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Association of plasma homocysteine, folic acid levels, and C677T polymorphism in methylene tetra hydrofolate reductase with risk of preeclampsia: a case-control study in Iranian women

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Summary

Introduction: Preeclampsia is the most dangerous hypertension with unknown etiology. Preeclampsia is a kind of pregnancy-specific syndrome. *Objective:* The aim of this study was to evaluate the correlation between C677T polymorphism of MTHFR gene, folic acid, and homocysteine serum levels in Iranian pregnant women with preeclampsia. *Materials and Methods:* This study was performed in 129 pre-eclamptic pregnant women and 125 control individuals and MTHFR gene (C677T polymorphism) was determined by PCR-RFLP method, and the plasma levels of the homocysteine and acid folic was measured by ELISA method. *Result:* The CC, CT, and TT genotypes were not significantly different in patients compared to control ($p = 0.614$). Low mean levels of homocysteine and folic acid in the preeclamptic cases were observed compared to control group. The levels of BMI, gestational age, and neonatal weight were statistically different in two groups and other variables revealed no significant difference between these groups. *Conclusion:* These findings showed that there was no correlation between the C677T polymorphism of MTHFR gene and preeclampsia but the TT genotype of C677T polymorphism seems to be a protective factor for preeclampsia. It is also concluded that in this study, homocysteine and folic acid serum levels and BMI are significantly affected in patients with preeclampsia compared to controls and can increase the risk of developing sever side effect to mothers and neonates.

Key words: Preeclampsia; Methylenetetrahydrofolate reductase (MTHFR); C677T polymorphism; Folic acid; Homocysteine.

Introduction

Preeclampsia is the most dangerous hypertension with unknown etiology. Preeclampsia is a kind of pregnancy-specific syndrome and one of pregnancy hypertensive disorders, which is manifested by blood pressure of 140/90 and above and concomitant proteinuria. It could affect almost all body organs and cause maternal and fetal morbidity and mortality [1-3]. Preeclampsia is diagnosed in 3.9% of all pregnancies and is the reason for 16% of all maternal mortalities in developed countries. However, its etiology is still unspecified and, in fact, one of the most critical and controversial unresolved issues in obstetrics area [1, 4]. Currently, researchers have concluded that the preeclampsia incidence rate is strongly influenced by ethnicity and hence genetic background contributes to its incidence, but environmental factors are also involved [4]. Ward *et al.* in their recent investigation found that more than 70 genes were studied in view of potential association with preeclampsia and reported the risk of preeclampsia development 20-40% in sufferers' daughters, 11-37% in their sisters, and 22-47% in twins [5, 6]. In the investigations conducted, numerous genes have been studied in view of association with preeclampsia. Seven of these genes are methylenetetrahydrofolate reductase (MTHFR), Factor V Leiden, angiotensinogen M235T, various human leukocyte antigens, eNOS (GLU 298ASP), factor II G20210, and angiotensin I-converting enzyme have been more extensively examined and a positive significant association was observed between the disease and the studied gene(s) in more than half of the studies [1]. Regarding the heterogeneity of preeclampsia syndrome and particularly other genetic and environmental factors that are involved in its complicated phenotypic incidence, it is improbable that a candidate gene is diagnosed as responsible for this disease [1]. MTHFR is essential enzyme in the metabolism of folic acid [7, 8], and mutations in encoding gene could affect enzyme reducing activity and increase homocysteine associated with vascular disease and preeclampsia. MTHFR gene is located on the short (p) arm of chromosome 1 at position 36.3 and encoded the protein called MTHFR enzyme, which converts 5, 10-methylenetetrahydrofolate into 5-methylenetetrahydrofolate that methylates homocysteine and converts it into methionine. This enzyme has a great contribution to regulating folate balance and to converting homocysteine into

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methionine. As a result, variation in its activity causes increase in homocysteine, which, in turn, plays a role in vascular injury to endothelium and hence preeclampsia incidence. Homocysteine is an amino acid containing sulphur, derived from demethylation [9]. Methionine is an essential amino acid for folate [10]. Folate is a water-soluble vitamin and is classified as B vitamins, play role in many biochemical processes of the blood, particularly homocysteine metabolism [11]. Some researchers have reported hyperhomocysteinemia as the main factor in venous and arterial thrombosis incidence and argued that variable folate rates could have a key contribution to the increase and/or decrease in blood homocysteine [12]. Plasma homocysteine concentration could be reduced by folic acid supplementation [13]. The genetic variations in MTHFR increase the genetic predisposition to arterial occlusive diseases, neural tube defects, colon cancer, acute leukemia, and preeclampsia [14-16]. The most prevalent mutation in MTHFR is replacement of cytosine by thymine (C→T) at nucleotide 677, which causes conversion of the amino acid alanine into valine and hence decreases MTHFR activity, causes MTHFR deficiency, and finally increases plasma homocysteine concentrations [3]. MTHFR C677T polymorphism in MTHFR gene has been associated with preeclampsia incidence in some of previous works [10, 17], while no association was found between this gene's polymorphism and preeclampsia incidence [12, 18, 19]. According to different findings of studies, the environmental factors affecting heterozygous phenotype incidence of preeclampsia and no study on this disease has been conducted in Chaharmahal Va Bakhtiari province (southwest of Iran). Therefore, the present study was performed to investigate C677T polymorphism of MTHFR gene frequency and its association with folic acid and homocysteine rate in pregnant women with preeclampsia compared to healthy individuals.

Materials and Methods

This case-controlled study was conducted in 129 women with preeclampsia as case group and 125 healthy pregnant women as control. All pregnant women (25- to 35-years-old) with preeclampsia diagnosis referred to Hajar Hospital in Shahrekord city from October 2011 to July 2011 were selected as case group (preeclamptic individual; blood pressure of higher than or equal to 140/90 mmHg after 20th week of pregnancy and proteinuria of +1 and higher in spot urine samples or more than 300 mg per 24-hours urine). The pregnant women with no blood pressure or preeclampsia referring for delivery and similar to the case group by gestational age were selected as control group. The mean of uric acid of studied patients with preeclampsia was 16.5 mg/dl. It should be noted that in this study, patients had not smoked and they had used folic acid and fersolin during prenatally. The method of measuring blood pressure was as follows: After ten minutes rest, while the patient's legs were hanging down and arms and hands were placed on the heart, blood pressure was measured [1]. The individuals with any medical or chronic disease such as cardiovascular diseases, hypertension, hepatic disease, hypo- and hyperthyroidism, infectious disease, diabetes, and renal diseases as well as multiple births, were excluded from the study. After obtaining approval of Ethics Committee and the individuals' consents and filling out the questionnaires, 5-ml of peripheral blood was taken and transferred into two separate 15 mm falcon tube, each containing 60 μL ethylenediaminetetraacetic acid (EDTA) 0.5 M. To implement the first step of the study, one of falcon tubes containing blood sample was surrounded by ice to cool down gradually and after centrifugation, the serum was isolated and serum level of folic acid and homocysteine was measured using enzyme-linked immunosorbent assay (ELISA). The data were gathered and entered into SPSS version 20 and were analyzed using descriptive statistics, correlation tests (*t*-test and chi-square, and p-value < 0.05 was considered as the level of significance. For investigation of MTHFR polymorphism total genomic DNA were extracted from peripheral blood samples of patients and controls using the phenol and chloroform standard procedure [17]. The quality of extracted DNA was quantified with a spectrophotometer at a wavelength of 260/280 nm and the extracted DNA was kept at -20°C until molecular analysis. Then, the type of polymorphism was determined using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). The forward and reverse oligonucleotide primers (MTHFR-F: 5′-CTTTGAGGCTGACCTGAAGC-3′ and MTHFR-R: 5′-TCACAAAGCGGAAGAATGTG-3′) used in this study for gene amplification were obtained from Cui *et al.* study in 2010 [18].

The PCR reaction in this study were performed in a 25-μL total volume in 0.5-ml tubes containing 1 μg of template DNA, 1 μM of each forward and reverse primers, 2 mM $MgCl_2$, 200 μM dNTP mix, 2.5 μL of 10X PCR buffer and 1 unit of *Taq* DNA polymerase. The samples were amplified in a gradient palm cycler and PCR temperature programs involved an initial denaturation at 96°C for six minutes, then 30 temperature cycles including 50 seconds at 95°C for pre-denaturation, 50 seconds at 57°C for annealing, one minute at 72°C for extension, and five minutes at 72°C for final extension [19]. A 104-bp fragment was proliferated using the necessary primers for MTHFR (C677T) polymorphism. For RLFP assay, the enzyme used in this study was *Hinf*I restriction enzymes, which was employed for cutting 1000 ng DNA in one unit of enzyme concentration. It should be mentioned that PCR product was digested at 37°C within one hour [16]. The PCR products and digested fragments were analyzed on 8% polyacrylamide gel electrophoresis (29:1 bis acrylamide/acrylamide) in 200 V and 40 mA for one hour. Then, polyacrylamide gel was stained by the silver nitrate staining method to confirm the presence of 104 bp band to identify C677T mutation and digested bands. Finally, the data were analyzed by SPSS version 20 using *t*-test and chi-square analyses.

Results

In this study, the frequency of C677T polymorphism in MTHFR gene and its association with folic acid and homocysteine rate in the pregnant women with preeclampsia were evaluated. The demographic information of 129 women with preeclampsia and 125 healthy pregnant women (control) are shown in Table 1.

In this study, there was a significant association between age and the disease incidence ($p < 0.001$), such as age advanced preeclampsia incidence increased in the patients. In addition, there was a significant association between BMI prior to pregnancy and preeclampsia incidence ($p < 0.002$); this indicated that the patients with high BMI prior to preg-

Table 1. — *Demographic data of patients and controls.*

Parameters	Patients (n=129) Mean ± SD	Control (n=125) Mean ± SD	*p*-value
Age (years)	5.5 ± 29.3	4.9 ± 26.2	> 0.001
Gestational age (weeks)	4.6 ± 35.6	2.8 ± 38.6	> 0.001
Birth weight (g)	0.809 ± 2.56	0.596 ± 3.05	> 0.001
Parity	1.35 ± 2.19	1.04 ± 1.81	< 0.017
Pre-pregnancy weight BMI	4.6 ± 25.22	3.6 ± 23.23	< 0.002
Hemoglobin (mg/dL)	1.35 ± 12.22	1.33 ± 12.11	0.609
	Fill (n / %)		
Employee	5 (3.9%)	11 (8.8%)	
Housekeeper	113 (87.6%)	107 (85.6%)	
	Education (n / %)		
Under-diploma	55 (42.62%)	49 (39.2%)	
Diploma	47 (36.43%)	53 (42.4%)	
Associate degree	17 (13.17%)	9 (7.2%)	
Bachelor	10 (7.75%)	14 (11.2%)	

Table 2. — *Comparison of serum level of folic acid and homocysteine in patients and matched control group*

Parameters	Patient Mean ± SD	Control Mean ± SD	*p*-value
Folic acid (ng/ml)	8.39 ± 3.6	9.96 ± 4.3	0.002
Homocysteine (µmol/L)	11.2± 4.8	9.9 ± 3.9	< 0.01

Note: Normal range of folic acid and homocysteine is respectively 4.6-18.8 ng/mL and 2.5-16 µmol/L.

Table 3. — *Genotypic and allele frequencies of C677T polymorphism of MTHFR gene in patients and controls.*

Genotype	Patients n=129 (%)	Control n=125 (%)	*p*-value < 0.05
CC	74 (57.4)	67 (53.6)	> 0.05
CT	38 (38.8)	50 (40)	> 0.05
TT	5 (3.9)	8 (6.4)	> 0.05
Allele frequency			
T	23.3%	26.4%	> 0.36
C	76.7%	73.6%	> 0.05

nancy developed preeclampsia more frequently. Nevertheless, preeclampsia had no significant association with ethnicity, job, and educational level ($p > 0.05$). In addition, the mean of gestational age was significantly low in the patients ($p < 0.001$). The mean of birth weight in the patients was significantly low ($p < 0.001$). The women with more pregnancies had significantly higher frequency of developing preeclampsia ($p < 0.017$). The mean uric acid in the patients with preeclampsia in this study was 5.16 mg/dL.

According to Table 2 in this study, folic acid serum level in the patients compared to control group was significantly low ($p = 0.002$). Furthermore, the homocysteine serum level was significantly high in the patients compared to the control group ($p < 0.01$).

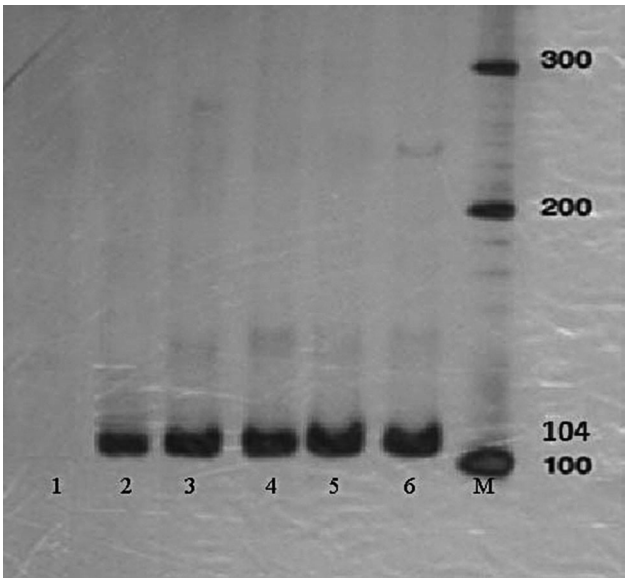

Figure 1. — The 8% polyacrylamide gel electrophoresis of PCR products using oligonucleotide primers for amplification of MTHFR gene (Line M is a 100 bp DNA ladder, line 1 is negative control (without DNA), and lines 2-6 are amplified samples, respectively).

In this study, the mean serum level of folic acid and homocysteine in all samples were not significantly associated with BMI, infant's weight, abortion, and stillbirth. However, mean serum homocysteine level was statistically significantly high in the patients with stillbirth history (0.09 vs. 0.02 µmol/L, $p = 0.01$). In addition, association between folic acid and homocysteine serum level was not found in all samples ($p = 0.056$), but the correlation coefficient was negative; such that either of them increased, the other decreased and vice-versa ($r = -0.012$). There was also no significant association between folic acid and homocysteine serum levels in the patients and control group separately, but the correlation coefficient was negative.

The amplification of MTHFR gene by PCR technique using specific and mutated oligonucleotide primers on polyacrylamide gel electrophoresis revealed fragments with the length size of 104 bp (Figure 1). The digestion of PCR products by *Hinf*I in patients and controls were showed that in presence of C677T polymorphism at 104 bp fragment, a cutting position is developed for this enzyme at nucleotide 677 due to C→T (G^ATTTC) and 104 bp fragment is converted into two 50-and 54-bp fragments due to cutting this enzyme. In CC genotype, cutting position for this enzyme was not observed and consequently the 104 bp fragment remained in the individual (Figure 2).

In this study, frequency of the CC, CT, and TT genotypes

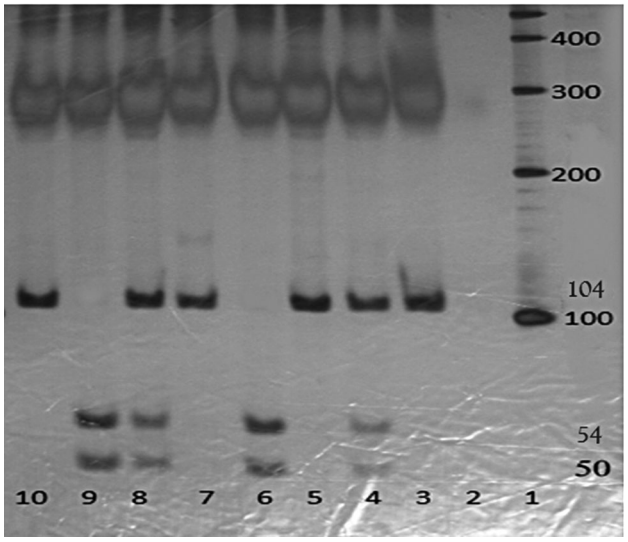

Figure 2. — Polyacrylamide gel electrophoresis of PCR products after digestion with *Hinf*I restriction enzyme (line 1 is a 100 bp DNA ladder, line 2 is negative control (no DNA), line 3 is positive control (uncut), lines 4 and 8 are heterozygous CT, lines 6 and 9 are homozygous CC, and lines 5, 7, and 10 are homozygous TT, respectively).

Table 4. — *Comparison of serum level of acid folic in each age group of patients and controls.*

Age group	95% confidence interval (CI)	*p*-value
15-20	-2.39, +6.67	0.33
20-25	-2.01, +1.96	0.97
25-30	-0.76, +2.9	0.246
30-35	-1.43, +3.2	0.44
35-40	-0.5, +5.8	0.09
40-45	-1.3, +4.6	0.06

Table 5. — *Comparison of homocysteine level of serum in each age group of patients and controls.*

Age group	95% confidence interval (CI)	*p*-value
15-20	-7.09, +3.5	0.49
20-25	-2.5, +1.42	0.58
25-30	-2.85, +0.72	0.23
30-35	-4.47, +0.73	0.15
35-40	-10.24, +2.56	0.22
40-45	-8.74, +4.31	0.37

of C677T polymorphism of MTHFR gene were 57.4%, 38.8%, and 3.9% in the patients and in the control group were 53.6%, 40%, and 6.4%, respectively, with no significant difference ($p > 0.05$). However, the frequency of T allele (TT genotype) in homozygous individuals was high in the control group compared to the patients, although not statistically significant (6.4% *vs*. 3.9%, $p = 0.36$). Also, the frequency of rare T allele was 23.3% in the patients and 26.4% in the control group. In the present study, odds ratio was calculated using logistic regression and interesting finding was that the CT and CC genotypes increased the risk of preeclampsia development 1.6 and 1.76 times compared to the *TT* genotype, respectively (Table 3). In addition, the association between C677T polymorphism of MTHFR gene and preeclampsia severity and age was not significant ($p = 0.051$ and 0.79, respectively).

Results indicated that C677T polymorphism of MTHFR gene had no significant relationship in serum level of folic acid ($p = 0.38$) and homocysteine ($p = 0.23$) (Tables 4-7) levels. The folic acid and homocysteine levels were compared in both healthy and patient groups, and there was no significant difference between the two groups. Minimal serum levels of folic acid and homocysteine detected in the CC genotype were 2.2 ng/ml and 31.4 µmol/L, respectively. The minimum mean of serum level of folic acid was 8.9 ng/ml in the *CC* genotype and maximum mean of serum level of homocysteine was 8.9 ng/ml in the TT genotype. In individuals with CC genotype, folate deficiency and hyperhomocysteinemia were more significant in the preeclamptic patient group compared to the control group. In addition, in the present study, TT and CT genotypes were not associated with folate deficiency and hyperhomocysteinemia in individuals, respectively.

Discussion

In this study, the C677T polymorphism of MTHFR gene was investigated in terms of its effective role in disease development and diagnosis with 95% CI in view of the genotypes frequency, and no significant difference was observed in this regard between the patients and healthy pregnant women. However, the high frequency of T allele (TT genotype) in the control group compared to the patients (6.4% *vs*. 3.9%) was observed. This genotype seems to contribute to decrease in preeclampsia risk, but this finding was not statistically significant in this study ($p > 0.36$). The findings of this study are similar to the Saravani *et al.* study in 2011-2012 in Zahedan city (Iran) [19]. They indicated that T allele is likely to assume a protective role against preeclampsia in Iranian populations. In addition, the mean folic acid serum levels in the patients (8.39 ng/dL) compared to the control group (9.96 ng/dL) were low. Furthermore, homocysteine serum level in the patients (11.2 µmol/L) compared to the control group (9.9 µmol/L) was statistically significant and high ($p < 0.01$). It is worth mentioning that all participants consumed folic acid in prenatal period. Therefore, according to these findings, it seems that preeclampsia could be prevented through increasing folic acid dosage received by pregnant women in prenatal pe-

Table 6. — *T677C polymorphism of MTHFR gene associated with serum level of folic acid.*

Genotype	Number	Max (μmol/L)	Min (μmol/L)	95% Confidence interval	Mean ± SD (μmol/L)	*p*-value
CC	140	20	2.2	8.2-9.5	8.9 ± 4.04	> 0.05
CT	99	20	2.9	8.5-10.1	9.3 ± 3.9	> 0.05
TT	13	19.7	4.7	7.3-13.3	10.3 ± 4.9	> 0.05
Total	252	20	2.2	8.6-9.6	9.1 ± 4.06	0.383

Table 7. — *T677C polymorphism of MTHFR gene associated with serum level of homocysteine.*

Genotype	Number	Max (μmol/L)	Min (μmol/L)	95% Confidence interval	Mean ± SD (μmol/L)	*p*-value
CC	140	31.4	2.5	10.1-11.7	10.9 ± 4.8	> 0.05
CT	99	23.4	2.8	9.2-10.7	10.02 ± 3.7	> 0.05
TT	13	21.2	4.4	8.27-14.7	11.4 ± 5.3	> 0.05
Total	252	31.4	2.5	10.05-11.1	10.6 ± 4.4	0.230

riod.

Investigation of C677T polymorphism of MTHFR gene by PCR-RFLP showed that frequency of the CC, CT, and TT genotypes are 57.4%, 38.8%, and 3.9% in the patients and in the control group are 53.6%, 40%, and 6.4%, respectively, with no significant difference ($p > 0.05$). The frequency of T allele (TT genotype) in homozygous individuals in the control group compared to the patients were high and not statistically significant ($p = 0.36$). Also, the CT and CC genotypes increased the risk of preeclampsia development 1.6 and 1.6 times compared to the TT genotype, respectively. Investigation of MTHFR conducted for the first time by Sohda *et al.* in 1997 in Japan, indicated a significant association between this gene and preeclampsia [23]. This finding is not consistent with the present study. In their study, in 67 patients and 98 healthy women, T allele frequency are 48% and 36%, respectively [24] while in the present study, the frequency of T allele in the patients (23.3%) and in the healthy individuals (26.4%) were less. In another study Grandone *et al.* in Italy concluded that the TT genotype was associated with preeclampsia, and this was consistent with the present study [13]. After that Powers *et al.* in USA did not find any association between this polymorphism and preeclampsia [25]. In a study by Rajkovic *et al.* in 2000 in Zimbabwe, no association was found between this polymorphism and preeclampsia [26]. In other studies conducted between 2000 and 2011 in the Netherlands [16], UK [26], and Australia [29], a lack of the association between this polymorphism and preeclampsia was reported. In another study by Aggarwal *et al.* in 2011 in northern India, the mutated MTHFR allele prevented preeclampsia and eclampsia paradoxically [13]. Perhaps, the most similar findings to present study could be found in the study of Canto *et al.* in 2007 in Mexico and Saravani *et al.* in 2011 in Zahedan, Iran [9, 19]. Canto *et al.* argued that C677T polymorphism of MTHFR gene contributed to a decrease risk of preeclampsia [9]. Also, the frequency of *CC*, CT, and TT genotypes of this polymorphism was not significantly different in two groups, but the frequency of homozygous TT individuals as well as T allele in the control group are increased, which contributed possibly to the decreased risk of preeclampsia by the authors' argument [19].

Investigating nitric oxide and homocysteine serum level in preeclampsia concluded that the tragic outcomes of this syndrome is only associated with homocysteine serum level but not to nitric oxide [27]. A study in Norway indicated that such infants' blood is influenced by high level of homocysteine in maternal blood and the unknown effects of this on infants should be discovered [28]. In the present study, the mean folic acid serum level was 9.96 and 8.39 ng/dL in the control group and patients, respectively, and folic acid serum level in the patients was low. Homocysteine serum level was 9.9 and 11.2 μmol/L in the control group and patients, respectively, and showed that homocysteine serum level was significantly high in the patients ($p < 0.01$). It is worth mentioning that all participants consumed folic acid in prenatal period. Therefore, serum level of folic acid and homocysteine are different in the two groups and folic acid serum level is low and homocysteine serum level is high in these patients. It seems that preeclampsia could be prevented through increasing folic acid dosage received by pregnant women in the prenatal period. However, the study in Venezuela showed that folic acid had no effect on prevention of preeclampsia [29]. Therefore, further interventional studies are recommended on this area. In the present study, a significant association between age and preeclampsia incidence, as well as homocysteine serum level was observed with aging homocysteine serum and preeclampsia incidence increase. Different studies have also indicated an association between age and homocysteine. For example, in the study of Erdemoglu *et al.* in Turkey, the older mothers had increased homocysteine serum level and developed preeclampsia [27]. Another investigation by Zeeman *et al.* indicated a positive correlation of chronic blood pressure with high homocysteine and aging [30]. In the present study, the CT and CC

genotypes increased preeclampsia development 1.6 and 1.7 times compared to the *TT* genotype. In addition, preeclampsia risk increased approximately twice in individuals with the CC genotype accompanied to folic acid deficiency compared to the control group (11 patients *vs*. six healthy individuals). Also, preeclampsia risks in individuals have CC genotype accompanied with hyper-homocysteinemia increased approximately twice compared to the control group (nine patients *vs*. five healthy controls). These results are high similar to the results of Yilmaz *et al.* study in 2004 in Turkey, which concluded that homocysteine blood level was partially high in preeclamptic individuals. Moreover, no allelic disorder affected plasma homocysteine concentration, but in the individuals with preeclampsia, with C677T polymorphism in MTHFR gene (unmutated gene), homocysteine level considerably increased compared to normal pregnancies, which rejects the hyper homocysteinemia due to allelic mutation in preeclampsia [31]. In a similar study by Hermann in 2006 in Syria, C677T polymorphism of MTHFR gene in pregnant women was not associated with preeclampsia while in the present study, if homocysteine concentration was above than 7.8 μmol/L, the risk of preeclampsia increased 21.6 times compared to individuals with homocysteine concentration below 5.2 μmol/L. In addition, low folate concentration founded to be associated with preeclampsia risk. Furthermore, the possibility of preeclampsia incidence increased from 1 to 4.8 in the women with *CC* genotype and folate deficiency, while its risk increased from 0.8 to only 1.5 in those with TT genotype and folate deficiency [32]. The findings of Laivuori *et al.* study in 2000 in Finland are similar to the present study and carriers of MTHFR T677 allele are not predisposed to preeclampsia. Meanwhile, they found that C→T in this allele caused a decline of MTHFR activity and hence an increase in plasma homocysteine concentration, particularly when the body folic acid-, B6-, and B12-deficient. Also, they argued that homocysteine level increased in preeclampsia [33]. In another research by Rajkovic *et al.* in Zimbabwe, no noticeable association was founded between MTHFR genotypes and preeclampsia risk; also, plasma concentration of folate, B12, and homocysteine were not significantly different in normotensive homozygosity and heterozygosity for MTHFR gene, but a strong association was discovered between maternal folate plasma concentration and preeclampsia risk, such that, in the women with folate concentration below 5.7 nmol/L, the risk of preeclampsia increased by 10.4 times and a clear association between B12 concentration and preeclampsia risk were not noted [26]. In the present study, direct significant correlation between BMI and preeclampsia was observed, which is similar to the findings of Kristin *et al.* in Norway and Rahimi *et al.* in Iran and their studies that showed that in case group, the mothers with preeclampsia, had high BMI compared to the control group [28, 31]. As observed in several studies, the findings are various in different regions. This inconsistency could be due to ethnic and racial disparities, nutritional habits, the difference in sample size and inclusion criteria, and concomitant effects of other gene variations on the single-nucleotide polymorphism of MTHFR gene in preeclampsia incidence [34]. In other words, the presence of other single-nucleotide polymorphisms in MTHFR gene and/or diversity in other genes is likely to affect expression, translation, and post-translational amendments of this gene and hence bring about these conditions. Therefore, further research is needed on various genes' effect on acute and preterm preeclampsia. Since preeclampsia incidence is most probably a result from involvement by numerous polymorphic genes, lack of the significant association between a gene polymorphism alone and a phenomenon like preeclampsia is not surprising [1]. Therefore, the logical trend for investigating the role of polymorphism in preeclampsia incidence more closely is simultaneous study of polymorphism status in different genes. Throughout such investigation, a better and more reliable assessment of the association of genetic mutations and gene polymorphism with preeclampsia pathogenesis could be achieved.

Conclusions

Preeclampsia is one of the main reasons of prenatal mortality and complications in both mothers and infants, and an acute disease which could lead to complications in most organs in short-term and morbidity in long term. As a result, this disease could cause serious mental and functional injuries to the patients and impose stupendous costs on them. Since most researchers have argued that some genetic background contributed to preeclampsia incidence, if we find the gene(s) involved in this disease, it could be prevented through gene therapy in the future. The logical trend for investigating the role of polymorphism in preeclampsia incidence more closely is simultaneous study of polymorphism status in different genes. Also, as vascular injuries have some role in this disease, high levels of homocysteine cause vascular injuries, and hyperhomocysteinemia is accompanied with low levels of folic acid, preeclampsia incidence could be prevented or minimized by prescribing high dosage of folic acid in prenatal period. In the present study, the frequency of C677T polymorphism of MTHFR gene genotypes not significantly different between patients and healthy individuals, but high frequency of homozygous *CC* genotype in the patients (57.4% *vs.* 53.6%) and high level of heterozygous CT and homozygous TT genotypes in healthy individuals (40% *vs.* 38.8% and 6.4% *vs.* 3.9%, respectively) observed. Furthermore, low level of folic acid and high percent of homocysteine in serum of the patients compared to the control group are observed. Therefore, homocysteine serum level could be declined through increasing folic acid dosage consumed by pregnant women and hence vascular injuries by extension of preeclampsia could

be prevented. In addition, according to these findings. it seems that the older pregnant women, who have high homocysteine serum level and BMI, are more likely to develop preeclampsia and should receive closer prenatal cares in order to face less maternal-fetal risks. Therefore, conducting complementary and interventional studies could be helpful to diagnosis and treat preeclampsia in Iranian population.

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