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ORIGINAL ARTICLE

Maternal serum Apelin 13 and *APLN* gene promoter variant -1860T > C in preeclampsia

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ABSTRACT

Objective: To evaluate the apelin (*APLN*) −1860 T > C (rs56204867) polymorphism and maternal serum apelin 13 levels in preeclampsia and its association with blood pressure.

Methods: This case-control study was conducted in department of Biochemistry, Sri Devaraj Urs Medical College, Karnataka, India. A total of 181 subjects were enrolled in the study from department of Department of Obstetrics and Gynecology. The recruited women were grouped as: Group–I ($n = 91$) cases with preeclampsia and Group–II ($n = 90$) normotensive healthy pregnant women as controls. Under aseptic conditions, the collected 5 mL blood was distributed for serum separation (3 mL) and genetic analysis (2 mL). Serum was stored at −80 °C after centrifugation at 3000 rpm for 10 min. The collected five mL urine sample was used for urinary protein analysis by dipstick method. The *APLN* gene −1860 T > C polymorphism and Apelin 13 levels were analyzed by molecular methods and ELISA technique respectively. Birth weight and demographic details were recorded.

Results: In the present study, no significant difference was observed for mean gestational age and maternal age. Systolic (158.7 ± 14.0 mmHg) and diastolic (104.9 ± 10.7 mmHg) blood pressure, and mean arterial pressure (MAP) (123.0 ± 11.1 mmHg) (p-value .001) were significantly increased in preeclamptic women compared with healthy pregnant women. Birth weight (2.4 ± 0.5 kg) (p-value .001) was significantly decreased in babies born to preeclamptic mothers. Birth weights were also expressed in centiles, according to Fenton Chart. Number of small for gestational age (SGA) babies were more in preeclampsia ($n = 55$) than healthy pregnant women ($n = 28$). Mean maternal serum apelin 13 (239.4 ± 126.3 pg/mL) (p-value .001) concentrations were significantly lower in preeclampsia compared with healthy controls. Maternal serum apelin 13 concentration in preeclampsia was negatively correlated with systolic blood pressure ($r = -0.235$), diastolic blood pressure ($r = -0.172$) and mean arterial pressure ($r = -0.206$). However, maternal serum apelin 13 levels showed insignificant positive correlation with age, gestational age and birth weight. The genotype and allele frequencies of *APLN* gene were found significant between study groups as in preeclampsia ($\chi^2 = 11.69$; df = 2; $p = .0028$ and $\chi 2 = 14.27$; df = 1; $p = .00013$ respectively). CC genotype and C allele of *APLN* − 1860 T > C site was high in preeclampsia.

Conclusion: Study concludes that preeclamptic women have low level of serum apelin 13 and −1860 T > C polymorphism at *APLN* gene promoter site with increased allelic frequency of CC genotype and C allele compared to normotensive pregnant women. And this evidence may link to cardiac complications in preeclamptic women after delivery in later stage.

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Angiotensin; Apelin -1860T > C polymorphism; Apelin 13; Preeclampsia; Vasoconstriction

Introduction

Preeclampsia, a potentially dangerous pregnancy disease, characterized by new onset of hypertension and proteinuria after twenty weeks of gestation. Globally, the incidence of preeclampsia is around 5–8% and India alone accounts for about 10.3% of pregnancies [1,2]. Preeclampsia is the leading cause of maternal, perinatal morbidity and mortality. It also causes preterm birth and intrauterine growth restriction [3]. The symptoms include persistent headache, blurred vision, epigastric pain, vomiting and edema [4]. This multisystem disorder has the possible underlying pathophysiology such as abnormal placentation with improper trophoblast invasion, shallow remodeling of spiral arteries and also maternal systemic inflammation, oxidative stress and metabolic changes links to

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vascular endothelial dysfunction and hemodynamic changes during pregnancy [5–6]. Even though, the precise mechanism of preeclampsia is unclear.

Several biomarkers and genetic evidences have been studied to early understanding of later onset of preeclampsia. The reliability of selection of biomarkers based on the consideration of single or in combination for preeclampsia determination [7]. Similarly, the gene polymorphism is also emphasized with respect to the development of preeclampsia. A few such genes are studied in relation to pregnancy complications such as Methylene tetrahydrofolatereductase (*MTHFR*) (C677T) gene, Factor V (Leiden), Angiotensinogen (*AGT*) (M235T), Human leukocyte antigens (*HLA* various), endothelial nitric oxide synthase (*NOS3*) (Glu298Asp) etc [8,9]. However, there is a need to screen for new biomarker and its genetic expression with respect to biological activity.

Human *APLN* gene is located on chromosome X at Xq25–26.1, comprises 3 exons and 1 intron, and this gene will be transcribed to a bioactive peptide, apelin (10). The biosynthesis involves activation of preproapelin by splicing mechanism to produce biologically active short peptides [Apelin 36, Apelin 17, Apelin 13 and (Pyr1) apelin 13] (Figure 1) [11–12]. All the active peptides exhibit agonistic activity on Apelin receptor (APJ/APLNR receptor). Among these peptides, apelin13 is the most active peptide responsible for the biological activity, it promotes vasodilation, through nitric oxide pathway [11].

Apelin expresses in cardiomyocytes, vascular smooth muscles, endothelial cells, adipose tissue, and in placenta [13,14]. Apelin is an angiogenic factor in endothelial cells, stimulates vessel growth and endothelial cell proliferation [15–16]. And also involved in the regulation of vascular bore size and integrity [17,18]. Apelin acts as a positive inotropic agent, involved in endothelium-dependent vasodilation, angiogenesis, cardiac contractility, apoptosis and reduction of vascular wall inflammation [19,20]. Studies have shown that apelin and its Angiotensin like 1 receptor (APJ) are being targeted to treat cardiovascular disease and hypertension [14,15]. But the role of apelin peptides in preeclampsia is not clearly established and seldom studies have also reported non-consistent apelin levels in preeclampsia condition [14,21,22]. To the best of our knowledge, the present study is the first investigation to examine the association between apelin −1860 T > C (rs56204867) gene polymorphism and serum apelin 13 levels in south Indian population on preeclamptic women. The aim of this study was to determine the association between *APLN* gene −1860 T > C polymorphism and serum apelin 13 levels in preeclampsia and normotensive pregnancy and its association with blood pressure.

Materials and methods

Study design

A case-control study was designed and conducted in Department of Biochemistry in collaboration with Department of Obstetrics and Gynecology, RL Jalappa Hospital and Research Center, a teaching hospital of Sri Devaraj Urs Medical College, a constituent of Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India. Sample size calculation was done by using SPSS, version 22.0, with 80% power and 95% confidence interval. A total of 181 subjects were enrolled in the study from department of Department of Obstetrics and Gynecology, after obtaining the approval from institutional ethics committee and written informed consent from study subjects. The recruited women were grouped as: Group–I ($n = 91$) cases with preeclampsia and Group–II ($n = 90$) normotensive healthy pregnant women as controls. The diagnosis of preeclampsia is considered based on the American College of Obstetricians and Gynecologists (ACOG) guidelines (ACOG practice bulletin 2013) [23].

Inclusion and exclusion criteria

The inclusion criteria of the study were primigravida, multigravida, singleton pregnancy and women aged from 18 to 35 years. The exclusion criteria were pregnant women with history of renal disease, liver disease, thyroid disorder, chronic systemic hypertension, gestational diabetes, hypertensive encephalopathy, cardiovascular diseases, pregnancy with fetal anomaly, smoking and malignancy conditions.

Sample collection

Five mL of venous blood sample was collected in a vaccutainer under aseptic conditions, aliquoted 3 mL of blood into a plain tube and 2 mL of blood into a tube containing EDTA from both preeclamptic and normotensive groups. To obtain clear serum to estimate apelin13, the aliquoted blood for this purpose was rested for 2 h at room temperature and centrifuged at 3000 rpm for 10 min. Thus, obtained clear serum was stored at −80 °C until testing. Remaining two mL of EDTA blood was used for genetic analysis.

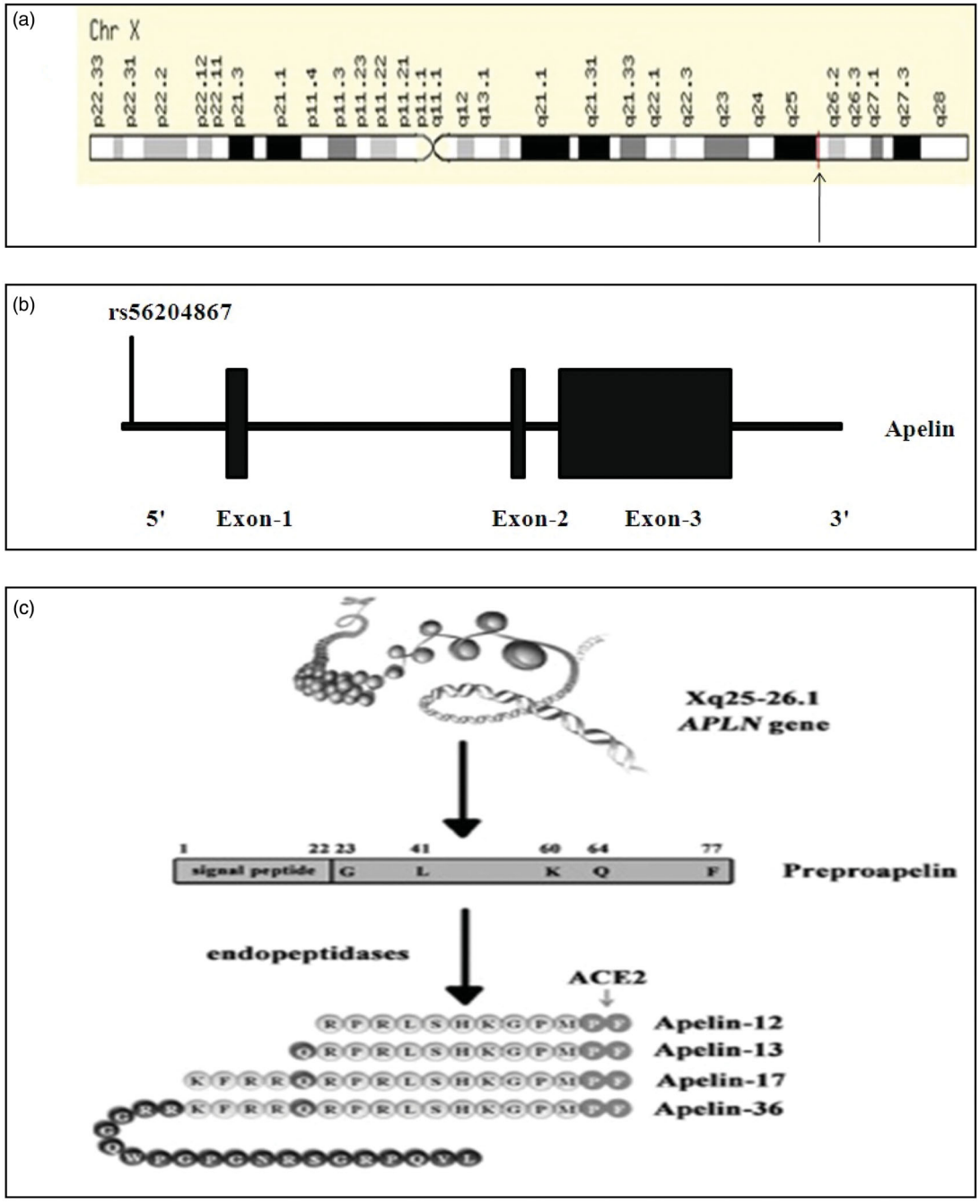

Figure 1. (a) Structure of chromosome X with location of *APLN* gene (arrow); (b) Structure of *APLN* gene showing exons, introns and with restriction site (11); (c) Expression of *APLN* gene to different isoforms of Apelin peptides (12).

Five mL urine sample was collected for urinary protein analysis by dipstick method. Mean arterial pressure (MAP) was calculated by using the formula: systolic pressure + [2 × diastolic pressure]/3.

Determination of Apelin 13

Human apelin 13 concentration in serum was measured by enzyme-linked immunosorbent assay (ELISA) technique as per the procedure supplied by Sincere Biotech Co.Ltd, Beijing, China (Human Apelin 13 kit catalogue No: E13652182).

Isolation of DNA from whole blood

Genomic DNA was isolated from the peripheral blood by salting out method [24]. The 2 mL of blood sample was transferred to 15 mL falcon tubes at room temperature. The volume was made up to 12 mL with erythrocyte lysis buffer (ELB) and vortexed vigorously for 2–3 min to remove any debris or clumps formed in

the tube. The tubes were centrifuged for10 min at 3000 rpm at 4 °C and supernatant was discarded. The obtained pellet was dissolved in ELB, centrifuged and supernatant was discarded. The pellet thus obtained was mixed using 0.27 mL of 20% sodium dodecyl sulfate (SDS) and 30 µL of proteinase K (20 mg/mL). The tubes were gently swirled, and incubated over night at 37 °C in water bath.

The pellet from the above step was mixed with 0.5 mL of 5 M NaCl and equal volume of Isopropyl alcohol, mixed, and swirled gently until the DNA strands were visible, which were carefully transferred to an eppendorf tubes containing 0.5 mL of 80% ethanol and centrifuged at 12,000 rpm for 7 min. This step was repeated twice to get clear DNA pellet and subjected for drying at room temperature. To this, 0.5 mL of Tris EDTA buffer (TE) was added and allowed at 65 °C in water bath for 30 min. Eppendorf tubes were parafilmed and placed on the rotator for solubilization of DNA in the Tris EDTA buffer. Finally, the obtained DNA was preserved at −80 °C until analysis.

Quantification and purity of DNA

The quality and quantity of the DNA samples were assessed by using UV spectrophotometer (Perkin Elmer Lambda 35) against TE buffer. TE buffer (48 µl) and DNA sample (2 µl) were mixed in cuvette and the absorbance was measured at 260 and 280 nm. The absorbance at 260 nm gives DNA concentration and 260/280 ratio gives the purity of DNA. DNA samples with 260/280 absorbance ratio between 1.7 and 1.9 were considered for PCR procedure.

Polymerase chain reaction (PCR)

The reference *APLN* promoter gene sequence was retrieved from Genbank (Accession No. NG_016718.1). To carry out PCR, sequence specific primer pairs were designed to amplify the promoter boundary regions of *APLN* gene with the help of Primer Quest tool, IDT DNA software. Polymerase chain reaction (PCR) was carried out with these specific primers (Table 1) [25]. The reaction contained 100 ng of genomic DNA, 10X PCR buffer, dNTPs (10 mM), each primer (10 picomole), $MgCl_2$ (1.5 mM), and 1 unit *Taq* DNA polymerase (Bangalore Genei, India) and the conditions followed were: initial denaturation at 95° C for 5 min followed by 28 cycles of denaturation at 95 °C for 30 s, annealing 56 °C for 30 s, 72 °C for 1 min and final extension at 72 °C for 5 min.

Table 1. Designed primers on *APLN* gene.

Name of primer	5′ to 3′ sequence
Forward primer	GGGGAACAGTGAAGGGAGAATGGT
Reverse primer	AGAAGCGGGTCCTGAAGTTGT TTG

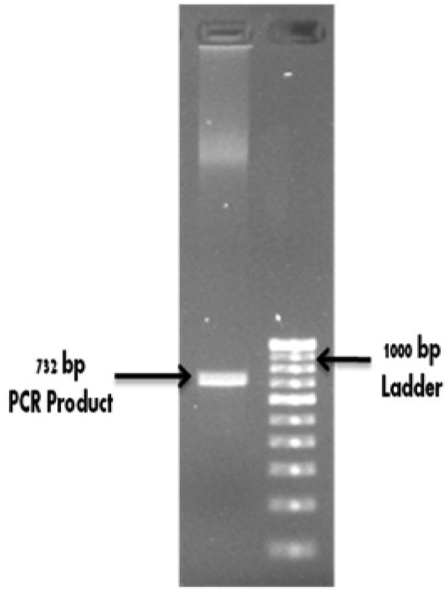

Figure 2. Agarose gel electrophoresis of PCR products.

Electrophoresis procedure

The PCR amplified products were subjected to agarose gel electrophoresis. The gel was prepared by mixing 1 g in 100 mL of 1xTE buffer in a microwave until dissolved completely. Allowed to cool down to 50 °C, mixed with 1.2 µL ethidiumbromide (EtBr). Placed freshly poured gel with comb sit at room temperature for 20–30 min, until get solidified. Loaded the loading dye mixed PCR products and DNA ladder in respective wells in the gel kept in pre-equlibrated electrophoresis unit. Gel was allowed to run using electric current of 130 V until the dye line covers approximately 75–80% of the way down the gel. Visualized the PCR separated products in gel documentation device under ultraviolet light (BIO-RAD, Gel Doc XR+) (Figure 2) [26].

Restriction fragment length polymorphism (RFLP)

The PCR product (10 µL) was digested with *Xho*I (New England Biolabs, China) in a 20 µL volume mixture containing 1 µL restriction enzyme, 2 µL CutSmart buffer and 7 µL water. The reaction mixture was incubated at 37 °C overnight. After incubation, digested products were mixed with 2 µL loading buffer. The electrophoresis was performed with ethidium bromide-stained 2% agarose gel. The size of the restriction fragments for RFLP was determined by using 1000 bp

DNA ladder. The differences in polymorphic allele were determined by using UV light (BIO-RAD, Gel Doc XR+) (Figure 3).

Statistical analysis

Study results were expressed as mean and standard deviation. Mann-Whitney *U* test was used for continuous non-normally distributed variables. Spearman's correlation (*r*) was applied for correlation of apelin 13 with study parameters. Fisher exact test was used for comparison of categorical variables and to test the genotype frequencies and Hardy- Weinberg equilibrium was used. Chi- square (χ^2) test was used for the comparison of the allele and genotype frequencies between preeclamptic cases and controls. The statistical analysis was performed using SPSS software, licensed version 22.0. The *p* value <.05 was considered as significant.

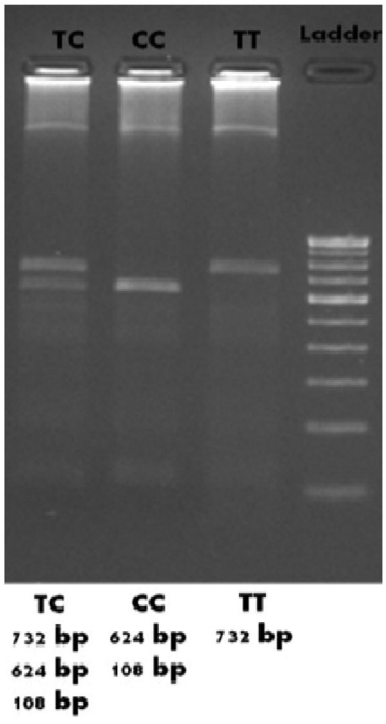

Figure 3. Agarose gel electrophoresis of RFLP products.

Results

Table 2 describes the demographic details of preeclampsia and normal pregnants group. No significant difference was observed for mean gestational age and maternal age. Systolic (158.7 ± 14.0 mmHg) and diastolic (104.9 ± 10.7 mmHg) blood pressure, and mean arterial pressure (MAP) (123.0 ± 11.1 mmHg) (*p*-value .001) were significantly increased in preeclamptic women compared with control group. Presence of proteinuria was seen in all preeclamptic cases. Birth weight (2.4 ± 0.5 kg) (*p*-value .001) was significantly decreased in babies born to preeclamptic mothers. Mean maternal serum apelin 13 (239.4 ± 126.3 pg/mL) (*p*-value .001) concentrations were significantly lower in preeclampsia compared with healthy controls.

By using the Fenton Chart, birth weights were expressed in centiles and compared between preeclampsia and healthy pregnant women. It was observed that <10th centile was observed in 55 (60.4%) cases compared to healthy controls 28 (31.1%), which was more in babies born to preeclamptic mothers, indicating increased small for gestational age (SGA) babies. Between 11th and 50th centile was observed in 25 (28.5%) cases compared with controls 49 (53.8%) and was more in controls. 51th–90th centile was observed in 10 (10.9%) cases compared to 12 (13.1%) in controls. More than 97th centile was observed in 1 (1%) in preeclampsia and 1 (1%) in healthy pregnant women (Table 3). Preeclampsia cases were divided into women with small for gestational age (SGA) *vs* women with appropriate for gestational age (AGA). It was observed that 55 (60.4%) babies were SGA and 36 (39.5%) were AGA in preeclamptic women.

Table 4 indicates maternal serum apelin 13 concentration in preeclampsia that was negatively correlated

Table 2. Baseline characteristics and maternal serum apelin 13 levels in preeclampsia and healthy pregnant women.

Parameters	Preeclampsia (*n* = 91) Mean ± SD	Healthy pregnant women (*n* = 90) Mean ± SD	*p*-value
Age (years)	22.6 ± 3.3	23.37 ± 3.27	.476
Primigravide (*n*, %)	85 (93.4%)	87 (95.6%)	–
Multigravide (*n*, %)	6 (6.5%)	3 (3.2%)	–
Gestational age at sampling (wks)	37.8 ± 2.3	38.81 ± 1.63	.762
SBP (mmHg)	158.7 ± 14.0	115.54 ± 7.83	.001*
DBP (mmHg)	104.9 ± 10.7	74.13 ± 6.49	.001*
MAP (mmHg)	123.0 ± 11.1	87.83 ± 6.24	.001*
Presence of proteinuria (*n*,%)	91 (100%)	Nil	–
Birth weight (kg)	2.4 ± 0.5	2.84 ± 0.49	.001*
Maternal serum Apelin 13 (pg/mL)	239.4 ± 126.3	498.4 ± 237.5	.001*

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MAP: Mean Arterial Pressure. *Significant.

with systolic blood pressure ($r = -0.235$), diastolic blood pressure ($r = -0.172$) and mean arterial pressure ($r = -0.206$). However, maternal serum apelin 13 levels showed insignificant positive correlation with age, gestational age and birth weight.

Tables 5 and 6 depict the distribution of genotype/allele frequencies between preeclampsia and control group. In this study context, 68 (74.72%) of preeclamptic cases and 84 (93.35%) of healthy pregnants were homozygote (TT), whereas 18 (19.78%) of preeclamptic cases and 5 (6.02%) of controls were heterozygote (TC), and also 05 (5.49%) of cases and 01 (1.0%) of controls were homozygous (CC) at this position were observed. The distribution of apelin-1860T > C genotype frequency was higher in preeclampsia in comparison with the healthy controls. In the allelic distribution, T allele was observed in 154 (86.61%) of preeclamptic cases and 178 (96.21%) of the controls. C allele was seen in 28 (15.38%) of preeclamptic cases and 7 (3.78%) of the controls. Hence, C allele distribution was found to be significant ($p = .00013$). The frequency of promoter *APLN* gene −1860 T > C polymorphism allele/genotypes is higher in cases than controls and appears as risk allele.

Table 3. Comparison of birth weight centiles in preeclampsia and healthy pregnant women.

Birth weight centiles	Preeclampsia ($n = 91$)	Healthy pregnant women ($n = 90$)
Below 10th Centile	55 (60.4%)	29 (31.8%)
Below 50th Centile	25 (28.5%)	49 (53.8%)
50–90th Centile	11 (12%)	12 (13.1%)

Table 4. Correlation of maternal serum apelin 13 with other study parameters.

Parameters	*r*-value	*p*-value
Age (years)	0.113	.284
Gestational age (wks)	0.048	.649
SBP	−0.235*	.025
DBP	−0.172*	.046
MAP	−0.206*	.050
Birth weight	0.116	.275

*Correlation is significant at the 0.05 level (2-tailed).

Discussion

Preeclampsia is a life-threatening disorder of pregnancy associated with altered vasoactive factors in maternal circulation accompanied by dysregulation of blood pressure and endothelial dysfunction [27]. The study results with respect to genetic analysis of apelin gene exhibited *APLN* − 1860 T > C (rs56204867) polymorphism with concomitant circulating low levels of apelin 13 peptide precisely more in preeclamptic women than normal pregnants. However, in support of this observation, blood pressure also altered proportionately.

Li et al. first reported *APLN* gene −1860 T > C (rs56204867) polymorphism at the promoter region in the Han Chinese hypertensive population [28]. Apelin −1860 T > C is documented in cardiovascular diseases with increased blood pressure and vascular complications [25]. However, similar observation needs to be screened with respect to preeclampsia. It has been shown that the apelin −1860 T > C gene polymorphism and plasma apelin concentration are related. The possible apelin genotype and allele frequencies of *APLN* gene were different between study groups ($\chi^2 = 11.69$; df = 2; $p = .0028$ and $\chi 2 = 14.27$; df = 1; $p = .00013$, respectively). CC genotype and C allele of *APLN* − 1860 T > C site were significantly higher in preeclampsia compared to healthy controls.

The apelin −1860 T > C polymorphism is established in hypertensive conditions like heart disease [25,29]. Though less information is available with respect to similar polymorphism in pregnancy hypertensive disorders. Thereby study results of −1860 T > C at promoter region and low levels of serum apelin apparently project the possible onset of cardiac problems in preeclamptic women after delivery [28].

The probable explanation for the above observation is that the up- regulation of vasoconstrictor and pro-inflammatory angiotensin II (ANG II) and angiotensin II receptor type 1 (AT1R) have direct influence for down regulation of apelin protein [30]. Apelin counter-regulates the actions of Ang II and variations in apelin and APJ

Table 5. Comparison of Apelin-1860T > C gene polymorphism in preeclampsia and healthy pregnant women.

Apelin T1860C genotype frequency	Preeclampsia ($n = 91$) (%)	Healthy pregnant women ($n = 90$) (%)	OR (95% CI)	*p*-value
TT	68 (74.72%)	84 (93.35%)	-	.0028
TC	18 (19.78)	5 (6.02%)		
CC	05 (5.49)	1 (1.20%)		
$\chi^2 = 11.69$; df = 2				
Allele				
T	154 (86.61%)	178(96.21%)	4.623 (1.965 − 10.88)	–
C	28 (15.38%)	7 (3.78 %)		.00013
$\chi 2 = 14.27$; df = 1				

OR: Odds ratio.

Table 6. Allele frequency/genotype frequencies and test of Hardy–Weinberg equilibrium.

	Preeclampsia		Healthy pregnant women	
f(T)	0.8461		0.9621	
f(C)	0.1538		0.0378	
	O	E	O	E
TT	68	65.15	84	83.13
TC	18	23.69	05	6.72
CC	05	2.15	01	0.136
	$\chi^2 = 32.23$, $p = .9202$		$\chi^2 = 5.93$, $p = .96$	

f = observed frequency of each allele (T or C); O = observed genotype numbers; E = expected genotype numbers under a Hardy- Weinberg equilibrium (HW) equilibrium assumption; χ^2 = Chi Square values.

genes may be associated with risk of hypertension [31]. The similar observation was also reported by Yamaleyeva et al. where they described up-regulation of ANG II/receptor in chorionic villi of preeclamptic women, suggested a paracrine role of local ANG II/AT1R the placenta. Evidenced by an *in vitro* study where administration of ANG II has negative impact on the release of apelin from chorionic villi. These research findings focus on the possible potential interaction between apelin and ANG II that can interfere with the formation of apelin in the placenta or its biological functions [27].

Endogenous apelin peptides upon interaction with APJ/AR causes endothelium dependent vasodilation by stimulating endothelial nitric oxide synthase (eNOS) by phosphorylation process at serine 1177 residue and produce nitric oxide [32]. This observation supported by research report of Jia et al., where the apelin showed triggering action on L-arginine transport and more nitric oxide production in aorta of the rats [33].

In normal pregnancy, placental apelin is more abundant during early gestation, suggesting its functional role during placentation in terms of favoring angiogenesis [34]. These results directly linked to the altered concentration of angiogenic and anti-angiogenic factors that are involved in preeclampsia complications [35–37]. The possible explanation might be due to impaired migration of invasive trophoblasts, defective vascular invasion and spiral artery formation [21].

The low apelin level substantially justified in a study conducted by Inuzuka et al., where they reported decreased expression of apelin gene captured as mRNA level in placenta of preeclamptic women [21]. Similarly, immuno-histochemical reports also presented decreased APJ receptors [14]. thereby lower levels of apelin affects fetal development is evident. Wang et al. observed in an animal model that administration of apelin 13 improves the expression of eNOS in placenta and increases both the plasma eNOS and nitric oxide levels. Therefore, maintenance of apelin 13 levels in preeclampsia found to be critical and essential for restoration of eNOS/NO system [38].

Accordingly, our study results demonstrated decreased levels of maternal serum apelin13 in preeclamptic women indicated elevation of blood pressure compared with control group, where, yet another few studies reported low level of apelin 13 in association with hypertensive disorders and cardiac diseases [39–41]. It has been reported that preeclamptic women have 2-fold increased risk to develop cardiovascular disease in future life. This may be due to chronic hypertension, renal disease, dyslipidemia, oxidative stress in preeclampsia, which may be associated with cardiovascular risk in preeclampsia. Therefore, the reduced apelin 13 levels may suggest compromised cardioprotective effects in preeclamptic women [42].

Considering the functional aspect of apelin 13, subcutaneous administration of recombinant apelin 13 to preeclampsia subjects might improve the associated pregnancy complications and fetal outcome. Even though, extrapolation of the similar strategy to the patient volunteers using recombinant apelin 13 is challenging in the management of disease that needs to be established. A few research reports emphasized a relationship of low levels of apelin 13 and cardiovascular complications such as coronary artery disease [43], coronary artery ectasia [44], heart patients with systolic left ventricular dysfunction [45]. The evidences also exist about the onset of cardiovascular complications by preeclamptic mother after delivery [46]. Nevertheless, information links between preeclampsia and cardiac complications after delivery period are limited. Hence, in this preliminary report, we postulate the genetic evidence of *APLN* − 1860 T > C (rs56204867) may be one of the risk factors involved in later development of cardiac complications in preeclamptic women after delivery that needs to be established.

Conclusion

The study results conclude that preeclamptic women have low level of serum apelin 13 and −1860 T > C (rs56204867) polymorphism at promoter site with increased allelic frequency of CC genotype and C allele compared to normotensive pregnant women. Maternal serum apelin 13 levels were negatively correlated with blood pressure in preeclampsia and this evidence may link to cardiac complications in preeclamptic women after delivery in later stage.

Limitations of the study

This study has the limitation with respect to sample size, screening for placental apelin expression and

sequence analysis of *APLN* gene. In addition to this, babies with SGA were observed in control subjects, could be due to confounding factors like economic status, life style, genetic and epigenetic factors. Therefore, further studies are recommended.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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