doi.org/10.36721/PJPS.2023.36.3.REG.749-753.1

# Effect of serum apelin and elabela levels in pregnancies complicated with preeclampsia in association to BMI, CRP and parity

**Humaira Khan[1], Sammiya Uraneb[1], Rehana Mushtaq[2] and Farhat Bano[1]**
[1]University of Health Sciences, Lahore, Pakistan
[2]Obs & Gynae Department, National Medical College and Teaching Hospital, Parsa, Birgunj, Nepal

**Abstract**: Hypertensive disorders of pregnancy are a serious and life-threatening condition often complicating the pregnancy leading to preeclampsia, eclampsia, maternal and neonatal mortality and morbidity. Preeclampsia is myriad of diseases starting from improper remodeling of spiral arteries, poor placentation leading to fatal consequences of intrauterine fetal and maternal death due to uncontrolled blood pressure. Thirty-three preeclamptic women in Group 1 and thirty-three healthy pregnant in group 2 has been taken in this research. Statistical analysis and evaluation were performed by utilizing SPSS version 23.0. Result of our research show significant decrease in serum apelin and elabela and association of these hormones with parity, body mass index (BMI), C-reactive protein (CRP) , systolic and diastolic blood pressure in preeclamptic women as compared to healthy pregnant while no significant association with age.

**Keywords**: Apelin (APLN), BMI, CRP, Elabela (ELA), ELISA, pre-eclampsia.

## INTRODUCTION

Preeclampsia (PE) is defined as onset of new hypertension after twenty weeks of gestation and proteinuria>0.3g/24h (Fox *et al*., 2019, Pankiewicz *et al*., 2019). Preeclampsia affects 2-8% of all pregnancies worldwide, accounting for 10%-15% of maternal death and morbidity (Belay Tolu *et al*., 2020) while that in Pakistan is documented to be between 2.4% to 5.6% (Soomro *et al*., 2019). Exact mechanism of pathophysiology of PE is still least understood however it is hypothesized that an imbalance between angiogenic and anti-angiogenic factors may be a causal factor (Jim and Karumanchi, 2017).

Number of biochemical markers have importance in prediction and treatment of preeclampsia. Apelin and elabela are one of them. Apelin identify before three decay act as endogenous ligand of the APJ orphan G protein-coupled receptor, which is an active cardiovascular peptide (angiotensin receptor-like1) (Briana and Malamitsi-Puchner, 2021). The role of Apelin in preeclamptic etiology has been the subject of numerous clinical research, all of which have found that patients with hypertensive disorders of pregnancy had much lower apelin expression than normal controls in their placentas (Deniz *et al*., 2019).

Elabela (ELA) is a placental and kidney hormone that encodes ligand for the apelin receptor (Lu *et al*., 2017). It was first discovered in human blastocysts and regulates the function of embryonic stem cell self-renewal (Pritchard *et al*., 2018). First-trimester placental tissue, which includes 8+3 weeks and term placentas, are the primary source of this hormone (Panaitescu *et al*., 2020, Zhou *et al*., 2019). Spiral artery remodeling is typically inadequate in PE, leading to shallow and poor placentation because of improper extra villous trophoblast invasion (Wang *et al*., 2019). It is possible that ELA activity enhances invasion and as a result it helps prevent PE from developing in pregnant females through improving spiral artery remodeling (Panaitescu *et al*., 2020).

The aim of present study is to find out the concentration of hormone and relate it with BMI, CRP in P.E and also with demographic data such as age, social status and education.

## MATERIALS AND METHODS

### *Study design and population*

The study design was comparative cross sectional and was approved by Ethical Review committee, University of Health Sciences, Lahore, Pakistan. Present research included two groups.

Group 1- included 33 preeclamptic pregnant women.
Group 2- included 33 healthy pregnant women.

Inclusion criteria of Preeclamptic Patients is as fallows:

i- Pregnant females of any age without any age limitation.
ii- All pregnant females more than 20 weeks of gestation diagnosed preeclamptic having increased blood pressure (≥140/90mmHg) measured at two separate occasions which are at least 6 hours apart and protein of >100mg/dl by urine analysis or >300milligram in a 24-hour urine collection, after 20 weeks of gestation were diagnosed as preeclamptic according to ACOG (Reddy *et al*., 2021) and were included in preeclamptic group (Rana *et al*., 2019).

**Corresponding author:* e-mail: farhatbano_2000@yahoo.com

The exclusion criteria were as follows: i-Severe anemia (<9g/dl), ii-Hepatic dysfunction (serum ALT>40 IU/L & Bilirubin>1.2mg/dl), iii-Renal dysfunction (serum creatinine>1.1mg/dl), iv-Females with gestational and pre-gestational diabetes (BSR>160mg/dl), v-Females with chronic hypertension, vi- Females with body mass index>30.

***Sample collection***

All participants gave their informed consent to participate in the study. Samples from all the participants were taken in delivery room. This was done to standardiz the protocols of study and to avoid the stress of simple vaginal delivery. A total 5ml of blood was drawn under aseptic conditions from the median cubital vein from anterior aspect of forearm of preeclamptic and healthy pregnant women at the time of delivery.

***Preparation of serum***

The blood was collected in gel containing serum separation tubes. After clotting the blood was centrifuged at 3000 rpm (revolutions per minute) for 10 minutes. The serum was transferred in properly labelled autoclaved eppendorff tubes and was stored at -80 degree Celsius for subsequent biochemical analysis. Serum apelin, elabela and serum CRP levels were measured by using commercially available kits on ELISA and proteinuria was measured by dipstick method.

## RESULTS

***Effects of demographic parameters in preeclamptic and healthy pregnant women***

Of the total 66 mothers in the study, 50% were preeclamptic and 50% were healthy pregnant mothers.

***Effect of Age in preeclamptic and healthy pregnant women***

There was no difference found between maternal age in preeclamptic and healthy pregnant women (table 1).

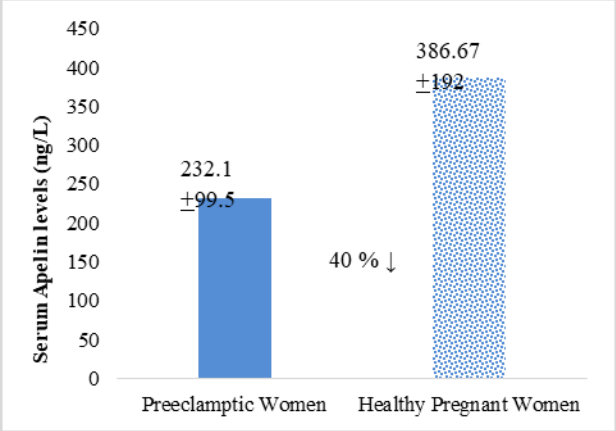


**Fig. 1**: Shows serum apelin (ng/L) of preeclamptic patients and healthy pregnant controls (p<0.001).

***Effect of systolic and diastolic blood pressure in preeclamptic and healthy pregnant women***

There was 47.02% and 46.34 % increase of systolic and diastolic blood pressure in preeclamptic women as compared to healthy pregnant women respectively (table 1).

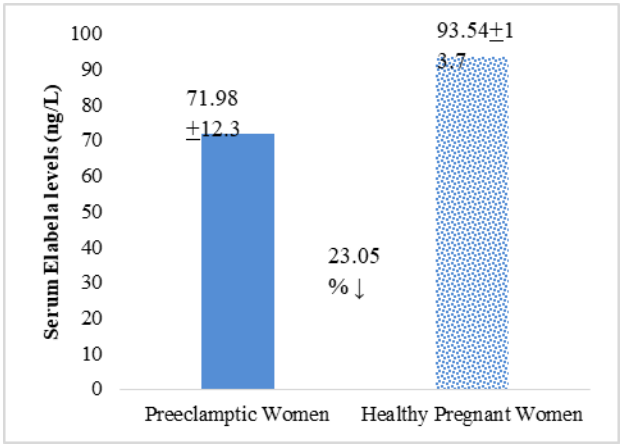


**Fig. 2**: Shows serum elabela (ng/L) of preeclamptic patients and healthy pregnant controls (p<0.001)

***Effect of BMI in preeclamptic and healthy pregnant women***

There was 20.34% increase in preeclamptic women as compared to healthy pregnant women (table 1).

***Biochemical parameters***

*Effect of serum CRP in preeclamptic and healthy pregnant women*

There was significant increase in serum CRP levels in preeclamptic patients (fig. 3) (p<0.05) (increased by 91.8%) as compared to healthy pregnant controls.

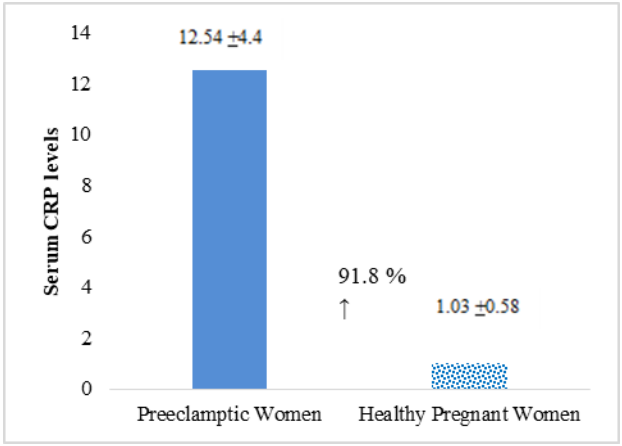


**Fig. 3**: Shows serum CRP (mg/dl) in PE patients and healthy pregnant control

***Effect of protein urea in preeclamptic and healthy pregnant women***

There was proteinuria present with mean (2.3±1.06) in preeclamptic while absent in healthy pregnant women.

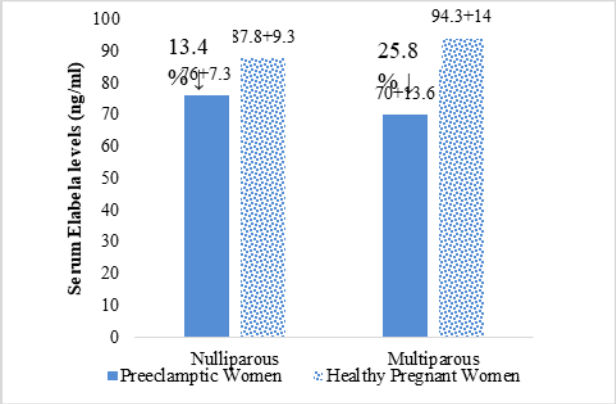


**Fig. 4**: Shows % difference between serum elabela among nulliparous and multiparous pregnant women with and without preeclampsia.

***Effect of serum Apelin in preeclamptic and healthy pregnant women***

There was significant decrease in serum apelin levels in preeclamptic patients (fig. 1) ($p<0.001$) (40% dec.) as compared to healthy pregnant controls.

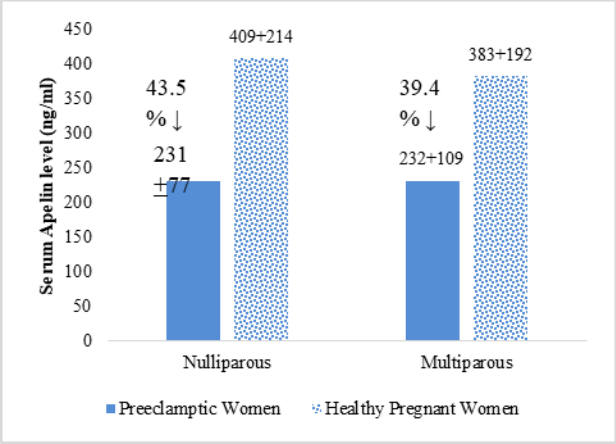


**Fig. 5**: Shows % difference between serum apelin in preeclamptic and healthy pregnant women in relation with parity.

***Effect of serum Elabela in preeclamptic and healthy pregnant women***

Serum elabela levels were significantly lower in preeclamptic patients (fig. 2) ($p<0.001$) (23.05% dec.) as compared to healthy pregnant control.

## DISCUSSION

Preeclampsia is a major cause of fetal mortality, premature birth, fetal growth restriction and intra-uterine diseases. This placental disorder originates with dysfunction of trophoblasts. Many studies have been conducted to evaluate and explain the possible role of serum apelin and elabela levels in the course of development of preeclampsia.

In our present research shows serum significant decrease in apelin levels in preeclamptic women as compared to healthy pregnant women (fig. 1, dec by 40%). The samples for our research study were collected in last trimester. This is in accordance with other studies (Deniz *et al*., 2019, Temur *et al*., 2022). Circulating apelin level decreases in the middle of pregnancy, but it tends to rise in healthy pregnancies towards the end of the third trimester. In normal pregnancy, increased apelin concentrations should be expected; however, decreased maternal apelin concentrations may play a role in the development of preeclampsia (Gürlek *et al*., 2020, Hamza *et al*., 2021). We collected blood samples from preeclamptic women between the 24$^{th}$ and 42$^{nd}$ week of pregnancy, during such a wide interval of time, the placental growth may have had an impact on the serum Apelin levels. Apelin levels may also be influenced by the degree of preeclampsia, elevated blood pressure, or the development of placental disease during the week of sample collection. Due to the fact that Apelin is expressed at varying levels during pregnancy (Hanssens *et al*., 2022), the results obtained in the early and late stages of pregnancy will differ.

Elabela has important role in development of placenta, the decrease in this novel hormone has gained important role in development of placenta. Our study shows 23.05% decrease of serum elabela level in preeclamptic women as compared to healthy pregnant controls (fig. 2). Elabela is mainly secreted from placenta and it helps in normal physiological processes carried out in placenta, so a decreased level will hamper the normal activity which is also the basis of preeclampsia development (Chen *et al*., 2020). Elabela exhibits a more promising effect on lowering blood pressure as compared to apelin. Elabela regulates blood pressure via APJ receptor (Li *et al*., 2020). It was reported that elabela knock-out mice showed decreased release of elabela causing proteinuria, hypertension and renal damage in preeclampsia mice resulting in placental vascular disruption, under perfusion of placenta and cause maternal systemic damage in preeclampsia (Pritchard *et al*., 2018). Complete absence of elabela resulted in disturbed feto-maternal circulation, underdeveloped vasculature, thin labyrinths, defective cardiac looping, increased apoptosis and decreased proliferation leading to death of about 50% of embryos (Papangeli and Chun, 2017). Elabela levels were considerably decreased in late-onset preeclamptic pregnancies compared to normal pregnancies (Ma *et al*., 2020). However, elabela concentrations were found to be higher in late-onset preeclampsia patients than in early-onset preeclampsia patients (Panaitescu *et al*., 2020). Blood pressure was decreased by injecting elabela and apelin in the experimental animals with hypertension. Apelin and elabela administration exerts an effect both in animal models and in humans (Li *et al*., 2020).

**Table 1**: shows BMI, CRP, correlation of nulliparity, multiparity, apelin and ELA levels in PE and healthy pregnant females.

| Parameters | Preeclamptic patients N=33 | Healthy-normal controls N=33 | p-value | % |
|---|---|---|---|---|
| | Mean ± SD | Mean ± SD | | |
| Age | 28.66±4.85 | 28.06±5.9 | >0.05 | 2.14 Inc. |
| Systolic blood pressure | 156.6±20.27 | 106.51±8.15 | <0.001 | 47.02Inc. |
| Diastolic blood pressure | 101.36±9.45 | 69.24±5.2 | <0.001 | 46.34 Inc. |
| BMI | 31.9±4.4 | 26.5±2.2 | <0.001 | 20.34 Inc. |
| Proteinuria | 2.3±1.06 | 0.00 | <0.001 | 100 Inc. |
| CRP (mg/dl) | 12.54±4.4 | 1.03±0.58 | <0.05 | 91.8 Inc. |

Our results show increase in systolic and diastolic blood pressure 47.06% and 46.34 % respectively (table 1) and proteinuria 2.3±1.06 in preeclamptic women and they are probably related with reduced elabela and apelin levels. Many studies have reported that the amount of apelin and elabela in circulation is low with high blood pressure and proteinuria. Elabela and apelin causes vasodilation which leads to low blood pressure (Deniz *et al*., 2019). Thus, in our preeclamptic cases with a similar mechanism hypertension may have arisen due to reduction in the amount of apelin and elabela which restricts vasodilation.

In current study, preeclamptic women have higher BMI (20.34% inc.) as compared to healthy pregnant women (fig. 3) (Babah *et al*., 2017, Ogunwole *et al*., 2021). The current study showed that serum CRP level was increased (figure, increased by 91.8%) in PE patients as compared to healthy pregnant controls. Serum CRP level and BMI was increased in preeclamptic patients (Raio *et al*., 2019). Preeclamptic women with higher BMI are at higher risk of developing systemic inflammation (Bernhardt *et al*., 2022). Similarly, elevated levels of CRP were observed in the early onset preeclampsia. (Ali *et al*., 2022). In our study we also found the positive correlation among BMI and CRP levels. The higher concentrations of these parameters are seemed to be involved in development of preeclampsia. We found the decreased concentration of serum apelin and elabela level in preeclamptic women with increased CRP level. It means there is negative correlation with apelin and elabela in preeclamptic women.

In current study we compared the levels of elabela in relation to parity in both groups and we revealed non-significant decrease among multiparous preeclamptic women (fig. 4, 25.8% decrease), while apelin decrease in nulliparous (fig. 5, 43.5% decrease). Multiparity was associated with low incidence of preeclampsia while high incidence observed in nulliparous women (Maeda *et al*., 2021). In our study decreased level of apelin might be due to nulliparity and reduced level of elabela is related to multiparity.

According to the findings of our study, early gestational levels of apelin and elabela can aid in the early diagnosis of preeclampsia. Increased BMI, CRP and nulliparity can raise the risk of PE later in pregnancy. These parameters apelin and elabela can help us in timely detection and screening out of patients at high risk.

## CONCLUSION

In our study we concluded that serum apelin and elabela levels are associated with BMI, CRP and parity in preeclamptic women. These are the major risk factors in pathogenesis of preeclampsia. By controlling these we can prevent preeclampsia. If we observe these parameters early in gestation, it might be helpful in early detection of preeclampsia in high-risk population.

## ACKNOWLEDGEMENT

Special gratitude to the University of Health Sciences, Lahore for its support during the whole project.

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