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THE INCIDENCE OF CHROMOSOMAL ABERATIONS DETECTED PRENATALLY USING AMNIOCENTESIS IN GENERAL HOSPITAL UŽICE DURING THE PERIOD 2006-2014

Abstract: Objective. The main objective of this paper is to present for the first time results of prenatal diagnosis, the incidence of chromosomal aberrations, in the General Hospital Uzice, with special reference to trisomy 21.

Methods. This study included a group of 749 pregnant women who underwent invasive prenatal diagnosis using amniocentesis between September 2006th to the August 2013th at the Department of Gynecology and Obstetrics. The total number of delivered women during the same time period was 12 447. Karyotype analysis of *in vitro* cultured fetal cells was performed using standard techniques on Cytogenetic Department, Clinical Center Kragujevac. All collected data were analyzed and even acknowledge the graphic.

Results. Cytogenetic analysis revealed 29 fetal chromosomal abnormalities (3,9%). From the total number of chromosomal aberrations was revealed 14 unbalanced chromosomal rearrangements (48%) and 15 balanced chromosomal aberrations (52%). The seven trisomy of chromosome 21, one triploidy, one deletion, one duplication, two mosaic trisomies, two numerical aberrations of sex chromosomes were detected. The balanced chromosomal rearrangements revealed 15 inversion of chromosome 9. Trisomy 21 makes 24% of all total chromosomal aberrations and 50% of unbalanced rearrangments. The incidence of Down syndrome prenatally dijagnostifikovanog is one of 107 amnion.

Keywords: prenatal diagnosis, amniocentesis, chromosomal aberrations.

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Introduction

Chromosome aberrations (lat. aberatio-deviation from normal) are changes in the genome that include whole chromosomes or parts of it, deviation from the normal structure, and the number and, or shape of chromosomes (1). Aberration can affect any chromosome or chromosomal region and clinical consequences depend on the nature of genetic material include in aberration and the extent of the affected region, as well as the number and nature of the affected genes. Chromosomal aberrations occur with an incidence of approximately 7.5%. However, chromosomal aberrations usually lead to large birth defects causing early spontaneous abortions so that the frequency of chromosomal aberrations at births is reduced to 0.6%. Recent studies have shown that 15% of congenital anomalies detected in children before the age of 1 year constitute chromosomal aberrations (2). Depending on whether they represent the change in the structure or number of chromosomes there are two types: numerical aberrations (changes in chromosome number) and structural aberrations (changes in chromosome structure). Structural aberrations occur as a consequence of chromosomal breaks where there may be loss of genetic material, moving a broken chromosome fragment to another chromosome or to another location within the same chromosome. As a result of these structural changes, there is a change in the linear arrangement of genes. Structural aberrations may be divided into balanced and unbalanced structural aberrations. The difference between balanced and unbalanced structural aberrations depends on whether the total amount of genetic material is altered or not. If the total amount of genetic material is unchanged, only is modified linear arrangement of genes, it is a balanced aberration. Holder of a balanced structural aberrations is phenotypically normal, but carry a high risk of getting an aberrant offspring. In the case of unbalanced aberrations change in gene dosage leads to genetic imbalances caused by deletion or duplication of certain genes, which results in the manifestation of pathological symptoms in carriers.

The most common numerical chromosomal aberration, with an incidence of approximately 1: 800, is a trisomy of chromosome 21 which is clinically manifested as Down syndrome (DS). It was first described by Thomas Langdon Down in 1866. Common clinical features of the patients with DS are an IQ between 40 and 50, heart anomalies accompanied in varying degrees anomalies of other organs, wide-set eyes, lack of skin elasticity, giving the appearance of excess skin on the body and flat face with small nose. Valentin et al in 1968 first discovered prenatally trisomy of chromosome 21 from a sample of amniotic fluid, and since then it is possible to do prenatal detection of Down syndrome in pregnant women by early amniocentesis (3). The most cases of Down syndrome are due to an extra chromosome 21 in fetus because of non-disjunction of chromosomes at meiosis in the mother (4). Only in very few cases occur so-called translocation Down arising as a consequence of the presence of Robertsonian translocation between chromosome 21 and another acrocentric chro-

mosomes in one of the parents. There was a relationship between age of pregnant women and the incidence of Down syndrome. Thus, for example, the probability that a woman of 30 years fathered a child with Down syndrome is 1: 910, for a woman of 35 years of 1: 350, whereas in women 40 years of age, this probability is significantly higher 1: 100. It should be noted that in 10-24% of pregnancies with trisomy 21 spontaneous abortions during the early gestation is very common, so the actual incidence of Down syndrome is higher. However that risk the 35 year old pregnant woman is 1: 270 (5). The most reliable method for the detection of Down syndrome is amniocentesis, which is performed between the 16th and 18th weeks of gestation, and the most common indication for amniocentesis are age of pregnant women (> 35). In addition to the age, indications for prenatal diagnosis are pathologic values of biochemical markers in the first and second trimester of pregnancy isolated from the serum of pregnant women. In the first trimester of pregnancy double test are determined by the value of the two markers (free beta-hCG free beta chorion gonadotropin and PAPP-pregnancy associate plasma protein A) and together with the results of NT thickness (nuchal translucency). On the basis of these values the risk is calculated for each pregnancy using the individual risk PRISKA standardized computer methods (9). Biochemical markers whose concentration are tested in the second trimestry (triple test AFP alpha-feto protein, HCG total chorion gonadotropin, and unconjugated estriol-uEstriola) are important in calculating individualnog risk for pregnant women in the assessment of the existence of the embryo with aneuploidy as well as the presence of of certain defects in the development of the neural tube (6).

In addition to trisomy of chromosome 21, others trisomies that occur most frequently in the population are trisomies of chromosomes 18, 13 as well as aneuploidy of sex chromosomes. Trisomy 18 or Edwards syndrome incidence is approximately 1: 5000 live births. The phenotype of carriers of trisomy 18 is characterized by mental retardation, intrauterine growth retardation, cranio - facial abnormalities, low set ears, small mouth, narrow palpebral fissures, short toe, esophageal atresia, tracheo - esophageal fistula, spina bifida (7,8). Trisomy 13 or Patau syndrome occurs with an incidence of about 1: 12 000 live births. For phenotype holder is characteristic microcephaly, neural anomalies, congenital heart anomalies, kidney anomalies and anomalies of genital organs (7,8). The changes in the number of sex chromosomes are the most common monosomy X chromosome known as Turner syndrome (45, X) as well as Klinefelter syndrome (47, XXY) (9,10).

The main objectives of this study were to determine the frequency of chromosomal aberrations detected prenatally using amniocentesis in the period from 2006 to 2014. in the Department of gynecology and obstetrics in the General Hospital Uzice, in order to evaluate importance of prenatal diagnosis to prevent the birth of children with Down syndrome in the first place, as well as other chromosomal aberrations. We emphasis the incidence of Down syndrome, prenatal detected in relation to the incidence of other chromosomal aberrations.

Material and Methods

This retrospective study included a group of 749 pregnant women who underwent invasive prenatal diagnosis at the Department of Gynecology and Obstetrics in the General Hospital Uzice in the period from 2006 to 2014 years.

Indications for prenatal diagnosis were age of pregnant women (over 35 years); results of biochemical analysis of fetal markers in maternal serum obtained by prenatal screening; genetic hereditary disease in the family; fetus or child born with multiple congenital anomalies; presence of balanced chromosomal rearrangements (reciprocal translocations, Robertsonian translocations, inversions) in one of the spouse.

Amniocytes were obtained by puncture of the amniotic fluid of pregnant women in the 16-18-th week of gestation. Amniocytes were cultured in vitro for 10-20 days. Chromosomes were isolated by standard techniques of preparation. After preparation, chromosome preparations were stained with the classical technique of staining technique and G tape and then analyzed under a light microscope. The failure of culturing cells occurs in less than 1%.

Before any intervention, pregnant woman was informed on Genetic about the type of intervention and the type of its implementation, and methods risk and possible complications. The women were informed that a given analysis examined only a specific problem, not the state of health of the fetus. The decision to continue or terminate the pregnancy decide a pregnant woman after detailed talks on the issue with a team of professionals who make a gynecologist, a pediatrician and geneticist, as well as the Ethics Committee which makes the final decision on the outcome of pregnancy. Statistical analysis included descriptive method. Presentation of data is given in the form of percentage values. The results are shown as graphs and tables.

Results

In our work in the period from September 2006 to August 2014, 749 amniocentesis were performed. This study included only patients in whom prenatal diagnosis using amniocentesis were performed at the Department of Gynecology and Obstetrics in the General Hospital Uzice. Total number of delivery during eight years amounted to 12 447 including a group of pregnant women who underwent amniocentesis. In the examined time period on average about 6% of pregnant women were included invasive prenatal diagnoses. Indications for invasive prenatal diagnosis are 63% of cases were age of pregnant women (pregnant women older than 35 years), followed by 34% of abnormal findings of biochemical markers screening of the first and second trimester of pregnancy. A positive family history is an indication only in 3% of cases (Figure 1).

Based on our results, it can be observed an increasing trend in the number of amniocentesis during seven years of screening. Since from September 2006, the num-

ber of amniocentesis was 16. In 2007, that number had increased and amounted to 72 pregnant women. In 2008, the number of amniocentesis was 79, in 2009 there were 66 amniocentesis. 2010 was done 89, 2011. There were 85 and 2012 amniocentesis done a 104, and 2013 amniocentesis were 149 in 2014, to August 89 was the same.

Cytogenetic analysis revealed 29 chromosomal aberrations (Table 1). It was detected 14 of unbalanced chromosomal aberrations (48%) and 15 balanced chromosomal aberrations (52%). It was detected seven trisomy of chromosome 21, one triploidy, one deletion, one duplication, two trisomy in the mosaic, two numerical aberrations of sex chromosomes. Also we discovered 15 balanced chromosomal rearrangements and inversions of chromosome 9. The total percentage of chromosomal aberrations in the test sample is 3.9% with inversions, or 1.9% without balanced chromosomal rearrangements. Trisomy of chromosome 21, as the most common aneuploidy, accounting for 24% of all total chromosomal aberrations and 50% of unbalanced chromosomal rearrangements. When setting the indication for invasive prenatal diagnostic criteria we used recommended medical indications as shown by the fact that for every 26 performed amniocentesis we revealed a chromosome abnormality.

Amniocentesis revealed a seven cases of Down syndrome. Cytogenetic analysis detected the presence of 47 chromosomes in the karyotype, trisomy of chromosome 21 (47, XX or 47, XY, +21). Indications for amniocentesis in four cases were age of pregnant women (35, 36, 38 and 42 years). While in the three cases indications were pathological values of double test. All seven pregnancies were terminated at the parents' request and in accordance with the decision of the Ethics Committee.

In addition to trisomy 21 in the examined time period cytogenetic analysis revealed one triploidy. Chromosomal aberrations 69 XXX is detected in the fetus after amniocentesis. The patient was 25 years old and amniocentesis was performed due to bad values of serum markers during routine biochemistry screening in the first trimester. Pregnancy is terminated on request of the patient and the Ethics Commission.

Prenatal was discovered a mosaic karyotype of chromosome 20 in a patient during a routine amniocentesis for prenatal analysis indicated by age of pregnant woman. Trisomy was detected in 53% of the analyzed cells. A detailed ultrasound examination did not show any changes in the developing fetus, as well as anomalies or developmental delay. Karyotype analysis of fetal blood from the baby during cordocentesis was not indicated the presence of a mosaic. Parent is informed in detail on Genetic counseling about the possible consequences of prenatally detected mosaic chromosome 20. They decided to keep the pregnancy, after delivery male baby with normal phenotype without clinical signs of anomalies was born. Numerical sex chromosome aberrations were detected in two cases. In pregnant women due to age, she underwent prenatal fetal karyotype analysis and the result showed 47 XYY karyotype fetus. Pregnancy is retained and the phenotype of a male newborn was normal. In the second case because of age underwent prenatal diagnosis of fetal phenotype obtained was a 47 XXX.

In addition to the a forementioned abnormalities we detected a deletion of the distal region of chromosome 1, a partial duplication of chromosome 22 and one trisomy of chromosome 17 in a mosaic in patients older than 35 years. Also we discovered 15 inversion of chromosome 9 with the breakpoint p11q13. To determine the origin of inversions whether they are *de novo* or inherited balanced aberration, in all cases of prenatally diagnosed inversion of chromosome 9, parents karyotyp were analyzed. All of the examined fetuses carried the inversion inherited from one parent, usually the mother. The clinical picture of children at birth was completely normal.

Discussion

Amniocentesis is the most commonly used invasive prenatal diagnostic methods. Amniocentesis of all invasive method has the lowest risk to the fetus and the pregnant woman and therefore represents the gold standard method in prenatal diagnosis. The method is in the Department of Gynecology and Obstetrics done routinely for fifteen years. We presented the results of the last eight years in the interval from September 2006 to August 2014. The rising trend in the number of amniocentesis annually in the investigated time period can be explained by the improvement of non-invasive methods of prenatal diagnosis, the introduction of biochemical markers screening as well as improving ultrasound screening, on the other hand the fact is an increasing number of older couples.

Incidence of pathological karyotypes with structural and numerical chromosomal aberrations in the examined time interval is approximately 1.9%. During invasive prenatal screening we detected 2% of balanced chromosomal rearrangements. The obtained results were compared with existing data. The frequency of chromosome aberrations at the Center for Medical Genetics, Children's Clinic in Novi Sad, which is very similar to 1.67%, and the frequency of chromosomal aberrations in the General Hospital of the Brcko District is 2.48% (11).

From the detected structural and numerical chromosome aberrations without balanced chromosomal rearrangements almost half or 50% is trisomy of chromosome 21. Prenatal detected four cases of trisomy have been in pregnant women older than 35 years, while three cases were discovered during screening of biochemical markers in the serum of pregnant women. All pregnancies were terminated at the request of the parents and the Ethics Committee. Based on our results, the incidence of Down syndrome is 1 in 107 amnion.

Based on these studies we can conclude that there is a correlation between maternal age and the incidence of Down syndrome. It is likely that prohibits the proper orientation of chromosomes due to less chiasmus with oocytes that have recently entered into meiosis, or are some factors external environment caused by mutations in genes involved in chromosome segregation.

An interesting fact is that it was detected a case of triploidy in fetus of pregnant women younger than 35 years in prenatal diagnosis indicated by pathological values of biochemical markers (12). The case of triploidy detected by amniocentesis due to poor findings biochemistry screening is very rare in prenatal diagnosis. In 5998 pregnant women analyzed at the Institute for Health Protection of Mother and Child Serbia "Dr Vikan Čupić" of 218 aberrant karyotype was present only one triploidy (13). The occurrence of triploidy with pregnant women younger than 35 years attests to the fact that triploidy occurs as a result of random events, usually by two sperms fertilize an egg. Triploidy is very rare with an incidence of about 2% in the fetus. In a small number of cases of pregnancy be brought to an end and my own children have multiple abnormalities (14).

Also it was discovered in a fetus with a change in the number of sex chromosomes (47 XYY). The incidence of XYY syndrome is 1: 1000 in the general population. The existence of the Y chromosome unlike other numerical aberrations of sex chromosomes does not affect the mental development of the patient. Six prospective US study showed that boys with karyotype 47XYY have an IQ of about 105.00 IQ. The phenotype of the holders of a an extra Y chromosome is without major anomalies except extremely high growth. The largest number of these men is normal, fertile and has normal offspring (15).

During karyotyping was discovered a mosaic trisomy of chromosome 20 in 53% of tested amniocytes. The carriers phenotype of mosaic chromosome 20 vary from completely normal, to the phenotype characteristic of cases of complete trisomy of chromosome 20. The presence of phenotypic anomalies depends on the percentage of trisomic cells detected prenatally (16). Although there is a greater degree of mosaicism baby was born without any visible structural anomalies.

Based on our study, the incidence of inversion of chromosome 9 with the breakpoint p11q13 is about 2%, which corresponds to the values characteristic of this inversion in the general population, 1.8% (17). All children born with inversion of chromosome 9 had a normal phenotype. Inversion of chromosome 9 (inv p11q13) has no phenotypic expression because it is affected by heterochromatic region of secondary constriction. Analyses have shown that inversion in any analyzed case in our study did not arise *de novo* but is inherited from one parent.

Conclusion

Propagating the opportunities provided by invasive prenatal diagnosis is essential in improving public health and preventing the birth of children with chromosomal aberrations. The incidence of chromosomal aberrations obtained by prenatal diagnosis is 3.9% in the analyzed time interval from September 2006 to August 2014, with 1,7 % of balanced chromosome aberrations. At the same time it was discovered seven

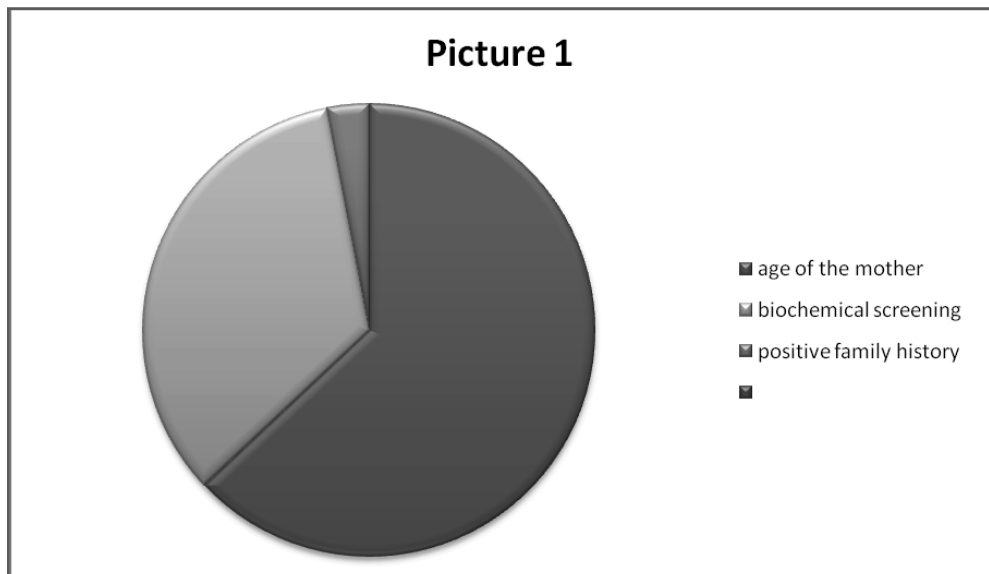
cases of trisomy 21 which highlights the great importance of applying methods of invasive prenatal diagnostic amniocentesis primarily in modern medicine.

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Figure 1. Graphical display that present the percentage of indications for prenatal diagnostics.



Number of detected chromosome aberrations, percentage of single chromosome abnormalities and maternal age.

Chromosomal aberration	Number of cases	% chromosomal aberrations	% age of mother ≥ 35
Trisomy 21	7	25	57
Other trisomies	2	7,2	100
Triploidy	1	3,6	0
Delecion	1	3,6	100
Duplication	1	3,6	100
Sex chromosome numerical aberrations	2	7,2	100
Inversion chromosome 9	15	54	60