

## Prenatal Screening for Chromosomal Abnormalities in Tabriz, North-West of Iran

Zahra Fardiyazar,<sup>1</sup> Shabnam Vazifekhhah\*<sup>1</sup>

1. Department of Obstetrics and Gynecology, Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

Article information	Abstract
<p>Article history: Received: 25 May 2012 Accepted: 20 July 2012 Available online: 11 Mar 2013 ZJRMS 2013; 15(9): 68-73</p> <p>Keywords: Maternal age Chromosomal aberrations Nuchal Translucency Trisomy 21</p> <p>*Corresponding author at: Obstetrician and Gynecologist, Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran E-mail: Vazifekhhahshabnam@gmail.com</p>	<p><b>Background:</b> Several studies have indicated that when compared to non-consanguineous marriage, consanguineous marriage may lead to a higher incidence of congenital abnormalities. The study was performed to evaluate few screening tests to estimate the risk of chromosomal abnormalities in the first trimester compared between familial and non-familial marriages.</p> <p><b>Materials and Methods:</b> In this cross sectional study, 300 pregnant women with singleton pregnancy presenting to Tabriz Al-Zahra hospital from 2007 to 2009 were enrolled as study population. The participants were evaluated about chromosomal malformations using a combination of NT (Nuchal Translucency), PAPP-A (Pregnancy-Associated Plasma Protein A), and free beta- human chorionic gonadotropin (<math>\beta</math>-hCG). In positive screening test results, the participants underwent fetal karyotyping using amniocentesis or chorionic villi sampling (CVS).</p> <p><b>Results:</b> Pregnancies with higher risk were observed more among non-consanguineous marriages. The maternal age was not found to be a determinant in this regard. NT and free <math>\beta</math>-hCG values (but not PAPP-A) were significantly different between the two study groups. The triple screening test had a sensitivity of 100%. There were two cases of Down syndrome both belonging to the maternal age less than 35 years and non-consanguineous marriages.</p> <p><b>Conclusion:</b> Considering that a statistically significant association was not observed between abnormal test results and pregnancy complications (<math>p=0.73</math>), it seems that it is essential to use screening tests in all pregnant women. Especially that the only two pregnancies with Down syndrome in our study were under 35 years of age.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

## Introduction

In developed countries, pregnant women take part in prenatal programs that screen for chromosomal disorders and major defects of the fetus [1]. Screening for chromosomal abnormalities has developed over the last decade. Screening test can be carried out biochemically, ultrasonographically, or by both modalities [2].

Ultrasonographic measurement of fetal nuchal translucency (NT), when combined with maternal age, can independently detect 77% of cases of trisomy 21, with a similar false positive rate of 5% [3]. Combining ultrasonography and biochemical markers constituted an improvement in screening techniques. Through first trimester screening via analysis of biochemical markers taken from maternal blood [e.g., pregnancy-associated plasma protein A (PAPP-A) and free beta- human chorionic gonadotropin ( $\beta$ -hCG)], a detection rate of 90% for the most important chromosomal abnormalities, with a false-positive rate of 5%, is attainable [1-3]. Screening by a combination of ultrasound markers and maternal serum  $\beta$ -hCG and PAPP-A can identify up to 97% of fetuses with trisomy 21 and other major chromosomal abnormalities [4-6]. By combining the parameters for NT, PAPP-A, and free  $\beta$ -hCG, and incorporating the maternal risk factor, FMF (Fetal Medicine Foundation) -certified software calculates individual specific risk estimations for

the most frequent aneuploidies with a false-positive rate of only 5%. FMF certified software is a strict managing system and quality assessment, which estimates risk of aneuploidies with using of maternal age, NT, and biochemical parameters [1-3]. Data was being entered in the software and the software was being calculated the risk of aneuploidies automatically.

Prenatal diagnosis requires either amniocentesis from 15 weeks of pregnancy or chorionic villous sampling from 11 weeks. However, the invasive tests are carried out only in pregnancies considered to be at high-risk [5]. It has been a matter of discussion to which invasive prenatal tests such as amniocentesis or chorionic villous sampling (CVS) should be offered [1]. A major goal of a screening test is to achieve maximum accuracy and minimum harm at low cost. The integrated test currently meets best these criteria [2]. In this study we evaluated the triple screening tests (NT, PAPP-A, and free  $\beta$ -hCG) to estimation the risk of chromosomal abnormalities during the first trimester compared between consanguineous and non-consanguineous marriages.

## Materials and Methods

This is a cross-sectional study performed on 300 pregnant women with singleton pregnancy presenting to

Tabriz Al-Zahra hospital and sub-specialty clinics of Tabriz Medical University from February 2007 to July 2009. There were 99 consanguineous and 201 non-consanguineous marriages.

Written informed consent was obtained from all enrollees, according to the criteria of the Ethical Committee of Tabriz University of Medical Sciences. Inclusion criteria were women with singleton pregnancy with a gestational age within 11 to 14 weeks. Exclusion criteria were refusing to participate in the study, obstetric emergencies leading to abortion or preterm delivery, and a gestational age higher than 14 weeks. The patients were assessed to record age, gravidity, parity, previous abortion, familial relation, calculated risk for chromosomal malformations, presence of chromosomal or structural abnormalities, performing amniocentesis or not, and outcome of pregnancy and delivery.

The participants were examined for chromosomal malformations using a combination of NT, PAPP-A, and free  $\beta$ -hCG. All laboratory tests and examinations were performed in Tabriz Al-Zahra hospital by trained and expert personnel.

For calculating the risk of pregnancy, these tests results and the maternal age were entered into the related software and the risk was obtained as a ratio. Then, the primitive ratio was obtained as the percent of abnormality in certain gestational age, and was multiplied in MoM (mili of mole) results or medium of mean curve. It was considered as "positive screening test result" if obtained ratio was more than 1 in 250.

In positive screening test results, the participants underwent fetal karyotyping using amniocentesis or chorionic villus sampling (CVS). The couples were evaluated about consanguineous marriage and non-consanguineous marriage, and pregnancy complications and outcomes. The collected data was analyzed by SPSS-15 statistical software using *t*-test, Mann-Whitney-*U*,  $\chi^2$  and Exact Fisher tests. The results were expressed as percent and mean with standard deviation. The *p*-Values of less than 0.05 were considered as statistically significant.

## Results

From 300 pregnant women, 99 had consanguineous and 201 had non-consanguineous marriages. The mean age of

studied women was  $31.9 \pm 7.5$  years in familial and  $32.9 \pm 7.3$  years in non-familial marriages (Statistically non-significant). Of all patients 48 had positive screening tests (high risk) and underwent amniocentesis; 252 were low risk and spared from amniocentesis. The patients had a mean age of  $30.56 \pm 6.59$  years in amniocentesis (high risk) group and  $30.67 \pm 6.49$  years in non- amniocentesis (low risk) group.

The characteristics of patients in these age groups are compared in table 1. The gravidity, parity, and abortion histories of patients in high risk and low risk groups have been compared in table 2. The average gravidity of patients was  $2.28 \pm 1.42$  in high risk group in comparison with  $2.39 \pm 1.40$  in low risk. The comparison of difference in independent groups indicate that the difference was non- significant (Table 2). The average parity of patients was  $0.84 \pm 0.86$  in group with amniocentesis in comparison with  $0.88 \pm 0.86$  in patients without amniocentesis ( $p=0.82$ ). The average abortion history of patients was  $0.47 \pm 0.95$  in group with amniocentesis in comparison with  $0.59 \pm 1.08$  in patients without amniocentesis ( $p=0.55$ ). In high risk group 6 couples (12.5%) had consanguineous marriage, and 42 marriages (87.5%) were non- consanguineous. These figures in low risk group were 87 (34.6%) and 165 (65.45%) ( $p=0.013$ ).

The average NT of patients was  $3.79 \pm 1.26$  in the high risk group (with amniocentesis) versus  $1.42 \pm 0.04$  in low risk group ( $p<0.001$ ). The high NT was effective in obtaining high risk level and performing the amniocentesis. The average free  $\beta$ -hCG of patients was  $59.60 \pm 32.44$  in high risk group (with amniocentesis) in comparison with  $70.14 \pm 30.80$  in patients without amniocentesis ( $p=0.04$ ). The average free  $\beta$ -hCG was effective in obtaining high risk level and performing the amniocentesis. The MoM was 1.3 in high risk group (with amniocentesis) in comparison with 1.01 in patients without amniocentesis ( $p=0.031$ ).

The average PAPP-A of patients was  $9.7 \pm 2.51$  in high risk group (with amniocentesis) in comparison with  $11.14 \pm 0.58$  in low risk group. The MoM was 0.32 in high risk group (with amniocentesis) in comparison with 0.37 in patients without amniocentesis. The pregnancy outcome and complications in high risk and low risk groups have been compared in table 3. In women with lower serum PAPP-A (<5th percentile or <0.4 MoM), neither Down syndrome nor abortion were observed.

**Table 1.** The comparison of characteristics of patients in two age groups

Variable	Status	$\leq 35$ years N(%)	$> 35$ years N(%)	<i>p</i> -Value
Family relation	Yes	73(31.4%)	20(29.4%)	0.85
	No	159(68.5%)	48(70.5%)	
Pregnancy complications	Yes	16(6.9%)	9(13.2%)	0.22
	No	216(93.1%)	59(86.7%)	
	Alive birth	224(96.5%)	65(95.5%)	
Pregnancy outcome	Intrauterine death	6(2.7%)	3(4.5%)	0.31
	Down syndrome	2(0.8%)	0(0%)	
Amniocentesis	Yes	37(16.0%)	11(16.1%)	0.56
	No	195(84.0%)	57(83.8%)	

**Table 2.** The comparison of gravidity, parity, and abortion histories of patients in two studied groups

Variable	Number	High risk (amniocentesis) N (%)	Low risk N(%)	Total N(%)
Gravida	1	19(39.5)	83(32.9)	102(34)
	2	4(8.3)	32(12.7)	36(12.0)
	3	16 (33.3)	94(37.3)	110(36.6)
	4	3(6.2)	20(7.9)	23(7.6)
	5	6(12.5)	23(9.1)	29(9.6)
	Total	48(100)	252(100)	300(100)
Parity	0	19(39.6)	83(32.9)	102(34)
	1	5(10.4)	46(17.8)	51(17)
	2	15(31.2)	92(36.1)	107(35.6)
	3	9(18.7)	31(11.9)	40(13.3)
	Total	48(100)	252(100)	300(100)
Abortion	0	36(75.0)	178(70.6)	214(71.3)
	1	4(8.3)	30(11.9)	34(11.3)
	2	6(12.5)	23(9.1)	29(9.6)
	3	0(0)	10(3.9)	10(3.3)
	4	2(4.1)	11(4.3)	13(4.3)
	Total	48(100)	252(100)	300(100)

**Table 3.** The comparison of pregnancy outcome and complications of patients in two studied groups

Variable	Status	High risk(amniocentesis) N(%)	Low risk N(%)	p-Value
Pregnancy	Normal	43(90.3)	207(82.1)	>0.05
	Complicated	5(10.4)	45(17.8)	
Sex of newborn	Boy	27(56.2)	142(56.4)	0.56
	Girl	21(43.7)	110(43.6)	
Pregnancy outcome	Alive birth	43(90.6)	247(98.1)	>0.05
	Stillbirth	1(2.0)	5(1.9)	
	Down syndrome	2(4.1)	0(0)	
	Gestational Htn	1(2.0)	9(3.5)	
	Preeclampsia	2(4.1)	5(1.9)	
	GD	0(0)	10(3.9)	
Pregnancy complications	Preterm labor	4(8.3)	10(3.9)	>0.05
	IUGR	3(6.2)	5(1.9)	
	Cholestasis	0(0)	3(1.1)	
	DVP	0(0)	3(1.1)	
	Oligohydramnios	2(4.1)	4(1.5)	

Htn, hypertension; GD, gestational diabetes; IUGR, intrauterine growth restriction; DVP, deep vein thrombosis

The serum PAPP in couples with consanguineous and non-consanguineous marriage was not statistically significant. Serum free  $\beta$ -hCG in couples with consanguineous ( $73.01 \pm 4.33$ ) and non-familial ( $66.41 \pm 2.53$ ) marriage was not statistically significant. The serum NT in couples with consanguineous ( $1.41 \pm 0.07$ ) and non-consanguineous ( $1.59 \pm 0.29$ ) marriage was not statistically significant. There was two Down syndrome in women with high serum NT. There was not statistically significant relation between abnormal test results and pregnancy complications. We achieved specificity and sensitivity values of 84% and 100% respectively for the triple test used in screening of chromosomal anomalies during the first trimester pregnancies. We didn't get any false negative result.

## Discussion

We studied 300 women with singleton pregnancies during the first trimester to evaluate the triple method for screening aneuploidy. Spencer et al. suggest that the detection rates of first trimester screening for trisomy 21 and other aneuploidies are far better than can be achieved

by second trimester serum screening [7]. First-trimester screening for trisomies 21 and 18 on the basis of maternal age, maternal serum free  $\beta$ -hCG and PAPP-A with NT thickness has good sensitivity at an acceptable false positive rate [3].

There are very few reports about screening performance of second trimester markers [8]. Regarding second trimester noninvasive testing, biochemical screening is more accurate in establishing risk than maternal age alone. As the value of first trimester screening becomes more evident and practical, we can anticipate that second trimester screening and invasive testing may be needed only in a minority of cases [9].

Miron et al. suggest that in prenatal screening for Down syndrome and trisomy 18, it is possible to identify NT threshold values above which biochemical screening provides no additional benefit. In pregnancies in which NT is above the established upper cut-offs, invasive prenatal screening can be offered without delay [10]. Chitty et al. suggest that in the diagnosis of chromosomal abnormalities after first trimester screening for trisomy 21, karyotyping only if the fetal NT thickness is increased would reduce the economic costs, provide rapid delivery

of results, and identify 99% of the clinically significant chromosomal abnormalities [11].

When NT levels increase above the 95th percentile, the chance that a healthy baby will be born, decreases [1]. The high detection rate for Down syndrome pregnancies which can be obtained by measuring fetal NT early in pregnancy can be increased by combining it with placental hormones (PAPP-A and free  $\beta$ -hCG) and maternal age ('combined test') [12]. Similarly we used the same method in our study.

There is an increasing popularity of first trimester targeted ultrasound examination with the potential advantage of an earlier diagnosis of fetal aneuploidy [13]. One major progress in fetal medicine in recent years is the increased sensitivity of sonographic screening for fetal malformations, due to technical improvement and also to a better training of professionals [14]. The most effective sonographic marker of trisomy 21 and other chromosomal defects is increased NT thickness at 11-14 weeks [8]. Maymon et al. used NT and biochemistry assessments in the first trimester triple test. Fetal karyotyping was performed by means of mid-gestation amniocentesis. NT and PAPP-A emerged as the most sensitive marker combination. The combined strategy yields a 60% detection rate (13.22) of the affected pregnancies and without any increase in the false-positive results [15].

Naidoo et al. conducted a study to determine the effectiveness of NT thickness screening in predicting aneuploidy and structural abnormalities in a South African population. They concluded that the use of these screening methods has enabled prenatal karyotyping to become cost effective, and allows concentration on pregnancies at highest risk for chromosomal abnormalities, regardless of age [16]. The data showed that screening between 10 and 14 weeks by combining the serum markers with NT measurement had a detection rate of 80% for a 5% false-positive rate, better than maternal age with two serum markers (62% for 5%) or maternal age with nuchal translucency measurement (63% for 5%). Meanwhile, these results provide a reasonable working estimate of screening performance using different combinations of these markers [17].

Krantz et al. assessed the effectiveness of free  $\beta$ -hCG, PAPP-A, and NT in a first-trimester screening study. Down syndrome screening using combined biochemistry and ultrasound resulted in a false-positive rate of 4.5% and detection rate of 87.5% in patients under age 35 years. In older patients, the false-positive rate was 14.3% and detection rate was 92%. Using modeling, at a fixed 5% false-positive rate, the Down syndrome detection rate was 91%. Conversely, at a fixed 70% Down syndrome detection rate, the false-positive rate was 1.4%. They concluded that first-trimester screening for Down syndrome and trisomy 18 is effective and offers substantial benefits to clinicians and patients [18]. We found two cases of Down syndrome which both belonged to the maternal age less than 35 years. So, it seems that it is essential to use screening tests in all pregnant women. Screening for trisomy 21 by a combination of maternal age, fetal NT thickness and maternal serum free  $\beta$ -hCG

and PAPP-A at 11 to 14 weeks of gestation is associated with a detection rate of 90% for a false-positive rate of 5% [19].

An improvement in prenatal screening for chromosomal defects has been achieved by combining sonography and biochemical markers. Analyzing markers taken from maternal blood such as PAPP-A and free  $\beta$ -hCG in combination with the ultrasound marker NT provides detection rates of 90% for the most important chromosomal anomalies. In addition, nuchal translucency is a marker for severe heart defects [1]. In a study on 100 trisomy 21 and 400 chromosomally normal singleton pregnancies at 11 to 14 weeks of gestation, ultrasound examination showed that there were no significant differences in median maternal age, gestational age, NT, free  $\beta$ -hCG MoM and PAPP-A MoM in trisomy 21 fetuses with and without a visible nasal bone (a sonographic marker). For a false-positive rate of 5%, it was estimated that screening with the four markers in combination with maternal age would be associated with a detection rate of 97%. They concluded that an integrated sonographic and biochemical test at 11 to 14 weeks can potentially identify about 90% of trisomy 21 fetuses for a false-positive rate of 0.5% [19]. In our study, NT and maternal serum free  $\beta$ -hCG were significantly higher in high risk group. Also, free  $\beta$ -hCG MoM was significantly higher in high risk group. However, although free PAPP-A and PAPP-A MoM were higher in high risk group, the differences were not significant. The increase in maternal age in recent years has intensified the effort to develop early non-invasive methods for screening for trisomy 21 and other chromosomal abnormalities in prenatal diagnosis. In the first trimester of pregnancy, maternal age, fetal NT, maternal serum free  $\beta$ -hCG and PAPP-A are used as screening markers [5]. In our study, the maternal age was not effective on pregnancy outcome and complications.

Dhaifalah et al. showed that use of the first trimester screening reduced the number of invasive genetic testing from 18% to 5%. First trimester screening for trisomy 21 and other aneuploidies has a high sensitivity with a low false positive rate and can be delivered in an efficient manner in a university hospital [5]. The results of Dhaifalah et al. suggest that a combination of maternal serum free  $\beta$ -hCG and PAPP-A with NT thickness at 11–14 weeks of gestation would identify about 90 % of trisomy 21 pregnancies for a 5 % false positive rate, which is far superior to the average sensitivity of 65 % achievable by second trimester biochemical screening. The evidence on first trimester screening for trisomy 21 by nuchal translucency and/or biochemical methods was sufficiently well developed to move out of the research phase into routine practice and that the detection rates by first trimester screening would be superior to those obtained with biochemical screening in the second trimester [5, 20].

There were two cases of Down syndrome which both were belonging to the non-consanguineous marriages. Similarly, in the Pouya et al. study, fragile X syndrome

(FXS) was found in 32 of the 508 families (6.3%), in 15.3% (19.124) of families with unrelated parents, and in 3.4% (13.384) of consanguineous families. Thus, the frequency of FXS seems to be higher in patients with unrelated parents [21]. Also, in our study, the values of NT, free  $\beta$ -hCG and PAPP-A were not different between consanguineous and non-consanguineous couples.

The value of all noninvasive prenatal tests must be viewed with the perspective of the consequences of invasive testing [9]. Prenatal diagnosis of chromosomal abnormalities can be accurately made by cytogenetic studies of samples obtained from invasive procedures, such as amniocentesis or CVS. Because these procedures are associated with a risk of miscarriage, the common approach is to perform non-invasive test to define an individual woman's risk of having a chromosomal abnormal pregnancy [2].

Recent advances in genetic technology have substantial implications for prenatal screening and diagnostic testing. The past year has also seen important changes in recommendations surrounding the genetic counseling that occurs in the provision of such testing. Testing for chromosomal abnormalities has seen the introduction of first-trimester screening, as well as strategies to improve detection through sequential testing [22]. A beneficial consequence of screening for trisomy 21 is the early diagnosis of trisomies 18 and 13, which are the second and third most common chromosomal abnormalities. First-trimester screening for trisomy 21 by maternal age, fetal NT thickness, fetal heart rate (FHR) and maternal serum free  $\beta$ -hCG and PAPP-A, can detect approximately 95% of trisomy 13 and 18 fetuses [23]. Detection rates in excess of 90% can be routinely achieved for Trisomy 21, Trisomy 13, Trisomy 18 using a combination of fetal NT thickness and maternal serum free  $\beta$ -hCG and PAPP-A at 11 to 13 weeks of gestation [24]. We achieved the specificity and sensitivity of 84% and 100% respectively for the triple test used for screening of chromosomal anomalies in first trimester pregnancies.

## References

- Eiben B, Glaubit R. First-trimester screening: An overview. *J Histochem Cytochem* 2005; 53(3): 281-3.
- Stojilkovic-Mikic T, Rodeck CH. Screening for chromosomal anomalies: First or second trimester, biochemical or ultrasound? *Ann Acad Med Singapore* 2003; 32(5): 583-8; quiz 588-9.
- Wapner R, Thom E, Simpson JL, et al. First-trimester screening for trisomies 21 and 18. *N Engl J Med* 2003; 349(15): 1405-13.
- Cicero S, Bindra R, Rembouskos G, et al. Integrated ultrasound and biochemical screening for trisomy 21 at 11 to 14 weeks. *Prenat Diagn* 2003; 23(4): 306-10.
- Dhaifalah I, Santavy J, Zapletalova J. Screening for chromosomal anomalies in the first trimester: A report on the first year of prospective screening for chromosomal anomalies in the first trimester in the Czech Republic. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2006; 150(2): 275-8.
- Kagan KO, Wright D, Maiz N, et al. Screening for trisomy 18 by maternal age, fetal nuchal translucency, free beta-

Low levels of maternal serum PAPP-A are related with abnormal karyotype and subsequent delivery of a small-for-gestational age (SGA) infant [25]. Low levels of maternal serum PAPP-A and free  $\beta$ -hCG and increased fetal NT are associated, in the absence of an abnormal karyotype, with an increased risk of impending fetal death. The likelihood ratio profiles provided at various levels of PAPP-A or free  $\beta$ -hCG may be of some help in counseling women with such results and raise awareness among health-care professionals for increased surveillance in such cases [26].

With the advent of first-trimester screening, it is important to reassure pregnant women that they will give birth to a healthy baby. Very accurate risk estimations can now be offered, and invasive procedures such as amniocentesis or CVS can be performed with more reliability. For these reasons, it is extremely important to offer genetic counseling to women and their partners so that they understand the limits and risks of first-trimester screening. Considering that there was not statistically significant relation between abnormal test results and pregnancy complications, it seems that it is essential to use screening tests in all pregnant women. Especially that the only two pregnancy with Down syndrome in our study were belonged to the maternal age less than 35 years.

## Acknowledgements

We would like to thank the Tabriz University of Medical Sciences for funding this investigation. The code of project is 87/3-9/5.

## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

## Conflict of Interest

The authors declare no conflict of interest.

## Funding/Support

Tabriz University of Medical Sciences.

- human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 2008; 32(4): 488-92.
- Spencer K, Spencer CE, Power M, et al. Screening for chromosomal abnormalities in the first trimester using ultrasound and maternal serum biochemistry in a one-stop clinic: A review of three years prospective experience. *BJOG* 2003; 110(3): 281-6.
- Cicero S, Sacchini C, Rembouskos G and Nicolaides KH. Sonographic markers of fetal aneuploidy: A review. *Placenta* 2003; 24 Suppl 2: S88-S98.
- Budorick NE, O'Boyle MK. Prenatal diagnosis for detection of aneuploidy: the options. *Radiol Clin North Am* 2003; 41(4): 695-708.
- Miron P, Cote YP, Lambert J. Nuchal translucency thresholds in prenatal screening for Down syndrome and trisomy 18. *J Obstet Gynaecol Can* 2009; 31(3): 227-35.
- Chitty LS, Kagan KO, Molina FS, et al. Fetal nuchal translucency scan and early prenatal diagnosis of chromosomal abnormalities by rapid aneuploidy

- screening: Observational study. *BMJ* 2006; 332(7539): 452-5.
12. Schuchter K, Hafner E, Stangl G, et al. The first trimester 'combined test' for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. *Prenat Diagn* 2002; 22(3): 211-5.
  13. Geipel A, Daiss T, Katalinic A, et al. Changing attitudes towards non-invasive aneuploidy screening at advanced maternal age in a German tertiary care center. *Ultraschall Med* 2007; 28(1): 67-70.
  14. Mahieu-Caputo D, Senat MV, Romana S, et al. What's new in fetal medicine? *Arch Pediatr* 2002; 9(2): 172-86.
  15. Maymon R, Betser M, Dreazen E, et al. A model for disclosing the first trimester part of an integrated Down's syndrome screening test. *Clin Genet* 2004; 65(2): 113-9.
  16. Naidoo P, Erasmus I, Jeeboth J, et al. Nuchal translucency as a method of first-trimester screening for aneuploidy. *S Afr Med J* 2008; 98(4): 295-9.
  17. Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in first-trimester screening for Down's syndrome. *Prenat Diagn* 1997; 17(9): 821-9.
  18. Krantz DA, Hallahan TW, Orlandi F, et al. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. *Obstet Gynecol* 2000; 96(2): 207-13.
  19. Cicero S, Bindra R, Rembouskos G, et al. Integrated ultrasound and biochemical screening for trisomy 21 using fetal nuchal translucency, absent fetal nasal bone, free beta-hCG and PAPP-A at 11 to 14 weeks. *Prenat Diagn* 2003; 23(4): 306-10.
  20. Spencer K, Souter V, Tul N, et al. A screening program for trisomy 21 at 10-14 weeks using fetal nuchal translucency, maternal serum free  $\beta$ -human chorionic gonadotropin and pregnancy-associated plasma protein-A. *Ultrasound Obstet Gynecol* 1999; 13(4): 231-7.
  21. Pouya AR, Abedini SS, Mansoorian N, et al. Fragile X syndrome screening of families with consanguineous and non-consanguineous parents in the Iranian population. *Eur J Med Genet* 2009; 52(4): 170-3.
  22. Norton ME. Genetic screening and counseling. *Curr Opin Obstet Gynecol* 2008; 20(2): 157-63.
  23. Kagan KO, Wright D, Valencia C, et al. Screening for trisomies 21, 18 and 13 by maternal age, fetal nuchal translucency, fetal heart rate, free beta-hCG and pregnancy-associated plasma protein-A. *Hum Reprod* 2008; 23(9): 1968-75.
  24. Spencer K. Aneuploidy screening in the first trimester. *Am J Med Genet C Semin Med Genet* 2007; 145(1): 18-32.
  25. Spencer K, Cowans NJ, Avgidou K, et al. First-trimester biochemical markers of aneuploidy and the prediction of small-for-gestational age fetuses. *Ultrasound Obstet Gynecol* 2008; 31(1): 15-9.
  26. Spencer K, Cowans NJ, Avgidou K and Nicolaides KH. First-trimester ultrasound and biochemical markers of aneuploidy and the prediction of impending fetal death. *Ultrasound Obstet Gynecol* 2006; 28(5): 637-43.