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Original Article

Prenatal diagnosis of Down syndrome: A 13-year retrospective study

Ana Vičić [a, *], Tomislav Hafner [a], Ivanka Bekavac Vlatković [a], Petra Korać [b], Dubravko Habek [a], Feodora Stipoljev [a, c]

[a] Department of Obstetrics and Gynecology, Clinical Hospital "Sveti Duh", Zagreb, Croatia
[b] Division of Molecular Biology, Department of Biology, Faculty of Science, University of Zagreb, Zagreb, Croatia
[c] Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

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ABSTRACT

Objective: The aim of this study is to summarize the experience on prenatal diagnosis of Down syndrome.
Materials and methods: The study includes a retrospective data analysis of 157 prenatally detected cases of Down syndrome, routinely diagnosed among 6448 prenatal investigations performed during a 13-year period (2002–2014) in a single tertiary center.
Results: The prevalence of diagnosed Down syndrome cases was 2.4%. Maternal age alone was indication for prenatal diagnosis in 47 cases (45.2%), increased first-/second-trimester biochemical screening test in 34 cases (21.7%), abnormal ultrasound examination in 69 cases (43.9%), positive familial history for chromosomal abnormalities in four cases, and high risk for trisomy 21 revealed by cell-free DNA testing in three cases. Ultrasound anomalies were present in total of 94 fetuses (59.8%). The most common abnormality was cystic hygroma found in 46 cases (29.3%). A regular form of Down syndrome (trisomy 21) was found in 147 cases (93.6%), Robertsonian translocation in six cases (3.8%), and mosaic form in four cases (2.6%).
Conclusion: In prenatal diagnosis of Down syndrome noninvasive screening methods are important for estimation of individual risks, in both, young population of woman and older mothers, while conventional and molecular cytogenetic methods are essential for definite diagnosis and proper genetic counseling.

Introduction

With the incidence estimated between one in 1000 to one in 700 live births, Down syndrome is the most common chromosomal abnormality, and considered one of the major congenital causes of intellectual disability in human population. Moreover, if we take into account pregnancies ending up with medically induced abortions and stillborn, the incidence of Down syndrome increases to approximately one in 450 births [1]. During the last 20 years, the increase of 10% in number of pregnancies with Down syndrome has been noticed in Europe, mainly due to increasing maternal age at the time of conception. However, development and the increasingly widespread practice of prenatal screening followed by terminations of pregnancies have resulted in stable live birth prevalence [2].

Prenatal diagnosis of Down syndrome comprises noninvasive screening methods which provide the risk estimation of having affected pregnancy, while the definite diagnosis is made by karyotyping of cultured fetal cells obtained by one of invasive procedures, mainly chorionic villus sampling (CVS) or amniocentesis. As the most common chromosomal aberration trisomy 21 is detected in approximately 1.6–3.2% of all prenatal karyotyping investigations performed [3,4] Over the years several screening strategies have been applied, and the methods used are maternal age assessment, first- and/or second-trimester ultrasound examinations, and maternal serum biochemical testing at the first and/or second trimester of pregnancy. Furthermore, in the last few years a noninvasive prenatal testing (NIPT) using analysis of cell-free fetal DNA from maternal plasma has been widely introduced [5]. In order to give parents better informed counseling and to minimize the risk of miscarriage associated with invasive procedures, the present

* Corresponding author. Department of Obstetrics and Gynecology, Clinical Hospital "Sveti Duh", Sveti Duh 64, 10000 Zagreb, Croatia. Fax: +385 1 3745 534.
E-mail address: vicic.ana@gmail.com (A. Vičić).

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guidelines and studies are directed toward calculation of individual risks for every pregnant woman and improvements in sensitivity ratios and reduction of false-positive results.

The aim of this study is to summarize the experience on prenatal diagnosis of Down syndrome, presenting a 13-year data collected in a single tertiary center.

Materials and methods

A retrospective survey covering a 13-year period from January 2002 to December 2014 at our Department included 157 prenatally detected cases of Down syndrome, routinely diagnosed after CVS, amniocentesis, cordocentesis or analysis of materials collected after termination of pregnancy (TOP). Throughout observed period, a total of 6448 fetal karyotyping analyses were performed. Indications for prenatal diagnosis are given in Table 1. Advanced maternal age was defined as 35 years or older at expected date of delivery. Abnormal ultrasound findings detected at first-trimester examination (11–13 + 6 weeks gestation) included nuchal lesions defined as increased nuchal translucency (NT) thickness or cystic hygroma (CH), abnormal ductus venosus (DV) flow and the absence of nasal bone. NT measurements were performed at mid–sagittal plane and compared to NT nomograms at a given gestational age. Increased NT thickness was considered ≥2.5 mm. CH was defined as a bilateral, mostly symmetric septated cystic structure located mainly in the occipital region of the neck, with or without associated anasarca. Ultrasound findings discovered during second-trimester examination included various major abnormalities and minor/soft markers associated with aneuploidies (Table 2).

Cytogenetic analysis was performed on cultured amniocytes, fetal blood cells, skin fibroblasts, or on short-term cytotrophoblast/mesenchymal stroma cultures, following European Cytogeneticists Association guidelines [6]. Fluorescence *in situ* hybridization (FISH) was carried out with commercially available 21q22.1 specific region probe (Kreatech Diagnostics, Netherlands), according to manufacturer's protocols.

Descriptive statistics were used for the analysis of collected data. Comparisons for categorical variables were made using Pearson χ^2 test, and for comparison of continuous variables between two groups, due to violation of normality assumption, nonparametric Mann–Whitney *U*-test was used. $P < 0.05$ was considered statistically significant.

Results

During a 13-year period (2002–2014) a total of 6448 prenatal investigations were performed, and Down syndrome was diagnosed in 157 cases (2,4%). In 91 cases (58.0%) the diagnosis was made after amniocentesis, in 54 (34.4%) after CVS, in eight (5.1%) after analysis of materials collected after TOP, and in four cases (2.5%) after cordocentesis. Indications for prenatal diagnosis are presented in Table 1. The mean maternal age was 35.9 years (SD 5.2 years, range 20–46 years). The diagnosis was made in 49 cases (31.2%) during the first trimester, in 107 cases (68.2%) during the second, and in one case (0.6%) in the third trimester of pregnancy. However, in a period from 2002 till 2007 in only 13.1% of cases karyotyping was performed in the first trimester, in comparison with the higher rate of early diagnosis assessment in 2008–2014 (42.7% cases) ($P < 0.0001$). The mean gestational age at the time of diagnosis in a period 2002–2007 was 17 weeks, and in 2008–2014 was 14 weeks and 6 days. Furthermore, during a period 2002–2007 61 cases with Down syndrome was detected among 3610 diagnostic procedures performed (1,7%), in comparison with detection rate of 3.3% in 2008–2014 (96 cases within 2838 investigations).

There was a statistically significant difference in gestational age when the diagnosis was performed between a group of women older than 35 and younger mothers (median gestation of 16.4 weeks vs. 15 weeks) ($P = 0.006$). In a group of mothers aged less than 35 ($n = 53$), the most common indication for prenatal diagnosis was abnormal ultrasound examination (60.4%), while in 30.2% of cases positive first- or second-trimester biochemical screenings (with or without ultrasound abnormalities) indicated fetal karyotyping (Table 1). In this group, ultrasound anomalies were found much more often (in 81,1% of fetuses) than in group of women aged 35 or older (53,8% of fetuses) ($P < 0.0001$, Pearson χ^2 test). Among all diagnosed Down syndrome cases, an abnormal first-/second-trimester ultrasound scan was observed in 94 fetuses (59.8%).

The most common ultrasound finding was CH ($n = 46$), in 12 cases associated with anasarca. Increased NT thickness was observed in 33 cases. As an additional finding, abnormal DV flow was found in 10 and absent nasal bone in six cases. Soft markers and major structural malformations diagnosed at second-trimester scan are summarized in Table 2. Furthermore, in five cases polyhydramnios was present, in two starfish amnion, and in one case amniotic band syndrome. Isolated soft markers were found in three fetuses, while in cases with echogenic intracardiac focus (EIF) and bilateral choroid plexus cysts (CPC) patients underwent amniocentesis due to positive maternal serum screening test, and in a case of pyelectasis because of positive familial history for chromosomal abnormalities.

Amniocentesis was performed in three dichorionic diamniotic (DCDA) twin pregnancies. The indication for prenatal diagnosis in two cases was ultrasound finding of CH present in a single twin, while cytogenetic analyses in both cases revealed trisomy 21 in affected twin, and normal karyotype in other fetus. In the third case amniocentesis was performed solely due to advanced maternal age, and the trisomy 21 was observed in one twin.

A regular form of Down syndrome (trisomy 21) was found in 147 cases (93.6%), Robertsonian translocation (RT) in six (3.8%), and mosaic form in four (2.6%), with the percentage of trisomic cells ranging from 5% to 33%. Male to female ratio (sex ratio, SR) was 1.9. Robertsonian translocation was of parental origin in two cases and *de novo* in four cases. In a 35-year-old patient, amniocentesis was performed at 17 weeks gestation after results of high risk for

Table 1
Indications for prenatal diagnosis in a group of women younger than 35, and in older mothers.

Maternal age	Indication No. of cases (%)					Total No. of cases (%)
	Maternal age alone	Combined screening[a]	Second-trimester maternal serum screening[a]	Ultrasound anomaly	Other[a]	
<35 years	0	10 (18.9)	6 (11.3)	32 (60.4)	5 (9.4)[b]	53 (100)
≥35 years	47 (45.2)	10 (9.6)	8 (7.7)	37 (35.6)	2 (1.9)[c]	104 (100)

[a] With or without abnormal ultrasound findings.
[b] Positive familial history for chromosomal abnormalities in three cases; high risk for trisomy 21 revealed by non-invasive prenatal test (NIPT) from maternal plasma in two cases.
[c] Positive familial history for chromosomal abnormalities in one case; high risk for trisomy 21 revealed by NIPT in one case.

Table 2
Abnormal ultrasound findings discovered during second-trimester examination.

Structural abnormalities	Number	Soft markers	Number
Cystic hygroma[a]	19	Hyperechogenic bowel	4
Anasarca	4	Choroid plexus cysts	3
Ventriculomegaly	5	Echogenic intracardiac focus	2
Cardiac defects[b]	3	Short femur	1
Duodenal atresia	2	Pyelectasis	1
Micrognathia	1		
Meningocele	1		
Omphalocele	1		
Brachycephaly	1		
Intrauterine growth restriction	1		

[a] With or without associated anasarca.
[b] One case of ventricular septal defect (VSD), one case of atrial septal defect (ASD), and one unspecified.

trisomy 21 obtained by NIPT. Cytogenetic analysis of cultured amniotic fluid cells revealed a 46,XY,inv(9),der(14; 21) (q10; q10),+21 karyotype, and parental karyotyping showed that mother was carrier of RT 14; 21. Retrospective analysis also revealed a cautionary case of mosaic Down syndrome. A 37-year-old G1P0 was referred for amniocentesis due to advanced maternal age and risk for trisomy 21 of 1:85, obtained by double test. Cytogenetic analysis revealed the proportion of trisomic cells of 5%. Subsequent amniocentesis was performed, and trisomy 21 was confirmed in two metaphases from two flasks (3.8%). However, FISH analysis on uncultured amniotic fluid cells revealed a trisomy 21 in 16.8% of analyzed cells. Two cases of mosaicism have also been observed after CVS. In the first, a 46,XX/47,XX,+21 karyotype was detected in both, short- and long-term cultures. In the second case, CVS was performed because of an increased NT of 5.2 mm and high risk for trisomies 13/18/21 (>1:5) obtained by combined screening. Cytogenetic analysis of short-term culture showed a 48,XX,+20,+21 karyotype in all analyzed metaphases, while long-term cultured mesenchymal stroma revealed trisomy 21 (47,XX,+21) in all cells. In all mosaic cases a karyotype was confirmed in fetal tissue after TOP. A case of double aneuploidy, 48,XXY,+21, was found in a fetus with CH measuring 7.9 mm.

Discussion

Definite prenatal diagnosis of Down syndrome requires the application of invasive techniques which are associated with risk of miscarriage in a range of 0.3–1%, depending on the type of procedure [7]. In order to identify the pregnancies with high risk for chromosomal defects, noninvasive screening methods are used. Although 66.2% of women in the present study were aged 35 or older, advanced maternal age alone was indication in only 29.9% of cases. A positive first- or second-trimester biochemical screening, or abnormal fetal ultrasound were present in 66.9% of cases (n = 105). These results emphasize the value of screening methods not only for detection of high-risk pregnancies within unselected population of younger women, but also for estimation of individual risks for women aged 35 or older. Furthermore, a widespread implementation of combined screening (Fig. 1), and probably more frequent conduction of first-trimester ultrasound examination alone have resulted in a significantly higher proportion of fetuses with Down syndrome detected during the first trimester in a period from 2008 till 2014, than in the earlier years. Also, a greater percentage of diagnosed trisomy 21 cases in respect to total number of performed invasive procedures in period 2008–2014 (3.3% vs. 1.7% in 2002–2007) indicate improvements in positive predictive value of screening strategies. Overall, the prevalence of detected Down syndrome cases of 2.4% in our study correlate with the detection rates of 1.6% and 3.2% reported by Comas et al. [3] and Jacobs et al. [4], respectively.

Recently, analysis of cell-free DNA (cfDNA) in maternal plasma has been proposed as a method with detection rate for trisomy 21 of about 99%, and the false-positive rate of 0.1% [8]. However, the cost of cfDNA testing is still considered as too high to be used as the primary screening method within general pregnancy population. Thus, it is proposed that it should be performed only in a group of women with high- or intermediate-risks, ascertained by conventional screening methods [5]. A case of RT of maternal origin revealed after NIPT in our population, emphasize the value of classical cytogenetic analysis, providing genetic counselors information for familial risk-estimation of having offspring with chromosomopathy in subsequent pregnancies.

We have differentiated between septated and nonseptated forms of nuchal thickening, although according to some authors [9] for first-trimester measurements the term *nuchal translucency* should be used, irrespective of whether the collection of fluid is septated, and whether it is confined to the neck or envelopes the whole fetus. However, Malone et al. [10] have suggested that CH should be distinguished from “simple” increased NT, since their study showed that CH cases are associated with significantly higher risks for fetal aneuploidy, cardiac malformation and fetal or neonatal death. They also proposed that first-trimester finding of CH could be used for Down syndrome risk assessment without additional maternal serum measurements or usage of risk-calculation software programs, and consequently unnecessary postponement of diagnosis. A finding of CH as the most common ultrasound anomaly present in 29.3% of Down syndrome fetuses in our study, correlates with the report of De Vigan et al. [11] in which CH was found in 32.6% of trisomy 21 cases.

According to the previous reports, major or structural abnormalities discovered during the second trimester are seen in approximately 20% of fetuses with Down syndrome [12]. A slightly higher proportion of 25.9% has been obtained in our study. This could be due to different gestational age at the time of ultrasound examinations, since most ultrasound scans in our study were performed before 18 weeks, and the commonest abnormal finding was CH. Isolated soft markers (EIF, pyelectasis and bilateral CPC) were observed in three cases. Although, soft markers are nonspecific, often transient and correlate with high false-positive rates, they are suggested to be useful for estimation of individual risks, if used in conjunction with other screening methods [13].

A cytogenetic finding of mosaicism discovered during prenatal diagnostics always presents a challenge in the interpretation of results, especially in the cases of cryptic mosaicism. One such case was disclosed in our retrospective study. A mosaicism with 5% and 3.8% trisomic cells was revealed by cytogenetic analysis of the first and subsequent amniotic fluid samples, respectively. However, FISH analysis on uncultured amniotic fluid cells showed a trisomy 21 in 16.8% of analyzed cells. This case emphasizes the crucial role of FISH analysis in ascertainment of cryptic mosaicisms, since they

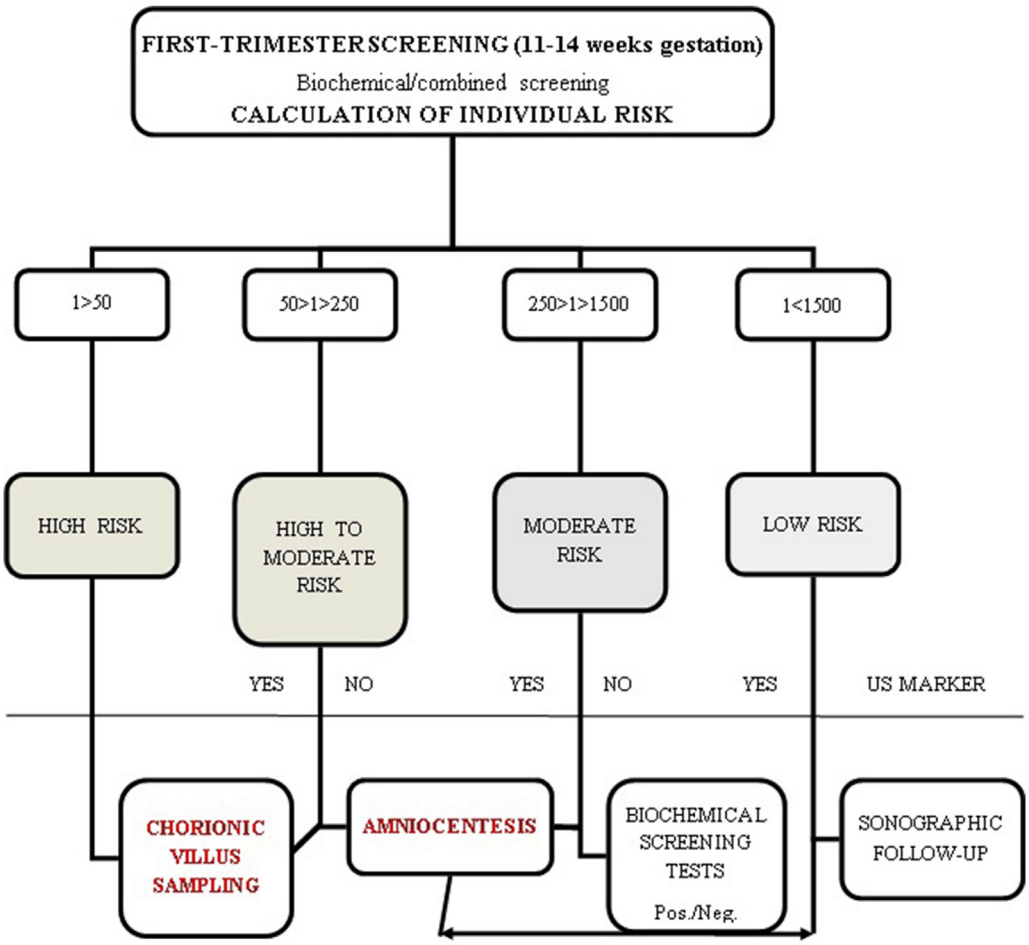

Fig. 1. Schematic diagram of algorithm used for an individual risk calculation during first-trimester screening.

may get unnoticed or misinterpreted as pseudomosaicisms. Furthermore, subculturing of amniotic fluid cells causes a descending proportion of trisomic cells with preferable growth of normal cell line. In such a manner, interphase FISH on uncultured amniocytes provides more accurate information on the trisomy 21 mosaic percentage, more precisely reflecting the degree of aneuploid cells present *in vivo* [14].

This study has also revealed an extremely rare case of mosaicism involving a cell line with double trisomy of 48,XX,+20,+21 detected in short-term culture, and a 47,XX,+21 karyotype found in long-term cultured mesenchymal stroma. The distribution of different cell lines detected at CVS depends upon the time when aberration occurred and type of cells involved. Theoretically, a double trisomy in our case could have originated from meiotic events leading to a 48,XX,+20,+21 karyotype present in zygote, or a zygote was initially trisomic for chromosome 21, and the trisomy 20 have occurred from postzygotic mitotic events in trophoblast cells. Though, when abnormal cell line is present in cytotrophoblast in non-mosaic state, the current aneuploidy most commonly originates from parental meiosis [15]. Thus, a probable mechanism in our case is that double trisomy was present in the zygote, and that extra chromosome 20 was lost (rescued) during early mitotic divisions of cells within inner cell mass. Also, there are two possible scenarios by which double trisomy could have occurred. The first is that nondisjunction of both extra chromosomes had happened during a single parent gametogenesis, while simultaneous parental nondisjunctions, with both gametes being disomic is less probable.

When a mosaicism is found in chorionic villi, cautiousness is needed regarding fetal involvement, but also for a possible impact of aneuploid cell line on placental dysfunction. An abnormal karyotype found only in cytotrophoblast cells, almost always means that current cell line is restricted only to placenta, and not present in the fetus. However, it is important to take into consideration that the presence of abnormal cell line in placental tissue carries an additional risk of fetal los and intrauterine growth restriction [16]. Furthermore, a mosaic trisomy 21 observed in CVS requires a special attention. In the case of mosaicism revealed in our study, a trisomic cell line was detected in both, short- and long-term culture. Although, a finding of mosaicism for other chromosomopathies in both, cytotrophoblast and mesenchymal stroma (confined placental mosaicism (CPM) type III or true fetal mosaicism (TFM) type VI) carries the risk of TFM of approximately 24%, the presence of placental generalized mosaicism for trisomy 21 is associated with risk of 72.7% [17].

In conclusion, noninvasive screening methods play a crucial role for estimation of individual risk of having chromosomally affected pregnancy, in both, young population of woman and older mothers, while prenatal invasive diagnosis through conventional and molecular cytogenetic methods is essential for definite diagnosis of Down syndrome and appropriate genetic counseling.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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