Comparison of Serum Calprotectin in Preeclamptic and Normotensive Pregnant Females

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ABSTRACT

Background: Preeclampsia is a syndrome characterized by raised blood pressure (140/90 mm Hg) and proteinuria of 100 mg/dl by urine analysis or > 300 mg in 24 hours urine collection following 20 weeks of pregnancy. It is one of the most serious complications of pregnancy with greatest impact in developing countries. Calprotectin is released by activated neutrophils.

Aim: To measure and compare the serum calprotectin and in pregnant femaleswith and without preeclampsia and to investigate the exact role of calprotectin in the etiology and pathophysiology of preeclampsia.

Study Type: Cross sectional comparative study

Method: Primigravida females with preeclampsia (Group A) and with normal blood pressure (Group B) of gestational age of 30-34 weeks were recruited from gynecological departments of Jinnah Hospital and Lady Willingdon Hospital, Lahore. In this cross sectional comparative study, a total of 90 subjects, 45 subjects from each group (A and B) were included for measuring serum calprotectin levels by ELISA technique.

Results: Preeclampsia is associated with a higher levels of Calprotetcin levels. The level of serum Calprotectin was significantly higher in patients as compared to the controls (p= 0.000). Another interesting finding was that the serum calprotectin levels increase with an increase in the gestational age of the preeclamptic females and this finding was statistically significant (p= 0.001). Also, serum calprotectin levels also increase with a rise in the systolic blood pressure (p= 0.016) and diastolic blood pressure (p= 0.010).

Key words: Preeclampsia, calprotectin, blood pressure, gestational age

INTRODUCTION

Preeclampsia (PE) is pregnancy specific syndrome which is characterized by persistent high blood pressure (more than 140/90 mm Hg) that is associated with high concentration of protein in the urine > 300mg/day by urine analysis[1] . The signs and symptoms of PE include high blood pressure, proteinuria, edema, headache, nausea and vomiting, changes in vision and shortness of breath[2].

Every year, pregnancy related complications cause death of half a million women and hypertensive disorders account for 16% of the maternal deaths in Africa and Asia[3]. After hemorrhage, the hypertensive disorders are the second major cause of direct maternal death. The incidence of preeclampsia is higher in middle and low income countries[4]. Approximately, 276 Pakistani pregnant females die for every 100,000 live births; and 10-15% of these deaths occur due to preeclampsia and eclampsia[5].

Preeclampsia causes a number of complications in both the mother and the fetus including placental abruption, preterm labor, intracranial hemorrhage, Low fetal birth weight and fetal growth restriction[6]. This disorder is characterized by extensive vascular endothelial dysfunction and vasospasm[7]. The pathology of the PE starts much earlier than the actual appearance of the clinical signs and symptoms. Many mechanisms have been postulated to play role in its pathology including abnormal decidualization and vascularization of the placenta, endothelial

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dysfunction, abnormal maternal immunological intolerance and inflammatory response[8]. It is obvious that a single mechanism responsible for the PE does not exist. Instead, several mechanisms can act together and even multiply each other and the exact etiology and pathophysiology is still unknown[9]. Hence, it can be stated that PE is a heterogeneous disorder new biomarkers and genetic markers may help in redefining the diagnosis and prognosis of disease, as well as, they may reveal the role of hidden risk factors.

Calprotectin (CALP) is newly identified cytosolic protein which is produced by myleomonocytic cells i.e. activated neutrophils, macrophages and monocytes[10]. It belongs to S-100 calgranulin family of proteins. It is composed of two subunits known as S100A8 and S100A9.The estimated weight of the two subunits is 8 and 14 KDa respectively[11]. It is released from activated neutrophils in conditions which are associated with increased immunological activity and inflammation such as rheumatoid arthritis, sepsis, inflammatory bowel disease, ulcerative colitis, tuberculosis, chronic bronchitis, cystic fibrosis, glomerulonephritis, bacterial infections and systemic lupus erythematosus[12] .

Systemic inflammation and leucocyte activation have long been established as one of the pathological causes of PE and since CALP is secreted by the neutrophils it is involved in promoting the inflammatory response in PE. Nowadays, calprotectin (CALP) is gaining increased attention as an inflammatory marker of PE. Furthermore, many enzymes including metalloproteinases (MMPs) are inhibited when CALP binds with Zn. MMPs are enzymes

which are required for invasion of the trophoblastic tissue into decidua. There are several subtypes of the MMPs such as MMP1-MMP-3, MMP-7, MMP-8 and MMP13 which help in the trophoblastic invasion. MMP-2 and MMP-9 are essential for the remodeling of the spiral arteries[13]. A dysregulation in the secretion of these enzymes or inhibition of their activity leads to abnormal placentation poor invasion of the trophoblastic cells into the decidua; damage to endothelial cells and failure of the remodeling of the spiral arteries leading to abnormal placentation and PE[14]. PE is associated with reduced trophoblastic invasion and decrease MMPs activity which is mediated by increased level of CALP[15]. The current evidence based on the recent studies suggests that serum CALP may be elevated in females with PE during the third trimester. However, further research is needed to confirm these findings and reach conclusion regarding role of CALP as a screening and surveillance biomarker in pregnant females who are at a risk of developing PE.

MATERIALS AND METHODS

After approval from UHS ethical review board, subjects were recruited from Gynecological departments of tertiary care hospitals of Lahore including Jinnah Hospital and Lady Willingdon Lahore. Written informed consent and all the relevant information (age, gestational age, lifestyle and dietary habits, clinical parameters, past medical and personal history) was taken and recorded on a subject data sheet. Blood samples of both the preeclamptic (Group A) and normotensive pregnant females (Group B) were taken using the convenient sampling technique under the aseptic conditions. Blood pressure and BMI were also recorded. Serum Calprotectin concentration (in pg/ml) was estimated by solid phase enzyme linked immunosorbent assay (ELISA) using commercially available human Calprotectin (CAL) ELISA kit (Glory Science Co., Ltd, USA), Catalog # 90405. The inclusion criteria for both the groups (A and B) was primigravida females of gestational age (30-34 weeks), singleton pregnancy, non-diabetic, no evidence of UTI or any other infection. The exclusion criteria for both the groups was multiple pregnancy, gestational age of less

than 30 weeks or more than 34 weeks, PIH, diabetic, smoker, any evidence of acute or chronic infection and subjects with fetal congenital anomalies.

Statistical analysis: The data were entered and analyzed using IBM SPSS version 22.0. Mean±Standard Deviation (SD) is given for normally distributed quantitative variables and Median±Inter Quartile Range (IQR) is given for non-normally distributed quantitative variables. Normal distribution of the data was checked by Shapiro-Wilk's statistics and if *p* value was ≤0.05, data were considered to be non-normally distributed. In case of normally distributed quantitative variables, Student "t" test was applied to compare group means with each other. In case of non-normally quantitative variables, non-parametric statistics i.e., Mann-Whitney U test was used to compare various variables between two groups. Pearson correlation (r) was used to observe correlation between normally distributed quantitative variables and Spearman's rho correlation (rho) was used to observe correlation between non-normally distributed quantitative variables. A *p*-value of ≤0.05 was considered statistically significant for all purposes.

RESULTS

Median IQR serum calprotectin levels of the control and patient group were 4532 (2421-8087) and 11025(11647) respectively. Mann Whitney U test was applied to see the statistical difference, which was statistically significant. P value was 0.00 as shown in the Table 1.

Mean ±SD systolic blood pressure of the controls and patients was 112.8± 10.62 and 156.86 ±8.27 mm Hg respectively. Mann-Whitney U test showed statistically significant difference (p value 0.000; Table, 1). Mean ±SD diastolic blood pressure of the apparently healthy pregnant females at the time of their presentation in the hospital was 74.0±10.27 while it was 106.9 ±11.66 of the preeclampsia females. There was a significant difference in the diastolic blood pressure of the apparently healthy pregnant females and preeclampsia patients (Mann-Whitney U test; p-value 0.000; Table, 1).

Table 1: Data distribution and comparison of all parameters between controls and patients

Parameter	Control	n	P values
	Mean ± SD	Mean ± SD	
Demographic parameters			
Age (years)	22.4±2.85	23.5±3.97	0.148 b
	22 (20-25)	23 (20.5-26)	
Gestational age (weeks)	32±1.44	31.68±1.32	0.179 a
	32 (31-33)	32 (30.5-33)	
Clinical parameters			
Systolic blood Pressure (mm Hg)	112.8±10.62	156.86±8.27	0.000* a
	112 (102-123)	156 (150-164)	
Diastolic Blood Pressure (mm Hg)	74±10.27	106.9±11.66	0.000* a
	72 (65-83)	106 (96-118)	
	253 (170-295)	221 (177-281.5)	
Biochemical Parameter			
Calprotectin (pg/mL)	5196.17±3484	10488±6283	0.000* a
	4532 (2421-8087)	11025 (4803.94-16451.09)	

[a]p-value generated by Mann-Whitney U Test
[b]p-value generated by Independent Sample "t"-Test
p-value ≤ 0.05 is considered statistically significant

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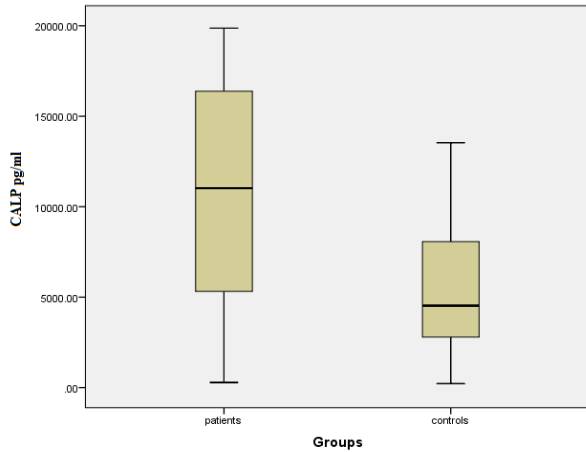
Box plot showing comparison serum calprotectin levels in preeclamptic pregnant patients and apparently healthy pregnant controls

Correlation analysis of demographic, clinical and biochemical parameters in the control and patient group:

Figure 1: Scatter plots showing significant correlation of systolic blood pressure and calprotectin in preeclampsia females
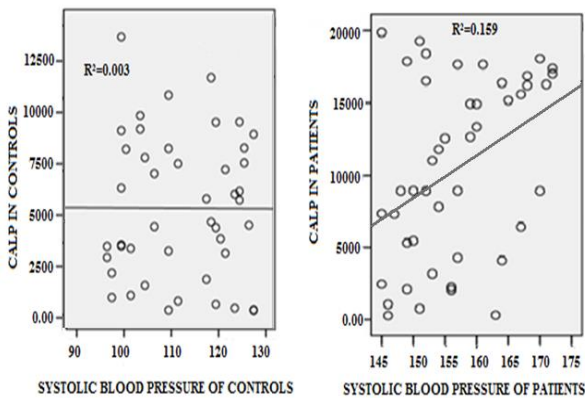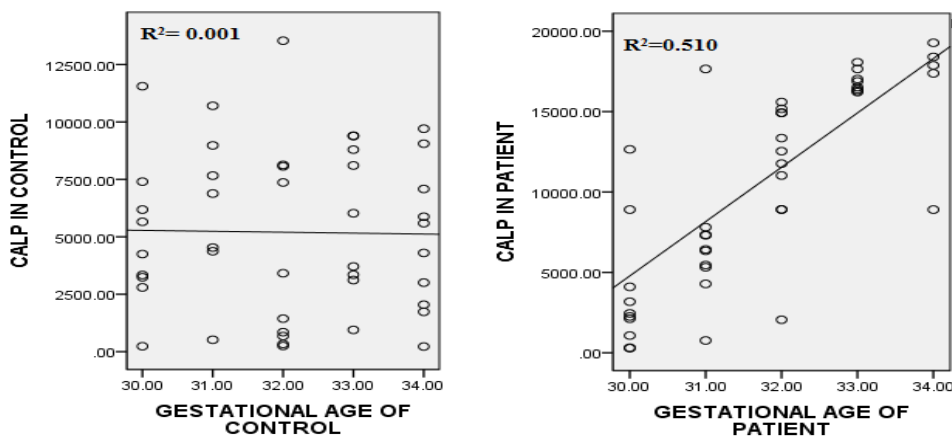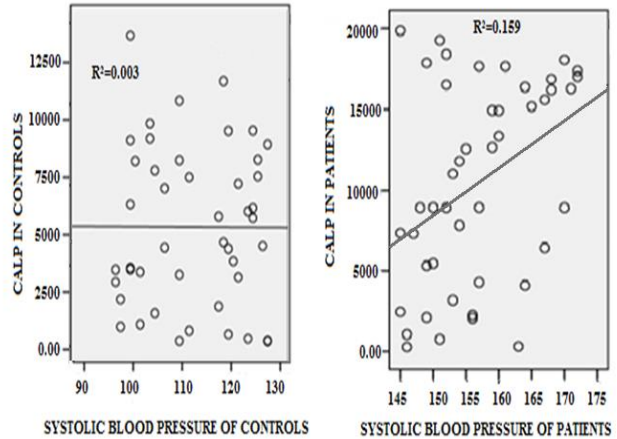

A significant positive correlation of systolic blood pressure with calprotectin was observed in the preeclamptic females (spearman's rho= 0.356; p value=0.016; Figure 1). Also, a Significant positive correlation of diastolic blood pressure was seen with calprotectin in cases as illustrated in figure 2 (spearman's rho=0.318; p value=0.010).

Figure 2: Scatter plots showing significant correlation of diastolic blood pressure and calprotectin in preeclampsia females

Significant positive correlation of gestational age with calprotectin was seen in the preeclampsia females as shown in figure 3 (spearman's rho=0.715; p value=0.00; Figure 30). No correlation was seen in control group

Figure 3: Scatter plots showing significant correlation of gestational age and calprotectin in preeclampsia females

ORIGINAL ARTICLE
DISCUSSION

This study included 90 primigravida females of gestational age 30-34 weeks and singleton pregnancy (45 diagnosed cases of preeclampsia- Group A and 45 normotensive healthy pregnant females- Group B). Both PE patients and healthy controls were age and gestational age matched. Measurements were taken for demographic parameters such as age, gestational age, height, weight and BMI; clinical parameter i.e. blood pressure; biochemical parameter i.e. serum Calprotectin.

In this study the median serum CALP level in patients of preeclampsia was higher 11025 pg/mL (4803.94-16451.09) as compared to normotensive pregnant females 4523 pg/mL (2421-8087) (p=0.001).Results of this study are in agreement with the previous studies where serum CALP levels were significantly elevated in preeclampsia group as compare to healthy pregnant control group (6,7,8,11). A recent study conducted by Li et al. (2018) in Beijing, China also reported substantial elevated levels of CALP in the preeclamptic pregnant women (2656.76 ±1724.56 µg/L) as compared to healthy normotensive pregnant females (1877.33± 905.69 µg/L; p=0.036) .Similar results were reported by Akçum et al. (2010) in which CALP levels in preeclamptic females were elevated 783 (478-928) µg/L compared with woman in the third trimester of healthy pregnancy 618 (343-878) µg/L (P= 0.001). This current study and findings of previous studies support that excessive maternal inflammatory response and release of CALP from activated leucocytes are hallmarks of this disorder.

In the present study, a significant positive correlation of serum CALP level was observed with gestational age (spearman's rho=0.715; P=0.001). This finding is similar to the studies done by Li et al. (2018) which found out that as duration of the pregnancy increases as indicated by gestational age the serum CALP level also increases. This clearly shows that serum CALP levels are directly proportional to the gestational age. This may be due to the reason that with advancing gestational age there is more severe excessive maternal inflammatory response, more placental hypoxia due to increase in size of placenta and leucocyte activation and release of CALP from activated leucocytes. Since CALP also acts as a pro-inflammatory factor therefore it triggers inflammation and its levels are higher in serum with the advancing gestational age .No such data are available in Pakistani women and we need to establish reference values of CALP throughout pregnancy, so that it may be used as a possible marker for anticipating PE.

Similarly, in this study a strong positive correlation of CALP was seen with systolic and diastolic blood pressure. This indicates that as the CALP level increases in preeclampsia females the blood pressure also increases. This finding manifests that a rise in the CALP level indicates severity of the disease and blood pressure corresponds well with the severity and disease activity[17].

CONCLUSION

In the present study we have concluded that there is a significant increase in the serum Calprotectin levels in preeclamptic females as compared to healthy pregnant females. Calprotectin is an inflammatory marker which can be used with diagnostic and prognostic value which has a significant role in the pathophysiology of PE. Serum calprotectin level increase with an increase in gestational age and severity of disease as indicated by rise in BP.

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