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Original Article

Biochemistry Section

Apelin 13 and Blood Pressure, Is there any Association in Pre-eclampsia? - A Case-control Study

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ABSTRACT

Introduction: Pre-eclampsia is a pregnancy specific disorder, characterised by the onset of hypertension and proteinuria. Pre-eclampsia is the leading cause of maternal, perinatal morbidity and mortality. The exact cause of pre-eclampsia is not known clearly and needs to be explored.

Aim: To evaluate the maternal serum apelin 13 levels among pre-eclampsia and healthy pregnant women and also, to find the association between apelin 13 and blood pressure.

Materials and Methods: A case-control study was conducted between Department of Biochemistry and Department of Obstetrics and Gynaecology, RL Jalappa Hospital and Research Centre, Kolar, Karnataka, India. After approval from the Institutional Ethics Committee and written informed consent from study subjects, a total of 270 pregnant women were recruited for this study. Among them, 135 pre-eclamptic women were considered as cases and 135 normotensive healthy pregnant women served as controls. According to the pre-eclampsia severity, cases were grouped into mild (n=47) and severe pre-eclampsia (n=88). Blood samples were collected from all the study subjects and was analysed for apelin 13 by Enzyme Linked Immunosorbent Assay (ELISA) method. Maternal and foetal adverse outcomes were recorded. Results were expressed as mean±Standard Deviation (SD). Categorical variables were expressed in percentages. Spearman's correlation was applied and $p<0.05$ was considered significant.

Results: The mean gestational age was 36.66±3.69 weeks which was, significantly low in pre-eclamptic women compared with healthy pregnant women. BMI (26.94±3.81 kg/m^2), systolic (157.82±15.14 mmHg), diastolic (101.68±11.02 mmHg) and Mean Arterial Pressure (MAP) (120.20±11.12 mmHg), pulse rate (88.14±5.82 bpm), Aspartate Transaminase (AST) (25.25±12.49 IU/L) and Alanine Transaminase (ALT) (19.01±10.95 IU/L) were significantly increased in pre-eclamptic women when compared with control group. Mean maternal serum apelin 13 (341.44±218.63 pg/mL) concentrations were significantly lower in pre-eclampsia compared with healthy pregnant women. Maternal serum apelin 13 concentrations were negatively correlated with Systolic Blood Pressure (SBP) (r = -0.196), Diastolic Blood Pressure (DBP) (r = -0.172) and MAP (r =-0.204). Adverse maternal outcomes such as epigastric pain 75 (55.55%), oedema 62 (45.92%) and persistent headache 35 (25.92%) were higher in pre-eclamptic group. Additionally, adverse foetal outcomes were more in pre-eclamptic cases including significantly decreased birth weight (2.40±0.65), babies requiring Neonatal Intensive Care Unit (NICU) admission were 54 (40%), preterm birth (≤37 wks) in 50 (37.03%), Respiratory Distress Syndrome (RDS) 31 (22.96%), Small for Gestational Age (SGA) in 4 (2.96%) and Intra Uterine Death (IUD) in 11 (8.14%) babies.

Conclusion: It was concluded from the present study that there was low maternal serum apelin 13 concentrations in pre-eclampsia and had negative correlation with blood pressure, suggesting its potential role in the pathophysiology of pre-eclampsia.

Keywords: Angiogenesis, Endothelial dysfunction, Foetal outcome, Vasodilation

INTRODUCTION

Pre-eclampsia is a pregnancy disorder, characterised by new onset hypertension with or without proteinuria. It is characterised by blood pressure ≥140/90 mmHg and proteinuria (0.3g/day) after 20 weeks of gestation. In 2019, it has been reported that pre-eclampsia affects 2-8% of pregnancies worldwide [1]. Whereas in India, the incidence of pregnancy induced hypertension is 10.3% [2].

Pre-eclampsia is the leading cause of maternal, perinatal morbidity and mortality [3]. The major risk factors of pre-eclampsia include chronic hypertension, prior pre-eclampsia, cardiovascular disease, renal disease, diabetes mellitus, multiple gestations, advanced maternal age (>40 years) and obesity [4,5].

The precise mechanism of pre-eclampsia origin is not clear. The pathophysiology of pre-eclampsia is characterised by abnormal placentation, shallow trophoblast invasion, remodeling of spiral arteries and also maternal systemic inflammation, metabolic and thrombotic responses, links to altered vascular function, results in multi-organ damage [1,6,7]. Further, which is responsible for endothelial dysfunction and vascular inflammatory response, which causes disturbance in the haemodynamic changes necessary for maternal adaptation to pregnancy [8].

Apelin is a bioactive peptide, synthesised from preproapelin (77 amino acids) nascent single peptide, with hydrophobic rich N-terminal region. Further, preproapelin in the endoplasmic reticulum cleaves to generate 55 amino acid proapelin, containing receptor binding sites. Proapelin generates several biologically active short peptides. The short peptides include apelin 36, apelin 17, apelin 13 and Pyroglutamate apelin 13 (Pyr1-apelin 13) [9]. All these apelin peptides exhibit agonistic activity on the apelin receptor (APJ). However, apelin 13 being the most active peptide responsible for the biological activity of apelin [5]. The shorter peptides are more potent activators of APJ. The activation of apelin peptides by APJ promotes vasodilation, through Nitric Oxide (NO) pathway and the APJ is being targeted to treat heart failure and hypertension [10].

Studies have shown that apelin and its APJ receptor are expressed in endothelial cells, adipose tissue, heart and syncytiotrophoblast cells of placenta [9,11]. Apelin is an angiogenic factor in endothelial cells, stimulates vessel growth and endothelial cell proliferation [11,12]. Further, apelinergic system has been previously shown to be involved in the regulation of vascular bore size and integrity [13,14]. Even though, the role of apelin peptides in pre-eclampsia is not clear, but studies have reported conflicting results on apelin peptides [10,15,16].

The accurate assessment of these apelin peptides is useful in order to establish its role in pre-eclampsia. However, previous studies have reported on the serum total apelin concentrations without discriminating the specific biologically active short peptides and also data is not available on the association of apelin with blood pressure and adverse pregnancy outcomes in pre-eclampsia [10,15]. Hence, the present study was focused: (a) To evaluate the maternal serum apelin 13 levels in pre-eclampsia and healthy pregnant women; (b) To find the association between apelin 13 and blood pressure.

MATERIALS AND METHODS

Study design

This case-control study was conducted in Department of Biochemistry in association with Department of Obstetrics and Gynaecology, RL Jalappa Hospital and Research Centre, Kolar, Karnataka, India, after obtaining the approval from the Institutional Ethics Committee (No. DMC/KLR/IEC/235/2019-20) and written informed consent from all study subjects The study duration was from November 2018 to December 2019. Sample size was calculated with 90% power and 95% confidence interval by using the formula $n=2Sp^2[Z_1-\alpha/2 + Z_1-\beta]2/\mu d^2$, $Sp^2 = S1^2 + S2^2/2$ with a prevalence of 7-10% [10]. The sample size arrived for each group was 135, that is 135 pre-eclamptic subjects and 135 normotensive healthy pregnant women. A total of 270 pregnant women were recruited for this study from Department of Obstetrics and Gynaecology, RL Jalappa Hospital and Research Centre, Kolar, Karnataka, India. Out of 270 pregnant women, 135 pregnant women with pre-eclampsia were included as cases and 135 age matched normotensive healthy pregnant women were considered as control group. According to the pre-eclampsia severity [17], cases were grouped into mild (n=47) and severe pre-eclampsia (n=88).

Diagnosis of pre-eclampsia

Pre-eclampsia was diagnosed with blood pressure of ≥140/90 mmHg noted for the first-time during pregnancy on two occasions at least four hours apart, after 20 weeks of gestation with proteinuria of ≥300 mg/24 hours or +1 by dipstick method in a random urine sample. Mild pre-eclampsia was considered when blood pressure of ≥140/90 mmHg or more on two occasions at least 4 hours apart after 20 weeks of gestation and with proteinuria (dipstick reading of +1). Severe pre-eclampsia was defined as the presence of any of the following criteria: SBP ≥160 mmHg or DBP ≥110 mmHg on two separate measurements, performed at six-hour intervals, elevated serum creatinine concentrations >1.1 mg/dL or doubling of the serum creatinine concentrations in the absence of other renal diseases, elevated liver transaminases to twice normal concentration, platelet count less than 100,000/microliter, headache, visual impairment, epigastric pain or pain in the right upper quadrant. (American College of Obstetricians and Gynecologists practice bulletin 2013) [17].

Inclusion Criteria

Cases: Pregnant women diagnosed with pre-eclampsia, primigravida and multigravida women were considered.

Controls: Age matched normotensive with primigravida, multigravida, singleton pregnancy, no foetal anomaly and non-smokers were considered. All the controls were recruited from Department of Obstetrics and Gynaecology, RL Jalappa Hospital and Research Centre, Kolar, Karnataka, India.

Exclusion Criteria

Cases: Pregnant women with twin pregnancy, history of renal disease, liver disease, thyroid disorder, chronic systemic hypertension, gestational diabetes, hypertensive encephalopathy, cardiovascular diseases, pregnancy with foetal anomaly, patients with history of smoking and malignancy conditions were excluded from the study.

Controls: Pregnant women with twin pregnancy, foetal anomaly, history of renal disease, liver disease, thyroid disorder, chronic systemic hypertension, gestational diabetes, cardiovascular diseases, and patients with smoking and malignant conditions were excluded.

Demographic, physical and clinical examinations were done for all the study subjects. All the study subjects were treated and followed-up after diagnosis of pre-eclampsia and delivered their babies in the hospital. Maternal adverse outcomes including thrombocytopenia, acute renal failure, HELLP syndrome (haemolysis, elevated liver enzymes, low platelet count), oedema, persistent headache, visual disturbances, epigastric pain, vomiting, elevated hepatic enzymes and eclampsia were recorded. Any adverse foetal outcomes including preterm birth, RDS, SGA, birth weight, newborns requiring NICU admission and IUD were recorded.

Under aseptic conditions, five mL of venous blood was collected from the pre-eclamptic and normotensive healthy pregnant women. Gestational age of the study subjects was between 20-40 weeks. The collected blood samples were allowed to stand for two hours and centrifuged at 3000 rpm for 10 minutes to obtain the clear serum. Thus, obtained clear serum was stored at -80°C until testing. Serum was used for the estimation of apelin 13, AST and ALT. Two ml of EDTA blood was used for platelet count. Five mL urine sample was collected for urinary protein analysis by dipstick method. Height and weight were recorded and Body Mass Index (BMI) was calculated as the weight divided by square of height (kg/m^2). A trained nurse was available at the time of examination and collection of blood samples. Blood pressure was measured by using calibrated sphygmomanometer. MAP was calculated by using the formula: systolic pressure + (2x diastolic pressure)/3.

Determination of maternal serum apelin13

Human apelin 13 concentration in serum was measured by ELISA technique as per the procedure supplied by Sincere Biotech Co., Ltd., Beijing, China (Human Apelin 13 kit catalogue No: E13652182) in Clinical Biochemistry lab. This assay was based on the principle of quantitative sandwich technique. Purified human apelin 13 antibody was pre-coated onto the microtiter plate wells; to make solid phase antibody, standards and samples were added. Apelin 13 present in the serum was bound to the human apelin 13 antibody which with Horse Radish Peroxidase (HRP) labeled, become antibody-antigen-enzyme-antibody complex, after removing any unbound substances by washing procedure, 3,3', 5,5'-Tetramethylbenzidine (TMB) substrate solution was then added. TMB substrate solution becomes blue colour at HRP enzyme-catalysed. The reaction was terminated by adding stop solution (2 mol/L sulfuric acid) and then colour changes to yellow, the absorbance of the colour was measured at 450nm in spectrophotometer. No significant cross-reactivity or interference between human apelin 13 and any other cytokines were seen. The sensitivity for apelin 13 was 13.5 pg/mL. The concentration of human apelin 13 in the samples was then determined by comparing with standard curve and represented as pg/ml.

STATISTICAL ANALYSIS

Results were expressed as mean±SD. Mann-Whitney U test was used for continuous non-normally distributed variables. Categorical variables were expressed in percentages. Spearman's correlation was applied. The level of significance was $p<0.05$. Analysis was performed using Statistical Package for the Social Sciences (SPSS) software, version 22.0.

RESULTS

The baseline characteristics and maternal serum apelin 13 concentrations of women with pre-eclampsia and control group are presented in [Table/Fig-1].

The mean baseline gestational age (36.66±3.69 weeks) was significantly low in pre-eclamptic women compared with control group. BMI (26.94±3.81 kg/m^2), SBP (157.82±15.14 mmHg), DBP (101.68±11.02 mmHg), MAP (120.20±11.12 mmHg), pulse rate (88.14±5.82 bpm) AST (25.25±12.49 IU/L) and ALT

Parameters	Pre-eclampsia (n=135) Mean±SD	Healthy pregnant women (n=135) Mean±SD	p-value†
Age (years)	23.31±3.73	23.37±3.27	0.518
Primigravida (n, %)	100 (74.07%)	109 (80.74%)	-
Multigravida (n, %)	35 (25.93%)	26 (19.26%)	-
Gestational age at sampling (wks)	36.66±3.69	38.81±1.63	0.001*
BMI (kg/m^2)	26.94±3.81	25.37±3.92	0.006*
SBP (mmHg)	157.82±15.14	115.54±7.83	0.001*
DBP (mmHg)	101.68±11.02	74.13±6.49	0.001*
MAP (mmHg)	120.20±11.12	87.83±6.24	0.001*
Pulse rate (bpm)	88.14±5.82	86.21±7.82	0.001*
Platelet count x (10^9/L)	232.69±81.83	241.94±63.68	0.511
Presence of proteinuria (n,%)	135 (100%)	Nil	-
Serum AST (IU/L)	25.25±12.49	20.44±7.41	0.001*
Serum ALT (IU/L)	19.01±10.95	13.47±6.72	0.001*
Birth weight (kg)	2.40±0.65	2.84±0.49	0.001*
Maternal serum Apelin 13 (pg/mL)	341.44±218.63	498.40±237.51	0.001*

[Table/Fig-1]: Baseline characteristics and maternal serum apelin 13 levels of women with pre-eclampsia and healthy pregnant women.
*Significant (p<0.05), † Mann-Whitney U test; BMI: Body mass index; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean Arterial Pressure; AST: Aspartate transaminase; ALT: Alanine transaminase

(19.01±10.95 IU/L) were significantly increased in pre-eclamptic women compared with control group. Presence of proteinuria was seen in all pre-eclamptic cases. Mean maternal serum apelin 13 (341.44±218.63pg/mL) concentrations were significantly lower in pre-eclampsia compared with healthy controls. In the subgroup analysis, maternal serum apelin 13 concentrations were low in severe pre-eclamptic women (312.76±218.02 pg/mL) in comparison with mild pre-eclampsia; difference in mean values were not statistically significant [Table/Fig-2].

Parameters	Mild pre-eclampsia (n=47) Mean ± SD	Severe pre-eclampsia (n=88) Mean ± SD	p-value†
Apelin 13 (pg/mL)	342.23±290.06	312.76±218.02	0.352

[Table/Fig-2]: Apelin 13 levels in women with mild and severe pre-eclampsia.
†Mann-Whitney U test

Adverse maternal outcomes were higher in pre-eclamptic group, such as epigastric pain 75 (55.55%), oedema 62 (45.92%) and persistent headache 35 (25.92%) as illustrated in [Table/Fig-3].

Maternal adverse outcomes (n, %)	Pre-eclampsia (n=135)	Healthy pregnant women (n=135)
Thrombocytopenia	7 (5.18%)	Nil
Acute renal failure	1 (0.74%)	Nil
HELLP Syndrome	10 (7.40%)	Nil
Eclampsia	4 (2.96%)	Nil
Oedema	62 (45.92%)	11 (8.14%)
Persistent headache	35 (25.92%)	Nil
Blurred vision	12 (8.88%)	Nil
Epigastric pain	75 (55.55%)	72 (53.33%)
Vomiting	29 (21.48%)	7 (5.18%)
Disturbed sleep	11 (8.14%)	Nil

[Table/Fig-3]: Maternal adverse outcomes of pre-eclamptic and healthy pregnant women.
HELLP Syndrome: Haemolysis, Elevated liver enzymes, Low Platelet count

Additionally, adverse foetal outcomes were more in pre-eclamptic cases including significantly decreased birth weight (2.40±0.65), babies requiring NICU admission were 54 (40%), preterm birth (<37 wks) in 50 (37.03%), RDS 31 (22.96%), SGA in 4 (2.96%) and IUD in 11 (8.14%) babies [Table/Fig-4].

Adverse foetal outcomes (n, %)*	Pre-eclampsia (n=135)	Healthy pregnant women (n=135)
Preterm birth (≤37 wks)	50 (37.03%)	24 (17.77%)
Respiratory distress syndrome (RDS)	31 (22.96%)	11 (8.14%)
Low Birth Weight (LBW)	68 (50.37%)	27 (20%)
Small for Gestational Age (SGA)	4 (2.96%)	2 (1.48%)
Newborns requiring NICU admission	54 (40%)	22 (16.29%)
Intrauterine death (IUD)	11 (8.14%)	Nil

[Table/Fig-4]: Adverse foetal outcomes of pre-eclamptic and healthy pregnant women.
* more than one adverse outcome; NICU: Neonatal Intensive Care Unit

Maternal serum apelin 13 concentrations were weakly negatively correlated with SBP (r= -0.196), DBP (r= -0.172) and MAP (r=-0.204). However, maternal serum apelin 13 did not correlated significantly with age, gestational age and BMI [Table/Fig-5].

Parameters	r-value	p-value
Age (years)	0.024	0.785
Gestational age (wks)	0.043	0.617
BMI (kg/m^2)	0.091	0.294
SBP (mmHg)	-0.196*	0.022
DBP (mmHg)	-0.172*	0.046
MAP (mmHg)	-0.204*	0.018

[Table/Fig-5]: Spearman's correlation of apelin 13 with other parameters.
*Spearman's correlation is significant at the 0.05 level (2-tailed)

DISCUSSION

Pre-eclampsia is a life-threatening pregnancy specific disorder and is associated with secretion of vasoconstrictor factors into maternal circulation to initiate endothelial dysfunction and vasoconstriction [18]. Furthermore, the therapeutic strategies used to manage the disease are inadequate. The results of the present study indicated that the circulating levels of apelin 13 were significantly lower in pre-eclamptic women compared with healthy controls.

Apelin peptide through APJ causes endothelium dependent vasodilation by stimulating endothelial Nitric Oxide Synthase (eNOS) phosphorylation at serine 1177 and releases NO, known as potent vasodilator [9]. In support of this, Jia YX et al., reported that apelin triggers L-arginine transport and increases the NO production in isolated aorta of the rat [19]. In normal pregnancy, placental apelin is more abundant during early gestation, suggesting its role in placentation [20]. Apelin is angiogenic factor that stimulates blood vessel growth and differentiation. Studies indicated that imbalance in the angiogenic/anti-angiogenic factors are involved in pre-eclampsia complications [21-23].

Therefore, decreased apelin levels might affect the migration of invasive trophoblasts along the spiral artery and impair their vascular invasion [24]. This abnormal spiral artery remodeling, results in high resistance uteroplacental circulation, as observed in pre-eclampsia [15].

A few studies reported reduced apelin levels in other hypertensive disorders and also cardiac diseases [25,26]. In a study done by Inzukua H et al., who reported significantly decreased apelin mRNA levels in placentas of pre-eclamptic women and immunohistochemical signals for apelin and APJ receptor were also decreased in pre-eclampsia [15]. Cobellis L et al., reported that apelin secretion from placenta is abundant, suggesting its role in regulation of foetal development and hence, placentation [20].

Deniz R et al., reported that elabela, apelin and NO concentrations were significantly reduced in mild and severe pre-eclampsia compared to healthy pregnant women. Similar findings were also observed in newborn venous-arterial cord blood samples [27]. Taha AS et al., reported that maternal serum apelin concentrations were significantly lower in pre-eclampsia compared to healthy pregnant women [28]. Reduced levels of apelin may therefore have deleterious

effects on development of the foetus. Wang C et al., found that apelin treatment improved the expression of eNOS in placenta and serum levels of NO and eNOS, which were all decreased in pre-eclamptic rats, suggesting that restoration of eNOS/NO pathway may be involved in the ameliorative effects of apelin on pre-eclampsia [29]. Aberrant angiogenesis and increased blood pressure are the hall marks of pre-eclampsia. Thus, one might expect decreased levels of apelin associated with angiogenic and hypotensive in pre-eclampsia. Accordingly, study results demonstrate decreased levels of apelin in pre-eclamptic women compared with control group.

Considering the effects of apelin especially on angiogenesis and vasodilation, administration of low doses of apelin to pre-eclamptic women may reduce the blood pressure and maternal/foetal outcome. In a study by Wang W et al., demonstrated that subcutaneous apelin 13 administration to pre-eclamptic rats improved the clinical findings of pre-eclampsia, relieved the maternal hypertension, proteinuria, improved foetal growth and outcomes [30]. Even though extrapolation of the similar strategy to the patient volunteers using recombinant apelin is challenging in the management of disease and needs to be established.

Limitation(s)

Major limitation of the present study was the small sample size. Apelin concentrations were measured only once at the time of diagnosis.

CONCLUSION(S)

The present study concludes that decreased apelin 13 concentrations in pre-eclampsia, is negatively correlated with blood pressure. Because of its direct activating effect on L-arginine/eNOS/NO pathway, apelin may restore this pathway and inhibition of oxidative stress may be involved in the ameliorative effect of apelin on pre-eclampsia. Further, apelinergic system should be investigated for its role in pre-eclampsia and treatment strategies for the pre-eclampsia treatment.

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