Polymorphisms of The *IL1-BETA-511T/C* Gene In Pregnant Women Complicated With Pre-eclampsia

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Abstract

Background: Pre-eclampsia (PE) is a relatively common, systemic pregnancy disorder characterized by the development of concurrent hypertension (> 140/90 mmHg) and proteinuria (> 300 mg/24 h) at \geq 20 weeks of gestation, that may also be associated with a myriad of other symptoms such as edema, headache, blurred vision, irritability, abdominal pain, and thrombocytopenia. **Aim:** Identify the association between single nucleotide polymorphisms (SNP) of IL-1Beta and the onset and severity of the disease. **Methods:** The extracted DNA was amplified for IL-1Beta RFLP in 60 clinically diagnosed preeclampsia pregnant women and 60 normotensive pregnant women. For statistical significance, OR was measured and all data were processed by using SPSS. **Results:** IL-1 Beta genotyping in PE pregnant women showed the following results TT genotype having 2.33 fold risk of having PE and etiological factor (0.57) while TC mutant genotype showed 1.125 fold risk of having PE etiological factor of (0.111), while homozygous CC genotype considered as protective genotype with protective factor of (0.674). T allele considered the etiological factor of (0.287) while C allele considered as protective allele with protective factor of (0.589). **Conclusion**: IL-1 Beta T allele considered as significant risk factor for having preeclampsia. The presence of IL-1 Beta C alleles protects against having preeclampsia.

Key words: Preeclampsia, IL-1Beta RFLP, IL-1Beta and Preeclampsia.

Introduction

Preeclampsia(PE) is a pregnancy-specific syndrome, characterized by the new onset hypertension and proteinuria after 20th week of gestation. PE occurs in about 5%–7% of all pregnancies in the world, which is a leading cause of the maternal and fetal mortality and morbidity (1). Although extensive efforts to evaluate the mechanisms and molecules of PE, the underlying pathogenesis of PE has not yet been fully elucidated. Epidemiological research indicated that the PE was associated with the family and genetic control, and inheritance appeared to have a major function in the pathology of this disease (2). Moreover, PE is a multiple gene disorder, affected by genetic and environmental factors, like interaction between maternal and fetal genes, which were important determinants of maternal disease susceptibility (3). Therefore, genetic factors cannot be ignored in the pathogenesis of PE. Despite the etiology of PE remains unknown, a growing evidences indicated that the inflammatory response might explain the development of the PE (3). It was reported that the normal pregnancy was regarded as a mild inflammatory state (4), while PE was considered to be an exaggerated inflammatory state (4,5). Furthermore, PE had been proposed to be a complicated systemic inflammatory response acting in network, which contained not only the endothelium but also the inflammatory immune cells, the clotting and the complementary systems, metabolic and other changes mainly regulated by cytokines (6). During pregnancy, many cytokines are secreted by immune cells and lymphocytes at the interface of trophoblast and decidua, which mainly mediate and regulate immunity, inflammation and hematopoiesis. Benyo et al indicated that several cytokines had been found to be increased in pregnant women with PE. The serum level of inflammation cytokines, one kind of cytokines, such as IL-1b, IL-2, IL-6 and IFN-c had been found to be higher in women with PE than in normotensive pregnant women (7–9), which leaded to harmful Th1 immunity, threatening pregnancy by generating cytotoxic factors that injured maternal endothelium, altered steroid hormones biosynthesis and affected other factors which were implicated in trophoblast invasion and maternal spiral artery remodeling (10,11). The production of inflammatory cytokines is regulated by the cytokine gene, thus, the cytokines gene polymorphism may play a key role in the development of PE. IL-1b is a pro-inflammatory cytokines, belonging to the IL-1 system, which is an important role in mammalian reproduction. It had been reported that IL-1b had been implicated in the pathogenesis of PE (12). Many researchers had found that the plasma level of IL-1b was elevated in preeclamptic women (9,13-15). And increased placental expression of IL-1b was also observed in several researches (13). IL-1b is located in 70–110 kb region of chromosome 2q13–21, including 7 exons and 6 introns. At least 20 SNPs have been reported in the region of IL-1b, among which -511 and-31 loci in the promoter region of IL-1b were two important single nucleotide polymorphisms, whose gene polymorphism could influence the gene transcription and lead to the function changes (16). Moreover, the SNPS 2511 and 231 cites have been repeatedly associated with cardiovascular diseases (16). In the previous studies, Hefler et al, **Lachmeijer** *et al.*, and Lin *et al.*, observed the genotypic distribution of the IL-1b at position +3954 in exon and the IL-1b-511 C/T polymorphism respectively in Hispanic population, Dutch population and Taiwanese women, similarly, they all found no significantly statistical at the loci between the PE and normal pregnant women (17–19).

Patients and methods

Patients

The current study was conducted during the period from first of February 2016 to the first of December 2016, 60 patient who attended the outpatient and inpatient department, were diagnosed by a women consultant in the Maternity and Childhood Teaching Hospital on the basis of blood pressure measurement and proteinuria elevation. All subjects within the age group of 16-40 years were selected for the study. The PE diagnosis was based on the criteria from the Report of the 'National High Blood Pressure Education Program, and 60 healthy control, were included in the study. Blood pressure was measured with a mercury sphygmomanometer and the Korotkov sound technique. Diastolic pressure was indicated by the Korotkov V. sound. All patients had proteinuria \geq 3+ or 4+ tested by dipstick in at least two random urine specimens obtained at least 4 h apart (according to the kit 3+=300mg/day, 4+=1000mg/day). Medical history, maternal age, gestational age were recorded.

Selection Criteria of Patients

Inclusion Criteria: All subjects within the age group of 16-40 years were selected for the study. PE was defined as persisting elevated diastolic blood pressure (\geq 90mmHg), a proteinuria (\geq 300 mg in a 24 urine sample) and the presence of edema. Subjects willing to participate were included in the study.

Exclusion criteria: Subjects with non-confirmed PE, essential hypertension, malaria, haemolytic anemia, any other infection such as urinary tract infection or upper respiratory tract infection, fetal death, renal disease, uterine malformation, *in vitro* fertilization treatment, placental abruption, infection, cancer, gestational diabetes mellitus, or any other systemic disease, including pre-existing hypertension, systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) were excluded from the present study.

Selection Criteria of Control groups

Inclusion Criteria: Normotensive pregnant women had systolic/diastolic blood pressure below 120/80 mmHg, normal protein urea and blood urea and no history of hypertension or proteinuria. All pregnant women showed gestational age from 20 to 40 weeks.

Exclusion criteria: Exclusion criteria for healthy control were chronic hypertension, homeostatic abnormalities, cancer, diabetes mellitus, cardiovascular, autoimmune, renal and hepatic diseases, and anticoagulant therapy. All normotensive pregnant women have no systemic disease or chronic disease . In addition they have no dead baby or history of dead baby.

Collection of Sample

Blood Sample: Blood samples were collected from pregnant women included in present study by venipuncture. A total six ml of blood were drawn from forearm vein, 4 ml is divided into 2 ml for (GOT, GPT and ALP) and 2 ml for blood urea and the rest 2 ml sample in EDTA tubes which was immediately deeply frozen for *IL-1BETA* SNP analysis.

Urine Collection: For pregnant women and in most identical situations, a midstream clean-catch technique is usually adequate. Ten ml of urine for protein urea detection was required.

Genotyping: DNA was extracted with standardized DNA-extracting protocols using AccuPrep®Genomic DNA extraction kit (Bioneer. Korea). The amount of used blood samples for the DNA extraction was 200 μ l. The PCRs were performed in a final volume of 50 μ l containing 40 μ l PCR buffer, and sense and antisense primers, 2.5 μ l of each. We used 5 μ l DNA per PCR reaction all mixed in master mix tubes AccuPowerTM PCR PreMix Taq DNA polymerase dNTPs (dATP, dCTP, dGTP, dTTP) Tris-HCl pH 9.0,KCl, MgCl₂ Stabilizer and Tracking dye. The investigated DNA sequences were amplified by the following primers: IL-1b -511 locus: forward: 5′- TGGCATTGATCTGGTTCATC - 3′ and reverse: 5′- GTTTAGGAATCTTCCCACTT -3′.The condition of PCRs was as follows: The PCR conditions of -511T/C polymorphism of IL-1b were initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s and final tension at 72°C for 7 min. Then the PCR fragment was digested by AluI enzyme at 37°C for 1 h in a reaction volume of

10 mL. Digested products were separated by electrophoresis on a 2.5% agarose gel and visualized by Goodview staining. The expected results of IL-1b-511T/C is digested into 3 expected results: TT showing one band with 518 bp, CC showing two bands with 403 bp and 115 bp, TC showing three bands with518 bp, 403 bp and 115 bp.

Data analysis: Hardy–Weinberg equilibrium of the tested SNP were calculated. Logistic regression analysis was used for the analysis of the association between IL-1b-511T/C genotype and risk of PE. Multiple linear regression analysis was used to test the association between carrier state of IL-1b-511T/C genotypes and diagnosis of hypertension and proteinuria. The associations were adjusted for maternal age, gestational age, All calculations were performed with the statistical software package SPSS 23.

Result

The results presented in this study were based on the analysis of a random 60 pregnant women with an established diagnosis of PE, their age ranged between 16 and 39 years with a mean of 27.75 years. Also a 60 apparently healthy pregnant as control group was selected according to maternal and gestational age of the patients group. Their age ranged between 16 and 40 years with a mean of 30.2 years. The patients group was further subdivided into two subgroups according to their gestational age at which the hypertension and proteinuria appear into early onset PE , and late onset PE. So, early onset PE started before 35 week of gestation and the late onset of PE appear after 35

week of gestation, the early onset represent 30 cases with gestational age range (24 - 34) weeks and mean of 27.9 while the late onset (30 cases) with gestational age range between (36- 39) week and mean of 36.7. In addition the gestational age mean of the studied group was statistically significant with (P<0.05), **table 1**.

| Preeclampsia Cases Healthy Control | Р |
|------------------------------------|---|

 Table (1): Description of the 3 Study Groups by Maternal Age, Gestational Age

 and Party.

| | | Early Onset | | Late Onset | | | | |
|----|-------------------------|-------------|------|------------|------|-------|-------|-----------|
| | | No. | % | No. | % | No. | % | |
| 1. | Maternal Age (years) | | | | | | | 0.042[NS] |
| | 16-26 | 11 | 36.7 | 18 | 60 | 29 | 48.3 | |
| | 27-37 | 12 | 40 | 5 | 16.7 | 13 | 21.7 | |
| | ≥38 | 7 | 23.3 | 7 | 23.3 | 18 | 30 | |
| | Range | 16-39 | | 17-37 | | 20-40 | | |
| | Mean | 30.6 | | 25.7 | | 30.2 | | |
| | SD | 7.28 | | 7.2 | | 7.79 | | |
| | SE | 1.32 | | 1.3 | | 1.006 | | |
| | Total | 30 | 100 | 30 | 100 | 60 | 100 | |
| 2. | Gestational Age (weeks) | | | | | | | < 0.05 |
| | <35 | 30 | 100 | - | - | 41 | 86.3 | |
| | ≥35 | - | - | 30 | 100 | 19 | 31.7 | |
| | Range | 24-34 | | 35-39 | | 23-39 | | |
| | Mean | 27.9 | | 36.7 | | 31.16 | | |
| | SD | 4.26 | | 0.9 | | 5.53 | | |
| | SE | 0.78 | | 0.2 | | .718 | | |
| | Total | 30 | 100 | 30 | 100 | 60 | 100 | |
| 3. | Parity | | | | | | | 0.178[NS] |
| | Multiparous | 11 | 36.6 | 14 | 46.6 | 43 | 71.44 | |
| | Uniparous | 19 | 63.4 | 16 | 53.4 | 17 | 28.8 | |
| | Total | 30 | 100 | 30 | 100 | 60 | 100 | |

No.=Number, SD=Stander Deviation, SE=Stander Error, NS=Non-Significant.

The frequency of PE patient who have history of PE in previous pregnancy (45%), and history of dead baby in pervious pregnancy was (35%) which represent a high percentage of cases. Family history frequency was represent (66.6%) which have sister or mother history of PE, as in **table 2**.

Table (2): Frequency Distribution of Preeclampsia Cases by family history, andPatient History.

| | | No. | % |
|----|------------------------------------|-----|------|
| 1. | Family history of Preeclampsia | | |
| | Negative | 20 | 33.3 |
| | Positive | 40 | 66.6 |
| | Total | 60 | 100 |
| 2. | Patient history of Preeclampsia in | | |
| | previous pregnancy | | |
| | Negative | 33 | 55 |
| | Positive | 27 | 45 |
| | Total | 60 | 100 |
| 3. | History of dead baby | | |
| | Negative | 39 | 65 |
| | Positive | 21 | 35 |
| | Total | 60 | 100 |

No.= Number.

The diastolic Blood pressure measurement of the PE patients ranged between 90 and 150, in the early onset PE the diastolic blood pressure ranged between100 and 110mm Hg and mean of (101 mm Hg), while in the late onset PE ranged between 90 and 150mm Hg with mean of (118 mm Hg) when compared to the healthy control group the diastolic blood pressure range between 70 and 80mm Hg with mean of (75.5 mm Hg). Diastolic blood pressure mean was statistically higher in PE patient when compared to the control group with P value (0.001). Regarding the systolic blood pressure in early onset PE patients ranged between 140 and 160 mmHg with mean of (163 mmHg), while in the late onset ranged between 130 and 200 mmHg with mean of (149 mmHg).

Systolic blood pressure mean was statistically higher in PE patient when compared to the control group with P value (≤ 0.01), **table 3**.

Table (3): The Difference Between Three Study Groups in Diastolic Blood Pressure and Systolic Blood Pressure.

SD=Stander Deviation, SE= Stander Error.

Protein urea measurement among early and late onset of PE ranged from 300-1000 mg/24hours with mean of (790) in early onset and (486.7) in late onset when compared to healthy control which still normal with statistically significant P value (<0.01), Blood urea measurement among PE patient's showed significantly higher elevation when compared with healthy control, in the early onset PE blood urea measurement ranged

| | | Early Onset | Late Onset | Healthy Control | P Value | | | | |
|----|---|-----------------------|------------------------|-----------------|---------|--|--|--|--|
| 1. | Diastolic blood pressure mm Hg | | | | | | | | |
| | Mean | 101 | 118 | 75.5 | 0.001 | | | | |
| | Range | 100-110 | 90-150 | 70-80 | | | | | |
| | SD | 3 | 26.8 | 5 | | | | | |
| | SE | 0.56 | 4.9 | 0.65 | | | | | |
| | Total | | | | | | | | |
| | Bonferonni t-test for difference in mean between: | | | | | | | | |
| | Cases (PE) x Healthy Controls P Value = 0.001 | | | | | | | | |
| 2. | Systolic blood pressure mm Hg | | | | | | | | |
| | Mean | 163 | 149 | 125.33 | ≤ 0.01 | | | | |
| | Range | 140-160 | 130-200 | 120-130 | | | | | |
| | SD | 27.7 | 5.5 | 5 | | | | | |
| | SE | 5 | 1 | 0.7 | | | | | |
| | Total | 30 | 30 | 60 | | | | | |
| | Bonferon | ni t-test for differe | nce in mean betw | veen: | | | | | |
| | Cases (PF | E) x Healthy Contr | ols P Value ≤ 0.0 | 1 | | | | | |

from 67-120 mg/dl while the late onset ranged from 57-113 mg/dl when compared with healthy control with statistically significant P value (<0.05). as in **table 4.**

Table (4): The Case-Control Difference in Mean Concentration of Protienurea and blood urea.

SD=Stander Deviation, SE= Stander Error

| | Preeclamp | sia cases | Healthy Control | P Value | | |
|---|-----------------------|------------|-----------------|---------|--|--|
| | Early Onset | Late Onset | | | | |
| Protein Urea Nitrogen n | ng/dL | | | < 0.01* | | |
| Range | Range 300-1000 | | 30-100 | | | |
| Mean 790 | | 486.7 | 41.7 | | | |
| SD | 326 | 314.8 | 26 | | | |
| SE 59.5 | | 57 | 3.4 | | | |
| Total | 30 | 30 | 60 | | | |
| Bonferonni t-test for difference in mean between: | | | | | | |
| Cases (Preeclampsia) x Healthy Controls <0.01 | | | | | | |
| Blood Urea Nitrogen m | ng/dl | | | < 0.05* | | |
| Range | 67-120 | 57-113 | 11-23 | | | |
| Mean | 92 | 85 | 17 | | | |
| SD | 14.9 | 13.9 | 4 | | | |
| SE | 2.6 | 2.5 | 0.5 | | | |
| Total | 30 | 30 | 60 | | | |
| Bonferonni t-test for difference in mean between: | | | | | | |
| Cases (Preeclampsia) x Healthy Controls <0.05 | | | | | | |

Genetic Study:

DNA Amplification: The products of successful binding between the extracted DNA and

specific primers for IL-1 Beta gene were detected by gel electrophoresis analysis using

DNA marker (100 bp DNA ladder) and the products size was 518bp for both patients and control groups.

Detection of *IL-1 Beta gene* **Polymorphism:** The distribution of *IL-1Beta -511* polymorphism was detected by PCR-RFLP technique, at this locus there're three genotype; homozygote (CC) at 403bp, and 115bp, heterozygous (TC) at 518bp and 403bp, 115bp and wild type (TT) which still undigested 518bp, Figure (3-5), the genotype distribution had no deviation from Hardy-Weinberg equilibrium in all study groups and agree with the reports of (3). Figure (1)



M TT TC CC

Figure (1): Agarose Gel Electrophoresis Image that Show the RFLP-PCR Product Analysis of *IL-1Beta* Gene by Using Alu I Restriction Enzyme from Some Blood Patient Samples and Healthy Control Sample . Where M: Marker (2000-50bp), Patient Samples as Homozygote (CC) at 403bp, 115bp. Patient Samples as Heterozygous (TC)

at 518bp and 403bp, and 115bp. Patient and Control that Appeared as Wild Type (TT) which Still Undigested.

As shown in **table (5)**, IL-1 Beta genotyping in PE pregnant women showed the following results TT genotype expressed (50%) of cases having 2.33 fold risk of having PE with P value of (0.04) and with etiological factor (0.57) while TC mutant genotype expressed (20%) and showed 1.125 fold risk of having PE with P value of (0.006) and etiological factor of (0.111), while homozygous CC genotype expressed (30%) and considered as protective genotype with P value of (<0.001) and protective factor of (0.589).

 Table (5): Distribution of Genotypes and Alleles of *IL-1 Beta Gene* in Case &

 Control.

| | PE Cases No.(%) | Healthy Control No.(%) | OR | Inverse OR | 95% CI OR | P Value | Adjusted P | EF | PF |
|---------------------|--------------------|------------------------------|-------|------------|-----------|---------|------------|-------|-------|
| IL-1beta (-511)Pol | ymorphism | | | | | | | | |
| TT | 30(50) | 18(30) | 2.3 | 0.4 | 1.1 -4.9 | 0.04 | 0.04 | 0.57 | *** |
| TC | 12(20) | 26(43.3) | 1.1 | 0.9 | 1.4-6.9 | 0.006 | 0.006 | 0.111 | *** |
| CC | 18(30) | 16(26.7) | 0.326 | 3.1 | 0.4-1.9 | < 0.001 | <0.001 | *** | 0.674 |
| Total | 60(100) | 60(100) | | | | | | | |
| Allele frequency (I | [L-1beta -511) | | | | | | | | |
| T-allele | 72(60) | 62(51.7) | 2.4 | 0.4 | 0.8 -2.3 | 0.001 | 0.001 | 0.287 | *** |
| C-allele | 48(40) | 58(48.3) | 0.4 | 2.4 | 0.4 - 1.2 | 0.001 | 0.001 | *** | 0.589 |
| Total | 120(100) | 120(100) | | | | | | | |

Discussion

Interlukine-1beta is located on Chromosome 2q13-21 at location 70-110kb, which include 7 exon and 6 intron (25). IL-1 β is a pro-inflammatory cytokine, secreted by monocytes, macrophages and epithelial cells, which implicates in a variety of activities (9, 22). IL-1 β has previously been found to increase the production of TNF-alpha, IL-6, HCG, which were proved to be associated with the development of PE (9, 22). IL-1 β may also involve in the oxidative stress linked with PE by stimulating the secretion of other lymphocytotropic cytokines and catabolic enzymes (3). In addition, IL-1 β has been considered to be a potential mediator of maternal endothelial dysfunction in PE (23). Beyond that, it has been shown that IL-1 β played an important role in abnormal extrarillous trophoblast invasion in PE (24). The positive association of *IL-1\beta -511* polymorphism with preeclampsia which is established against preeclamptic pregnant women and normotensive pregnant women as control group indicating that the T-511C polymorphism carry a significant risk for preeclampsia. There must be other risk factors or complementary mechanisms for the risky genes to operate and cause preeclampsia among preeclampsia cases.

The *IL-1* β -511 genotypes were assessed for their role in predicting the risk of having preeclampsia, compared with healthy control group. **Table 5** showed the *IL-1* β - 511 genotypes, had significant predictive power. The T allele had the strongest association P=0.001 and significantly increases the risk of having preeclampsia by 2.4 times compared to normotensive pregnant women. In the other hand C allele had significant association and considered as protective allele among Preeclamptic pregnant women (40%, OR= 0.4). Both the heterozygous TC and the wild type TT genotypes increase the risk of the disease by 1.1times, p=0.006 and 2.3 times (P =0.04), respectively. While the homozygous CC genotype showed a statistically significant protective effect. Occurrence reduces the risk of having preeclampsia by 3.1 times .The diagnostic criteria for preeclampsia that studied in present study were the same as previous studies investigating for the role of IL-1 β gene polymorphisms in the pathogenesis of PE (3,19).

These results were highly comparable with a study conducted by **Wang** *et al.*, which investigated the relationship between the -511T/C polymorphisms of the IL-1 β and PE in Chinese population. It was worthwhile to note that the polymorphisms in the promoter region of the IL-1 β was associated with the genesis of the PE (P<0.05). After calculation of the value of OR, the pregnant women bearing the TT genotype of the -511

T/C polymorphism of the IL-1 β was more susceptibility of experiencing the PE (X² = 9.479, P =0.002, OR= 1.716, 95%CI =1.215–2.424). As for allele, the -511 T allele was observed to be associated with a high risk of the PE, in the similar manner, also found evidences that the polymorphisms at the position -511 in the promoter region of the IL-1 β were associated with PE. The IL-1 β promote genotype TC were associated with the development of PE. Polymorphic analysis data of -511C/T in IL-1b studied in **Wang** *et al.*, ,not only the genotypic distribution but also the allele frequency did show a significantly statistical difference among case and control groups (for genotypic distribution X² = 9.687, P = 0.008; for allele frequency X² = 8.557, P= 0.003).The subjects carrying the TT genotype had 1.716 fold risk of the PE compared with the women with TC and CC genotypes (X² = 9.479, P =0.002, OR= 1.716, 95%CI = 1.215–2.424). The results of allele also showed that the T allele might be a risk factor of the PE (X² = 8.557, P= 0.003, OR= 1.4, 95%CI = 1.117–1.754) (3).

Kang *et al.*, revealed that there was no any role of the polymorphism of the IL-1 β - 511T/C in the pathogenesis of PE, among Taiwanese Population and also conducted that the homozygotes (T/T) could not be observed in both severe preeclampsia and control groups and the allele frequency of T alleles was only 2.1% in women with severe preeclampsia group and 1.7% in normal controls (19). Moreover, another two experiments also found the similar phenomenon respectively in Hispanic women (17) and in Holland population (20). Which were totally disagreed with the present study results. Because the ethnic difference might play an important role in disease-gene association studies, we cannot adopt the conclusion from other countries without our own studies in the Iraqi's pregnant women, while in the present study the bulk of cases in the TT

genotype in preeclamptic cases. The reason for the present results could be the complex interactions among genetic and environmental factors (18).

Nasr *et al.*, have found evidences that the polymorphism at the position -511 in the promoter region of the IL-1b was not associated with PE in Egyptian population. Concerning -511T/C gene polymorphisms,there was statistically significant difference between PE cases and healthy control studied regarding TT genotype (p value = 0.0196), but there were no statistically significant differences between PE cases and healthy control regarding CC or TC genotypes (p value = 0.3304 and 0.2660 respectively). which have disagreed with the present study results(21).

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