Original Article

First Trimester Screening Tests Pregnancy and Trisomy 13 Syndrome, Sex Chromosome Aneuploidy in Iran: A Cross-Sectional Study

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Abstract

Background: Trisomy 13 (T13) and sex chromosome aneuploidies (SCA) are the vital causes of congenital malformations. This study was performed to identify the T13 and SCA with screening tests in the first trimester of pregnancy.

Materials and Methods: In this cross-sectional study, first-trimester combined screening was conducted on 2100 pregnant women referred to Narges Genetics Laboratory, Ahvaz, Iran. Evaluating the first trimester screening tests, including nuchal translucency (NT), crown–rump length (CRL) and pregnancy-associated plasma protein-A (PAPP-A), and free beta of human chorionic gonadotropin (fβhCG) was performed. For a definitive diagnosis of T13 and SCA syndrome, fetal karyotype was evaluated.

Results: The average NT and CRL in high-risk group for T13 were 5.96 mm and 61.7 mm respectively and in high-risk groups for SCA were 3.7 mm and 75.9 mm, respectively. Significant correlation was observed among NT, CRL and T13, SCA ($P<0.05$). The average serum fβhCG and PAAP-A levels in high-risk group for T13 were 0.42 and 0.31, respectively. Significant correlation was observed between decrease fβhCG, PAPP-A and T13 levels and increase fβhCG levels and SCA levels ($P<0.05$). No Significant correlation was observed between PAPP-A levels and SCA levels ($P>0.05$).

Conclusion: Using special software and karyotype testing, the prenatal screening tests based on the maternal age and gestational age in the first trimester of pregnancy may determine the major risk of fetal chromosomal abnormalities.

Keywords: Chromosomal Anomaly, Karyotype, Prenatal diagnosis, Trisomy 13

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Introduction

In general, some births have chromosomal abnormalities during the fetal period or fetal development due to genetic factors such as genetic chromosomal or genetic abnormalities, resulting in abortion, intrauterine death, and shortened postnatal lifespan (1, 2). Chromosomal abnormalities cause more than 50% of spontaneous abortions (3). Therefore, in the last few decades, most researchers have paid more attention to prenatal screening tests for diagnosis of chromosomal abnormalities and their global effects. In developing countries, prenatal screening tests are widely used and validated in clinical practice to prevent birth defects and improve the quality of the birth population (4). Different prenatal screening tests are available based on the methodology and algorithms used for data analysis, the mother's trimester of pregnancy, and the kind of ailment in concern (5). Prenatal diagnosis of genetic disorders mostly is recommended in following situations: the maternal age of 35 years or above; positive first or second trimester screening test results, and increased risk of fetal aneuploidies in terms of family history (6, 7).

Meanwhile, the first trimester spontaneous abortions (9-12 weeks) occur in 15 to 20% of all clinically recognized pregnancies (3). Therefore, screening of the first trimester of pregnancy is done to diagnose congenital anomalies in most countries with 90% detection rate and 5% false-positive rate (8). Patau syndrome (T13) is one of the most common autosomal aneuploidies in newborns (birth incidence is about 1/5000 live births). More than 50 percent of infants with Trisomy 13 (T13) have severe heart, brain and spinal cord disorders, severe mental and physical disabilities, and cleft lip and palate, according to a 23-year study conducted at a public hospital in Brazil.

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These fetuses die in utero and will not live to be one year old if born (9). Also, infants with T13 are exposed to very low birth weight due to multiple genetic problems (10). Therefore, prenatal screening tests are one of the practical ways to prevent and reduce the birth rate of infants with T13 (11). It includes nuchal translucency (NT), crown–rump length (CRL), beta human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A) (12, 13).

On the other hand (sex chromosome aneuploidies; SCA) including, 45x (Turner syndrome) where chromosome deficiency x and 47xxy (Klinefelter syndrome) indicate the presence of extra chromosomes x (14). In some cases, the symptoms of people with SCA can be mild or without mental retardation; some have a fixed phenotype including physical abnormalities, learning disabilities, and infertility (15). First-trimester prenatal screening tests for Klinefelter syndrome and Turner syndrome assessment age mother of fetal NT and maternal levels freeβ-hCG and levels PAPP-A at 11+0 to 13+6 weeks of pregnancy is now an effective screening programin maternal serum (16). Increase levels of multiple of the median (MOM) freeβ-hCG and decrease levels PAPP-A in maternal serum can be an important indicator for the following Klinefelter syndrome and Turner syndrome (17). Therefore, our study investigated the implementation of prenatal screening tests for the accuracy of T13 and SCA abnormalities in pregnancies in southern Iran. The purpose of this study was prenatal diagnosis of T13 and SCA based on the evaluation of early pregnancy screening tests (NT, CRL, PAPPA, fβhCG). This is because of studies on fetuses with T13 have been less studied due to spontaneous abortions and also SCAs very common.

Materials and Methods

This was an analytic cross-sectional study performed on 2100 patients in 2021 (gestational age of 11+0 to 13+6 weeks) undergoing prenatal screening tests at Narges Genetics Laboratory, Ahvaz, Iran. Inclusion criteria included: first trimester of pregnancy, single fetus, and normal pregnancy and exclusion criteria including twin fetuses and multiple pregnancies using artificial methods with a history of maternal diseases such as diabetes, hypertension, hyperlipidemia, and aborted fetuses. Written consent was obtained consciously from all participants in this study, and also this project approved by the Ethics Committee of Behbhan Azad University, Behbhan, Iran (IR.IAU.BEHBAHAN.REC.1400.002) and is registered.

According to Fetal Medicine Foundation (FMF), all pregnant women were checked with a certified ultrasound to determine their gestational age. The required information was recorded in the questionnaire form, which included the mother's age and weight, multiple births, history of specific underlying disease, family history of genetic disease, family relationship between parents, history of giving birth to a baby with a congenital anomaly, and smoking at the time of screening and genetic tests. There were 1950 cases in the low-risk group and 150 cases in the high-risk group. Transabdominal and optionally transvaginal ultrasonography was performed by an ultrasound examiner for evaluation major fetal defects and measure fetal CRL and NT. Experiments have been performed using professional techniques described by the FMF (www.fetalmedicine.com) and recorded in a questionnaire. Afterwards, 5 mL venous blood mother were collected and kept at room temperature (18-25°C) for 30 minutes, and centrifuged for amount 2500 g for 15 minutes (4ºC). Sera samples were stored at -20ºC to analysis. To analyze the samples, seramaternal samples were analyzed for MOM PAPP-A and MOM fβ-hCG concentrations were assessed by automated analyzer time resolved fluorescence immune analyzer using appropriate reagents (PerkinElmer, Gaithersburg, MD, USA). Closed immunoassay analyzers are sophisticated platforms for measuring biochemical biomarkers (13). In the final stage, to diagnose chromosomal anomaly T13, Klinefelter syndrome, and Turner syndrome karyotype examinations were performed.

The statistical analysis was done using the SPSS version 18.0 soft ware Inc, Chicago, IL. The results were expressed as the means and standard deviations and performed with analysis of variance (ANOVA) followed by Dunnett's new multiple range test, and values of $P<0.05$ were considered statistically significant.

Results

The comparison was made between low-risk and high-risk cases, pregnant women under 35 and over 35 years of age to assess T13 and SCA. Classification was performed based on age maternal body mass index (BMI), fetus NT, and CRL are shown in Table 1. Prenatal screening tests predicted 150 pregnant women were included in high-risk and 1950 cases in low-risk group betweenT13 and SCA, receptively. A value of Under and Over 35 years for age, ≥2.5 mm for NT, and 45≥ mm for CRL meant high-risk group based on routine prenatal screening tests. No significant differences in maternal age and BMI were evident in low and high-risk groups T13 (>35 and ≤ 35 years) (P=0.451 and P=0.461). No significant differences in the maternal age and BMI were evident in low and high-risk groups SCA (>35 and ≤ 35 years) (P=0.421 and P=0.471). On the other hand, NT and CRL variables in T13 and SCA have a significance level of less than 0.05, so that the P value for NT and CRL variables in T13 and SCA is equal to 0.0001. It means that these variables have a significant difference among 4 groups so that in T13, NT variable in the high-risk group has the highest mean (5.96 ± 0.9) and in the low-risk group has the lowest mean (1.61 ± 0.4), CRL variable has the highest mean (66.8 ± 4.8) in the group over 35 years and the lowest mean in the low risk group (51.6 ± 10.8). In this analysis, NT variable in SCA had the highest mean (4.01 ± 1.7) in the high risk group over 35 years and had the lowest mean (1.72 ± 0.4) in the low risk group, CRL variable had the highest mean (66.8 ± 4.8) in the high-risk group over 35 years and the lowest mean (55.1 ± 6.6) in the low risk group.

Table 2 shows the maternal biochemical markers of with the chromosomal anomaly (T13 and SCA). The free β-hCG and PAPP-A contents was were significantly increased in T13 cases in the low-risk group in the comparison of high-risk group. MoM fβhCG variable has the highest mean (1.22 ± 0.63) in the low risk group and MoM PAPP-A variable in the low risk group has the highest mean with P=0.0001(1.22 ± 0.63). The free β-hCG levels were significantly decreased in SCA cases in the low-risk group in comparison of high-risk group, the variable MoM fβhCG with P=0.04 has the highest mean (1.83 ± 1.36) in the high-risk group of 35 years. PAPP-A content was not significant in SCA cases in the low-risk group compared to the high-risk group with P=0.07. In the first trimester screening for T13 and SCA anomalies by maternal age, the fetal characteristics NT and CRL, and maternal blood biochemical markers free β-hCG and PAPP-A about 2.86% of fetuses with T13 anomaly can be identified and about 3.33% of fetuses with SCA.

Of the 2100 pregnant women who were screened in group T13, there were 1067 cases in the low-risk group and 81 cases in the high-risk group, of whom 70 followed the karyotype test (number of fetuses with T13 n=3). In group SCA, there were 883 cases in the low-risk group and 69 cases in the high-risk group, of whom 62 followed the karyotype test (number of fetuses with SCA; n=5, Klinefelter syndrome; n=3, Turner syndrome; n=2). The detection rate and false positive were 100 and 3% for T13 and 100 and 2% for SCA, respectively (Table 3). Table 4 shows the formula for calculation the percentage of the amount false positive and detection rate.

Table 1: Prenatal screening test gestational age, BMI, NT,CRL for detection of trisomy 13 (T13) and sex chromosome aneuploidies (SCA) at risk pregnant womenand her fetuses

Chromosomal anomaly	Characteristics	Groups				P value
		Low risk	High risk	High risk under 35 years (n=85)	High risk over 35 years (n=65)	
T13	Age (Y)	33.9 ± 4.7	34.3 ± 2.5	29.1 ± 2.3	37.7 ± 2.2	0.451
	BMI (kg/m^2)	64.7 ± 10.7	66.2 ± 10.4	63.2 ± 9.2	65.2 ± 10.2	0.461
	NT (mm)	1.61±0.4^a	5.96 ± 0.9^b	3.24 ± 0.5^b	4.13 ± 0.5^b	0.001*
	CRL (mm)	51.6 ± 10.8^c	61.7 ± 4.4^d	60.6 ± 4.4^d	66.8 ± 4.8^d	0.001*
SCA	Age (Y)	30.2 ± 5.2	33.1 ± 4.4	24.9 ± 3.1	37.7 ± 1.5	0.421
	BMI (kg/m^2)	68.5 ± 14.2	67.7 ± 10.3	70.1 ± 10.1	72.3 ± 11.1	0.471
	NT (mm)	1.72 ± 0.4^a	3.7 ± 0.6^b	3.38 ±5.9^b	4.01 ± 1.7^b	0.001*
	CRL (mm)	55.1 ± 6.6^c	75.5 ± 9.7^d	63. 4 ± 6.4^d	75.9 ± 11.7^d	0.001*

Data are presented as mean ± SD. Analysis of variance (ANOVA) followed by Dunnet's new multiple range test. The letters (a-d) show significant difference between group low risk with high risk, high risk under and over years 35. BMI; Body mass index, NT; Nuchal translucency, CRL; Crown–rump length, and *; There is a significant difference (P<0.001) between low risk with high risk, high risk under and over 35 years groups.

Table 2: Maternal biochemical markers in MoM fβhCG and MoM PAPP-A pregnant woman with trisomy 13 (T13) and sex chromosome aneuploidies (SCA) cases

Chromosomal anomaly	Biochemical markers	Groups				P value
		Low risk	High risk	High risk under 35 years	High risk over 35 years	
T13	MoM fβhCG (ng/mL)	1.02 ± 0.71^a	0.42 ± 0.55^b	0.41 ± 0.53^b	0.41 ± 0.42^b	0.001*
	MoM PAPP-A (mg/L)	1.22 ± 0.63^c	0.31 ± 0.38^d	0.30 ± 0.21^d	0.31 ± 0.32^d	0.001*
SCA	MoM fβhCG (ng/mL)	0.96 ± 0.77^a	1.74 ± 0.92^b	1.83 ± 1.36^b	1.31 ± 0.79^b	0.04**
	MoM PAPP-A (mg/L)	1.21 ± 0.62	0.67 ± 0.58	0.84 ± 0.68	0.72 ± 0.41	0.07¶

Data are presented as mean ± SD. One-way analysis of variance (ANOVA) followed by Dunnet's new multiple range test. The letters (a-d) show significant difference between group low risk with high risk, high risk under and over years 35. fβ-hCG; Free beta-human chorionic gonadotropin, PAPP-A; Pregnancy-associated plasma protein-A, MoM; Multiple of the median, *; There is a significant difference (P<0.001) between low risk with high risk, high risk under and over 35 years groups, **; There is a significant difference (P<0.05) between low risk with high risk, high risk under and over 35 years groups, and ¶; Not significant (P>0.05) between low risk with high risk, high risk under and over 35 years groups.

Table 3: Outcome of amniocentesis, karyotype examinations and, detection rates and false positive in pregnant women

Group	Total	Karyotype examinations	Culture of amniotic fluid		False positive (%)	Detection rete (%)
			Abnorma karyotype	Normal karyotype		
High risk (T13)	81	70	n=3	n=67	3	100
Low risk (T13)	1067	-	-			
High risk (SCA)	69	62	Klinefelter syndrome n=3	n=30		
			Turner syndrome n=2	n=27	2	100
Low risk (SCA)	883	-	-			

T13; Trisomy 13 and SCA; Sex chromosome aneuploidies.

Table 4: Formula calculation the percentage of the amount false positive and detection rate

Karyotype examinations – Abnorma karyotype ÷ Total number of participants × 100	False negative %
Abnormal karyotype ÷ Abnormal karyotype + False negative × 100	Detection rete %

Nuchal translucency test measurements shows ultrasound picture of in 12-14 week gestation fetus (Figs.1, 2).

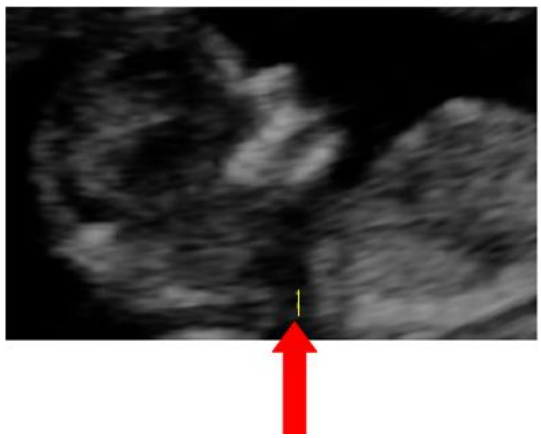

Fig.1: Shows a normal fetus.

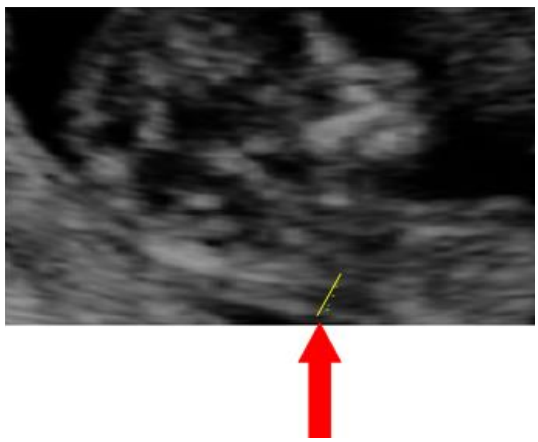

Fig.2: Shows fetus with chromosomal anomaly, demonstrating increased nuchal translucency thickness.

Discussion

The birth of babies with a birth defect has many cultural, economic and social impacts on the family and society. In addition, the risk of giving birth to a baby with congenital and structural abnormalities is 3-5% in any pregnancy, so screening tests are required in the first semester of pregnancy (18, 19). As a result, medical teams are focused on improving pregnancy outcomes by detecting these abnormalities as soon as possible and providing appropriate counseling to couples (20).

The present study showed that by performing screening in the first trimester of pregnancy, and measuring fetal NT and CRL levels as well as serum levels of maternal blood mother markers PAPP-A and fβhCG, T13 and SCA can be detected. In the present study, 150 participants were in the high-risk group, and after examining the karyotype, 8 abnormal karyotypes were observed. Concretely chromosomal anomaly associated with increased fetuses NT thickness and CRL characteristics in the first trimester gestational. Consequently, all these practical benefits (high sensitivity and specificity) suggested that measurements of NT thickness and CRL characteristics can calculate the risk of fetus chromosomal anomalyin at the first trimester gestational. In agreement with the present findings, multiple studies showed the diagnosis of chromosomal anomaly can be made by prenatal screening tests (like NT thickness and serum marker PAPP-A and fβhCG) (21-23). In a study similar to the present study, Carrara et al. (24) and Engell et al. (25) of diagnosis T13 after screening tests in the first trimester of pregnancy, they stated that the mean NT of high-risk group was higher than that of the low-risk group. In addition, after serum analysis of maternal blood markers, the mean fβhCG and PAPPA were significantly lower in high-risk subjects compared to the low-risk group. Studies have shown that PAPPA is associated with a variety of physiological and pathological processes through the regulation of local concentrations of insulin-like growth factor (26).

Our results show that chromosomal abnormalities are associated with low levels of PAPPA in maternal blood during the first trimester. It may increase the risk of T13 by inducing the development of chromosomal abnormalities. On the other hand, according to the results of biochemical markers, in the case of T13, a significant decrease in free βhCG levels was found at maternal serum pregnancy levels. Similar to our results, previous studies stated that in T13 anomaly reduced in the levels of free β-hCG and PAPP-A in maternal serum pregnancy.

Decreasing the marker serum levels PAPP-A which indirectly reflected the presence of a chromosomal anomaly in fetus's body cells (27). Difference between the present study and the study of Piazze et al. (28) indicates that there is no significant relationship between NT screening in the first trimester of pregnancy, PAPP-A and fβhCG levels, and the incidence of and chromosomal abnormalities. In a study similar to the present study, screening tests in the first trimester of pregnancy were used to diagnose the abnormality SCA, which was a significant relationship between increase size NT and increase serum marker level fβhCG that was revealing with an anomaly SCA (29). In a study by Hai Long et al. (30), the NT test was recommended for pregnant women due to its low risk and availability. It was also reported that the NT score of a foetation with SCA was higher than that of a control (30-32). The results of our study showed that fetuses with SCA abnormalities were associated with elevated maternal NT and elevated serum fβhCG marker levels. Similar to the results of present study, the study of Shiefa et al. (17) showed the size NT 4.76 and amount of the marker serum fβhCG 1.11 in patients with SCA.

Meanwhile, based on screening tests in the first trimester of pregnancy similar to the present study, In the

study of Mak et al. (33) and Viuff et al. (34) there was a significant relationship between increasing fetal NT thickness increased serum marker fβhCG in women over 35 years and decreasing the marker serum levels PAPP-A by about 50% compared to the low-risk group in fetus with SCA. Moreover, for detecting all above categories of aneuploidies, the combination of measurement NT, levels fβhCG, levels PAPP-A, and the maternal age background risk were found adequate, with a 74% detection rate for a 5% false-positive rate (35). In the study Kazemi et al. (36) similar to the present study, a false positive rate for diagnosing chromosomal abnormalities in the first trimester of pregnancy was 2-3%. A study by Wright et al. (37), similar to the present study for the diagnosis of Tr13 the first trimester of pregnancy, the detection rate was 99% and in a study by Lu et al. (38), the detection rate of SCA was 90%. One of the strengths of this study is the large number of samples that help identifying women at risk of having children with chromosomal abnormalities. One of the limitations of our study was the inability to obtain karyotype confirmation of women in the high-risk group refusing further testing. Further research needs to consider the possible roles of other parameters during the screening of the first semester, as people of different ethnicities live in southwestern, Iran.

Conclusion

Utilizing particular software, prenatal screening tests based on mother age information and gestational age in the first trimester of pregnancy reduced the baseline risk for fetal chromosomal abnormalities. In addition, the findings provide an unique mechanistic approach to prenatal screening tests that may be used safely in Iran's clinical situation.

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Authors' Contributions

M.H., M.B.; Contributed to conception and design, and performed data collection. M.H., M.B., A.R.; Contributed to all experimental work, data and statistical analysis, and interpretation of data, were responsible for overall supervision. M.H;. Drafted the manuscript, which was revised by M.B. All authors read and approved the final manuscript.

References

1. Duque JAP, Ferreira CF, Zachia SA, Sanseverino MTV, Gus R, Magalhães JAA. The natural history of pregnancies with prenatal diagnosis of Trisomy 18 orTrisomy 13: retrospective cases of a 23-year experience in a Brazilian publichospital. Genet Mol Biol. 2019; 42 Suppl 1: 286-296.
2. Deng C, Zhu Q, Liu S, Liu J, Bai T, Jing X, et al. Clinical application of noninvasive prenatal screening for sex chromosome aneuploidies in 50,301 pregnancies: initial experience in a Chinese hospital. Sci Rep. 2019; 9: 7767.
3. Witters G, Van Robays J, Willekes C, Coumans A, Peeters H, Gyselaers W, et al. Trisomy 13, 18, 21, Triploidy and Turner syndrome: the 5T's. Look at the hands. Facts Views Vis Obgyn. 2011; 3(1): 15-21.
4. Zheng Y, Wan S, Dang Y, Song T, Chen B, Zhang J. Non-invasive prenatal testing for detection of trisomy 13, 18, 21 and sex chromosome aneuploidies in 8594 cases. Ginekol Pol. 2019; 90(5): 270-276.
5. Yaron Y, Ochshorn Y, Tsabari S, Shira AB. First-trimester nuchal translucency and maternal serum free beta-hCG and PAPP-A can detect triploidy and determine the parental origin. Prenat Diagn. 2004; 24: 445-450.
6. Jezela-Stanek A, Krajewska-Walasek M. Noninvasive prenatal testing of aneuploidies: where are we now? Rev Bras Ginecol Obstet. 2014; 36(9): 383-386.
7. Allen EG, Freeman SB, Druschel C, Hobbs CA, O'Leary LA, Romitti PA, et al. Maternal age and risk for trisomy.21 assessed by the origin of chromosome nondisjunction: a report from the atlanta and national down syndrome projects. Hum Genet. 2009; 125(1): 41-52.
8. Malan V, Bussières L, Winer N, Jais JP, Baptiste A, Le Lorc'h M, et al. Effect of cell-free DNA screening vs direct invasive diagnosis on miscarriage rates in women with pregnancies at high risk of trisomy 21. JAMA. 2018; 320(6): 557-565.
9. Morris JK, Savva GM. The risk of fetal loss following a prenatal diagnosis of trisomy 13 or trisomy 18. Am J Med Genet A. 2008; 146A(7): 827-832.
10. Kawasaki H, Yamada T, Takahashi Y, Nakayama T, Wada T, Kosugi S. The short-term mortality and morbidity of very low birth weight infants with trisomy 18 or trisomy 13 in Japan. J Hum Genet. 2020; 66(3): 273-285.
11. Bringman JJ. Invasive prenatal genetic testing: a Catholic healthcare provider's perspective. Linacre Q. 2014; 81(4): 302-313.
12. Syngelaki A, Guerra L, Ceccacci I, Efeturk T, Nicolaides KH. Impact of holoprosencephaly, exomphalos, megacystis and high NTT in first trimester screening for chromosomal abnormalities. Ultrasound Obstet Gynecol. 2017; 50(1): 45-48.
13. Dolatkhah M, Noori S, Khavari-Nejad RA, Rahnamaye Farzami M. First-trimester combined screening for trisomies 21, 18, and 13 by three closed chemiluminescence immunoassay analyzers (an experiment on Iranian pregnant women). Iran J Neonatol. 2020; 11(3): 71-79.
14. Catic A, Gurbeta L, Kurtovic-Kozaric A, Mehmedbasic S, Badnjevic A. Application of neural networks for classification of patau, edwards, down, turner and klinefelter syndrome based on first trimester maternal serum screening data, ultrasonographic findings and patient demographics. BMC Med Genomics. 2018; 11(1): 19.
15. Palomaki GE, Lee JE, Canick JA, McDowell GA, Donnenfeld AE. Technical standards and guidelines: prenatal screening for Down syndrome that includes first-trimester biochemistry and/or ultrasound measurements. Genet Med. 2009; 11(9): 669-681.
16. Guex N, Iseli C, Syngelaki A, Deluen C, Pescia G, Nicolaides KH, et al. A robust 2nd generation genome-wide test for fetal aneuploidy based on shotgun sequencing cell-free DNA in maternal blood. Prenat Diagn. 2013; 33(7): 707-710.
17. Shiefa S, Amargandhi M, Bhupendra J, Moulali S, Kristine T. First trimester maternal serum screening using biochemical markers PAPP-A and free b-hCG for down syndrome, patau syndrome and edward syndrome. Ind J Clin Biochem. 2013; 28(1): 3-12.
18. Shahshahan Z, Azami N. The diagnostic value of combined test for trisomy 21 and 18 screening in over 35-years old pregnant women in the gestational age of 9-14 Weeks. J Isfahan Med Sch. 2013; 30: 400-407.
19. Hawk AF, Saller DN. Screening for fetal aneuploidy: is maternal age relevant? Clin Obstet Gynecol. 2012: 55(1): 217-225.
20. Seyyedkavoosi E, Younessi S, Farhud D. Screening of fetal chromosome aneuploidies in the first and second trimester of 125,170 Iranian pregnant women. Iran J Public Health. 2015; 44(6): 791-796.
21. Xue S, Yan H, Chen J, Li N, Wang J, Liu Y, et al. Genetic examination for fetuses with increased fetal nuchal translucency by genomic technology. Cytogenet Genome Res. 2020; 160(2): 57-62.
22. Kagan KO, Wright D, Valencia C, Maiz N, Nicolaides KH. Screening for trisomy's 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): 1968-1975.

23. Huang T, Dennis A, Meschino WS, Rashid S, Mak-Tam E, Cuckle H. First trimester screening for Down syndrome using nuchal translucency, maternal serum pregnancy-associated plasma protein A, free-β human chorionic gonadotrophin, placental growth factor, and α-fetoprotein. Prenat Diagn. 2015; 35(7): 709-716.
24. Carrara J, Vivanti A, Jani JC, Demain A, Costa J, Benachi A. Usefulness and reliability of cell free fetal DNA screening for main trisomies in case of atypical profile on first serum screening. J Transl Med. 2019; 1(1): 398.
25. Engell AE, Carlsson ER, Jørgensen FS, Sørensen S. Comparison of two immunoassay systems for hCGβ and PAPP-A in prenatal screening for trisomy 21, 18, and 13 in the first trimester. Pract Lab Med. 2017; 9: 18-23.
26. Karaaslan O, Seckin KD, Kadirogullari P, Kiyak H, Bestel M, Simsek E, et al. The utility of pregnancy associated plasma protein-A MoM values in prediction of term respiratory distress syndrome. J Obstet Gynaecol. 2020; 40(2): 200-204.
27. Spencer K. Aneuploidy screening in the first trimester. Am J Med Genet C Semin Med Genet. 2007; 145C(1): 18-32.
28. Piazze J, Anceschi MM, Cerekja A, La Torre R, Pala A, Papa A, et al. Nuchal translucency as a predictor of adverse pregnancy outcome. Int J Gynaecol Obstet. 2007; 98(1): 5-9.
29. Kolarski M, Ahmetovic B, Beres M, Topic R, Nikic V, Kavecan I, et al. Genetic counseling and prenatal diagnosis of triploidy during the second trimester of pregnancy. Med Arch. 2017; 71(2): 144-147.
30. Hai Long N, Danh Cuong T, Toan Anh N. Relation between increased fetal Nuchal translucency thickness and chromosomal defects in northern Vietnam. Cureus. 2021; 13(10): e18446.
31. Xue SH, Yan H, Chen J, Li N, Wang J, Liu Y. genetic examination for fetuses with increased fetal nuchal translucency by genomic technology. Cytogenet Genome Res. 2020; 160(2): 1-6.
32. Younesi S, Taheri Amin MM, Hantoushzadeh S, Saadati P, Jamali S, Modarressi MH, et al. Karyotype analysis of amniotic fluid cells and report of chromosomal abnormalities in 15,401 cases of Iranian women. Sci Rep. 2021; 11(1): 19402.
33. Mak A, Lee H, Poon CF, Kwok SL, Ma T, Chan KYK, et al. Factors associated with common and atypical chromosome abnormalities after positive combined first-trimester screening in Chinese women: a retrospective cohort study. BMC Pregnancy Childbirth. 2019; 19(1): 55.
34. Viuff MH, Stochholm K, Uldbjerg N, Nielsen BB; Danish Fetal Medicine Study Group, Gravholt CH. Only a minority of sex chromosomeabnormalities are detected by anational prenatal screening programfor down syndrome. Hum Reprod. 2015; 30(10): 2419-2426.
35. Maymon R, Sharony R, Grinshpun-Cohen J, Itzhaky D, Herman A, Reish O. The best marker combination using the integrated screening test approach for detecting various chromosomal aneuploidies. J Perinat Med. 2005; 33(5): 392-398.
36. Kazemi M, Salehi M, Kheirollahi M. Down syndrome: current status, challenges and future perspectives. Int J Mol Cell Med. 2016; 5(3): 125-133.
37. Wright D, Syngelaki A, Bradbury L, Akolekar R, Nicolaides KH. First-trimester screening for trisomies 21, 18 and 13 by ultrasound and biochemical testing. Fetal Diagn Ther. 2014; 35(2): 118-126.
38. Lu X, Wang C, Sun Y, Tang Y, Tong K, Zhu J. Noninvasive prenatal testing for assessing foetal sex chromosome aneuploidy: a retrospective study of 45,773 cases. Mol Cytogenet. 2021; 14(1): 1.