The Importance of Screening and Prenatal Diagnosis in the Identification of the Numerical Chromosomal Abnormalities

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ABSTRACT

Background and aims: *The obstetric care of a pregnancy, as it is practiced today, includes non-invasive screening approaches as well as invasive procedures for the definitive prenatal diagnosis of fetal disorders correlations between indications for prenatal cytogenetic diagnosis and results of the chromosomal analysis made upon fetal cells. The aim of our study was to evaluate the correlations between the screening test results and results of chromosomal analysis on fetal cells.*

Methods: *Amniotic fluid samples from 1159 pregnant women were studied with the rapid FISH method and the cytogenetic analysis (karyotype). The results from both methods were compared.*

Results: *The indications to perform prenatal cytogenetic diagnosis for numerical chromosomal abnormalities were: abnormal results of double or triple test, advanced maternal age, fetal abnormality detected through ultrasound examination, and positive family history for chromosomal anomalies. In our study we identified 30 cases with abnormal numeric chromosomes (18 cases of trisomy 21, 4 cases of trisomy 18, 3 cases of trisomy X, 1 case of monosomy, 2 cases of trisomy XYY, 1 case of trisomy XXY and 1 case of triploidy).*

Conclusions: *This report confirms the importance of screening and the cytogenetic diagnosis in the identification of the numerical chromosomal abnormalities.*

Keywords: aneuploidy, prenatal screening, prenatal diagnosis, amniotic fluid

INTRODUCTION

Diagnosis of chromosomal abnormalities in fetus is one of the most important challenges in modern perinatology. The most common chromosomal abnormalities in newborns are trisomies 21, 18, 13, monosomy X and other sex chromosome aneuploidies (1). These aneuploidies can account for up to 95% of live-born chromosomal abnormalities (2). Prenatal diagnosis employs a variety of techniques to determine the health and condition of an unborn fetus.

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Methods of prenatal diagnosis can be divided into non-invasive and invasive techniques.

Non-invasive methods include ultrasound and biochemical screening from maternal blood. Maternal serum screening in the second trimester has now been available for over two decades. More recently, first trimester screening tests offer women the opportunity of early screening for fetal aneuploidy and the option of earlier diagnosis.

Invasive testing is advised for pregnancies that bear a high risk of being affected by a chromosomal aberration from family and individual history.

Non-invasive techniques

In the first trimester of pregnancy, screening by a combination of ultrasound markers (the nuchal translucency -NT) and maternal serum β-hCG (human chorionic gonadotropin) and PAPP-A (pregnancy associated plasma protein - A) can identify up to 97% of fetuses with trisomy 21 and other major chromosomal abnormalities (3). Collection of blood for biochemical analysis is performed between 9 and 13 6/7 weeks' gestation (4,5).

In trisomy 21, during the first trimester of pregnancy, the maternal serum concentration of free β-hCG is increased and PAPP-A is decreased (6,7). In trisomies 18 and 13 maternal serum free β-hCG and PAPP-A are decreased (8).

The big breakthrough in first trimester screening was the advent of the nuchal translucency (NT) measurement. Between 11 and 14 weeks, a clearly demarcated fluid-filled space can be seen behind the fetal neck. This space is present in all fetuses. An increased NT measurement is significantly associated with trisomy 21 and other forms of aneuploidy (5). NT measurement alone has a detection rate for Down Syndrome (DS) of 70% with a 5% false positive rate. Other sonographic findings are being investigated as potential markers for DS. Absence of the nasal bone is associated with DS but its value as a screening test in the general population is controversial.

Second-trimester maternal serum testing includes the triple and quadruple screens. Multiple marker screening is used in the second trimester (15–20 weeks) to screen for trisomies 21 and 18 as well as open neural tube defects. The triple screen is the measurement of alpha fetoprotein (AFP), human chorionic gonadotropin (hCG), unconjugated estriol (uE3), levels in maternal serum (9). This combination of markers can detect approximately 60% of cases of fetal Down syndrome with a false positive rate of approximately 4% (10). The addition of inhibin A testing to the triple screen yielded the quadruple screen (11,12). The values of these parameters can be influenced by the presence of maternal diabetes type 1, smoking and pregnancy-related weight gain (13). In most cases of DS, the AFP and uE3 levels are lower, whereas hCG and dimeric inhibin-A levels are higher.

Ultrasonography may also be used for screening in the second trimester, either alone or as an adjunct to maternal serum testing. The use of ultrasound for prenatal diagnosis is appealing for many reasons. Its safety and noninvasive characteristics are certainly two of its most desirable traits.

Second trimester ultrasonography may identify fetal anatomic defects, such as congenital heart defect or markers suggestive of fetal aneuploidy like a thickened nuchal fold, absent nasal bone, renal pyelectasis, or echogenic bowel.

The advantages of this non invasive method are the aiming to reduce the number of women undergoing invasive prenatal diagnosis, as well as increase the proportion of Down's syndrome detection.

Invasive techniques

Prenatal diagnosis of chromosomal abnormalities is currently accomplished by invasive techniques, such as amniocentesis and chorionic villus sampling (CVS).

CVS is performed in the first trimester from 10 through 13 weeks' gestation, whereas amniocentesis can be performed starting at 15 weeks' gestation.

Fetal chromosome analysis has been traditionally performed using Giemsa banding (G-banding) of cultured cells in metaphase and is considered the gold standard detection method (14). This technique is accurate and reliable allowing the detection of a variety of numerical and structural aberrations. The diagnostic accuracy of karyotyping with amniocentesis is 99.4–99.8% (15) and for CVS 97.5–99.6% (16).

The primary disadvantage of the conventional cytogenetics is that the prenatal tissue must be cultured for several days prior to analysis. It

takes 10 days to obtain results and has a culture failure rate of about 1% (17).

Advances in molecular genetics, using either fluorescence in situ hybridization (FISH) (18) or quantitative fluorescence-polymerase chain reaction (QF-PCR) (19), can be applied to give karyotype results within one or two days. Fluorescence in situ hybridization on uncultured amniotic fluid cells using chromosome-specific DNA probes offers the opportunity for rapid screening of aneuploidies and has become an integral part of the current practice in many clinical cytogenetics laboratories. Aneuploidies involving chromosomes 13, 18, 21, X and Y account for the majority of all chromosome abnormalities in live-born infants. Rapid diagnosis of fetal chromosome anomalies may facilitate clinical decision making, especially when a fetal abnormality is detected late in pregnancy.

This study aimed at different aspects of pregnancy associated chromosomal abnormalities, such as prenatal screening, frequency assessment, cytogenetic analysis issues, correlations between indications and results. ❏

MATERIALS AND METHODS

Amniocentesis is the most common invasive prenatal procedure for the detection of fetal chromosomal abnormalities.

Indications used to classify the pregnant patients as high-risk pregnancies for prenatal diagnosis were as follows: abnormal maternal serum screening (37.45%), advanced maternal age (AMA) (≥35 years; 14.06%), abnormal ultrasonographic (US) findings (3.97%), family history of chromosomal abnormalities (1.73%), abnormal ultrasonographic (US) findings + family history of chromosomal abnormalities (0.09%), abnormal maternal serum screening +AMA+ family history of chromosomal abnormalities (0.86%), abnormal maternal serum screening + family history of chromosomal abnormalities (1.12%), AMA+ family history of chromosomal abnormalities (0.95%), AMA +abnormal US findings (1.98%), abnormal maternal serum screening +AMA (25.88%), abnormal maternal serum screening +AMA + abnormal US findings (1.98%), abnormal maternal serum screening + abnormal US findings (2.93%) and others (6.99%).

Investigation for chromosomal anomalies was routinely performed by cytogenetic analysis and FISH. The traditional "gold standard" for prenatal diagnosis of chromosome abnormalities is metaphase analysis by G- banding. ❏

RESULTS AND DISCUSSION

The prenatal screening results (the maternal serum screening and echography) and personal data were taken from the patients' files that were performed amniocentesis for cytogenetic diagnosis; most of these patients were considered high risk patients. Between April 2009 and December 2010, 1159 patients of the Medlife Clinic with positive screening tests were performed cytogenetic tests.

The patients that were recommended amniocentesis procedure were 20-45 years old with the following distribution: younger or at 20 (0.43%), 21-25 years old (4.57%), 26-30 years old (22.95%), 31-35 years old (34.51%), 36-40 years old (32.01%) and 41-45 years old (5.52%).

Regarding the gestational age, the amniocentesis was performed between 13 and 25 weeks of gestation, with a peak at 16-20 weeks. The most frequent indications for amniocentesis were: abnormal maternal serum screening, advanced maternal age, abnormal ultrasonographic (US) findings and family history of chromosomal abnormalities.

The amniocentesis was followed by the karyotype analysis and the FISH test analysis. From a total number of 1159 pregnant women, 131 of them opted for conventional karyotype, 181 of them requested the FISH test, and 847 patients requested both tests.

Regarding the results of the karyotype performed on embryo-fetal products, in 92.94% of the cases the analysis showed normal karyotype but 2.56% of the fetuses had abnormalities of the chromosomal numbers, with the following distribution: 17 cases of trisomy 21 (the most common finding 1,74%), 2 cases of trisomy 18 (0.2%), 3 cases of trisomy X (0.31%), 1 cases of trisomy XXY (0.1%), 1 case of trisomy XYY (0.1%) and 1 case of triploidy (0.1%). Structural chromosomal abnormalities (inversions and translocations) were found in 4.50% of cases.

The FISH analysis showed that 97.47% of the pregnancies had normal chromosomes and only 2.53% of the cases had abnormalities in the number of the chromosomes. Chromosomal abnormalities identified by FISH technique

were 19 cases (1. 85%) of autosomal aneuplodies and 7 cases (0.68%) of gonosomal aneuplodies.

For the numeric abnormalities, it is critical to do a correlation between the karyotype and FISH interphasic diagnosis.

In our study we identified 30 cases with abnormal numeric chromosomes, and we confirmed the results in 21 of the cases, using both diagnosis techniques. Four cases were identified only by doing the karyotype, because FISH could not be done; for the rest of the cases, we used FISH because the patients did not want the karyotype to be performed. The main reasons we failed to use the results from FISH test were the following: first, the amniotic fluid was contaminated with blood when it should have been clear, and secondly, problems with the technique such as the absence of hybridization or abnormalities in the process of hybridization that did not allow us to obtain a proper number of cells to analyze.

There were no instances of false – positive (abnormal report, by FISH shown to be normal on cytogenetics) or false – negative (normal result by FISH, diagnosed as aneuploid for the tested chromosomes by cytogenetics) autosomal or sex chromosomal results. Similar results were obtained by Lim, Pergament, Sung – Hee in their studies (20-22). Chromosomal abnormality, such as inversion and translocations were not be detected by interphase FISH analysis (23).

Correlations between the screening test results and the prenatal diagnosis results

From the total number of patients (1159), only 2.59% patients were pregnant with fetuses that had numerical chromosomal abnormalities.

Other studies showed a higher incidence of the numerical abnormalities: 4.61- 4.85% (20, 21). Other studies had similar results: 2.01% (22).

Analyzing both the maternal age and the abnormalities in karyotype (Table 1), we conclude that the percentage of the pregnancies with trisomy 21 was higher for the pregnant mothers that were 41-45 years old (3.1%) than for those that were 26-30 years old (1.8%). Similar results were reported by Sung – Hee et al: 2.17% for pregnant mothers on 41-45 years old and 1.23% for pregnant mothers on 26-30 years old. This observation indicates that there is a risk of trisomy 21 that is increasing with the age of the mother. In the case of the other chromosomal abnormalities (trisomy 18, trisomy X, trisomy XYY, triploidy), these were more frequently seen in the pregnant mothers who were 26-30 years old (5 cases) rather than when the mothers were 41-45 years old (no cases). We conclude that there is no correlation between these anomalies and maternal age.

In the 1980s, amniocentesis was used primarily for those in advanced maternal age groups, at least 35 years old. Yang reported that the most common indication of amniocentesis for rapid prenatal diagnosis of chromosomal aneuploidies by FISH was due to advanced maternal age (24).

As seen in Table 2, we used a combination of methods for the detection of various syndromes. The maternal serum screening was a critical step in detecting Down syndrome, when it was requested as single indication or combined with AMA and abnormal US findings. From a total number of 814 cases with positive double or/and triple test, after using prenatal diagnosis methods, only 21 cases were confirmed as having numerical chromosomal abnormalities. The echography was extremely

Maternal age (years)	No. of pacients (%)	No. of aneuploidies detected by FISH /Conventional Cytogenetics								
		Trisomy 21	Trisomy 18	Trisomy 13	Trisomy X	Monosomy	Trisomy XYY	Trisomy XXY	Triploidy	Total
≤20	5 (0.43)	0	0	0	0	1	0	0	0	1
21-25	53 (4.57)	0	1	0	1	0	0	0	0	2
26-30	**266 (22.95)**	**5**	1	0	1	0	2	0	1	10
31-35	400 (34.51)	5	2	0	0	0	0	0	0	7
36-40	**371 (32.01)**	**6**	0	0	1	0	0	1	0	8
41-45	64 (5.52)	2	0	0	0	0	0	0	0	2
TOTAL	1159 (100)	18	4	0	3	1	2	1	1	30

TABLE 1. The distribution of the abnormal numbers of chromosomes that were identified by FISH and/or conventional karyotype analysis, as a function of maternal age

Indications	No. of pacients (%)	No. of aneuploidies detected by FISH /Conventional Cytogenetics								
		Trisomy 21	Trisomy 18	Trisomy 13	Trisomy X	Monosomy	Trisomy XYY	Trisomy XXY	Triploidy	Total
Abnormal maternal serum screening	**434 (37.45)**	6	1	0	2	0	1	0	0	10
Advanced maternal age (AMA) (≥35 years)	163 (14.06)	2	0	0	0	0	0	1	0	3
Abnormal ultrasonographic (US) findings	**46 (3.97)**	0	2	0	0	1	1	0	1	5
Abnormal maternal serum screening +AMA	**300 (25.88)**	5	0	0	0	0	0	0	0	5
Abnormal maternal serum screening +AMA + Abnormal US findings	23 (1.98)	1	0	0	0	0	0	0	0	1
Abnormal maternal serum screening + Abnormal US findings	34 (2.93)	4	1	0	0	0	0	0	0	5
Others	159 (13.71)	0	0	0	1	0	0	0	0	1
TOTAL	1159	18	4	0	3	1	2	1	1	30

TABLE 2. The analysis of the recommendations for amniocentesis that conducted to the discovery of the numerical chromosomal abnormalities

helpful in identification of the Turner syndrome and triploidy. In literature, the majority of the fetuses with abnormal chromosomes had also abnormal US findings (6.5%, 8.9%, 5.3%) (25-27). In our study, 11 out of 127 cases that presented echographic abnormalities had also abnormal chromosomes, indicating that the amniocentesis had a high predictive value (8.66%). ❑

CONCLUSIONS

The prenatal screening is the first step towards a prenatal diagnosis of the congenital abnormalities. The goal of the screening is to identify the fetuses at high risk to have a congenital abnormality; after the screening they will be further investigated using invasive methods such as amniocentesis and the biopsy of the chorial villi. Using the screening tests allow us to avoid these potential damaging procedures for the unaffected fetuses. ❑

CONFLICTS OF INTEREST

None to declare.

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