RESEARCH PAPER

Physiological predictions and the role of IL-10 -819 promoter polymorphism in preeclampsia

Shno Sabah Tahir[1,3] and Mudhir Sabir Shekha [1,2,3]

1 Department of Biology, College of Science, Salahaddin University-Erbil, 44001 Erbil, Kurdistan-Region, Iraq
2 Department of Pathological Analysis, College of Science, Knowledge University, Erbil 074016, Kurdistan-Region of Iraq, Iraq
3 Research Centre, Salahaddin University-Erbil, 44001 Erbil, Kurdistan-Region, Iraq

A B S T R A C T:

Preeclampsia is a multisystem pregnancy-specific syndrome that affects some of pregnancies, which remains a leading cause of maternal and perinatal morbidity and mortality worldwide. Preeclampsia primarily provokes life-threatening, especially to the fetus. The aim of this study was to estimate some physiological and biochemical markers of preeclampsia and investigate the association between IL-10 -819 polymorphism and preeclampsia.

A total of 52 pregnant women with preeclampsia and 35 women with normal pregnancy attended the high-risk unit of Erbil Maternity and Pediatric Governmental Hospital, KRG, Iraq, were considered in the present study. During the regular pregnancy check-ups, blood pressure, occurrence of gestational hypertension (early or late onset), preeclampsia, were also documented. Remarkably, serum Urea, Creatinine, Alkaline Phosphatase (ALP) and Mean Platelet Volume (MPV) were significantly increased in preeclampsia patients. In contrast, Direct Bilirubin, Glucose, GPT, GOT and Platelet count were not significant between preeclampsia and control women. Additionally, Mean Arterial Pressure (MAP) and Systolic Blood Pressure (SBP) were significantly elevated. Furthermore, genotyping of IL-10 T-819 C promoter polymorphism was carried out for all participants using a standard Amplification Refractory Mutation System (ARMS) PCR. Genotypic distribution of the control and patient groups were compared with values predicted by Hardy-Weinberg equilibrium using χ^2 test. Interestingly, there were significant differences in (IL)-10 (-819) T/C genotype distribution frequency between preeclampsia and control groups. **Conclusion** The present study suggests that the IL-10 T-819 C gene promoter polymorphism might be a major genetic regulator in the etiology of increased risk of preeclampsia.

INTRODUCTION:

Preeclampsia is the most commonly confronted medical complication of pregnancy, which has an adverse impact on 3% to 5% of all pregnancies (Wang et al., 2002). It is characterized by a complex hypertensive disorder ($\geq$140/$\geq$90 mmHg) along with proteinuria ($\geq$0.3 g/24 h), which leads to maternal and fetal mortality/ morbidity. Preeclampsia takes place after 20 weeks of gestation as far as 6 weeks postpartum, also leads to perinatal death, preterm birth, intrauterine growth restriction (IUGR) and in a number of cases, intrauterine death (IUD) of the fetus (Salimi et al., 2014, Tannetta et al., 2013).

Preeclampsia is a multisystem disorder that may trigger eclampsia, renal failure, pulmonary edema, stroke and death. The first preclinical phase comprises deficient remodeling of the uteroplacental circulation during the 8th–18th week of gestation, results in dysfunctional perfusion and placental oxidative stress. The second clinical phase, which initiates after the 20th week, includes systemic vascular inflammation. This has been shown to be an extension of a broader maternal systemic inflammatory response intrinsic to normal pregnancies, but more severe in preeclampsia, including endothelial dysfunction, clotting, and complement disturbances (Tannetta et al., 2013). Despite the thorough studies, the underlying pathophysiology of preeclampsia is still indistinct (Wang et al., 2002).

* **Corresponding Author:**
Shno Sabah Tahir
 E-mail: mudhir.shekha@su.edu.krd

Previous studies have shown the role of various cytokines in defective placental invasion and endothelial damage in preeclampsia (Udenze et al., 2015). Wegmann *et al.* have reported that T-helper type 2 (Th2) cell is responsible for a successful pregnancy, nevertheless the survival rate of the fetus is secured by the inhibition of T-helper type 1 (Th1) cell responses. Therefore, a Th1/Th2 ratio is required for a decent placentation. Th1 cells produce proinflammatory cytokines such as interleukin (IL)-2, interferon (IFN)-γ and tumour necrosis factor (TNF)-α that are engaged in cell-mediated responses and delayed type hypersensitivity reactions. Conversely, the anti-inflammatory cytokines such as IL-4, IL-5, IL-10 and IL-13 are generated by Th2 cells that elicitate humoral immunity (Mosmann and Sad, 1996). Th1 and Th2 coordinate their functions in a regular way to generate a balance in the immune system (Liberman et al., 2003, Matsuzaki et al., 2005). Interleukin-10 (IL-10) is a potent pleiotropic cytokine, plays a crucial role in Th2 immunity, which is located on human chromosome 1 (1q31–1q32) (Eskdale et al., 1997, Kim et al., 1992). IL-10 plays a vital role in maintaining equilibrium of anti-inflammatory and proinflammatory setting at the fetal-maternal interface (Kalkunte et al., 2011). Numerous single nucleotide polymorphisms (SNPs) positioned in the IL-10 both of proximal (-1082A/G, -819T/C, and -592A/C) and distal regions of promoter control the transcriptional rate of propagating IL-10 (Eskdale et al., 1998, D'Alfonso et al., 2000, Mörmann et al., 2004).

Genotypic modifications in the human IL-10 promoter justify remarkable inter-individual differences in IL-10 production and may conduct individual susceptibility to autoimmune diseases. Sowmya *et al.* have suggested that IL-10 T-819 C gene promoter polymorphism can be a major genetic regulator in the etiology of preeclampsia (Sowmya et al., 2014a). The T/C polymorphism at position -819 has been related to high/low IL-10 production rank. With this in mind, the current study is designed to assess the role of IL-10 (-819 T/C) gene promoter polymorphism in the etiology of preeclampsia.

Materials and methods

Selection of cases and controls

A total of 52 pregnant women with early-onset preeclampsia and 35 of age-matched women with normal pregnancy attended the high-risk unit of Erbil Maternity and Pediatric Governmental Hospital were considered in the present study during the year 2019. Women with no complications throughout their gestational period, such as infections, fetal anomalies, hypertension and diabetes were considered as the control subjects. Information regarding the demographic features such as age, parity, systolic blood pressure, diastolic blood pressure, smoking status, gestational age, family history and consanguinity, etc. were obtained from all the subjects with the help of a standard structured questionnaire. The study was approved by the general director of Health.

Criteria for patients

Inclusion criteria A case was defined as follows: preeclampsia was diagnosed with minimum criteria of blood pressure >130/90 mm Hg on two occasions, 6 h apart, and onset of proteinuria >2 + by dipstick test in urine samples, and those who showed blood pressure >150/100 mm Hg and proteinuria >3 + by dipstick test in urine samples were considered to be patients with severe pre-eclampsia.
Exclusion criteria. . Patients with a previous history of intrauterine fetal deaths and other complications were not considered for the study.

Criteria for controls

Inclusion criteria The inclusion criteria were pregnant women with a gestational age of more than 20 weeks, normal blood pressure, normal fetal growth and with no other physiological abnormalities. Controls were selected randomly at the same time as the case selection. The controls were administered the same questionnaire.

Exclusion criteria. Pregnant women with heart problems, with previous history of eclampsia or blood pressure were not included, as per the normal standard, in the study.

Sample collection

Five milliliters of the venous blood was collected from all the subjects for biochemical and molecular analysis and aliquoted in plain and EDTA vacutainers. Serum and plasma was separated after centrifugation at 1,500 rpm for 10 min. All the samples were stored at -20 °C for further analysis.

Determination of IL-10 Polymorphism (DNA extraction and genotyping)

The genomic DNA was extracted from blood according to the protocol of Primeprep Genomic DNA extraction Kit/Korea. Twenty microliter of proteinase K was added to 1.5ml tube then 200μL of whole blood sample mixed with it. Two hundred microliter of GB buffer was added and mixed well by vortex. The samples were incubated at 56C° around 10 minutes. After incubation 200μL of Absolute ethanol was added with well pulse vortexing. Then transfer the lysate to the spin column. The samples were centrifuged at 10,000 rpm for 1 minute. Five hundred microliter of GW1 buffer was added then centrifuged at 10,000 rpm for 1 minute. The flow through was discarded and transfer the spin column to new collection tube. The excess ethanol was removes by more centrifugation at 12,000 rpm for 1 to 2 minutes. Two hundred microliter of GE buffer was added and incubate at room temperature for 1 minute.Eventually DNA was eluted by centrifugation 10,000 rpm for 1 minute. The extracted DNA samples were stored at -20C° (Sowmya et al., 2014). The isolated DNA was subjected to a standard amplification refractory mutation system polymerase chain reaction (ARMS-PCR) (Perrey et al., 1999). Briefly, two complementary reactions were established for each allele consisting of target DNA: allele-specific ARMS primers and a common primer(CR). A 223-bp region in the IL-10 gene promoter was targeted for amplification. The primers used are as follows: common reverse AGG ATG TGT TCC AGG CTC CT; C forward CCC TTG TACAGG TGA TGT AAC; and T forward ACC CTT GTA CAG GTG ATG TAA T. The optimized reaction conditions for the amplification was performed in 10 μl with 25–50 ng of DNA sample, 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 2 μM of each specific/common primers, and 0.25 units of Taq DNA polymerase. The cycling conditions were as follows: an initial denaturation at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, 64.2 °C for 50 s, and 72 °C for 1 min 30 s. The final extension step was at 72 °C for 5 min. The PCR products were separated by electrophoresis on an agarose gel (2 %) stained with ethidium bromide. The gel was visualized under ultraviolet light with a 100-bp ladder. Results were crosschecked with internal positive (410 bp of β globin gene) and negative controls (Millipore water). Ten percent of the samples were randomly taken, and the assay was repeated, and no bias in the genotyping was found. The findings were similar on a replicative study with the results being 100 % concordant.

Statistical analysis

Genotype distribution in the control and case groups were compared with values predicted by Hardy-Weinberg equilibrium using χ2 test. Discrete variables were expressed as counts (%) and were compared by the χ2 test. Odd ratios (OR) and their 95% confidence intervals were used to measure the strength of association between IL-10 gene polymorphism and preeclampsia. Data of the physiological and biochemical were described as percentages and mean± SD (standard deviation) for parametric. Level of significance between preeclampsia and normal women was analyzed using independent t-test

Results

Demographics between preeclampsia and control groups

In total 87 patients' data was analyzed. The mean age was approximately similar between the two groups, being 26.6±6.93 for control group and 30.14±7.48 for Preeclampsia group. There were no significant differences in age between preeclampsia patient and control groups.

The mean of diastolic blood pressure in the preeclamptic women was (104.4±13.0 mmHg); while in the control group was (79.17±12.01 mmHg); with P.value (<0.0001). The mean of systolic blood pressure in the preeclamptic women was (166.3±20.08 mmHg); while in the control group was (116.7±28.75 mmHg); with P.value (<0.0001). The mean of arterial pressure in the women with pre-eclampsia was (123.3±18.49 mmHg) versus (91.67±17.35 mmHg) in the women with normal pregnancy with very high significant difference P. Value (0.0004) (P < 0.001; Table 1.

The mean of serum urea in the women with pre-eclampsia was (32.52±7.883 mg/dl) versus (19±11.11 mg/dl) in the women with normal pregnancy with very high significant difference. Serum creatinine in the women with pre-eclampsia was (0.676±0.199 mg/dl) versus

(0.524±0.134 mg/dl) in the women with normal pregnancy with P.value (0.0405) Table 1.

The mean of serum ALP in the women with pre-eclampsia was (331.6±67.75 mg/dl) versus (249.1±19.95mg/dl) in the women with normal pregnancy with P.value (0.047) Table 1.

The mean of S. Glucose in the women with pre-eclampsia was (0.247±0.190 g/dl) versus (84.16±19.58 g/dl) in the women with normal pregnancy with no significant difference. The mean of S. Direct Bilirubin in the women with pre-eclampsia was (0.247±0.190) versus (0.170±0.112) in the women with normal pregnancy with no significant difference. Table 1.

The mean of S. GOT and GPT in the women with pre-eclampsia were (14.78±9.266) and (21.05±7.829) versus (10.96±1.686 and 20.02±3.345) in the women with normal pregnancy with no significant difference respectively as shown in Table 1.

The mean of PLTs in the women with pre-eclampsia was (233.9±53.33 X 10^9/l) versus (210.7±78.76 X 10^9/l) in the women with normal pregnancy; with no significant difference. The mean of MPV in the women with pre-eclampsia was (8.981± 0.9537) versus (8.981± 0.9537) in the women with normal pregnancy; with P. value (0.0365), as seen in Table 1.

Furthermore, the allele and genotype frequencies of the IL-10 promoter polymorphisms at positions −819 T/C were detected by T-ARMS-PCR method and were compared between preeclampsia cases and healthy control women. The data reported in Table 3 illustrate allele and genotype distributions of different promoter polymorphisms of IL-10.

The frequencies of the genotypes in Co-dominant CC, CT, and TT were 41.18, 37.25, and 21.57 % in women with preeclampsia and 22.86, 28.57 and 48.57 % in control subjects, respectively. The frequencies of the genotypes in dominant were 41.18 and 58.82 % in women with preeclampsia and 22.86 and 77.14 % in control subjects, respectively. The IL-10 −819 T/C recessive genotype distribution frequencies were 62.07 and 37.93% in women with preeclampsia and 70.18 and 29.82 % in control groups, respectively. The IL-10 −819 T/C over-dominant genotype distribution frequencies were 62.75 and 37.25 % in women with preeclampsia and 71.43 and 28.57 % in control groups, respectively while the distribution frequencies of interleukin (IL)-10 (-819) T/C alleles were 57.75 and 42.25 % in women with preeclampsia patients and 48.72 and 51.28 % in control groups.

There were significant differences in (IL)-10 (-819) T/C genotype distribution frequency between preeclampsia and control groups. There were statistical differences in the distribution of genotypic frequencies between the patient and control groups when compared with different models: over-dominant model: CT *vs.* CC+TT (OR=1.484, 95 % CI= 0.5646-3.712, *P*= 0.4027) and recessive model: TT *vs.* CT+CC (OR= 1.438, 95 % CI= 0.5426-3.577, *P*= 0.4482). In contrast, there were no significant differences in the distribution of genotypic frequencies between the preeclampsia and control groups when compared with different models: codominant model: CC *vs.* TT (OR=0.2465, 95 % CI=0.087-0.71) and CC *vs.* CT (OR=0.724, 95 % CI=0.2582-2.262); and dominant model: CC *vs.* CT+TT (OR=0.4233, 95 % CI=0.1589-1.158).

Regarding to allele frequency of the IL-10-1082 T/C allele between preeclampsia and healthy control groups (X^2= 0.8276, P= 0.3630) there was no statistical difference in allele frequency of IL-10-819 C/T and it was not associated with preeclampsia patients.

Genotypes expressed as CC (C) and TT (T) in the homozygote alleles while TC in the heterozygous allele. Using the two pairs primers to the homozygous CC and TT the genotypes and the heterozygote genotype, a single band of 233 bp was produced.

Table 1. Demographic features in women with preeclampsia during pregnancy and women with normal pregnancy

Parameters	Control n(35)	Preeclampsia n(52)	P-Value
Mean age(years)	26.6±6.93	30.14±7.48	NS
SBP (mmHg)	116.7±28.75	166.3±20.08	<0.0001 ***
DBP (mmHg)	79.17±12.01	104.4±13.0	<0.0001 ***
MAP (mmHg	91.67±17.35	123.3±18.49	0.0004 ***

Table 2. Biochemical and hematological characteristics in controls and patients

Parameters	Control n(35)	Preeclampsia n(52)	t-test	P-Value
S. Urea	19±11.11	32.52±7.883	t=3.703	0.0007 ***
S. Creatinine	0.524±0.134	0.676±0.199	t=2.125	0.0405 *
S. ALP	249.1±19.95	331.6±67.75	t=2.072	0.047 *
S. Direct Bilirubin	0.170±0.112	0.247±0.190	t=0.5748	0.570
S. Glucose	84.16±19.58	93.88±22.54	t=1.177	0.2463
S. GPT	10.96±1.686	14.78±9.266	t=0.9048,	0.3746
S. GOT	20.02±3.345	21.05±7.829	t=0.2863	0.7765
Platelet count	210.7±78.76	233.9±53.33	t=1.281	0.2057
MPV	8.981± 0.9537	9.605± 0.9839	t=2.147	0.0365*

Table 3. Distribution Frequencies of interleukin (IL)-10 (-819) Genotypes and Alleles in control and patients

Genotypes and alleles	Control group n (%)	Patient group n (%)	OR (95% CI)	X^2-test P-value
Co-dominant				
C/C	8 (22.86%)	21 (41.18%)		X^2=7.178
C/T	10 (28.57%)	19 (37.25%)	0.724 (0.2582-2.262)	
T/T	17(48.57%)	11 (21.57%)	0.2465 (0.087-0.71)	P=0.0276*
Dominant				
C/C	8 (22.86%)	21 (41.18%)		X^2=3.117
C/T + T/T	27 (77.14%)	30 (58.82%)	0.4233 (0.1589-1.158)	P=0.0775
Recessive				
C/C+ C/T	40 (70.18%)	18 (62.07%)		X^2= 0.5752
T/T	17 (29.82%)	11 (37.93%)	1.438 (0.5426-3.577)	P= 0.4482
Over-dominant				
C/C+ T/T	25 (71.43%)	32 (62.75%)		X^2= 0.7002
C/T	10 (28.57%)	19 (37.25%)	1.484 (0.5646-3.712)	P= 0.4027
Alleles				
C	19 (48.72%)	41 (57.75%)	0.6951 (0.3061-1.554)	X^2= 0.8276
T	20 (51.28%)	30 (42.25%)		P= 0.3630

*P value < 0·05. OR = odds ratio; CI = confidence interval.

Discussion

Preeclampsia is the most widespread pregnancy specific complication that yet situates as one of the considerable obstetric disorders. Preeclampsia is a placenta- dependent pregnancy disorder. Preeclampsia disease is characterized as exaggerated response to maternal inflammation, maybe destine against foreign fetal antigens that induce a series proceedings including: defection in the spiral artery remodeling, placental infarction and release of pro-inflammatory cytokines, invasion of surface trophoblast, and placental fragments in the systemic circulation (Gupte and Wagh, 2014, Neiger, 2017, Sabnavis et al., 2013).

Present research was assumed to estimate some physiological and biochemical parameters in pre-eclampsia.

Our observations are in agreement with the work of (Macdonald-Wallis et al., 2012) they present that increased SBP, DBP and MAP in patients with pre-eclampsia. Preeclampsia causes an increment in peripheral vascular resistance and vasoconstriction (Roberts et al., 1991, Schobel et al., 1996a). An increase in the activity of

sympathetic vasoconstrictor has been demonstrated with measurements of muscle sympathetic nerve tone, which may lead to endothelial dysfunction (Greenwood et al., 2003, Savvidou et al., 2003, Schobel et al., 1996b). Endothelial dysfunction that is known to occur in preeclampsia as this is an important step in the development of atherosclerosis in patients with chronic hypertension (Cipolla, 2007).

Creatinine, urea and uric acid are non-protein nitrogenous metabolites that are cleared from the body by the kidney following glomerular filtration. Measurements of plasma or serum concentration of these metabolites are commonly used as indicators of kidney function and other conditions (Gowda et al., 2010). Therefore, their determination in serum during pregnancy is of a major importance to diagnose kidney function especially in women with preeclampsia signs (Müller-Deile and Schiffer, 2014, Tangren et al., 2018). This would be used to evaluate kidney function as well as the possibility of a secondary source of urea or of the nitrogen part of urea

increase (Blood urea nitrogen) in plasma (Weiner et al., 2015).

In the present study, the serum urea and creatinine levels were higher in pre-eclamptic when compared to the normotensives. Our observation was similar to the previous study by Hassan et al and Vyakaranm et al as they describe raised blood urea and creatinine during pregnancy as a known feature of pre-eclampsia (Hassan et al., 1991, Vyakaranam et al., 2015).

One of the most commonly accepted explanations of elevated Serum Urea and Creatinine has been thought to be due to increase reabsorption and decrease excretion in proximal tubules, similar to the physiologic response to hypovolemia.

In the present study, the serum ALP was significantly higher in preeclampsia, which was similar to those seen in other studies (Dabare et al., 1999, Okesina et al., 1995, Rajagambeeram et al., 2014).

Alkaline phosphatase is an important enzyme involved in the transport of sugar and phosphate across the trophoblast cell membranes (She et al., 2000). The elevated levels of serum ALP in preeclampsia may be attributed to placental dysfunction, which results in increased serum levels of this enzyme. Shedding of syncytiotrophoblast into the maternal circulation is a normal part of pregnancy, but is increased during pre-eclampsia. In pre-eclampsia, this process of syncytio-trophoblast renewal is overactive and complicated by necrosis and apo necrosis of the syncytio-trophoblast particles (Hutchinson et al., 2009)

Bilirubin comes from haemoglobin (Hgb) as red blood cells (RBCs) breakdown either through physiological regeneration at the end of normal lifespan or as a consequence of pathologic hemolysis. Hgb releases heme and is converted inside macrophages to biliverdin, which is then converted into unconjugated bilirubin (indirect bilirubin) that travels through the liver, where, it combines with glucuronic acid to form conjugated bilirubin (also known as bilirubin diglucuronide or direct bilirubin (Odhiambo et al., 2015). In relation to direct bilirubin level, the result was confirmed that direct bilirubin level in preeclamptic pregnant women had not obvious difference compared to normotensive pregnant women our results are compatible with results of

(Hassanpour and Karami, 2018, Kasraeian et al., 2018)

In this study, there were a non-significant increase in serum AST and ALT concentration in preeclampsia compared to normal pregnant women. There is no agreement on the effect of preeclampsia on serum AST and ALT. In a few studies, AST and/ or ALT levels slightly increase in the third trimester (Mutua et al., 2018). However, in most studies, AST and ALT levels remain within the normal range for non-pregnant state (Westbrook et al., 2016).

Mean platelet volume is a routinely measured marker of platelet size, with established predictive value in a variety of cardiovascular disorders (Choi et al., 2016). The present research demonstrated that preeclampsia is associated with a significant increase of MPV. Our results are in consistent with study of (Monteith et al., 2018) they detailed that there is a significant increase in MPV at time of diagnosis preeclampsia therefore the mean platelet volume can represents a promising biomarker for the detection and follow-up of patients that develop preeclampsia (Bellos et al., 2018).

The results of the data analysis showed platelet count non significantly changed in preeclampsia comparing with control. Our data are compatible with work of (Bellos et al., 2018) and incompatible with experiment of (Neiger et al., 1992) their findings suggest the existence of subclinical thrombocytopenia in preeclamptic women whose platelet values are within normal range.

The mean of S. Glucose in the women with pre-eclampsia was not significant compared to women with normal pregnancy. The current results are compatible with previous study that has shown no significant alteration in fasting blood glucose between preeclampsia and control (Babu et al., 2014).

The IL-10 gene promoter is highly polymorphic with multiple single nucleotide polymorphic sites. The three most important ones are -819 (C/T), -1082 (A/G), and -592 (C/A) (Jiang et al., 2015). In the present study (our case – control study), established that the genotype distribution of the IL-10 gene promoter -819 (C/T) to pregnant women with preeclampsia in of Erbil Maternity and Pediatric Governmental Hospital is reliable with the results of a previous study

(Perrey et al., 1999) Therefore, this gene might conceivably be a candidate susceptibility gene in pre-eclampsia (Sowmya et al., 2014b).

According to the result of our study, as shown in figure 1. promoter gene -819 (C/T) polymorphism represented a statistically significant positive association with pre-eclampsia. The polymorphic CT-genotype and TT frequencies as an over-dominant and recessive model were significantly greater while polymorphic CC-genotype frequency was not significant in pregnant women with preeclampsia compared to control women. These positive associations of over-dominant and recessive models indicate that this C>T polymorphisms might increase the predisposition possibility of preeclampsia women. Our results agree with previous reports which correlate women with CT genotype are at high risk to develop preeclampsia in pregnant women. The conflicting results from different populations were reported. Our observation is in concordance with the study by Sowmya et al (Sowmya et al., 2014a) in an Indian population, which revealed high association with the promoter gene -819 (C/T) polymorphism of preeclampsia in pregnant women to high production of IL-10.

Additionally, our data showed that the promoter -819 locus CC genotype frequency and the polymorphic CT genotype and TT frequencies were statistically not significant association with pre-eclampsia under condition of co-dominant, dominant and alleles C and T models. On the other hand, Kamali-Sarvestani et al. have demonstrated that the genotype of IL-10 promoter genotype and allele frequency in an Iranian population has no significant difference of the polymorphism between the patient and the control group (Kamali et al., 2007). Likewise, some studies have investigated the correlation of promoter polymorphisms with the circulating levels and placental levels of IL-10. Moreover, have shown that the genotype of IL-10 promoter may not play a significant role in the circulating of IL-10 levels, but has an effect on the placental levels of IL-10, suggesting that IL-10 plays an important role in proper placentation (Makris et al., 2006, Sowmya et al., 2014b).

Conclusion

The present research suggests that the IL-10 T-819 C gene promoter polymorphism can be a major genetic regulator in the etiology of increased risk of preeclampsia.
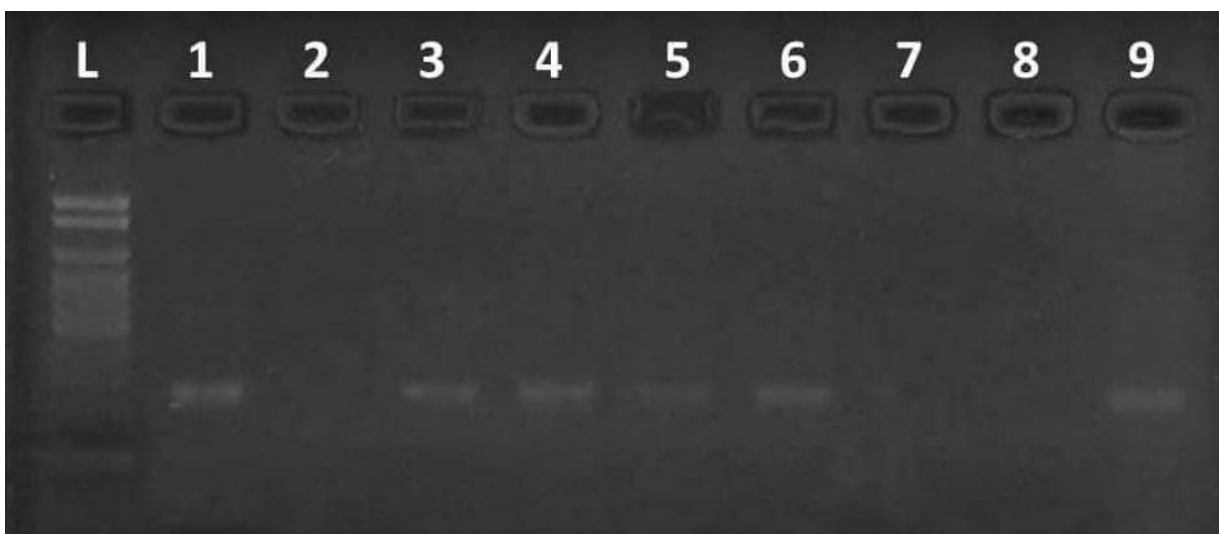
Acknowledgment

Figure 1: Genotyping of interleukin 10 promoter region -819 locus. L:DNA Ladder of 1kb, Lane: 1,3,4, homozygous C/C; Lane:2 Patient sample not amplified with allele C primer; Lane :5,6, homozygous T/T, Lane:7-8 Preclampsia samples not amplified with allele T primer; Lane:9,heterozygous C/T.

Footnotes

Disclosure: No conflict of interest is reported at the current time.

References

BABU, M. S., BOBBY, Z., HABEEBULAH, S., SRILATHA, K., LALITHA, V. & NIRANJAN, G. K. 2014. Fasting insulin levels , lipid profile and proteinuria an index of cardiovascular risk in euglycemic preeclampsia. *Conference Proceedings*.

BELLOS, I., FITROU, G., PERGIALIOTIS, V., PAPANTONIOU, N. & DASKALAKIS, G. 2018. Mean platelet volume values in preeclampsia: A systematic review and meta-analysis. *Pregnancy Hypertens,* 13**,** 174-180.

CHOI, D.-H., KANG, S.-H. & SONG, H. 2016. Mean platelet volume: a potential biomarker of the risk and prognosis of heart disease. *The Korean journal of internal medicine,* 31**,** 1009-1017.

CIPOLLA, M. J. 2007. Cerebrovascular function in pregnancy and eclampsia. *Hypertension,* 50**,** 14-24.

D'ALFONSO, S., RAMPI, M., ROLANDO, V., GIORDANO, M. & MOMIGLIANO-RICHIARDI, P. 2000. New polymorphisms in the IL-10 promoter region. *Genes Immun,* 1**,** 231-3.

DABARE, A., NOURI, A., CANNELL, H., MOSS, T., NIGAM, A. & OLIVER, R. J. U. I. 1999. Profile of placental alkaline phosphatase expression in human malignancies: effect of tumour cell activation on alkaline phosphatase expression. 63**,** 168-174.

ESKDALE, J., GALLAGHER, G., VERWEIJ, C. L., KEIJSERS, V., WESTENDORP, R. G. & HUIZINGA, T. W. 1998. Interleukin 10 secretion in relation to human IL-10 locus haplotypes. *Proc Natl Acad Sci U S A,* 95**,** 9465-70.

ESKDALE, J., KUBE, D., TESCH, H. & GALLAGHER, G. 1997. Mapping of the human IL10 gene and further characterization of the 5' flanking sequence. *Immunogenetics,* 46**,** 120-8.

GOWDA, S., DESAI, P. B., KULKARNI, S. S., HULL, V. V., MATH, A. A. K. & VERNEKAR, S. N. 2010. Markers of renal function tests. *North American journal of medical sciences,* 2**,** 170-173.

GREENWOOD, J. P., SCOTT, E. M., WALKER, J. J., STOKER, J. B. & MARY, D. A. J. A. J. O. H. 2003. The magnitude of sympathetic hyperactivity in pregnancy-induced hypertension and preeclampsia. 16**,** 194-199.

GUPTE, S. & WAGH, G. 2014. Preeclampsia-eclampsia. *J Obstet Gynaecol India,* 64**,** 4-13.

HASSAN, T. J., SADARUDDIN, A. & JAFAREY, S. N. 1991. Serum calcium, urea and uric acid levels in pre-eclampsia. *J Pak Med Assoc,* 41**,** 183-5.

HASSANPOUR, S. & KARAMI, S. 2018. Evaluation of Hepatic Biomarkers in Pregnant Women with Preeclampsia. *Gynecology & Obstetrics,* 08.

HUTCHINSON, E. S., BROWNBILL, P., JONES, N. W., ABRAHAMS, V. M., BAKER, P. N., SIBLEY, C. P. & CROCKER, I. P. 2009. Utero-placental haemodynamics in the pathogenesis of pre-eclampsia. *Placenta,* 30**,** 634-41.

JIANG, X.-H., LIN, K.-X., ZHANG, Y.-X., CHEN, R.-H. & LIU, N. 2015. Correlating interleukin-10 promoter gene polymorphisms with human cerebral infarction onset. *Neural regeneration research,* 10**,** 1809-1813.

KALKUNTE, S., NEVERS, T., NORRIS, W. E. & SHARMA, S. 2011. Vascular IL-10: a protective role in preeclampsia. *J Reprod Immunol,* 88**,** 165-9.

KAMALI, E., KIANY, S., GHARESI-FARD, B. & ROBATI, M. 2007. Association study of IL-10 and IFN-γ gene polymorphisms in Iranian women with preeclampsia. *Journal of reproductive immunology,* 72**,** 118-26.

KASRAEIAN, M., ASADI, N., VAFAEI, H., ZAMANPOUR, T., SHAHRAKI, H. R. & BAZRAFSHAN, K. 2018. Evaluation of serum biomarkers for detection of preeclampsia severity in pregnant women. *Pakistan journal of medical sciences,* 34**,** 869-873.

KIM, J. M., BRANNAN, C. I., COPELAND, N. G., JENKINS, N. A., KHAN, T. A. & MOORE, K. W. 1992. Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. *J Immunol,* 148**,** 3618-23.

LIBERMAN, A. C., REFOJO, D. & ARZT, E. 2003. Cytokine signaling/transcription factor cross-talk in T cell activation and Th1-Th2 differentiation. *Arch Immunol Ther Exp (Warsz),* 51**,** 351-65.

MACDONALD-WALLIS, C., LAWLOR, D. A., FRASER, A., MAY, M., NELSON, S. M. & TILLING, K. 2012. Blood pressure change in normotensive, gestational hypertensive, preeclamptic, and essential hypertensive pregnancies. *Hypertension (Dallas, Tex. : 1979),* 59**,** 1241-1248.

MAKRIS, A., XU, B., YU, B., THORNTON, C. & HENNESSY, A. 2006. Placental deficiency of interleukin-10 (IL-10) in preeclampsia and its relationship to an IL10 promoter polymorphism. *Placenta,* 27**,** 445-51.

MATSUZAKI, J., TSUJI, T., IMAZEKI, I., IKEDA, H. & NISHIMURA, T. 2005. Immunosteroid as a regulator for Th1/Th2 balance: its possible role in autoimmune diseases. *Autoimmunity,* 38**,** 369-75.

MONTEITH, C., EGAN, K., O'CONNOR, H., MAGUIRE, P., KEVANE, B., SZKLANNA, P. B., COOLEY, S., MALONE, F. & AINLE, F. N. 2018. Early onset preeclampsia is associated with an elevated mean platelet volume (MPV) and a greater rise in MPV from time of booking compared with pregnant controls: results of the CAPE study. *J Perinat Med,* 46**,** 1010-1015.

MOSMANN, T. R. & SAD, S. 1996. The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today,* 17**,** 138-46.

MUTUA, D., NJAGI, E. & ORINDA, G. 2018. Liver Function Tests in Normal Pregnant Women. *Journal of Liver,* 07.

MÖRMANN, M., RIETH, H., HUA, T. D., ASSOHOU, C., ROUPELIEVA, M., HU, S. L., KREMSNER, P.

G., LUTY, A. J. & KUBE, D. 2004. Mosaics of gene variations in the Interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used. *Genes Immun,* 5**,** 246-55.

MÜLLER-DEILE, J. & SCHIFFER, M. 2014. Preeclampsia from a renal point of view: Insides into disease models, biomarkers and therapy. *World journal of nephrology,* 3**,** 169-181.

NEIGER, R. 2017. Long-Term Effects of Pregnancy Complications on Maternal Health: A Review. *J Clin Med,* 6.

NEIGER, R., CONTAG, S. A. & COUSTAN, D. R. 1992. Preeclampsia effect on platelet count. *Am J Perinatol,* 9**,** 378-80.

ODHIAMBO, C., OYARO, B., ODIPO, R., OTIENO, F., ALEMNJI, G., WILLIAMSON, J. & ZEH, C. 2015. Evaluation of Locally Established Reference Intervals for Hematology and Biochemistry Parameters in Western Kenya. *PLoS ONE,* 10**,** e0123140.

OKESINA, A., DONALDSON, D., LASCELLES, P., MORRIS, P. J. I. J. O. G. & OBSTETRICS 1995. Effect of gestational age on levels of serum alkaline phosphatase isoenzymes in healthy pregnant women. 48**,** 25-29.

PERREY, C., TURNER, S. J., PRAVICA, V., HOWELL, W. M. & HUTCHINSON, I. V. 1999. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. *Transpl Immunol,* 7**,** 127-8.

RAJAGAMBEERAM, R., ABU RAGHAVAN, S., GHOSH, S., BASU, S., RAMASAMY, R. & MURUGAIYAN, S. B. 2014. Diagnostic utility of heat stable alkaline phosphatase in hypertensive disorders of pregnancy. *Journal of clinical and diagnostic research : JCDR,* 8**,** CC10-CC13.

ROBERTS, J. M., TAYLOR, R. N. & GOLDFIEN, A. 1991. Clinical and biochemical evidence of endothelial cell dysfunction in the pregnancy syndrome preeclampsia. *Am J Hypertens,* 4**,** 700-8.

SABNAVIS, S., RAMAIAH, A., SUNITHA, T., NALLARI, P., JYOTHY, A. & VENKATESHWARI, A. 2013. Evaluation of Interleukin-10 (G-1082A) Promoter Polymorphism in Preeclampsia %J JRI. *Journal of Reproduction & Infertility,* 14**,** 62-67.

SALIMI, S., FARAJIAN-MASHHADI, F., NAGHAVI, A., MOKHTARI, M., SHAHRAKIPOUR, M., SARAVANI, M. & YAGHMAEI, M. 2014. Different profile of serum leptin between early onset and late onset preeclampsia. *Dis Markers,* 2014**,** 628476.

SAVVIDOU, M. D., HINGORANI, A. D., TSIKAS, D., FRÖLICH, J. C., VALLANCE, P. & NICOLAIDES, K. H. J. T. L. 2003. Endothelial dysfunction and raised plasma concentrations of asymmetric dimethylarginine in pregnant women who subsequently develop pre-eclampsia. 361**,** 1511-1517.

SCHOBEL, H. P., FISCHER, T., HEUSZER, K., GEIGER, H. & SCHMIEDER, R. E. 1996a. Preeclampsia -- a state of sympathetic overactivity. *N Engl J Med,* 335**,** 1480-5.

SCHOBEL, H. P., FISCHER, T., HEUSZER, K., GEIGER, H. & SCHMIEDER, R. E. J. N. E. J. O. M. 1996b. Preeclampsia—a state of sympathetic overactivity. 335**,** 1480-1485.

SHE, Q.-B., MUKHERJEE, J. J., HUANG, J.-S., CRILLY, K. S. & KISS, Z. J. F. L. 2000. Growth factor-like effects of placental alkaline phosphatase in human fetus and mouse embryo fibroblasts. 469**,** 163-167.

SOWMYA, S., RAMAIAH, A., SUNITHA, T., NALLARI, P., JYOTHY, A. & VENKATESHWARI, A. 2014a. Role of IL-10 -819(t/c) promoter polymorphism in preeclampsia. *Inflammation,* 37**,** 1022-7.

SOWMYA, S., SRI MANJARI, K., RAMAIAH, A., SUNITHA, T., NALLARI, P., JYOTHY, A. & VENKATESHWARI, A. 2014b. Interleukin 10 gene promoter polymorphisms in women with early-onset pre-eclampsia. *Clin Exp Immunol,* 178**,** 334-41.

TANGREN, J. S., WAN MD ADNAN, W. A. H., POWE, C. E., ECKER, J., BRAMHAM, K., HLADUNEWICH, M. A., ANKERS, E., KARUMANCHI, S. A. & THADHANI, R. 2018. Risk of Preeclampsia and Pregnancy Complications in Women With a History of Acute Kidney Injury. *Hypertension,* 72**,** 451-459.

TANNETTA, D. S., DRAGOVIC, R. A., GARDINER, C., REDMAN, C. W. & SARGENT, I. L. 2013. Characterisation of syncytiotrophoblast vesicles in normal pregnancy and pre-eclampsia: expression of Flt-1 and endoglin. *PLoS One,* 8**,** e56754.

UDENZE, I., AMADI, C., AWOLOLA, N. & MAKWE, C. C. 2015. The role of cytokines as inflammatory mediators in preeclampsia. *Pan Afr Med J,* 20**,** 219.

VYAKARANAM, S., BHONGIR, A. V., PATLOLLA, D., CHINTAPALLY, R. J. I. J. O. M. S. & HEALTH, P. 2015. Study of serum uric acid and creatinine in hypertensive disorders of pregnancy. 4**,** 1424.

WANG, J. X., KNOTTNERUS, A. M., SCHUIT, G., NORMAN, R. J., CHAN, A. & DEKKER, G. A. 2002. Surgically obtained sperm, and risk of gestational hypertension and pre-eclampsia. *Lancet,* 359**,** 673-4.

WEINER, I. D., MITCH, W. E. & SANDS, J. M. 2015. Urea and Ammonia Metabolism and the Control of Renal Nitrogen Excretion. *Clinical journal of the American Society of Nephrology : CJASN,* 10**,** 1444-1458.

WESTBROOK, R. H., DUSHEIKO, G. & WILLIAMSON, C. 2016. Pregnancy and liver disease. *Journal of Hepatology,* 64**,** 933-945.