Endothelial nitric oxide synthase gene Glu298Asp polymorphism and risk of preeclampsia in South East of Iran

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Preeclampsia (PE) is the most serious complication of pregnancy that causes maternal and fetal morbidity and mortality. Although the exact pathophysiology of PE is unknown, a large number of studies have shown that abnormalities in nitric oxide (NO) synthesis may contribute to the development of this disorder. There are some evidences that polymorphisms of the endothelial nitric oxide synthase (eNOS) gene affect NO production and have been associated with hypertension and PE in some populations. Therefore the aim of this study was to assess the relation of the Glu298Asp eNOS polymorphism and PE in an Iranian population. We compared the frequency of the Glu298Asp polymorphism in 147 women with PE and 137 healthy pregnant control subjects by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method. The frequencies of Glu298Asp genotypes were significantly different between PE women and controls (p < 0.001). The frequency of Asp allele was 0.32 in PE patients and 0.20 in controls and was significantly different (p < 0.001). The risk of PE was 2.4 fold in pregnant women with Asp allele. In conclusion, the Asp allele could be a risk factor for PE in South East of Iran.

Key words: Nitric oxide synthase, polymorphism, preeclampsia, pregnancy.

INTRODUCTION

Preeclampsia (PE) is one of the most severe problems of pregnancy and has a familial predisposition. Although the exact pathophysiology of preeclampsia is not clear, several maternal genetic variations, in conjunction with environmental factors, may predispose to the development of the disease (Salonen et al., 2000). Genetic factors and mutilation of nitric oxide (NO)–mediated vasodilation appear to have important roles in progress of PE (Salonen et al., 2000; Broughton et al., 2001). The role of endothelial nitric oxide synthase (eNOS) gene as a candidate gene for the development of PE has been investigated by many studies (Arngrimsson et al., 1997; Lade et al., 1999; Yoshimura et al., 2000; Tempfer et al., 2001). Furthermore, some evidences demonstrated familial pregnancy-induced hypertension associated with a locus in the region of chromosome 7q36, which also encodes the eNOS gene (Lade et al., 1999; Lewis et al., 1999). The eNOS is expressed in the endothelium, encoded by a 26 exon gene with a total size of 21 kb and encodes a mRNA of 4052 nucleotides (Marsden et al., 1993). The eNOS gene has a common polymorphism at position 298 (Glu298Asp) which has been associated with both altered NO production (Wang et al., 1997) and with vascular disorders including hypertension (Benjafield and Morris, 1999), myocardial infarction (Hibi et al.,...
1998), coronary artery spasm (Yoshimura et al., 2000), stroke (Elbaz et al., 2000), and renal disease (Noiri et al., 2002). Yoshimura et al. (2000) reported the association of the Asp allele with severe PE in Japanese but they did not observe any association between this polymorphism and PE in Bangladesh (Yoshimura et al., 2003). There are conflicting results about the correlation between Glu298 Asp polymorphism and PE in different ethnic groups. In Eastern Finland and USA, it was revealed that there was no evidence for the association between this polymorphism and PE (Hakli et al., 2003; Landau et al., 2004) while Tempfer et al. (2004) showed a significant differences between them in USA.

Since there is not any report in Iranian population, the aim of this study was to investigate the association between Glu298 Asp polymorphism of the eNOS gene and PE in South East of Iran.

MATERIALS AND METHODS

Subjects

With Institutional Review Board approval and written informed consent from all subjects, we obtained blood samples for genotyping from women delivering at Ali-ebn-abitaleb hospital in Zahedan. Women were considered to have PE if they met the 1996/2000 American College of Obstetricians and Gynecologists criteria for the definition of PE: Systolic blood pressure > 140 mm Hg or diastolic blood pressure > 90 mm Hg, occurring on at least two occasions 6 h apart, with proteinuria > 0.3 g/L in a 24 h specimen, or a proteinuria dipstick reading of 2+ on a random urine collection, with no pre-pregnancy history of essential hypertension or hypertension before 20 weeks gestation. Blood pressures were determined using the automated blood pressure module of a Hewlett-Packard M1176A model 66 (Hewlett-Packard, Andover, MA) with the patient in the supine position. A total of 147 women with PE were genotyped and compared with 137 healthy normotensive women delivering at term.

DNA genotyping

Blood samples were obtained and genomic DNA was isolated from peripheral blood by DNA extraction Kit (Roche, Germany). The eNOS polymorphism of interest, Glu298Asp, was detected using a polymerase chain reaction (PCR)–based restriction enzyme analysis in Zahedan Cellular and Molecular Research Center.

In brief, this exonic fragment was amplified by PCR with a forward primer 5’- GAC CCT GGA GAT GAA GGC AGG AGA-3’ and reverse primer 5’- ACC TCC AGG ATG TTG TAG CGG TGA-3’ (Hibi et al., 1998). The reaction performed according to the protocol as previously described, except for the annealing step which was at 60°C (Salimi et al., 2010). The 517 bp PCR product was digested overnight at 37°C with 10 units of the BanII restriction enzyme (Fermentas, Lithuania). The digested PCR products were run on 2% agarose gel and visualized by ethidium bromide staining. The G wild type allele was not digested and produced a 517 bp fragment, whereas the variant T allele was digested into 346 and 171 bp fragments (Figure 1).

Figure 1. The Glu298Asp polymorphism of eNOS gene was shown by electrophoresis on 2% agarose gel. Lane 3: 50-bp DNA ladder; lane 2 and 7: Glu/Glu genotype; lane 5 and 6: Glu/Asp genotype; lane 1, 4, 8 to 10: Asp/Asp genotype.

Statistical analysis

All statistical analyses were performed with SPSS V-11.5. The differences between groups were examined by χ² tests or an independent student t-test for quantitative parameters. Allele frequencies were calculated by the gene counting method. The frequencies of the alleles and genotypes were analyzed between
Table 1. Demographic characteristics of preeclamptic patients and controls.

| Demographic characteristic | Preeclampsia, N = 147 | Controls, N = 137 | p Value |
|----------------------------|------------------------|-------------------|---------|
| Age (year)                 | 28.1±7.7               | 26.3±6.1          | NS      |
| Gestational age (weeks)    | 36.6 ± 3.6             | 38.2 ± 2.8        | 0.002   |
| Birth weight (g)           | 2789 ± 829             | 2993 ± 666        | NS      |
| Diastolic blood pressure (mm Hg) | 96 ± 8.7            | 69.7 ± 9.1        | 0.0001  |
| Systolic blood pressure (mm Hg) | 152.2 ± 14.5         | 111.9 ± 11.9      | 0.0001  |
| Primiparity (%)            | 0.42                   | 0.29              | 0.015   |
| Family history of preeclampsia (%) | 0.31                | 0.37              | NS      |

| Race                       |            |                  |         |
|----------------------------|------------|------------------|---------|
| Persian (%)                | 25         | 39               |         |
| Baloch (%)                 | 44         | 43               | 0.011   |
| Afghan (%)                 | 31         | 18               |         |

NS, Not significant; gestational age at onset of preeclampsia.

Table 2. Genotype and allele frequencies of Glu298Asp polymorphism of the eNOS gene in preeclamptic patients and controls.

| 4b/a polymorphism          | Pre-eclampsia, N = 147 | Controls, N = 137 | χ² | P Value | OR (95%) |
|----------------------------|-------------------------|-------------------|----|---------|----------|
| Glu/Glu, n (%)             | 61 (41.5)               | 86(63)            |    |         |          |
| Glu/Asp, n (%)             | 78(53)                  | 48(35)            | 13.3| 0.001   |          |
| Asp/Asp, n (%)             | 8(5.5)                  | 3(2)              |    |         |          |
| Glu/Asp + Asp/Asp (%)      | 86(58.5)                | 51(37)            | 12.8| 0.0001  | 2.4(1.5 - 3.8) |
| Glu (%)                    | 68                      | 80                | 11.1| 0.001   |          |
| Asp (%)                    | 32                      | 20                |    |         |          |

The frequencies of Glu/Glu, Glu/Asp and Asp/Asp genotypes were 46, 47 and 10% in PE patients and 63, 35 and 2 in healthy pregnant women, respectively and were significantly different (p < 0.001). The frequency of Asp allele was 0.32 in PE patients and 0.20 in controls and was significantly different (p < 0.001). The risk of PE was 2.4 fold in pregnant women with Asp allele (Glu/Asp + Asp/Asp) in contrast to control women without Asp allele (OR, 2.4 [95% CI, 1.5 to 3.8]; P = 0.0001).

There was no correlation between Glu298Asp polymorphism of eNOS gene and the onset and severity of PE. Moreover, there was no variation in Glu298Asp polymorphism in different races. Multiple regression analysis revealed that Afghan race, gravity and presence of Asp allele were independent risk factors of PE (Table 3).

RESULTS

The clinical and biochemical parameters of the controls and PE women are shown in Table 1. Maternal age and family history of PE did not differ significantly between two groups. As expected, systolic and diastolic blood pressure and primiparity were significantly higher and gestational age was significantly lower in PE women. Although birth weight was lower in PE women, the difference was not significant.

The frequencies of ethnic groups (Persian, Balooch and Afghan) were significantly different between PE women and controls (P = 0.03) and the risk of PE was two fold in Afghan women in contrast to other groups (OR, 2 [95% CI, 1.1 to 3.8]; P = 0.01).

Allele frequencies of Glu298Asp polymorphism were in Hardy Weinberg equilibrium. The distribution of genotype and allele frequencies of Glu298Asp polymorphism of eNOS gene was compared between PE patients and controls (Table 2).

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DISCUSSION

PE is the most serious complication of pregnancy influencing both fetal and maternal morbidity and
In the present study, we found that the Glu298Asp polymorphism and the T-786C polymorphism (4a/b, Glu298Asp and T-786C) and assessed the synergic effect of these SNPs. Serrano et al. (2004) in Colombia concluded that the eNOS Glu298Asp polymorphism and the common G894T polymorphism of eNOS gene may be protective against PE in a Chinese population, in contrast to the results in the Japanese population.

In another study, a significant difference in the allele frequency and genotype distribution of this polymorphism and PE have been shown in USA (Tempfer et al., 2001). Furthermore, other investigators evaluated the relation of three common polymorphisms of eNOS gene (4a/b, Glu298Asp and T-786C) and assessed synergic effect of these SNPs. Serrano et al. (2004) in Colombia concluded that the eNOS Glu298Asp polymorphism and the Asp298-786C-4b haplotype are risk factors for PE; in consistent with our results, they found in women homozygous for the Asp298 allele, the adjusted OR for PE was 4.60 (95% confidence interval [CI], 1.73 to 12.22) compared with carriers of the Glu298 allele. Sandrim et al. (2008) in Brazil showed that the haplotype 'C Glu a' was more common in women with gestational hypertension and PE than in healthy controls. Recent study in

**Table 3. Multiple logistic regression analysis with forward stepwise selection (Wald).**

| Risk factor                  | B   | S. E. | Wald | df | Sig.  | Exp (B) | 95% CI        | Lower | Upper |
|-----------------------------|-----|-------|------|----|-------|---------|--------------|-------|-------|
| Afghan race                 | 0.8 | 0.3   | 7.1  | 1  | 0.008 | 1.5     | 2.5          | 4     |       |
| Primigravida                | 0.72| 0.27  | 7.4  | 1  | 0.007 | 2.1     | 1.2          | 3.5   |       |
| Glu/Asp + Asp/Asp genotype  | 0.95| 0.25  | 13.9 | 1  | 0.001 | 2.4     | 1.6          | 4.2   |       |
| Constant                    | 0.89| 0.3   | 8.6  | 1  | 0.003 | 2.4     |              |       |       |

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India showed that there was no relation between individual eNOS gene polymorphisms and hypertensive disorders of pregnancy in North Indian women. But the presence of rare alleles at all the three sites in eNOS seemed to increase the risk of PE (Aggrawal et al., 2010). What we have seen in South East of Iran was in agreement with studies in Colombia, USA and Japan and was in contrast to with studies in Finland, Korea, United Kingdom and India.

This discrepancy is common in association studies and is due to the unlikely genetic and environmental backgrounds, inclusion and exclusion criteria for PE women and controls and sample size volume; however, with attention to meta analysis study, it is more probable that the small study volume is the most important reason of this discrepancy.

In conclusion, this study demonstrated that maternal eNOS Glu298Asp polymorphism is associated with PE and the frequency of Asp allele was significantly higher in PE patients than healthy controls. The risk of PE was 2.4 fold in pregnant women with Asp allele contrast to control women without Asp allele in the South East of Iran.

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