International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

# Genetic Aspect of Iraqi PregnantWomen with Pre-Eclampsia

**Salih M. Al-Khafaji[1], Anwar M. Al-janabi[2], BasimaAl-ghzaly[3], Shehab A .Faris[4]**

[1, 4]Department ofAnatomy&Histology,College of Medicine, Kufa University

[2]Department of Clinical Biochemistry,College of Medicine, Kufa University

[3]Department of Gynecology,College of Medicine, Kufa University

**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 serumhomocysteine 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 patientsthan the subjects of control groups(P<0.0001) and the risk of PE was higher by 20.9 folds in homozygous 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 pregnancyproblemdescribed by hypertension and proteinuria and usually develops after20 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 withdiabetesor 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 methylenetetrahydrofolate 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). The5, 10-MTHFR enzymescatalyze the conversion of 5, 10-methylenetetrahydro-folate 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 positon of 677 in gene encoding methylenetetrahydrofolate 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 IraqiPE 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±SD31.75 ± 3.1 and the healthy pregnant control group ranged from 29 -38years, with mean±SD33.5 ±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 specimenswere 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 drown into tubes free of anticoagulant material, these tubes were centrifuged for 10min at 3000 rpm, and serum were separated and stored at -17°Cuntilthe 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 polymerasechain 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/ µlTaq 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 andwasrun at 70 V for 120 min, after electrophoresis, the digested products were photographed under UV light after separation ob 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 genotype 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.

| *Parameters* | *PE (n= 52) Mean ±SD* | *Control(n= 50) Mean ±SD* | *P Value* |
|---|---|---|---|
| **Maternal age (years)** | 31.75 ± 3.1 | 33.5± 3.3 | 0.69 |
| **Gestational age (weeks)** | 32.1 ±3.6 | 31.8 ± 3.8 | 0.95 |
| **BMI (kg/m$^2$)** | 28 ± 1.5 | 25 ± 2.1 | 0.24 |
| **Homocysteine (µM)** | 18.7±2.1 | 10.5 ±1.7 | 0.0032 |
| **Urine. Protein (mg/dl.)** | 44.9 ±2.6 | 25.6± 2.3 | 0.03 |
| **Systolic Bp(mm Hg)** | 155 ±4 | 118±5 | 0.0001 |
| **Diastolic Bp(mm Hg)** | 95± 5 | 75± 3 | 0.001 |

P<0.05: statistically significant.

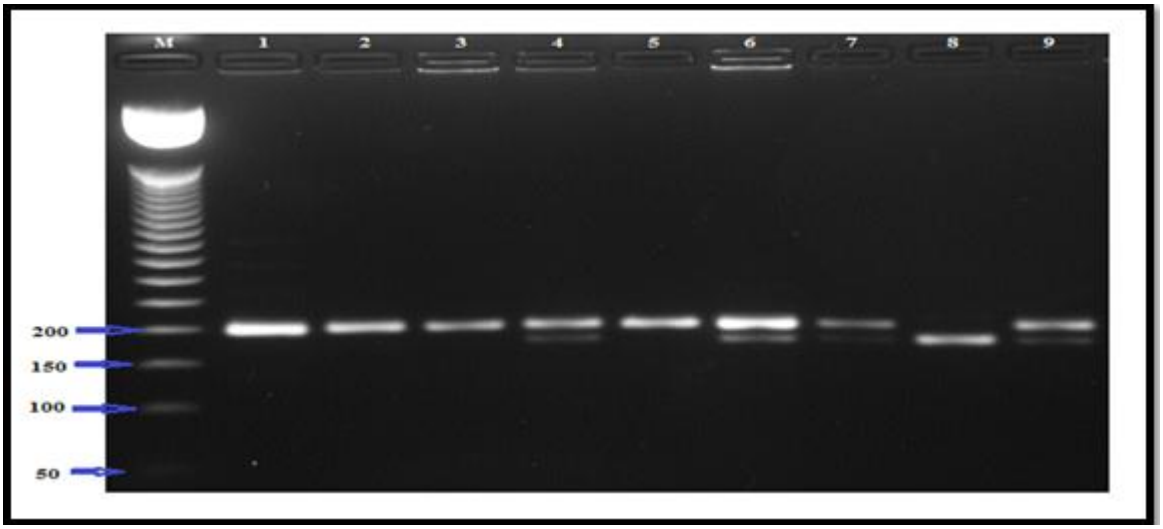


**Figure 1:** The HinfI restriction digested PCR product profile of MTHFR gene. Lane M: DNA Marker, Lane1,2, 3 and 5 CC genotype, Lane 4,6,7and 9 show CT genotype, Lane 8 shows TT genotype.

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 heathy 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,CTand 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-eclapsia 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 heathy group.

| *SNPs Genotype/ Allele frequency* | *PE Patients No=55* | *Healthy control No=60* | *OR* | *(95%CI)* | *P-Value* |
|---|---|---|---|---|---|
| **CC** | 10(19.2%) | 19(38%) | 1 | Reference | ------- |
| **CT** | 20(44.2%) | 29(58%) | 1.31 | 0.50-3.4 | 0.57 |
| **TT** | 22(36%) | 2(4%) | 20.9 | 4.06-107.4 | 0.0003 |
| **C** | 40(38.5) | 67(67%) | 1 | Reference | ------- |
| **T** | 64(61.5%) | 33(33%) | 3.2 | 1.8-5.7 | 0.0001 |

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 celldysfunction(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 bigobstetric problem in Iraq, up to our knowledge, role of the genetic polymorphism in PE have not well studied.

Our investigation revealed that there are no statistical differences in some of clinical characteristics between PE patients and healthy groups with respect maternalage, 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 significantlyeffective 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 ofhigher 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 etal.(29),Stiefelet 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 deferent 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

[1] Steegers L, Van Dadelszen P, Duvekot R. Pre-eclampsia. Lancet 2010; 376:631-644.
[2] Bellamy L, Casas JP, Hingoran AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: Systematic review and meta-analysis .Br Med J 2007: 335-974.
[3] Esplin MS, Fausett MB, Fraser A, Kerber R, Mineau G, Carrillo J, Varner MW.Paternal and maternal components of the predisposition to preeclampsia.NEngl J Med 2001;344:867–872.
[4] Nilsson E, Salonen RH, Cnattingius S, Lichtenstein P. The importance of genetic andenvironmental effects for pre-eclampsia and gestational hypertension: a familystudy. BJOG 2004;111:200–206.
[5] Cincotta RB, Brennecke SP. Family history of pre-eclampsia as a predictor forpre-eclampsia in primigravidas. Int J GynaecolObstet 1998;60:23–27.
[6] Bilano VL, Ota E, Ganchimeg T, Mori R, Souza JP: Risk factors of preeclampsia/eclampsia and its adverse outcomes in low- and middle incomecountries: a WHO secondary analysis. PLoS One 2014, 9:e91198.
[7] Young BC, Levine RJ, Karumanchi SA: Pathogenesis of pre-eclampsia. AnnuRevPathol 2010, 5:173-92.
[8] Duckitt K, Harrington D: Risk factors for pre-eclampsia at antenatalbooking: systematic review of controlled studies. BMJ 2005, 330:565.
[9] Irinyenikan TA, Roberts OA, Arowojola A. Serum lipid levels in in pregnant normotensive and gestational hypertensive women in Ibadan, anigeria,Annals of biological research 2013; 4(4): 204-208.
[10] Bhaskar S, Ganesane M, Chandak GR, Mani R, Idris MM, et,al. Association of PON1 and AOPAS gene polymorphisms in a cohort of Indian patients having coronary artery disease with and without type2 diabetes. Genet Test Biomarkers.2011: 15(7-8): 507-512.
[11] Ueland PM, Hustad S, Schneede J, Refsum H, Vollset SE. Biological and clinical implications of MTHFR C677T polymorphisms. Trend pharma Col Sci 2011; 22(4): 195-201.
[12] Hickey SE, Curry CJ, Toriello HV. ACMG practice Guideline: lack of evidence for MTHFR polymorphisms testing. Genet Med 2013; 15(2):153 -156.
[13] Hague WM. Homocysteine and pregnancy. Best Pract Res ClinObstetGynaecol2003 ; 17(13): 459-469.
[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.
[15] Santos- ReboncasCB, Correa JC, Bonomo A, Fintelman-Rodrigues N, Moura KC, Rodrigues CS, Santos JM, Pimentel MM. The impact of folate pathway polymorphisms combined to nutritional deficiency as maternal predisposition factor Down syndrome. Disease markers .2008; 25(3): 149-57.
[16] Len Fant C, Gifford J RW,Zuspan F. Nuritional high blood pressure on education program working group report on high blood pressure in pregnancy. Consensus Rep Am J ObstetGynecol 1990; 163: 1689-712.
[17] Salih M. Al-Khafaji . Evaluation of FokI polymorphisms of VDR gene in Iraqi patients with colorectal cancer.International Journal of Advanced Research . 2015; 3 (4) :1194-1198.
[18] Ch.Kalyankumer and Jamil K.MethylenetetrahydrofolateReductase(MTHFR)C677 and A1298C polymorphisms and breast cancer.International Journal of cancer Research in South Indian Pop. 2006; 2(2):143-151.
[19] Chan X, Gan T, Liao Z, Chen S, Xiao J. Foxp3 (-IATT) polymorphisms contributes to the susceptipility of pre-eclampsia. Los one2013; 8(4): e59696.
[20] Lin R, Lei Y, Yuan Z, Ju H, Li D. Angiotensinogen gene M 235 T and T 174 polymorphisms and susceptibility of pre-eclampsia: meta-analysis. Ann Hum Genet 2012;76(5): 377-386.
[21] Ray JG, LaskinCA. Folic acid and homocysteine metabolic defects and riskof placental abruption, pre-eclampsia and spontaneous preganacy loss: asystematic review, Placenta.1999;20: 519-529.
[22] Malinow MR, Bostom AG, Krauss RM. Homocystiene diet and cardiovascular disease: astatement for healthcare profesionals from the Nutrition committee, American Heart Association. Circulation.1999,99; 178-182..
[23] L, Rai R : Thrombophilia and pregnancy Loss .J Reprod Immunol.2002;55:163-80.
[24] Wang XM, Wu HY, Quxj J. Methyletetrahydrofolate reductase(MTHFR) gene polymorphism and risk of pre eclampsia: an update meta-analysis based on 51 studies .Arch Med Res.2013;17(6):159-768.
[25] Rojas JC, Luna M, Rangel-Neva H, Banos D, Collados MT. Genetic thrompophilia and Markers of endothelial activation in patients with pre eclampsia. Ginecol.Obstet Mex 2010;78(8):401.
[26] Williams MA, Sanchez SE, Zhang C, Bazul V. Methylenetetrahydrofolate reducta 677 C-T polymorphism and plasma folate in relation to pre-eclampsia risk amonPeruvian women. J Matern Fetal Neonatal Med 2004;15(5):337.
[27] Yilmaz H, Unlu¨ c¸erc¸i Y, Gu¨ rdo¨ l F, Isbilen E, Isbir T. Association of pre-eclampsia with hyperhomocysteinemia and methylenetetrahydrofolate reductase gene C677T polymorphism in a Turkish population. Aust N Z J ObstetGynaecol 2004;44(5):423–7.

[28] Yalinkaya A, Erdemoglu M, Akdeniz N, Kale A, Kale E. The relationship between thrombophilic mutations and preeclampsia: a prospective case-control study. Ann Saudi Med 2006;26(2):105–9.
[29] Canto P, Canto-Cetina T, Jua´ rez-Vela´ zquez R, Rosas-Vargas H, Rangel-Villalobos H, Canizales-Quinteros S, Vela´zquez-Wong AC, Villarreal-Molina MT, Ferna´ndez G, Coral-Va´zquez R. Methylenetetrahydrofolate reductase C677T and glutathione S-transferase P1 A313G are associated with a reduced risk of preeclampsia in Maya-Mestizo women. Hypertens Res 2008;31(5): 1015–9.
[30] Stiefel P, Miranda ML, Bellido LM, Luna J, Jime´nez L, Pamies E, de Frutos PG and Villar J. Genotype of the CYBA promoter - 930A/G, polymorphism C677T of the MTHFR and APOE genotype in patients with hypertensive disorders of pregnancy: an observational study. Med Clin (Barc). 2009 Nov 7;133(17):657-61. Epub 2009 Oct 24.

.