Prenatal Screening of Cytogenetic Anomalies - A Ten Year Retrospective Study on 1510 Cases

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ABSTRACT

Introduction: Prenatal diagnostic is a diagnostic method which is used to prove the presence of chromosome changes, a large number of metabolic disorders and other morphological fetus abnormalities. Prenatal genetic testing mostly refers to the molecular genetic and cytogenetic methods used during pregnancy to diagnose genetic fetal conditions.

Aim: To investigate the existence and incidence of cytogenetics abnormalities in fetuses.

Material and Methods: The retrospective research is based on cytogenetic analysis of the 1510 amniotic fluid samples collected from pregnant women sent to the cytogenetic laboratory from January, 2012 to December, 2022.

Results: The karyotype without visible structural and numerical changes was detected in 96.8% (1462/1510) cases. The fetal karyotype was abnormal in 3.2 % (48/1510) of the cases. Trisomy 21 was the most frequent chromosome aberration detected in 1.12% (17/1510) cases followed by pericentric inversion 9 (10/1510; 0.66%) and trisomy 18 (4/1510; 0.26%). Mosaics were detected in five cases (5/1510; 0.33%). Comparing the prevalence of chromosome abnormalities according to maternal age, we come to know the prevalence of chromosome aberrations in the group of females above age 35 (26/790; 17.2/1000) is higher than in the group of females under age 25 (7/95; 4.63/1000), but not significantly different (P≈ 0.09).

Conclusion: Conventional cytogenetics maintains its role as a powerful diagnostic tool in detecting chromosomal changes during prenatal screening.

Keywords: Cytogenetic aberrations, invasive prenatal diagnostic, maternal age.

I. INTRODUCTION

According to the World Health Organization statistics, genetic and congenital disorders occur in about 2%-5% of all live births. These changes cause about 50% of childhood deaths in developing countries [1]. They are also responsible for perinatal and neonatal mortality in the world, mostly in developing countries [2]. Genetic abnormalities, except mortality, cause mental retardation, blindness and deafness. Some of them in most cases cause aesthetic defects which do not affect the quality of life of the individual and do not interfere with body function [3]. One of the most important causes of birth defects are chromosomal abnormalities [4]. For that reason, any prenatal screening program is so important. In the prenatal diagnosis of possible fetal anomalies, a large number of methods are in use. They can be divided into two groups: non-invasive and invasive. The most widely used non-invasive method is ultrasonography during pregnancy which can be combined with biochemical markers including the double-marker test, the triple-marker test, or invasive procedures such as amniocentesis [5].

A significant step in the early diagnosis of fetal abnormalities and karyotype changes is the introduction of transvaginal ultrasound examination at the end of the first trimester of pregnancy. The goal is the search for morphological anomalies, as well as ultrasound indicators of chromosomal aberrations based on phenotypic and structural characteristics that were found to be highly correlated with chromosomopathies [6]. If positive markers are found it is suggested to perform a biochemical screening. If according to the results of this test, the risk is greater than 1:100, karyotyping is suggested [7]. In pregnant women older than 35 years or in those who already have children with chromosomal abnormalities, biochemical screening should not be done because there is already an indication for cytogenetic analysis [8].

Reliable diagnosis of karyotype abnormalities is possible only by cytogenetic analysis of fetal cells. Between the 16th and 18th week, under ultrasound control, a sample of amniotic fluid is taken transabdominal to perform karyotyping of fetal cells. Analysis of cultured cells from the amniotic fluid by cytogenetics methods is still considered the gold prenatal diagnostics standard. Using this method, it is possible detection of structural and numerical chromosome aberrations, as well as possible mosaicism [9].
In this retrospective study, we aimed to investigate the presence of chromosome aberrations and their incidence in fetuses, using amniotic fluid as a sample.

II. PATIENTS AND METHODS

A. Patients

The retrospective study was carried out at the University Clinical Center Tuzla (UCC), Department of pathology, for the period between January, 2012 to December, 2022. A laboratory history, including maternal age, indications for prenatal diagnosis (amniocentesis) and cytogenetics examination results were obtained from 1510 pregnant women. The median age of all participating women was 33.2 years (18 to 43). The indications for invasive prenatal diagnostic are shown in Table I.

B. Karyotyping

Prenatal cytogenetic analysis was done according to the standard adopted laboratory protocol. At least 15-25 mL of amniotic fluid, received in the laboratory is centrifuged at 1000 RPM/10 minutes. Finally, we used a cell pellet containing 2.4 mL Amniomed plus complete medium (Euroclone, Italy). Whenever possible, four in situ cultures were set up, including one more additional backup culture. All cultures were grown on 35x10 mm cell culture Petri dishes with cover glass. The cultures were incubated at 37°C with 5% CO₂ for 7 to 9 days, changing the complete medium every second day. The cultures were usually harvested when at least 5 amniocyte colonies were seen on each cover glass in Petri dishes. After exposing the amniocytes to colcemid solution and hypotonic suspension (sodium citrate/potassium chloride), cells were fixed with a mixture of acetic acid and methanol. The coverslips are removed from the Petri dishes, then glued to the slides and dried at the temperature of 90°C for one hour. Chromosome slides are then stained using the Giemsa-Trypsin banding method. At least 15 metaphase cells per patient were completely analyzed. The karyotypes were interpreted according to An International System for Human Cytogenetic Nomenclature 2016[10].

C. Statistical Analysis

We used Microsoft Excel 2010 for data analysis. Descriptive statistics were processed using the Chi-square test ($\chi^2$), at the level of statistical significance ($P<0.05$).

III. RESULTS

From 2012 to 2022, a total of 1510 amniotic fluid samples underwent prenatal cytogenetic diagnostics. The majority of samples had karyotype without visible structural and numerical changes (1462; 96.8%). The fetal karyotype was abnormal in 48/1510 (3.2%) of the cases.

Among abnormal karyotypes, the most frequent chromosome aberration was trisomy 21 (17/1510; 1.12%), followed by pericentric inversion 9 (10/1510; 0.66%) and trisomy 18 (4/1510; 0.26%). Mosaics were detected in five...
cases. A summary of the chromosomal aberrations found in amniotic fluid samples and their prevalence is shown in Table II.

Out of 48 cases where chromosomal aberrations were detected, 35 (72.9%) cases had abnormal ultrasound findings including nuchal translucency, 25 (52.1%) of these cases were studied due to advanced maternal age, 5 (10.4%) had pathological prenatal tests (triple, double or combined) and 2 (4.2%) cases had a previous history of children with genetic disorders or burdened family history (Down syndrome in family, previous offspring born with malformation, spontaneous abortion).

We also compared the prevalence of chromosome abnormalities according to maternal age. The prevalence of chromosome aberrations in the group of females above age 35 (26/790; 17.2/1000) is higher than in the group of females under age 25 (7/95; 4.63/1000), but not significant (P= 0.09) (Table III).

### IV. DISCUSSION

Prenatal screening can be divided into non-invasive and invasive tests. Non-invasive prenatal examination, including biochemical tests from maternal blood and ultrasound examination, can detect many fetal malformations, which reduce the birth of children with phenotype defects and also affect the reduction of perinatal morbidity and mortality. Special attention is also put on invasive tests, including chorionic villus sampling, amniocentesis and cordocentesis [11]. These tests are more sensitive regarding the detection of chromosome abnormalities [12]. However, the birth of children with chromosomal abnormalities is still a major problem even in countries with highly developed prenatal care.

Although the aim of our study was to investigate the existence and prevalence of cytogenetics abnormalities in fetuses, we also paid attention to the pregnant women who were referred for amniocentesis.

As in many other studies [13], we found the most prominent indication for prenatal diagnostic (amniocentesis) was the advanced maternal age. Some other authors conclude that the most important indicator for prenatal diagnostic is abnormal ultrasound findings or maternal serum screening tests [14], [15]. Correlating the karyotype abnormalities and maternal age (Table III), we conclude that the total prevalence of cytogenetics abnormalities increased by almost 4-times in women aged ≥ 35 years compared with those aged <25 years. These findings are in concordance with most references [8], [16]. Reference [17], in his research, also reported the abnormal karyotype and advanced maternal age have a statistically significant correlation with \( r = 0.4783 \) (P < 0.01). However, some of the authors [12] believe that there is no correlation between chromosome anomalies and maternal age.

Data derived from our study show 3.2% (48/1510) of unborn fetuses that had an abnormal karyotype. These data are mostly similar to other relevant publications, while in some cases they are significantly different (Table IV).

### TABLE IV: A COMPARATIVE OVERVIEW OF THE FREQUENCY OF ABERRANT KARYOTYPES IN OUR STUDY WITH DATA DERIVED FROM THE LITERATURE

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample size</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference [18]</td>
<td>6278</td>
<td>2.0</td>
</tr>
<tr>
<td>Reference [19]</td>
<td>1234</td>
<td>2.19</td>
</tr>
<tr>
<td>Reference [20]</td>
<td>29883</td>
<td>2.9</td>
</tr>
<tr>
<td>Reference [21]</td>
<td>564</td>
<td>5.5</td>
</tr>
<tr>
<td>Reference [22]</td>
<td>1728</td>
<td>7.2</td>
</tr>
<tr>
<td>Reference [23]</td>
<td>2500</td>
<td>8.24</td>
</tr>
<tr>
<td>Reference [24]</td>
<td>1441</td>
<td>4.55</td>
</tr>
<tr>
<td>Present study</td>
<td>1510</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Our study revealed that the majority of the karyotype abnormalities were numerical (30/48; 62.5%), which is consistent with the research findings obtained from Europe [25]. The most common autosomal chromosome aneuploidy detected was trisomy 21 (11.3/1000; 35.41%), followed by trisomy 18 (2.6/1000; 8.3%). Numerical aberrations of the gonosomal chromosomes were found only in two cases (1.3/1000; 4.2%) (Table II). A retrospective analysis of 283890 perinatal infants, referred for amniocentesis, showed that the most commonly detected chromosome aneuploidy was trisomy 21 (67/1000; 59.6%), followed by trisomies 18 (11/1000; 10%) and 13 (2/1000; 1.9%) [26]. In a review, [25] also reported the majority of chromosome aberrations found in fetuses are trisomies, mostly of the chromosomes 13, 18 and 21. We also noticed one 47,XY,+20/46,XY mosaic case (2.1%). According to available literature, most cases with trisomy 20 mosaicism detected during prenatal cytogenetic screening have a good chance of a normal outcome [27]. These are mostly prenatal cases where less than 50% of cells containing trisomy 20 were detected by karyotype analysis [28], [29] as in our study.

The majority of the remaining cases (18/48; 37.5%) were diagnosed to have structural chromosome aberrations without knowledge of whether a parental chromosomal aberration was present. Here we mean first of all the pericentric inversion of chromosome 9, which some authors consider a normal variant in the population [10]. In our study, two male karyotypes were containing Robertsonian translocation in a balanced state. This finding correlates with data from other studies. Namely, several researchers conclude that in more than 75 per cent of cases, Robertsonian translocation occurs in male carriers and arises as a result mostly of alternate segregation mode during the meiotic division [30], [31]. Four more balanced and two unbalanced chromosome aberrations of different autosome and sex chromosomes were presented in our study (Table II). In both cases, carriers of balanced or unbalanced chromosome changes have an increased risk to have at least a phenotypic abnormality.

Reciprocal translocations or numerical chromosome changes which are found in the fetal karyotype occur during gametogenesis as a result of wrong segregation at meiosis.

**TABLE III: CORRELATION BETWEEN MATERNAL AGE WITH CHROMOSOMAL ANEUPLOIDY AND STRUCTURAL CHROMOSOME ABERRATIONS**

<table>
<thead>
<tr>
<th>Age group</th>
<th>Fetuses with karyotypic changes (n)</th>
<th>Numerical chromosome changes</th>
<th>Structural chromosome changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>Prevalence (1000)</td>
<td>n</td>
<td>Prevalence (1000)</td>
</tr>
<tr>
<td>&lt;25</td>
<td>95</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>25-35</td>
<td>625</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>≥35</td>
<td>790</td>
<td>26</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>1510</td>
<td>48</td>
<td>30</td>
</tr>
</tbody>
</table>

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These unbalanced gametes may lead to recurrent abortuses and multiple malformations in carriers [32]. So, in all of these cases genetic counselling is required, including all future pregnancies in which risk is increased.

In recent times, in fear of spontaneous abortions, we have witnessed that many pregnant women choose non-invasive prenatal tests instead of invasive screening. Some of them avoid prenatal screening altogether due to religious reasons. Although it must still be an individual decision of the pregnant woman and her partner, patients should be counselled and informed about the advantage of all of these methods and their limitations.

Our study supports cytogenetics analysis of amniotic fluid because it effectively reveals chromosomal changes during the prenatals period, reducing the rate of abnormal karyotypes in live-born children and also minimising social as well as family trauma of children with mental or psychophysical dysfunction.

V. CONCLUSION

Although we are living in the era of modern molecular genetics techniques, conventional cytogenetics maintains its role as a powerful diagnostic tool in detecting chromosomal changes during prenatal screening.

CONFLICT OF INTEREST

The authors declare that they don’t have any conflict of interest.

REFERENCES