. In contrast, high levels of PAPP-A lead to IGF-binding protein being broken down more rapidly than usual and increases in circulating IGF, without any particular harmful effect.[22,23,31] The main function of hCG is the maintenance of the corpus luteum and secretion of progesterone in early pregnancy.

The prevalence of an enlarged NT in the unaffected euploid fetus group was 2.2%, which was less than the prevalence of 4.4% reported in another multicenter study.[24] This difference probably reflects the different compositions of the study populations that is a mixed population versus a tertiary reference hospital population. Moreover, in this study 97% of screened pregnancies resulted in an unaffected outcome, greater than the reported 63-92% in similar studies.[4-6] In our study population, the rate of adverse outcomes was 3.0%, and nearly 60% were associated with an enlarged NT, a finding that is consistent with previous reports.[4-6] An isolated bilateral clubfoot, an isolated bilateral symmetrical ventriculomegaly and two cases of fetal minor heart defects (small interventricular septal defects) were detected at the second trimester scan. By detecting both minor and major defects, there are certain limitations to the first trimester screening, which makes an anatomic evaluation necessary in the second trimester as seen in other studies.[4,5,32]

It has been reported that a low first trimester measurement of CRL is linked with adverse perinatal outcome in singleton pregnancies.[33] Measurement of CRL discordance has been shown to correlate with perinatal complications in twins, as well.[34] In contrast, this study did not find any statistical association between low CRL and adverse perinatal outcome as has been reported in a previous study.[35] Discrepancies are probably due to an overestimation of the age of the fetus at the time of the scan. This can occur either because of incorrect menstrual dates or because of delayed ovulation in the conception cycle.

One of the strengths of this study is that we followed a screening strategy in which six markers were integrated into one patient visit. The majority of studies investigating associations with adverse perinatal outcomes have only evaluated the NT value alone[4-7] or the PAPP-A alone[15] or in combination with β-hCG.[13,14,16,20-23] Moreover, long-term patient follow-up decreases the error in classifying perinatal outcomes. In a few similar studies, the follow-up period was not reported[30] or was short term.[7] A limitation of our study is the small sample size, which may have limited our ability to detect adverse perinatal outcome. The number of live births in Panama during the study period was 472,851.[36] It would take a multicenter study in all hospitals in Panama and in the Central American Region to increase external validity. This is a difficult task because of the small number of certified sonographers in the region, and the dearth of prenatal diagnosis screening programs across the region. Low sample size also diminished the capacity to stratify the cases of increased NT into subgroups and conduct a more detailed analysis of these cases, as has been reported previously.[7,9]

This study represents the first report of first trimester ultrasound and maternal serum markers and their relationship to pregnancy outcomes in Panama. Ongoing studies in our group are focused on comparing first and second trimester ultrasound markers, with calculated likelihood ratios for each marker and risk estimates during the second trimester. Further studies will examine the predictive utility of integrated serum and ultrasound markers in our population to predict preeclampsia, preterm birth, and stillbirth in a group of high-risk patients.

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AUTHORS' CONTRIBUTION

Each author's contribution was as follows: TTH conceived and designed the study, and wrote the initial draft of the

manuscript. SS contributed to the study design and data collection. AS assisted in data collection and initial analysis. GBB conducted the statistical analysis, interpreted the data and wrote the final draft of the manuscript. All authors have read and approved the content of the manuscript.

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