

Susceptibility to preeclampsia is associated with a 50-bp insertion/deletion polymorphism at the promoter region of the *SOD1* gene

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Abstract

Objective: There is much evidence that oxidative stress is involved in the pathogenesis of preeclampsia (PE). A 50 bp insertion/deletion (Ins/Del) functional polymorphism in the promoter region of *SOD1* has been reported. Due to a total lack of data, the aim of this study was to investigate the association between the *SOD1* (Ins/Del) polymorphism and the risk of PE.

Material and Methods: The current hospital-based case-control study included a total of 172 preeclamptic and 171 non-preeclamptic pregnancies. Genotyping was performed using the polymerase chain reaction method.

Results: Statistical analysis revealed that the Del/Del genotype significantly correlated with susceptibility to PE [odds ratio (OR): 6.53, 95% confidence interval (CI): 1.43-29.7, $p=0.015$]. Since maternal body mass index, family history of PE in first degree relatives, and educational levels were statistically associated with the susceptibility to PE, further analyses were carried out in order to estimate the adjusted ORs. After adjustment for aforementioned variables, the Del/Del genotype increased the risk of PE (OR: 5.98, 95% CI: 1.21-29.5, $p=0.028$).

Conclusion: The 50 bp Ins/Del in promoter region of the *SOD1* gene could be an intriguing susceptibility factor for developing preeclampsia in Iranian Caucasians. (J Turk Ger Gynecol Assoc 2021; 22: 268-72)

Keywords: Ins/Del, polymorphism, preeclampsia, susceptibility, superoxide dismutase

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Introduction

Preeclampsia (PE) affects approximately 5-8% of pregnancies and greatly contributes to morbidity/mortality of mother and fetus (1). Preeclampsia is defined as proteinuria and hypertension after 20 weeks of gestation. Furthermore, preeclamptic women are at risk for developing cardiovascular disease (2). The detailed etiology of PE remains unknown and thus precise prediction and prevention are difficult. Genetic components and environmental factors are known to be involved in the etiology of PE, and therefore PE is highly heritable (3,4). Many genetic association studies of candidate genetic polymorphisms have been performed to investigate the genetic background of PE (5).

Reactive oxygen species (ROS) lead to oxidation of numerous biomolecules, including DNA (6). Oxidative stress can occur due to an imbalance between production of ROS and antioxidant capacity. There is plentiful evidence that such conditions are implicated in the pathogenesis of PE (4,7-9). Antioxidant enzymatic system comprises a number of gene families, such as the family SODs [(EC 1.15.1.1) (EC 1.15.1.1) EC 1.15.1.1)]. In mammals, SODs have been classified into three different isoforms, including *SOD1* (MIM: 147450).

A 50 bp insertion/deletion (Ins/Del) in the *SOD1* promoter region has been reported (10). This is a functional polymorphism and alters gene expression with the Del allele resulting in lower *SOD1* mRNA levels (11). Association between the *SOD1*



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expression level and PE has been investigated (12). *SOD1* expression is significantly down-regulated under oxidative stress, for example it is decreased in morphine treated human SH-SY5Y cells (13), and in cells exposed to electromagnetic fields (14). *SOD1* Ins/Del genetic variation is related with complex human diseases associated with oxidative stress, such as breast and gastric cancers, age of onset of bipolar disorder, and cardiovascular disease (15-19).

We hypothesized that the Ins/Del genetic variation in *SOD1*, which is involved in cellular detoxification, may represent a good candidate for susceptibility to PE. Since no data has been published on the association between the *SOD1* (Ins/Del) polymorphism and the risk of PE, a hospital-based, case-control study was carried out and is presented here.

Material and Methods

Participants

This was a hospital-based case-control study. A total of 172 preeclamptic and 171 non-preeclamptic pregnancies, as healthy control group, were included in the study. The participants were recruited from the delivery ward at Zainabeieh, Hafez and Dena Hospitals (Shiraz, Fars province, south-west Iran). DNA extraction, genotype determination and statistical analysis were done in our research laboratory. The preeclamptic and non-preeclamptic women were matched with each other by age. PE was defined as persistent blood pressure above 140/90 mmHg and proteinuria of more than 0.3 g/24 hours, developing after 20 weeks of gestation. Exclusion criteria were twin pregnancies, fetal growth retardation, altered renal function, recurrent miscarriages, and placental abruption. The patients were not classified according to the severity of PE.

Body mass index (BMI, kg/m²) was defined as weight (kg) divided by square of height (m²). Data on age, family history of PE among first-degree relatives, educational level, gravidity, physical activity, interval from last pregnancy, and smoking habits were obtained through interviews with participants. As the gene pool of the Iranian population is heterogeneous (20,21), participants were selected from Caucasian Persian/Muslims living in Fars province, south-west Iran.

The minimum sample size that would be necessary to identify a significant difference in genotype distribution between PE and normal pregnancies was estimated using QUANTO software (<http://biostats.usc.edu/oftware>). Assuming 15% frequency for the D allele, equal number of cases and controls, Rg: 1.8, $\alpha=0.05$, and $\beta=0.80$, minimally, 149 patients would be required. In the present study 172 PE and 171 normal pregnancies were included.

The current study was approved by the Ethical Committee of Shiraz University (approval number: SU.DB-9630578). This study was conducted in accordance with the Declaration of

Helsinki. All pregnant women signed an informed consent before the study.

Genotyping

DNA was extracted from whole blood using a previously described method and stored at -20 °C until use (22). Genotyping analysis was carried out based on a polymerase chain reaction (PCR) method using specific primers as described previously (19). The PCR reaction was performed using 0.4 pmol/ μ L of each primer, 1.5 mM of MgCl₂, 0.2 mM each of desoxynucleotide triphosphates, 0.12 U/ μ L of Taq DNA polymerase (SinaGene, Iran) and genomic DNA as template. The reaction was pre-incubated for 5 minutes at 95 °C for denaturation, followed by 35 seconds at 95 °C, 30 seconds at 60 °C, and 35 seconds at 72 °C, for 31 cycles and a final extension at 72 °C for 10 minutes. The amplified DNA was electrophoresed in 1.5% agarose gel, under constant current of 90 volts for 40-60 minutes. Gels were stained and visualized by ethidium bromide staining under ultraviolet illumination.

Statistical analysis

Hardy-Weinberg equilibrium was investigated in the healthy controls and PE patients by χ^2 analysis. Continuous clinical characteristics were compared between the PE cases and non-PE controls, using Student's t-test. We performed unconditional logistic regression analysis to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) for assessment of differences in the prevalence of characteristics between PE cases and non-PE controls.

The following variables were used in the multivariate logistic regression analysis: maternal age (years); maternal prepregnancy BMI (kg/m²); interval from last pregnancy (years); parity [nulliparous (0), multiparous (1)]; family history of PE in first degree relatives [negative family history (0), positive family history (1)]; smoking during pregnancy [no (0), yes (1)]; physical activity before pregnancy [no (0), yes (1)]; maternal educational level [high school or less (0), University (1)]; and the genotypes of the Ins/Del polymorphism [Del/Del (2), Del/Ins (1), and Ins/Ins (0)]. Statistical analyses were carried out using SPSS, version 25 software (IBM Inc., Armonk, NY, USA). Statistical significance was defined as p-value <0.05.

Results

Descriptive characteristics of the PE and control groups are shown in Table 1, 2, containing continuous and binary variables, respectively. Preeclamptic mothers compared to control subjects had significantly higher BMI (p<0.001) (Table 1). The mean interval from last pregnancy was statistically similar between normal pregnancies and PE outcomes. A positive history for PE among first degree relatives (OR: 8.25,

95% CI: 3.39-20.0, $p < 0.001$) and higher educational levels (OR: 3.77, 95% CI: 2.01-7.08, $p < 0.001$) were associated with risk of PE. However, neither smoking nor gravidity were associated with PE (Table 2). A higher percentage of controls had some history of smoking compared to the PE group, but due to the very low frequency of smokers, the difference was not significant.

Figure 1 shows the genotypes of the study polymorphism by electrophoresis of the PCR products on 1.5% agarose gel. The Ins/Del alleles have 297 and 247 bp, respectively. Table 3 summarizes the distribution of genotypes of the *SOD1* Ins/Del genetic variation in PE and normal pregnancies. The genotypes were in Hardy-Weinberg equilibrium in control subjects ($\chi^2 = 0.02$, $df = 1$, $p = 0.880$). However, there was significant deviation between

observed and expected genotypic frequencies in the PE group ($\chi^2 = 11.9$, $df = 1$, $p < 0.001$). Statistical analysis indicated that the Del/Del genotype was significantly correlated with susceptibility to PE (OR: 6.53, 95% CI: 1.43-29.7, $p = 0.015$). The Del allele increased the risk of PE (OR: 1.67, 95% CI: 1.08-2.58, $p = 0.020$). The risk of PE significantly increased as a function of the number of Del alleles (χ^2 for trend: 4.75, $p = 0.029$).

Since maternal BMI, family history of PE in first degree relatives, and educational levels were statistically associated with the risk of PE, further analyses were carried out in order to estimate the adjusted ORs. After adjustment for these variables, the Del/Del genotype significantly increased the susceptibility to PE (OR: 5.98, 95% CI: 1.21-29.5, $p = 0.028$).

Table 1. Comparison of continuous characteristics between preeclampsia patients and healthy controls

Characteristics	Controls		PE		Results of comparisons		
	n	Mean ± SD	n	Mean ± SD	t	df	p
Age (years)	171	26.8±4.7	172	27.7±4.7	1.83	341	0.068
BMI (kg/m ²)	137	27.7±4.3	145	30.7±5.3	5.2	280	<0.001
Interval from last pregnancy (years)	87	4.6±3.1	77	5.3±4.2	1.22	163	0.222

SD: Standard deviation, PE: Preeclampsia

Table 2. Comparison of binary characteristics between preeclampsia patients and healthy controls

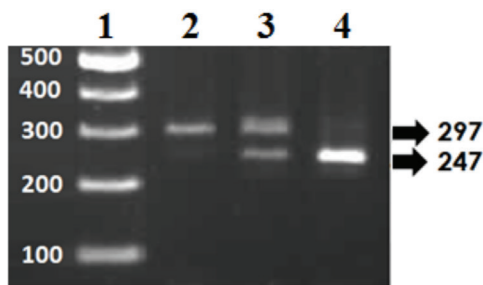
Characteristics	Controls, n (%)	Cases, n (%)	OR	95% CI	p
Parity					
Nulliparous	75 (43.9)	90 (52.3)	1.0	-	-
Multiparous	96 (56.1)	82 (47.7)	1.40	0.91-2.14	0.117
Family history of PE in first degree of relatives					
No	161 (94.2)	130 (75.6)	1.0	-	-
Yes	6 (3.5)	40 (23.3)	8.25	3.39-20.0	<0.001
Missing data	4 (2.3)	2 (1.1)	-	-	-
Smoking habit					
No	157 (91.8)	168 (97.7)	1.0	-	-
Yes	7 (4.1)	4 (2.3)	0.53	0.15-1.85	0.324
Missing data	7 (4.1)	0 (0)	-	-	-
Physical activity					
No	147 (86.0)	142 (82.6)	1.0	-	-
Yes	21 (12.3)	29 (16.9)	1.43	0.77-2.62	0.249
Missing data	3 (1.7)	1 (0.6)	-	-	-
Maternal education levels					
High school and lower	154 (90.0)	125 (72.7)	1.0	-	-
University	15 (8.8)	46 (26.7)	3.77	2.01-7.08	<0.001
Missing data	2 (1.2)	1 (0.6)	-	-	-
Gender of fetus					
Males	83 (48.5)	83 (48.3)	1.0	-	-
Females	83 (48.5)	81 (47.1)	0.97	0.63-1.50	0.912
Missing data	5 (2.9)	8 (4.6)	-	-	-

OR: Odds ratio, CI: Confidence interval, PE: Preeclampsia

Table 3. Association between the 50 bp Ins/Del genetic polymorphisms of the SOD1 and susceptibility to preeclampsia

Genotypes/Alleles	Controls	Cases	OR	95% CI	p	OR*	95% CI	p
Ins/Ins	134	123	1.0	-	-	1.0	-	-
Ins/Del	35	37	1.15	0.68-1.94	0.597	1.15	0.60-2.22	0.658
Del/Del	2	12	6.53	1.43-29.7	0.015	5.98	1.21-29.5	0.028
Ins	303	283	1.0	-	-	-	-	-
Del	39	61	1.67	1.08-258	0.020	-	-	-

*: Adjusted odds ratio for maternal body mass index, family history of PE among first degree relatives, and educational level, OR: Odds ratio, CI: Confidence interval, PE: Preeclampsia

**Figure 1. Polymerase chain reaction products of 50 bp insertion/deletion (Ins/Del) polymorphism at the promoter region of the SOD1 gene. Lanes 1, 2, 3, and 4 are 100 bp DNA ladder, Ins/Ins, Ins/Del and Del/Del genotypes, respectively**

Discussion

These results showed that maternal BMI, interval from last pregnancy, family history of PE among first degree relatives, and educational levels were significantly associated with PE, which were similar to previous studies (1,4,5,23-25).

It is well established that oxidative stress is associated with the pathogenesis of PE (4,7-9). SOD1 is involved in the antioxidant system and it has several genetic polymorphisms, including the 50 bp Ins/Del functional polymorphism in its promoter region (10). Investigation of the association between the SOD1 Ins/Del functional polymorphism and the risk of PE was the main aim of this study. The Del allele is reported to significantly decrease promoter activity (11). Therefore, it was suggested that individuals having the Del/Del genotype would have lower antioxidant capacity compared to the Ins/Ins genotype (11). The Del/Del genotype showed positive correlation with susceptibility to PE, which supported our hypothesis. Previously, reduced expression of SOD1 in peripheral blood mononuclear cells of PE patients had been reported (12). In addition, a significantly lower level of the SOD1 mRNA in trophoblast cells isolated from placentas of PE pregnancies has been reported (26,27). These reports are in keeping with the current findings. Investigation of the 50 bp Ins/Del genetic variation in the promoter region of SOD1 may improve the prediction of susceptibility to PE and

may aid in the design and development of new markers and treatment strategies.

The present case-control study has some limitations. First, it is well established that numerous environmental factors have significant association with the risk of PE (4,5). In the present study we did not investigate these factors alone and their possible gene-environment interactions. Second, patients were not classified according to the severity of PE. Third, the SOD1 gene has other polymorphisms such as the A251G polymorphism (rs2070424) which is associated with some multifactorial traits (28-30). In the present study only the SOD1 Ins/Del genetic polymorphism was investigated. It is highly likely that ethnicity may impact associations in complex human traits (31-33). Therefore, replication of the current study with a larger sample size and in other ethnic groups is suggested, as well as investigating the relationship between risk of PE and both environmental and genetic factors, simultaneously.

Conclusion

The 50 bp Ins/Del functional polymorphism in the promoter region of the SOD1 gene appears to be an intriguing susceptibility factor for the development of preeclampsia in Iranian Caucasians. Further, larger studies are required to confirm and expand upon these findings.

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Ethical Committee Approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The current study was approved by the Ethical Committee of Shiraz University (approval number: SU.DB-9630578).

Informed Consent: Informed consent was obtained from all patients.

Peer-review: Externally peer-reviewed.

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References

- Raymond D, Peterson E. A critical review of early-onset and late-onset preeclampsia. *Obstet Gynecol Surv* 2011; 66: 497-506.
- Chen CW, Jaffe IZ, Karumanchi SA. Pre-eclampsia and cardiovascular disease. *Cardiovasc Res* 2014; 101: 579-86.
- Roten LT, Thomsen LC, Gundersen AS, Fenstad MH, Odland ML, Strand KM, et al. The Norwegian preeclampsia family cohort study: a new resource for investigating genetic aspects and heritability of preeclampsia and related phenotypes. *BMC Pregnancy Childbirth* 2015; 15: 319.
- Dasinger JH, Abais-Battad JM, Mattson DL. Influences of environmental factors during preeclampsia. *Am J Physiol Regul Integr Comp Physiol* 2020; 319: R26-32.
- Giannakou K, Evangelou E, Papatheodorou SI. Genetic and non-genetic risk factors for pre-eclampsia: umbrella review of systematic reviews and meta-analyses of observational studies. *Ultrasound Obstet Gynecol* 2018; 51: 720-30.
- Blasiak J, Szaflik JP. DNA damage and repair in age-related macular degeneration. *Front Biosci (Landmark Ed)* 2011; 16: 1291-301.
- D'Souza V, Rani A, Patil V, Pisal H, Randhir K, Mehendale S, et al. Increased oxidative stress from early pregnancy in women who develop preeclampsia. *Clin Exp Hypertens* 2016; 38: 225-32.
- Matsubara K, Higaki T, Matsubara Y, Nawa A. Nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. *Int J Mol Sci* 2015; 16: 4600-14.
- Taysi S, Tascan AS, Ugur MG, Demir M. Radicals, oxidative/nitrosative stress and preeclampsia. *Mini Rev Med Chem* 2019; 19: 178-93.
- Broom WJ, Greenway M, Sadri-Vakili G, Russ C, Auwarter KE, Glajch KE, et al. 50bp deletion in the promoter for superoxide dismutase 1 (SOD1) reduces SOD1 expression in vitro and may correlate with increased age of onset of sporadic amyotrophic lateral sclerosis. *Amyotroph Lateral Scler* 2008; 9: 229-37.
- Saify K, Saadat M. Influence of a 50bp Ins/Del polymorphism at promoter of the superoxide dismutase-1 on gene expression and risk of heroin dependency. *Environ Health Prev Med* 2017; 22: 4.
- Martinez-Fierro ML, Garza-Veloz I, Carrillo-Sanchez K, Martinez-Gaytan V, Cortes-Flores R, Ochoa-Torres MA, et al. Expression levels of seven candidate genes in human peripheral blood mononuclear cells and their association with preeclampsia. *Hypertens Pregnancy* 2014; 33: 191-203.
- Saify K, Saadat I, Saadat M. Down-regulation of antioxidant genes in human SH-SY5Y cells after treatment with morphine. *Life Sci* 2016; 144: 26-9.
- Mahmoudinasab H, Sanie-Jahromi F, Saadat M. Effects of extremely low-frequency electromagnetic field on expression levels of some antioxidant genes in human MCF-7 cells. *Mol Biol Res Commun* 2016; 5: 77-85.
- Gallegos-Arreola MP, Ramirez-Hernandez MA, Figuera LE, Zúñiga-González GM, Puebla-Pérez AM. The rs2234694 and 50 bp Insertion/Deletion polymorphisms of the SOD1 gene are associated with breast cancer risk in a Mexican population. *Eur Rev Med Pharmacol Sci* 2020; 24: 8017-27.
- Eftekhari A, Peivand Z, Saadat I, Saadat M. Association between genetic polymorphisms in superoxide dismutase gene family and risk of gastric cancer. *Pathol Oncol Res* 2020; 26: 335-9.
- Kordestanian N, Saadat M. A 50-bp Ins/Del polymorphism at the promoter region of the superoxide dismutase-1 and bipolar disorder type 1. *Nord J Psychiatry* 2017; 71: 570-3.
- Darroudi S, Tajbakhsh A, Esmaily H, Ghazizadeh H, Zamani P, Sadabadi F, et al. 50bp deletion in promoter superoxide dismutase 1 gene and increasing risk of cardiovascular disease in Mashhad stroke and heart atherosclerotic disorder cohort study. *Biofactors* 2020; 46: 55-63.
- Eskandari-Nasab E, Kharazi-Nejad E, Nakhaee A, Afzali M, Tabatabaei SP, Tirgar-Fakheri K, et al. 50-bp Ins/Del polymorphism of SOD1 is associated with increased risk of cardiovascular disease. *Acta Med Iran* 2014; 52: 591-5.
- Rafiee L, Saadat I, Saadat M. Glutathione S-transferase genetic polymorphisms (GSTM1, GSTT1 and GSTO2) in three Iranian populations. *Mol Biol Rep* 2010; 37: 155-8.
- Nasseri G, Zahedi T, Mousavi-Kazerooni F, Saadat M. Prevalence of null genotypes of glutathione s-transferase T1 (GSTT1) and M1 (GSTM1) in seven Iranian populations. *Iran J Public Health* 2015; 44: 1655-61.
- Newton CR. Mutational analysis: known mutations. In: McPherson MJ, Hames D, Taylor GR (eds). *PCR2: a practical approach*. IRL-Press, Oxford; 1995. pp. 219-22.
- Bobić MV, Habek D, Habek JČ. Perinatal epidemiological risk factors for preeclampsia. *Acta Clin Croat* 2015; 54: 9-13.
- Luo ZC, Julien P, Wei SQ, Audibert F, Fraser WD; Maternal and Infant Research on Oxidative Stress (MIROS) study group. Association of pre-eclampsia with SOD2 Ala16Val polymorphism among mother-father-infant triads. *Int J Gynaecol Obstet* 2018; 142: 221-7.
- Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet*. 2005; 365: 785-99.
- Wang Y, Walsh SW. Antioxidant activities and mRNA expression of superoxide dismutase, catalase, and glutathione peroxidase in normal and preeclamptic placentas. *J Soc Gynecol Investig* 1996; 3: 179-84.
- Wang Y, Walsh SW. Increased superoxide generation is associated with decreased superoxide dismutase activity and mRNA expression in placental trophoblast cells in pre-eclampsia. *Placenta* 2001; 22: 206-12.
- Ebrahimpour S, Saadat I. Association of CAT C-262T and SOD1 A251G single nucleotide polymorphisms susceptible to gastric cancer. *Mol Biol Res Commun* 2014; 3: 223-9.
- Jamhiri I, Saadat I, Omidvari S. Genetic polymorphisms of superoxide dismutase-1 A251G and catalase C-262T with the risk of colorectal cancer. *Mol Biol Res Commun* 2017; 6: 85-90.
- Zendehboodi Z, Saberikia Z. Association of temperament with genetic polymorphisms in SOD1, GSTM1 and GSTT1 genes. *Mol Biol Res Commun* 2021; 10: 33-8.
- Saadat M. Genetic polymorphisms of glutathione S-transferase T1 (GSTT1) and susceptibility to gastric cancer: a meta-analysis. *Cancer Sci* 2006; 97: 505-9.
- Saadat M. Apolipoprotein E (APOE) polymorphisms and susceptibility to breast cancer: a meta-analysis. *Cancer Res Treat* 2012; 44: 121-6.
- Saadat M. Haplotype analysis of XRCC1 (at codons 194 and 399) and susceptibility to breast cancer, a meta-analysis of the literatures. *Breast Cancer Res Treat* 2010; 124: 785-91.