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Impact of Oxidative Stress on Maternal Serum Apelin 13 and Endothelial Nitric Oxide Synthase in Preeclampsia

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Preeclampsia (PE) is the most common hypertensive disease of pregnancy, leads to maternal, perinatal morbidity and mortality, which accounts for 2-8% of pregnancies. Preeclampsia is characterized by new onset of hypertension and proteinuria after 20 weeks of gestation.The exact cause of preeclampsia is not clear. Aim of this study is to investigate the association between maternal serum apelin13, endothelial nitric oxide synthase, markers of oxidative stress in healthypregnant womenand preeclamptic women. This prospective studycomprises 140 pregnant womenconsists of 70 preeclamptic women treated as cases and 70 normotensive healthy pregnant women as controls. Five mL blood sample was collected, centrifuged to obtain serum/plasma and was stored at -80°C for further testing. Plasma was used for Ferric reducing antioxidant power (FRAP) assay and complete blood countwas done. Routine parameters like random blood sugar, renal profile, liver enzymes, lactate dehydrogenase (LDH), malondialdehyde (MDA), nitric oxide (NO), apelin 13 and endothelial nitric oxide synthase (eNOS)were also analyzed. Corresponding urine sample was tested for protein. Study results showed lower gestational age (36.99±3.48 weeks) and demographic details such as elevated blood pressure [systolic (156.80±13.71 mmHg),diastolic (101.97±10.70 mmHg),and mean arterial pressure (120.88±11.02 mmHg)], BMI (27.42±3.80 kg/m²) and pulse rate (87.68±5.74 bpm) were observed in cases than controls. The biological markers namely serum MDA (18.57±7.52ìmoles/L) levels were significantly increased and nitric oxide (6.47±1.22ìmoles/L), FRAP (1292.10±525.38 mmol/L), apelin 13(312.42±189.00pg/ml) andeNOS(5.07±2.30 ng/ml)levels were significantly decreased in cases.Mean arterial pressure was negatively correlatedwithApelin 13 (r=-0.179), NO (r=-0.065), FRAP (r=-0.169), and birth weight (r=-0.281) and eNOS (r= 0.013), MDA (r= 0.022) were positively correlated with mean arterial pressure. The study concludes that reduced levels of apelin 13, eNOS, FRAP,NO and high oxidative stress, contribute to pathogenesis of preeclampsia and adverse perinatal outcome. It also demandssufficient evidence for the functional role of apelin 13 as a target in hypertension regulation.

Keywords: Angiogenesis, Hypertension, Malondialdehyde, Nitric Oxide.

Preeclampsia (PE) is apregnancy specific complication and it causes maternal, perinatal morbidity and mortality, which accounts for 2-8% of pregnancies [1,2]. The incidence of preeclampsia in India varies from 5% to 10%[3].

This disease is characterized by new onset of hypertension and proteinuria after 20 weeks of gestational period[1].The exact cause of preeclampsia is not clear. However, it includes abnormal placentation, vascular endothelial dysfunction, oxidative stress, inflammation and impaired angiogenesis [4,5]. Oxidative stress contributes further in the pathophysiology of preeclampsia. Increased oxidative stress has influence on endothelial dysfunction, systemic vascular resistance, leading to decreased placental perfusion and aggravates placental ischemia-reperfusion injury [6-8].

Apelin protein encoded by *APLN* gene located on chromosome Xq25-26.1[9]. The apelinergic system role in cardiovascular homeostasisis studied [10].However, apelinrole in preeclampsia is poorly understood. The apelin and APJ/AR expression are widespread in human tissues.High levels were seen in placenta, suggesting its potential production from placenta[11-13].Biosynthesis includes apelin precursor processing into many biologically active short peptides. They are apelin precursor (77 amino acid residues), proapelin (55 amino acids) further into short apelins (apelin-36, apelin-17, apelin- 13, and apelin 12), and are potentially biological activator for APJ [14-15]. These peptides have distinct activities and shorter apelinpeptides are more potent activators for APJ [16].The activated apelinpeptides promote vasodilatation through L-arginine/eNOS/NO pathway[17].Moreover, it induces cardiac contractility, angiogenesis and suppresses aortic inflammation[18]. It is believed that altered expression of apelin impairs angiogenesis process and linked to preeclampsia complications[19].

Endothelial nitric oxide synthase (eNOS) catalyzes the conversion of L-arginine into nitric oxide (NO) molecule, which is a potent vasodilator,regulates blood pressure, anti-atherogenic, prevents cell adhesion and platelet aggregation[20-22]. In the placenta, the high expression of NOS is seen in syncytiotrophoblast, villous endothelium, macrophages and eNOS being the predominant isoform[23-25]. A few studies have reported conflicting results about either decreased eNOS levels or unchanged [26, 27]and differences in eNOS staining between preeclampsia and normal pregnancy. A recent study report also given clue about abnormalities in eNOS/NO pathway, but the role ofeNOS in preeclampsia needs to be established[28].Hence, measurement of oxidative stress helps to understand the balance between oxidants and antioxidants and also investigation with the maternal serum apelin13, endothelial nitric oxide synthase (eNOS) association in healthy pregnant and preeclamptic women.

MATERIALS AND METHODS

Prospective case-control study design approved by Institutional Ethics Committee conducted in Department of Biochemistry in collaboration with Department of Obstetrics and Gynecology of RL Jalappa Hospital, attached to Sri Devaraj Urs Medical College, Karnataka, India. Study participants were enrolled after obtaining written informed consent. The study comprised of 140primigravida pregnant, among them 70 were normotensive pregnant women as controls and 70 preeclamptic women were considered as cases. Demographic details and clinical history were collected for all subjects.

Pregnant with history of renal disease, liver disease, thyroid disorder, chronic systemic hypertension, gestational diabetes, hypertensive encephalopathy, cardiovascular diseases, pregnancy with fetal anomaly, multiple pregnancies, patients with smoking history and malignancy conditions were excluded. Subjects were followed until delivery. Any adverse perinatal outcomes including respiratory distress syndrome (RDS), intrauterine death (IUD), and birth weight were recorded.

Preeclampsia diagnosis

Preeclampsia was diagnosed according to the ACOG guidelines (ACOG practice bulletin 2013),with blood pressure of systolic e”140 and diastolic e”90 mmHg noted for the first time during pregnancy on 2 occasions at least 4 hours apart, after 20 weeks of gestation with proteinuria of e”300 mg/24 hours or 1+ proteinby dipstick method or in the absence of proteinuria, the following findings were considered; thrombocytopenia (a platelet count <100,000/iL), renal insufficiency, hepatic dysfunction, symptoms associated with cerebral or ophthalmic and edema [29].

Under aseptic conditions, 5 ml venous blood was collected, aliquoted into plain tube (3 mL) and EDTA (2mL) tubes from the subjects visiting to Department of Obstetrics and Gynecology for antenatal check-up. Blood samples were allowed to stand for 30 minutes, centrifuged at 3000 rpm

for 10 minutes to obtain the clear serum. Thus, obtained clear serum was stored at -80°C for further testing. Complete blood count was done by using EDTA blood. Plasma was used for the FRAP assay. Serum was used for the estimation of RBS (GOD-POD),urea (urease), creatinine (sarcosine oxidase), uric acid (uricase), AST (IFCC), ALT (IFCC), LDH (kinetic), malondialdehyde (MDA) (Thiobarbituric acid reactive substances), nitric oxide (Griess reaction), and apelin 13, eNOS by ELISA. Five mL of corresponding urine sample was collected for urinary protein analysis by dipstick method.

Table 1. Comparison of baseline characteristics between preeclampsia and healthy pregnant women

Parameters	Preeclampsia (n=70) Mean ± SD	Healthy pregnant women(n=70) Mean ± SD	*P*- value
Age (years)	23.05±3.75	23.01±3.09	0.941
Gestational age (weeks)	36.99±3.48	38.65±1.80	0.001*
BMI (kg/m^2)	27.42±3.80	25.83±3.00	0.007*
SBP (mmHg)	156.80±13.71	115.14±6.67	0.000*
DBP (mmHg)	101.97±10.70	74.00±6.46	0.000*
MAP (mmHg)	120.88±11.02	87.55±5.80	0.000*
Pulse rate (bpm)	87.68±5.74	84.88±7.77	0.017*
Hematological parameters			
Hemoglobin (%)	11.13±2.01	11.34±1.82	0.533
WBC (10^3/ìL)	12.93±3.71	13.63±4.21	0.303
Platelet count x (10^9/L)	230.33±84.65	237.20±56.47	0.755
Biochemical Parameters			
RBS (mg/dL)	83.02±22.29	81.54±14.92	0.750
Serum Urea (mg/dL)	16.15±6.58	14.61±4.70	0.117
Serum Creatinine (mg/dL)	1.04±0.16	0.49±0.11	0.148
Serum uric acid (mg/dL)	5.97±1.84	4.85±1.55	0.000*
Serum AST (IU/L)	17 (22 - 29)	16 (20 - 25)	0.067
Serum ALT (IU/L)	12.75 (17 - 22)	7.75 (13 - 18)	0.000*
Serum LDH (IU/L)	313.01±161.50	172.50±59.17	0.000*

*Significant
BMI: Body Mass Index, SBP: Systolic Blood Pressure, DBP: Diastolic Blood Pressure, MAP: Mean Arterial Pressure, WBC: White Blood Cell, RBS: Random Blood Sugar, AST: Aspartate Transaminase, ALT: Alanine Transaminase, LDH: Lactate Dehydrogenase

Table 2. Comparison of biochemical parameters between preeclampsia and healthy pregnant women

Parameters	Preeclampsia (n=70) Mean ± SD	Healthy pregnant women (n=70) Mean ± SD	*P*-value
Serum MDA (ìmoles/L)	18.57±7.52	9.49±5.13	0.000*
Plasma FRAP (mmol/L)	1292.10±525.38	1982.40±152.89	0.000*
Serum NO (ìmoles/L)	6.47±1.22	10.78±3.94	0.000*
Apelin 13 (pg/mL)	312.42±189.00	490.42±269.95	0.000*
eNOS (ng/mL)	5.07±2.30	7.05±2.58	0.000*

* Significant
MDA: Malondialdehyde,FRAP: Ferric Reducing Ability of Plasma, NO: Nitric Oxide, eNOS: endothelial Nitric Oxide Synthase

Body mass index (BMI) was calculated and fetal outcome was recorded.

Determination of maternal serum apelin-13

The serum concentration of apelin13 (SiNCERE Catalogue NoÿE13652182, China) in each sample was detected by using sandwich ELISA kitas per the manufacturer's instructions.

Determination of maternal serum eNOS

The serum concentration of eNOS(SiNCERE Catalogue NoÿE13650645, China) in each sample was detected by using sandwich ELISA kit as per the manufacturer's instructions.

Statistical Analysis

The data was represented as Mean ± SD. Mann-Whitney *U* test was used for non-normally distributed variables. Spearman's correlation was used to find the association of variables. Interquartile ranges were used for skewed parameters. P value <0.05 considered as significant. Data was analyzed by using SPSS software, version 22.0

RESULTS

No significant change in maternal age. Gestational age (36.99±3.48 weeks) was low in preeclampsia compared with healthy pregnant. Systolic (156.80±13.71 mmHg) and diastolic (101.97±10.70 mmHg), mean arterial pressure (120.88±11.02 mmHg), BMI (27.42±3.80 kg/m^2) and pulse rate (87.68±5.74 bpm) were significantly increased in preeclampsia compared with controls(Table 1). Mean levels of serum MDA (18.57±7.52ìmoles/L), uric acid (5.97±1.84 mg/dL), ALT (12.75 [17-22 IU/L]), and LDH (313.01±161.50 IU/L) were increased significantly in preeclampsia compared to control group. Mean levels of serum NO (6.47±1.22ìmoles/L) and FRAP (1292.10±525.38 mmol/L) were decreased significantly in preeclamptic women compared with control group. Mean level of maternal serum apelin13 (312.42±189.00pg/mL) and eNOS (5.07±2.30 ng/mL) were decreased significantly in preeclamptic women compared with control group (Table2).

MDA (r= 0.022), eNOS (r= 0.013), UA (r= 0.295), AST (r= 0.173), ALT (r= 0.109) and LDH (r= 0.211) were positively correlated with mean arterial pressure and FRAP (r=- 0.169), NO (r=-0.065), apelin 13 (r=-0.179) and birth weight (r=-0.281) were negatively correlated with mean arterial pressure. Among the correlations, serum uric acid and birth weight was significant (Table 3).

Birth weight (2.50±0.64 kg) of the babies born to preeclamptic mother was decreased significantly compared with control group. Among the babies born to preeclamptic mothers, RDS and IUD were observed in 13 (18.57%) and 5 (7.14%) respectively. In normotensive mothers, RDS in 8 (11.42%) babies and IUD nil (Table 4).

Table 3. Correlation of mean arterial pressure with study parameters

Parameters	*r*- value	*p*-value
MDA	0.022	0.856
FRAP	-0.169	0.162
NO	-0.065	0.593
Apelin 13	-0.179	0.137
eNOS	0.013	0.917
UA	0.295*	0.013
AST	0.173	0.153
ALT	0.109	0.371
LDH	0.211	0.079
BW	-0.281*	0.018

* Correlation is significant at the 0.01 level (2-tailed)

DISCUSSION

Preeclampsia is a life-threatening pregnancy-specific disorder with no current treatment. In the pathophysiology of preeclampsia, role of placenta is crucial and is considered as a source

Table 4. Comparison of birth weight and fetal complications of babies born to mothers with preeclampsia and healthy pregnant women

Parameters	Preeclampsia (n=70)	Healthy pregnant women (n=70)
Birth weight (Kg)	2.45±0.63	2.82±0.49[a]*
RDS	13 (18.57%)	8 (11.42%)
IUD	5 (7.14%)	Nil

a: Mean±SD (p<0.002), * Significant,
RDS: Respiratory distress syndrome, IUD: Intrauterine death

of inflammation and release of vasoconstrictor molecules to initiate endothelial cell injury, endothelial dysfunction and vasoconstriction[14].

In preeclampsia, altered spiral artery remodeling and improper invasion of trophoblasts, an inadequate blood perfusion, lead to ischemia and reperfusion injury, which increase ROS generation[30]. Serum MDA levels represents oxidative stress, and was significantly elevated in preeclampsia compared with healthy pregnant women and correlated positively with mean arterial pressure. Total antioxidant status was decreased significantly in preeclampsia compared with healthy pregnant women and negatively correlated with mean arterial pressure. This indicates that increased lipid peroxidation is associated with decreased antioxidant protective/compensatory adaptation in preeclampsia [31,32].

In this study, we documented the decreased maternal serum apelin 13 levels in preeclamptic women compared with control group and correlated negatively with mean arterial pressure. This indicates apelin 13 is moderately successful marker to differentiate patients with preeclampsia from healthy pregnant women. Apelin is a vasodilator and exhibits positive inotropic activity in cardiovascular system through increasing release of nitric oxide from endothelium and by antagonizing the activity of angiotensin II [15]. Studies reported reduced apelin levels in other hypertensive disorders and also cardiac diseases [33,34]. Inzukua*et al*., reported that apelin mRNA levels were reduced significantly in preeclamptic placentas and immunohistochemical signals for apelin and APJ receptor were also decreased [19].Cobellis L *et al*., reported that in normal pregnancy, placental apelin is more abundant during early gestation, suggesting its role in the regulation of fetal development and placentation [12]. Reduced levels of apelin may therefore have deleterious effects on development of the fetus[5]. Apelin is involved in the direct activation of L-arginine/eNOS/NO pathway [17].Wang C *et al*., found that apelin treatment improved the expression of eNOS in placenta and serum levels of nitric oxide and eNOS, which were all decreased in preeclamptic rats, suggesting that restoration of eNOS/NO pathway may be involved in the ameliorative effects of apelin on preeclampsia[35]. Aberrant angiogenesis and increased blood pressure are the hall marks of preeclampsia. Thus, one might expect decreased levels of apelin, which is angiogenic and hypotensive in preeclampsia. This observation was supported by our study results and demonstrates decreased apelin levels in preeclampsia compared with healthy pregnant women.

In this study, eNOS levels were reduced significantly in preeclampsia compared with healthy pregnant women and positively correlated with mean arterial pressure. It has been reported that eNOS in the mother and in the fetus contribute utero-placental vascular changes and elevated uterine arterial blood flow,whereas preeclampsia is associated with abnormal placentation and abnormalities in the eNOS/NO pathway[20].Kim YJ *et al*., reported that placental expression of eNOS is significantly low in preeclamptic syncytiotro phoblasts compared to normal syncytiotrophoblasts[36].Zawiejska A *et al*., reported that significantly reduced serum eNOS levels in women with severe preeclampsia[37]. Carolina *et al*., reported that *exvivo*-derived syncytiotrobhoblast extracellular microvesicles (STBMV) and syncytiotrobhoblast extracellular exosomes (STBEX) isolated from placental perfused lobes to have low activity of eNOS in preeclampsia compared with controls. Similarly, *invivo*-derived plasma STBMV analyzed by flow cytometry showed less STBMV bound eNOS expression in preeclampsia compared with normal pregnancy. This may contribute to the low nitric oxide levels in preeclampsia[38].In support of this, Choi JW *et al*., reported that nitric oxide levels in preeclampsia were significantly lower than the normal pregnant women. During normal pregnancy, the production of nitric oxide increases with advancing gestation and decreases in preeclampsia [39].

Low birth weight was seen in babies born to preeclamptic mothers compared to healthy pregnant women and negatively correlated with mean arterial pressure. Nadkarni*et al*., reported, incidence of low birth weight to be 52% among mothers with pregnancy-induced hypertension [40]. Odegard*et al*., reported that, preeclampsia and severe preeclampsia were linked with a 5% and 12% reduction in birth weight, respectively [41]. We observed 18.57% of babies with RDS and IUD to be 7.14% which is supported by Nadkarni*et al*.,

reported 10% incidence of RDS among babies born to preeclampsia mothers [40].

Limitations of the study

The study has limitation with respect to small size; preeclampsia subjects were not stratified based on severity of the disease, apelin and eNOS levels were measured only after the diagnosis of preeclampsia but not in early onset of preeclampsia.

CONCLUSION

The study results conclude that reduced levels of apelin 13, eNOS, NO and elevated oxidative stress highly contribute to the process of pathophysiology of preeclampsia and results in adverse perinatal outcome.

Conflict of interest

There are no conflicts of interest.

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