

Original Research Article

Correlation of High sensitive C-reactive protein levels with Mean Arterial Pressure and Serum Calcium in women with mild and Severe Pre-eclampsia

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 severity of PE.

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 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-eclamptics and severe pre-eclamptic women). Significant positive correlation is found between hs-CRP with MAP, while negative association is detected among hs-CRP and serum calcium.

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

27 **Key-Words:** *Pre-eclampsia, hs-CRP, mean arterial pressure, urinary protein, calcium.*

28 **1. INTRODUCTION:-**

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

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

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

46 Recent studies indicate that alteration in trace elements may play a role in causing pre-
47 eclampsia. In pre-eclamptic women, significant low serum calcium levels has been observed.⁵

48 It has been detected that decreased calcium level stimulates parathyroid hormone and rennin

49 release and also induces vasoconstriction by increasing its level in vascular smooth muscle ,
50 thereby causes high blood pressure.^{6,7}

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

55 **1.1 OBJECTIVES OF THE STUDY:-**

56

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

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63 **2. MATERIALS AND METHODS:-**

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65 The study was conducted at biochemistry department, Parul Institute of Medical
66 Science & Research, Vadodara (gujarat) with prior ethical approval by institutional
67 ethical experimentation committee as per Helsinki declaration of 1975 that was revised
68 in 2000.⁸

69 During this study, normal primigravidas and pre-eclamptic women were referred to
70 obstetrics and gynecology clinic of Parul Sevashram hospital, vadodara (gujarat) from
71 august 2017 to june 2018 were enrolled.

72

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 were 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 definition⁹ 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 ACOG; 2013 criteria⁹ 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.

3 ml blood sample and 24 hrs. urine sample of each subject was collected in plain vial.

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. 1973¹²

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 $p \leq 0.05$.

3. RESULTS:-

The demographic and biochemical characteristics of the groups are summarized in table

1. 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 highly significant difference when compared between both mild and severe patients ($p < 0.0001$, table-2).

As evident by table-2, mean serum calcium levels were lower in pre-eclamptic groups (mild & severe) in comparison to controls, and their difference were highly significant ($p < 0.0001$, table-2). Statistically highly significant results were also found when compared between mild and severe pre-eclampsics ($p < 0.0001$, 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 & figures – A, B, C). 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 & figures – D, E, F).

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Table-1. Demographic and biochemical profile of study subjects

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

149 Group I—Healthy Pregnant Women

BMI—body mass index

150 Group II—Mild Pre-eclamptic Women

MAP—mean arterial pressure

151 Group III-- Severe Pre-eclamptic Women

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153 **Table -2. Comparison of demographic and biochemical variables among control and**
 154 **cases-**

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

155 One way ANOVA test was applied.

156 Among the groups for each parameter means with different letters horizontally have
 157 significant difference at $p \leq 0.05$.

158 NS—non significant

159 **Table-3 Correlation of hs-CRP with S. Calcium and Mean Arterial Pressure among**
 160 **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

Figure (A): Correlation between Serum High Sensitivity- C-Reactive Protein (mg/l) values & Mean Arterial Pressure (mmHg) values in healthy controls

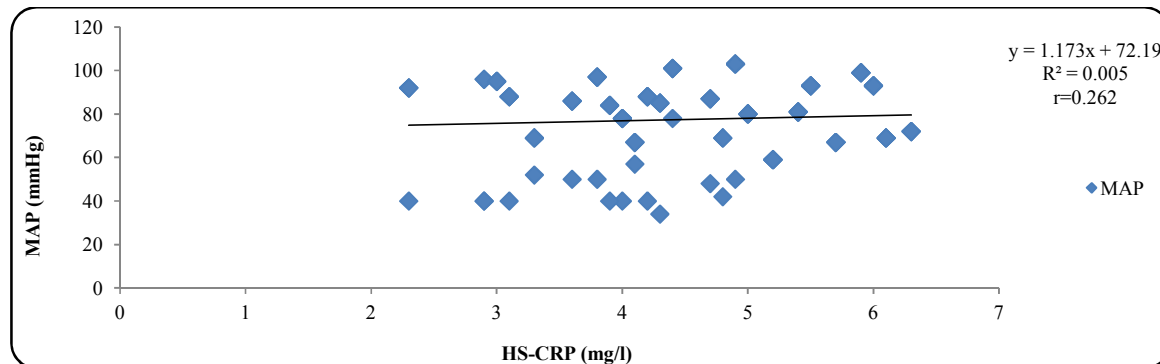


Figure (B): Correlation between Serum High Sensitivity- C-Reactive Protein (mg/l) values & Mean Arterial Pressure (mmHg) values in mild pre-eclamptic women

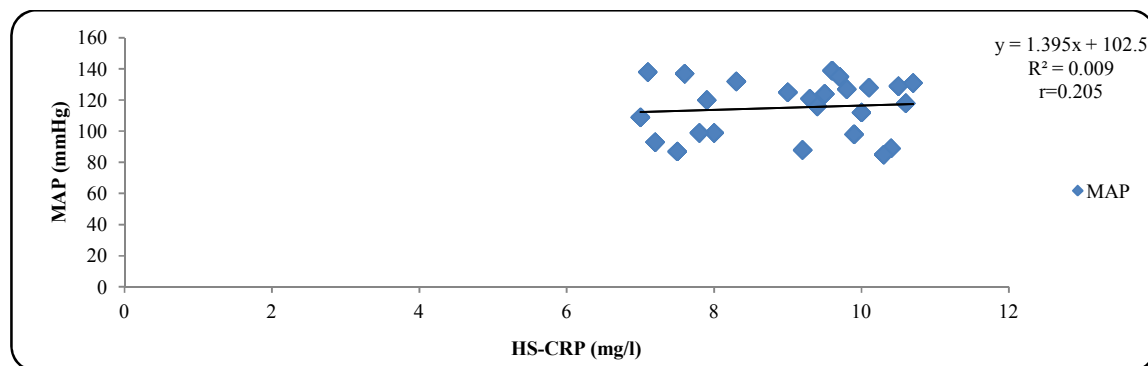


Figure (C): Correlation of Serum High Sensitivity- C-Reactive Protein (mg/l) values with Mean Arterial Pressure (mmHg) values in severe pre-eclampsics

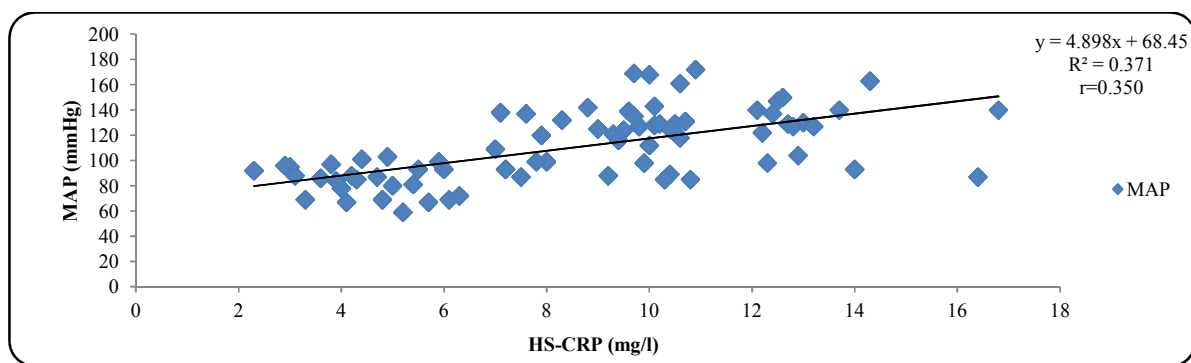


Figure (D): Correlation of Serum High Sensitivity- C-Reactive Protein (mg/l) values with S. Calcium (mg/dl) in healthy pregnant women

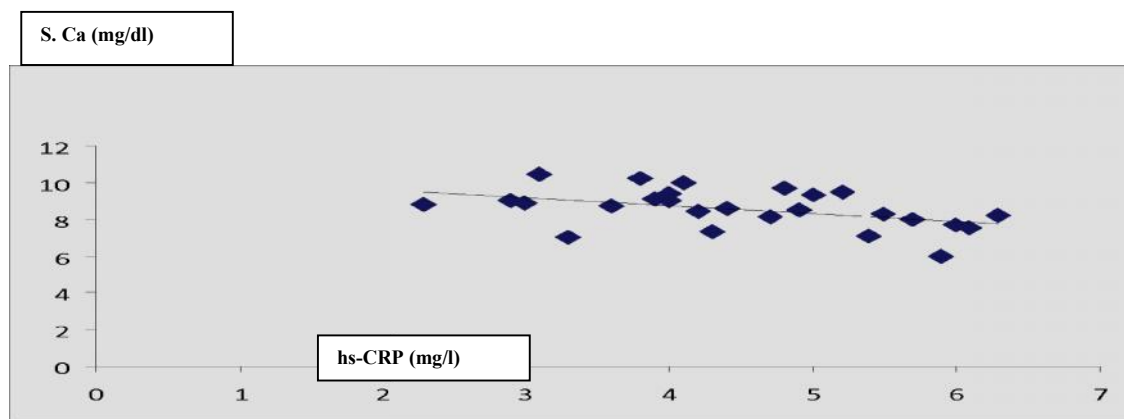


Figure (E): Correlation of Serum High Sensitivity- C-Reactive Protein (mg/l) values with S. Calcium (mg/dl) in mild pre eclamptic patients

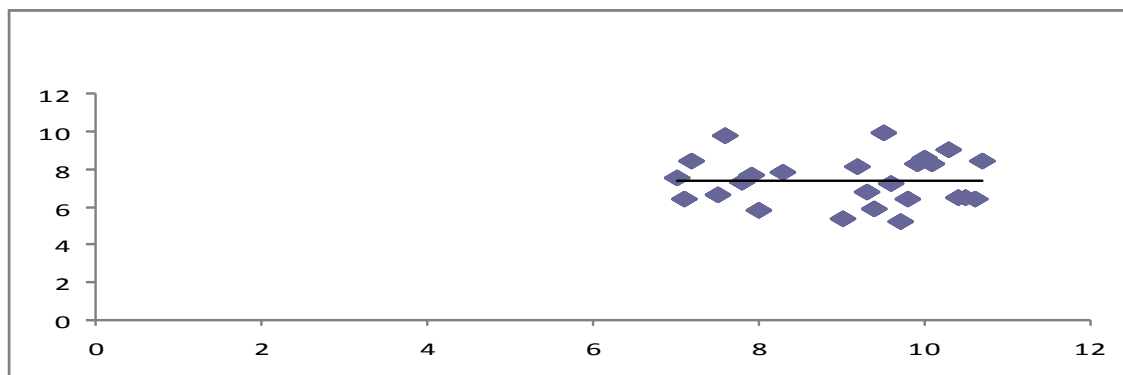
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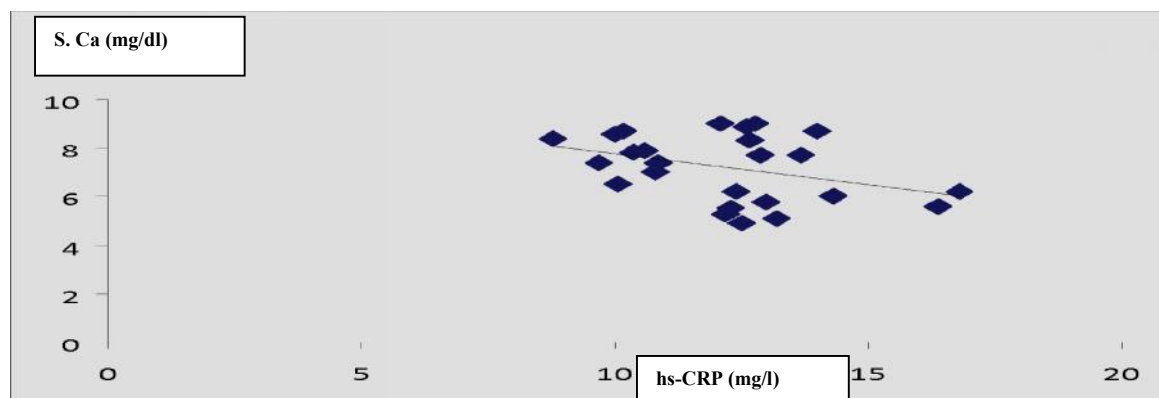
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189 **Figure (F): Correlation of Serum High Sensitivity- C-Reactive Protein (mg/l) values with S. Calcium**
 190 **(mg/dl) in severe pre eclamptic patients**

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192 4. DISCUSSION:-

193 Many studies reported that pre-eclampsia is not only associated with higher rate of morbidity
 194 and mortality but also it is considered as a risk factor of vascular diseases both for mother and
 195 fetus. According to these evidence, the rate of stroke is higher among primigravidas with PE,
 196 and in their children, vulnerability to depression is higher and cognitive ability is lower than
 197 other children, respectively.¹³ On the contrary, it is felt that preventative strategies for
 198 reducing the maternal adverse consequences of PE and its related complications are currently
 199 sub optimal may be due to that PE and its severity could not predict clinically. Thus, this

200 study designed to evaluate the role and correlation of this marker (hs-CRP) with
201 inflammation in this field.¹⁴

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

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

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

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

220 It has been found that there is increase in pro-inflammatory markers in normal pregnancy,
221 but in the pre-eclamptic women, it has been suggested that the cause of serum hs-CRP
222 increase may be because of reduced plasma volume in these patients. Thus, our results
223 indicates presence of inflammation in pre-eclamptic women associated with increase in levels
224 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 intra-cellular 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 with other studies on hypertensive disorders in pregnancy.^{22, 23} The theory behind this observation, may be the fact that when there is 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 rise in systolic and diastolic blood pressure.²⁴ it has been speculated that hypocalcemia may result from 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 study done by Sakurako K-K et al.²¹

5. CONCLUSION:-

The findings of our study determined that higher level of hs-CRP in pregnant women with PE than those with normal pregnancy could potentially explain the exaggerated inflammation in PE. Regarding significantly increased level of hs-CRP in severe PE than mild PE, we could

suggest that hs-CRP is more appropriate marker to investigate pregnant women with severe PE and its clinical usefulness will be superior in this regard.

In context to 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 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 relation between inflammation and reduced calcium level in pre-eclampsia.

ETHICAL APPROVAL

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

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