



Correlation of High Sensitive C-reactive Protein levels with Mean Arterial Pressure and Serum Calcium in Women with Mild and Severe Pre-eclampsia

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Authors' contributions

This work was carried out in collaboration between all authors. Author PB designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors SJ and AKB managed the analyses of the study. Authors IAS and GGK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Low grade systemic inflammation is considered as a risk factor for the progression of inflammation related disorders such as pre-eclampsia (PE). It is characterized by hypertension, proteinuria and edema during pregnancy. High sensitive C-reactive protein (hs-CRP) is a useful marker of inflammation that is able to predict the risk of PE. Urinary protein and trace elements like serum calcium may also play an important role in evaluating the severity of PE.

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Aim: To compare the mean results of serum levels of hs-CRP and calcium in mild and severe pre-eclamptic women with healthy pregnant women and to investigate the relationship, if any between levels of serum hs-CRP with mean arterial pressure (MAP) and serum calcium.

Material and Methods: A case control study was performed on 50-50 primigravidas with mild and severe PE as cases as per clinical guidelines and 50 healthy pregnant women as controls. They were all age and parity matched primigravidas at the third trimester of pregnancy.

Results: By using ANOVA, statistically highly significant mean values of hs-CRP and serum calcium were observed among all groups (healthy pregnant women, mild pre-eclamptic and severe pre-eclamptic women). Significant positive correlation is found between hs-CRP with MAP, while the negative association is detected among hs-CRP and serum calcium.

Conclusion: Serum hs-CRP may be feasible to be used as a sensitive biomarker for determining women at risk of PE. There is a significant influence of calcium supplementation during the antenatal period and the occurrence of pregnancy induced hypertension.

Keywords: Pre-eclampsia; hs-CRP; mean arterial pressure; urinary protein; calcium.

1. INTRODUCTION

Pre-eclampsia is a multisystem disorder of pregnancy and a significant cause of maternal morbidity and mortality. It affects around 5% to 8% of all pregnant women [1]. As per the revised definition of pre-eclampsia in 2014, it is defined as hypertension developing after 20 weeks' gestation with one or more of the following: proteinuria, maternal organ dysfunction which includes hepatic, renal, neurological, hematological complications and fetal growth restriction [2].

Recent clinical and biochemical evidences suggest that disturbances in the normal endothelial cell function may be a primary role in the pathogenesis of pre-eclampsia and is accompanied by elevated levels of inflammatory markers. Levels of such markers have been found to be higher in women with pre-eclampsia than those in normal pregnancy [3].

Amongst these markers, C-reactive protein (CRP) is considered as a sensitive marker of systemic inflammation. It is synthesized primarily in response to infection and tissue injury in liver cells. Studies have shown that hs-CRP determination is more sensitive than measurement of CRP and is a more sensitive marker of inflammation. Recently, studies have been conducted to find the relationship between serum hs-CRP levels and pre-eclampsia. As hs-CRP levels predict the development of coronary heart disease, attempts have been made to determine its predictive value in the development of pre-eclampsia [3, 4].

Recent studies indicate that alteration in trace elements may play a role in causing pre-

eclampsia. In pre-eclamptic women, significant low serum calcium levels have been observed [5]. It has been detected that decreased calcium level stimulates parathyroid hormone and renin release and also induces vasoconstriction by increasing its level in vascular smooth muscle, thereby causes high blood pressure [6,7]. This Study was conducted after taking ethical approval from concerned authority as per protocol [8].

Therefore, studies on inflammatory markers, trace elements and pre-eclampsia are of significant importance for academicians, health planners as well as treating physicians. Thus, the present study was undertaken to estimate hs-CRP levels and serum calcium in pre-eclampsia and to find out the possible correlation among them.

1.1 Objectives of the Study

- To measure and compare levels of high sensitive C-reactive protein, urinary protein and serum calcium in normal pregnancy, mild and severe pre-eclampsia.
- To study the correlation of high sensitive C-reactive protein and serum calcium among all three groups.

2. MATERIALS AND METHODS

The study was conducted at biochemistry department, Parul Institute of Medical Science & Research, Vadodara (Gujarat).

During this study, normal primigravidas and pre-eclamptic women were referred to the obstetrics and gynaecology clinic of Parul Sevashram

hospital, Vadodara (Gujarat) from August 2017 to June 2018 were enrolled.

A diagnosed case control study was done with 50 mild and 50 severe primigravidas of pre-eclampsia which were compared to age and parity matched 50 healthy primigravidas. Their gestational age was ranging from 28-40 weeks. They were classified as follows:-

- Group I: 50 healthy pregnant women
- Group II: 50 mild Pre-eclamptic women
- Group III: 50 severe Pre-eclamptic women

2.1 Inclusion Criteria

- All third trimester primigravidas without any complications were included as controls.
- All mild pre-eclamptic third trimester pregnant women were taken according to the 2013 definition [9] of American College of Obstetrics and Gynecology: (1) Blood pressure > 140/90 mmHg for two readings 6 hours apart; (2) Proteinuria > 300 mg/24 hours or +1 dipstick; (3) Edema.
- All severe pre-eclamptic third trimester primigravidas were considered as American College of Obstetrics and Gynecology (ACOG); 2013 criteria [9] as: Blood pressure > 160/110 mmHg for two readings 6 hours apart, proteinuria > 5 gm/24 hours or +2, +3 dipstick, serum creatinine > 1.2 mg/dl, platelets <100,000/mm³, microangiopathic hemolysis, elevated liver enzymes, epigastric pain or right quadrant pain, persistent headache or other cerebral or visual disturbances, intrauterine growth restriction (IUGR), pulmonary edema and oliguria.

2.2 Exclusion Criteria

- Pregnant women with any sign of infection, multiple pregnancies, and premature rupture of membranes, symptomatic inflammatory diseases, diabetes mellitus and chronic renal or hepatic disease were excluded.

2.3 Methods

Anthropometric Measurements: BMI and mean arterial pressure were calculated.

Venous blood sample (3 ml) was collected in plain vacutainer from each individual by after an overnight fast of 12 hours. The tubes are placed

in a rack safely for 15 minutes to prevent hemolysis as well as for blood clotting to occur. For the urinary protein, 2-5 ml of 24-hour urine sample of each subject was collected with the help of preservative boric acid crystals (10 gm/24 hour collections) in a sterile urine container. The serum and urine samples were separated by centrifugation for 15 min at 1500 rpm. All the samples were stored at -20°C for further analysis.

The following laboratory investigations were done from blood and urine sample in controls and cases:

- Serum hs-CRP and urinary protein levels were quantified by Immuno-turbidimetric assay and Pyrogallol red method respectively [10,11].
- Serum calcium was determined by modified ortho cresolphthalein complexone (OCPC) method Barnett et al. 1973 [12].

2.4 Statistical Analysis

All the data analysis was done by using Microsoft Excel and the SPSS (statistical package for social science) version 24.0 windows software. Mean, standard deviation (SD) and one way ANOVA (analysis of variance) were calculated. ANOVA was used to compare the groups. Statistical results were considered significant at $p < 0.05$.

3. RESULTS

The demographic and biochemical characteristics of the groups are summarized in Table 1. The difference in mean levels of gestational age and BMI between all three (controls, mild PE and severe PE) groups were found to be non-significant ($p > 0.05$, Table 2).

As per Table 2, mean arterial blood pressure levels in mild and severe pre-eclamptic groups are significantly higher as compared to control group ($p < 0.0001$) and mild pre-eclamptic women have significantly lower mean MAP levels than severe subjects ($p < 0.0001$).

Serum hs-CRP and urinary protein excretion levels were higher in both pre-eclamptic groups in comparison to controls ($p < 0.0001$, Table 2). Mean values of both parameters were showed a highly significant difference when compared between both mild and severe patients ($p < 0.0001$, Table 2).

Table-1. Demographic and biochemical profile of study subjects

Variables	Group I (n=50)		Group II (n=50)		Group III (n=50)	
	Mean ± SD	95 % CI	Mean ± SD	95 % CI	Mean ± SD	95 % CI
Gestational age (weeks)	33.60 ± 3.96	32.81-34.39	34.28 ± 3.56	33.65-34.91	33.76 ± 3.64	32.92-34.60
BMI (Kg/m ²)	29.68 ± 5.41	28.20-30.35	30.08 ± 6.65	28.91-31.25	29.88 ± 6.08	28.50-31.26
MAP (mm Hg)	83.92 ± 12.17	81.51-86.33	115.16 ± 17.64	112.04-118.28	133.12 ± 24.61	127.46- 138.78
Urinary protein (mg/24 hrs)	129.40 ± 37.26	0.234	1386.00 ± 859.78	4.822	4188.00 ± 1417.26	10.26
hs-CRP (mg/l)	4.50 ± 1.09	4.28-4.71	9.06 ± 1.20	8.84-9.27	12.22 ± 1.93	11.77-12.66
Serum calcium (mg/dl)	8.54±1.05	8.34-8.75	7.37±1.26	7.14-7.59	7.18±1.34	6.88-7.49

Group I—Healthy Pregnant Women; BMI—body mass index
 Group II—Mild Pre-eclamptic Women; MAP—mean arterial pressure
 Group III-- Severe Pre-eclamptic Women

Table 2. Comparison of demographic and biochemical variables among control and cases

Variables	Group -1	Group-2	Group-3	p-value
	Mean ± SD	Mean ± SD	Mean ± SD	
Gestational age (weeks)	33.60 ± 3.96	34.28 ± 3.56	33.76 ± 3.64	0.73 (NS)
BMI (Kg/m ²)	29.68 ± 5.41	30.08 ± 6.65	29.88 ± 6.08	0.35 (NS)
MAP(mm Hg)	83.92 ± 12.17	115.16 ± 17.64	133.12 ± 24.61	0.0001
Urinary protein(mg/ 24 hrs)	129.40 ± 37.26	1386.00 ± 859.78	4188.00 ± 1417.26	0.0001
hs-CRP (mg/l)	4.50 ± 1.09	9.06 ± 1.20	12.22 ± 1.93	0.0001
Serum calcium (mg/dl)	8.54±1.05	7.37 ± 1.26	7.18±1.34	0.0001

One way ANOVA test was applied.

Among the groups for each parameter means with different letters horizontally have a significant difference at p≤0.05.

NS— non-significant

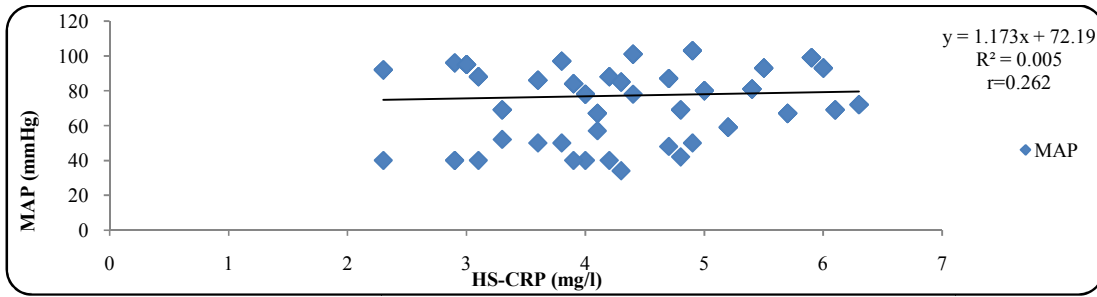


Fig. 1. Correlation between serum high sensitivity- C-reactive protein (mg/l) values & Mean arterial pressure (mmHg) values in healthy controls

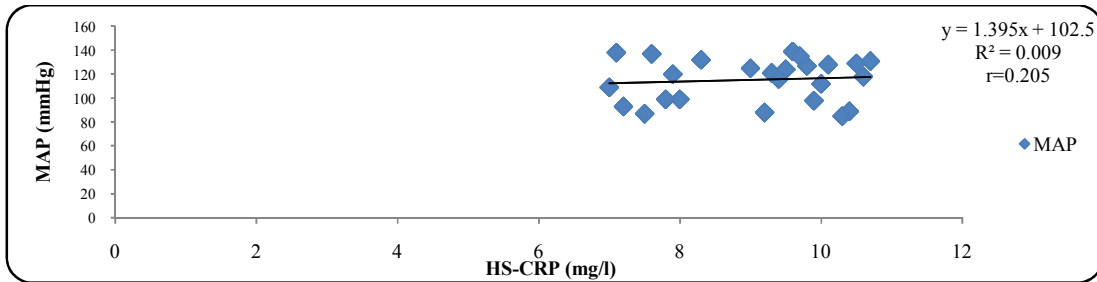


Fig. 2. Correlation between serum high sensitivity- C-reactive protein (mg/l) values & mean arterial pressure (mmHg) values in mild pre-eclamptic women

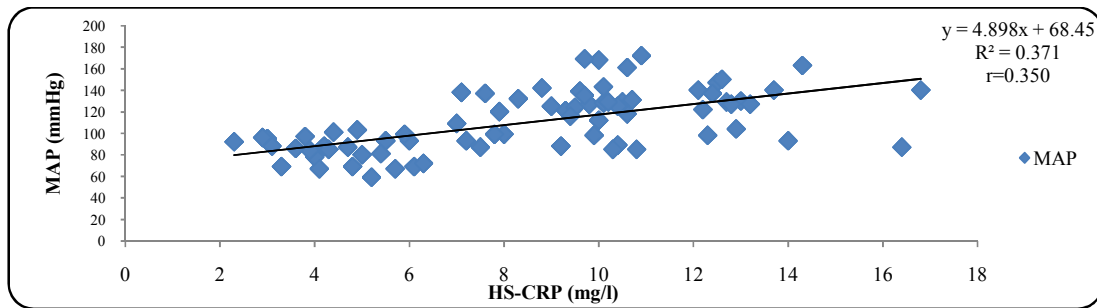


Fig. 3. Correlation of serum high sensitivity- C-reactive protein (mg/l) values with mean arterial pressure (mmHg) values in severe pre-eclamptic

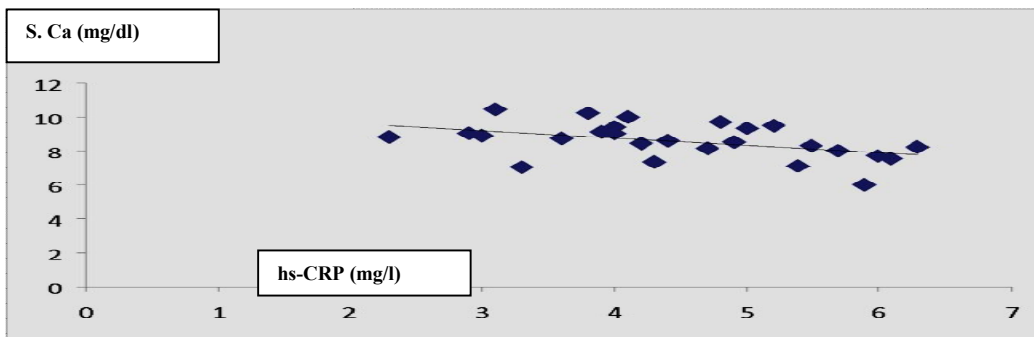


Fig. 4. Correlation of serum high sensitivity- C-reactive protein (mg/l) values with S. calcium (mg/dl) in healthy pregnant women

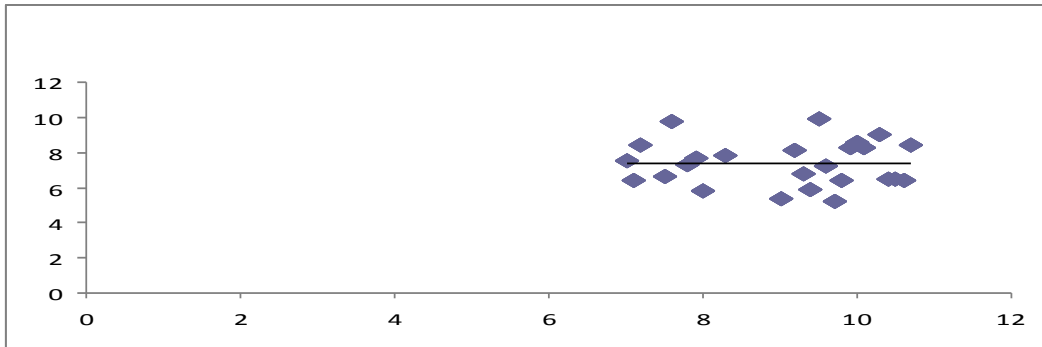


Fig. 5. Correlation of serum high sensitivity- C-reactive protein (mg/l) values with S. calcium (mg/dl) in mild preeclamptic patients

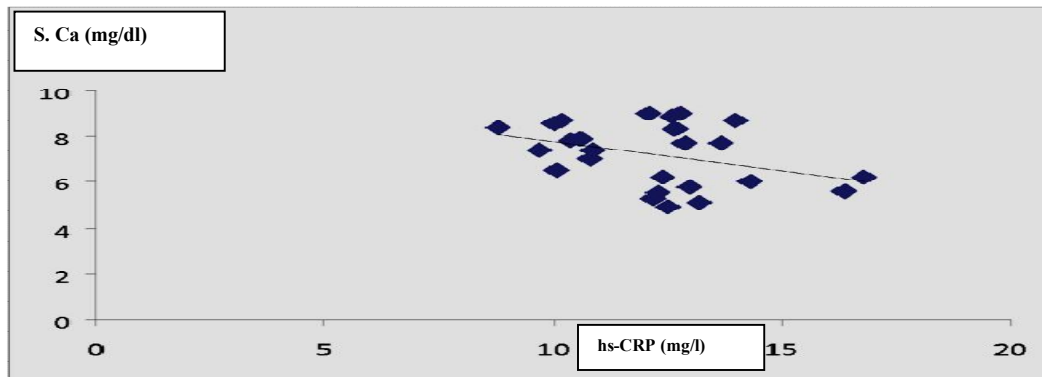


Fig. 6. Correlation of serum high sensitivity- C-reactive protein (mg/l) values with S. calcium (mg/dl) in severe pre eclamptic patients

Table 3. Correlation of hs-CRP with S. calcium and mean arterial pressure among different groups

	Serum calcium		Mean arterial pressure	
	r	p-two-tailed	R	p-two-tailed
Group I	-0.453	0.000	0.262	0.000
Group II	-0.010	0.455	0.095	0.027
Group III	-0.367	0.001	0.350	0.925

r = Pearson's correlation coefficient

As evident by Table 2, mean serum calcium levels were lower in pre-eclamptic groups (mild & severe) in comparison to controls, and their difference was highly significant ($p < 0.0001$, Table 2). Statistically highly significant results were also found when compared between mild and severe pre-eclamptic ($p < 0.0001$, Table 2). Severe patients had lower values than mild subjects.

In mild and severe Pre-eclamptic groups as well as in control, correlation analysis tests showed positive associations when hs-CRP was

correlated with mean arterial pressure ($r = 0.262, 0.095, 0.350$) (Table 3 & Figs. – 1,2,3). Negative correlations were detected between serum hs-CRP levels and serum calcium levels in controls and cases ($r = -0.453, -0.010, -0.367$) (Table 3 & Figs. 4,5,6).

4. DISCUSSION

Many studies reported that pre-eclampsia is not only associated with a higher rate of morbidity and mortality but also it is considered as a risk factor for vascular diseases both for mother and

fetus. According to this evidence, the rate of stroke is higher among primigravidas with PE, and in their children, vulnerability to depression is higher and cognitive ability is lower than other children respectively [13]. On the contrary, it is felt that preventative strategies for reducing the maternal adverse consequences of PE and its related complications are currently sub optimal may be due to that PE and its severity could not predict clinically. Thus, this study designed to evaluate the role and correlation of this marker (hs-CRP) with inflammation in this field [14].

The findings of our study show that there is no significant difference in gestational age and BMI in women with pre-eclampsia (mild and severe) and normal healthy pregnant women ($p=0.7$, $p=0.3$). However, in contrast to this, there are studies conducted by Rajkovic A et al. [15] and Dantas et al. [16] showing an association between elevated BMI and pre-eclampsia.

Hypertensive disorders, characterized by an increase in blood pressure, are the most common medical complications, occurring during pregnancy. In accordance with this, in our study, we investigated that mean level of blood pressure (mmHg) was significantly raised in women with pre-eclampsia when compared with controls which were comparable to the findings of Baksu A et al. [17], Powers RW et al. [18] and Ephraim et al. [19]. They also detected similar results.

Moreover, we found that there was a significant increase in urinary protein levels in women with pre-eclampsia ($p<0.001$) as compared to normal pregnant women. The cause of proteinuria in pre-eclampsia may be due to renal glomerular endotheliosis which leads to impaired glomerular perfusion and filtration [20].

In this study, we found that serum hs-CRP levels were significantly higher in severe pre-eclamptic women as compared to healthy pregnant women ($p=0.0001$). Our results are in accordance with studies conducted by S. Kumru et al. and Dr.Kanak Kanti Mandal et al., which reports that serum hs-CRP levels increase in women with severe pre-eclampsia when compared with normal pregnant women [3,4].

It has been found that there is an increase in pro-inflammatory markers in normal pregnancy, but in the pre-eclamptic women, it has been suggested that the cause of serum hs-CRP increase may be because of reduced plasma

volume in these patients. Thus, our results indicate the presence of inflammation in pre-eclamptic women associated with increase in levels of hs-CRP [3].

Sakurako K-K et al. showed a significant inverse association between dietary calcium intake and serum hs-CRP concentrations in their study [21]. According to them, calcium has been reported to inhibit gene expression of inflammatory cytokines in adipocytes by inhibiting calcitriol, which could have anti-inflammatory effects by increasing intracellular calcium levels.

Our study assessed the levels of serum calcium in women with pre-eclampsia compared to that in normal pregnancy and we found these were significantly reduced in women with pre-eclampsia than controls. This observation of reduced serum calcium levels is similar to other studies on hypertensive disorders in pregnancy. [22,23]. The theory behind this observation, maybe the fact that when there are decrease in serum calcium levels, the intracellular calcium level increases, which leads to constriction of smooth muscles in blood vessels and increase in vascular resistance, leading to rising in systolic and diastolic blood pressure. [24] it has been speculated that hypocalcemia may result from the decreased dietary intake, decreased intestinal absorption, increased calcium uptake by the fetus and placenta and increased tubular reabsorption [25].

We found a positive correlation between serum levels of hs-CRP and diastolic blood pressure in both pre-eclamptic groups, while on the other hand, a negative relationship was seen between serum hs-CRP and serum calcium which was similar as the study done by Sakurako K-K et al. [21].

5. CONCLUSION

The findings of our study determined that a higher level of hs-CRP in pregnant women with PE than those with normal pregnancy could potentially explain the exaggerated inflammation in PE. Regarding the significantly increased level of hs-CRP in severe PE than mild PE, we could suggest that hs-CRP is a more appropriate marker to investigate pregnant women with severe PE and its clinical usefulness will be superior in this regard.

In context of the above findings, we suggest that serum hs-CRP levels can reflect the severity of

pre-eclampsia especially by means of proteinuria and blood pressure. Also, we detected that there was a significant negative correlation between hs-CRP levels and serum calcium levels. To date, this is the first study to find such correlation which shows that there is a relation between inflammation and reduced calcium level in pre-eclampsia.

CONSENT

As per international standard or university standard written participant consent has been collected and preserved by the authors.

ETHICAL APPROVAL

As per revised Helsinki declaration of 2000 and university standard written ethical permission has been taken and preserved by the author. [8]

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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