MATERNAL SERUM SCREENING - AS A PRENATAL TESTING CHOICE

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ABSTRACT

Maternal serum screening is prenatal blood genetic test which in combination with highly specialized ultrasound scan of fetal morphology is a non invasive approach for evaluation of congenital anomalies. The aim of the present announcement is to report our first results of the started in 2005 maternal serum screening for Down syndrome, neural tube defects and ventral wall defects as an approach for genetic prophylaxis. Maternal serum screening is a program for prenatal evaluation of the risk for the most common chromosome disorders in all pregnant women. It is performed in the Medical genetic laboratory in “St. Marina” University hospital – Varna and for now is the only unit outside the capital, where the screening is performed for the region of North-Eastern Bulgaria. In 15 months (January 2005 – march 2006) 324 women are evaluated (251 of them from Varna and 73 – from the region). 220 of them are under 35 years of age and 104 – over this age. The biochemical test is dual – for hAFP and Free hCG? and software calculates risk, based on gestational age, pregnant’s weight and her age. High risk (higher than 1:250) was evaluated in 11 women (5%) under 35 years of age and in 24 (23.1%) - over 35 years of age. In 63.6% in the first group and 75.0% in the second group amniocentesis and cytogenetic investigation were performed and normal results were found.

Keywords: maternal serum screening, Down syndrome, neural tube defects, ventral wall defects

INTRODUCTION

Every pregnant woman wonders about the health of unborn baby and the possibility of birth defects. Women 35 and older may be especially concerned because certain birth defects (such as Down syndrome) are more common in pregnancies of older women. The prenatal tests can detect some birth defects but not all of them. Down syndrome, open neural tube defects, abdominal wall defects, trisomy 18 and other chromosomal defects are some of the birth defects found during testing. First, a woman needs to describe if she wants a screening test or a diagnostic test. A diagnostic test (amniocentesis, chorionic villus sampling - CVS) can tell whether or not the fetus actually has a certain birth defect, but as an invasive procedure it is not safe. A screening test (dual or triple blood test) estimates the chances (risk) of the fetus having a certain birth defect (Fig1). If the risk is high, a woman can then choose to have a diagnostic test (1).

Who can help the woman make this decision?
Before deciding between a screening test and a diagnostic test, a woman who will be 36 years or older at delivery

<table>
<thead>
<tr>
<th>Age 35, For every 100 women who would deliver a Down syndrome child...</th>
<th>The Screening program Detects 71 of these Down syndrome pregnancies And misses 29 of them.</th>
</tr>
</thead>
<tbody>
<tr>
<td>At the age 37, For every 100 women who would deliver a Down syndrome child...</td>
<td>The screening program Detects 79 of these Down syndrome pregnancies And misses 21 of them.</td>
</tr>
<tr>
<td>At age 39, For every 100 women who would deliver a Down syndrome child...</td>
<td>The screening Program Detects 87 of these Down syndrome pregnancies And misses 13 of them.</td>
</tr>
</tbody>
</table>

Fig 1. Sensitivity of the screening test related to maternal age

should talk to her doctor who refers her to a genetic counselor. A genetic counselor can explain the choices in detail and answer any questions.

For many years, the only prenatal test for birth defects in women 55 years and older was amniocentesis and CVS has also been offered. There is now another choice: Maternal Serum Screening. The blood test result is combined with a
woman’s age to estimate her own personal risk for having a fetus with Down syndrome. Knowing this risk can help a woman decide whether to have amniocentesis. The blood test result also provides information about the risk of open neural tube defects, abdominal wall defects and most cases of trisomy 18. If the result is positive, diagnostic follow-up tests are provided.

What does the blood screening test involve?
A small amount of venous blood is taken from the pregnant woman’s arm. The mother’s blood (serum) is checked for two items: the amount of AFP (alpha-fetoprotein) and HCG (free beta human chorionic gonadotropin) as independent measurements. These substances are made by the fetus and the mother’s placenta. In deriving a risk estimate, complex formulas take into account the mother’s weight, age, and fetal age. Once the blood test results are determined, a risk factor is calculated based on the “normal” blood tests of the testing laboratory (2). The average of normals is called “population median” so that we use the median values of the reference laboratory is Sofia The results are reported as “Multiples of the Median (MoM)” and the “average” value is therefore 1.0 MoM. Down syndrome pregnancies have lower levels of AFP, so their levels would be below the average, and therefore less than 1.0 MoM. Likewise, hCG in a Down syndrome pregnancy would be greater than 1.0 MoM. The laboratory gets all results in this way and as a total, risk factor is calculated by a software program of the Fluorimeter Delphia. The result of the blood test is sent to the genetic counselor and/or the patient’s doctor within 1 week. The calculated risk is used to modify the risk already statistically calculated based on the mother’s age alone. The cut-off for high risk versus low-risk has been set at 1 in 250; less than that is high-risk, higher than that is low-risk. The risk for the cut-off has to do with the risk of miscarriage from amniocentesis (3,4).

When is the blood screening test done?
The blood test can only be done reliably between 15 and 20 weeks of pregnancy, but it is most accurate between the 16th and the 18th week. The best time is 16-17 weeks. A very important consideration in the screening test is the age of the fetus (in gestation weeks and days). The correct analysis of the different components depends on knowing the gestational age precisely because at each week of pregnancy there are different amounts of AFP and hCG in the mother’s blood. The best way to determine that is by ultrasound.

What does the “screen negative” result mean?
It means that the risk for certain birth defects is low enough and does not consider follow-up tests necessary. Since the blood test is just screening test, there is still a chance that the fetus may have a problem – even when the test result is negative (5,6). A “screen negative” result (low risk < 1 to 250) is the most common result and it varies with a woman’s age. The way the test is set up, the serum screening test has a false-negative rate of 35%, and so will only detect about 65% of all fetuses with Down syndrome; it accurately predicts 70-90% of fetuses with Down syndrome in women 35 and older. “Screen negative” result for neural tube defects and abdominal wall defects are not influenced by age. About 98% of women of all ages have a “screen negative” (low risk) result.

What does a “screen positive” result mean?
It means that there is an increased risk for certain birth defects in this pregnancy (such as Down syndrome, neural tube defect, abdominal wall defect, trisomy 18). Most of the time, however, the reason for the result is not a birth defect. Normally 5-6% of women < 36 year old and 40% of women > 36 may have false “screen positive” result. The most common reason for a screen positive result include:
- the due date is earlier or later than thought, or
- there is more than one fetus (twins, triplets),
- fetal death
- the substances in the blood varied more than usual without any known problem.

To determine the reason for the “screen positive” result, the follow-up diagnostic tests are offered. Most women with “screen positive” results will have normal follow-up tests and healthy babies. The woman is called by her doctor or geneticist. She is offered diagnostic services at the prenatal diagnostic clinic. The follow-up service includes:
- Genetic counseling – a professional counselor discusses the pregnancy and the family medical history. Questions are answered to help the family make decisions about further testing.
  - Ultrasound – a picture of the fetus is made using sound waves. This picture shows the age of the fetus, twin pregnancy, and detects certain birth defects by the diagnostic investigation “fetal morphology”.
  - Amniocentesis – a small amount of fluid is taken out of the uterus by experienced doctors at the Prenatal diagnostic centers and fast DNA with/without cytogenetic analyses are provided.

Women may refuse any of the services at any time. If the follow-up tests show that the fetus has a birth defect, information is given to the women by the doctor. The genetic counselor discusses the type of the birth defect that has been found, options for continuing or ending the pregnancy, any available treatment.

What birth defects may be found through follow-up testing?
Down syndrome, open neural tube defects, abdominal wall defects, and some other birth defects (trisomy 18). Among women who have the dual blood test and follow-up tests 97% of the cases of anencephaly and 80% of the cases of open spina bifida are found
- 85% of the cases of abdominal wall defects
- 50% or more of the cases of trisomy 18

Down syndrome
Down syndrome is a common cause of mental retardation and can occur in the fetus of a woman of any age. As women get older, the chances increase for delivering a baby with Down syndrome, but the vast majority of Down patients (75-80%) are born from young mothers (4,7). On average the detection of Down syndrome by the screening test
varies with a woman’s age. The test and the follow-up testing can detect most, but not all, pregnancies with Down syndrome (fig 2). It is never 100% successful in detecting Down syndrome pregnancies. Only the diagnostic tests by amniocentesis and CVS find almost 100% of them.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>- Low cost</td>
<td>- False positive screen results 5-7% for pregnancies at women’s age &lt;35 years and 35% at the age &gt;35.</td>
</tr>
<tr>
<td>- Safe, non-invasive</td>
<td>- False-negative results according to women’s age - 10 to 35%</td>
</tr>
<tr>
<td>- Detects 70% of Down syndrome pregnancies at women’s age &lt; 35 and up to 90% at the age &gt; 35</td>
<td>- Not recommended in multiple pregnancies</td>
</tr>
<tr>
<td>- Detects between 80% and 97% of all cases with NTD</td>
<td>- Little time for parental decisions</td>
</tr>
<tr>
<td>- Useful when amniocentesis not possible (technically difficult or too late)</td>
<td></td>
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<td>- Relatively rapid results</td>
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</table>

**Fig 2. Advantages and Disadvantages of the screening test**

**Neural Tube Defects (NTDs)**

Because 90-95% of NTD births occurs in the absence of a family history of the condition, the safe, non-invasive population screening procedure for NTD is highly desirable. The neural tube is completely formed by 5 weeks after conception. Anencephaly (when most of the brain does not develop) causes death of the fetus or newborn. Spina bifida (an opening in the spine) often causes paralysis of the legs, loss of bowel and bladder control, hydrocephaly, requiring surgery.

**Abdominal Wall defects**

Fetuses with abnormal opening on the abdomen, with intestines and other organs formed outside the body, where surgery often corrects the defect. These birth defects do not occur very often. However, if there is one of these birth defects, the screening test helps detect it. Ultrasonography completes the diagnostic process of neural tube and abdominal wall defects.

**Can the expanded screening program detect every type of birth defects?**

No. There are birth defects, which cannot be detected. Even when the blood test is “screen negative”, there is still a chance the fetus may have a problem.

Each woman should consider her prenatal testing choices carefully (5).

Women who decide to have the blood test must sign the consent form and have blood drawn between 15 and 20 weeks.

- Women who decide to have amniocentesis or CVS should make an appointment at a Prenatal Diagnostic center (University clinics of obstetrics and gynecology in Sofia, Plovdiv, just starting in Varna).

- Women should see a genetic counselor if they need help deciding between a screening test and diagnostic tests.

- Women can decide to have no prenatal testing.

**First MSAFP screening results in the University hospital of Varna**

Our test results are based on clinical studies and follow-up of 324 pregnant women, who have been studied at the Maternal screening laboratory, University hospital “St. Marina” Varna for the period January 2005 to March 2006 incl. (Fig 3). There were 220 women at the age <35 and 104 - > 35. The false positive results were 5.9% and 23.1% respectively, close to the expected ones. None of the high-risk pregnant women delivered a Down syndrome affected child, though 6 in each group refused follow-up by amniocentesis (only diagnostic ultrasound for fetal morphology provided). A peculiar fact is the pregnancy in 28 year old Down syndrome patient with free trisomy 21 whose serum screening result was the best one – a risk of <1 to 10 000, the karyotype from amniocytes was normal and a normal baby was successfully born. However examination was ongoing and soon after march 2006 the first Down syndrome pregnancy rate was drawn in a 36 year old mother, free trisomy 21 was confirmed by amniocentesis both in Sofia and Varna cytogenetic laboratories. Two screen positive result considered very high risk for NTD (anencephaly and open spina bifida). The pregnancies were terminated after ultrasound diagnosis.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number</th>
<th>Risk for Down s-me</th>
<th>Risk for NTD</th>
<th>w/o AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women &lt;35</td>
<td>220***</td>
<td>13 (5,9%)*</td>
<td>2</td>
<td>6**</td>
</tr>
<tr>
<td>Women &gt;35</td>
<td>104</td>
<td>24 (23,1%)*</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

* 5-7% false positive at age <35 ; 35% at age >35 by literature data
** - 2 terminated pregnancies with NTD incl.
*** - 1 pregnant women with free trisomy herself and serum risk

**Fig 3. First results from the MSAFP screening in Varna**

**CONCLUSION**

Maternal serum alpha-fetoprotein and beta chorion-gonadotropin measurements have expanded the option of population screening for Down syndrome; NTD abdominal wall defects and some other birth defects. The two indicators together can identify approximately 65% (with a false-positive rate of 5%). The use of additional tests, including ultrasonography rate can further increase sensitivity. This non-invasive screening approach in the second trimester entails virtually no risk but increases sensitivity for detection of common birth defects.
REFERENCE