Genetic Aspect of Iraqi Pregnant Women with Pre-Eclampsia

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Abstract: Introduction: One of serious problems of pregnancy complication is the Pre-eclampsia syndrome (PE) which mostly leads to increase morbidity and mortality in mother and fetus. Objective: we aimed to study and evaluate the genetic polymorphism contribution of MTHFR gene in preeclampsia patients and healthy pregnant women. Methods: We examined maternal age, gestational age, BMI, serum homocysteine concentration, proteinuria, systolic blood pressure, diastolic blood pressure and MTHFR (C677T) gene polymorphism in 52 PE and 50 healthy pregnant women. After DNA extraction from blood samples of all participants the PCR-RFLP technique were applied for detection of MTHFR gene polymorphism. Results: We found the means of serum homocysteine concentration, proteinuria, systolic blood pressure, diastolic blood pressure which were significantly higher in PE patients compared to healthy control (P<0.05). The miner allele frequency of T genotype was significantly higher in PE patients than the subjects of control groups (P<0.0001) and the risk of PE was higher by 20.9 folds in homoygous allele genotype (TT) when compared with wild genotype (CC) (OR 20.9, 95% CI= 4.06-107.4 P= 0.003). Conclusion: We concluded that the TT genotype of MTHFR gene is increased the risk of PE in pregnant patients of Iraqi population.

Keywords: MTHFR, Pre-eclampsia, Polymorphism, SNP, Iraq

1. Introduction

Pre-eclampsia is serious pregnancy problem described by hypertension and proteinuria and usually develops after 20 weeks of pregnancy. Pre-eclampsia affects 5-8% of pregnancies and is a leading cause of maternal death and contributes significantly to premature deliveries (1). Furthermore, women with pre-eclampsia have an increased risk of developing cardiovascular disorders later in their life (2). The etiology of pre-eclampsia is still unknown, but genetic factors have been implicated since the syndrome shows a familial tendency. Published reports of pedigree analysis suggest that development of pre-eclampsia may be based on a single recessive gene or dominant gene with incomplete penetrance (3, 4, and 5). The common risk factors accompanying with PE are advanced maternal age, history of PE, obesity, multiple pregnancies, and women with diabetes or gestational diabetes (6, 7, 8, and 9).

The interaction of genetic component with the environment factors play important role in development of pre-eclampsia. Indeed, Genetic polymorphisms are markers of biological diversity and genetic variations, which correlate with specific phenotype, are sometimes associated with the development of human disease in different ethnic groups (10). Polymorphisms of methyleneetetrahydrofolate reductase (MTHFR) gene have been evaluated in numerous studies to assess its association with several complex disorders, MTHFR is critical folate -metabolizing enzyme which requires riboflavin as its cofactor (11). The 5, 10-MTHFR enzymes catalyze the conversion of 5, 10-methyleneetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulating form of folate, and substrate for homocysteine remethylation to methionine (12). It is well-known that there are genetic polymorphisms of enzymes involved in metabolism of homocysteine (13). Indeed, the homozygous form of mutation which result from conversion of cytosine to thymine at the position of 677 in gene encoding methyleneetetrahydrofolate reductase results in reduced production of 5- methyltetrahydrofolate (12). Furthermore, 677 C>T polymorphism in the gene for MTHFR that show a high frequency heterozygous CT genotypes and homozygous TT genotype with characteristics of low folate levels, which necessary as factors that compromise oocyte maturation before conception and successful DNA methylation and demethylation, causing point mutations, chromosome breakage, defective chromosome recombination, and may set the stage for genetically conditioned high risk conception, with increased risk for maternal chromosomal non-disjunction, which is the main cause of fetal chromosomal aneuploidy (14, 15).

We proposed that the MTHFR C677T mutation occur more frequently in Iraqi PE mothers compared to healthy pregnant control.

2. Materials and Methods

Between January 2015 and June 2015 a total of 52 pregnant patients with pre-eclampsia in the second and third trimester, in parallel with 50 healthy pregnant women, who admitted the clinic of Al Zahra Teaching hospital in Al Najaf Province were subjected to the present study. The age of patients was ranged from 28-37 years, with mean±SD 31.75 ±3.1 and the healthy pregnant control group ranged from 29-38 years, with mean±SD 33.5 ±3.3, all women were primigravida, body mass index (20-33) Kg/m².

Pre-eclampsia was defined using the criteria of gestational hypertension, proteinuria after pregnancy. Gestational hypertension was defined as increase of blood pressure
140/190 mm Hg 20 weeks of gestation if earlier blood pressure were not know according to (The consensus Report of the American Working Group on High Blood Pressure in Pregnancy and other working Group of the German Society of Obstetrics and Gynecology)(16). Proteinuria was defined as >300 mg/24hour urine collection or one plus urine dipstick. The specimens were taken after written informed consent obtained from all participants. This study, including the consent protocol, was approved by the Medical ethics committee Faculty of Medicine / Kufa University.

A total of 5 milliliter maternal venous blood samples were collected from all women. 1 ml of blood sample wasdrowning in EDTA tube for DNA extraction. Another 4 ml of blood samples were drawn into tubes free of anticoagulant material, these tubes were centrifuged for 10min at 3000 rpm, and serum were separated and stored at -17°C until the assayed was performed. Total plasma homocysteine was analyzed according to the manufacturer procedure for determination by using microplate enzyme immunoassay ELISA kit method of Biorad laboratories. DNA extraction that has been published previously (17). The DNA was amplified by polymerase chain reaction using primers described by Ch.Kalyankumer and Jamil K. (18). The primers used for PCR–RFLP were Forward 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' and Reverse 5'-AGG ACG GTG CGG TGA GAG TG-3' resulting in a PCR product of 198bp. The amplification was accomplished with a 25 μl reaction mixture containing 5 μl of (10-100) ng template DNA, 1.5 μl(1.5)pmol of each primer, 12.5 μl master mix contains of (2.5 μl 10 mM dNTPs, 1.5 μl of 20 mM MgCl2, 0.3 μl of 5 U/ μl Taq polymerase with 2.5 μl of 10X Taq Buffer) (Promega, USA). The reaction volume completed by addition of nuclease free water. PCR conditions were as follows: Initial denaturation at 94°C for 6 minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, extension at 72°C for 45 seconds and final extension at 72°C for 5 minutes. The polymorphism was detected by enzymatic digestion of the initial polymerase chain reaction product with HinfI at 37°C restrictaseHinfI (Promega,USA) at 37°C for 4 hrs. The resulting of DNAfragments was separated on 3% agarose gel and was run at 70 V for 120 min, after electrophoresis, the digested products were photographed under UV light after separation of bands by agarose Gel electrophoresis. Accordingly, Samples who lack the mutation appeared one 198bp fragment, sample with heterozygous for the mutation revealed both 198bp and 175bp fragments, and homozygous sample revealed one 175 fragment Fig.(1).

### Statistical Analyses

Statistical analyses were performed using the SPSS software package ( revision 20 Inc., Chicago,USA) Data are expressed as means ±SD. Differences in distribution of genotye or alleles between patients and control were tested using the Chi-square statistic. Odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated to estimate the risk of pre-eclampsia. Values of P > 0.05 were considered statistically significant.

### 3. The Results

The principle characteristics of females in current study are presented in table (1). One hundred and two women are participated in this study, the study population divided into two groups are first group include 52 patients with pre-eclampsia, and second group of 50 pregnant women with normal pregnancy, clinical feature and demographic characteristics for pre-eclampsia PE and control study group are demonstrated in table(1), the mean and standard deviation of the age, gestational age and body mass index in PE and control group, statistical analysis revealed that there is no significant differences(p>0.05). While a comparison between two groups by the mean and SD for other clinical features such as the serumhomocysteinelevel and proteinuria, SBP as well as DBP there were significantly different between two study groups (P<0.05).

### Table 1: Clinical features of PE patients and healthy control group.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>PE (n=52)</th>
<th>Control(n=50)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>Mean ±SD</td>
<td>Mean ±SD</td>
<td>0.69</td>
</tr>
<tr>
<td>31.75 ± 1.3</td>
<td>33.5 ± 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>32.1 ±3.6</td>
<td>31.8 ± 3.8</td>
<td>0.95</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28 ± 1.5</td>
<td>25 ± 2.1</td>
<td>0.24</td>
</tr>
<tr>
<td>Homocysteine (μM)</td>
<td>18.7±2.1</td>
<td>10.5 ±1.7</td>
<td>0.0032</td>
</tr>
<tr>
<td>Urine Protein (mg/dl)</td>
<td>44.9 ±2.6</td>
<td>25.6 ±2.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Systolic Bp(mm Hg)</td>
<td>155 ±4</td>
<td>118±5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Diastolic Bp(mm Hg)</td>
<td>95±5</td>
<td>75±3</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P<0.05: statistically significant.
Figure 1: The HinfI restriction digested PCR product profile of MTHFR gene. Lane M: DNA Marker, Lane1, 2, 3 and 5 CC genotype, Lane 4, 6, 7 and 9 show CT genotype, Lane 8 shows TT genotype.

Nucleotide substitution at locus 677 in exon 5 region of MTHFR gene is studied using SNP by the use of PCR-RFLP technique. The allele frequency and genotypes of the C677T MTHFR gene polymorphism in patients are consistent with HWE, P= 0.176 while in healthy group are inconsistent with HWE, P= 0.027. The allele frequency and genotype of SNP of the MTHFR gene in PE patients 19.2% for CC, 44.2% for CT and 36% for TT respectively, whereas in control group CC, CT and TT variants are at frequency of 38%, 58% and 67% respectively. The allele frequency obtained in the patients for C are (38.5%) and for T are (61.5%), whereas in the control group for C are (67.5%) and for T are (33%). The allele and genotypes frequency of C677T in TT variants 22 (36%) were significantly increased (p<0.05) by twenty one folds in homozygous genotype of pregnant women complained pre-eclampsia when compared with wild genotype pre-eclampsia women (OR 20.9, 95% CI= 4.06 -107.4 P= 0.003).

Table 2: Allele frequency and genotype of SNPs Analysis among women with Pre-eclampsia and healthy group.

<table>
<thead>
<tr>
<th>SNPs Genotype/ Allele frequency</th>
<th>PE Patients No=55</th>
<th>Healthy control No=60</th>
<th>OR (95%CI)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>10(19.2%)</td>
<td>19(38%)</td>
<td>1</td>
<td>Reference</td>
</tr>
<tr>
<td>CT</td>
<td>20(44.2%)</td>
<td>29(58%)</td>
<td>1.31</td>
<td>0.50 - 3.4</td>
</tr>
<tr>
<td>TT</td>
<td>22(36%)</td>
<td>2(4%)</td>
<td>20.9</td>
<td>4.06 -107.4</td>
</tr>
<tr>
<td>C</td>
<td>40(38.5)</td>
<td>67(67%)</td>
<td>1</td>
<td>Reference</td>
</tr>
<tr>
<td>T</td>
<td>64(61.5%)</td>
<td>33(33%)</td>
<td>3.2</td>
<td>1.8 -5.7</td>
</tr>
</tbody>
</table>

Table 2: Allele frequency and genotype of SNPs Analysis among women with Pre-eclampsia and healthy group.

OR= Odds ratio, CI=Confidence interval, SNP= Single Nucleotide Polymorphisms

4. Discussion

This study is case control study, we summarized possible association of mutation polymorphisms of MTHFR (677 C>T) gene with pre-eclampsia patients and healthy pregnant subjects in Iraqis women. Usually, PE has been associated with insufficient trophoblast invasion of maternal spiral arteries, impaired placental perfusion, and wide spread endothelial cell dysfunction(19). The Causes behind these clinical variations remain unclear. PE believed to be a multifactorial disorder with important role of genetic factor (20). In fact, PE is a big obstetric problem in Iraq, up to our knowledge, role of the genetic polymorphism in PE have not been well studied.

Our investigation revealed that there are no statistical differences in some of clinical characteristics between PE patients and healthy groups with respect maternal age, gestational age, or BMI while the other, statistical analysis showed that there is a high significant difference in two groups as regard to homocysteine, proteinuria, systolic and diastolic blood pressure. It is clear, that there are firm implications of having homocysteine level raises are important for women. In fact, elevations of homocysteine levels have been observed more frequent among women with certain pregnancy complication, such as preeclampsia (21).

Our results also showed that homozygous mutated TT genotypes of C677T and T allele polymorphisms was higher in study group of PE patients compared to healthy control. Indeed, homozygous mutation for the 677 C>T in gene for MTHFR, caused decrease synthesis of 5-methyltetrahydrofolate, the primary methyl donor in conversion of homocysteine to methionine which lead to increase of homocysteine in plasma (22). 677 C→T mutation is responsible for reduced MTHFR activity, and it is found significantly effective only in recessive homozygous state (23). On the other hand, the association between recessive homozygous 677C→T in MTHFR gene and PE, and the presence of higher 677C→T mutations in MTHFR gene among women with PE, compared to healthy women, indicated that mutation in this gene might be a risk factor for pregnancy.
This result is consistent with similar studies of Caucasian, East Asian (24), and Mexican women with pre-eclampsia population (25). In contrast, Williams et al.(26),Yilmaz et al.(27),Yalinkaya et al.(28),Canto et al.(29),Stiefel et al.(30) reported no association between pre-eclampsia and MTHFR gene mutation.

The discrepant results of published studies on disease associations of genetic MTHFR polymorphism presumably due to the prevalence of variation of MTHFR polymorphisms in different ethnic populations. In conclusion, C677T polymorphism of MTHFR gene was found associated with development of pre-eclampsia among Iraqi pregnant women. We recommended further studies which conducted with chromosomes abnormalities and large sample size of different races of Iraqi population is still needed.

References

[14] Martinez-Frias ML. The Biochemical structure and function of methylenetetrahydrofolate reductase provide the rationale to interpret the epidemiological results on risk for infants with down syndrome American Journal of Medical Genetics .2006 Jun 1; 146A(11):1477-82.
