Genetics and Molecular Research

Investigations into the association between polymorphisms in the interleukin-10 gene and risk of early-onset preeclampsia

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Genet. Mol. Res. 14 (4): 19323-19328 (2015)
Received August 3, 2015
Accepted October 2, 2015
Published December 29, 2015
DOI http://dx.doi.org/10.4238/2015.December.29.42

ABSTRACT. In this case-control study, we assessed the influence of *IL-10* -1082A/G and -819T/C on the development of preeclampsia. The *IL-10* -1082A/G and -819T/C polymorphisms were assessed by polymerase chain reaction-restriction fragment length polymorphism. The genotype distributions of the *IL-10* -1082A/G and -819T/C polymorphisms in the control subjects were in conformance with Hardy-Weinberg equilibrium (HWE; P = 0.46 and 0.17). Unconditional logistic regression analyses revealed that individuals carrying the CC genotype of *IL-10* -819T/C were associated with an increased risk of preeclampsia compared to the TT genotype. The odds ratio (95% confidence interval) for the CC genotype of *IL-10* -819T/C was 1.71 (1.07-3.27) compared to the TT genotype.

In conclusion, the results of our study indicated that the *IL-10* -819T/C polymorphism was associated with an increased risk of preeclampsia in a Chinese population.

Key words: Interleukin-10; Polymorphism; Early-onset preeclampsia

INTRODUCTION

Preeclampsia is a multi-system disorder unique to pregnancy, which is characterized by high hypertension and proteinuria above twenty weeks' gestation (Redman and Sargent, 2005; Sibai et al., 2005). This disease would contribute to the preterm birth and intrauterine growth restriction, and increase the risk of perinatal materno-fetal morbidity and mortality. The preeclampsia is involved in many environmental factors, such as preexisting hypertension, diabetes and obesity as well as smoking (Wang et al., 2002; Chelbi et al., 2007). Moreover, many genetic factors also contribute to the development of this disease, such as interleukin (IL)-6, NLRP1 and MTHFR genes (Pontillo et al., 2015; Sowmya et al., 2015; Wu et al., 2015).

Previous studies have reported that altered concentrations of many cytokines may contribute to the defective placental invasion and endothelial damage in preeclampsia. Several previous studies have reported that IL-1β, IL-4, IL-1α, and IL-6 (Bayoumy et al., 2013; Chen et al., 2014; Kang et al., 2014; Wang et al., 2014). IL-10 gene has an important role in Th2 immunity, and is located on human chromosome 1 (1q31-1q32) (Kim et al., 1992; Eskdale et al., 1997). Two common single nucleotide polymorphisms (SNPs) in the *IL-10* gene, such as -1082A/G and -819T/C, locate regions of the promoter region and regulate the levels of circulating IL-10 (D'Alfonso et al., 2000; Mormann et al., 2004). Several studies have reported the correlation between polymorphisms in the *IL-10* gene and susceptibility to preeclampsia; however, the results have been inconsistent (Pissetti et al., 2014; Sowmya et al., 2014a,b; Yang et al., 2014). In this case-control study, we conducted a case-control study to assess the influence of *IL-10* -1082A/G and -819T/C in the development of preeclampsia.

MATERIAL AND METHODS

Patients

A total of 177 women suffering from preeclampsia above 20 weeks of gestation were enrolled in this case-control study from the Eighth People's Hospital of Qingdao between March 2012 and March 2014. Preeclampsia were defined as gestation more than 20 weeks, blood pressure ≥140/90 mmHg and presence of proteinuria. Patients with a previous history of intrauterine fetal deaths were excluded from the study. In addition, 182 control subjects at the >20 weeks gestation stage were collected at our hospital during the same period. The excluded criteria of the controls were those who had a chronic hypertension or a history of renal, autoimmune, metabolic, or cardiovascular disease.

Demographic and clinical data were collected from a self-designed questionnaire and medical record. Written informed consents were obtained from patients and control subjects prior to the study. The study was previously approved by the Institutional Research Ethics Committee of the Eighth People's Hospital of Qingdao.

DNA extraction and genotyping

Two milliliters of peripheral blood was collected from the patients prior to their treatment in EDTA-anticoagulant tubes. Genomic DNA was extracted from the blood, using the QIAamp DNA MAX Kit (Qiagen, Hilden, Germany). The IL-10 -1082A/G and -819T/C polymorphisms were assessed using the polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) assay. The following forward and reverse primers were used in this assay: IL-10 -1082A/G: 5'-TGATACTATCTCGTCTTTGATCG-3' and 5'-TCCGGCTTGTGAATGGCTGA-3'; IL-10 -819T/C: 5'-TGAATGTATGTGCTGGAGATCC-3' and 5'-GGTGAGCGTGCCTTCGGC-3', respectively. The PCR conditions were set as follows: 95°C for 5 min for the initial denaturation, following 30 cycles of denaturation at 95°C for 30 s, annealing at 62°C for 45 s, extension at 72°C for 30 s and final extension at 72°C for 5 min. The PCR products were confirmed via electrophoresis on a 2% agarose gel stained with ethidium bromide, which was ubsequently visualized under UV light.

Statistical analysis

The demographic and clinical data were expressed as the mean ± standard deviation and frequency and percentage. Conformance with the Hardy-Weinberg equilibrium (HWE) was tested using the standard χ^2 test or the Fisher exact test. Unconditional logistic regression analysis was used to evaluate the association between *IL-10* -1082A/G and -819T/C polymorphisms and risk of preeclampsia; the results of this analysis were expressed as odds ratio (ORs) and corresponding 95% confidence intervals (95%CI). A two-tailed P value <0.05 was considered to be statistically significant. All statistical tests were performed on the SPSS 21.0 package (SPSS Inc., Chicago, IL, USA).

RESULTS

We observed no significant differences in the age of the preeclampsia patients and controls. Preeclampsia patients were more likely to have a higher gestational age, BMI, systolic blood pressure, and diastolic blood pressure, and lower number of weeks up to delivery and undergo Caesarean delivery, compared to the control subjects (Table 1).

Table 1. Baseline characteristics of preeclampsia patients and controls.

Variables	Patients (N = 177)	%	Controls (N = 182)	%	*t* or χ^2 test	P value
Age (years)	28.20 ± 4.75		27.45 ± 4.96		1.46	0.07
Gestational age (weeks)	26.46 ± 3.84		25.62 ± 4.10		2.01	0.02
BMI (kg/m^2)	29.51 ± 4.20		28.20 ± 3.95		3.05	0.001
Systolic blood pressure (mmHg)	148.30 ± 11.85		114.23 ± 12.23		26.80	<0.001
Diastolic blood pressure (mmHg)	97.43 ± 18.40		70.52 ± 13.45		15.85	<0.001
Delivery weeks	35.65 ± 3.60		39.10 ± 1.42		12.01	<0.001
Mode of delivery						
Normal	80.712	45.6	104.286	57.3		
Caesarean	96.288	54.4	77.714	42.7	12.01	<0.001

The χ^2 test revealed no significant differences in the genetic frequencies of *IL-10* -1082A/G and -819T/C between the preeclampsia patients and control subjects. The genotype distributions of the *IL-10* -1082A/G and -819T/C polymorphisms were consistent with the HWE in the controls (P value for HWE: 0.46 and 0.17, respectively; Table 2). Moreover, the minor allele frequencies

(MAFs) of *IL-10* -1082A/G and -819T/C polymorphisms were similar to the MAFs of dbSNPs (http://www.ncbi.nlm.nih.gov/snp/).

Table 2. Genotype frequencies of *IL-10* -1082A/G and -819T/C polymorphisms in preeclampsia patients and controls.

IL-10 gene	Patients	%	Controls	%	χ^2 test	P value	P value for HWE	Minor allele frequency	
								In controls	In database
-1082A/G									
AA	83	46.89	93	51.1					
AG	77	43.5	77	42.31					
GG	17	9.6	12	6.59	1.36	0.51	0.46	0.2775	0.2722
-819T/C									
TT	54	30.51	68	37.36					
TC	80	45.2	79	43.41					
CC	43	24.29	35	19.23	2.36	0.31	0.17	0.4093	0.4347

HWE = Hardy-Weinberg equilibrium.

Unconditional logistic regression analyses indicated that individuals carrying the CC genotype of the *IL-10* -819T/C were associated with an increased risk of preeclampsia, compared to those expressing the TT genotype. The OR (95%CI) for the CC genotype of *IL-10* -819T/C was 1.71 (1.07-3.27) compared to the TT genotype (Table 3). However, the *IL-10* -1082A/G polymorphism showed no significant association with the risk of preeclampsia.

Table 3. Association between *IL-10* -1082A/G and -819T/C polymorphisms and risk of preeclampsia.

IL-10 gene	Patients	%	Controls	%	OR (95%CI)[1]	P value
-1082A/G						
AA	83	46.89	93	51.1	Ref.	
AG	77	43.5	77	42.31	1.26 (0.82-1.89)	0.56
GG	17	9.6	12	6.59	1.66 (0.72-4.15)	0.21
AG+GG	94	53.1	89	48.9	1.18 (0.77-1.83)	0.43
-819T/C						
TT	54	30.51	68	37.36	Ref.	
TC	80	45.20	79	43.41	1.36 (0.77-2.32)	0.26
CC	43	24.29	35	19.23	1.71 (1.07-3.27)	0.09
TC+CC	123	69.49	114	62.64	1.36 (0.86-2.16)	0.17

[1]Adjusted for age, gestational age, body mass index, systolic blood pressure, diastolic blood pressure, delivery weeks and mode of delivery. OR = odds ratio; CI = confidence interval.

DISCUSSION

In the present study, we investigated the impact of gene polymorphisms with risk of preeclampsia in a sample of Chinese population. Our findings showed a significant association between. In this study, the CC genotype of *IL-10* -819T/C was found to be associated with an increased risk of preeclampsia in a Chinese population.

Previous studies have assessed the correlation between *IL-10* -1082A/G and -819T/C polymorphisms and the susceptiblity to preeclampsia (Daher et al., 2006; Makris et al., 2006; Mirahmadian et al., 2008; Stonek et al., 2008; de Lima et al., 2009; Vural et al., 2010; Sowmya et al., 2014a,b, 2015). Daher et al. (2006) suggests that *IL-10* -1082A/G polymorphism was associated with the risk of preeclampsia. Vural et al. (2010) reported that the AA genotype of *IL-10*

-1082A/G had 3.38-fold increased risk of developing preeclampsia compared to the GG genotype. Similarly, Sowmya et al. (2014a) conducted a study in an Indian population, they suggested that the *IL-10* -819T/C gene polymorphism could contribute to the risk of preeclampsia. Sowmya et al. (2014b) conducted another study in Indian population, and they reported that *IL-10* -819T/C and -592A/C played an important role in the pathogenesis of early-onset preelampsia. Pissetti et al. (2014) suggested that *IL-10* -1082A/G polymorphism was correlated with the development of preeclampsia. However, other studies, such as the one conducted by Stonek et al. (2008), have reported the lack of any association between the *IL-10* gene polymorphism and risk of preeclampsia. Additionally, de Lima et al. (2009) did not find any significant association between the *IL-10* -1082A/G and -819T/C polymorphisms and the development of preeclampsia. A recent meta-analysis pooled with eleven studies, and it suggests that *IL-10* -1082 G/A, -819 C/T and -592 C/A polymorphisms are unlikely to be associated with pre-eclampsia (Lee et al., 2014). In our study, we found that the *IL-10* -819T/C polymorphism contributes to the development of preeclampsia. Further studies with large sample size are greatly needed to confirm our finding.

We identified three limitations in our study. Firstly, patients with preeclampsia and the control subjects were selected from a single hospital, resulting in a selection bias; that is, these subjects may not be representative of other populations. Secondly, other genetic polymorphisms might have influenced the risk of preeclampsia in addition to *IL-10* gene. Finally, the sample size is relatively small, which may influence the statistical power of identifying differences between the groups.

In conclusion, the results of our study indicate an association between the *IL-10* -819T/C polymorphism and an increased risk of preeclampsia in a Chinese population.

Conflicts of interest

The authors declare no conflict of interest.

REFERENCES

Bayoumy NM, Al-Sharaidh AS, Babay ZH, Abdulgader AM (2013). The role of interleukin-6 promoter polymorphism -174G/C in Saudi women with hypertensive disorders of pregnancy. *Saudi Med. J.* 34: 689-94.

Chelbi ST, Mondon F, Jammes H, Buffat C, et al. (2007). Expressional and epigenetic alterations of placental serine protease inhibitors: SERPINA3 is a potential marker of preeclampsia. *Hypertension* 49: 76-83.

Chen P, Zhou B, Zhang K, Zhang Z, et al (2014). An insertion-deletion polymorphism in interleukin-1α gene associated with susceptibility to preeclampsia. *Hypertens. Pregnancy* 33:395-401.

D'Alfonso S, Rampi M, Rolando V, Giordano M, et al. (2000). New polymorphisms in the IL-10 promoter region. *Genes Immun.* 1: 231-233.

Daher S, Sass N, Oliveira LG and Mattar R (2006). Cytokine genotyping in preeclampsia. *Am. J. Reprod. Immunol.* 55: 130-135.

de Lima TH, Sass N, Mattar R, Moron AF, et al. (2009). Cytokine gene polymorphisms in preeclampsia and eclampsia. *Hypertens. Res.* 32: 565-569.

Eskdale J, Kube D, Tesch H (1997). Mapping of the human IL10 gene and further characterization of the 5′ flanking sequence. *Immunogenetics* 46:120-128.

Kang L, Chen CH, Yu CH, Chang CH, et al (2014). An association study of interleukin-4 gene and preeclampsia in Taiwan. *Taiwan J. Obstet. Gynecol.* 53: 215-219.

Kim JM, Brannan CI, Copeland NG (1992). Structure of the mouse IL-10 gene and chromosomal localization of the mouse and human genes. *J. Immunol.* 148: 3618-3623.

Lee YH, Kim JH, Song GG (2014). Meta-analysis of associations between interleukin-10 polymorphisms and susceptibility to pre-eclampsia. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 182: 202-207.

Makris A, Xu B, Yu B, Thornton C, et al (2006). Placental deficiency of interleukin-10 (IL-10) in preeclampsia and its relationship to an IL10 promoter polymorphism. *Placenta* 27: 445-451.

Mirahmadian M, Kalantar F, Heidari G, Safdarian L, et al. (2008). Association of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in Iranian patients with pre-eclampsia. *Am. J. Reprod. Immunol.* 60: 179-185.

Mörmann M, Rieth H, Hua TD, Assohou C, et al. (2004). Mosaics of gene variations in the Interleukin-10 gene promoter affect interleukin-10 production depending on the stimulation used. *Genes Immun.* 5: 246-255.

Pissetti CW, Bianco TM, Tanaka SC, Nascentes GA, et al. (2014). Protective role of the G allele of the polymorphism in the Interleukin 10 gene (-1082G/A) against the development of preeclampsia. *Rev. Bras. Ginecol. Obstet.* 36: 456-460.

Pontillo A, Reis EC, Bricher PN, Vianna P, et al (2015). NLRP1 L155H polymorphism is a risk factor for preeclampsia development. *Am. J. Reprod. Immunol.* 73: 577-581.

Redman CW and Sargent IL (2005). Latest advances in understanding preeclampsia. *Science* 308: 1592-1594.

Sibai B, Dekker G and Kupferminc M (2005). Pre-eclampsia. *Lancet* 365: 785-799.

Sowmya S, Ramaiah A, Sunitha T, Nallari P, et al. (2014a). Role of IL-10 -819(t/c) promoter polymorphism in preeclampsia. *Inflammation* 37: 1022-1027.

Sowmya S, Sri Manjari K, Ramaiah A, Sunitha T, et al. (2014b). Interleukin 10 gene promoter polymorphisms in women with early-onset pre-eclampsia. *Clin. Exp. Immunol.* 178: 334-341.

Sowmya S, Ramaiah A, Nallari P, Jyothy A, et al. (2015). Role of IL-6 -174(G/C) promoter polymorphism in the etiology of early-onset preeclampsia. *Inflamm. Res.* 64: 433-439.

Stonek F, Hafner E, Metzenbauer M, Katharina S, et al. (2008). 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.* 77: 85-90.

Vural P, Degirmencioglu S, Saral NY, Demirkan A, et al. (2010). Tumor necrosis factor alpha, interleukin-6 and interleukin-10 polymorphisms in preeclampsia. *J. Obstet. Gynaecol. Res.* 36: 64-71.

Wang JX, Knottnerus AM, Schuit G, Norman RJ, et al. (2002). Surgically obtained sperm, and risk of gestational hypertension and pre-eclampsia. *Lancet* 359: 673-674.

Wang X, Jiang F, Liang Y, Xu L, et al. (2014). Interleukin-1β-31C/T and -511T/C polymorphisms were associated with preeclampsia in Chinese Han population. *PLoS One* 9: e106919.

Wu X, Yang K, Tang X, Sa Y, et al. (2015). Folate metabolism gene polymorphisms MTHFR C677T and A1298C and risk for preeclampsia: a meta-analysis. *J. Assist. Reprod. Genet.* 32: 797-805.

Yang W, Zhu Z, Wang J, Ye W, et al. (2014). Evaluation of association of maternal IL-10 polymorphisms with risk of preeclampsia by a meta-analysis. *J. Cell Mol. Med.* 18: 2466-2477.