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The Relationship between Serum Bimarkers with the Results of Down Syndrome Screening and Fetal Karyotype in High Risk Pregnant Women

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Abstract

Introduction: Considering the many problems of a child with Down syndrome, early diagnosis allows parents to prepare for the birth and care of these children or to suggest termination of pregnancy. This study aimed to investigate associations between serum levels of folic acid and Superoxide dismutase (SOD1) with the results of the first trimester Down syndrome screening and fetal karyotype in high-risk pregnant women.

Methods: In this cross-sectional study, 232 women with high-risk pregnancies who had positive Down syndrome screening and undergone amniocentesis were selected through purposive sampling at the gestational age of 14-20 weeks. After obtaining an informed written consent form, the questionnaires related to the research were filled and the information on NT ultrasound and biochemical screening tests was extracted from the patients' files. We measured serum levels of folic acid and SOD1 in all participants using the ELISA method. Statistical analysis was done by applying a multivariate logistic regression model by backward strategy. The Hosmer-Lemeshow test was utilized for better goodness of fit for the logistic regression model. In this study, p-value of <0.05 was considered statistically significant.

Results: In our study, 97% of participants consumed folic acid, serum level measurements revealed, 6.9% of participants had low folic acid levels, and 5.6% of Down syndrome positive screenings had a positive karyotype. The mean (SD) serum levels of superoxide dismutase enzyme (SOD-1) measured in the present study in the participants was 297.40 (75.55) U/ml. There were no significant relationship between serum levels of folic acid [odds ratio (OR) (95% CI): 0.125 (0.001 to 31.42); P=0.461] and SOD1 levels [OR (95% CI): 0.99 (0.976 to 1.01); P=0.799] with fetal karyotype results (P>0.05).

Conclusions: These findings demonstrate that serum folic acid and SOD1 concentration is not the predictive markers of Down syndrome karyotype in high-risk pregnant women who have positive Down syndrome screening through a double marker test.

INTRODUCTION

Down syndrome (DS) is a type of mental retardation and the most common human chromosomal abnormality in which 3 copies of chromosome 21 are usually produced instead of 2 [1, 2]. It occurs in 1:500 of all pregnancies and 1:800 to 1:1000 of live births [3]. Special facial features, severe mental retardation, and stunted growth are the main clinical manifestations of trisomy 21 [4]. General hypotonia, short stature, head defects, low set eyes, epicanthic fold, and large tongue are other clinical manifestations of trisomy 21 [4, 5].

The chance of the fetus having this genetic disorder is assessed using a combination of Down syndrome screening results, ultrasound parameters, the mother's age, gestational age, and mother's weight [2]. The most effective screening method for trisomy 21 is a combination of the mother's age, measurement of fetal nuchal translucency thickness by ultrasonography, and biochemical tests on mother's blood including free beta-human chorionic gonadotropin (βhCG) and pregnancy-associated plasma protein-A (PAPP-A) at 11-13 weeks of gestation with about 90% diagnosis accuracy and a 5% false positive rate [6]. However, the positive screening results should be confirmed using diagnostic tests such as amniocentesis or chorionic villus sampling (CVS).

Abnormal folate metabolism has been recognized as a maternal risk factor for Down syndrome in different populations [7]. Studies have shown that folate deficiency can lead to aneuploidy of chromosome 21 [8]. Folic acid or folate is a member of the vitamin B family [9] and acts as the receiver and donor of one-carbon units during the synthesis of precursors of nucleic acids and protein [10]. Folate deficiency is associated with DNA hypomethylation, DNA damage, chromosomal instability, abnormal chromosome segregation, and aneuploidy of chromosome 21 [7].

The results of a study in Turkey noted that DNA hypomethylation was associated with spontaneous chromosomal damage and low levels of serum folate [11]. A study in Japan showed that low levels of serum folic acid and increased plasma homocysteine could contribute to the development of Down syndrome [12]. Therefore, serum folate concentration may be a reliable diagnostic test, especially if performed in relation to Red Blood Cell (RBC) folate levels [13].

Oxidative stress is a phenomenon that is often discussed in relation to many diseases [14] and these oxidative stress conditions can occur in early pregnancy [15]. An increase in the amount of oxidative stress in people with Down syndrome has been confirmed in several studies [14].

Superoxide dismutase enzyme (SOD) is one of the most important antioxidant enzymes that occur in the body in the form of three isoforms. The gene of this enzyme is located in the distal part of chromosome 21 (Tan et al. 1973) [14] and it seems that the extra SOD1 gene is responsible for some manifestations of DS [15].

The activity of SOD in the amniotic fluid of DS pregnancies is higher than in normal cases [16]. The increased expression of the superoxide dismutase gene is one of the main factors in the production of hydroxyl radicals and the occurrence of oxidative stress in Down's syndrome [16, 17].

Since blood sampling is a relatively non-invasive method, it is considered a preferred method for evaluating health status by measuring blood metabolites and making conclusions based on the measurements [18]. The data from blood tests may help in understanding the physiology and biochemistry of Down syndrome compared to prenatal diagnostic tests [19]. Consequently, this study aimed to evaluate the relationship between some serum biochemical markers and the results of first-trimester screening tests and fetal karyotype in high-risk pregnant mothers visiting Al-Zahra teaching hospital in Tabriz, Iran.

METHODS

The present study was a descriptive-analytic cross-sectional study. The study was approved by the Ethics Committee for Research with the code: 2018.IR.TBZMED.REC.1397.458. The research population was pregnant women at the gestational age of 14-20 weeks whose first-trimester screening (NT ultrasound and double marker test) results indicated they were at high risk for having a fetus with Down syndrome and in 2018-2019, they had referred to Al-Zahra Educational hospital of Tabriz, Department of Fetal Medicine, to perform amniocentesis. Therefore, they visited the fetal medicine ward at Al-Zahra teaching hospital to undergo amniocentesis. The minimum sample size was calculated for a confidence level of 95% and taking into consideration the p-values of previous studies (NT ultrasound sensitivity=74%, sensitivity of the double marker test=63%, sensitivity of amniocentesis= 100%), and Cohen's d of 0.1). Since the calculated sample size was larger based on the sensitivity of the double marker test compared to the other estimates, n=230 was the selected sample size. Eventually, 232 women were eligible for the study [20]. The sampling method was purposive and the selection of samples was based on the inclusion criteria. Informed written consent was obtained from all the participants. Personal information, midwifery history, and a questionnaire related to the risk factors of Down syndrome were filled out in face-to-face interviews. Then the information on NT ultrasound and biochemical screening tests was extracted from the patients' files and recorded in the relevant

questionnaires. Before amniocentesis, about 5 mL of venous blood was drawn from each participant, transferred into a tube free of any anticoagulant, and sent to the laboratory where the serum was separated by centrifugation at 3000 rpm for 5 min. The separated sera were poured into micro tubes and immediately stored at -70 °C until the tests were performed. Before analysis, samples were thawed to 25 ºC for five minutes. Serum folic acid levels were measured employing ELISA and using Monobind folate ELISA kits (Saman Tajhiz Noor Co., Iran) by product code: 7525-300. Also, the activity of the SOD1 was determined by the ELISA method and using the Nasdox kit (Navand Lab Kit).

Amniocentesis was performed on non-fasting women with no local anesthesia under aseptic conditions by fetal medicine specialists using an amniocentesis needle at Al-Zahra teaching Hospital in Tabriz. The collected samples of amniotic fluid were sent to a reputable genetic lab in Tabriz and the results were reported in 2-3 weeks. In this step, the researcher evaluated the amniocentesis results of the participants who brought their fetal karyotype results to Al-Zahra teaching Hospital and recorded them in the questionnaires. Otherwise, the researcher called the participants, and obtained the amniocentesis results and recorded them in the questionnaires.

The obtained data were analyzed using descriptive (frequency, percent, mean, and standard deviation) and analytical statistics in SPSS ver 23.0 (SPSS/ ver 23 (IBM SPSS Statistics, IBM Corporation, Chicago, IL). Logistic regression analysis was used to determine the relationship between the serum levels of folic acid and SOD with the results of the first-trimester screening for Down syndrome. The Hosmer-Lemeshow test was utilized for better goodness of fit for the logistic regression model. In this study, *p*-value of <0.05 was considered statistically significant.

RESULTS

232 eligible pregnant women were enrolled in the study. The mean (SD) maternal age was 33.97 (6.73). Two-thirds (66.4%) of participants were in 2nd or 3rd gravid. The mean (SD) gestational age by first sonography was 111.03 (8.20) days. Previous child abnormalities were reported by 6 (2.6%) women. The other personal-social, midwifery, and Down syndrome risk factors characteristics have been listed in Tables 1 and 2.

The results of fetal karyotyping showed that 219 participants (94.4%) had fetuses with a normal karyotype and 13 of them (5.6%) had fetuses with a Down syndrome karyotype. The mean (SD) of ultrasound (NT screening) score for Down syndrome was 2.07(0.91) and the mean (SD) values of biochemical double marker screening tests were 2.48(2.68) mIU/mL for PAPP-A and 64.83 (43.76) ng/mL for free βhCG (Table 3).

The results indicated that 97% of the participants took folic acid with a mean dose of 1.89 mg/day and a dose of 1 mg/day in the majority of women. The mean serum folic acid level of the participants was 0.585 ng/ml. And the mean (SD) serum levels of superoxide dismutase enzyme (SOD-1) measured in the present study in the participants was 297.40 (75.55) U/ml.

There was no relationship between serum levels of folic acid [odds ratio (OR) (95% CI): 0.125 (0.001 to 31.42); p=0.461] and SOD [OR (95% CI): 0.99 (0.976 to 1.01); p=0.799] with fetal karyotype results (Table 4).

Table 1. Characteristics of study participants (n=232)

Variables		N (%)
Ethnicity		
	Tork	219 (94.4)
	Fars	0 (0)
	Kord	13 (5.6)
Habitat		
	City	169 (72.8)
	Village	63 (27.2)
Income		
	Less than expenses	103 (44.4)
	Equal expenses	129 (55.6)
	More than expenses	0 (0)
Gestational age by first sonography (day) #		111.03 (8.20)
Gravida		
	1	42 (18.1)
	2,3	154 (66.4)
	4, more	36 (15.5)
Para		
	0	52 (22.4)
	1	107 (46.1)
	2, more	73 (31.5)
Alive		
	0	53 (22.8)
	1	108 (46.6)
	2, more	71 (30.6)
Abortion		

0	165 (71.1)
1	46 (19.8)
2,more	21 (9.1)
Route of delivery	
No childbirth	53 (22.8)
NVD (Normal Vaginal Delivery)	99 (42.7)
C/S (Caesarean/Section)	71 (30.6)
NVD & C/S	9 (3.9)
Acid folic take	
Yes	225 (97)
No	7 (3)
Number of Acid folic consumed (daily) #	0.97 (0.18)
Dose of Acid folic consumed (daily) (mg) #	1.89 (2.46)
Duration of Acid folic consumption (monthly) #	3.63 (2.71)

For These variables, Mean (Std. Deviation) is reported

Table 2. The characteristics associated with Down syndrome risk factors (n=232)

Variables	N (%)	Variables	N (%)
Maternal age #	33.97 (6.73)	Contraceptive used	0 (0)
<20	10 (4.3)	Contraceptive used	0 (0)
20-30	53 (22.8)	Contraceptive used	0 (0)
31-40	133 (57.3)	Contraceptive used	0 (0)
>40	36 (15.5)	Contraceptive used	0 (0)
Maternal weight (kg) #	71.29 (12.80)	Maternal smoking	0 (0)
Maternal BMI #	27.68 (4.85)	X-ray radiation	4 (1.7)
<18.5	6 (2.6)	X-ray radiation	4 (1.7)
18.5-24.9	64 (27.6)	X-ray radiation	4 (1.7)
25-29.9	95 (40.9)	X-ray radiation	4 (1.7)
≥30	67 (28.9)	X-ray radiation	4 (1.7)
Maternal job		Father job	
House wife	211 (90.9)	free job	136 (58.6)
Working at home	3 (1.3)	public sector employee	28 (12.1)
Working outside the home	18 (7.8)	private sector employee	11 (4.7)
		retired	4 (1.7)
		unemployed	5 (2.2)
		manual worker	48 (20.7)
Maternal special diet	18 (7.8)	Father age #	37.60 (6.90)
Previous child abnormalities	6 (2.6)	Father smoking	68 (29.3)
Maternal relatives abnormality	3 (1.3)	Duration father smoking (year) #	3.79 (6.91)
Infertility history	20 (8.6)	Number father cigarette #	3.16 (6.57)
Duration of infertility (year) #	0.48 (1.90)	Father special diet	0 (0)
Assisted reproductive technique	19 (8.2)	Father relatives abnormality	4 (1.7)
Kind of assisted reproductive techniques		Special Drugs used	77 (33.2)
No assisted	213 (91.8)		
IVF	2 (0.9)		
IUI	5 (2.2)		
Amp Hcg & drugs	12 (5.2)		

For These variables, Mean (Std. Deviation) is reported

Table 3. Down syndrome screening results in the first trimester of pregnancy and fetal karyotype results and serum levels of folic acid and SOD1 in the participants (n=232)

Variables	Mean (SD)
NT (nuchal translusnecy) (mm)	2.07 (0.91)
PAPP-A (miu/ml)	2.48 (2.68)
PAPP-A (MOM)	0.80 (0.63)
Free BHCG (ng/ml)	64.83 (43.76)
Free BHCG (MOM)	2.15 (1.27)
Karyotype #	
46 xx	108 (46.6)
46 xy	111 (47.8)
47 xx	4 (1.7)
47 xy	9 (3.9)
Serum levels of folic acid	0.58 (0.20)
Serum levels of SOD1	297.40 (75.55)

For This variable, number (percent) is reported

Table 4. Logistic regression analysis results to predict fetal karyotype results through serum levels of folic acid and superoxide dismutase (SOD) (n=232)

Variables	OR*	95% CI** for Odds		P-VALUE
		LOWER	UPPER	
Serum level of folic acid	0.125	0.001	31.42	0.461
Serum level of SOD1	0.99	0.976	1.01	0.799

Hosmer and Lemeshow Test: Chi-square= 2.623 df= 8 p= 0.956

*odds ratio, **95% confidence interval, Lower: Lower Bound for 95% C.I. for OR Upper: Upper Bound for 95% C.I. for OR

DISCUSSION

The present study was conducted on 232 high-risk women visiting the hospital for an amniocentesis test. All participants were at the gestational age of 14-20 weeks and the results of the first-trimester screening tests and ultrasound indicated a high risk for the existence of Down syndrome in the fetuses. Two hundred nineteen of the fetuses had normal karyotypes and 13 Down syndrome karyotypes.

Some studies have correlated abnormal metabolism and deficiency of folic acid to aneuploidy of chromosome 21 [7, 8]. In the present study, no association was found between the serum levels of folic acid in pregnant mothers and Down syndrome in their fetuses. In addition, 97% of the participants took folic acid and their mean serum level of folic acid was 0.585 ng/mL.

According to the literature, folate deficiency may lead to certain genetic mutations. Consequently, researchers have concluded that folate deficiency can greatly affect the prevalence of Down syndrome, and mothers of children with Down syndrome have significantly higher levels of homocysteine and lower levels of folate in their blood as well as a higher prevalence of mutations in the methylenetetrahydrofolate reductase gene which is necessary for folate metabolism [21]. Takamura *et al.* showed that the plasma levels of homocysteine were significantly higher and the serum levels of folic acid were significantly lower in these mothers than the control group. They concluded that maternal folate status may be associated with the occurrence of Down syndrome in Japan [12]. The results of this study are not consistent with our study. The reason for the difference may be that we did not examine the genetic mutation and homocysteine level, and also the sample size of mothers with Down syndrome fetuses in our study was very low.

Hollis et al. showed that there was no relationship between the lack of folic acid supplementation and maternal non-disjunction among all case mothers. However, when the participants were categorized according to the mothers' age and meiotic errors, a significant relationship was found between lack of folic acid supplementation and meiosis II nondisjunction errors in older mothers [22]. Although the method of this study is different from our study, their results were consistent in terms of the lack of relationship between folic acid and Down syndrome, but in terms of the increase in error in older mothers, the results are not consistent.

Also, the results of some studies regarding genetic polymorphisms in the folic acid metabolic pathway showed that some polymorphisms were a risk factor for Down syndrome and some were not [23, 24]. We did not investigate genetic polymorphisms in the present study.

The lack of a relationship in our study can be attributed to the fact that only a low percentage of the participants had positive karyotypes, and a larger sample size is required to observe such a relationship. In the present research, most participants took acid folic and hence serum level was high. Therefore, no relationship was found between folic acid and karyotype results.

The studies conducted on the serum level of superoxide dismutase enzyme and the prevalence of Down's syndrome are very limited, but the studies conducted show the relationship between various markers of oxidative stress and Down's syndrome. In the present study, the mean (SD) serum level of SOD1 was 297.40 (75.55) and there was no significant association between the serum levels of SOD-1 and the results of fetal karyotype. Our findings partially agree with the results of the study by Pennings et al. They have pointed out that in our study, an increase in SOD1 levels was observed in the placenta of people with DS pregnancy. But the serum level of SOD1 did not show a significant difference and also did not contribute to the improvement of the Down syndrome risk prediction model. This suggests that SOD1 is probably not of interest as a serum biomarker for DS pregnancies [25]. Due to that the level of SOD1 in the placenta and DNA of the participants was not investigated in the present study, therefore, the first part of the Pennings study results cannot be compared and discussed with the present study.

However, some studies introduced serum levels of SOD and other oxidative stress as predictive biomarkers of Down syndrome, which are not consistent with the results of our study. It may be that the results of these studies are not consistent with the present study, the difference in the study method or the difference in the activity level of SOD1 enzyme in amniotic fluid and serum. Results of the study conducted in Turkey, have shown that SOD enzyme activity in the amniotic fluid of Down syndrome fetuses were significantly increased compared to the control group [16]. The results of Zafrilla et al.'s study in 2014 by measuring several biochemical parameters showed that there was a statistically significant difference between the activities of antioxidants in people with Down syndrome compared to the control group [26].

The main limitation of the present study was its cross-sectional nature and hence the relationship between variables is not necessarily a causal one. The majority of participants (97%) took the folic acid supplements and hence it was not possible to clearly determine the relationship between the serum levels of folic acid and the results of fetal karyotype. Another limitation of this study was the small number of pregnancies with Down syndrome considering the sample size.

CONCLUSION

Our investigation noted that the serum levels of folic acid and SOD1 were not able to predict the existence of Down syndrome karyotype in the fetus, and case-control studies with the two groups of high-risk pregnant women with normal fetal karyotype and with Down syndrome fetal karyotype should be designed so that the differences can be clearly observed.

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CONFLICT OF INTEREST

The authors declare no conflict of interest in this study.

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AUTHOR CONTRIBUTION

Hejazi: 40% Sampling and writing articles; Shahnazi: 20% Supervision of article writing; Farshbaf: 20% data analysis; Mahdipour: 10% Analysis of experiments; Abbasalizadeh: 10% Cooperation in sampling.

ETHICAL CONSIDERATION

1. Obtaining approval from the Research Council of the Department of Midwifery, Faculty of Nursing and Midwifery, Tabriz
2. Obtaining permission to conduct research from the Research Ethics Committee of Tabriz University of Medical Sciences
3. Obtaining written consent from research participants
4. All the people were assured that all their personal information will remain confidential
5. The possibility of opting out of continuing cooperation in the research for the participants
6. Full compliance with ethical principles in the use of other research and sources

The Ethic code of this study is: 2018.IR.TBZMED.REC.1397.458

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