REVIEW ARTICLE

Understanding Biochemical Markers for Antenatal Screening

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ABSTRACT

Screening tests to identify fetuses at risk for aneuploidies should be offered to all pregnant women.

It should be remembered that screening tests can not diagnose a birth defect; they can only indicate an increased risk. An abnormal screening test result simply mean that additional testing is recommended.

Maternal serum biochemical markers in first trimester are free beta-hCG and PAPP-A. In second trimester the markers are AFP, UE3, hCG (Triple test) and inhibin added to these three markers forming the quadruple test. Level of cell free fetal DNA and fetal cells in maternal circulation are some of the molecular techniques for prenatal diagnosis of aneuploidies.

Keywords: Aneuploidy, Screening, Prenatal diagnosis, Nuchal translucency, Triple test, Quadruple test.

INTRODUCTION

Screening tests identify individuals broadly as high-risk (proceed to diagnostic procedures) or low-risk. Screening tests to identify fetuses at risk for aneuploidies should be offered to all pregnant women. Maternal age of over 35 years is no longer accepted as a cut off to offer screening tests to pregnant women.

The aim of prenatal screening program is to further refine a women's risk of carrying a fetus with chromosomal disease beyond her age related risks. In a screening test, there is considerable degree of overlap between affected and non-affected pregnancies. Therefore, a positive result in a screening test only places the patient in a high-risk group and does not in any way imply that fetus is affected.

Pre-test and post-test counseling prior to biochemical screening tests, interpretation of the results, options available and the implications of the test are essential, and hence genetic counseling must be offered to the family prior to prenatal testing.

SCREENING FOR FETAL ANEUPLOIDES

Maternal serum screening by biochemical markers and ultrasound form the mainstay of non-invasive prenatal screening.

- Biochemical Markers
- Ultrasound Markers.

Biochemical Markers

- Human chorionic gonadotropin (hCG)
- Free β-subunit of hCG (Fβ-hCG)
- Alpha-fetoprotein (AFP)
- Unconjugated estriol (uE3)
- Pregnancy-associated plasma protein A (PAPP-A)
- Inhibin-A.

Ultrasound Markers

Nuchal translucency (NT) has emerged as the most sensitive ultrasound marker for detection of chromosomal anomalies in the first trimester. Nuchal translucency (NT) measurements are done between 10 and 14 weeks of gestation.

First Trimester Biochemical Screening

Although many markers have been studied in the first trimester, two robust markers suggested are β-hCG and PAPP-A. Both are measured between 9 and 136/7 weeks' gestation (CRL 24-84 mm). hCG has been measured as intact (i-hCG), α-hCG, total (t-hCG), (β-hCG) and free β-hCG (Fβ-hCG). Meta-analysis of several studies showed that free p-hCG was better marker as compared to (i-hCG). PAPP-A levels are decreased and hCG increased in pregnancies at risk for Down's syndrome.

With these two markers combined together with maternal age, the detection rates are 67% for a false-positive rate of 5%.

Combined first trimester biochemical and ultrasound screening offered a detection rate of 85% with a false-positive rate of 5%. PAPP-A is currently the single best serum marker with a 42% detection rate for a 5% false-positive rate.

Numerous factors affect the levels of maternal serum markers irrespective of the gestational age, which should be taken into account while calculating risks. These include maternal weight, tendency to decrease due to greater blood volume, a number of fetuses, smoking, ethnicity, gravidity and parity, previous screening results, assisted reproduction, pregnancy complications, and diabetes (lower levels). Most programs usually include correction for maternal weight and diabetic status.

Second Trimester Biochemical Screening

Traditionally at 16 to 22 weeks, the concentration of alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin (hCG) in the "triple screen," and additionally inhibin-A in the "quadruple screen" are measured and the composite risk for neural tube defect, trisomy 21 and trisomy 18 is estimated.

In twin pregnancies, the overall sensitivity of second trimester screening is lower and only approximately 50% affected fetuses may be identified.

Maternal Serum Markers

- First Trimester
  - PAPP-A
  - Free beta-hCG
- Second Trimester
  - AFP
  - uE3
  - hCG
  - Inhibin-A

AFP is synthesized early in gestation by the fetal yolk sac and later by the fetal gastrointestinal tract and the liver. It normally circulates in fetal serum and passes into fetal urine, and thus into amniotic fluid. AFP passes into the maternal circulation via placental circulation. AFP is found in steadily increasing quantities in maternal serum after 12 weeks. Open fetal body wall defects uncovered by integument permit additional AFP to leak into the amniotic fluid.

The markers profile of a pregnancy with Down syndrome in 1st trimester is:
- NT High
- Fβ-hCG High (2.0 × normal)
- PAPP-A Low (0.4 × normal).

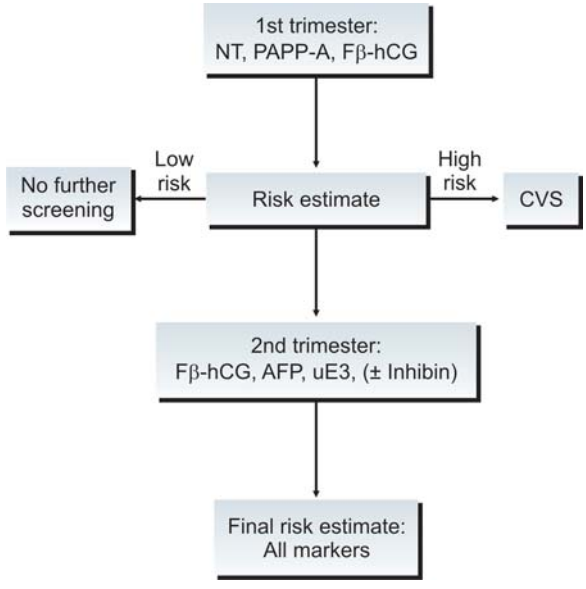
The markers profile of a pregnancy with Down syndrome in 2nd trimester is:
- AFP (0.75 × normal)
- uE3 (0.72 × normal)
- hCG (2.0 × normal)
- Inhibin-A (2.0 × normal).

Those favoring first trimester screening argue that:
1. Screening at this gestation allows for termination of an affected pregnancy at an earlier stage with less psychological burden.
2. The test is efficient although some identified affected pregnancies would miscarry spontaneously.
3. An early normal result gives reassurance to the women.
4. The efficiency of the first trimester result should mean that single markers of Down's syndrome in later pregnancy can be ignored.

Comparative performance of different screening modalities:

Test	*Sensitivity (%)*	*False positive rate (%)*
• Nuchal translucency	68	5
• First trimester combined screening	85-87	5
• Quadruple test	81	5
• Triple test	65-69	5
• Full integrated	95	5

First and second trimester evaluation of Risk Research Consortium Trial, NEJM 2005, 11. Approach for screening of aneuploidies:



Biochemical marker profile in second trimester:

Marker	Aneuploidies			
	T21	*T18*	*T13*	*Turner*
AFP	Low	Unchanged	Increase	Decrease
hCG	High	Very low	Normal	Very high
uE3	Low	Low	Normal	Decrease
Inhibin-A	High	Unchanged	Normal	Very high

FASTER trial (2003) concluded that combining both 1st and 2nd trimester screening the fully integrated test yielded as Down's syndrome detection rate of 90% at screen positive rate of 5.4%.

Serum, urine and ultrasound screening study (SURUSS) suggested that NT has a 60% detection rate for Down's syndrome (false-positive rate of 5%) at 10 weeks' gestation. NT has a poor performance as a screening test for Down's syndrome on its own or with maternal age alone.

New biochemical markers:
- Pregnancy-specific beta 1-glycoprotein (Sp1)—Time window: 7 to 12 weeks

- Invasive trophoblastic antigen (ITA, a highly glycosylated form of hCG) Time window: 15 to 20 weeks
- ADAM-12 (A disintegrin and metalloprotease) Proteolytic function against IGFBP-3 and IGFBP-5-regulates bioavailability of IGF-1-ADAM-12 is reduced in pregnancies with Down syndrome and this is more pronounced earlier in pregnancy (Laigaard et al 2006).

Weeks	8-9	10-11	12-13
ADAM-12 (MoMs)	0.12	0.50	0.93

New strategy in 1st trimester:

At 8 to 10 weeks: PAPP-A ADAM-12
At 12 to 13 weeks: NT + Fb-hCG

Molecular techniques in prenatal diagnosis: This technique opens new horizon for noninvasive prenatal testing. Various types of fetal cells have been identified in maternal circulation. These can be

- Free fetal cells in maternal circulation
- Free nucleic acids (DNA and RNA) in maternal circulation.

Fetal cells in maternal circulation: Nucleated red blood cells could be used for prenatal diagnosis of fetal aneuploidies. With an aneuploid fetus, Bianchi et al (1997) have reported a six-fold increase in the number of fetal cells in the maternal blood but the isolation techniques are highly complex, so it has limited application today.

Cell free fetal DNA in maternal circulation:

- Studies demonstrated that Down syndrome pregnancies exhibit a 1.7-fold higher serum level of cff-DNA than normal pregnancies.
- Farina et al 2003 found that when added to the quadruple screening test in the 2nd trimester, fetal DNA increased the detection rate for Down syndrome from 81 to 86% at a 5% FPR.

The technique has been tried successfully in fetal sexing for X-linked disorders and fetal Rh grouping in Rh iso-immunization. Success in diagnosis of other single gene disorders has also been reported.

The main limitation of the cff-DNA, is the use of Y-chromosome sequences (mostly SRY gene) as biomarkers, and thus restricting the detection to pregnancies carrying only male fetuses.

CONCLUSION

Screening tests may have a role to play in high-risk cases. Combined screening by ultrasound and first trimester biochemical markers give best results. Only screen positive cases could be taken for invasive testing.

The cost of prenatal diagnostic services is only a fraction of the expense involved in looking after the children born with incurable disability due to chromosome abnormalities.

New ultrasound and biochemical markers on the horizon will vastly improve the sensitivity of these screening tests in coming future. In the light of all these advances, an informed choice of the woman remains the mainstay of the antenatal screening programs.

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