Utility of Maternal Serum Analyte Screening in the Era of Cell-free Fetal DNA

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Abstract

Background: To determine whether in the era of cell-free fetal DNA (cfDNA), first and second trimester maternal serum analytes will be obsolete or whether they have a role in predicting adverse pregnancy outcomes.

Methods: Retrospective cohort study using maternal serum analyte results from first trimester and sequential screening tests from 1/2002 to 12/2012. Abnormal serum analytes included low pregnancy-associated plasma protein-A (PAPP-A), defined as ≤ 0.4 multiples of the median (MoM), and elevated alpha fetal protein (AFP), defined as ≥2.5 MoM. Patients were assigned into 4 groups: both low PAPP-A and elevated AFP (BOTH), an isolated elevated AFP (AFP), an isolated low PAPP-A (PAPPA), and those with normal results (NML).

Results: 328 patients with abnormal analytes were matched 2:1 to patients with normal analytes, leaving 984 included. Compared to the other groups, the BOTH group (N=5) delivered earlier, had lower birth weights, and had a higher incidence of preterm birth and growth restriction. The AFP group (N=70) had the highest percentage of miscarriage <20 weeks. The PAPP-A group (N=254) conferred an increased risk of gestational hypertension/preeclampsia.

Conclusions: The importance of maternal serum analytes and their link to poor pregnancy outcomes should not be forgotten in the era of cfDNA.

Introduction

Maternal serum analyte tests are established screening methods for aneuploidy in all pregnant women. The tests detect various biochemical analytes in the first and second trimesters. First trimester analytes include pregnancy-associated plasma protein-A (PAPP-A) and free beta-human chorionic gonadotropin (βhCG), while second trimester analytes include unconjugated estriol (uE3), human chorionic gonadotropin (hCG), alpha fetal protein (AFP), and inhibin A. In addition to its use in aneuploidy detection, AFP alone can be used to detect open neural tube defects (ONTD) in the second trimester.

In the absence of aneuploidy or ONTD, abnormal serum analytes have been associated with adverse pregnancy outcomes [1]. For example, numerous studies [2-6], have shown an association with low PAPP-A to intrauterine growth restriction (IUGR), preterm birth, low birth weight, stillbirth, neonatal death, miscarriage, pre eclampsia, and gestational hypertension. Similarly, high levels of AFP are associated with the above complications, as well as abnormal placentaion [7].

Noninvasive prenatal screening (NIPS) utilizes circulating free fetal DNA, or cell-free fetal DNA (cfDNA) within maternal serum to detect aneuploidy. The use of this screening test has grown due to its advantage of having a higher sensitivity and specificity compared to maternal serum analytes [8,9]. However, a disadvantage of NIPS thus far, is its inability to predict adverse pregnancy outcomes.

Due to the increased use of NIPS, there will likely be a decline in maternal analyte screening. We sought to determine whether in the era of cell-free fetal DNA, first and second trimester maternal serum analytes will be obsolete or whether they still have a role in predicting adverse pregnancy outcomes.

Methods

This is a retrospective cohort study conducted via a review of an electronic database of maternal serum analyte results from first trimester and sequential screening tests from January 2002 to December 2012 at a single hospital. The study was approved by the Mount Sinai Roosevelt Institutional Review Board (IRB 13-026X); patient consent was waived as this was a retrospective study. Eligible patients included those who had abnormal maternal serum analytes sent through our lab during the aforementioned dates. The diagnosis of abnormal maternal serum analytes refers to the patient’s PAPP-A and AFP results only. A low PAPP-A value was considered abnormal and was defined as ≤ 0.4 multiples of the median (MoM), while an AFP value was abnormal if it was elevated, defined as ≥ 2.5 MoM. Women with pre-gestational diabetes, multiple gestations, and chromosomal and/or structural abnormalities were excluded. Patients with normal analytes were matched by age and dates of analytes drawn to women with abnormal analytes in a 2:1 fashion. The patients were placed into four groups based on their results. 1) included those patients with both a low PAPP-A and an elevated AFP (BOTH); 2) included those with an elevated AFP (AFP); 3) those with a low PAPP-A (PAPPA); and 4) those with normal analytes (NML). All groups were followed until delivery. Charts were reviewed and data was collected including maternal demographics, delivery information, and neonatal outcomes.

The primary outcome of our study was to determine the distribution of abnormal analytes in our population. Secondary pregnancy outcomes included gestational age (GA) at delivery, birth weight, birth weight percentile, and the incidence of preterm birth (PTB), preterm premature rupture of membranes (PPROM), IUGR, low birth weight (LBW), spontaneous abortion (SAB or miscarriage), intrauterine fetal demise (IUFD), gestational diabetes (GDM), and gestational hypertension (GHTN)/preeclampsia. We defined PTB as delivery <32 weeks GA, SAB if it occurred <20 weeks, and IUFD if it occurred ≥ 20 weeks GA. IUGR was defined as an estimated fetal weight at last sonogram of <10% and/or the abdominal circumference <10%, while low birth weight was <2500g at birth. Statistical analysis was performed using Kruskal-Wallis, Fisher’s exact test, and Student’s t-test to compare pregnancy outcomes among the four groups as appropriate.

Results

From January 2002 to December 2012, 11,958 women had screening tests. There were 370 women with abnormal analytes. Forty-two women were excluded (28 patients with multiples, 2 diabetics, 12 patients with chromosomal/structural abnormalities), leaving 328 women who were matched to 656 patients with normal analytes. A total of 984 patients were included in the analysis. Of women with abnormal analytes, the majority of patients, 253 (77%), were in the PAPP-A group, and the smallest group was the BOTH group, with 5 (2%) patients.

Maternal demographics were compared: there was no significant difference in the median maternal age among the groups but there were significant differences in the MoM of PAPP-A and AFP (Table 1) as anticipated. Additionally, there was a significant difference found...
Utility of Maternal Serum Analyte Screening in the Era of Cell-free Fetal DNA


Data represented as median (range) compared using Kruskal-Wallis test.

### Table 2: Pregnancy Outcomes.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>BOTH (N=5)</th>
<th>AFP (N=70)</th>
<th>PAPP-A (N=254)</th>
<th>NML (N=656)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA delivery (weeks)</td>
<td>32.16 (5.52)*</td>
<td>36.27 (6.86)*</td>
<td>38.73 (2.25)</td>
<td>39.14 (3.37)</td>
<td>0.0471</td>
</tr>
<tr>
<td>PTB</td>
<td>20 (19)*</td>
<td>14 (11)*</td>
<td>2 (4)</td>
<td>2 (13)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PPROM</td>
<td>20 (1)*</td>
<td>7 (5)*</td>
<td>6 (14)*</td>
<td>3 (17)</td>
<td>0.0118</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1583.60 (1077.56)*</td>
<td>2937.66 (812.95)*</td>
<td>3126.49 (593.40)*</td>
<td>3269.43 (578.81)*</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Birth weight %</td>
<td>17.00 (10.32)*</td>
<td>17.00 (10.32)*</td>
<td>37.62 (25.97)*</td>
<td>43.16 (26.78)</td>
<td>0.0005</td>
</tr>
<tr>
<td>IUGR</td>
<td>40 (2)*</td>
<td>17 (12)*</td>
<td>5 (13)*</td>
<td>2 (11)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Low birth weight</td>
<td>60 (3)*</td>
<td>20 (13)*</td>
<td>12 (31)*</td>
<td>5 (35)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SAB</td>
<td>0 (0)</td>
<td>9 (6)*</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IUFD</td>
<td>0 (0)</td>
<td>1 (1)</td>
<td>1 (2)</td>
<td>1 (6)</td>
<td>0.735</td>
</tr>
<tr>
<td>GDM</td>
<td>0 (0)</td>
<td>10 (7)</td>
<td>6 (16)</td>
<td>7 (43)</td>
<td>0.6622</td>
</tr>
<tr>
<td>GHTN/Preeclampsia</td>
<td>20 (1)</td>
<td>7 (5)</td>
<td>10 (26)*</td>
<td>5 (32)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Continuous data shown as mean (SD) compared using Student’s t-test and categorical data shown as percent (N) compared using Fisher’s exact test.

*Indicates statistically significant comparison to the NML group.

Discussion

Two percent of the patients in this study had a combination of both a low PAPP-A and a high AFP on maternal serum analyte screening; with an overall incidence of 0.04% (5/11,598 study population). These women were more likely to deliver at an earlier gestational age, to have a preterm birth, to have GHTN/preeclampsia and to have a low birth weight infant. Twenty-one percent of the patients in the study (0.6% overall of the study population, 70/11,598) had an isolated high AFP on screening and were more likely: to have a spontaneous miscarriage less than 20 weeks gestation, especially compared to controls and those with only low PAPP-A, and to have a comparably higher incidence of GHTN/preeclampsia when compared to the BOTH group. Unlike previous studies, our population with an isolated low PAPP-A did not appear to have an increased risk of poor pregnancy outcomes (no significant difference in gestational age at delivery or rate of PTB compared to controls). In fact, the only notable pregnancy complications were lower birth weights (and higher incidence of IUGR) and gestational hypertension/preeclampsia at a higher rate than controls. All of these women would not have been identified using cell-free fetal DNA as a screening test for aneuploidy.

In the past, the only way to detect aneuploidy was by invasive testing on women of advanced maternal age. However, with the advancements in maternal serum tests and ultrasound, routine noninvasive screening for aneuploidy and ONTD is now offered to all pregnant women [10]. The integrated and sequential screening tests which combine first and second trimester analytes with the nuchal translucency (NT)
have excellent detection rate for aneuploidy, especially for Down syndrome (94-96%) [11]. There are 2 blood draws in the integrated and sequential screens: the first part is done at 11-13 weeks gestation with the NT and the second part is done at 15-21 weeks (depending on the lab). Maternal serum screening is advantageous in that it is not invasive, but given that these tests are for screening only, confirmation of the diagnosis is needed with invasive testing, such as amniocentesis or chorionic villus sampling. A disadvantage of integrated screening is that the results are obtained at later gestational ages; and affirmation of the screening results is further delayed by the need to confirm the results with invasive testing.

However, even if the diagnosis of aneuploidy or ONTD is excluded with invasive testing, several previously mentioned studies have established that women with abnormal maternal serum analytes are at increased risk for adverse pregnancy outcomes. The pathogenesis of these adverse outcomes is theoretically attributable to 2 mechanisms. Firstly, PAPP-A is a protease for insulin-like growth factor (IGF) binding protein-4. IGF is produced by the syncytiotrophoblasts and aids in regulation of fetal growth. Low PAPP-A leads to low free IGF and therefore disrupts adequate fetal development in the first trimester, leading to poor pregnancy outcomes [12]. Secondly, AFP is a fetal oncoytic protein excreted in fetal urine and transported to maternal circulation through transporters in the placental interface. Disruption in these transporters from abnormal placental development leads to elevated maternal serum AFP levels, and augments poor placentation and growth.

Noninvasive prenatal screening is a new medical advancement that can screen for aneuploidy, sex chromosomal abnormalities, and various pathologic microdeletions. The technology utilizes the normal presence of circulating cell-free fetal DNA within the maternal vasculature. A maternal blood sample is taken and cell-free fetal DNA is identified and screened for abnormalities. There are generally 2 approaches to the technique; massively parallel genomic sequencing that can calculate DNA fragments and chromosome selective sequencing [13-16]. The test has been validated in several studies on women at high risk for aneuploidy, and found to have a sensitivity and specificity for Trisomy 21 of 99%, and slightly less for Trisomies 18 and 13 [17]. In addition to its high detection rate and noninvasive nature, cfDNA can be performed as early as 10 weeks gestation. Several professional societies have endorsed NIPS for women at high risk of aneuploidy with the following indications: ≥ 35 years of age at delivery, ultrasonographic findings indicating an increased risk of aneuploidy, history of a prior pregnancy with a trisomy, positive test result for aneuploidy, and parental balanced Robertsonian translocation [18]. It is also important to remember that cfDNA is still a screening test and, like maternal serum analyte screening, needs confirmation with invasive diagnostic testing.

When comparing maternal serum analytes to cfDNA, it is easy to see the appeal in the latter as it can be done earlier in pregnancy with a higher detection rate for aneuploidy. Many at- risk patients and their providers are opting to only send cfDNA, without maternal serum analytes, as it may appear as screening for duplicate information. This thought process is counter-intuitive as maternal serum analytes have a dual purpose. In addition to screening for aneuploidy, as stated earlier, abnormal serum analytes in the absence of aneuploidy and ONTD, are associated with adverse pregnancy outcomes. If only cfDNA is sent, the status of the maternal serum analytes will be unknown. This could place the patient at high risk for these outcomes and without proper follow up in the pregnancy for adverse consequences such as preterm birth, growth restriction, and low birth weight. Although many of these adverse outcomes cannot be prevented with prophylactic measures, some of these outcomes can be screened for with more frequent visits and fetal surveillance; for example, intrauterine growth restriction with sonograms or gestational hypertension with prenatal visits.

There are limitations to our study. It is retrospective and by nature of the design, there is a risk of selection bias. Secondly, there is a small amount of patients in the BOTH group with both abnormal PAPP-A and AFP. It is difficult to extrapolate conclusions from this group to the general population. Thirdly, in our cohort, those patients with low PAPP-A did not appear to have as many increased risks of adverse outcomes which contradict previous studies. The strengths of our study include its large sample size overall and the use of a control group to determine the baseline risk of these adverse outcomes in women with normal serum analytes.

In conclusion, the importance of maternal serum analytes and their link to poor placental function and pregnancy outcomes, specifically preterm birth, PPROM, low birth weight, IUGR, miscarriage, and GHTN/preeclampsia should not be forgotten in the era of cfDNA. We advocate that maternal serum analytes, particularly PAPP-A and AFP, should be routinely sent in addition to cfDNA in order to detect women who are at risk for poor pregnancy outcomes. These women can be followed more closely with antenatal surveillance, particularly if both analytes are abnormal. Further studies are needed to determine the frequency of this surveillance, as well as interventions that can be used to decrease the risk of these outcomes.

**Ethical Approval**

The study was approved by the Mount Sinai Roosevelt Institutional Review Board (IRB 13-026X); patient consent was waived as this was a retrospective study and did not cause undue risk to the patients.

**Conflicts of Interest**

The authors report no declarations of interest.

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**Authorship**

The guaranteeing author is Kimberly Herrera; she guarantees the manuscript’s accuracy. All of the authors contributed to the design, methods, and writing of the manuscript.

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