

Research article (Award paper)

Diagnostic accuracy of maternal serum endothelial-derived nitric oxide synthase (eNOS) and its correlation with birth outcomes, in preeclampsia - a case-control studySangappa Virupaxappa Kashinakunti^{1,2}, Basavaraj Devaranavadi³, Manjula Rangappa⁴, Ashalata Mallapur⁵¹PhD Scholar, ³Professor and Head, Department of Biochemistry, Shri B M Patil Medical College, BLDE University, Vijayapur-586101, Karnataka, India²Professor, Department of Biochemistry, ⁴Professor, Department of Community Medicine, ⁵Professor and Head Department of Obstetrics and Gynaecology, S. Nijalingappa Medical College, Navanagar, Bagalkot-587102 Karnataka, India

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ABSTRACT

Introduction and Aim: Preeclampsia (PE) results from impaired placentation, leading to placental hypoxia and dysfunction and abnormalities in the endothelial nitric oxide synthase (eNOS)-nitric oxide pathway. The present study was undertaken to estimate and compare maternal serum eNOS concentrations in women with PE and normal healthy pregnant women, to know the correlation of eNOS level with severity of PE, to find the best cut-off value for the diagnosis of PE and to study its association of eNOS levels with the birth outcome.

Material and Methods: This was a case-control study, conducted at a tertiary care hospital. The study was approved by the Institutional ethics committee. Informed consent was obtained from all the participants. Primigravida (singleton) pregnant women aged 18-35 years, 20 or more weeks of gestation, diagnosed as PE and classified as mild/severe PE based on American college of obstetricians and gynecologists guidelines were selected for the study. eNOS was estimated by the ELISA method. All the participants were visited again to note the outcomes.

Results: The maternal serum eNOS in PE was significantly lower than the control group ($p=0.019$). The best cut-off value was 187.25pg/ml to diagnose PE, area under the ROC curve (AUC) 0.61, sensitivity 95.1%, specificity 37.5% and diagnostic accuracy was 66,67%. Birth outcomes did not have a significant correlation with eNOS.

Conclusion: eNOS was significantly lower in PE, it can be used as a diagnostic marker with the best cut of value of 187.25 pg/ml.

Keywords: Preeclampsia; nitric oxide synthase; diagnosis.

INTRODUCTION

Preeclampsia (PE), constitute a unique, complex clinical condition of human pregnancy, is characterized by the development of de-novo hypertension and proteinuria after 20 weeks or above gestation in patients free from any clinical disease, but it can occur at any time during labor, or even up to 6 weeks after delivery. PE is a multi-organ involvement targets the endothelium of the brain, liver, kidneys and the coagulation system and impairs a short-term perinatal outcome and remote prognosis of cardiovascular disease for the mother. Termination of pregnancy remains the only curative treatment in severe PE. Management of patients with PE must be individualized and must balance the maternal and fetal risks (1-3).

It has been suggested that the root cause of PE is the placenta. Its functions are vascular development and blood flow, which depend on proper trophoblast growth and differentiation. According to the studies, preeclampsia results from impaired placentation early in the beginning of the pregnancy, leading to placental hypoxia and dysfunction and abnormalities in the endothelial nitric oxide synthase (eNOS)-nitric oxide pathway (4-6). eNOS is localized mainly in the plasma membrane, and a small amount is present in

the cytosol also. Golgi apparatus acts as the main source of eNOS. The eNOS is a key enzyme of the cardiovascular system that contributes to vascular homeostasis through tightly regulated NO production (7,8). Endothelial cells release NO, a potent vasodilator. Nitric oxide is required for regulation of the vascular tone and hence maintains blood pressure. The placental blood flow is regulated by NO and it also participates in trophoblast invasion and development of the placenta. As NO is a highly reactive and short-lived molecule. It is clearly not known whether eNOS deficiency plays an important role in pathogenesis of preeclampsia. Many studies suggest that insufficient nitric oxide synthesis or NO bioavailability, may contribute to increased blood pressure, systemic vascular resistance, and sensitivity to the pressors. However, studies conducted among various ethnic groups, which resulted in mixed or inconclusive results (9-12).

The current study was undertaken to estimate and compare maternal serum concentrations of endothelial nitric oxide synthase (eNOS) in women with PE in comparison to healthy normotensive pregnant women. and also to find the correlation between eNOS level with severity of PE, the best

cutoff value for the diagnosis of PE and to study its association of eNOS levels with the birth outcome.

MATERIALS AND METHODS

This is a case-control study, conducted in the department of obstetrics and gynecology and department of biochemistry at tertiary care hospital, Karnataka India, from Jan 2019 to Jun 2019. The study was approved by the Institutional ethics committee. Informed consent was obtained from all the study participants.

Primigravida (singleton) pregnant women aged 18-35 years, 20 or more weeks of gestation, diagnosed as PE and classified as mild/severe PE based on American college of obstetricians and gynecologists' guidelines (13) were selected for the study. Patients with premature rupture of membranes, chorioamnionitis, multiple gestations, Rh isoimmunization, fetal anomalies, intrauterine fetal death, chronic inflammatory diseases, history of diabetes mellitus, history of systemic hypertension, cardiovascular or renal diseases and chronic inflammatory diseases were excluded from the study. Healthy pregnant women, matched for age, gravida and gestational weeks with the cases were selected as controls.

All participants were subjected to history taking and followed by general physical examination. Abdominal examination was performed for fundal level, lie and presentation of the fetus. Blood samples were collected by venipuncture in two tubes, 2ml with anticoagulant and 2 ml without anticoagulant. Complete blood count, blood grouping and rhesus factor were done. After clotting, the samples were

centrifuged and serum was pipetted and the following parameters were measured blood glucose, liver function tests, urea, serum creatinine, uric acid and eNOS. Sample for eNOS measurement was stored at -20° C till estimation. eNOS was estimated by the ELISA method the kits were supplied by Bioassay Technologies Laboratory. Urine analysis for proteinuria was done. All the participants were visited again at the outcome of the pregnancy and the outcome findings were noted.

Sample size calculation was done using Open Epi software version 2.3:1, retrospectively with 83.47% power of the study, using case severity into consideration, the sample size calculated was 39~ 45. Hence, 45 preeclampsia cases and 45 normal gestation controls were included in the study.

Analysis was done using SPSS software version 19. Unpaired 't' test for quantitative data and Pearson's correlation tests were applied. P<0.05 will be considered statistically significant. ROC curve analysis for eNOS was done to find the optimum cutoff value for the diagnosis of PE. Tests of validity namely sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of eNOS were calculated with 95% confidence intervals.

RESULTS

In the present study, there was no significant difference in maternal age, gestational weeks. The systolic, diastolic blood pressure, SGOT were significantly higher in PE patients than normal pregnant women (Table 1).

Table 1: Demographic characteristics of controls and cases

	Controls	Cases	p
Age in years	22.55±3.22	22.83±4.23	0.94
Gestational Weeks	37.88±2.87	35.83±4.77	0.085
SBP mmHg	118.51±10.65	158.45±18.85	0.000
DBP mmHg	75.40±8.75	102.30±9.20	0.000
Platelet	228792.84±49502.22	200888.45±82528.64	0.078
Creatinine mg/dl	0.72±0.14	0.76±0.10	0.600
AST IU/L	24.27±10.38	44.53±62.24	0.044
ALT IU/L	15.85±10.18	36.37±68.25	0.067
Birth weight in Kg	2.88±0.67	2.16±0.85	0.000
APGAR 1min	7.82±0.770	6.49±2.62	0.016
APGAR 5min	8.94±0.40	7.69±2.85	0.020

SBP: Systolic blood pressure, DBP: Diastolic blood pressure, AST: Aspartate transaminase, ALT: Alanine transaminase

Table 2: eNOS in controls and cases

	Controls	Cases	p
eNOS pg/ml	173.36±187.68	99.87±56.58	0.019

eNOS: Endothelial derived nitric oxide synthase

The maternal serum eNOS in PE was significantly lower than the control group(p=0.019).

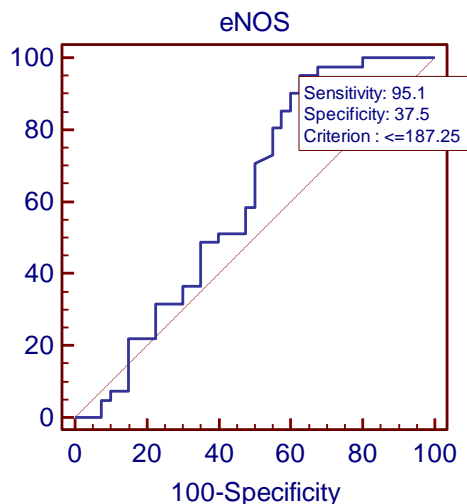


Fig. 1: The best cutoff value of eNOS for the diagnosis of PE by ROC curve

Table 3: Sensitivity, Specificity, Positive Predictive Value, Negative Predictive Value and Diagnostic Accuracy of eNOS in PE patients

Sensitivity	95.1%	(48.69, 71.94 ¹)
Specificity	37.5%	(65.66, 96.71 ¹)
Positive Predictive Value	95.12%	(83.86, 98.65 ¹)
Negative Predictive Value	37.50%	(24.22, 52.97 ¹)
Diagnostic Accuracy	66.67%	(55.85, 75.97 ¹)

The best cut-off value was 187.25 pg/ml to diagnose PE, area under the ROC curve (AUC) 0.61, sensitivity 95.1%, specificity 37.5% and diagnostic accuracy was 66.67% (Fig. 1, Table 3).

Table 4: eNOS in mild and severe PE patients

	Mild PE	Severe PE	p
eNOS pg/ml	101.18±60.63	95.76±40.94	0.824

eNOS: Endothelial derived nitric oxide synthase, PE: Preeclampsia.

There was decrease in eNOS in severe PE cases than mild PE cases but it was not statistically significant (p=0.824) (Table 4).

Table 5: Correlation of eNOS with the severity of PE and birth outcomes

	Severity of PE	Birth weight	APGAR 1min	APGAR 5min
r	0.036	-0.029	-0.112	-0.142
p	0.824	0.868	0.530	0.422

eNOS: Endothelial derived nitric oxide synthase, PE: Preeclampsia

Birth outcomes did not have a significant correlation with eNOS (Table 5).

DISCUSSION

NO is synthesized from the reduction of L-arginine to L-citrulline by the NOS enzyme, which has three isoforms: nNOS or neuronal, iNOS, the inducible and eNOS endothelial NOS, here BH4 acts as a coenzyme, promotes dimerization and activity of NOS. Decreased concentration of the substrate L-arginine and increased concentration of the endogenous NOS inhibitor asymmetric dimethylarginine (ADMA) may interfere with eNOS activity in preeclampsia (14,15).

In the present study, there was a significant decrease in eNOS in PE patients compared to the normal pregnant women (p=0.019). Kim *et al.*, and Khalil *et al.*, found lower expression of eNOS in the syncytiotrophoblast, reduced concentrations of L-arginine, and unchanged ADMA in the serum of women with pregnancies complicated by

preeclampsia (16,17). A cohort study conducted in Greek showed that the plasma ADMA levels in PE women were found to be higher (18) than the control group. Maranzana *et al.*, and Laskowska *et al.*, showed that the levels of serum eNOS were lower in women with PE than in the healthy women from the control group, but it was not statistically significant (p=0.238899). The mean values of serum eNOS were 181.30±178.44 U/ml in the PE group and 217.74±265.11 U/ml in the control group (19,20). The levels of serum endothelial nitric oxide synthase were lower in women with pregnancies complicated by severe PE than in the healthy women from the control group, but these differences were not statistically significant (p = 0.118770). The mean values of serum eNOS were 134.06 ± 76.73 U/ml in the Pre group and 187.70 ± 165.41 U/ml in the Control group (21). Myatt *et al.*, (5) observed intense expression of eNOS in placentas from pregnancies

complicated by preeclampsia. Schiessl *et al.*, found significantly increased placental expression of endothelial nitric oxide synthase in pregnancies complicated by preeclampsia (22). In a Brazilian cohort study, plasma ADMA concentration was more in PE women and plasma NO concentration was found lower in comparison with normotensive women (23).

The uncoupling of eNOS has also been shown as a source of superoxide formation and this leads to reduce NO production. When eNOS cofactor, tetrahydrobiopterin(BH4) is low or when post-translational changes regulate eNOS function. It has been demonstrated that various inflammatory makers like TNF- α and CRP are increased in plasma and placenta from PE women. TNF- α an inflammatory marker increased in PE, which downregulates eNOS and mitochondrial biogenesis leading to mitochondrial dysfunction and elevated ROS. Conversely, CRP is another inflammatory marker also increased in PE, indirectly downregulates BH4 production, leading to uncoupling of eNOS catalysed reaction and leads the formation of peroxynitrite. Animal studies have shown that BH4 supplementation in pE increased the concentration of NO (14,15,24).

In the present also there was low levels of maternal serum concentration of eNOS in severe PE as compared to mild PE but it was not statistically significant ($p=0,824$). The unchanged levels of eNOS in severe PE. A small increase in eNOS in mild hypertension PE patients, whereas in a subgroup with severe hypertension, concentrations of these markers were greatly reduced. Studies have shown elevated ADMA levels in early-onset PE may suggest a relationship between the severity of the disease and determining the time of PE clinical manifestations (2,12,25).

In the current study the best cut-off value was 187.25 pg/ml to diagnose PE, area under the ROC curve (AUC) 0.61, sensitivity 95.1%, specificity 37.5% and diagnostic accuracy was 66.67% (Figure 1, Table 3). Wender-Ozegowska *et al.*, in their study, found that eNOS AUC was 0.57 best cut-off was 0.34 with a sensitivity of 78.6% specificity 40% (1). Our study did not show any correlation of eNOS with the severity of PE and birth outcome viz birth weight and APGAR score.

Further studies are required with gene expression along with serum estimation of NO, eNOS, L-arginine, ASDA, BH4, oxidative stress markers and inflammatory markers may give the role of NO and eNOS in the pathogenesis of PE.

CONCLUSION

The eNOS was significantly lower in PE patients than normal healthy pregnant women, it can be used as tool diagnose PE with the best cut-off value of

187.25 pg/ml, with sensitivity 95.1%, specificity 37.5% and diagnostic accuracy of 66.67%.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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