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Association between endoglin/transforming growth factor beta receptors 1, 2 gene polymorphisms and the level of soluble endoglin with preeclampsia in Egyptian women

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ABSTRACT

Introduction: Preeclampsia (PE) is a pregnancy-specific hypertensive disease whose etiopathogenesis remains unclear.

Objectives: This study was designed to assess association between PE and 3 single nucleotide polymorphisms (SNPs): *ENG*(G/A) rs11792480, *TGFβR1*(A/C) rs10739778 and *TGFβR2*(G/A) rs6550005, beside circulating soluble endoglin (sENG), oxidative stress biomarkers and nitric oxide (NO) in Egyptian women.

Methods: The study included 75 preeclamptic women stratified into 4 clinical subgroups and 50 normotensive pregnant women. Genotyping was performed by real time polymerase chain reaction-Taqman allelic discrimination.

Results: Preeclamptic women showed significantly increased sENG and malondialdehyde (MDA), decreased total antioxidant capacity (TAC), endothelial nitric oxide synthase (eNOS) and NO, without change in transforming growth factor beta 1 (TGFβ1) versus controls. Moreover, sENG was significantly higher in severe and early than mild and late PE. Higher MDA and lower TAC and NO were observed in severe than mild PE. *ENG*(G/A) and *TGFβR2*(G/A) showed no association with PE. However, CC genotype of *TGFβR1*(A/C) was more frequent in controls than either PE, early-onset or severe revealing a reduced PE risk in CC genotype versus AA or AA + AC. Importantly, patients carrying AA genotype had higher SBP and MDA with lower TAC, gestational age at delivery (GA) and birth weight than those carrying CC genotype.

Conclusions: Excessive sENG release with decreased eNOS/NO may be involved in PE pathogenesis. Women who carry C allele or CC genotype of *TGFβR1*(A/C) may be less prone to develop PE.

1. Introduction

Preeclampsia (PE) is a pregnancy-specific disorder that affects about 5–8% of pregnancies worldwide and is a major cause of maternal and fetal morbidity and mortality [1]. The pathogenesis of PE is not fully understood; however, it is believed to be initiated by reduced placental perfusion secondary to inadequate trophoblast invasion and defective spiral artery remodelling. The persisted state of placental underperfusion produces placental hypoxia and local oxidative stress, resulting in endothelial dysfunction, leading to the onset of the clinical symptoms of PE [2]. Growing evidence indicates that transforming growth factor beta (TGFβ) and its co-receptor endoglin (ENG) can be involved in PE pathogenesis through activation of endothelial cell pathway [3].

ENG is a *trans*-membrane glycoprotein that serves as a co-receptor of TGFβ signaling system. It is expressed on vascular endothelial cells [4], synctyiotrophoblasts and invasive cytotrophoblasts [5]. ENG modulates TGFβ signal transduction via interaction with a receptor complex comprised of TGFβ receptor (TGFβR) type I and II [6]. After activation of this complex, TGFβ signals are transmitted from the cell-surface receptors to the nucleus regulating endothelial nitric oxide synthase (eNOS) expression and activation [7,8]. ENG may be involved in the regulation of placental implantation and spiral artery remodelling during pregnancy through regulation of nitric oxide (NO) dependent vasodilatation [9].

Importantly, inhibition of ENG translation in first trimester improves the invasive capacity of trophoblast cells and increases placental perfusion during pregnancy [10]. In PE, ENG gene expression has been

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List of abbreviations

χ^2	Chi-square
95% CI	95% confidence interval
ANOVA	Analysis of variance
BP	blood pressure
DBP	Diastolic blood pressure
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme linked immunosorbant assay
ENG	Endoglin
eNOS	Endothelial nitric oxide synthase
GA	Gestational age
HWE	Hardy Weinberg equilibrium
MDA	Malondialdehyde
NO	Nitric oxide
NOx	Nitrate-nitrite
OR	Odds ratio
PCR	Polymerase chain reaction
PE	Preeclampsia
SBP	Systolic blood pressure
SE	Standard error
sENG	Soluble endoglin
SNPs	Single nucleotide polymorphisms
SPSS	Statistical Package for the Social Science
TAC	Total antioxidant capacity
TGFβ1	Transforming growth factor beta 1
TGFβR1	Transforming growth factor beta receptor 1
TGFβR2	Transforming growth factor beta receptor 2

reported to be increased in placenta and/or cellular/non-cellular components of blood [11]. Soluble endoglin (sENG) is also released into circulation after cleavage of *trans*-membrane ENG by matrix metalloproteinase-14. This sENG is believed to play an anti-angiogenic effect by limiting the availability of TGFβ1 and other members of the TGFβ family as bone morphogenetic proteins to their receptors, thereby preventing downstream signaling in activation of eNOS and vasodilatation [12,13].

PE is a multifactorial disorder that results from complex interactions between genetic and environmental factors [14]. Genetic factors are believed to be among the main risk factors for PE development [15], a widespread research for identifying an association between a number of genes and susceptibility to development of the disease has been attempted among which, ENG pathway genetic variations have been part of these investigations. In USA, Bell et al. [16] had examined a number of single nucleotide polymorphisms (SNPs) in ENG pathway among American black and white women to assess their possible association with PE development, their results suggested that the pathway's involvement in PE differed in whites and blacks, with *ENG* and *TGFβR2* being associated in whites and *TGFβ1*, *TGFβR1* and *TGFβR2* being associated in blacks. In addition, Schmella et al. [17] had validated the association of ENG pathway genetic variation with PE in Norwegian and Latina populations providing further support for the involvement of ENG pathway in PE. In Egyptian women the analysis of ENG pathway genetic variation and its possible association with development of PE have not yet been investigated. Therefore, the present study was directed to examine the possible association between PE and 3 SNPs: *ENG*(G/A) rs11792480, *TGFβR1*(A/C) rs10739778 and *TGFβR2*(G/A) rs6550005, beside the circulating level of sENG, oxidative stress biomarkers and NO in various clinical manifestations of PE among Egyptian women.

2. Patients and methods

2.1. Studied populations

This study included 125 pregnant women recruited from EL-Galaa teaching hospital of Obstetrics and Gynecology, Cairo, Egypt between April 2016 to December 2016. Of these 125 women, 75 were preeclamptic patients and 50 were normotensive pregnant women matched for age and gestational age (GA) at sampling, where their mean ages were 28.09 ± 0.63 and 27.98 ± 0.78 years, respectively. All women provided informed consent before sample collection and use for research purposes under the protocol approved by the ethical committee of Faculty of Pharmacy, Cairo University (BC 1728).

The criteria for defining PE were systolic blood pressure (SBP) ≥ 140 mmHg and/or diastolic blood pressure (DBP) ≥ 90 mmHg on at least two occasions 4 h to 1 week apart, in the presence of proteinuria (≥300 mg/24 h or ≥ +1 on a standard urine dipstick) arising only after 20 weeks of gestation. On the other side, controls had BP ≤ 120/80 with no history of hypertension or proteinuria. The preeclamptic women (n = 75) were divided into subgroups based on clinical manifestations: the disease onset-time [early (n = 34) vs. late (n = 41)] and severity [severe (n = 52) vs. mild (n = 23)]. Early-onset PE was defined as clinical manifestations occurring < 34 weeks and late-onset PE ≥ 34 weeks. Severe PE was defined by presence of one or more of the following criteria: SBP ≥160 mmHg and/or DBP ≥110 mmHg, proteinuria (≥2 g/24 h or at least +2 or more by dipstick), persistent headache, visual disturbances, upper abdominal pain, serum creatinine and transaminase elevation, thrombocytopenia and fetal growth retardation as described by the American college of obstetricians and gynecologists [18]. Exclusion criteria common for the patients and controls were chronic hypertension, chronic renal diseases, diabetes mellitus and multiple pregnancies.

Table 1
Demographic, clinical and biochemical measurements of the studied populations.

Variable	Controls n = 50	Preeclampsia n = 75
Age (years)	28.12 ± 0.81	28.09 ± 0.63
SBP (mmHg)	114.40 ± 1.21	154.40 ± 1.72***
DBP (mmHg)	75.40 ± 0.95	99.07 ± 1.025***
Proteinuria		
+1 n (%)	–	13 (17.33%)
+2 n (%)		25 (33.33%)
+3 n (%)		35 (46.66%)
+4 n (%)		2 (2.66%)
Parity n (%)		
Nulliparous	14 (28%)	37 (49.33%)
Multiparous	36 (72%)	38 (50.66%)
GA at delivery (weeks)	38.28 ± 0.13	34.90 ± 0.34***
BMI (Kg/m^2)	33.01 ± 0.36	34.61 ± 0.36**
Birth weight (g)	3109 ± 30.84	2201 ± 77.30***
AST (U/L)	19.62 ± 0.89	24.59 ± 1.17**
ALT (U/L)	17.04 ± 1.08	22.3 ± 1.38**
Creatinine (mg/dL)	0.74 ± 0.02	0.79 ± 0.02
Uric acid (mg/dL)	4.27 ± 0.15	5.48 ± 0.18***
Hemoglobin (g/dL)	11.02 ± 0.18	10.81 ± 0.13
Platelets $x10^3/\mu L$	241 ± 8.87	210.30 ± 8.74*
sENG (pg/mL)	1696 ± 28.37	1974 ± 44.13***
MDA(nmol/mL)	6.78 ± 0.48	12.56 ± 0.58***
TAC (μmol/L)	331.50 ± 11.72	247.30 ± 7.83***
eNOS(pg/mL)	175.20 ± 2.75	153.40 ± 2.53***
NOx (μmol/L)	121.40 ± 3.31	89.43 ± 2.90***
TGFβ1(ng/L)	695.30 ± 13.57	677.40 ± 7.87

Qualitative data were presented as numbers & percentages, quantitative data were presented as mean ± SE, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, GA: gestational age, BMI: body mass index, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, sENG: soluble endoglin, MDA: malondialdehyde, TAC: total antioxidant capacity, eNOS: endothelial nitric oxide synthase, NOx: nitric oxide and TGFβ1: transforming growth factor beta 1, significantly different at *P < 0.05, **P < 0.01 and ***P < 0.001.

Table 2
Demographic and clinical characteristics of preeclampsia subgroups.

Variable	Preeclamptic women			
	Early (n = 34)	Late (n = 41)	Mild (n = 23)	Severe (n = 52)
Age (years)	27.38 ± 0.82	28.68 ± 0.93	28.39 ± 1.16	27.96 ± 0.75
SBP (mmHg)	155 ± 2.902	155.10 ± 2.01	142.60 ± 0.93	160.60 ± 1.98 [e]
DBP (mmHg)	100 ± 1.52	100.20 ± 1.37	93.04 ± 0.98	103.30 ± 1.15 [e]
Parity n (%)				
Nulliparous	20 (58.82%)	17 (41.46%)	10 (43.47%)	27 (51.92%)
Multiparous	14 (41.17%)	24 (58.53%)	13 (56.52%)	25 (48.07%)
GA at delivery (weeks)	32.74 ± 0.49 [b]	36.61 ± 0.25	35.91 ± 0.52	34.38 ± 0.42 [c]
BMI (Kg/m^2)	32.59 ± 0.38 [b]	36.09 ± 0.43	34.95 ± 0.59	34.45 ± 0.45
Birth weight (g)	1748 ± 111.10 [b]	2576 ± 63.28	2493 ± 110.10	2071 ± 95.50 [c]
AST (U/L)	25.30 ± 1.86	24.37 ± 1.49	18.13 ± 1.38	27.57 ± 1.41 [e]
ALT (U/L)	24.22 ± 2.34	23.11 ± 1.78	13.68 ± 1.35	24.46 ± 1.68 [e]
Creatinine (mg/dL)	0.83 ± 0.03	0.76 ± 0.02	0.72 ± 0.03	0.82 ± 0.02 [c]
Uric acid (mg/dL)	6.04 ± 0.31 [a]	5.15 ± 0.19	4.53 ± 0.30	6.002 ± 0.205 [e]
Hemoglobin (g/dL)	10.68 ± 0.209	10.91 ± 0.17	11.03 ± 0.203	10.74 ± 0.14
Platelets $x10^3/\mu L$	201.70 ± 12.76	220.10 ± 12.03	245.60 ± 17.57	194.80 ± 9.24 [d]

Qualitative data were presented as numbers & percentages, quantitative data were presented as mean ± SE, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, GA: gestational age, BMI: body mass index, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, significantly different at [a]$P < 0.05$ and [b]$P < 0.001$: versus late preeclamptic women, [c]$P < 0.05$, [d]$P < 0.01$ and [e]$P < 0.001$: versus mild preeclamptic women.

2.2. Blood sampling

Immediately after collecting 5 ml of venous blood from each participant in EDTA-coated tubes, they were divided into 2 portions, one portion for DNA extraction and genotyping and the other was centrifuged at 3000 rpm for 15 min at 4 °C to collect plasma and the separated plasma was stored at −80 °C until further analysis.

2.3. DNA extraction and genotyping

Genomic DNA was extracted from whole blood of patients and controls using a commericially available kit according to the manufacturer's instructions (QIamp DNA blood minikit, Qiagen, Germany). DNA samples were quantitated using the NanoDrop 2000c spectrophotometer at 260 nm (Thermo Fisher Scientific, Waltham, MA, USA) and stored at −20 °C. Genotyping was performed by real time polymerase chain reaction (PCR)-Taqman allelic discrimination using Viia 7 real time PCR system (Applied biosystems). 3 SNPs: *ENG*(G/A) rs11792480, *TGFβR1*(A/C) rs10739778 and *TGFβR2*(G/A) rs6550005 were analyzed in the extracted DNA by using specific primers and Taqman probes (Taqman genotyping assays, Applied Biosystems, USA). PCR reactions were carried out with an initial denaturation step of 10 min at 95 °C, followed by 40 cycles at 95 °C for 15 s, annealing and extension at 60 °C for 1 min.

2.4. Biochemical analysis

Plasma concentrations of sENG, eNOS and TGFβ1 were determined using enzyme linked immunosorbant assay (ELISA) kits (Glory Science Ltd, USA) by BioTek ELISA reader at wavelength 450 nm for all of them according to the manufacturer's instructions. Their concentrations were presented as pg/mL for both sENG and eNOS and ng/L for TGFβ1. Plasma malondialdehyde (MDA) and total antioxidant capacity (TAC) concentrations were determined colorimetrically using the method of Mihara and Uchiyama [19] and Benzie and Strain [20], respectively. Plasma NOx (nitrate/nitrite) concentration was determined colorimetrically using the method of Miranda et al. [21] with small modification using zinc sulfate instead of ethanol for precipitation of plasma proteins [22]. Their concentrations were presented as nmol/mL for MDA and μmol/L for both TAC and NOx.

2.5. Statistical analysis

Parametric data were reported as mean ± standard error (SE). Comparisons between 2 groups were made by unpaired student t-test for parametric variables. Comparisons between 3 continuous variables were determined by one way analysis of variance (ANOVA) followed by post hoc (Tukey test). Non Parametric data were statistically described in terms of frequencies (number of cases) and percentages. Comparisons between the study groups were done using Chi-square (χ^2) test. Genetic analysis was performed by calculating the allelic frequency, calculating the odds ratio (OR) with the 95% confidence interval (95% CI) and Hardy Weinberg equilibrium (HWE) analysis. $P < 0.05$ was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows and Graphad prism 6.

3. Results

3.1. Demographic, clinical and biochemical assessment

The demographic, clinical and biochemical measurements of controls and patients are shown in Table 1. No statistical significant differences were observed in maternal age and GA at sampling. As expected, higher systolic and diastolic blood pressures were found in PE, in addition to proteinuria was found only in patients. Importantly, preeclamptic women showed significantly increased plasma levels of sENG and MDA, decreased plasma levels of TAC, eNOS and NOx, without significant changes in TGFβ1 levels when compared to controls. The clinical differences in PE subgroups are summarized in Table 2. In preeclamptic patients, the significant elevation of sENG level was mainly contributed by its significant increase in both severe and early-onset PE groups, where sENG level was significantly higher in severe than mild PE and in early-than late-onset PE (Fig. 1A). The present study demonstrated a close relationship between degree of oxidative stress and the severity of disease where MDA and TAC levels differed significantly being higher and lower respectively in severe than mild PE. On the other hand, no significant differences in MDA and TAC levels were found between early- and late-onset PE (Fig. 1B and C). Moreover, no significant difference was detected in eNOS level when comparing severe to mild and early to late PE (Fig. 1D). Whereas, NOx level was significantly lower in severe than mild PE (Fig. 1E). With respect to TGFβ1, no significant differences were found in their plasma

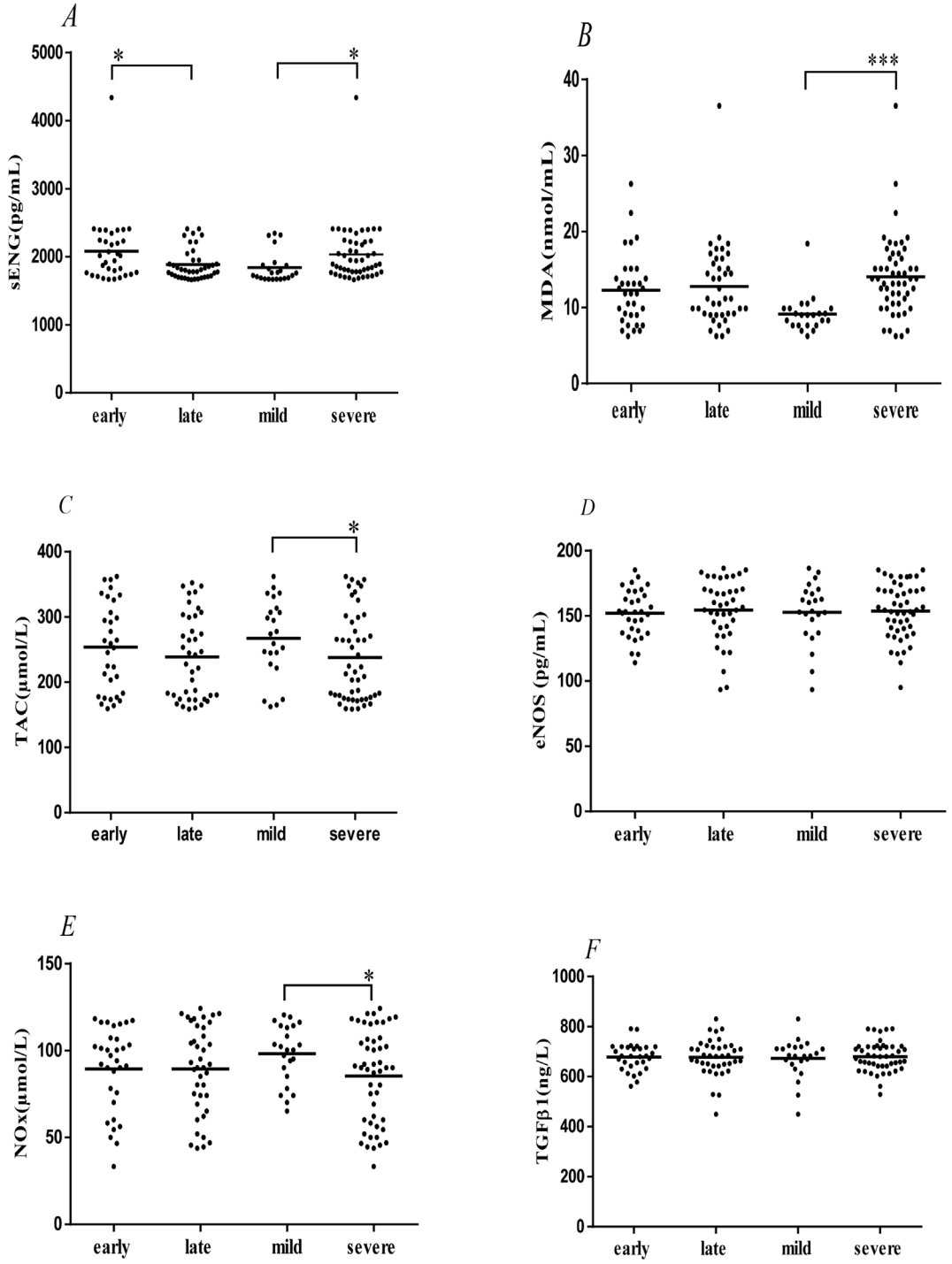

Fig. 1. Plasma levels of sENG (A), MDA (B), TAC (C), eNOS (D), NOx (E) and TGFβ1(F) in different preeclampsia subgroups, horizontal bars represent mean values, sENG: soluble endoglin, MDA: malondialdehyde, TAC: total antioxidant capacity, eNOS: endothelial nitric oxide synthase, NOx: nitric oxide and TGFβ1: transforming growth factor beta 1, significantly different at *P < 0.05 and ***P < 0.001.

levels between different subgroups based on severity and onset-time of the disease (Fig. 1F).

3.2. Genetic analysis

The observed genotype distributions of *ENG*(G/A) rs11792480 and *TGFβR2*(G/A) rs6550005 were in agreement with **HWE** in PE and controls. On the other side, *TGFβR1*(A/C) rs10739778 was in agreement with **HWE** in PE but not in controls. Results of genotypic association tests are shown in Table 3, upon comparing patients to controls, neither *ENG*(G/A) nor *TGFβR2*(G/A) showed any significant difference in co-dominant, dominant and recessive models and in allele frequencies distribution indicating no association of these genotypes with PE risk. With respect to *TGFβR1*(A/C), the homozygous CC genotype was more frequent in controls than PE (26% vs. 8%, respectively) and significantly reduced PE risk was found to be associated with CC genotype in co-dominant and recessive models (CC vs. AA: OR = 0.26, 95% CI = 0.08–0.78, P = 0.01; CC vs. AA + AC: OR = 0.24, 95% CI = 0.08–0.705, P = 0.006), in addition to a less frequency of C allele in PE (28%) than controls (41%) (OR = 0.56, 95% CI = 0.32–0.95,

Table 3
Genotype and allele frequencies of *ENG* (G/A) rs11792480, *TGFβR1* (A/C) rs10739778 and *TGFβR2* (G/A) rs6550005 among controls and patients and their association with preeclampsia risk.

Genetic Models/Alleles	Genotype (s)	Controls n (%)	PE n (%)	OR (95%CI)	P value
ENG(G/A)					
Co-dominant model	GG	21 (42%)	30 (40%)	Ref.	–
	GA	21 (42%)	36 (48%)	1.20 (0.55–2.605)	0.6
	AA	8 (16%)	9 (12%)	0.78 (0.26–2.37)	0.6
Dominant model	GG	21 (42%)	30 (40%)	Ref.	–
	GA + AA	29 (58%)	45 (60%)	1.08 (0.52–2.24)	0.8
Recessive model	GG + GA	42 (84%)	66 (88%)	Ref.	–
	AA	8 (16%)	9 (12%)	0.71 (0.25–2.002)	0.5
Alleles	G	63 (63%)	96 (64%)	Ref.	–
	A	37 (37%)	54 (36%)	0.95 (0.56–1.62)	0.8
TGFβR1(A/C)					
Co-dominant model	AA	22 (44%)	39 (52%)	Ref.	–
	AC	15 (30%)	30 (40%)	1.12 (0.501–2.53)	0.7
	CC	13 (26%)	6 (8%)*	**0.26 (0.08–0.78)**	**0.01**
Dominant model	AA	22 (44%)	39 (52%)	Ref.	–
	AC + CC	28 (56%)	36 (48%)	0.72 (0.35–1.48)	0.3
Recessive model	AA + AC	37 (74%)	69 (92%)	Ref.	–
	CC	13 (26%)	6 (8%)**	**0.24 (0.08–0.705)**	**0.006**
Alleles	A	59 (59%)	108 (72%)	Ref.	
	C	41 (41%)	42 (28%)*	**0.56 (0.32–0.95)**	**0.03**
TGFβR2(G/A)					
Co-dominant model	GG	26 (52%)	46 (61.3%)	Ref.	–
	GA	20 (40%)	25 (33.3%)	0.706 (0.33–1.51)	0.3
	AA	4 (8%)	4 (5.3%)	0.56 (0.13–2.45)	0.4
Dominant model	GG	26 (52%)	46 (61.3%)	Ref.	–
	GA + AA	24 (48%)	29 (38.7%)	0.68 (0.33–1.408)	0.3
Recessive model	GG + GA	46 (92%)	71 (94.7%)	Ref.	–
	AA	4 (8%)	4 (5.3%)	0.64 (0.15–2.72)	0.5
Alleles	G	72 (72%)	117 (78%)	Ref.	–
	A	28 (28%)	33 (22%)	0.72 (0.405–1.29)	0.2

rs: reference single nucleotide polymorphism (SNP), OR: odds ratio, CI: confidence interval, Ref.: reference, significantly different at *P < 0.05 and **P < 0.01.

P = 0.03).

Analyses of *TGFβR1* A/C genotypes in the clinical subgroups of PE are demonstrated in Table 4. Concerning disease onset-time, CC genotype was less frequent in early-onset PE than controls (5.9% vs. 26%, respectively) and significantly lower early-onset PE risk was found to be associated with CC genotype in co-dominant and recessive models than controls (CC vs. AA: OR = 0.16, 95% CI = 0.03–0.84, P = 0.01; CC vs. AA + AC: OR = 0.17, 95% CI = 0.03–0.84, P = 0.01). In addition, C allele was less frequent in early-onset PE than controls (OR = 0.44, 95% CI = 0.22–0.88, P = 0.01). Regarding disease severity, CC genotype was less frequent in severe PE (1.9%) than either mild PE (21.7%) or controls (26%) and significantly lower severe PE risk was found to be associated with CC genotype in co-dominant and recessive models than mild PE (CC vs. AA: OR = 0.06, 95% CI = 0.007–0.66, P = 0.005; CC vs. AA + AC: OR = 0.07, 95% CI = 0.007–0.64, P = 0.004) and controls (CC vs. AA: OR = 0.05, 95% CI = 0.007–0.48, P = 0.001; CC vs. AA + AC: OR = 0.05, 95% CI = 0.006–0.44, P = 0.0004). In addition, C allele was less frequently counted in severe PE than either mild PE or controls (OR = 0.46, 95% CI = 0.22–0.98, P = 0.04 and OR = 0.43, 95% CI = 0.23–0.79, P = 0.006, respectively). These findings revealed that C allele might have a protective effect against PE and might be associated with a lower risk of developing either severe or early-onset PE.

3.3. Clinical characteristics and biochemical measurements in PE women among different TGFβR1 genotypes

As shown in Table 5, PE patients carrying AA genotype had significantly higher SBP and MDA and lower TAC level than those carrying CC genotype. On the other side, patients carrying AC genotype manifested significantly higher DBP and insignificantly higher SBP than those carrying CC genotype. With respect to pregnancy outcome, patients carrying AA genotype manifested the lowest GA at delivery resulting in delivery of neonates with the lowest birth weight. These results revealed that patients expressing CC genotype manifested less elevated BP as well as moderate modified pregnancy outcome and biochemical changes compared to those expressing AA genotype.

4. Discussion

PE development is widely accepted to be initiated by reduced placental perfusion secondary to inadequate placental implantation, which produces hypoxia with subsequent release of placental factors including sENG and local oxidative stress resulting in endothelial dysfunction [2]. sENG is believed to exert an anti-angiogenic effect in PE by limiting the availability of TGFβ1 to its receptors blocking the downstream signaling in activation of eNOS expression and vasodilatation [13]. In the present study, a marked elevation of plasma sENG together with severe decline of eNOS and NOx levels were evidenced in preeclamptic women. Regarding severity or onset-time of the disease, sENG level was significantly higher in severe than mild PE consistent with work of Perucci et al. [13] and Sachan et al. [23]; in addition, its level was significantly higher in early-than late-onset PE in agreement with the finding of Aldika Akbar et al. [24].

Disruption of the eNOS-NO pathway plays an important role in the impairment of uteroplacental adaptations during pregnancy [25]. Reduced eNOS and NOx levels observed in present preeclamptic women are responsible for the widespread vasoconstriction and hypertension recognized in PE [26]. However, conflicting data about NO alteration have been reported in PE showing either an increase [26,27], or a decrease [28,29] or even no change [30] in its level. Regarding severity or onset-time of the disease, no significant difference in eNOS level was evidenced between PE subgroups in the present study. These data are in agreement with the observations of Laskowska et al. [31] which revealed no significant difference in eNOS level between early- and late-onset PE. Whereas, NOx level was significantly lower in severe than

Table 4
Genotype and allele frequencies of *TGFβR1*(A/C) rs10739778 in the investigated Preeclampsia subgroups and its association with preeclampsia risk.

	Genotype n (%)			Genetic models								Alleles	
				Co-dominant			Dominant		Recessive		A vs. C		
	AA	AC	CC	AA vs. AC		AA vs. CC		AA vs. AC + CC		AA + AC vs. CC		OR (95% CI)	P value
				OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value		
PE													
Early	20(58.8)	12(35.3)	2(5.9)	0.63 (0.24–1.66)	0.3	0.47 (0.07–2.903)	0.4	0.605 (0.24–1.51)	0.2	0.57 (0.09–3.36)	**0.5**	0.66 (0.32–1.37)	0.2
				0.88 (0.33–2.32)	0.7	**0.16 (0.03–0.84)***	**0.01**	0.55 (0.22–1.32)	0.1	**0.17 (0.03–0.84)***	**0.01**	**0.44 (0.22–0.88)***	**0.01**
Late	19(46.3)	18(43.9)	4(9.8)	1.38 (0.55–3.48)	0.4	0.35(0.09–1.27)	0.1	0.91 (0.39–2.08)	0.8	0.307(0.09–1.03)	0.06	0.66(0.36–1.23)	0.1
Mild	10(43.5)	8(34.8)	5(21.7)	1.17 (0.37–3.66)	0.7	0.84 (0.23–3.02)	0.7	1.02 (0.37–2.76)	0.9	0.79 (0.24–2.56)	0.6	0.92 (0.45–1.88)	0.8
Severe	29(55.8)	22(42.3)	1(1.9)	0.94 (0.32–2.80)	0.9	**0.06 (0.007–0.66)**[b]	**0.005**	0.61 (0.22–1.64)	0.3	**0.07 (0.007–0.64)**[b]	**0.004**	**0.46 (0.22–0.98)**[a]	**0.04**
				1.11 (0.47–2.62)	0.8	**0.05 (0.007–0.48)****	**0.001**	0.62 (0.28–1.36)	0.2	**0.05 (0.006–0.44)*****	**0.0004**	**0.43 (0.23–0.79)****	**0.006**
Control	22 (44)	15(30)	13(26)										

Table 5
Clinical and biochemical measurements in Preeclampsia patients among different *TGFβR1* genotypes.

Genotypes	AA	AC	CC
Number	39	30	6
SBP (mmHg)	156.20 ± 2.71*	155 ± 2.18	140 ± 0
DBP (mmHg)	100 ± 1.34	102.30 ± 1.63[a]	91.67 ± 1.66
GA at delivery (weeks)	34.28 ± 0.49*	35.10 ± 0.51	37.37 ± 0.67
Birth weight (g)	2077 ± 110.10*	2247 ± 116.50	2773 ± 159.70
BMI (Kg/m^2)	33.78 ± 0.49	35.10 ± 0.51	34.62 ± 1.46
sENG (pg/mL)	2133 ± 112.50	1903 ± 44.22	1864 ± 105.20
MDA (nmol/mL)	13.63 ± 0.95*	11.99 ± 0.63	8.44 ± 0.86
TAC (μmol/L)	239.20 ± 11.96*	244.80 ± 10.80	308.60 ± 15.06
eNOS (pg/mL)	151.90 ± 3.86	153.10 ± 3.72	163.90 ± 6.39
NOx (μmol/L)	90.09 ± 4.71	87.41 ± 3.75	95.60 ± 9.22
TGFβ1(ng/L)	683.10 ± 10.97	671.90 ± 12.24	671.30 ± 32.82

Data represented as mean ± SE, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, GA: gestational age, BMI: body mass index, sENG: soluble endoglin, MDA: malondialdehyde, TAC: total antioxidant capacity, eNOS: endothelial nitric oxide synthase, NOx: nitric oxide and TGFβ1: transforming growth factor beta 1, significantly different at *P < 0.05: AA versus CC and [a]P < 0.05: AC versus CC.

mild PE in the present study consistent with the work of Mao et al. [32].

TGFβ1 is a multifunctional cytokine involved in the regulation of trophoblast invasion, proliferation and differentiation [3], suggesting its role in PE pathogenesis, but the results about its levels in PE are inconsistent. In the present study and in accordance with previous work [13,33], no significant difference was observed in TGFβ1 in preeclamptic group compared to controls and among PE subgroups. However, elevation [34,35] or decline [3] in TGFβ1 have been recorded by others. Such controversy in the foregoing results may be due to differences in gestational age at sampling, severity, variation in procedures for obtaining and processing blood samples and assay techniques in the different studies [13].

In the current investigation, PE patients were found in state of oxidative stress indicated by marked increment in plasma MDA together with a remarkable decrement in plasma TAC as compared with controls. These results are in agreement with the finding of Kashinakunti et al. [36] and Adeniji and Oparinde [37]. In fact, oxidative stress is a consequence of disease process and activity in PE patients where oxidative damage is thought to be of vascular sources, since PE seemed to be associated with defective spiral artery remodelling [38], richness of placenta in macrophages [39], besides activation of NADPH oxidase [40]. Under these conditions, increased free radicals production, enhanced peroxidative damage to various tissue components and depletion of endogenous antioxidants would be expected to trigger greater oxidative changes with reduced NO bioavailability among our patients. Moreover, the present study demonstrated a close relationship between degree of oxidative stress as well as sENG levels and the severity of disease, where higher levels of oxidative stress markers and sENG were observed in severe PE group. Recently, Varejckova et al. [41] and Vitverova et al. [42] have suggested that sENG might aggravate endothelial dysfunction via interference with membrane ENG/eNOS dependent pathway along with induction of pro-inflammatory markers in endothelial cells.

In this study, we attempted to establish an association between 3 polymorphisms of ENG pathway: *ENG*(G/A) rs11792480, *TGFβR1*(A/C) rs10739778 and *TGFβR2*(G/A) rs6550005 and the prevalence of PE in a sample of preeclamptic Egyptian women. Results of our study showed only a genetic variability in *TGFβR1*(A/C) rs10739778, where a significant difference was observed in the prevalence of CC genotype between preeclamptic women and controls. The CC genotype was less identified in PE (8%) than controls (26%), in addition to a less frequency of C allele in PE (28%) than in controls (41%), thus CC genotype turned out to be a protective factor for initiation of PE. This finding is in

accordance with Bell et al. [16] who reported a less frequency of C allele in PE (20.7%) than controls (43.3%) in American black population. However, our finding disagrees with their observation in American white population, where they did not find any significant difference in allele frequency distribution. Moreover, assessment of CC genotype association with onset-time and severity of the disease revealed a less frequency of CC genotype in early-onset PE (5.9%) than controls (26%) and in severe PE (1.9%) than either mild PE (21.7%) or controls revealing a significant lower association of early-onset and severe PE risk with CC genotype. Furthermore, women carrying AA genotype had significantly higher SBP and MDA and lower TAC level with lower GA at delivery and birth weight than those carrying CC genotype. These data emphasize the protective effect of CC genotype against PE pathogenesis.

On the other side, no significant association was observed in allele frequency distribution of *ENG*(G/A) rs11792480 and *TGFβR2*(G/A) rs6550005 between PE and controls in the present study. Result about *ENG*(G/A) rs11792480 is in accordance with the findings of Schmella et al. [17] in Norwegian and Latina populations along with those of Bell et al. [16] in American black population. However, it is in contradictory to Bell et al. [16] result in American white population, where they showed that the A allele was more frequent in controls. Whereas, our result of *TGFβR2*(G/A) rs6550005 is in agreement with Schmella et al. [17] in Norwegian and Latina populations; however, it is different from that of Bell et al. [16] in both American white and black populations in which the A allele was more frequent in white controls and black PE subgroups.

There are some limitations in the current study. First, our findings are based on a small sample size which may influence the statistical power to find differences between groups. Second, we have selected some SNPs in ENG pathway genes, this may have introduced some bias which can be resolved by including more SNPs in genes of ENG pathway in future.

In conclusion, it seems that excessive sENG release in the circulation with subsequent decreased eNOS and NO as well as increased oxidative stress may be involved in PE pathogenesis. Moreover, our study demonstrates that women who carry C allele or CC genotype of *TGFβR1*(A/C) rs10739778 are less prone to develop PE or its pathogenic outcomes than those carrying A allele or AA genotype. Further collaborative research in larger diverse populations are needed to elucidate the contributory role of ENG pathway genetic variation in PE by performing genetic wide association studies.

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Declaration of interest

The authors have declared that no competing interests exist.

rs: reference single nucleotide polymorphism (SNP), OR: odds ratio, CI: confidence interval, significantly different at *P < 0.05, **P < 0.01 and ***P < 0.001: versus controls, $^{a}P < 0.05$ and $^{b}P < 0.01$: versus mild preeclamptic women.

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