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Association of Parental Age and the Type of Down Syndrome on the Territory of Bosnia and Herzegovina

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ABSTRACT

Background: Advanced paternal and/or maternal age is a classic risk factor for Down syndrome. The aim of the study was to investigate the frequency of Down syndrome types in children and its association with maternal and paternal age in Bosnia and Herzegovina. **Subjects and Methods:** The cross sectional, observational study included 127 children, 49 girls and 78 boys, aged 1-180 months suspected to have Down syndrome, admitted to the Centre for Genetics, Faculty of Medicine University of Sarajevo, for cytogenetic analysis and differential diagnosis of Down syndrome during the period from January 2010 to May 2015. Standard method of 72 hours cultivation of peripheral blood lymphocytes has been applied. The accepted level of statistical significance was $p<0.05$. **Study Results:** The most common type of Down syndrome was standard trisomy (86.6%), comparing to translocation and mosaicism (7.1%; 6.3%, respectively). The highest frequency of Down syndrome cases was in mother and father's group from 30-39 years old (57; 57 children, respectively) compared to mother and father's groups with younger than 30 (44; 29, respectively) and 40 and older (26; 41, respectively). The significant difference was found in maternal age between translocation and mosaicism groups ($p=0.036$). Difference between parental years and type of Down syndrome was significant when Standard trisomy 21 and translocation ($p=0.045$), as well as mosaicism and translocation ($p=0.036$), were compared. **Conclusion:** The most common type of Down syndrome was standard trisomy 21, with highest occurrence in parents from 30 to 39 years old. Parents were the youngest in translocation group. Obtained results suggest that multidisciplinary approach to identifying the trigger for trisomy appearance and the influence of maternal age is required.

Key words: advanced age, trisomy 21, translocation, mosaicism.

1. INTRODUCTION

Down syndrome (DS) and it's chromosomal basis is the most common genetic reason for congenital malformations in the human population (1). It has been recognized in 1933 (2), occurring with an incidence of around 1/733 newborns (3). Some people with Down syndrome are mosaics with only a proportion of trisomy 21 (T21) cells, and a minority have the relevant translocation from the chromosome 21 to another chromosome. The third, and the most common type of Down syndrome is standard trisomy 21, with an extra small chromosome, in approximately 95% of individuals (4,5). Two facts are worth consideration, in these 95% of children with Down syndrome, their extra chromosome is mothers origin, and in more than 80% of cases, the nondisjunction happened during the first meiotic division which completely takes place in the ovary (5). The most intensively studied etiological factor for the occurrence of this trisomy is

advanced maternal age (6), while other risk factors are less well established. The question is, what goes wrong during the meiotic divisions of oocytes ovulated later in reproductive lifespan?

Current understanding of the pathways leading to female meiotic errors is based on genetic studies on trisomy cases (7). All studies indicate that the incidence of female origin aneuploidy slowly increases after the age of 35 years, and dramatically after the age of 38 years (8).

Advanced paternal and/or maternal age is a classic risk factor for Down syndrome. Other risk factors include consanguinity, region (urban or rural) of residence of the family. Besides these, the parental exposure to drugs or chemicals, the habits of father, prenatal scanning, and mothers reproductive functions are potential risk factors for Down syndrome.

The study aim was to investigate the frequency of Down syndrome types in children and its association with maternal and paternal age in Bosnia and Herzegovina. Also, the aim was to investigate possible differences between maternal, paternal and parental ages, according to the Down syndrome type.

2. SUBJECTS AND METHODS

The cross sectional, observational study included 127 children, 49 girls and 78 boys, aged 1-180 months suspected to have Down syndrome, admitted to the Centre for Genetics, Faculty of Medicine University of Sarajevo, for cytogenetic analyses and differential diagnosis of Down syndrome during the period from January 2010 to May 2015.

Children's blood samples were taken in order to perform cytogenetic analysis to confirm or dismiss Down syndrome diagnosis and determinate the type of aneuploidy.

The data about case maternal and paternal ages were also obtained and the parental age was calculated by summing given mother and father's age (9). After obtaining the results of cytogenetic analyses the parental age groups were formed, as less than thirty, from thirty to forty, and forty and older (10)diagnosis, and subsequent termination of Down's syndrome pregnancies.

Standard method of 72 hours cultivation of peripheral blood lymphocytes has been applied. Instructions and rules given by International System of Human Chromosomal Nomenclature (ISCN) were followed when the cytogenetic analysis was performed (11).

The informed consent was signed by parents and all performed procedures were in accordance with ethical approval from the Faculty of Medicine University of Sarajevo. Kolmogorov-Smirnov test was used to test data normality. The data were presented as the median with range of first and third quartiles because their normality was not satisfied. The categorical variables were presented as the absolute numbers of cases and percentage.

The differences between groups were tested using chi square test for categorical and Mann-Whitney test for numerical variables. The confidence level was set at $p<0.05$. Statistical calculation was performed by using of software SPSS for Windows (version 19.0. SPSS Chicago, IL).

Type of Down syndrome, frequency		Maternal age (years)			Paternal age (years)		
		<30	≥30-39	≥40	<30	≥30-39	≥40
		Group1	Group2	Group3	Group1	Group2	Group3
Standard trisomy 21,n	110	37	51	22	23	50	37
%	86.6%	33.6%	46.4%	20.0%	20.9%	45.5%	33.6%
Translocation,n	9	6	2	1	4	4	1
%	7.1%	66.7%	22.2%	11.1%	44.4%	44.4%	11.1%
Mosaicism,n	8	1	4	3	2	3	3
%	6.3%	12.5%	50.0%	37.5%	25.0%	37.5%	37.5%
Total,n	127	44	57	26	29	57	41
%		34.6%	44.9%	20.5%	22.8%	44.9%	32.3%
Chi-Square test		X^2=6.49 p=0.166			X^2=3.52 p=0.475		

Table 1. Frequency of Down syndrome type according to parental age groups. n-Number of subjects; %-percentage of subjects in each group; p-probability; X^2-Chi-Square test

3. RESULTS

Chromosome abnormalities investigation confirmed Down syndrome diagnosis in all 127 children.

The characteristics of DS children and their parents are demonstrated in Table 1. No associations between mother and father's age and the type of Down syndrome (X2=6.49; p=0.166; X2=3.52; p=0.475, respectively) were found. The highest frequency of Down syndrome cases was in mother's group 2 (57 children) compared to groups 1 and 3 (44; 26, respectively). Considering father's age, the highest frequency of DS cases has also been noted in the group 2 (57 children), comparing to groups 3 and 1 (41; 29, respectively). The most common type of Down syndrome was standard trisomy 21 (86.6%), comparing to translocation and mosaicism (7.1%; 6.3%, respectively).

To represent whether the maternal, paternal or parental ages are different according to Down syndrome type, results are given in figure 1.

The median maternal age in all Down syndrome children was 33.0 (27.0-38.0) years, the youngest mother was 15 and the oldest 56 years old. The similar paternal age has been noticed, 35.0 (30.0-41.0) years, with the youngest father aged 17, and the oldest 58. Parental years of all Down syndrome children was 68.0 (57.0-80.0).

The oldest mothers were in the mosaic group of children 35.50 (30.25-41.50), comparing to the standard and translocation type (33.0 (27.0-38.0), 28.0 (23.0-31.0), respectively). The similar distribution was in the father's age, the oldest ones were in the mosaicism group 36.0 (30.0-45.75), comparing to standard and translocation groups (35.0 (30.0-41.0), 30.0 (27.5-32.5), respectively). With the parental ages, similar results were found. The oldest parents were in mosaicism compared to standard and translocation groups (71.5 (60.25-87.5) vs. 68.0 (57.0-80.0) vs. 57.0 (50.5-62.0), respectively).

The significant difference was found only in maternal age between translocation and mosaicism groups (p=0.036), but mothers of children with standard Down syndrome type were not statistically significantly older

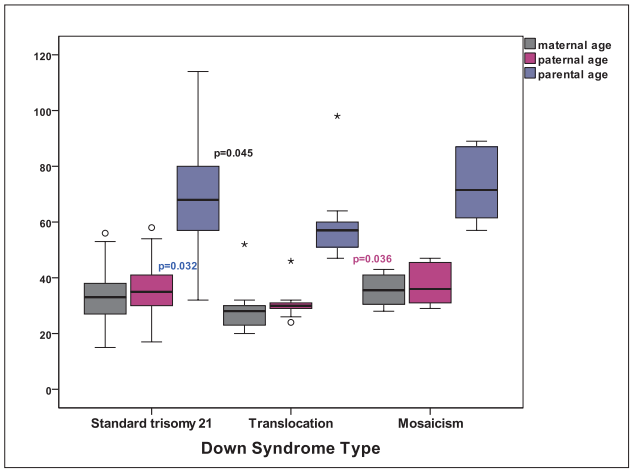

Figure 1. Association of Down syndrome type and parental age. Box-and-whisker plots present the medians of parental ages in Standard trisomy, Translocation and Mosaicism. Each bar shows upper and lower quartile, while the square and its central bar indicate interquartile range and median. P-probability; * and o-outliers

or younger compared to children with translocation and mosaicism (p=0.72, p=0.29; respectively).

Figure 1 also shows that no statistically significant difference was found between father's age among translocation and mosaicism types and standard trisomy 21 and mosaicism (p=0.059, p=0.50, respectively), but the significant difference was noted between trisomy 21 and translocation (p=0.032).

Difference between the sum of parental years and the type of Down syndrome was significant when standard trisomy 21 and translocation (p=0.045), as well as mosaicism and translocation (p=0.036), were compared.

4. DISCUSSION

A number of hypotheses could be the answer to the question what could possibly go wrong during meiotic division of oocytes related to advanced maternal age. The most interesting hypothesis about occurrence of chromosomal nondisjunction is compromised microcirculation in perifollicular capillary bed, caused by hormonal imbalance. As a result of decreased volume, reduced blood flow occurs through that area and leads to deficit of oxygen and increase of carbon dioxide and anaerobic products inside the follicle. Furthermore, intracellular pH causes displacement of a chromosome in the spindle and its nondisjunction. The compromised microcirculation hypothesis explains why women of all reproductive ages may have a Down syndrome child (12,13).

Also, age-related chromosome cohesion loss in oocytes may be female-specific as a cause of aneuploidy (14). Another hypothesis concerning the link between maternal age and nondisjunction is normal biological ovarian aging that is accompanied by changes in the level of circulating reproductive hormones and a decline in the number of antral follicles maturing per cycle. One of the factors that could impact biological ovary aging is cigarette consumption (15).

There is also a hypothesis that higher parity is associated with an increased risk of giving birth to a Down's syndrome infant for both, women under 35 years of age and for women aged 35 years or more.

An increased risk of aneuploidy is also present in women who have had many spontaneous (and intended) abortions. Maternal health and reproductive potential have a great significance in the occurrence of Down syndrome. Studies have also shown that the bigger the number of abortions and the younger the mother, the higher the relative risk of a Down syndrome birth, compared with the women of the same age with no previous abortions (17,18).

However there are contradicting reports regarding the maternal and paternal ages and the risk for chromosomal aneuploidy. Findings from our study only partially agree with above mentioned results and they reveal that 44 cases (34.6% of the studied Down syndrome children) had younger mothers, whose age was less than 30 years. Possible reason why mothers of younger age on the territory of Bosnia and Herzegovina have children with DS is that only advanced-age mothers are submitted for amniocentesis, and according to the studies, a large percentage of DS pregnancies are terminated by abortion.

Another possible explanation of younger mothers having more children with DS is usage of alcohol, tobacco, environmental toxins and drugs can induce chromosomal non-disjunction (16). Young mothers-to be are likely to be sleep deprived, have imbalanced diet in order not to put too much weight and those are, very often, unintended pregnancies which are all leading to bad pregnancy habits. Habitual risk factors are more common among young mothers and are often correlated with unwanted pregnancies.

Studies demonstrate that father's age also makes a difference when calculating the chance for genetic abnormalities. Male biological clock is also ticking for Down syndrome (19). Our study showed that highest percentage of DS children are in the group of fathers aged 30 and more and older than 40 (44.9%, 32.3%, respectively). The reasons why younger fathers (22.8% of cases) have children with DS are probably the same as those when talked about young mothers. Still, there is one additional reason, a high possibility of younger man having older partner could also contribute to such a high prevalence of DS children. It is wide-known that older men produce more sperm with aneuploidy (1).

The small increase in the number of older mothers' effects the number of Down's syndrome pregnancies because the risk of a pregnancy affected with aneuploidy is highly increased for older mothers; the risk for a mother aged 40 years is 16 times of that compared to 25 year-old mother. The annual number of live births with the Down's syndrome diagnosis has remained fairly steady, as the number of terminated pregnancies balance those resulting from the increase related to the age. This plateau will not necessarily remain if the increase of maternal age continues and the proportion of parents that accepts screening and opting for a pregnancy termination remains steady, or decreases. However, the number of women who decide to terminate the pregnancy, when they receive Down's syndrome diagnosis, has remained

constant at 92% throughout the life of the register (10) diagnosis, and subsequent termination of Down's syndrome pregnancies. Since younger women usually do not undergo screening, the possibility of the diagnosis is reduced and they cannot decide whether to terminate or deliver pregnancy to the end.

When the maternal, paternal or parental ages and the Down syndrome types were observed, the results were unexpected. Both, mothers and fathers were the youngest in translocation type of Down syndrome. The possible explanation is that a type of translocation (the one that cannot be inherited) is more common in young parents to be and that's why the screening should be offered to both, mothers with advanced age as well as young mothers.

5. CONCLUSION

The most common type of Down syndrome among children in Bosnia and Herzegovina is standard trisomy 21, and it is also the most common in both, mother and father's age group from 30 to 39 years old.

When maternal and paternal ages were considered, significant difference was found between translocation and mosaicism in mothers, and between standard trisomy and translocation in fathers. Both parents were the youngest in translocation group.

Advanced paternal and maternal age is one of many other risk factors for developing Down syndrome.

Results of our study show the higher occurrence of DS in younger women, and that is why multidisciplinary approach to identifying the trigger for trisomy appearance and the influence of maternal age is required. Molecular approach in addition to the classical ones of genetics, cell biology, biochemistry, epidemiology, cytogenetics, and physiology, may also be considered.

- **Author's contribution:** First author, Mia Sotonica, gave substantial contribution to conception and design, and interpretation of the data needed for this manuscript. Critical revision for important intellectual content was performed by Mirela Mackic Djurovic as well as performing genetic analysis. Emina Kiseljakovic participated in paper design, but also did the final review of the paper. Data analysis was done by Sabaheta Hasic. Radivoj Jadric drafted the article. Final revision before publishing was done by Slavka Ibrulj.
- **Conflict of interest:** none declared

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