
Screening Options and Role of Biochemical Markers in the Risk Assessment of Down Syndrome, Trisomy 18, and Neural Tube Defects

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Abstract: Prenatal screening to identify genetic disorders gaining importance for the past few years. The goal of present maternal serum screening programs is to identify women at increased risk of having a baby affected with Down syndrome, trisomy 18 or neural tube defects and that will benefit from diagnostic testing. The ultimate goal is to have fewer invasive procedures as well as fewer procedure related losses of normal fetuses. The options available are first trimester, second trimester, and sequential screening. When a woman at high-risk or with a positive screen is identified, genetic counseling and a diagnostic procedure such a chorionic villus sampling (CVS) in the first trimester or amniocentesis in the second trimester is offered to determine if the fetus has the genetic defect. The first trimester screening (Double marker) which has a detection rate slightly higher than second trimester (Triple marker) and results will be available early in the pregnancy. If a woman wants to know her risk with highest detection rate, the combination of first and second trimester screening in a sequential manner should be the test of choice. In this review, we highlighted the options available for screening, detection rate, benefits and limitations of the test and hope this will provide useful information for physicians order such screening.

Keywords: Screening Options, Risk Assessment, Biochemical Markers, Down Syndrome, Trisomy 18, Neural Tube Defects

1. Introduction

Prenatal screening for Down syndrome (trisomy 21), Edwards syndrome (trisomy 18), and neural tube defects during the first and second trimester of pregnancy has become an established part of obstetric practice in many countries including India. Maternal serum screening has some limitations. The results provide an aneuploidy risk but not a diagnosis. Chorionic villus sampling or amniocentesis needs to be performed to determine whether the fetus is affected by Down syndrome or trisomy 18 [1]. Ultrasound and measurement of amniotic fluid alpha-fetoprotein (AFP) is used in the diagnosis of neural tube defects [2]. Although the current screening approaches have relatively high detection rate, about 5% to 20% of affected babies will not be

identified [3]. In addition, not all chromosomal conditions will be detected which is in contrast to CVS or amniocentesis where all autosomal trisomies are detected.

In the past, maternal age 35 years or older at the time of delivery was used to identify women at high-risk and who will benefit from diagnostic procedures. The birth prevalence of Down syndrome increases with maternal age. The risk of having children with the disorder is 1 in 1250 for women under the age of 25 years, increasing to 1 in 30 at age 44 (Figure 1). However, age alone is a poor risk predictor for fetal defects [4]. The introduction of biochemical screening in combination with the woman's age has facilitated the refinement of a woman risk to carry an affected fetus [5]. In a statement published in 2007, the American College of Obstetricians and Gynecologists (ACOG) recommended that

screening and invasive diagnostic testing should be offered to all women before 20 weeks of gestation, regardless of maternal age.

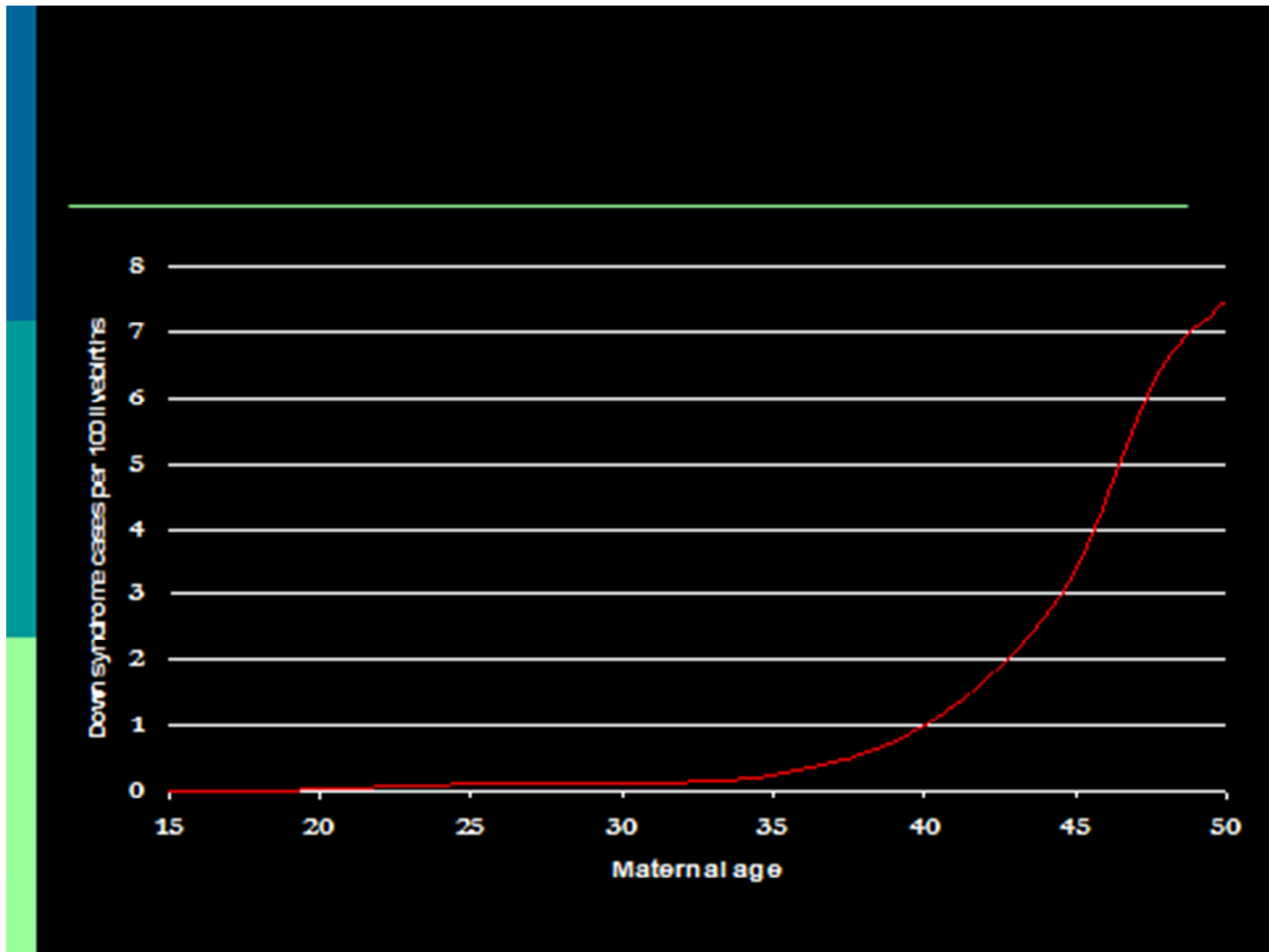


Figure 1. Birth prevalence of Down syndrome according to maternal age.

2. Materials and Methods

2.1. Screening Options

Two options are available for maternal serum screening. The options are first trimester and second trimester screening [6]. It is important to keep in mind that these two options are screening tests. This means that a screen-negative result does not guarantee the absence of fetal defects and a screen-positive will need additional tests like CVS or amniocentesis to determine if a genetic defect is present.

For the 2 screening options, the laboratory uses a mathematical model to calculate a woman risk of having a baby with Down syndrome, trisomy 18 or neural tube defect. This mathematical model takes into consideration the maternal age, the serum levels of various biochemical markers and, in the case of first trimester, the fetus ultrasound measurements [7]. In addition, a number of factors play an important role in the calculation of the risk as they will affect the values of the maternal serum biochemical analytes. This includes gestational age, weight, race [8], smoking, diabetic status [9] of the individual, the number of

fetuses present, and whether IVF treatment [10] was used for conceiving. In some rare cases like ovum donor pregnancy aneuploidy risk calculations, the use of the age of the ovum donor instead of the ovum recipient reduces the false-positive rate and improves screening efficacy. Inaccurate information can lead to significant alterations in the estimated risk. This is why it is so important that accurate information is provided when the sample is submitted to the laboratory.

2.2. Biochemical Markers Used for Assessment of Risk

First trimester screening is performed between 10 and 14 weeks of gestation. It provides a risk for Down syndrome and trisomy 18. The markers used on the risk calculation are Pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG). The third marker is the fetal nuchal translucency (NT), a fluid-containing area behind the fetal neck which is performed by ultrasound [11, 12]. The nuchal translucency measurement needs to be performed between 10 weeks and 13 weeks 6 days of gestation which is equivalent to a crown-rump length between 38 and 84 millimeters. The majority of fetuses with

Down syndrome have an increase NT measurement when compared to normal fetuses of the same gestational age [13].

3. Results

Decreased levels of PAPP-A before the 14th week of

gestation are associated with an increased risk for Down syndrome and trisomy 18. Whereas increased levels of hCG are associated with an increased risk of Down syndrome. Levels of free beta hCG decreases and PAPP-A increases when gestational age advances from 10-13 weeks in low risk pregnancy women (Table 1).

Table 1. Mean values of biochemical markers in first trimester of low risk pregnancy.

Gestational Age	Free Beta hCG ng/mL	PAPP-A mIU/mL
10 weeks (n=18)	51.51 (27.85-75.17)	1.43 (0.51-2.35)
11 weeks (n=25)	41.07 (14.71-67.43)	2.74 (0.87-4.61)
12 weeks (n=25)	41.85 (16.77-66.93)	4.34 (2.29-6.39)
13 weeks (n=25)	40.31 (18.22-62.4)	4.44 (2.49-6.39)

For first trimester serum screening, a screen-positive or screen-negative result is based on the laboratory-specific cutoffs. For the first trimester, Down syndrome risk calculation is based on a cutoff of 1 in 250. A screen-positive interpretation is provided if the calculated risk is equal to or higher than 1 in 250. Using this cutoff, the Down syndrome detection rate is 85% and the false-positive rate is 5%. For trisomy 18, risk estimates of 1 in 100 or higher are reported as screen positives. In the first trimester, the detection rate for trisomy 18 is approximately 80% and the false-positive rate is less than 1.0%.

All the screening approaches have benefits and limitations that need to be taken into consideration. In first trimester screening, the woman learns early in the pregnancy if she is at high risk, and earlier genetic counseling and diagnostic

procedures such as CVS could be offered. In addition if the woman decides to terminate the pregnancy the risk of complications is less during early pregnancy compared to the second trimester. Since neural tube defects could not be provided in the first trimester screening, another sample needs to be tested during the second trimester for measuring maternal alpha-fetoprotein and determine the risk for neural tube defects.

Second trimester screening is performed between 14 weeks and 22 weeks of gestation. The serum biochemical markers used to calculate a risk estimate are: AFP, unconjugated estriol (uE3), hCG and inhibin-A. hCG levels decreases and AFP and uE3 levels increases when gestational age increases from 14 –19 weeks in low risk pregnancy women (Table 2).

Table 2. Mean values of biochemical markers in second trimester of low risk pregnancy..

Gestational Age	AFP IU/mL	hCG mIU/mL	uE3 ng/mL
14 weeks (n=25)	30.11 (21.48-38.74)	52187 (31512-72862)	1.04 (0.55-1.49)
15 weeks (n=25)	30.53 (19.44-41.62)	34455 (20654-48256)	1.56 (0.91-2.21)
16 weeks (n=25)	42.36 (30.33-54.39)	25552 (13694-37410)	2.10 (1.24-2.96)
17 weeks (n=25)	49.05 (32.41-65.69)	21715 (11607-31823)	3.15 (1.77-4.53)
18 weeks (n=25)	53.4 (36.62-70.18)	20202 (10399-30005)	3.56 (2.37-4.75)
19 weeks (n=25)	55.71 (32.64-78.78)	16294 (9293-23295)	4.43 (2.78-6.08)

AFP is used on the risk calculation of the all 3 conditions. Low levels of AFP are seen in Down syndrome and trisomy 18 pregnancies, whereas increases are seen in neural tube defects. Unconjugated estriol, an estrogen produced by the placenta, is decreased in the majority of Down syndrome or

trisomy 18 pregnancies. hCG is normally increased in Down syndrome pregnancies and decreased in trisomy 18 pregnancies. Inhibin-A is only used to calculate the risk for Down syndrome, as in these cases it tends to be increased (Table 3).

Table 3. Changes in maternal serum biochemical markers in Down syndrome, trisomy 18, and neural tube defects.

	AFP	uE3	Total hCG	Inhibin-A
Neural tube defects	↑	N/A	N/A	N/A
Down syndrome (Trisomy 21)	↓	↓	↑	↑
Edwards syndrome (Trisomy 18)	↓	↓	↓	N/A

The concentration of first and second trimester biochemical markers changed to MoM values during risk calculation. The median concentration of PAPP-A is decreased by nearly two-thirds and free beta hCG is increased by two and half times in first trimester affected pregnancies. Total hCG and Inhibin-A increased by two times in pregnancies affected by Down syndrome in second trimester (Figure 2).



Figure 2. Changes in Median MoM values in Down syndrome in first and second trimester.

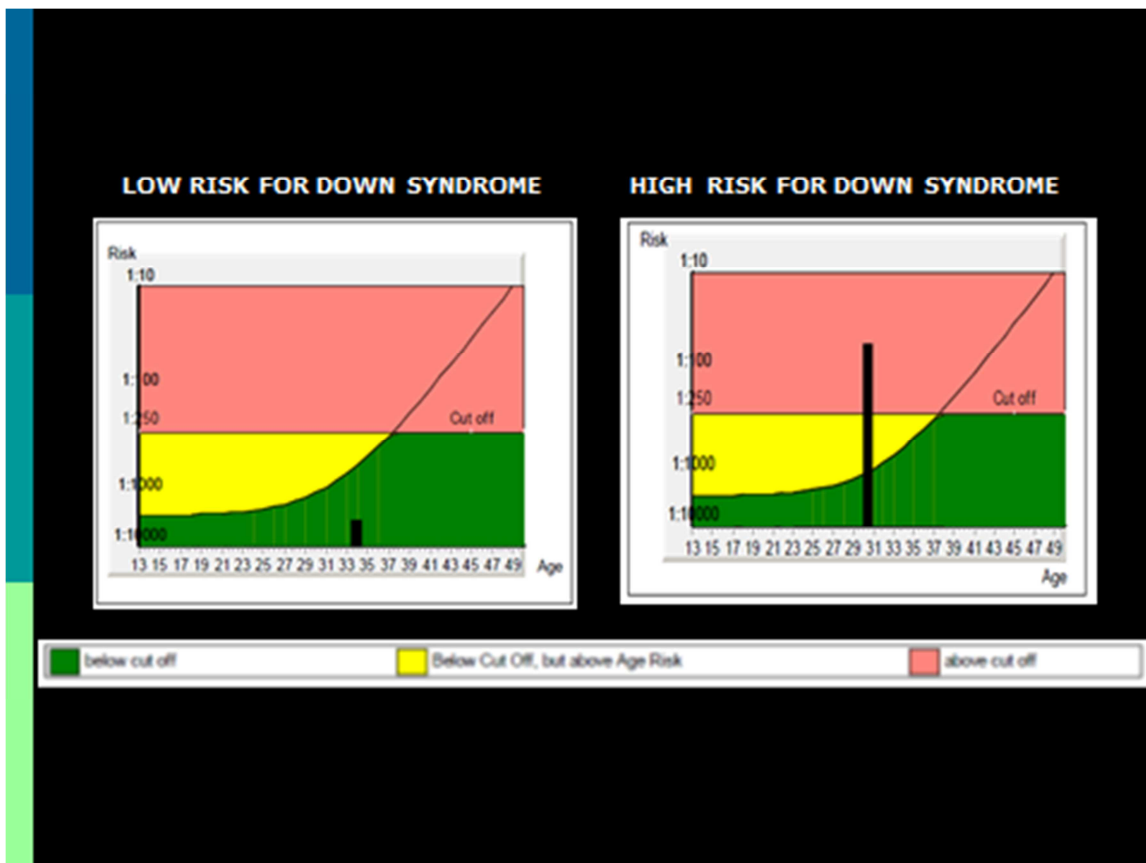


Figure 3. Summary of calculated risk for Down syndrome.

In contrast to first trimester screening, second trimester screening provides risk estimates for Down syndrome, trisomy 18 and also neural tube defects. For Down syndrome, a screen-positive or negative interpretation and a numeric risk is provided based on the cut-off of equal or higher than 1 in 250. Using this cutoff, the detection rate for Down syndrome is approximately 81% and the false-positive rate is approximately 5%. For trisomy 18, risk estimates of 1 in 100 or higher are reported as screen positives. The detection rate for trisomy 18 is 80% with a false-positive rate of less than 1% for the second trimester screening. For neural tube defects, only AFP is used for the risk assessment. An AFP multiple of the median (MoM) equal to or higher than 2.5 is reported as screen positive. Second trimester screening has a detection rate for neural tube defect of approximately 80% with a false-positive rate of 1% to 3%. Software specifically designed for prenatal risk assessment for Down syndrome, trisomy 18, and neural tube defects is able to provide MoMs corrected for variable factors. Once the corrected MoM value is obtained, the likelihood ratio is calculated for each of these values, and the combination of all likelihood ratios with maternal age related risk yields the final risk assessment (Figure 3).

4. Discussion

Second trimester screening using 3 markers (Triple marker or Triple screen) or 4 markers (Quad test or Quad screen) is the best option for women presenting for prenatal care later in the pregnancy. The Quad screen is similar to the triple screen test, except the likelihood of identifying pregnancies at risk for Down syndrome is higher through the evaluation of Inhibin-A levels and the false positive rate of the test is also lower [14]. Second trimester screening tends to have a slightly lower detection rate for Down syndrome compared to first trimester.

Sequential Screening

Sequential screening is a type of cross trimester screening that has been introduced to increase the detection rate and provide the lowest false-positive rate [15-17]. The markers used in the risk calculation include: NT and PAPP-A which are measured during the first trimester and AFP, unconjugated estriol, hCG and inhibin-A which are measured in the second trimester. In sequential screening, once NT and PAPP-A information is available, a risk calculation is performed. If the screen is positive, results are reported and this is considered the end of the screening test. In contrast, if the screen is negative, a second sample is collected in the second trimester and new risk estimate is generated.

The first measurements are performed at 11 to 13 weeks of gestation and include an NT measurement by ultrasound and a PAPP-A measurement in serum. Based on the laboratory's predetermined cutoffs, a screen-positive or screen-negative result is generated. If the screen is positive, meaning that the woman is considered to be at high risk of carrying an affected fetus, the results will be reported immediately to give the woman the option to undergo early genetic

counseling and diagnostic testing. If the woman had a negative screen in the first trimester, the results are not provided. A second sample needs to be sent to the lab between 15 and 22 gestational weeks for measurement of AFP, estriol, hCG and inhibin-A. A risk calculation using 6 markers is provided on the second trimester test. Based on the set of cutoffs used for sequential screening, the majority of women will complete the second part of the test providing a higher detection rate and a lower false-positive rate.

Calculation of the risk estimate for Down syndrome is based on 2 cutoffs. In the first trimester, risk estimate values of greater than or equal to 1 in 50 are interpreted as positive and a report will be issued. If the risk is below 1 in 50, a report will not be issued until the second sample is received in the second trimester. In the second trimester, the cutoff used for Down syndrome is equal to or higher than 1 in 270. Using this approach, the overall detection rate for Down syndrome is approximately 90% with a false-positive rate of approximately 4%. Trisomy 18 risk estimates greater than or equal to 1 in 100 are reported as positive in the first part of the screening when the Down syndrome risk is also increased. If the risk is below 1 in 100, the woman will continue to the second part of the screening. In the second trimester, the cutoff used for trisomy 18 is also 1 in 100. Using this approach, the overall detection rate for trisomy 18 is approximately 90% with a false-positive rate of less than 0.5%. Neural tube defect calculation is the same as the second trimester screening. A multiple of the median (or MOM) equal or higher than 2.5 is reported as screen positive. The detection rate in second trimester screening for neural tube defect is approximately 80% with a false-positive rate of approximately 1 to 3%.

One of the benefits of sequential screening is that if the woman is at high risk of having an affected fetus, the risk information is provided right away in the first trimester so genetic counseling and diagnostic testing is provided early in the pregnancy. In addition, if the decision of pregnancy termination is made, this could be performed earlier and with fewer risks. If the woman is consider low risk after the first risk calculation, continuation to the second part of the test provides a higher detection rate.

Recent Advances

The cell-free fetal DNA (cffDNA) test is a new test that may be used to assess the risk of a pregnant woman's developing fetus having a chromosome disorder, such as Down syndrome or Edward's syndrome [18-22]. Cell-free fetal DNA is genetic material that is released by the placenta and circulates in a woman's blood during pregnancy. It is present in small quantities starting in the first trimester and increases throughout pregnancy. The ACOG currently recommends that the cffDNA test be offered to women at an increased risk for trisomy 18 or 21 and does not recommend this testing for low-risk pregnancies because there are not yet sufficient data on its use in these cases [23]. Few studies have shown that the cffDNA test can be more specific and sensitive than the routine tests in high-risk women, ACOG

currently recommends that the cfDNA test be offered separately, not incorporated into routine prenatal testing. The cfDNA test is also used as a screening test, not a diagnostic test. If there are abnormal findings in cfDNA testing, then invasive confirmatory testing procedures such as CVS or amniocentesis may be indicated to diagnose a chromosome abnormality.

5. Conclusion

To summarize maternal serum screening options, it is recommended that regardless of which test is offered for prenatal screening, information about the detection rate, false-positive rate, and the benefits and limitations of the test should be available to patients so they could make an informed decision. If a woman wants to know her risk of having an affected child early in pregnancy, the best option will be first trimester screening (Double marker) which has a detection rate slightly higher than second trimester and results will be available early in the pregnancy. In contrast, for those women that present later in the pregnancy, second trimester screening using the 3 biochemical markers (Triple marker) or 4 biochemical markers (QUAD screening) is the best available maternal serum screening option. But if a woman wants to know her risk with highest detection rate, then sequential screening should be the test of choice as it provides a higher detection rate and lower false-positive rate compared to either first trimester or second trimester screening options.

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