Original Article

The Relationship between Screening Markers in the First Trimester of Pregnancy and Chromosome Aberrations

Maryam Mirsafaie, Lida Moghaddam-Banaem, Majid Kheirollahi[1]

Department of Midwifery and Reproductive Health, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran, [1]Department of Genetics and Molecular Biology, School of Medicine, Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background: This study was designed and performed to investigate the relationship between fetal chromosome aberrations and screening markers in the first trimester of pregnancy in order to prevent the birth of infants with chromosome aberrations with early prenatal diagnosis. **Methods:** We conducted an analytic cross-sectional study on result of chromosomal culture of 762 pregnant women with high-risk combined screening test from December 2018 to June 2020 and analyzed by SPSS program. **Results:** There was a significant relationship between chromosome structural abnormalities with free beta-human chorionic gonadotropin (free β-hCG) values equal to and higher than 1.5 multiples of the median (MoM) (*P*: 0.05). The highest incidence of disorder in number of chromosomes with abnormal nuchal translucency (NT) percentiles (≥99%) was seen ($P < 0.001$). It also shows that the cumulative number of chromosome aberrations of 25 (78.12%) occurred in individuals with a NT less than 99th percentile and at the same time a risk of 1/50≤ risk <1/10. **Discussion:** According to the results, Comparative Genomic Hybridization (CGH) array method is recommended to detect structural abnormalities in chromosomes in samples with NT ≥3.5. In addition, it is noteworthy that chromosomal structural abnormalities occur in free β-hCG ≥1.5 MoM. **Conclusion:** Due to the frequency of chromosomal structural disorders and its effect on the incidence of fetal abnormalities, the study of chromosomal structural disorders is recommended.

Keywords: *Chromosome aberrations, first trimester pregnancy, human chorionic gonadotropin beta subunit, pregnancy-associated plasma protein-A, nuchal translucency*

Introduction

Incomplete fetal development is a serious complication during pregnancy, which is a major cause of perinatal morbidity and mortality. Early detection of fetuses at risk of growth-restriction provides better monitoring, thus managing optimization, which has been shown to reduce the risk of adverse fetal consequences.[1]

Prenatal screening for trisomy disorders based on analysis of biochemical markers in the mother's serum has become part of prenatal care in many countries.[2]

The propensity for screening during pregnancy for chromosomal trisomies has focused on the first trimester. Among the biochemical markers examined, only maternal serum free β-hCG and pregnancy-associated plasma protein-A (PAPP-A) have been observed to be of value.

Amniotic fluid, fetal blood, and chorionic villi are the types of samples that currently used for distinguish for chromosome abnormalities.[3]

Chromosomal abnormality occurs when there is a disorder in the number or structure of the chromosomes. Indeed, the chromosomal alterations seen during prenatal testing were included autosomal or sex chromosome aneuploidy, triploidy, balanced or unbalanced structural rearrangements, deletions, and duplications and mosaicism.[3] it has been well established that these changes can lead to disturbance in the amount or arrangement of the genetic information in the cells, so that it may lead to growth retardation or influence the performance of the body systems, such as infertility, spontaneous abortions, stillbirths, congenital anomalies , mental retardation, and pathogenesis of malignancy. However most fetuses with some chromosomal abnormality, especially numeral anomalies, do not usually survive, but some may be born with these abnormalities.[4]

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Address for correspondence:
Dr. Lida Moghaddam-Banaem, Department of Midwifery and Reproductive Health, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.
E-mail: moghaddamb@modares.ac.ir
Dr. Majid Kheirollahi, Department of Genetics and Molecular Biology, School of Medicine, Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran.
E-mail: mkheirollahi@med.mui.ac.ir

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Studies have shown that any type of trisomy, including trisomies 21, 13, and 18, is associated with increasing maternal age, increased fetal nuchal translucency (NT), and decreased PAPP-A, but in trisomy 21, free β-hCG increases in serum, whereas in trisomy 18 and 13, free β-hCG is reduced.[2] In addition, PAPP-A and β-hCG values are independent factors in the diagnosis of trisomy 21, and if a combination of these two markers is used with NT ultrasound, provide the suitable conditions for prenatal diagnosis of trisomy 21.[5]

Some recent studies on prenatal screening have suggested that testing the double marker in first trimester screening helps to identify 90% risk for Down syndrome and 94% for other chromosomal defects including Patau syndrome, Edward syndrome, triploidy, and Turner syndrome.[2]

Fetal aneuploidy risk can be assessed based on the combination of maternal age, previous family history, biochemical tests of the mother's serum, and fetal ultrasound indicators.[6] High-risk women can receive genetic counseling, genetic diagnosis tests, and follow-up care.[7] Despite the significant effects of chromosomal abnormalities on fetal outcomes, the study of these chromosomal numerical and structural changes has not been performed simultaneously. Thus, this study was designed to investigate the relationship between all types of fetal chromosomal aberrations and screening markers in the first trimester of pregnancy to prevent the birth of infants with chromosomal aberrations with early prenatal diagnosis and reassuring pregnant mothers about the health of the fetus.

Methods

We conducted an analytic cross-sectional study on 762 pregnant women who participated in a prenatal screening program in Isfahan province between December 2018 and June 2020.

Inclusion criteria consisted of doing pregnancy screening tests (first trimester). Performing amniocentesis and chromosome culture (karyotype) at the request of the relevant specialist, Iranian citizenship, maternal singleton pregnancy, availability information of pregnant women, absence of chronic and systemic diseases (cardiovascular, respiratory, renal, etc.) in the mother. Only women with complete information about ultrasound of fetal growth at gestational age from 11 to 13 weeks and 6 days were assessed. PAPP-A and free β-hCG biomarkers, NT and NT percent rates were recorded.

All participants with high risk of chromosome aberrations underwent amniocentesis by an obstetrics and gynecology specialist, and chromosomal culture was performed in the Gene Azma Medical Genetics Laboratory. All the results of the study of embryonic chromosomal aberrations detected after amniocentesis and chromosomal culture in the laboratory were recorded in an Excel program. Chromosome aberrations were classified into two categories: numerical and structural. Structural chromosome aberrations include two categories of *de novo* and non *de novo*.

For further analysis, data transfer from Excel to SPSS software (version 20) was performed. To analyze the data, χ^2 method was used to compare the data qualitatively and Mann–Whitney U test and Kruskal–Wallis test were used to compare quantitative data.

Results

In this study, the median of PAPP-A in normal chromosomal culture was 0.66 ± 0.52. In addition, the median of PAPP-A in chromosomal cultures with number and structural defects was 0.50 ± 0.33 and 0.63 ± 0.51 multiples of the median (MoM), respectively. Kruskal–Wallis test was used to evaluate of difference PAPP-A in these three groups and a significant difference was observed between chromosomal cultures with number defects and normal chromosomes ($P < 0.005$). Besides in cases diagnosed with Down syndrome, the mean PAPP-A was 4825 ± 0.20272 MoM and the median was 0.5100 MoM. The mean of free β-hCG for Down syndrome was 2.6764 ± 1.45164 MoM and the median was 2,500 MoM. The mean and the median of NT in Down syndrome cases was 2.0082 ± 0.49518 mm 2.0600, respectively.

A noteworthy point in this study is the existence of a significant relationship between chromosome structural abnormalities with free β-hCG values higher than 1.5 MoM (P: 0.05).

No chromosomal structural abnormalities were observed in 29 individuals in the abnormal NT group. Although, the highest rate of disorders 6 (20.7%) was observed in group of disorders of number of chromosomes ($P \leq 0.001$).

The highest incidence of disorder in number of chromosomes was in 12 samples (10.3%) with abnormal NT percentiles (≥99%) ($P < 0.001$), whereas the highest incidence of structural disorders was observed in 31 pregnancy (4.8%) with normal percentiles (<99%) (P: 0.04).

Table 1 compares the chromosome aberrations (numerical and structural disorders) between individuals with NT percentile equal to and above 99 with all pregnant women with NT percentile less than 99 in different final risks.

As seen in the table, out of 117 people with NT percentile above 99, 104 pregnant women (88.88%) with normal chromosomal culture and 12 person (10.3%) with disorders of number of chromosomes and only 1 case (0.9%) with *de novo* chromosome structural disorder were identified ($P = 0.002$).

Also, out of 17 pregnant women who had a NT percentile of less than 99 and a final risk greater than 1:10, 12 (76.5%) with normal chromosomal culture and 4 (23.5%) with

Table 1: Evaluation and compare the incidence of chromosomal abnormalities based on final risk and NT percentile

Group characteristics	NT ≥99% (117) *P*: 0.002	NT <99% (17) 1/10≤ risk *P*: 0.003	NT<99% (119) 1/50≤ risk <1/10 *P*: 0.03	NT <99% (119) 1/100≤ risk <1/50 *P*: 0.36	NT <99% (254) 1/250≤ risk <1/100 *P*: 0.16	NT <99% (131) 1/250> risk *P*: 0.1
Normal	104 (88.88%)	12 (76.5%)	103 (84.9%)	115 (95.8%)	241 (93.8%)	136 (93.9%)
Numeric	12 (10.3%)	4 (23.5%)	9 (7.6%)	2 (1.7%)	4 (1.7%)	1 (0.8%)
Down syndrome	12	3	8	2	2	0
Others	0	1	1	0	2	1
De novo structural	1 (0.9%)	1 (5.9%)	6 (5%)	2 (1.7%)	7 (2.9%)	6 (46%)
Numeric cumulative	12 (37.5%)	16 (50%)	25 (78.125%)	27 (84.375%)	31 (96.875%)	32 (100%)
Structural cumulative	1 (43.47%)	2 (8.695%)	8 (34.782%)	10 (43.478%)	17 (73.913%)	23 (100%)
Total cumulative	13 (23.63%)	18 (32.72%)	33 (60%)	37 (67.27%)	48 (87.27%)	55 (100%)

number impairment and 1 (5.9%) with *de novo* structural chromosomal disorder were detected ($P = 0.003$).

The cumulative number of chromosome aberrations was 25 (78.12%) in individuals with a NT less than 99th percentile and at the same time a risk of 1/50≤ risk <1/10. Also, the cumulative number of chromosomal structural disorders was 8 (34.78%) in individuals within this threshold.

The cumulative total number of chromosome aberrations (numerical and structural disorders) identified in 33 (60%) pregnant women with NT less than 99th percentile and a final risk identified as 1/50≤ risk <1/10. Chi-square test was used which showed a significant difference between different groups ($P < 0.03$) [Table 1].

Discussion

In this study, 762 pregnant women were participated. The risk of Down syndrome was considered 1: 250 and based on the results, 35 people (4.6%) with a number of chromosomal disorders including 28 cases (3.8%) with Down syndrome, three cases (0.4%) with trisomy 13 and the same number of triploids. In addition, 31 patients (4.1%) with structural disorders including three patients (0.4%) with chromosomal translocation, three patients (0.4%) with chromosomal duplication, three patients with deletion, 12 patients (1.6%) with mosaicism, ten patients (1.3%) with inversion and one patient (0.1%) as chromosome markers were identified. A total of 55 patients (7.2%) were identified with a change in chromosomal heterochromatin region. Meanwhile, in a study by Yaron *et al*., they examined the association between decreased PAPP-A in the first trimester and predicted adverse pregnancy outcomes in 1,622 pregnant women and the risk of Down syndrome was considered 1: 380 in this study. According to their results, 33 people had chromosomal disorders including Down syndrome, trisomy 13 and 18 and triploidy and X chromosomal disorders.[8] Compared to the results of our study, the percentage of embryos identified with a number of chromosome aberrations in the study was very low. However, their study did not examine the fetal chromosomal structural abnormalities.

In our study, PAPP-A levels were significantly reduced in a number of cases and a significant relationship was observed between this biomarker and a number of chromosomal disorders. The results are reported in a study by Patil *et al*. (2014), similar to our study.[9] Thus, a decrease in PAPP-A is an important marker for the detection of abnormalities in the number of chromosomes. In this study, no significant association was found between PAPP-A changes and chromosomal structural disorders, although no study was found in this area.

In another study by Hsu *et al*. to evaluate high levels of maternal serum free β-hCG in pregnancies with Down syndrome, the median and mean values of free beta-hCG in Down syndrome pregnancies were 2.56 and 2.01 MoM, respectively, and was significantly different from unaffected pregnancies,[10] whereas in our study the characteristics were 2.5 and 2.67, but no significant difference was observed between pregnancies with chromosomal disorders and normal chromosomes. Free β-hCG may not be a good marker for identifying a number of chromosomal abnormalities in our population.

Also in a study conducted by Ziolkowska *et al*. in 2019, a significant relationship was observed between free β-hCG values equal to or above the threshold of 1.5 MoM with chromosomal abnormalities.[5] But in our study this relationship was not found. Interestingly, there was significant relationship between free β-hCG values equal to or above the threshold of 1.5 MoM with structural disorders, so that 82.8% of cases with structural abnormalities were found with free β-hCG ≥1.5 MoM. Unfortunately, this issue regarding the threshold has not been considered in their study. Therefore, by considering the appropriate threshold for free β-hCG, structural and numerical abnormalities of chromosomes may be detected simultaneously using these two markers (free β-hCG and PAPP-A).

In a study conducted by Yaron *et al.* and Shiefa *et al.* in 2004 and 2013, a significant relationship was found between NT values and chromosomal abnormalities, with a median of 1.03 and 2.67 mm, respectively.[5,11] This correlation was also seen in our study and the median NT

was 2.02 mm. Therefore, it may be appropriate to consider a 2 mm threshold for NT in our population.

In the present study, due to the type of method (Giemsa binding), it may not be possible to identify microdeletions in chromosomes in samples with NT ≥3.5, whereas in a study conducted by Grande *et al*. in 2015, CGH array method was able to identify structural disorders in this group and therefore, it is recommended to detect microdeletions abnormalities in chromosomes in samples with NT ≥3.5.[12]

According to the results, the highest accumulation of chromosomal numerical and structural aberrations was observed in pregnant women with a percentage of NT less than 99 and a risk of 1/50≤ risk <1/10 which was observed 26.08% increase in the diagnosis of structural chromosome aberrations occurs up this distance. Also, the highest cumulative total number of chromosomal disorders (number and structure) identified in the group of pregnant women with NT less than 99th percentile and the final risk is ≥1: 50, which is equivalent to 27.28% of all disorders in this threshold. Therefore, based on the results, overall, a threshold of 1/50 seems to be the most appropriate risk for recommending amniocentesis.

According to this study, the mean value of NT in disorders is 2.01. For this reason, for a more complete diagnosis of chromosomal aberrations, it is recommended that the NT threshold be 2 mm and therefore, amniocentesis performed at 95th percentile or higher.

Another study in 2019 by Ziolkowska *et al*. in Poland aimed to evaluate the clinical utility of biochemical parameters (β-hCG, PAPP-A) and ultrasound (NT) in the first trimester of pregnancy for 251 pregnant women. For high--risk pregnancies (risk greater than 1: 300), amniocentesis was performed for trisomy 21 and according to the results, 217 patients had normal chromosome cultures and 34 patients had trisomy 21. 85% of cases with trisomy 21 had elevated free β-hCG levels more than 1.5 MoM and only 52.94% had 0.001 <PAPP-A <0.5. In addition, in this study have shown that not all fetuses with Down syndrome have elevated NT levels.[5] In our study, 72.4% of trisomy 21 cases had increased levels of free β-hCG ≥1.5 and only 52.94% of them had PAPP-A ≤0.5. Interestingly, only 17.2% of cases with trisomy 21 had increased levels of NT ≥3.5, whereas in 82.8% of them, the NT level did not increase. All the mentioned results of this study are consistent with our study.

Another study conducted by Sadłecki*et al*. in Poland (2014) to assess genetic amniocentesis indices in 632 pregnant women. The mean age of mothers undergoing amniocentesis was 34 years, 47.9% of patients were under 35 years old, and 52.1% of patients were over 35 years old. Abnormal ultrasound findings and first trimester screening were the reason of amniocentesis in mothers under 35 years of age. According to the results, 74 chromosomal aberrations were reported and trisomy 13 or any other abnormal karyotype was observed in both age groups. Complications related to amniocentesis (abortion or intrauterine death) were observed in nine patients (1.42%). According to this study, if amniocentesis is performed correctly between 15 and 20 weeks of pregnancy, it is a safe method for mother and fetus.[13] In our study, the mean age of mothers undergoing amniocentesis was 33.56 ± 5.85 years, 58.7% of patients were less than 35 years, and 41.2% were more than 35 years. The cause of amniocentesis in both groups was due to abnormalities reported in the first trimester screening. The highest number of Down syndrome identified in the age group was less and equal to 35 years with 20 (69%) and the highest number of *de novo* chromosomal structural disorders identified with 5 (55.6%) belonged to the same age group. In this study, six abortions after amniocentesis (72 h) were observed, of which two cases were due to amniocentesis (3.4%) and four cases of abortions had markers that were also found in cases of intrauterine death (normal karyotype, NT ≥99th and PAPP-A <0.26) has been seen and were much lower compared to the mentioned study (that examined a smaller number of samples). Certainly, more specialized training for relevant specialists in the field of amniotic fluid sampling can be used to further reduce the rate of abortion caused by amniocentesis sampling. Therefore, performing amniocentesis at 15–20 weeks of pregnancy to identify chromosomal aberrations is a harmless method for mother and fetus .

In conclusion, according to the results obtained based on the median values of PAPP-A, free β-hCG and NT in comparison normal chromosomal cultures with Down syndrome seems to consider 0.5 MoM, 2.5 MoM and 2 mm, respectively, are the most appropriate thresholds.

It is also recommended to perform CGH array for pregnant women with NT ≥3.5 or NT ≥99th.

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Conflicts of interest

There are no conflicts of interest.

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References

1. Kirkegaard I, Henriksen T, Uldbjerg N. Early fetal growth, PAPP-A and free β-hCG in relation to risk of delivering a small-for-gestational age infant. Ultrasound Obstet Gynecol 2011;37:341-7.
2. Shiefa S, Amargandhi M, Bhupendra J, Moulali S, Kristine T. First trimester maternal serum screening using biochemical markers PAPP-A and free β-hCG for down syndrome, patau syndrome and edward syndrome. Indian J Clin Biochem 2013;28:3-12.

3. Bilancia CG, Ganapathi M, Levy B. 24-Prenatal diagnosis of chromosome abnormalities. In: Pandya PP, Oepkes D, Sebire NJ, Wapner RJ, editors. Fetal Medicine (Third Edition). London: Elsevier; 2020. p. 233-46.e3.
4. Khandekar S, Dive A, Munde P. Chromosomal abnormalities-a review. Central India J Dent Sci. 2013;4:35-40.
5. Ziolkowska K, Dydowicz P, Sobkowski M, Tobola-Wrobel K, Wysocka E, Pietryga M. The clinical usefulness of biochemical (free β-hCG, PAPP-A) and ultrasound (nuchal translucency) parameters in prenatal screening of trisomy 21 in the first trimester of pregnancy. Ginekol Pol 2019;90:161-6.
6. Rink BD, Norton ME, Screening for fetal aneuploidy. Semin Perinatol. 2016;40:35-43. doi: 10.1053/j.semperi.2015.11.006. Epub 2015 Dec 25.
7. Benn P, Borrell A, Crossley J, Cuckle H, Dugoff L, Gross S *et al*., Aneuploidy screening: a position statement from a committee on behalfof the Board of the International Society for Prenatal Diagnosis, January 2011. Prenat Diagn 2011;31:519-22.
8. Yaron Y, Heifetz S, Ochshorn Y, Lehavi O, Orr-Urtreger A. Decreased first trimester PAPP-A is a predictor of adverse pregnancy outcome. Prenat Diagn 2002;22:778-82.
9. Patil M, Panchanadikar T, Wagh G. Variation of papp-a level in the first trimester of pregnancy and its clinical outcome. J Obstet Gynecol India 2014;64:116-9.
10. Hsu J, Ou Y, Chen K, Hsieh T, Soong Y. High maternal serum free beta-hCG levels in Down syndrome pregnancies: A preliminary report. Changgeng Yi Xue Za Zhi. 1996;19:36-41.
11. Yaron Y, Ochshorn Y, Tsabari S, Shira AB. First-trimester nuchal translucency and maternal serum free β-hCG and PAPP-A can detect triploidy and determine the parental origin. Prenat Diagn 2004;24:445-50.
12. Grande M, Jansen F, Blumenfeld Y, Fisher A, Odibo A, Haak M, *et al*. Genomic microarray in fetuses with increased nuchal translucency and normal karyotype: A systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;46:650-8.
13. Sadłecki P, Walentowicz-Sadłecka M, Pasińska M, Adamczak R, Grabiec M. Indications for genetic amniocentesis investigated at the Department of Gynecology, Obstetrics, and Oncologic Gynecology, Nicolaus Copernicus University, Collegium Medicum, Bydgoszcz. Ginekol Pol 2014;85:420.