

# Early Amniocentesis as a Method of Choice in Diagnosing Gynecological Diseases

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## Original paper

### ABSTRACT

**Introduction:** The aim of prenatal diagnosis is to detect fetal structural and genetic abnormalities. Used are different medical methods, procedures, processes and techniques. For this reason we can speak about the prevention and detection of hereditary diseases and congenital anomalies in the unborn fetus. **Material and methods:** The authors analyzed the results of early amniocentesis tests performed during 2009 in Institute for Gynecology, Infertility and Perinatology "Mehmedbasic" in Sarajevo. Performed is 299 analysis of amniotic fluid after amnion puncture done in the Institute or at the Clinic of Gynecology and Obstetrics (GAK) Sarajevo. **Results and Discussion:** Indica-

tions for the performance of early amniocentesis were: age greater over 35 (84.9%), positive ultrasound markers (1.6%), positive biochemical markers (5.6%) and positive family history for hereditary diseases (7.9%). Detected was 19 pathological kariograms or very high 7% of the total annual number of amniocentesis. An analysis of the distribution of pregnant women in relation to the indication of the result of cytogenetic analysis for each table made positive predictive value (PPV). For indicator age PPV was 0.11, 0.66 for ultrasound markers, for biochemical markers 0.13, for other indications—0.04. The logistic regression model (odds -ratio 11.234 ) indicate a positive ultrasound findings in relation to the year indicates that the risk to gain abnormal fetal

karyotype 13 times higher when using only age as an indication for early amniocentesis. Of the 19 pathological kariogram largest number refers to M.Down (10), Sy. Edwards was detected in 2 patients, Sy. Klinefelter in 3, mosaicism in 3 and translocation gene in two of the fetus. **Conclusion:** The authors would like to acknowledge a very high percentage of pathological kariogram risk groups, the extension of indications for RAC indicate the value of ultrasound markers as a good screening methods and the need for social incentives to perform screening tests and early amniocentesis in B&H in order to prevent genetic abnormalities. **Key words:** prenatal diagnostics, early amniocentesis, genetic abnormalities.

## 1. INTRODUCTION

The aim of prenatal diagnosis is to detect fetal structural and genetic abnormalities (1, 2, 3). Used are different medical methods, procedures, processes and techniques (4, 5, 6). For this reason we can speak about the prevention and detection of hereditary diseases and congenital anomalies in the unborn fetus (7, 8, 9, 10, 11, 12). Some changes can be registered on chromosome level (chromosome mutations) or at the level of DNA (genetic or genomic mutations), which in turn can produce somatic malformations (13, 14, 15, 16). Reflections on genetics as a possible cause of fetal abnormalities date back to Aristotle (3). Aristotle believed that women are the future characteristics of the child resource for „material“ and men for „movement“. In the writings of other nations and cultures, too, we find similar thinking. Location gene was determined in 1908 when Tomas Morgan identified genes as parts of

the chromosomes. In 1911 Morgan and his colleagues presented the first genetic map (3). The structure of DNA was determined in 1953. Since then, started the rapid development of genetic and molecular biology. Interest in the genetic health of the child and congenital defects developed in the near future (3). Chromosome abnormalities are recurrent miscarriage represented as a percentage of 50%. From conception to birth incidence of chromosome abnormalities decreased from 20% to 0.5-1% at birth (3, 8, 9). The incidence of chromosome abnormalities in infants per 10,000 births amounting to a total of 90. From individual aberration trisomy 21 is 15, trisomy 18 is 3, sex chromosomes about 10 (47, XXY) structural autosomal aberrations encountered from 10-30 cases per 10,000 children (3, 9). In the prenatal diagnosis during the last 10 years was introduced a number of techniques and procedures. They are divided into invasive

and non-invasive. Standard non-invasive methods are (8, 9, 14, 15, 16): Serum markers; Serum tests; Ultrasound. Serum markers are used as screening tests during the first and second trimesters of pregnancy (3, 5, 16). Alpha fetoprotein (AFP); Human chorionic gonadotropin (hCG); Serum estradiol E3; Pregnancy-associated plasma protein A (PAPP-A).

**Serum tests.** They are also called biochemical genetic markers. The specificity and sensitivity of these tests to Morbus Down is about 60% and in combination with ultrasound detection rate reaches 90% (5). The mothers pose a risk for many chromosome defects. Cut-off between high and low risk is about 1:250. The lower value of this relationship is a high risk of Down syndrome, the higher value, in turn means less risk.

**Bi test.** Uses two markers: alpha-fetoprotein and total hCG. Its value is about 55% (6.5% false positives). In interpreting the values of this test are used the mother and the gestational age (3).

**Double test.** In this test are used as markers free beta hCG and PAPP-A test. Added to them is the risk of mother's age. The accuracy is around 62% (3, 15).

**Triple test.** In case of triple test as markers are used free E3, beta hCG and AFP. It is used around 16 gestation week. Value of the test ranges from 58% -91% (5).

**Ultrasound diagnosis.** The essence of this method is the fact that when reviewing also the neonatologists use physical characteristics of a newborn with Down syndrome. Knowing the changes in structure as a result of trisomy 13, 18 and 21, it is possible to use ultrasound markers to diagnose fetal anomalies of these genes. Wladimiroff (1988) notes the diagnostic accuracy of 75% and in combination with biochemical markers accuracy raises up to 90% (3, 9). It should be noted that the markers cannot reach diagnosis, but they are good screening method.

**Nuchal Translucency (NT, NN).** Substrate of these changes is the accumulated fluid in the subcutaneous tissue in cervical region. Examinations is done from 11 to 14 week of gestation in the sagittal section of the fetus, which is in the neutral position, where CRL amount of 45-85 mm (11, 12) for the cut-off is taken the value of 3 mm in first trimester, compared 6 mm in the second trimester of pregnancy. When are taken into account the mother's NT with a minimum risk of 1:100, then the value of this method increases to 78%.

**Cystic hygroma (CH).** This marker is displayed as a multicystic formations located at the rear. The finding is frequently associated with ascites, generalized hydrops and then it indicates 75% possibility to diagnose Turner syndrome (monosomia-X).

**Plexus cysts chorioideus (CHP).** Bilateral cysts show a greater connection with trisomias 13, 18 and 21. The most commonly diagnosed is trisomy 18 (Edwards syndrome)(3).

**Nasal bone abnormalities.** Lack of ossification of the nasal bone is a parameter for detecting Sy. Down (3, 15). The sensitivity of the method is about 85%.

**Omphalocele.** Herniation greater than 7 mm is considered suspicious

sign on chromosopathy and indications for karyotyping (3). Hyperechoic bowel, duodenal atresia, heart defects are also on a similar principle, markers on the genetic structure of fetal abnormalities. Invasive methods: Amniocentesis; Biopsy chorionic; Cardiocentesis; Fetoscopy.

## 2. MATERIAL AND METHODS

The study was conducted in the Institute of Gynecology, Infertility and Perinatology "Mehmedbasic" Sarajevo in the period from January 1st-December 31st 2009. This is the first medical institution in B&H as a whole for all methods of cytogenetic (amniotic fluid, blood, tissue). Indications for early amniocentesis are conducted according to the principles of modern perinatology. These indications are as follows: pregnant women older than 35 years, old married couple over 70 years, congenital genetic abnormalities in immediate family in previous pregnancies and positive ultrasound and biochemical markers (cut-off 1:199, or 1:299).

Amniocentesis was conducted in the gestational age of 16-20 weeks. According to these indications, the given gestational age was performed puncture of the amnion in the Institute of Gynecology, Infertility and Perinatology „Mehmedbasic“ and Gynecology and Obstetrics Clinic, Clinical Center Sarajevo (Sarajevo Canton for the insured patients, according to the treaty of the Institute and the Health Insurance Fund of the Canton Sarajevo). Before the procedure, an interview was conducted with the spouses; they were presented a risk of surgery (0.2 to 0.5%) and the time when they will get a cario-gram report. Before performing surgery early amniocentesis done a detailed scan which detects heart rate, verify gestational age, localization of placenta, amniotic fluid pockets and place for the puncture. Then we empty the bladder, perform disinfection of the lower abdomen (povidone iodide). Specifies the puncture site is running a "free hand" technique. As puncture needle size are used 20-22 gauge. Ultrasound verification is done on the appliance General Electric 730 Volusion Exp, with convex probe 3.5-7 MHz (multidimensional

scanning). The ultrasound probe, in order to implement the principles of asepsis and antisepsis was placed in sterile bags. Under the control of the paper is done puncture amnion and aspirated into the first syringe with 2 ml of amniotic fluid (reject it for possible contamination of breast tissue cells) and then into another syringe was aspirated 20 ml of amniotic fluid, which is then submitted to cytogenetic laboratory of the Institute on cytogenetic analysis. After intervention verify the cardiac activity of the fruit, determine relative quiescence. In the case of Rh isoimmunisation injected mother within 36 hours of 50-150 cc Rhogam.

Cytogenetic analysis implies that it is performed by direct in situ methods, and work subculture amniocytes. Staining (banding) of preparation is done on trypsin GTG stripes. Cariogram image is then transmitted to the PC program "Metasistem" after which agrees cario-gram unborn fetus. Grants are issued by finding a cario-gram image is given to the patient. In the case of pathological or borderline cario-gram findings, spouses are invited to genetic advice to the Institute where their multidisciplinary team (genetics, obstetrician and biologist) explains relevant medical facts regarding the findings.

## 3. RESULTS

During the 2009 in the Institute „Mehmedbasic“ is performed a total of 299 amniocentesis. Table 1 shows the demographic characteristics of the respondents. Amniocentesis is usually performed in pregnant women older than 35 years (254 or 84.9%). In other words, age of pregnant women was the most common indication for amniocentesis of pregnant women younger than 35 years (a total of 45 or 15.05%). The indication for amniocentesis in this group was positive ultrasound or biochemical markers, immediate family history on hereditary diseases. Employed women were present in 98 or 32.8%, which was characteristic of the social moment in our country. Pregnant women who smoked during pregnancy, regardless of the doctors' recommendations, are represented in a

very high percentage (172 or 59.2%). The high number of pregnant women who smoke may be explained by failure to comply with the law banning smoking in certain public places and the lack of a general campaign against smoking in pregnancy.

Table 2 provides graphical presentation of the age distribution of pregnant women according to individual indications for performing early amniocentesis. Age, as we have previously expressed the most common indication for amniocentesis. The average age in this group was 38.9 years. The average age of the ultrasound as indications for RAC is 31.9 years, the average age for the indications “triple test” is 34.9 years. For indications of a positive family history of a genetic disease is 34.1 years. This shows that the indications for the performance of RAC “stretched” and include greater range of ages with RAC. On Table 3 are presented data on the distribution of frequencies of pregnant women in relation to the indication for amniocentesis. There is a clear and important to the overall distribution of age and pregnant women in the age which is performed RAC. The range is from 21 years to 45 years, which is extremely important, because this group “escapes” from the traditional indications – “Maternal age older than 35 years”. On the Table 4 data were analyzed from pathologically cariograms. Most often it is finding Morbus Down. However, other types of genetic abnormalities are present in relatively small sample is detected by the variety of pathological cytogenetic forms.

Table 5 analyzes the distribution frequency of pregnant women in relation to the indications according to result of cytogenetic analysis with a positive predictive value of the test. Of the total number of amniocentesis (299), the largest number of those is done because age of the pregnant women was over 35 years (254 or 85%), a positive ultrasound marker was the indication in 5 pregnant women, or about 2%, a positive triple test indicated the RAC in 17 cases or 5%, a positive family history

No. of pregnant women	Age		Employed	Smokers
	>35	<35		
299	254 (84.9%)	45 (15.05%)	98 (32.8%)	172 (24.08%)

Table 1. Review of the pregnant women demographic characteristics

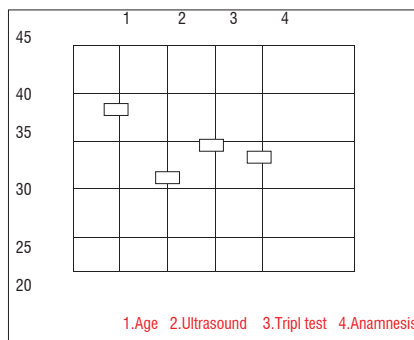


Table 3. Distribution of pregnant women according to age and indications for amniocentesis

of a close family member in 23 cases, or 8%. In all 299 amniocentesis no complications or miscarriage were recorded. There were no repeated punctures due to failed sampling or non-sterile procedure. The highest positive predictive value as a test indicative of pathological values of ultrasound markers (0.66). The positive predictive value for indication age is 0.06, the triple test of 0.13, positive family history 0.07. Logistic regression models were recorded odds-ratio of 11.234 to indicate positive ultrasound as marker in relation to age, which means that the risk of abnormal karyotype obtained with this indication is 13 times higher than the risk which have pregnant women where there is only one indication for RAC. At the same model suggests that indications “triple test” in relation to age is only 0.657, which is logical because this marker indicates the possibility of a genetic predisposition and not the disease. Overall positive predictive values for all amniocentesis performed in this study was 0.92.

#### 4. DISCUSSION

The goal of this retrospective-descriptive study is to point out the reasons for performing early amniocentesis in our country, to draw attention to some peculiarities of our population in this sense, to introduce new medical methods such as screening tests, and so on.

Overall positive predictive value for all RAC is 0.92 and slightly lower

than the value that obtained Howe et al (2000) or Guantiu et al. (2002) (3). Age as an indication of RAC in our material is indicated in 85 % of cases. Similar values are found by many authors (Guantiu 81%, 2002, Chauouni et al 66%, 2001) (3). In this indication field, located 14 pathological cariogram or 5.5% of the total compared to the number of which is an indication of age. This result coincides with results of many researchers: Chaubouni et al. (2001)–3.95%, Dupont and Carles (2003)–3.2% (3, 6). Otherwise, the risk for this age (35 – 45 years is 0.8 to 7 % (Hook, 1992).

Years	Age	Ultrasound	Triple test	Anamnesis
20				
21		1		
22				
23				
24		1		
25				1
26		1		2
27				2
28				1
29			1	2
30		1	2	2
31			2	2
32			2	1
33		1	2	2
34	33		2	2
35	43		3	2
36	30		2	1
37	31		1	2
38	36			1
39	55			1
40	38			1
41	21			
42	6			
43	2			
44	1			
45	1			
Total:	254 (84.9%)	5 (1.6%)	17 (5.6%)	23 (7.9%)

Table 2. Average age of pregnant women according to indication

Most common pathological substrate was trisomy 21 in 10 cases or 53%. This clearly indicates that age is the only indication of the limited security and that the risk of cariogram abnormality at a younger age is also very high. In this sense the study by Loncar et al. (1995) who finds that 75 % of Down syndrome occurs in women younger than 35 years.

Positive ultrasound markers as an indication were the reasons for the RAC in 2% of cases in our study. Other authors report a higher percentage of patients with this method as an indication for the introduction of RAC. Chabouni et al. found in their study this indication in 8.2%, while Hal-

Syndromes	Cytogenetic findings	N
Sy. Down	47, xx+21	5
	47, xy+21	5
Sy. Edwards	47, xx+18	2
Sy. Klinefelter	47, xxy	3
Mosaicism	46, xy / 45x	1
	46, xy / 47, xy+20/44, xy	1
Translocation	46, xy,t(5;18)	1
Super woman	48 xxx	1

**Table 4.** Analysis of pathological cytogenetic findings after RAC

	Positive	Negative	PPV	Total
Age	14	240	0.06	254
Ultrasound	2	3	0.66	5
Triple test	2	15	0.13	17
Other	1	22	0.07	23
Total	19	280	0.92	299

**Table 5.** Distribution of pregnant women frequencies in relation to indication and the result of cytogenetic analysis (positive/negative) with positive predictive value of the test

liday (1998) states the percentage of 20% (3). Of the 5 cases that indicated the ultrasound marker 2 are pathological and both indicated the pathological value of nuchal translucency - 66% of the total number within the set of methods, i.e. -10.5% of the total number of pathological kariogram. It is a known fact that in addition to markers with high probability indicates trisomy. These values range from 45% (Baena et al. 2003) to 85%, which shows Oats (2000) (3). Triple test as an indication of RAC in our material yielded one positive result of 22 made for this pathological marker or 4.5% and 5.2% of the total number (19) pathological kariograms. Similar or slightly lower observations in their results found Dupont and Carles (2004) of 2.1%, Nyber et al. (1995) of 5.3% (3, 6). These values refer to the same level of cut-off (1:250). In our study, there were 95% false-positive results in this test. Benn et al (1995) and Nyberg et al. (1995) reached the same results (95% and 94.7%) (1, 6, 14). We also analyzed the number of high 7% of pathological kariogram findings in our sample. Specifically Mueller and Young (1998) (3) gave the incidence of chromosome abnormalities in newborns - it is (in all abnormalities) 90 cases per 10,000 live births or 0.9%. These facts all show and need no comment. These facts are no doubt the reason for the maximum engagement of all relevant medical factors, the entire community to support the introduction of early amniocentesis to all pregnant

women in Bosnia and Herzegovina, within indication areas conducted in our research (3).

## 5. CONCLUSIONS

Based on the obtained results it is possible to draw conclusions on the basis of which it would be possible to define the guidelines in which direction, medically safe, rational and appropriate use of the available medical methods in the field of prenatal diagnosis. This would allow patients better health services and health funds rational consumption. Pregnant women age over 35 years is the risk for chromosome abnormalities. In our study it is 5.5%. The risk of performing RAC is very low (0.02%), which is negligible compared to the health benefit that is gained by performing RAC for this indication area. Ultrasound markers as screening method to detect chromosome abnormalities in our study showed complete validity of this method. Specifically, the use of this marker is a risk of leakage of pathological kariogram reduced 13 times. Given the fact that this method is noninvasive, simple as a screening method is highly relied upon, it is necessary to conduct a wide-education specialist gynecologist for the reference implementation of this method. Biochemical markers as a method gave modest results, but are still recommended in particular, stricter cuff-off area. Cumulative rate of studied methods values of prenatal diagnosis gives greater diagnostic validity. So it is recommended for practical applications. Very high incidence of pathological kariogram within risk groups formed using standard methods (age) and modern screening methods (ultrasound and biochemical markers) suggest couple crucial facts: The need for involvement of the social medical community in order to methods of early amniocentesis in B&H to be widely available to patients and gynecologists; The need to conduct training of gynecologists in screening methods such as ultrasound markers on chromosome abnormalities; The need for legally verified medical procedure (medically safe, financially rational), for the benefit of our pregnant women and our children.

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