ORIGINAL ARTICLE

Interrelationship between endothelial nitric oxide synthase with serum nitric oxide and endothelin1 levels in preeclamptic women

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Abstract

Background: Preeclampsia, a major hypertensive disorder of pregnancy is presumed to be due to imbalance between Nitric Oxide (NO) and Endothelin-1(ET-1). The serum ET-1 appear to have positive correlation with the severity of preeclampsia. Endothelial Nitric Oxide Synthase (eNOS) activity appear to be significantly decreased in affected endothelial cells of preeclamptic women and thus NO levels. *Aims and Objectives*: To estimate serum eNOS levels, serum NO and ET-1 levels in preeclamptic women and to determine the correlation between NOS with serum NO and ET-1 levels in preeclamptic women. *Materials and Methods*: An observational cross sectional study was conducted after obtaining ethical clearance from institutional ethics committee with 40 normal pregnant and 40 preecclamptic women from antenatal care unit. Blood samples collected from both the groups were analyzed for levels of ET-1, eNOS and NO and comparison was done. *Results*: A statistically significant difference was observed in the mean ET-1(pg/ml), NO-(umol/L), and eNOS (pg/mL) over groups. There was a significant positive correlation between eNOS (pg/mL) and NO (umol/L) among cases. *Conclusion:* Increased concentration of ET-1, reduction in NO and eNOS in serum can be the predictive factors of preeclampsia near third trimester.

Keywords: endothelin-1 (ET-1), Endothelial Nitric Oxide Synthase (eNOS), Nitric Oxide (NO), oxidative stress, preeclampsia

Introduction

Incidence of hypertensive disorders of pregnancy are as high as 10% of overall pregnancies. These are defined as new onset hypertension: Systolic Blood Pressure (SBP) of ≥ 140 mmHg or Diastolic Blood Pressure (DBP) of ≥ 90 mmHg after 20 weeks pregnancy as per the guidelines stated by International Society for the Study of Hypertension in Pregnancy (ISSHP) [1]. This broad definition consists of chronic hypertension, gestational hypertension and Preeclampsia (PE) as well [1-2].

The above have significant impacts on maternal and foetal morbidity either immediate or long-term. The pregnant women with preeclampsia have two-to-four-fold higher risk of developing long-term hypertension, cardiovascular mortality and also the risk of stroke. Whereas the foetus has the antenatal risks of Intra-Uterine Growth Restriction (IUGR), preterm birth, oligohydramnios, foetal distress, placental abruption, and also in-utero death [3-5].

The placentation and endothelial dysfunction are observed to play a key role in the pathophysiology of preeclampsia. Nitric Oxide (NO) and Endothelin-1 (ET-1), being endogenous counter regulators of vascular tone, imbalance among these is crucial in the progression of preeclampsia [6]. The serum ET-1, being the common end pathway, has been proved to be having positive correlation with the severity of preeclampsia. Hence, serum ET-1 levels could be a prognostic marker [6-7]. Studies have shown that Endothelial Nitric Oxide Synthase (eNOS) activity is markedly reduced in the endothelial cells of women with preeclampsia, leading to lower levels of NO, the vasodilatory factor normally produced through eNOS action. In the placental blood vessels, NO reduces the bioavailability of ET-1 even under physiological conditions. Early in the pathological process, NO does not play a role in suppressing ET-1. However, as pregnancy advances, persistently elevated ET-1 triggers a negative-feedback response that activates eNOS, resulting in increased NO production [8].

No clear evidence has been found regarding the relationship between ET-1 and NO in preeclamptic women. Therefore, we conducted this clinical study to measure serum eNOS, NO, and ET-1 levels in patients with preeclampsia and to assess how eNOS correlates with both NO, and ET-1 in this population.

Material and Methods

The present case-control study was conducted after obtaining ethical clearance from institutional ethics committee, RRMCH. Eighty singleton pregnant women were included in the study from antenatal care unit of department of Obstetrics and Gynecology, RajaRajeshwari Medical College and Hospital. Sample size calculation was done using prevalence of hypertensive disorders of pregnancy as 11% as per the study by Dhinwa *et al*. [9]. With 95% power of the study and 10% allowable error, sample size calculated was 38. Sample size of 40 per group were recruited. Of 80 samples, 40 were pregnant women with preeclampsia with blood pressure of ≥ 140/90 mmHg after 20 weeks of gestation accompanied by proteinuria and 40 were healthy pregnant ladies. Pregnant women with gestational hypertension, multiple gestation, fetal malformations, diabetes mellitus, thyroid disorders, autoimmune diseases, or chronic infectious diseases were excluded. Sample collection was done by drawing 5ml venous blood under aseptic precautions where in 3 ml of blood was transferred into a clot activator tube and 2 ml of blood into EDTA tube from both cases and controls. Collected samples were allowed to stand for 2 hours and centrifuged at 3000 rpm for 10 minutes and was stored at -80˚C until analysis. Serum levels of NO, ET-1 and eNOS were estimated among all the participants using Multiskan Gomicroplate spectrophotometer. NO levels were estimated using colorimertic assay kit from Elabscience. Double distilled water was used as blank. ET-1 levels were measured using RayBio Human Endothelin-1 ELISA kit. Assay's diluent C was used as blank. eNOS levels were measured using Elabscience® Human NOS3/eNOS ELISA Kit. All assays were performed as per the manufacturer's instructions.

Data were analyzed using GraphPad Prism version 10.3.1. Independent "t" test was used for comparing the distributions of different variables between the groups and correlation were done using Pearson correlation. Value of $p < 0.05$ was considered as statistically significant.

Results

With p values of 0.2669 and 0.4188 obtained from independent t test, we observed no significant statistical difference in the distribution of mothers' age and gestational age respectively as depicted in table 1 over groups acknowledging comparability within the groups. About 57.5% of mothers in control group and 42.5% mothers in the study group were primigravida.

Table 2 describes the distribution of variables with statistical significance. There is significant difference in the mean Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP), ET-1 (pg/ml), NO- (umol/L) and eNOS (pg/mL) over groups. The eNOS concentration and NO among controls were 2168.65 ± 467.28 pg/ml and 3.7 ± 2.25 umol/L, respectively whereas in case group, it was found to be, 1933.98 ± 468.57 pg/ml and 2.6 ± 1.47 umol/L respectively (Figures 1 and 2) with significant difference compared to controls ($p < 0.05$). Our key parameter, ET-1, was found to be significantly elevated in the preeclamptic group (Figure 3). ET-1 levels were 1.17 ± 0.18 pg/mL in controls and 1.81 ± 1.38 pg/mL in cases, with a p-value of 0.005. As shown in Table 3, no significant correlation was observed between ET-1 and eNOS or between ET-1 and NO in either group. However, a significant positive correlation ($p = 0.011$) was identified between eNOS and NO in the preeclamptic group, as illustrated in the scatter plot (Figure 4).

Table 1: Distribution of maternal age and gestational age

Variables	Groups		*p*
	Control (n=40)	Case (n=40)	
Age (years)	26.15 ± 3.96	27.28 ± 4.97	0.267
Gestational age (weeks)	31 ± 4.7	31.85 ± 5.2	0.419

Table 2: Distribution of variables with statistical significance

Variables	Groups		*p*
	Control (n=40)	Case (n=40)	
SBP (mmHg)	117.7 ± 9.5	149.3 ± 7.42	**<0.001**
DBP (mmHg)	79.6 ± 9.18	93.65 ± 9.17	**<0.001**
eNOS concentration (pg/mL)	2168.65 ± 467.28	1933.975 ± 468.57	**0.028**
NO (umol/L)	3.7 ± 2.25	2.6 ± 1.47	**0.011**
ET-1 (pg/ml)	1.17 ± 0.185	1.81 ± 1.378	**0.005**

SBP – systolic blood pressure; DBP – diastolic blood pressure; eNOS – endothelial nitric oxide synthase; NO – nitric oxide; ET-1 – endothelin-1

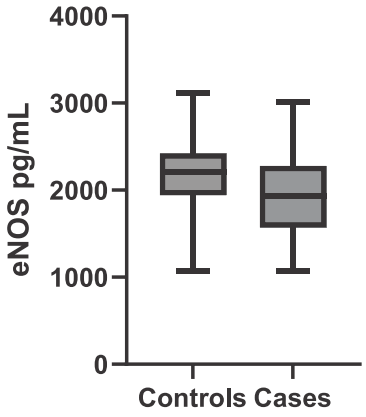

Figure 1: Mean plot of eNOS Concentration

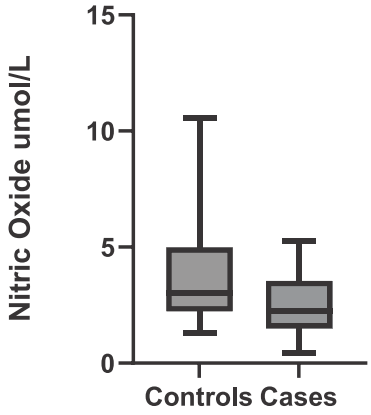

Figure 2: Mean plot of Nitric Oxide l

eNOS – endothelial nitric oxide synthase

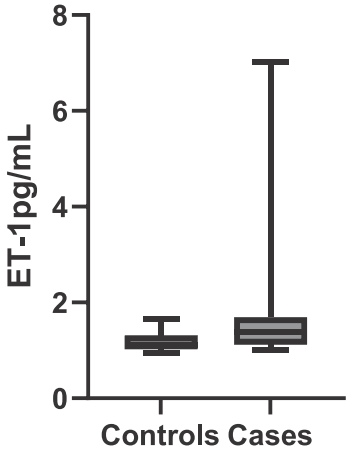

Figure 3: Mean plot of ET-1 levels

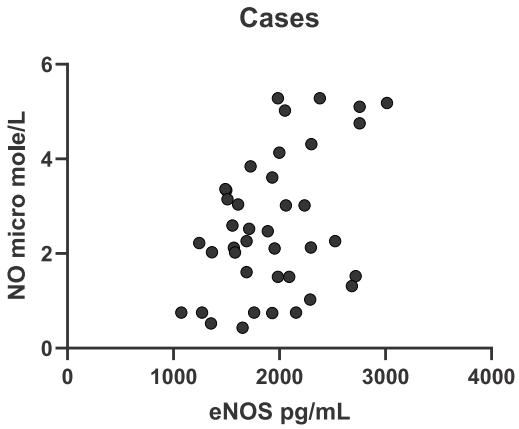

Figure 4: Correlation between eNOS & NO among cases

eNOS – endothelial nitric oxide synthase; NO – nitric oxide; ET-1 – endothelin-1

Table 3: Pearson Correlation between the parameters

	Et1 (pg/mL) & eNOS (pg/mL)		ET1 (pg/mL) & NO (umol/L)		eNOS (pg/mL) & NO (umol/L)	
	r	*p*	r	*p*	r	*p*
Controls (n=40)	0.2307	0.1522	-0.1872	0.2474	0.0617	0.705
Cases (n=40)	-0.2204	0.1718	-0.04707	0.773	0.3999	**0.011**
Controls & Cases (n=80)	-0.1995	0.0761	-0.1314	0.2452	0.2477	**0.028**

eNOS – endothelial nitric oxide synthase; NO – nitric oxide; ET-1 – endothelin-1

Discussion

Even with the advancement in field of medicine, there has been a gradual increased incidence of preeclampsia since few years. This could be due to various underlying causes but the adverse maternal and fetal outcomes could be avoided with few pre-diagnostic tests and hence the suggested measures. As the ET, eNOS and NO have been evidenced to be involved in pathophysiology of preeclampsia, the present study was aimed to analyze the correlation between these parameters among women with preeclampsia. In the present study we had included 80 pregnant women with singleton pregnancy. Majority of the studies [10,11] we came across used placental tissue for analyzing ET-1, eNOS and NO, whereas in the present study, we had considered venous blood as the study sample for biochemical tests as the sample can be conveniently obtained prenatally as compared to placental tissue. Of 80 participants, 40 healthy pregnant females were described as controls and the rest 40 women with preeclampsia were deemed to be cases. The average gestational age of our cases and controls was 31 ± 4.7 weeks and 31.8 ± 5 weeks, with no significant difference. In Khaing *et al*., the gestational age among both groups were between 28 to 29 weeks [10]. On analyzing the blood pressures, we found that there was significant ($p < 0.001$) increase in SBP as well as DBP among women with preeclampsia. The mean SBP was 117.7 ± 9.5 mmHg and 149.3 ± 7.4 mm Hg among controls and cases respectively, whereas the mean DBP was 79.6 ± 9.18 mmHg in control group and 93.65 ± 9.17 mmHg in case group. Similar to the present study, even Khaing *et al*., had observed significant increase in SBP as well as DBP among their case group, which was as high as 156 ± 13.5 mmHg and 100 ± 14.1 mmHg respectively [10]. Choi *et al*. explain that expression of eNOS and NO concentration increase with gestation until third trimester; hence the analysis at third trimester as conducted by our clinical trial would suffice in predicting the outcome [12].

Our primary parameter, ET-1, was significantly elevated in the case group. ET-1 levels measured 1.17 ± 0.18 pg/mL in controls and 1.81 ± 1.38 pg/mL in cases, with a p-value of 0.005. Additionally, eNOS and NO levels in controls were 2168.65 ± 467.28 pg/mL and 3.7 ± 2.25 μmol/L, respectively, whereas in the case group they were lower, at 1933.98 ± 468.57 pg/mL and 2.6 ± 1.47 μmol/L, both showing significant differences compared with controls ($p < 0.05$). Overall, the data indicate a significant rise in ET-1 among cases, accompanied by a corresponding and statistically significant decrease in both eNOS and NO levels. Like our study, Khaing *et al*., also had reported significantly higher serum levels of ET-1 among their pre-eclamptic group as compared to their controls. Unlike our observation, they had reported expression of eNOS being significantly higher in preeclamptic group. As they had included direct placental tissue after delivery, the values of ET-1 and eNOS might show the deviation from our study in which we had collected maternal venous blood for analysis [10]. Wang *et al*., also observed significant reduction in eNOS among the preeclamptic placental tissue they had considered in their study. The reason behind reduction in eNOS expression might be due to the increased oxidative stress leading to generation of free radicals which in turn cause oxidation of tetrahydrobiopterin and thus resulting in uncoupling of eNOS [13]. A study by Sayyed *et al*. stated that oxidative stress is the cause of endothelial dysfunction leading on to preecclampsia [14]. Gandham *et al.* reported a negative correlation between eNOS and NO levels with blood pressure in women with

preeclampsia, supporting the presence of endothelial dysfunction [15]. In an earlier study, they also demonstrated that serum NO and ferric reducing antioxidant power were significantly lower in women with preeclampsia compared with controls [16]. Additionally, Rotheneder et al., along with several independent studies, have documented upregulation of ET-1 gene expression and elevated ET-1 levels in preeclampsia [17]. On analyzing the correlation between ET-1 and eNOS, and ET-1 and NO, we found that they were negatively related but there was no significant correlation observed on statistical basis.

On biochemical aspect, it could be interpreted that, with ET-1 levels raised, eNOS and NO got reduced. Additionally there was a significant positive correlation between eNOS and NO among the case group with correlation coefficient of 0.399 and p value of 0.011. The same correlation was not seen in healthy participants group alone but when clubbed together and analyzed, we could note significant difference ($p = 0.028$). Like the present study, even Khaing *et al.* also did not find any significant correlation between ET-1 and eNOS [10]. A recent review by Ssengonzi *et al*., elaborated about increased expression on ET-1 and decreased expression on eNOS related genes among preeclamptic models of animals and they further have opined similar outcome among humans as well, but the correlation between these was not established in their study [18]. Fondjo *et al*. also have reported reduction in NO being positively correlating with eNOS expression. This study was most reliable to compare with ours as the sample collected for biochemical tests was venous blood as we did, unlike the other clinical evidences who had considered placental tissue [19]. Also, Shaheen *et al* reported significant reduction in NO, which had positive association with eNOS expression among their preeclamptic patients [20]. Additionally, the importance of assessing NO levels during pregnancy is supported by Turan et al., who demonstrated that circulating NO plays a vital role in maintaining fetoplacental hemodynamics, ensuring proper placental perfusion and fetal oxygenation [21]. Based on these findings, ET-1, eNOS, and NO may serve as valuable predictive markers for both maternal and fetal outcomes in preeclamptic pregnancies. Most available evidence focuses on ET-1 and eNOS gene expression in placental tissue, with little attention given to their levels in blood or serum. This highlights the need for additional case-control or cross-sectional studies, as blood collection is far simpler and safer than obtaining placental samples. This context underscores the uniqueness and importance of our work. In the future, we aim to expand our research by examining stored specimens for gene expression to better understand the potential genetic interactions involved in the pathogenesis of preeclampsia.

Conclusion

From the above analysis, we would like to conclude that increased concentration of ET-1, reduction in NO and eNOS in serum can be the predictive factors of preeclampsia near third trimester. Also, there is significant positive correlation between eNOS and NO in women with preeclampsia. Though statistically not significant, there could be a negative biochemical correlation between ET-1 and NO among the women with preeclampsia. Further study with increase in the sample size might show significant correlation among the currently used variables of the study.

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