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Ten-year trends in prevalence of Down syndrome in a developing country: impact of the maternal age and prenatal screening

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ABSTRACT

Objective: This study examines trends in total and live birth prevalence of trisomy 21 (T21) with regard to increasing maternal age and the introduction of prenatal diagnosis in Bosnia and Herzegovina.
Method: The prenatal detection was introduced in January 2008 in 3 hospitals and assessed until December 31, 2015. In this study, 99 fetuses and 330 babies were diagnosed with T21 in the studied period.
Results: On average, each year 33 T21 individuals were born and 13 T21 fetuses were diagnosed prenatally. The calculated incidence for the live born T21 individuals in Bosnia is 1:999. The live-birth prevalence of T21 was 9.6 per 10,000 births and the total prevalence of T21 was 19.1. The total T21 prevalence increases exponentially with the advanced maternal age. Prenatal T21 prevalence is 1.29 per 10,000 births for mothers <35, but increases exponentially with increasing age (32 for >40 years). The most common indications for invasive prenatal testing were ultrasound screening combined with biochemical serum analysis followed by the advanced maternal age.
Conclusion: The prevalence of liveborn Down syndrome children remained constant. Despite the fact that increasing maternal age in the last decade contributed to the rise in the total T21 prevalence, the effect of the introduction of prenatal diagnosis on the live-birth T21 prevalence of T21 was minimal, leading to the conclusion that the prenatal screening has to be improved in developing countries.

Introduction

The major cause of Down syndrome is trisomy of chromosome 21 (T21), accounting for about 95% of cases. Trisomy 21 occurs after the meiotic nondisjunction of chromosomes that fail to separate during gametogenesis. Frequency of meiotic nondisjunction increases in women with age [1]. Currently, Down syndrome is one of the most common birth defects, affecting about one in every 750–1100 live births [1].

In Europe, the average age of pregnant women has been increasing for the past 40 years [2]. More than one million births in Europe have been to mothers older than 35 years of age in 2009 [3]. Studies have shown that the only known risk factor for trisomy 21 is the advanced maternal age [4,5]. Prenatal diagnosis of trisomy 21 includes non-invasive screening (fetal ultrasound and biochemical serum analysis) and invasive methods (amniocentesis and chorionic villi sampling). Advanced maternal age has been the primary indicator for amniocentesis, but new biochemical serum tests are now widely used for the detection of chromosomal abnormalities [6]. Besides those most common indications, prenatal ultrasound screening has improved over the years and has one of the highest detection rates for fetuses with chromosomal abnormalities [7].

No study in Bosnia and Herzegovina has ever evaluated the T21 total or live birth prevalence. As a developing country [8], the T21 diagnostics have been introduced recently—the T21 prenatal diagnosis has been available since 2008. Thus, we set to evaluate

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the effectiveness of prenatal diagnosis by calculating the trends in T21 prevalence in Bosnia in order to create future guidelines for prenatal genetic diagnosis. The present and future T21 live birth prevalence is of practical significance for planning services and prioritizing resources to support individuals with T21. In our study we analyze trends in T21 total and live birth prevalence in the period from 2005 to 2015. We also examine whether these trends could be explained by increasing maternal age or by prenatal diagnosis.

Materials and methods

Data collection

Prenatal and postnatal data on the number of T21 pregnancies and births was collected from 4 cites: University Clinical Centers in the cities of Tuzla, Sarajevo and Banja Luka, and Medical Faculty of the University of Sarajevo (postnatal data only). Data on the annual total live births, annual births according to maternal age, and annual still deaths for the Federation of Bosnia and Herzegovina (FBH) and Republic of Srpska (RS) were obtained from publically available data on websites of Federal Statistical Institute and Institute of Statistics of Republic of Srpska, respectively. The study was approved by IRB boards of University Clinical Center Sarajevo, Tuzla and Banja Luka.

Postnatal patient samples

Postnatal diagnosis of Down syndrome has been assessed starting in January 01, 2005 in Banja Luka and Sarajevo (Medical Faculty, University of Sarajevo). Additional laboratories in Sarajevo and Tuzla started postnatal diagnostics in 2008. All data has been collected from January 01, 2005 to December 31, 2015. Collected data consisted of the analysis of peripheral blood samples from patients with suspected Down syndrome. Postnatal samples were analyzed by karyotyping and/or FISH (fluorescence in situ hybridization).

Prenatal patient samples

Since 2008, University Clinical Centers Tuzla, Sarajevo and Banjaluka performed prenatal diagnosis of frequent aneuploidies including trisomy 21 from amniotic fluid samples. Karyotyping, FISH or QF-PCR methods were used for analysis of amniotic fluid samples.

Karyotype analysis of amniotic fluid and peripheral blood was done using standard manufacturer's protocols (Euroclone, Milano, Italy). Cultivation of amniotic fluid sample for chromosome analysis was performed by the method of long-term cell cultures, where "flask" (Sarajevo, Banjaluka) and "coverslip" (Tuzla) techniques were used. For cultivating of samples, commercial medium for amniotic fluid cultivation was used (Amniomed, Euroclone, Milano, Italy and Amniomax, Invitrogen). Peripheral blood was cultured in 5 ml of Chromosome Kit P medium (Euroclone, Milano, Italy) for 72 h in 37 °C incubator with 5% CO_2. Slides were stained using standard protocols for G-banding—routine technique in classical cytogenetics [8]. Karyotype analysis was performed on Olympus BX61 microscope using karyotyping software Cytovision (Cytovysion, AB Imaging, Germany; Sarajevo) and AxioVision (Carl Zeiss, Oberkochen, Germany; Banja Luka). For each sample, 20 cells were analysed and recorded in the Cytovysion program.

Fluorescence in situ hybridization was performed on the slides prepared for karyotype analysis (Sarajevo). Briefly, slides were dehydrated using different percentages of ethanol, dried and FISH probe was applied and incubated in Hybridizer (Dako, Colorado, USA) according to the manufacturer's instructions (Vysis, Abbott, *Abbott Park*, *Illinois*, USA). For FISH analysis, AneuVysion (Vysis) commercial DNA test kits for enumeration of chromosomes 13, 18, 21, X and Y was used according to manufacturer's instructions. Analysis was performed according to standard protocols for FISH analysis. Cells were analyzed using fluorescent microscope (Olympus BX61, Olympus, Tokyo, Japan/ Hamburg, Germany) with Cytovision software (Cytovysion, AB Imaging, Germany). For each analyzed sample at least 30 interphase nuclei were counted.

For QF-PCR analysis (Sarajevo), fetal DNA from amniotic fluid sample was extracted using Qiagen Qiamp DNA Mini Kit, according the manufacturer instructions. Molgentix Aneufast QF PCR kit for amplification of 21 loci on chromosomes X, Y, 13, 18 and 21. Fragmental analysis was done on ABI 3130 Genetic Analyzer, using Run 3130 Data Collection software. Data analysis and Electropherogram creation was done using GeneMapper ID v3.2 software.

Statistical analysis

"Live birth prevalence" is defined as the number of live-born babies with T21 in 10,000 live births. "Total T21 prevalence" is defined as the proportion of babies that would be live-born with Down syndrome in the absence of elective pregnancy terminations, following a prenatal diagnosis of T21.

Total T21 prevalence was calculated as:

$$\frac{[\text{T21 Live birth (LB) in FBH and RS}] + [\text{prenatally diagnosed T21 in 3 clinical centers}] \times 10,000}{[\text{Total LB in FBH and RS}] + [\text{still births in FBH and RS}]}$$

LB T21 prevalence per 10,000 births was calculated as:

$$\frac{[\text{T21 Live birth(LB) in FBHand RS}] \times 10,000}{[\text{Total LB in FBH and RS}]}$$

Statistical data for Federation of Bosnia and Herzegovina (FBH) and Republic of Srpska (RS) were obtained from Federal Statistical Institute and Institute of Statistics of Republic of Srpska, respectively. Statistical analysis was conducted using SPSS v.21 (IBM, Armonk, NY, USA).

Results

In Bosnia and Herzegovina, the postnatal detection of trisomy 21 has been assessed since 2005 and prenatal detection since January 01, 2008. This study included all centers that diagnose T21 prenatally and postnatally in the period from January 01, 2005 to December 31, 2015. Fig. 1A shows the numbers of T21 cases detected prenatally and postnatally each year since 2005. Prenatally, there were 99 fetuses diagnosed in the 10-year period (55 males and 44 females). Postnatally, there were 330 babies diagnosed (179 males and 151 females) in the analyzed period (Supplementary Table S1). Supplementary Figs. S1 and S2 show the regional distribution of postnatally and prenatally diagnosed DS in Banja Luka, Tuzla, and Sarajevo. On average, each year 33 T21 individuals were born and 13 DS fetuses were diagnosed prenatally (Supplementary Table S1).

The 10-year trend in the total number of live born T21 individuals has been decreasing, while the number of prenatally diagnosed T21 have been increasing (Fig. 1). Fig. 1B showed the total number of annual T21 calculated by live births and prenatally diagnosed T21. The calculated incidence for the live born T21 individuals in Bosnia and Herzegovina is 1:999. Most Down syndrome children were diagnosed in the first year of life; however, there were 14 individuals who were diagnosed at