

RESEARCH ARTICLE

Assessment of Inflammatory Markers in Preeclampsia

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ABSTRACT

Aim: Pregnancies, including preeclampsia, are low-grade systemic inflammation which has been associated with complications. Endothelial dysfunction in preeclampsia is accompanied by elevated levels of inflammatory markers and cytokines such as high sensitivity C-reactive protein (hs-CRP), tumor necrosis factor- α (TNF- α) and interleukin-10 (IL-10). Our aim in this study is to assess the levels of these above markers in preeclampsia and to predict its severity.

Materials and methods: A case-control study was performed on 100 pregnant women with preeclampsia as cases (50 mild and 50 severe) 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: Significantly increased ($p < 0.001$) serum levels of hs-CRP, TNF- α and IL-10 were found in cases of mild and severe preeclamptic when compared to controls. In severe preeclamptics, all these parameters were also found highly significant in comparison to mild.

Conclusion and clinical significance: The observed high levels of hs-CRP, TNF- α , and IL-10 in preeclamptic women indicate that these markers can be used in identifying the severity of preeclampsia and it can help the clinician in their diagnosis, treatment, and management.

Keywords: High sensitivity C-reactive protein (hs-CRP), Interleukin-10 (IL-10), Preeclampsia, Tumor necrosis factor- α (TNF- α).

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INTRODUCTION

Preeclampsia is one of the hypertensive disorders of pregnancy. It is a major cause of maternal and perinatal

mortality and morbidity and is a pregnancy-specific hypertensive disease with multisystem involvement. It is a disorder of widespread vascular endothelial malfunction and vasospasm that occurs after 20 weeks of gestation and can present as late as 4 to 6 weeks postpartum.¹

Hypertension is one of the most common medical problem encountered during pregnancy, complicating up to 10% of pregnancies.² Preeclampsia occurs in 3 to 6% of all pregnancies and the incidence is 1.5 to 2 times higher in first-time pregnancies.³ The incidence of preeclampsia has been increased by 25% in the United States during the past two decades. In India, the incidence of preeclampsia is reported to be 8 to 10% among pregnant women. According to Dubey L, the prevalence of hypertensive disorders of pregnancy was 7.8% with preeclampsia in 5.4% of the study population in India. Preeclampsia is a leading cause of maternal and perinatal morbidity and mortality, with an estimated 50000-60000 preeclampsia related deaths per year worldwide.^{4,5}

Preeclampsia syndrome, in many cases, is thought to cause by a shallowly implanted placenta which becomes hypoxic, leading to an immune reaction characterized by secretion of upregulated inflammatory mediators from the placenta and acting on the vascular endothelium.⁶ It is characterized by widespread endothelial dysfunction throughout the maternal circulation resulting in hypertension attributable to vasoconstriction, proteinuria due to glomerular damage and edema as a result of increased vascular permeability.⁷ Inflammatory cells are activated in preeclampsia and localized to the site of vascular injury and associated with higher levels of pro-inflammatory molecules, cytokines, and adhesion molecules.

- One of the most important objectives and sensitive indexes of overall inflammatory activity in the body is hs-CRP.⁸ The hepatic synthesis of hs-CRP increases in response to inflammatory cytokines such as IL-10 and IL-6 which are responsible for inflammatory reactions and maternal endothelial activation in pre-eclampsia.^{9,10}
- The IL-10 is a potent pleiotropic cytokine, which has the dual ability of immune-suppression or immune-stimulation via the production of pro-inflammatory cytokines by the inhibition of T-helper 1 (Th1) lymphocytes and stimulation of B and Th2 lymphocytes and thus downregulates the inflammatory response.¹¹

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- The TNF- α is a 17 kD polypeptide cytokine produced in preeclampsia by neutrophils, monocytes, and placenta. Cytokines are involved in normal pregnancy and labor. It regulates trophoblast proliferation and differentiation, cell adhesion, tissue remodeling, apoptosis of villous trophoblast and trophoblast hormone production.¹² The low level of uterine TNF- α is beneficial to pregnancy, whereas elevated concentrations are detrimental.

If preeclampsia is not diagnosed or treated timely, it can progress to maternal multi-organ failure, coagulopathy, intrauterine fetal demise and finally maternal death. So, being very sensitive markers of inflammatory activity in the body, determination of all these parameters can be used as a marker of low-grade inflammation and further help in detecting pathophysiological process in pregnancy, to predict adverse pregnancy outcome and try preventive therapies on time.

MATERIALS AND METHODS

The study was conducted at Mahila Chikitsalaya, Jawahar Lal Nehru Medical College and associated group of hospitals, Ajmer (Rajasthan) with prior ethical approval by institutional ethical experimentation committee and as per Helsinki declaration of 1975 that was revised in 2000.¹³

A diagnosed case-control study with (age and parity-matched) 100 primigravidas of preeclampsia (50 mild and 50 severe) and 50 healthy primigravidas was done. Their gestational age was ranging from 28 to 40 weeks. They were grouped as follows:

Group I: 50 healthy pregnant women

Group II: 50 mild preeclamptic women

Group III: 50 severe preeclamptic women

Inclusion Criteria

All third-trimester primigravidas without any complications were included as controls.

All mild preeclamptic third-trimester pregnant women were taken according to American College of Obstetrics and Gynecology ACOG, 2013 criteria: (1) blood pressure > 140/90 mmHg for two readings 6 hours apart; (2) proteinuria > 300 mg/24 hours or +1 dipstick; (3) edema.¹⁴

All severe preeclamptic third trimester pregnant women were considered as ACOG, 2013 criteria (3) 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.

Exclusion Criteria

Patients with multiple pregnancies, chronic hypertension, and premature rupture of membranes, symptomatic inflammatory diseases, diabetes mellitus, and chronic renal disease were excluded.

Anthropometric Measurements: Body human index (BMI) and mean arterial pressure were calculated.

Three mL blood sample and 24 hours urine sample of each subject were collected in a plain vial. The serum and urine samples were separated by centrifugation for 15 minutes at 1500 rpm. All the samples were stored at -20° C for further analysis.

- Serum IL-10 and TNF- α concentrations were measured by sandwich enzyme-linked immunosorbent assay (ELISA) method (Bio-Rad Model 680 microplate reader and washer).^{15,16}
- Serum hs-CRP and urinary protein levels were quantified by Immuno-turbidimetric assay and pyrogallol red method respectively.^{17,18}

Statistical Analysis

All the data analysis was done by using Microsoft Excel and the Statistical Package for Social Science (SPSS) version 24.0 windows software, mean, standard deviation (SD) and analysis of variance (ANOVA) were calculated. The analysis was, and statistical results were considered significant at $p \leq 0.05$.

RESULTS

The demographic profile of the study subjects is presented in Table 1 which shows the elevation in BMI and gestational age is nonsignificant.

The biochemical parameters of the subjects are tabulated in Table 2.

Table 1: Demographic profile of study subjects

Variables	Group I (n = 50)		Group II (n = 50)		Group III (Cn = 50)		p-value
	Mean \pm SD	95% CI	Mean \pm SD	95% CI	Mean \pm SD	95% CI	
Gestational							
Age (weeks)	33.60 \pm 3.96	32.81–34.39	34.28 \pm 3.56	33.65–34.91	33.76 \pm 3.64	32.92–34.60	0.7
BMI (Kg/m ²)	29.68 \pm 5.41	28.20–30.35	30.08 \pm 6.65	28.91–31.25	29.88 \pm 6.08	28.50–31.26	0.3

Table 2: Biochemical parameters showing the following results

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
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 hours)	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
TNF-α (pg/mL)	6.78 ± 1.13	6.55–7.00	31.57 ± 9.77	29.84–33.30	64.69 ± 11.90	61.95–67.43
IL-10 (pg/mL)	4.38 ± 0.99	4.18–4.58	9.16 ± 1.96	8.80–9.50	12.88 ± 2.11	12.39–13.36

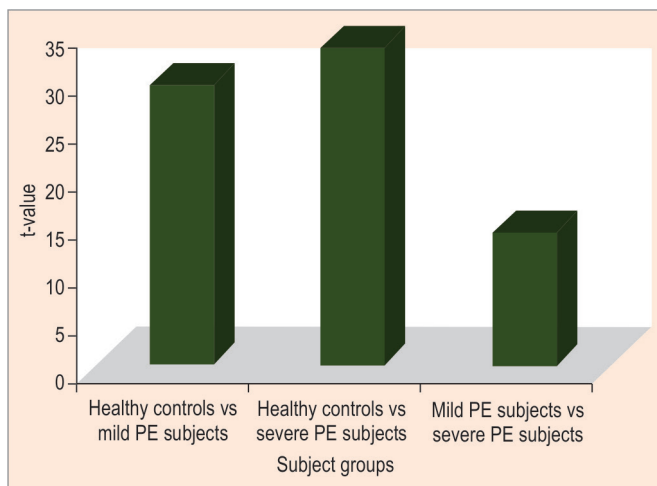


Fig. 1: Comparison of mean serum high sensitivity–C-reactive protein (mg/l) among various groups of subjects

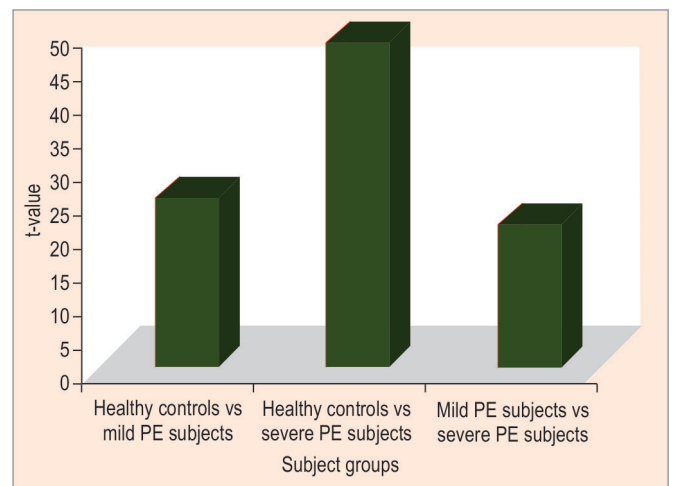


Fig. 2: Comparison of mean serum tumor necrosis factor–alpha (pg/mL) among various groups of subjects

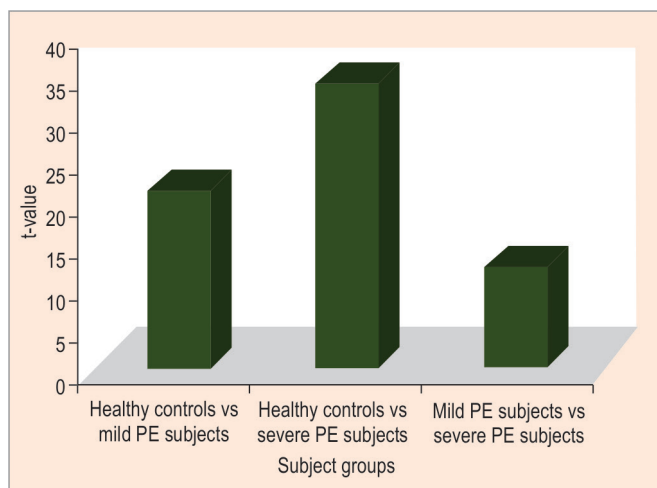


Fig. 3: Comparison of mean serum interleukin (pg/mL) among various groups of subjects

A significant elevation (according to ANOVA) in MAP, urinary protein, hs-CRP, TNF-alpha and IL-10 were observed in mild and severe preeclamptic women when compared in all groups as shown in Table 3. These data are also projected in Figures 1 to 3.

Among the groups for each parameter, many with different letters horizontally have significant differences at $p \leq 0.05$ using ANOVA.

The significantly high values ($p < 0.001$) of hs-CRP, TNF- α , and IL-10 levels were obtained in severely pree-

clamptic patients compared to mild and healthy controls in the present study which signify that these parameters can be used for interpreting complications of pregnancy.

DISCUSSION

This study was carried out to examine the extent of inflammation in toxemia of pregnancy and also to evaluate the role of inflammatory markers in predicting the severity of preeclampsia. Because of the proposed influence of the maternal immune system on placentation and the subsequent systemic reactions, it is of great interest to study the maternal inflammatory response during preeclampsia in the hope of achieving a better understanding of this puzzling disease.

In the present study, serum hs-CRP in different groups were 4.5 ± 1.09 , 9.06 ± 1.20 , 12.22 ± 1.93 and this matches with the similar study conducted by Nanda et al. on 70 pregnant women which showed a mean level of 2.78 ± 1.45 and the p -value < 0.001 .¹⁹ Oancea et al. published a study with 120 pregnant women showed statistically significant hs-CRP levels in patients with preeclampsia compared to controls.²⁰

Levels of serum TNF- α in control were 6.78 ± 1.13 , in mild 31.57 ± 9.77 and severe preeclamptics were 64.69 ± 11.90 and our study matches with the study done by Fozia et al. which showed a significant increase

Table 3: Comparison of biochemical variables among control and cases

Variables	Group 1	Group 2	Group 3	p-value
	Mean \pm SD	Mean \pm SD	Mean \pm SD	
MAP (mm Hg)	83.92 \pm 12.17	115.16 \pm 17.64	133.12 \pm 24.61	0.0001
Urinary protein (mg/24 hours)	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
TNF- α (pg/mL)	6.78 \pm 1.13	31.57 \pm 9.77	64.69 \pm 11.90	0.0001
IL-10 (pg/mL)	4.38 \pm 0.99	9.16 \pm 1.96	12.88 \pm 2.11	0.0001

One way ANOVA test was applied.

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

in TNF- α level in preeclamptics. A study conducted by Sharma et al. showed a statistically significant increase in serum TNF- α in preeclamptics ($p < 0.001$).²¹

Serum IL-10 values were 4.38 ± 0.99 , 9.16 ± 1.96 , 12.88 ± 2.11 in three groups respectively (Table 1). Fathollah et al. conducted a similar study in Iran and showed comparable results of TNF- α with our study $p < 0.001$.²² But considering the levels of IL-10, it is contradictory to what we have obtained in our study. In his study, the serum IL-10 levels were significantly high in normotensive pregnant women compared to preeclamptics. A study done by Lau et al. showed similar results with our study. The IL-10 levels in preeclamptics were significantly elevated.²³ Jonsson et al. observed no significant difference in IL-10 levels between normotensive and pre-eclamptic women.²⁴

The probable reasons for elevated pro-inflammatory markers like hs-CRP in preeclamptics, although some amount is associated with normal pregnancy, were reduced plasma volume and effect of BMI.²⁵ Some studies proposed a direct effect of CRP on human endothelial cells by inducing expression of adhesion molecules. CRP may also contribute to thrombosis by inducing human monocytes to synthesize tissue factor, a potent procoagulant.²⁶ These observations imply that a subclinical inflammation has a role in the pathogenesis of preeclampsia.²⁷

Preeclamptic patients show an alarming increase proinflammatory cytokines like IL-10 and TNF- in their serum (Fig. 1).²⁸ These inflammatory mediators promote systemic vascular damage, particularly in the kidney, results in the characteristic proteinuria and hypertension of the maternal syndrome of preeclampsia.²⁹

Interleukin-10 (IL-10) and TNF- α markedly upregulate IL-messenger RNA (mRNA) and protein expression by the resident decidual cells. This augmented expression of decidual IL-10 may play a key role in the inflammation in preeclamptics.³⁰

The high concentration of IL-10 in women with preeclampsia, as found in this study, maybe because preeclampsia is a manifestation of immune rejection, which IL-10 tries to suppress.

CONCLUSION AND CLINICAL SIGNIFICANCE

From the study mentioned above and many other studies, it was found that increased blood levels of inflammatory markers (hs-CRP, TNF- α , and IL-10) reflect endothelial dysfunction with inflammatory process in the preeclamptic complication of pregnancy.

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