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REVIEW ARTICLE

Integrated Screening for Chromosomal Anomalies: Strategies in Developing Countries

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ABSTRACT

Ultrasound screening has advantages over maternal serum screening. These include confirmation of embryo viability, accurate assessment of gestational age, early diagnosis of multiple pregnancies and identification of chorionicity, the detection of major structural abnormalities, major defects of the heart and great arteries, skeletal dysplasias and genetic syndrome and measurement of NT thickness in assessing the risk for Down's syndrome. Nuchal translucency (NT) has emerged as the most sensitive ultrasound marker for detection of chromosomal anomalies in the first trimester. However, the use of ultrasound in routine screening still faces problems with reliability and quality control. Combination of maternal age, NT and first and biochemical second-trimester markers is known as the integrated test. A major goal of screening tests is to achieve high detection rate and low false-positive rate at a low cost. The integrated test best meets these criteria. It could achieve a detection rate of 85% for a false-positive rate of 1.2%. It has a much better positive predictive value and, therefore, fewer amniocentesis and fewer losses of normal fetuses. Only screen-positive cases could be taken for invasive testing.

Keywords: Integrated screening, First trimester and second trimester biochemical screening.

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. Antenatal screening for chromosomal anomalies other than by maternal age alone has changed significantly for the last 20 years. Maternal age of over 35 years is no longer accepted as a cut-off to offer screening tests to pregnant women.

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

In a developing country like India, there are two sets of population: One who has access to all medical facilities and the other who is neither affordable nor seeks any antenatal care. In such population screening for chromosomal anomalies is generally not possible.

SCREENING FOR FETAL ANEUPLOIDIES

Maternal serum screening by biochemical markers and ultrasound form the mainstay of noninvasive prenatal screening.

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 of 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 β-hCG was a better marker as compared to i-hCG. PAPP-A levels are decreased and hCG increased in pregnancies at risk for Down's syndrome.[1-3]

With these two markers, combined together with maternal age, the detection rates are 67% for a false-positive rate of 5%.[4]

PAPP-A is currently the single best serum marker with a 42% detection rate for a 5% false-positive rate.

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 preganancies 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.

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.

Ultrasound screening has advantages over maternal serum screening. These include confirmation of embryo viability, accurate assessment of gestational age, early diagnosis of multiple pregnancies and identification of chorionicity, the detection of major structural abnormalities, major defects of

the heart and great arteries, skeletal dysplasias and genetic syndrome and measurement of NT thickness in assessing the risk for Down's syndrome.[8] However, the use of ultrasound in routine screening still faces problems with reliability and quality control.

Cicero et al[5] suggested that inclusion of the nasal bone yielded a 90% detection rate with reduction in the false-positive rate from 5 to 0.5%.

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. 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), 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.

In twin pregnancies, the overall sensitivity of second trimester screening is lower and only approximately 50% affected fetuses may be identified.[7]

Maternal serum markers

- First trimester: PAPP-A, free beta-hCG
- Second trimester: AFP, uE3, hCG, Inhibin-A

Combined Test

In the late first trimester, combining the measurement of fetal NT thickness with maternal serum biochemical markers and maternal age is referred to as the combined test. Combined first trimester biochemical screening and ultrasound offers a detection rate of 85% with a false-positive rate of 5%. With maternal age, fetal NT thickness and maternal serum free β-hCG and PAPP-A have a detection rate of trisomy 21 to be 90%, for a false-positive rate of 5%.[6] This screening test also detects 90% of other chromosomal anomalies, including trisomy 13, trisomy 18, Turner's syndrome and triploidy. Women with borderline risk based on PAPP-A, free β-hCG and NT are offered a more specialist scan to determine, among other things nasal bone hypoplasia and reassess the risk. Nasal bone hypoplasia is a very powerful marker of aneuploidy but requires appropriate training not generally available.

Integrated Test

Combination of maternal age, NT and first and second-trimester biochemical markers is known as the integrated test. It could achieve a detection rate of 85% for a false-positive rate of 1.2%. It consists of two steps. First, measurements of NT thickness and PAPP-A in the late first trimester (about 12 weeks) are taken. Second, the quadruple test is performed in the early second trimester (about 15 weeks). A single risk figure is then obtained. If NT measurement is not available or reliable, serum integrated test (using only PAPP-A in the late first trimester) and the quadruple test in the early second trimester are useful. At a detection rate of 85%, the false-positive rate for the serum integrated test is 2.7%.[6]

A major goal of screening tests is to achieve maximum accuracy (high detection rate) and minimum harm (low false-positive rate) at a low cost. The integrated test best meets these criteria and is closely linked with the best and most widely available diagnostic test, i.e. amniocentesis. It has several advantages. Besides being safe and efficacious, it allows women more time for decision-making. It also allows affected pregnancies to miscarry spontaneously, rather than making those women go through the anguish of terminating a wanted pregnancy. It has a much better positive predictive value and, therefore, fewer amniocentesis and fewer losses of normal fetuses.[6]

The markers profile of a pregnancy with Down's syndrome in 1st trimester is:

NT	High
Fb-hCG	High (2.0 × normal)
PAPP-A	Low (0.4 × normal)

The markers profile of a pregnancy with Down's syndrome in 2nd trimester is:

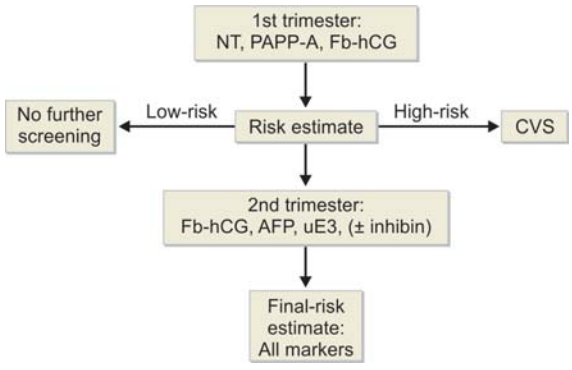
AFP (0.75 × normal)
uE3 (0.72 × normal)
hCG (2.0 × normal)
Inhibin-A (2.0 × normal)

Comparative performance of different screening modalities

Test	*Sensitivity*	*False-positive rate*
Nuchal translucency	68%	5%
First trimester	85-87%	5%
Combined screening		
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

	Aneuploidies			
Marker	*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 combined both 1st- and 2nd-trimester screening the fully integrated test yielded a 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 of gestation. NT has a poor performance as a screening test for Down's syndrome on its own or with maternal age alone.[8] uE3 is of value in the detection of trisomy 18, Smith-Lemli-Opitz syndrome and placental sulphatase deficiency where uE3 levels are extremely low. Incidentally, levels are also slightly lowered in spina bifida and more so in anencephaly, but the changes are much less than for AFP.[9]

Prevention of Down's Syndrome

Primary Prevention Strategies

1. Avoiding reproduction at advanced maternal age
2. Preimplantation genetic diagnosis for couples who are at high risk of Down's syndrome
3. Folic acid supplementation.

A strategy of completing the family before a maternal age of 30 could more than halve the birth prevalence of this disorder. Women with a high priori risk should have access to pre-implantation genetic diagnosis, which can lead to a reasonably high pregnancy rate with an extremely low risk of Down's syndrome (DS).[10]

Secondary Prevention of Down's Syndrome

It is done through antenatal screening followed by invasive prenatal diagnosis and termination of affected pregnancies. In the past, women were selected for prenatal diagnosis on the basis of high risk—largely advanced maternal age or family history. But, this had little impact on birth prevalence since most cases occur without any specific etiology.

New Biochemical Markers

Several other markers are presently under investigation. These are as follows:

- Pregnancy-specific beta 1-glycoprotein (SP1)
  - Time window: 7 to 12 weeks
- Invasive trophoblastic antigen[6] (ITA—a highly glycosylated form of hCG)
  - Time window: 15 to 20 weeks
- ADAM-12 (Á disintergin and metalloprotease)
  - As a first-trimester screening marker of trisomy.[11] It has a proteolytic function against IGFBP-3 and IGFBP-5. It regulates bioavailability of IGF-1
  - ADAM-12 is reduced in pregnancies with DS and this is more pronounced earlier in pregnancy (Laigaard et al 2006).

Wortelboer EJ et al concluded that the screening performance for DS did not greatly improve adding ADAM-12s. ADAM-12s could be an additional biochemical marker for first-trimester screening for trisomies other than DS.[11]

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

New strategy in 1st trimester	
At 8-10 weeks:	PAPP-A, ADAM-12
then at 12-13 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[12]

Nucleated red blood cells could be used for prenatal diagnosis of fetal aneuploidies. With an aneuploid fetus, Bianchi et al (1997) have reported a sixfold 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 (CFF) DNA in Maternal Circulation[10]

- Studies demonstrated that DS 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 DS 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 isoimmunization. Success in diagnosis of other single gene disorders has also been reported.[7]

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. While the diagnosis of Mendelian disorders may be possible by fetal cells or cell-free DNA, it is very likely that aneuploidy detection will be seen as a screening test to modify risk as a predicate for invasive diagnostic procedures.

Much research is still needed before this can be used as a noninvasive test for prenatal diagnosis.

CONCLUSION

Screening tests may have a role to play in high-risk cases but in a developing country like India only if the population seeks antenatal care then based on maternal age, history and examination sceening by way of ultrasonographic soft markers is suggested and if there is a suspicion first trimester biochemical tests or second trimester biochemical tests should be advised depending on the gestational age at which the patient seeks antenatal care.

Combined screening by ultrasound and first trimester biochemical markers gives best results.

Only screen-positive cases could be taken for invasive testing. The aneuploidy risk should be calculated on the basis of age, nuchal translucency, biochemical screening and anomaly scan. If the calculated risk for aneuploidy exceeds 1:380, an invasive karyotyping procedure should be done.[13] This approach will reduce the unnecessary invasive tests, reduce abortion rate and will increase the detection rate of aneuploidy.

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 the 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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