RISK ASSESSMENT PROGRAM FOR FETAL ANEUPLOIDIES AT THE MUNICIPAL CLINICAL EMERGENCY HOSPITAL IN TIMISOARA

Dan Navolan¹, Ioana Ciohăt², Simona Farcas³, Victor Dumitrascu⁴, Cristina Gug⁵, Maria Puiu⁶, Valerica Belengeanu⁷

REZUMAT

Objective: Evaluarea programului de estimare a riscului de aneuploidii fetale derulat in cadrul Spitalului Municipal de Urgenta Timisoara. Indicatorii primari de rezultat au fost rata de rezultate false pozitive si rata de detectie. Material si metode: Determinarile de triplu test (AFP, hCG, E3 neconjugat) si ecografia de morfologie fetala au fost efectuate la 2169 gravides. Riscul de aneuploidii a fost calculat pe baza unei formule matematice standard incluzand varsta, rezultatele biochimice si ecografice. Gravidite cu risc crescut au fost selectate prin cariotip fetal efectuat la unul din cele patru laboratoare de genetica din regiune. Au fost contabilizate rezultatele de cariotip normal si patologic.

ABSTRACT

Objective: To analyze the risk assessment program for fetal aneuploidies done by the Municipal Clinical Emergency Hospital Timisoara. The primary indicators are: the rate of false positive results and the detection rate. Materials and methods: A triple test determination (AFP, hCG, Free E3) and a fetal morphology ultrasound was performed on 2169 gravidas. The aneuploidy risk was assessed based on a standard mathematical formula including biochemical and ultrasound results. High risk gravidas received amniocentesis and a fetal karyotype in one of the four genetic centers in our area. The antenatal and postnatal diagnosed cases were counted. Results: The age of gravidas was 29.3+/−4.5 years (Median+/−DS) and the gestational age at the moment of screening was 16.3+/−1.1 weeks. 43 gravidas had an age-risk and 164 gravidas a biochemical risk higher than 1:100 while 184 gravidas had an age-risk and 163 gravidas a biochemical risk higher than 1:250. The biochemical risk was adapted based on second trimester ultrasound. In the evaluated cases, we performed 62 amniocentesis, from which 4 were positive: 3 Down syndrome and 1 Turner syndrome. There were one newborn with Down syndrome (due to poor doctor – patient communication). The rate of false positive cases was 2.85% and the detection rate was 80%. Conclusions: The combined assessment of aneuploidies based on age, biochemical triple test parameters and the second trimester ultrasound remains applicable method in our system as long as not all gravidas come for screening during the first trimester of pregnancy.

Key Words: diagnostic antenatal, triplu test, screening, amniocenteza, sindrom Down

INTRODUCTION

Developing and efficient method of risk assessment (screening antenatal) for fetal aneuploidies, malformations and pregnancy associated pathology is a priority for all specialists in the materno-fetal field.¹ The risk assessments methods developed progressively from the ones based on the age of the mothers, the maternal AFP concentration, the triple test, the triple test associated with the second trimester ultrasound, the first trimester bi-test screening, and the first trimester ultrasound.² By applying these risk assessment methods, there is the possibility to select the gravidas with a high risk of fetal aneuploidies.
and malformations. Usually these cases are explored invasively in order to collect cells or fetal tissue (chorionic villus sampling, amniocentesis, and cordocentesis) in order to have a genetic or enzyme analysis. The genetic or enzyme diagnosis is quite sure, having an accuracy percentage of more than 99%.

The invasive maneuvers have a risk because they are associated in a percentage of about 0.5% with a spontaneous abortion happening after the membrane rupture, amniocorial infection, or the beginning of abortion labor. For this reason, there are efforts made in order to optimize screening methods: the main result indicators that need to be improved are the rate results for the false positive ones and the detection rate.

Because the number of cases with aneuploidies is small compared to the cases invasively investigated, it is considered that the rate of false positive results is equal to the rate of invasive maneuvers in the lot that benefited from the screening. The detection rate refers to the percentage of positive cases discovered from the detected number of cases cumulated with the undetected ones.

The more efficient the risk assessment for the aneuploidies method is, the smaller the rate of false positive results and the higher the detection rate. An important role in establishing the rate of false positive results is given by the risk threshold value (cut-off) from which the performance of an invasive maneuver is recommended. Historically, the value has been established at 1:250 because it represented about 5% of the gravidas population, which means the pregnant women over 35. But presently, this percentage has changed. The modern algorithms of the Fetal Medicine Foundation include, for the calculated risk determined, a risk assessment for the first trimester other cut-off limits: 1:100 and 1:1000. The cases with a higher risk than 1:100 are explored through methods using high resolution ultrasound (nasal bone, venous duct Doppler analysis, tricuspid regurgitation) and are reclassified and the ones with a risk over 1:1000 are not invasively explored.

A similar screening method can be applied in the second trimester of pregnancy after the biochemical analysis (the triple test). The biochemical risk is adapted considering the presence of secondary signs for Down syndrome (the thickness of the nuchal fold, the femur and humerus length, the anterior-posterior diameter of the ureteral pelvis, the intestinal hyperecogenity, the presence of hyperecogene intracardiac focus, nasal bone size, and the presence of certain major malformations). The indication for an invasive maneuver is established after recalculating the biochemical risk based on morphologic fetal ultrasound.

Establishing the accurate gestational age is of great importance in risk assessment. If there is a difference of less than 4 days between the chronological age and the ultrasound age in the first trimester or less than 7 days in the second trimester, the chronological age has priority. If there is a discrepancy of over 4, respectively 7 days, we will consider the ultrasound age.

Nowadays there is great hope in developing non-invasive antenatal diagnostic methods that are based on detecting nucleic acids and fetal cells in the maternal circulation. These techniques, although applicable in certain cases are expensive at present and still inefficient for large-scale use.

**MATERIALS AND METHODS**

There was data retrospectively analyzed from 2169 gravidas that undergone from May 1st 2008 to December 1st 2010 for the risk assessment program based on the triple test and a second trimester ultrasound in the materno-fetal medicine department of the Women's Health Clinic at the Municipal Hospital in Timisoara. The gravidas were tested within the 14-23 weeks of pregnancy by analyzing the seric concentrations of AFP, hCG and Free Estriol by the chemiluminescence method, using a ImmuliteOne Machine (DPC, Diagnostic Products Corporation, Los Angeles, USA). The obtained results were reported to the gestation age corresponding medians and interpreted by using the PRISCA software, Version 4 (Typolog Software, Tomesch, Germany). The dating of the pregnancy and the acquired data storage has been performed through the ASTRAIA software, the materno-fetal module (Astraia GmbH, Munich, Germany). In order to establish the gestational age we used the protocol suggested by Chervenak et. al. All the pregnant women who are tested by triple test also undergo a morphological ultrasound. The biochemical risk through the triple test was adapted according to the presence or absence of minor signs of Down syndrome according to the Nicolaides protocol. The gravidas, who were evaluated with a high risk after the maternal biochemical assessment and the morphological ultrasound, were explored invasively. The cells were cultivated and evaluated in order to obtain a karyogram. In order to follow all the cases investigated through fetal karyotype or at birth, there was data collected from the four genetic laboratories in the area, and the data was confronted with the list.
of pregnant women that benefited from the triple test in our facility. The result indicators followed for the antenatal screening were: (1) the rate of false positive results) the rate of amniocentesis for the gravidas who benefited from the antenatal screening and (2) the detection rate (the percentage of newborn babies with Down syndrome detected before birth).

RESULTS

1. The age of pregnant women in the risk assessment program for aneuploidies (screening).

The age of the pregnant women varied between 16 years and 10 months old and 46 years and 6 months old (Mean±/DS; 29 years and 4 months ± 4 years and 7 months).

2. The gestational age of the pregnant women at the moment of risk assessment for aneuploidies (screening) and the manner of dating the gestational age.

The gestational age of the pregnant women varied between minimum 14 and maximum 22 gestational weeks and 5 days (Mean±/DS; 16 weeks and 3 days ± 1 week and 2 days). The gestational age was dated in 1433 gravidas by Last Menstrual Period (LMP), in 391 gravidas by Crown Rump Length (CRL), in 163 cases by Biparietal Diameter (DBP), in 75 cases by combined second trimester ultrasound, in 5 cases by conception date and in 102 gravidas the method was not specified.

3. The aneuploidies risk assessed based on the pregnant woman’s age.

It is known that the risk of aneuploidies increases with the age of the gravida. By analyzing the age-risk of the gravidas in the studied group, we notice that a number of 43 (1,98%) gravidas had an age-risk higher than 1:100 and a number of 184 (8,48%) gravidas had an age-risk higher than 1:250.

4. The aneuploidies risk based on biochemical parameters (triple test).

The biochemical screening methods have the role of increasing the detection rate for the cases with the same rate of false positive results compared to the screening performed based on age. In the gravidas evaluated for the aneuploidies risk it was noticed that a number of 49 (2.25%) gravidas had a biochemical-risk higher than 1:100 and a number of 163 (7,51%) gravidas had a biochemical-risk higher than 1:250.

5. Pregnant women with risk, invasively investigated by amniocentesis after applying the biochemical and ultrasound screening program.

In the period of time between May 1st 2008 and December 1st 2010 there were 62 amniocentesis performed. The rate of invasive explorations was of 2.85%. There were 3 cases diagnosed with Down syndrome, and one case of Turner syndrome 45,X0. The cases were distributed as follows: out of 16 amniocenteses performed on gravidas with a biochemical risk higher than 1:100, 4 had abnormal fetal karyotype and 12 had normal karyotype; out of 22 amniocenteses performed on gravidas with a risk score between 1:100 and 1:250 all of them had a normal fetal karyotype and there was one new-born with SD; out of 24 cases of amniocenteses performed on gravidas with a lower risk than 1:250 all had normal fetal karyotype and there was no case of new born with Down syndrome. (Table 1)

6. The newborns from the gravidas who followed the risk assessment program for aneuploidies (ultrasound and biochemical screening).

The data was collected from the neonatology department of our hospital and from all the four genetic laboratories in the area, which provide diagnosis for the population in the whole region, so it is very improbable that data from a newborn was missed (dropped-out). Between August 1st 2008 and June 30th 2011 there was only one baby born with trisomy 21, with the calculated triple-test risk of 1:227.

7. The false positive result rate and the detection rate.

The false positive rate was 2.85% while the detection rate was 80%. Because of the small incidence of this disease in the population, the calculated rates must be regarded carefully.

Table 1. Down Syndrome fetuses and newborn distribution according aneuploidies risk evaluation by triple test.

<table>
<thead>
<tr>
<th>Risk</th>
<th>No. gravida</th>
<th>No. amniocentesis</th>
<th>No. aneuploid fetuses</th>
<th>No. aneuploid newborns</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1:100</td>
<td>49 (2.25%)</td>
<td>16 (32.6%)</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>1:100−1:250</td>
<td>114 (5.25%)</td>
<td>22 (19.3%)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>&lt; 1:250</td>
<td>2006 (92.48%)</td>
<td>24 (1.2%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2169</td>
<td>62 (2.8%)</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

Dan Navolan et al 45
DISCUSSION

The main scientific trend in the materno-fetal area presently promotes screening during the first trimester as the gold-standard.\(^\text{10,11}\) Performing this screening requires machines, an adequate infrastructure and a concentration of the cases in a specialized center in order to cumulate the necessary number of cases for making the investment lucrative and also acquiring the necessary expertise. Another important aspect is making general medicine physicians and the pregnant women aware of the usefulness of performing the screening for aneuploidies starting with the first trimester of the pregnancy. In order to efficiently perform this program, physicians need to be properly trained, because the screening program needs to be well documented in order to make possible the auditing of a qualitative screening. In this paper the analysis was made upon the risk assessment program for aneuploidies in the second trimester of pregnancy, performed based on the seric biochemistry and the second-trimester fetal ultrasound.

The average age of the pregnant women is 29, approximately 5 years older compared to the age of pregnant women 20 years ago (unpublished data). Theoretically, due to the increase in age of pregnant women at the moment of conception, without an antenatal screening program, the percent of fetuses with aneuploidies could be expected to be higher compared to the number of cases diagnosed at birth 20 years ago. This evolution shows the necessity of developing a proper infrastructure in order to diagnose before birth and especially for the future, since it is expected that the age of women at first birth will continue to increase.

The gestational age when pregnant women came to the screening program was 16 weeks. In our center, there is also the possibility of risk assessment through a bitest at 11+4-13+6 weeks of pregnancy, but the number of pregnancies that come in at this age is much lower. We need to make an effort to increase the number of cases that are risk assessed during the first trimester.

The accuracy of establishing the gestational age is extremely important. It needs to be done according to known protocols. Establishing gestational age based on the fetal craniocaudal length needs to be the elective method for determining gestational age in the first trimester of pregnancy.\(^\text{8}\)

Establishing an adequate cut-off contributes to defining the rate of false positive cases and the detection rate. In the second trimester the assessment needs to be done sequentially, a biochemical screening, followed by an ultrasound screening. Implementing an ultrasound assessment algorithm and calculation in order to adapt the risk according to ultrasound results is useful for selecting the cases with aneuploidies risk.

The amniocenteses rate was 2.85%, a low rate determined mainly by addressing the issue towards the ultrasound, where the risk score was adapted based on the first trimester ultrasound results (previously performed nuchal translucence) and a second trimester ultrasound. The reported detection rate is 80%, 4 diagnosed cases and 1 undiagnosed case.

It is interesting that most of the cases with aneuploidies are within the group of gravidas with a biochemical risk higher than 1:100. The only case of a new-born undiagnosed was for a pregnant woman with a biochemical risk of 1:227; in this lot there was no case of fetal aneuploidies diagnosed before birth through amniocentesis, although the percentage of amniocentesis was 19.3% of the cases included in this category.

Results show that there were 80% of cases detected with aneuploidies. The sensitivity of screening could be improved by a better documentation of the cases, by applying a more sensitive and more specific method (bitest) and by consistently applying a standardized algorithm of ultrasound by the physicians trained in this area.

CONCLUSIONS

Due to the fact that the age of the gravidas at the moment of conception rose significantly in the last several years, and the tendency is a continual increase, it is necessary to develop medical services in the area of risk evaluation for aneuploidies and fetal malformations in the years to come.

Most of the gravidas come for a biochemical screening at the gestational age of 16 weeks. In order to apply an earlier method of screening, pregnant women must be included in the risk assessment programs from the gestational age of 12 weeks, the moment the inclusion of the thickness of nuchal translucence in calculating risk could increase the sensibility and the specificity of the screening.

In order to increase the detection rate of aneuploidies, the access to invasive methods needs to be encouraged where necessary, especially since there is a national program in this area.

Results show in fact that presently the triple test is not considered the method with the highest sensibility and specificity, this method remains though, a valid one for screening in situations where other previous methods are not accessed by the population on a large scale.\(^\text{12}\) (Table 1)
In order to have an adequate quality control, it is necessary to have a structured documentation of all the cases, perhaps through an informatics network which connects all the centers that perform these investigations in order to assess them in an integrated pattern. We notice that the first genetic amniocentesis was performed in our center in 2002.13,14

REFERENCES