Extracellular superoxide dismutase (SOD3) ALA40THR genetic polymorphism in correlation to Doppler flow indices in the Egyptian preeclamptic patients

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Background
Preeclampsia (PE) is a common pregnancy-specific complication, characterized by hypertension and significant proteinuria at or after 20 weeks of pregnancy; it affects ~ 5–10% of pregnant women worldwide and remains the second leading cause of maternal and perinatal morbidity and mortality. Depletion of antioxidant enzymes such as superoxide dismutase (SOD) might be associated with the development of PE.

Objective
This study aimed to investigate the correlation of Ala40Thr polymorphism of SOD3 gene with the severity of PE and its correlation to fetal Doppler flow indices.

Materials and methods
The study was conducted in 250 pregnant women, divided into three groups: 130 controls, 60 mild PE cases, and 60 severe PE cases. They all underwent transabdominal ultrasound for assessment of resistance index and pulsatility index. The polymorphism was detected by PCR restriction fragment length polymorphism.

Results
There was a significant difference in the SOD3 Ala40Thr polymorphism between preeclamptic patients and controls, with higher risk of mutant GG variant compared with the wild-type AA in patients. The gestational age at delivery and pulsatility index were found to be significantly different among the three genotypes, with higher pulsatility index in severe cases compared with mild cases.

Conclusion
Follow-up of resistance index and pulsatility index with genotyping of SOD3 can give a clue for early prediction of PE avoiding preterm labor and intrauterine growth restriction.

Keywords:
preeclampsia, superoxide dismutase, transabdominal ultrasound

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Introduction

Preeclampsia (PE) is a typical pregnancy-specific complication, represented by critical proteinuria and hypertension at or following 20 weeks of pregnancy, with incidence of ~ 5–10% of all pregnant women and remains the second driving reason of maternal and perinatal morbidity and mortality [1,2].

The only perfect therapy is to deliver the embryo and placenta. The choice to deliver includes matching the advantage of the extrafetal development against the maternal threat of progression of the disease. Thus, it is significant to perfectly assess both the maternal and fetal consequences of PE in pregnant women [3].

The diagnosis of PE depends on blood pressure and kidney function changes in a normal blood pressure pregnant woman after 20 weeks of pregnancy. Protein (mg)/creatinine (mg) ratio of greater than 0.3 or protein greater than 3 g in a 24h urine sample indicates the progress of proteinuria, whereas values of systolic blood pressure (SBP) greater than 140 mmHg and diastolic blood pressure (DBP) greater than 90 mm represent the progress of hypertension [3].

Although the growing evidence suggests that the pathogenesis of PE includes placental ischemia, which triggers systemic inflammation and maternal endothelial dysfunction, the exact cause of PE is not understood well [4].

The pathogenesis of PE is related to elevated oxidative stress and ischemia and/or placental hypoxia. Production of soluble factors from the ischemic placenta into the maternal blood stream plays a critical

role in the endothelial function change, which is the most considerable feature of the disease [5].

Oxidative stress is indicated by increased amount of reactive oxygen species (ROS) leading to tissue damage and is related to the progress of several diseases including hypertension. Superoxide dismutase (SODs) are the first significant line of antioxidant defence systems by removing damaging ROS from the cellular environment. Extracellular superoxide dismutase or EC-SOD (SOD3) is the main SOD in extracellular matrix [6].

EC-SOD plays a very important role in controlling oxidant stress and inflammation. A reduction in the activity of antioxidant SOD results in ROS accumulation, which leads to direct damaging effects on the endothelium and other vessel components, with effects on vessel tonus [7].

The EC-SOD gene is located on the 4p15 chromosomal region and consists of three exons and two introns [8]. The coding sequence is entirely located within exon 3. In this gene, several single nucleotide polymorphisms (SNPs) have been identified, including a transition mutation of G to A at position 172 resulting in change of alanine to threonine (Ala40Thr). This substation leads to oxidative stress with an imbalance in the oxidant/antioxidant activity, so it seems to play a central role in the pathogenesis of PE [9].

The aim of the present study was to investigate the correlation of SOD3 Ala40Thr gene polymorphism with severity of PE and its correlation to fetal Doppler flow indices.

Materials and methods

All participants were recruited from the Obstetrics Outpatient Clinic and Emergency Ward at Kasr El Ainy, Cairo University. The study was conducted on 250 pregnant (20–40 years old) women with gestational age greater than or equal to 28 weeks (28–40 weeks). The control group included 130 normotensive pregnant women not having any medical diseases. It was considered by absence of hypertension (SBP <140 mmHg and DBP <90 mmHg) and absence of proteinuria. They are matched with preeclamptic patients for maternal age, parity, and gestational age upon sampling.

The preeclamptic patients represented 120 cases, divided into two groups according to the disease severity: 60 cases of mild PE and 60 cases of severe PE. Mild PE cases are selected by the presence of SBP greater than or equal to 140 and less than 160 mmHg, DBP greater than or equal to 90 and less than 110 mmHg, and mild proteinuria + 1, with mild or no associated symptoms [10].

Severe PE cases were considered by the following criteria: symptoms such as persistent headache, epigastric pain, visual disorders, and oliguria less than 30 ml/h; signs such as sustained SBP of more than or equal to 160 mmHg or a sustained DBP of more than or equal to 110 mmHg or rise of SBP by 30 mmHg and DBP by 15 mmHg on two different occasions; investigations, such as proteinuria measured as +2 or more dipstick or 24 h urine collection with more than or equal to 2 g, oliguria or creatinine more than or equal to 1.2 mg; or laboratory findings characteristic of HELLP syndrome (elevated liver enzymes and low platelet count), and fundus findings of papilledema or retinal exudates [10].

Exclusion criteria were age less than 20 years or more than or equal to 40 years; women who were complicated with multiple gestations; women with fetal congenital malformation; women with fetal chromosomal disorders; and women with maternal history of cardiovascular, renal, or other hypertension-associated diseases, or gestational/pregestational diabetes, or autoimmunological diseases.

All patients were subjected to detailed history taking, complete physical examination, routine baseline investigations, blood pressure measurement, and Doppler studies using the apparatus (GE Voluson 730 Pro (General Electric, GE medical systems Tecnicare Healthcare Company. 8311 NW 64th. Street, No.6, Miami, FL 33166, USA) with Doppler unit and convex linear transducer 3.5 MHz) determining resistance index (RI) and pulsatility index (PI) of umbilical artery (UA).

RI is calculated as (peak systolic velocity – end-diastolic velocity)/peak systolic velocity [(PSV-EDV)/PSV)]. Normal (low resistance) is RI less than 0.55. High resistance may be bilateral notches RI more than 0.55 or unilateral notches RI more than 0.65. PI is calculated as (peak systolic velocity – end diastolic velocity)/mean velocity [(PSV – EDV)/Vm].

PCR restriction fragment length polymorphism (RFLP) assay was done for detection of Ala40Thr genetic polymorphisms as follows:

Sample collection for PCR-RFLP

Overall, 3 ml of venous blood was collected on ethylene diamine tetra-acetic acid by sterile venipuncture using a sterile vacutainer tube. Samples were stored at –20°C until DNA extraction.

Genotyping for PCR-RFLP

DNA extraction was done by QIAamp DNA Blood Mini kit provided by Qiagen (QIAGEN Manchester Limited, Skeleton House, Lloyd Street North, Manchester M156SH, United Kingdom). (catalog no. 51106).

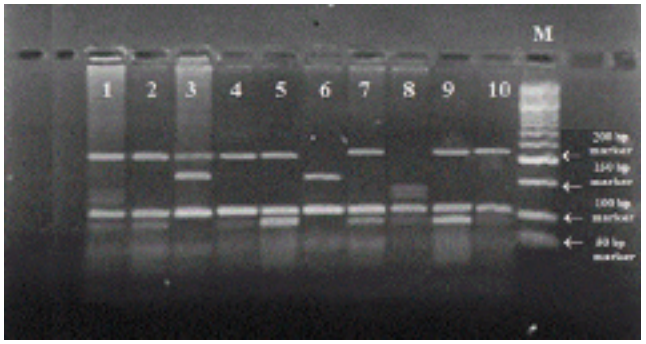
For DNA amplification, ready-to-use Dream Taq Green PCR Master Mix (2x) (Fermentas, Lithuania) was used. All reactions were performed in a total volume of 25 μl. Primers were provided by Fermentas–Lithuania with forward sequence: 5′-GCG ATA ATG GGG TCC CTG AGA T-3′ and reverse sequence: 5′-GCT GCC GGA AGA GGA CGA C-3′. The thermocycler program was conducted for amplification of Ala40Thr gene with initial denaturation at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 30 s, and then final extension step at 72°C for 10 min.

The amplified product (356 bp) was digested by FD Hha1 enzyme provided by thermo scientific protocols. After incubation with the restriction enzyme, the wild genotype produced AA bands of 213, 106, and 73 bp. The homozygous variant produced GG bands of 163, 106, 48, and 37 bp, whereas the heterozygous variant produced AG bands of 213, 163, 106, 48, and 37 bp. Visualization of the restricted fragments was done by ethidium bromide-stained agarose gel electrophoresis (Fig. 1) [11].

Data were analyzed using IBM SPSS advanced statistics version 17 (SPSS Inc., Chicago, Illinois, USA). Numerical data were expressed as mean and SD or median and range as appropriate. Qualitative data were expressed as frequency and percentage. χ^2 test or Fisher's exact test was used to examine the relation between qualitative variables. For quantitative not normally distributed data, comparison between two groups was done using Mann–Whitney test/Wilcoxon rank sum. Comparison between three groups was done using Kruskal–Wallis test (nonparametric analysis of variance). Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation. All tests were two-tailed. A *P* value less than 0.05 was considered significant.

The study was approved by Ethical Committee of Faculty of Medicine, Cairo University. All procedures performed in the study involving human participants were in accordance with the ethical standards of the Faculty's Ethical Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Moreover, all the required tests were conducted with the understanding and the consent of each participant.

Figure 1



Identification of SOD3 Ala40Thr genotypes by PCR- restriction fragment length polymorphism.

Results

The demographic data (age and parity) of the controls and cases are matched, with *P* value more than 0.05 (Table 1).

The gestational age at delivery (GA) was earlier in severe and mild cases compared with controls (Table 2). There was a significant difference in GA between control and mild cases as well as between controls and severe cases, with *P* value less than 0.001 in both. However, there was no difference between mild and severe cases, with *P* value 0.7.

Regarding fetal birth weight (FBW), it was lower in severe and mild cases compared with controls (Table 2). This was obvious between controls and severe cases, with *P* value 0.001. However, this difference was not clear between controls and mild cases and between mild and severe cases, with *P* value 0.192 and 0.135, respectively.

Regarding the clinical characteristics, SBP and DBP in controls were lower than in mild PE and in severe cases; this was highly statistically significantly different, with *P* value less than 0.001 (Table 2).

Regarding the Doppler flow indices, RI showed higher median value in severe and mild cases compared with controls, as shown in Table 2. That was clear when comparing RI in controls with mild cases as well as with severe cases, with *P* value less than 0.001 in both

Table 1 Demographic data of the controls and cases

	Control	Mild	Severe	*P**
Age (mean±SD)	28.7±4.89	30.03±3.89	30.03±3.52	0.242
Number of primigravida	70	20	32	0.151
Number of multipara	60	40	28	

**P* value is significant if <0.05.

Table 2 Clinical characteristics of the controls and cases

	Control (median/range)	Mild (median/range)	Severe (median/range)	*P**
SBP (mmHg)	120 (95-130)	145 (140-150)	180 (140-200)	<0.001
DBP (mmHg)	80 (60-90)	97.5 (90-100)	110 (95-120)	<0.001
GA (weeks)	37 (36-39)	35 (31-37)	35.5 (30-39)	<0.001
FBW (g)	2700 (2600-2850)	2670 (1688-2930)	2530 (1550-2950)	0.005
RI	0.56 (0.5-0.6)	0.69 (0.50-0.90)	0.7 (0.56-0.9)	<0.001
PI	1.1 (1-1.2)	1.1 (1-1.2)	1.1 (1-1.2)	0.126

DBP, diastolic blood pressure; FBW, fetal birth weight; GA, gestational age; SBP, systolic blood pressure. **P* value is significant if <0.05.

Table 3 Results of genotypes and alleles of controls and cases

	Control	Mild	Severe	*P**
Genotype [*n* (%)]				
AA	74 (56.9)	26 (43.3)	24 (40)	
AG	50 (38.5)	34 (56.7)	24 (40)	0.013
GG	6 (4.6)	0	12 (20)	
Allele (%)				
A	76.2	71.7	60	0.073
G	23.8	28.3	40	

**P* value is significant if<0.05.

comparisons. In contrast, there was no statistically significant difference in RI between mild and severe cases, with *P* value 0.132.

The PI was statistically significant higher in severe cases compared with mild cases, with *P* value 0.044. There was no statistically significant difference regarding it on comparison among the three groups. Moreover, no difference was seen between the controls and mild cases and the controls and severe cases, with *P* value 0.412 and 0.139, respectively.

Results of SOD3 Ala40Thr genotyping

There was a statistically significant difference regarding gene polymorphism between the control, mild, and severe cases, with a *P* value 0.013 (Table 3). The mutant homozygous genotype GG showed higher risk compared with wild-type genotype AA, with *P* value 0.007.

Regarding the comparison of controls with severe cases, the frequency of gene polymorphisms AA, AG, and GG showed statistically significant difference, with *P* value 0.043. The mutant genotype GG showed higher risk in severe cases rather than in controls; this was statistically significant (*P* = 0.02, OR = 6.167, and 95% CI = 1.334–28.514). The mutant allele G showed higher risk compared with wild allele; this was statistically significant (*P* = 0.022, OR = 2.129 and 95% CI = 1.106–4.1).

Comparing the mild cases with severe cases, the frequency of gene polymorphisms AA, AG, and GG showed statistically significant difference, with *P* value 0.032. The mutant genotype GG showed higher risk in severe cases rather than in mild cases; this was statistically significant (*P* = 0.028, OR = 0.48, and 95% CI = 0.319–0.722).

The GA was lower in the mutant genotypes AG and GG compared with wild-type genotype AA. This was statistically significant, especially when compared between heterozygous mutant genotype AG with wild-type genotype AA (Table 3). The EFW and RI in the three genotypes showed no statistically significant difference (Table 4).

The PI showed statistically significant difference among the three genotype, which was obvious between homozygous mutant genotype GG and wild-type genotype AA (Table 4).

Discussion

PE is a multisystem pregnancy-induced hypertensive disorder**.** Oxidant imbalance could play a central role in pathogenesis of PE. Specific biomarker detection for high-risk pregnancy early before the onset of the disease is very precious. SOD is one of the primary antioxidant system protections that catalyze removal of oxidant radicals [12].

The correlation of SOD Ala40Thr gene polymorphism with severity of PE and its relation to Doppler studies of UA was investigated in our study. The age in our work ranged between 20 and 42 years, with a mean age of 31 years. This was inconsistent with the study of Pongrojpaw *et al.* [13], where the mean age was 36.8 years. The younger age of the patients in our study could be related to our culture in having early pregnancy at a young age.

The GA for the majority of our controls was 37–41 weeks, with a mean value of 39 weeks, and for cases was 33–36 weeks, with a mean value of 34.5 weeks. This came in agreement with the work of Yu *et al.* [14], who reported that the mean value for GA for unaffected cases was 40 weeks; however, for those who developed PE was 37 weeks. The earlier time of delivery in our study might indicate the higher degree of severity in our patients who developed PE, and this

Table 4 Results of SOD3 Ala40Thr genotypes with the clinical characteristics

	AA (median/ range)	AG (median/ range)	GG (median/ range)	*P** comparison 3 genotypes	*P** comparison AA and AG	*P** comparison AA and GG
GA (weeks)	37 (34-37)	36 (30-38)	36 (32-39)	0.021	0.009	0.104
FBW (g)	2700 (1950-2950)	2660 (1550-2930)	2660 (2100-2844)	0.382	0.418	0.148
RI	0.59 (0.5-0.86)	0.6 (0.5-0.9)	0.69 (0.5-0.9)	0.226	0.280	0.140
PI	1.1 (1-1.1)	1.1 (1-1.2)	1.1 (1.1-1.2)	0.023	0.144	0.007

FBW, fetal birth weight; GA, gestational age. **P* value is significant if<0.05.

shows that our work also revealed significantly lower estimated FBW in the preeclamptic patients compared with the controls.

Regarding RI and PI in the umbilical cord Doppler flowmetry, our study revealed that preeclamptic patients have higher RI than the control group ($P < 0.001$) but the PI showed no significant difference. Aali *et al.* [15], in agreement with our study, showed higher RI in patients with PE than the control group ($P < 0.001$), but in contrast to our work, they demonstrated PI also higher in patients with PE than the control group ($P < 0.001$).

This is explained as in the fetal risk in PE, abnormal umbilical flow patterns, especially by serial measurements in combination with fetal biometry, can predict chronic placental dysfunction with intrauterine growth restriction (IUGR) [11]. In the normal fetus, the resistance to flow (impedance) decreases in the UA due to increased numbers of tertiary stem villi as the placenta matures. The UA impedance indices increase when there is decreased end-diastolic flow owing to reduced placental perfusion and uteroplacental insufficiency as is seen in IUGR [16].

In the present study, Ala40Thr gene polymorphism genotype frequencies in controls and mild and severe cases showed statistically significant difference, with no difference regarding allelic frequency.

There was no statistically significant difference regarding genotypes and allele frequencies between mild cases of PE and the controls. However, a statistically significant difference was estimated between the control group and severe PE cases as well as between mild and severe cases, with higher risk of the mutant genotype GG and allele G to severe PE. The mutant genotype GG increases the risk of severe PE by 6 folds (OR = 6.167 and 95% CI = 1.334–28.514).

There was no statistically significant difference regarding FBW and RI among the three genotypes. GA and PI showed statistically significant difference between the wild and mutant genotypes.

In contrast to our finding, Rosta *et al.*[17] demonstrated no significant differences in the genotype and allele frequencies of the SOD3 Ala40Thr polymorphism between preeclamptic patients and control subjects. The mutant allele carriers of this polymorphism showed an increased risk for severe fetal growth restriction-complicated PE (OR: 6.07, 95% CI: 1.33–27.8, $P = 0.020$).

The Ala40Thr substitution is located in the amino-terminal domain of EC-SOD. It was observed to be associated with increase liability to type 2 diabetes, especially, if associated with insulin resistance and hypertension. Hypertension and insulin resistance are also features of the maternal syndrome of PE [18]. However, our finding that the SOD3 Ala40Thr SNP was associated with PE especially if the disease was complicated by preterm labor with IUGR in severe PE suggests the role of this polymorphism in the liability to uteroplacental insufficiency and resultant impaired fetal growth rather than the hypertensive maternal syndrome of PE.

As this substation leads to oxidative stress with an imbalance in the oxidant/antioxidant activity, subsequent impaired placental perfusions complicated by PE/IUGR are commonly observed. So Ala40Thr gene polymorphism seems to play a central role in the pathogenesis of PE [9].

As shown in animal experiment, preinfusion of antioxidants SOD and catalase countered the evolution of IUGR by reperfusion of ischemia [19]. Antioxidant levels were found to be markedly diminished in maternal plasma, umbilical cord, and placenta of group affected with IUGR than in control group [20]. Moreover, SOD activity in placentas of pregnancies with PE alone was found to be higher than in placentas from pregnancies complicated by PE and IUGR [21].

Several studies have found the high frequency of SOD3 SNPs in pregnancies with PE, with diminished levels of antioxidants, such as vitamin E, SOD, and glutathione peroxidase in the placental tissues of those women [22]. In the presence of reduced levels of SOD, SO (super oxide) interacts with NO, which results in a strong oxidant named peroxy-nitrite (ONOO-), that causes lipid peroxidation [23].

Yuvaci *et al.*[24] found lower native/total thiol ratios in the patients of PE than control with existence of high

oxidative stress. Moreover they revealed that patients of severe PE had more oxidative stress than those of mild PE.

Despite some limitations, mainly lack of financial supports that limit the number of our studied groups, this study still contributes to the understanding of the role of SOD3 polymorphisms in the development of PE and provides insight to their frequencies in Egypt. A larger number of enrolled cases and detection of other polymorphisms for SOD are recommended in future studies.

Conclusion

Our study suggests that SOD3 Ala40Thr gene polymorphism has important role in development of PE. Mutant variant was more encountered in severe cases. Earlier GA, lower FBW, and higher RI were observed in our preeclamptic cases. Higher PI and earlier GA were noticed in the mutant genotypes. Follow-up of RI and PI with SOD3 genotyping can be used as early predictors of PE, with avoidance of preterm labor and IUGR.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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