

Evaluation of Interleukin-10 (G-1082A) Promoter Polymorphism in Preeclampsia

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Abstract

Background: Preeclampsia is a pregnancy-specific syndrome that may be life-threatening, especially to the fetus. Several causes have been reported that may have a possible role in the development of the disorder. Interleukin-10 affect maternal intravascular inflammation, as well as endothelial dysfunction. The aim of this study was to investigate the association between IL-10 G-1082A polymorphism and preeclampsia.

Methods: A total of eighty-eight pregnant women with preeclampsia and 100 women with normal pregnancy attending the Gynecological unit of Government Maternity Hospital, Petlaburz, Hyderabad, India, were considered for the study. A standard amplification refractory mutation system (ARMS) PCR was carried out for genotyping IL-10 G-1082A promoter polymorphism in all the participants. Genotypic distribution of the control and patient groups were compared with values predicted by Hardy-Weinberg equilibrium using χ^2 test. Odd ratios (OR) and their respective 95% confidence intervals were used to measure the strength of association between IL-10 gene polymorphism and preeclampsia.

Results: The frequencies of IL-10 G-1082A genotypes, GG, GA and AA, were 17.8%, 41.09% and 41.09% in women with preeclampsia and 25%, 28% and 47% in the controls respectively. There was no significant difference in the distribution of genotypes and alleles of IL-10 G-1082A between the two groups (Test power=0.66).

Conclusion: The present study suggests that the IL-10 G-1082A gene promoter polymorphism is not a major genetic regulator in the etiology of preeclampsia.

Keywords: ARMS PCR, Cytokines, Interleukin-10, Polymorphism, Preeclampsia.

To cite this article: Sowmya S, Ramaiah A, Sunitha T, Nallari P, Jyothy A, Venkateshwari A. Evaluation of Interleukin-10 (G-1082A) Promoter Polymorphism in Preeclampsia. *J Reprod Infertil.* 2013;14(2):62-66.

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Received: Aug. 6, 2012

Accepted: Nov. 17, 2012

Introduction

Preeclampsia is the most frequently encountered medical complication of pregnancy, causing considerable maternal and fetal morbidity and mortality (1). Preeclampsia affects approximately 5% of all pregnancies and in up to 10% to 20% of nulliparous women (2). Despite intensive studies, the underlying pathophysiology of preeclampsia is still unclear. Placental ischemia caused by the inadequate endometrial invasion of trophoblast and endothelial damage is considered to play a crucial role in the pathogenesis of pre-

eclampsia (3, 4). The failure of communication between trophoblast cells and decidual immune cells in the placenta, results in poor placentation which leads to poor remodeling of spiral arteries, systemic inflammatory response, dysfunctional maternal endothelium, poor placental oxygenation and hypoxic conditions. These events precede the clinically recognizable symptoms of maternal disease, such as hypertension, proteinuria and edema (5).

Interleukin-10 (IL-10) is a potent pleiotropic cy-

tokine, located on chromosome 1q31-32, consisting of five exons and four introns. It has the dual ability of immunosuppression or immunostimulation via the production of pro-inflammatory cytokines by the inhibition of T-helper 1 (Th1) lymphocytes and stimulation of B lymphocytes and Th2 lymphocytes, and thus downregulates the inflammatory response (6). Various single nucleotide polymorphisms (SNPs) have been described in the IL-10 promoter region, both of distal and proximal promoter regions, with functional significance (7). Genotypic variations in the human interleukin-10 (IL-10) promoter accounts for marked inter-individual variations in IL-10 production and may influence individual susceptibility to autoimmune diseases. The G/A polymorphism at position -1082 has been linked to high/low IL-10 production status. In view of the above, we aimed to evaluate the role of IL-10 (-1082 G/A) gene promoter polymorphism in the etiology of preeclampsia.

Methods

Selection of cases and controls: A total of 88 pregnant women with preeclampsia and 100 women with normal pregnancy attending the Gynecological unit of Government Maternity Hospital were considered for the present study during the year 2011-12. Information regarding the demographic features such as age, marital history, parity, gestational age, family history, consanguinity, etc were obtained from all the subjects with the help of a standard proforma questionnaire.

Inclusion criteria: The patients showing blood pressure $>130/90$ mm Hg on two occasions, 6 hr apart, and onset of proteinuria $>2+$ by dip stick test in urine sample were considered as preeclamptic and those who showed blood pressure $>150/100$ mm Hg and proteinuria $>3+$ by dipstick test in urine sample were considered as patients with severe preeclampsia.

Exclusion criteria: Women with no complications throughout their gestational period, like infections, fetal anomalies, hypertension and diabetes were considered as the control subjects. Patients with previous history of intrauterine fetal deaths and other complications were not considered for the study.

Sample collection: The venous blood (5 ml) was collected from all the participants for biochemical and molecular analysis and aliquoted in plain and EDTA vacutainers. Serum and plasma was separated after centrifugation at 1500 rpm for 10 min.

All the samples were stored at -20°C for further analysis.

Determination of IL-10 polymorphism: The genomic DNA was extracted from the samples using salting out method described by Lahiri et al. (8). The isolated DNA was subjected to a standard amplification refractory mutation system polymerase chain reaction (ARMS-PCR) (9). Briefly, two complementary reactions were established for each allele consisting of target DNA: allele specific ARMS primers (FG for G allele and FA for A allele) and the common primer (CF). A 169 bp region in the IL-10 gene promoter was targeted for amplification. The primers used are as follows: a common (CF) anti-sense primer 5'-GTA AGC TTC TGT GGC TGG AGT C-3'; (FG) Sense 5'-AAC ACT ACT AAG GCT TCT TTG GGT G-3' G-primer; (FA) Sense 5'-AAC ACT ACT AAG GCT TCT TTG GGT A-3'.

The optimized reaction conditions consisted of 40 ng of genomic DNA in a reaction volume of 15 μl containing 1X reaction buffer, 1.5 μM MgCl_2 , 30 μM of each dNTP, 0.16 μM of each primer, and 0.3 U of Taq DNA polymerase. The cycling conditions were as follows: an initial denaturation at 94°C for 4 min, followed by 35 cycles at 94°C for 30 s, 61°C for 20 s, and 72°C for 30 s. The final extension step was at 72°C for 6 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. All the collected samples were successfully genotyped and confirmed with DNA sequencing. Results were cross-checked with internal positive (796 bp of HLA gene) and negative controls (Millipore water). Ten percent of the samples were randomly taken, and the assay was repeated and found no bias in the genotyping. The findings were similar on 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 χ^2 test. Discrete variables were expressed as counts (%) and were compared by the χ^2 test. Odds ratios (OR) and their 95% confidence intervals were used to measure the strength of association between IL-10 gene polymorphism and preeclampsia.

Results

A total of 88 pregnant patients with preeclampsia and 100 controls with normal pregnancy were in-

Table 1. Distribution of genotypes and allelic frequencies in patients with preeclampsia and controls

Co-dominant	Cases N (%)	Controls N (%)	χ^2	p-value	OR (95% CI)
GG	13 (17.8)	25 (25)	--	--	--
GA	30 (41.09)	28 (28)	2.183	0.139	0.48 (0.20-1.13)
AA	30 (41.09)	47(47)	0.08	0.77	0.814 (0.3618-1.834)
Dominant					
GG	13 (17.8)	25 (25)	--	--	--
GA + AA	60 (82.18)	75 (75)	0.8883	0.3459	0.65 (0.3067-1.370)
Recessive					
GA + GG	43 (59.7)	53 (53)	--	--	--
AA	30 (41.09)	47 (47)	0.3805	0.537	1.271 (0.690-2.338)
Overdominant					
GG+AA	43 (59.7)	72 (72)	--	--	--
GA	30 (41.09)	28 (28)	2.686	0.1012	0.5574 (0.2943-1.056)
Allele Frequency					
G	56 (38.35)	78 (39)	--	--	--
A	90 (61.6)	122 (61)	0.0009	0.99	0.97 (0.62-1.5)

cluded in this study. The demographic characteristics of patients and controls revealed a significant difference with respect to age ($p=0.0013$), number of children ($p=0.0076$) and blood pressure ($p=0.0001$). However, there was no variation with regard to mode of delivery, fetal growth and edema. Fifty-nine percent of the patients and 14% of the controls showed consanguinity.

The genotypic distribution of -1082 G/A IL-10 polymorphism are illustrated in table 1. The frequencies of the genotypes GG, GA and AA, were 17.8%, 41.09% and 41.09% in patients with preeclampsia, and 25%, 28% and 47% in controls, respectively. There was no statistical difference in the distribution of genotypic or allelic frequencies between the patient and control groups (Test power=0.66) for GG vs. AA (OR=0.814, 95% CI=0.3618–1.834), GG vs. GA+AA (OR=0.65, 95% CI=0.3067–1.370), GA vs. GG+AA (OR=0.5574, 95% CI=0.2943–1.056), AA vs. GA+GG (OR= 1.271, 95% CI=0.690–2.338) and G allele vs. T allele (OR=0.97, 95% CI=0.62–1.5).

Discussion

Preeclampsia is the most common pregnancy-specific complication that still ranks as one of the major obstetric problems. Preeclampsia is a placenta-dependent pregnancy disorder. Preeclampsia syndrome is described as excessive maternal inflammatory responses, perhaps directed against foreign fetal antigens that induce a chain of events including surface trophoblast invasion, defective

spiral artery remodeling, placental infarction and release of pro-inflammatory cytokines and placental fragments in the systemic circulation.

A normal pregnancy is accompanied by an inflammatory response which produces a state of mild systemic inflammation, completed with activation of multi-components of inflammatory network. These inflammatory changes may progress to the point of circulatory decompensation and endothelial dysfunction, resulting in one or more pregnancy complications, including preeclampsia (10, 11).

Current theories regarding the immune response in preeclampsia focus on the phenomenon of T-cell differentiation into two types of helper cells: type 1 (Th1) and type 2 (Th2). T-helper type 1 cells produce pro-inflammatory cytokines and are involved in cell-mediated immunity, while T-helper type 2 cells produce anti-inflammatory, immuno-suppressor cytokines and are involved in B-cell humoral immunity (12). Wegmann et al. (13) first proposed the hypothesis that Th1 responses are suppressed in normal pregnancy to prevent immune rejection of fetus. In preeclampsia an imbalance in the Th1/Th2 ratio has been proposed with deviation towards Th1 response (14, 15). Th1 type cytokines, such as TNF- α , IFN- γ and IL-2, are pro-inflammatory cytokines which induce trophoblastic apoptosis, and restrain differentiation of trophoblast. Th2 type cytokines, such as IL-10, are anti-inflammatory cytokines, and act as autocrine inhibitors of cytotrophoblast invasion

in the uterine wall. Roth and Fisher stated that high concentrations of IL-10 reduces the trophoblast invasion which in turn is a major cause of preeclampsia (16).

Thus, the role of IL-10 in the immune response is to inhibit the production of various pro-inflammatory cytokines produced by a large number of different immune cells (17). The inter-individual differences in IL-10 production are largely under genetic control; 50–75% of the variation can be explained by genetic factors as demonstrated in twin studies (18, 19). It is also plausible that intrauterine cytokine milieu and balance of vascular factors may modulate the effects of hypoxic levels of oxygen. Hypoxia affects the cytokine balance by reducing interleukin IL-10 production and inducing IL-6 and IL-8 in placenta and trophoblast (20). Rees et al. carried out a study on patients with preeclampsia and indicated that the -1082A allele confers a two-fold increase in transcriptional activity of the IL-10 promoter compared to the G allele (21). There was marked inter-individual variation in IL-10 production by peripheral blood mononuclear cells in vitro, with no consistent effect of genotype.

IL-10 promoter SNP genotype frequencies appear to exhibit different distributions according to ethnicity. The present study revealed no association of IL-10 -1082 polymorphism in patients with preeclampsia compared to the controls. A number of studies on IL-10 polymorphism in preeclampsia have revealed conflicting results. Vural et al. (22) in Istanbulite population and Daher et al. (23) in white women demonstrated a significant association of IL-10 genotype and allele frequency between controls and patients. On the contrary, studies in Austrian population by Stonek et al. (24) and in Iranian population by Kamali Sarvestani et al. (25) revealed no association. The work done by Makris et al. (26) in Australian population demonstrated that even though the IL-10 genotype was AA, there was no significant lowering of IL-10 levels in maternal serum but when observed in placental tissue revealed a significant decrease in IL-10 concentration in placenta proving that IL-10 plays an important role in the placental formation. Most of the studies have either shown a significant association with A allele or no association with neither of the alleles. The latest meta-analysis conducted by Xie et al. (27), also concluded no association between IL-10 -1082A/G, and preeclampsia.

Conclusion

In conclusion, the present study suggests that the IL-10 -1082 promoter polymorphism is not a major genetic regulator in the etiology of preeclampsia in the studied ethnic group. However, larger sample size has to be analyzed to confirm the same.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Schobel HP, Fischer T, Heuszer K, Geiger H, Schmieder RE. Preeclampsia -- a state of sympathetic overactivity. *N Engl J Med.* 1996;335(20):1480-5.
2. Rochat RW, Koonin LM, Atrash HK, Jewett JF. Maternal mortality in the United States: report from the Maternal Mortality Collaborative. *Obstet Gynecol.* 1988;72(1):91-7.
3. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. *Science.* 2005;308(5728):1592-4.
4. Roberts JM, Gammill HS. Preeclampsia: recent insights. *Hypertension.* 2005;46(6):1243-9.
5. Redman CW, Sacks GP, Sargent IL. Preeclampsia: an excessive maternal inflammatory response to pregnancy. *Am J Obstet Gynecol.* 1990;180:499-506.
6. Xue H, Lin B, An J, Zhu Y, Huang G. Interleukin-10-819 promoter polymorphism in association with gastric cancer risk. *BMC Cancer.* 2012;12:102.
7. de Jong BA, Westendorp RG, Eskdale J, Uitdehaag BM, Huizinga TW. Frequency of functional interleukin-10 promoter polymorphism is different between relapse-onset and primary progressive multiple sclerosis. *Hum Immunol.* 2002;63(4):281-5.
8. Lahiri DK, Nurnberger JI Jr. A rapid non-enzymatic method for the preparation of HMW DNA from blood for RFLP studies. *Nucleic Acids Res.* 1991;19(19):5444.
9. Perrey C, Turner SJ, Pravica V, Howell WM, Hutchinson IV. ARMS-PCR methodologies to determine IL-10, TNF-alpha, TNF-beta and TGF-beta 1 gene polymorphisms. *Transpl Immunol.* 1999;7(2):127-8.
10. Celik H, Avi B, Alper T. Comparison of maternal serum levels of interleukin-10, interleukin-12, and interleukin-2 in normal and preeclamptic pregnancies. *Pregnancy Hypertens Int J Women Cardiovasc Health.* 2012;2:39-42.
11. Roberts JM, Hubel CA. Is oxidative stress the link in the two-stage model of pre-eclampsia? *Lancet.* 1999;354(9181):788-9.

12. Orange S, Horvath J, Hennessy A. Preeclampsia is associated with a reduced interleukin-10 production from peripheral blood mononuclear cells. *Hypertens Pregnancy*. 2003;22(1):1-8.
13. Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? *Immunol Today*. 1993;14(7):353-6.
14. Darmochwal-Kolarz D, Leszczynska-Gorzela B, Rolinski J, Oleszczuk J. T helper 1- and T helper 2-type cytokine imbalance in pregnant women with pre-eclampsia. *Eur J Obstet Gynecol Reprod Biol*. 1999;86(2):165-70.
15. Saito S, Umekage H, Sakamoto Y, Sakai M, Tanebe K, Sasaki Y, et al. Increased T-helper-1-type immunity and decreased T-helper-2-type immunity in patients with preeclampsia. *Am J Reprod Immunol*. 1999;41(5):297-306.
16. Roth I, Fisher SJ. IL-10 is an autocrine inhibitor of human placental cytotrophoblast MMP-9 production and invasion. *Dev Biol*. 1999;205(1):194-204.
17. Groux H, Cottrez F. The complex role of interleukin-10 in autoimmunity. *J Autoimmun*. 2003;20(4):281-5.
18. Westendorp RG, Langermans JA, Huizinga TW, Verweij CL, Sturk A. Genetic influence on cytokine production in meningococcal disease. *Lancet*. 1997;349(9069):1912-3.
19. de Craen AJ, Posthuma D, Remarque EJ, van den Biggelaar AH, Westendorp RG, Boomsma DI. Heritability estimates of innate immunity: an extended twin study. *Genes Immun*. 2005;6(2): 167-70.
20. Bowen RS, Gu Y, Zhang Y, Lewis DF, Wang Y. Hypoxia promotes interleukin-6 and -8 but reduces interleukin-10 production by placental trophoblast cells from preeclamptic pregnancies. *J Soc Gynecol Investig*. 2005;12(6):428-32.
21. Rees LE, Wood NA, Gillespie KM, Lai KN, Gaston K, Mathieson PW. The interleukin-10-1082 G/A polymorphism: allele frequency in different populations and functional significance. *Cell Mol Life Sci*. 2002;59(3):560-9.
22. Vural P, Degirmencioglu S, Saral NY, Demirkan A, Akgul C, Yildirim G, et al. Tumor necrosis factor alpha, interleukin-6 and interleukin-10 polymorphisms in preeclampsia. *J Obstet Gynaecol Res*. 2010;36(1):64-71.
23. Daher S, Sass N, Oliveira LG, Mattar R. Cytokine genotyping in preeclampsia. *Am J Reprod Immunol*. 2006;55(2):130-5.
24. Stonek F, Hafner E, Metzenbauer M, Katharina S, Stümpflen I, Schneeberger C, et al. Absence of an association of tumor necrosis factor (TNF)-alpha G308A, interleukin-6 (IL-6) G174C and interleukin-10 (IL-10) G1082A polymorphism in women with preeclampsia. *J Reprod Immunol*. 2008;77(1):85-90.
25. Kamali-Sarvestani E, Kiany S, Gharesi-Fard B, Robati M. Association study of IL-10 and IFN-gamma gene polymorphisms in Iranian women with preeclampsia. *J Reprod Immunol*. 2006;72(1-2):118-26.
26. Makris A, Xu B, Yu B, Thornton C, Hennessy A. Placental deficiency of interleukin-10 (IL-10) in preeclampsia and its relationship to an IL10 promoter polymorphism. *Placenta*. 2006;27(4-5):445-51.
27. Xie C, Yao MZ, Liu JB, Xiong LK. A meta-analysis of tumor necrosis factor-alpha, interleukin-6, and interleukin-10 in preeclampsia. *Cytokine*. 2011; 56(3):550-9.