Original Research Article

Serum CRP levels in pre-eclampsia and its association with microalbuminuria

Mitasha Nagori[1],*, Madhubala Chauhan[1]

[1]Dept. of Obstetrics and Gynaecology, RNT Medical College, Udaipur, Rajasthan, India

A B S T R A C T

Introduction: Hypertensive disorders complicating pregnancy are common and form one of the deadly triad, along with haemorrhage and infection; that contribute greatly to maternal morbidity and mortality. Maternal morbidity remains increased with pre-eclampsia, which continues to be one of the leading causes for the admission of pregnant women to intensive care units in the developed world.

Aim & Objectives: 1. To measure the levels of S. CRP and urine albumin levels in women with pre-eclampsia; 2. To evaluate the association of S. CRP levels and albuminuria in pre-eclampsia.

Materials and Methods: Hospital based study conducted at PDZH, RNT Medical College, Udaipur. All 100 Pre-eclampsia women attending OPD and labour room of PDZH were evaluated within a period of one year.

Results: There was statistically significant increase in the levels of serum CRP and Albumin excretion in urine, and also there was a significant positive correlation between CRP and Albuminuria (p<0.01).

Conclusions: The immune activation related to endothelial dysfunction, the inflammatory mechanisms that lead to vasospasm, and the inflammatory response associated with the presence of necrotic placental cells in the uterine and placental bed. Serum CRP and Albuminuria are positive markers of inflammation and can be used as utility parameters for the assessment of pre-eclampsia.

1. Introduction

Hypertensive disorders complicating pregnancy are common and form one of the deadly triad, along with haemorrhage and infection; that contribute greatly to maternal morbidity and mortality. How pregnancy incites or aggravates hypertension remains unsolved despite decades of intensive research. Indeed hypertensive disorders remain among the most significant and intriguing unsolved problems in obstetrics. [1]

Maternal morbidity remains increased with pre-eclampsia, which continues to be one of the leading causes for the admission of pregnant women to intensive care units in the developed world. In developing countries, where inadequate prenatal care limits the pre-eclampsia surveillance, maternal mortality is common, though has reduced to a low level due to early and free referral, easy transportation, free investigations and free medicines under JSSK scheme. [2]

The pathogenesis of pre-eclampsia is best explained by the two stage hypothesis. Pre-eclampsia is a two stage disorder. Stage 1 is caused by faulty endovascular trophoblastic remodelling that downstreams and causes the stage two of a systemic clinical syndrome. Due to incomplete trophoblastic invasion, the abnormally narrow spiral arteriolar lumen, likely impairs the placental blood flow. Diminished perfusion and hypoxic environment eventually leads to release of placental debris or microparticles. In response to placental factors released, a cascade of events begins.

Thus the second stage begins which under the influence of pre-existing medical conditions causes an exaggerated endothelial cell activation and a generalized hyper-inflammatory state. Endothelial injury or activation

* Corresponding author.
E-mail address: mitasha286@gmail.com (M. Nagori).

causes vascular constriction with increased resistance and subsequent hypertension. Endothelial cell damage also causes interstitial leakage through which blood constituents, including platelets and fibrinogen, are deposited subendothelially which is manifested as thrombocytopenia. The much larger venous circuit is similarly involved, and with diminished blood flow because of maldistribution, ischemia of surrounding tissues can lead to necrosis, haemorrhage, and end organ failure.

There is increasing evidence that pre-eclampsia is a systemic inflammatory disease.[3] Inherent to the inflammatory process is the occurrence of an acute phase response. This response is induced by pro-inflammatory cytokines (Interleukin 1 and 6) which are released from the inflamed tissue by inflammatory and/or parenchymal cells. This in turn stimulates the liver to synthesize a number of acute phase proteins. CRP is a hepatically derived classical acute phase reactant.[4] It is an objective and sensitive index of overall inflammatory activity in the body. Plasma CRP levels rise in cases of acute infection, malignancy and inflammatory diseases. It can bind to chromatin (released from apoptotic or necrotic cells) and to small nuclear ribo-nucleoprotein particles. It has been proposed that CRP acts as a scavenger and is responsible for the clearance of membranes and nuclear antigens. It has been suggested that CRP, in accordance with its proposed function, may play a role in eliciting the inflammatory response characteristics of pre-eclampsia.[5]

The understanding of the underlying factors that explain the pathogenesis of pre-eclampsia and the early identification of the patients at risk of the disease will help in the development of preventative or therapeutic interventions, aimed to reduce the associated morbidity and mortality during pregnancy, but also the long-term severe problems that pre-eclampsia produce or is associated with.[6]

Pre-eclampsia is one of the major conditions causing maternal morbidity and mortality throughout the world. It is a common complication of pregnancy in India. CRP, a sensitive marker of tissue damage and inflammation is proposed to play a role in eliciting the inflammatory response of pre-eclampsia. Elevated CRP levels indicate systemic inflammation. Inflammation apparently increases the likelihood of increased glomerular leakage of albumin in response to blood pressure. This glomerular leakage may involve either increased transmission of systemic BP or decreased barrier function of glomerulus.

2. Aim and Objectives

1. To measure the levels of S. CRP and urine albumin levels in women with pre-eclampsia.
2. To evaluate the association of S CRP levels and albuminuria in pre-eclampsia.

3. Materials and Methods

This is a hospital based cross-sectional study conducted at PDZH, RNT Medical College, Udaipur. All Pre-eclampsia women attending OPD and labour room of PDZH were evaluated within a period of one year. The study comprised of 100 pregnant women of age 18-35 years with GA >20weeks.

3.1. Inclusion criteria

Pregnant women of 18-35years, presenting themselves for antenatal care with gestational age >20weeks, BP$\geq$140/90mm Hg, proteinuria $\geq$ +1 of singleton pregnancy.

3.2. Exclusion criteria

Patients in active labour, with essential hypertension, h/o acute or chronic liver diseases, on drugs affecting coagulation, PROM or any medical diseases like DM, renal disease, hypo or hyperthyroidism, cardiovascular disease, auto-immune disease, drug abuse were excluded from the study.

Also women with severe anaemia (Hb<6g/dl), fetal infections or fetal congenital malformation, with neoplastic disorder, alcohol and cigarette smoking, with urinary tract infection, multiple gestation and eclampsia on admission were excluded.

CBC, Red Cell Indices, LFT, RFT, Coagulation profile, Serum CRP, Protein dipstick test, Urine ACR, 24 hour urine protein, Serum TSH, Random blood sugar were investigated.

After a thorough history taking and clinical examination, the procedure was explained to the subjects and an informed consent was obtained. Blood pressure and proteinuria noted. General physical and systemic examination including the obstetric examination was done. Per speculum examination was done to look for any evidence of vaginal infection clinically.

A qualitative test for urinary protein was carried out on a random midstream clean catch sample of urine using dipstick method. Protein by dipstick method was graded as follows: Traces = 0.15-0.3g/dl (+1 = >0.3g/dl; +2 = 1g/dl; +3 = 3g/dl).

The test was repeated on additional sample of urine immediately and if the subsequent tests show 1+ or more, quantitative tests for proteinuria were carried out.

3.3. Assessment of protein in urine/24 hours

24 hour urine collection was done using the clear white plastic 4litre container that was given to patients. All women were instructed to adhere to their normal diet and physical activity.

Urinary albumin is usually measured by an immunochemical method such as immunonephelometry, immunoturbidimetry, enzyme-linked immunosorbent assays (ELISA) or radioimmunodiffusion. Recently, a number of studies have used high-performance liquid chromatography (HPLC) for urinary albumin measurement in different populations, and demonstrated that the level of albumin detected in the urine by HPLC, when compared with conventional assays, is significantly greater because HPLC is able to measure both immunoreactive and immunounreactive intact albumin.

3.4. Nephelometry method

Nephelometric analysis is based on measuring the weakening of intensity of a luminous flux when it passes through a solution containing particles in suspension. The intensity decreases owing to absorption and scattering of light. The principle of nephelometry is based on the scattering or absorption of light by solid or colloidal particles suspended in solution. Light is passed through the sample solution (suspended particles) directly and the amount of scattered radiation is measured generally at $90°C$. The measurement of intensity of scattered light as a function of concentration of dispersed phase is the basis of analysis of nephelometry. In nephelometry, incident and scattered light are of same wavelength. As the amount of light scattered depends on size of the particles in the solution, hence correct results will depend upon method of preparing the suspensions and on reproducibility of their optical properties. All the instruments are placed inside a systematic automated analyser where the centrifuged sample of urine is placed and the volume of 24 hours collection is fed each time in the analyser.

The method measures the shift in the absorption spectrum from 460-600nm of the complex that occurs at acid pH between pyrogallol red-molybdate (PRM) and the basic amino groups of urine proteins. The intensity of the coloured complex formed is proportional to the concentration of proteins in urine sample.urine protein(pH 2.5) → PRM-Protein complex.

Instrument:- Semi-automated Photometer (Humalyzer 3000) German, Automatic pipettes to measure reagent and sample volume.

4. Results

The Table 1 shows distribution of cases according to age.55% of the women were in the age group of 21-25 years with mean age of the women in the study was 24.57 with standard distribution of 3.32.

Table 1: Distribution of cases according to age groups

Age groups	Number of patients	Percentage
19-20	9	9%
21-25	55	55%
26-30	31	31%
>30	5	5%
Total	N=100	100%
Mean $\pm$ SD	24.57 $\pm$ 3.32	
Median	24	
Min-Max	19-33	

4.1. Distribution of cases according to socioeconomic status

The Table 1 shows distribution of cases according to socioeconomic status. Most of the women belonged to middle class (52%) followed by lower socioeconomic status (44%). This could be because of free medical facilities being provided by JSSK scheme in our institution. Only 4% belonged to the upper class.
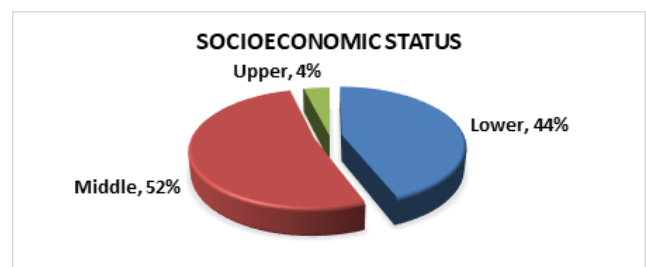
Fig. 1:

Table 2: Distribution of cases according to severity of pre-eclampsia

Severity of Pre-eclampsia	No. of Patients	Percentage
Mild Pre-eclampsia ($\geq$140/90 to 159/109)	63	63%
Severe Pre-eclampsia ($\geq$160/110)	37	37%
Total	N=100	100%

When the cases were grouped according to severity of pre-eclampsia, 63% belonged to mild pre-eclampsia group and the rest were in the severe pre-eclamptic group.

The Table 3 shows distribution of cases according to severity of albumin excretion in urine per day. 51% had mild albuminuria, 44% showed moderate albuminuria and remaining 5% had severe albuminuria.

The Table 4 shows that the difference in systolic as well as diastolic blood pressure between mild and severe pre-eclamptic patients were very highly significant (p-value < 0.001).

Table 3: Distribution of cases according to severity of albuminuria

24 Hr Urine Protein Range	No. of Patients	Percentage
Mild Albuminuria (300- 999 mg/Day)	51	51%
Moderate Albuminuria (1000-2999 mg/Day)	44	44%
Severe Albuminuria ($\geq$3000mg/Day)	5	5%
Total	N=100	100%

**Table 4:** Mean level of blood pressure among mild and severe pre-eclamptic patients

Blood Pressure	Mild Pre-eclampsia Mean±SD	Severe Pre-eclampsia Mean±SD	P Value
Systolic (mmHg)	147.78±6.22	170.65±8.98	<0.0001
Diastolic (mmHg)	93.59±4.60	112.16±4.17	<0.0001
CRP (mg/dl)	7.66±3.15	13.53±4.03	<0.0001

It is observed that mean level of serum CRP in mild pre-eclampsia patients was 7.66 and mean level of serum CRP in severe pre-eclampsia patients was 13.53. The difference in serum CRP level between mild and severe pre-eclamptic patients was found to be very highly significant (p <0.001).

A significant positive correlation between serum CRP level and blood pressure (systolic and diastolic) in both mild and severe pre-eclamptic patients. Similarly a significant positive correlation between albuminuria and blood pressure (mmHg) (systolic and diastolic) in both mild and severe pre-eclamptic patients.

The Table 6 shows a significant positive correlation between Serum CRP level and Albuminuria in Pre-eclampsia.

It was observed that among 100 patients, 58% had vaginal delivery and caesarean section was done in 42% pre-eclamptic patients.
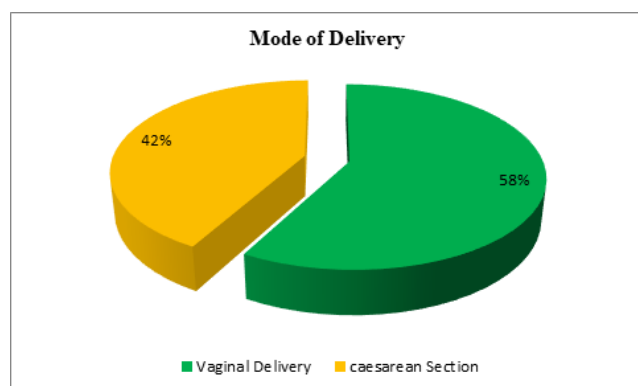
Fig. 2:

The Table 7 shows that the birth weight of 48% newborn of pre-eclamptic mothers and 52% newborn of pre-eclamptic mothers were less than 2.5kg and more than 2.5kg respectively.

5. Discussion

Hypertensive disorders of pregnancy which frequently manifest as pre-eclampsia continues to exert an enormous toll in developing countries like India and also in Western society. Despite progress in its prevention, detection and treatment, it continues to be the leading cause of maternal death. Research over last decade has proven the role of oxidative stress and inflammation in pathophysiology of pre-eclampsia. Various traditional and newer biomarkers were suggested for diagnosis and prognosis of pre-eclampsia.

Mean age of mothers in our study is 24.57 $\pm$ 3.32 years which is an appropriate and healthy trend regarding reproductive career of the women in the community. This was comparable to a study done by Rinehart et al. (1999)[7] in which patients admitted for pre-eclampsia had a mean age of 25±6.5years. Similar studies were done on women with pre-eclampsia and the mean age was found to be 25 years by Maryam Asgharnia (2013),[8] Shazia Majid Khan et al. (2014)[9] 26.83± 9.32 years and Savita Rani Singhal et al. (2014),[10] the mean gestational age was 24.58 $\pm$ 3.90 years.

Similar to our study, in studies done by Somnathan et al. (2003),[11] Tara et al. (2008),[12] and Adelberg et al. (2001),[13] a proteinuria of +3 or more was present in only 16%, 12% and 18% of the subjects respectively. In a study done by Savita Rani Singhal et al. (2014), the level of proteinuria was either +1 or +2 in 92% of cases, and only 8% of the cases had proteinuria of +3.

In our study, there was statistically significant increase in the serum levels of CRP in cases (p <0.001). Lohsoonthorn V[14] and co-researchers in their prospective study found that serum levels of CRP were significantly increased in patients with pre-eclampsia. The study also demonstrated the use of serum CRP levels in early pregnancy to clarify the temporal relationship between elevated maternal CRP and subsequent risk of preterm delivery. Sacks GP and co-workers did a study which included pregnant women who were being treated by an in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) at the IVF unit in Hammersmith

Table 5: Correlation of Serum CRP level and Albuminuria with Blood Pressure in mild and severe pre-eclamptic patients

Parameter		CRP (mg/dl) Correlation (r) (p value)	Albuminuria Correlation (r) (p value)
Mild Pre-eclampsia	Systolic Blood Pressure (mmHg)	0.424 (<0.01)	0.507 (<0.01)
	Diastolic Blood Pressure (mmHg)	0.464 (<0.01)	0.611 (<0.01)
Severe Pre-eclampsia	Systolic Blood Pressure (mmHg)	0.682 (<0.01)	0.607 (<0.01)
	Diastolic Blood Pressure (mmHg)	0.426 (<0.01)	0.595 (<0.01)

Table 6: Correlation of serum CRP level with albuminuria in pre-eclampsia

Parameter	Albuminuria (mg/day) Correlation coefficient (r)	P Value
CRP(mg/dl)	0.872	<0.01

Table 7: Neonatal outcome according to birth weight

Birth weight	No of cases	Percentage
<2.5 Kg	48	48%
>2.5 Kg	52	52%
Total	N=100	100%

Hospital. This study provided the evidence for a maternal inflammatory response with raised CRP levels as early as 4 weeks gestation.

Mohammadi B et al.[15] in their prospective cohort study in 2010, showed that there is significant relationship between CRP levels and mild and severe pre-eclampsia cases. CRP, as an inflammatory marker was not statistically significantly increased in pre-eclampsia compared to the control group which was demonstrated by Stefenovic M[16] and co-workers in 2009. Apart from infections, inflammation, and trauma, factors associated with increased levels of CRP include obesity, cigarette smoking, hormone use, metabolic syndrome and cardiovascular disease.

In the present study, Serum CRP concentration was found to be significantly higher (p <0.001) in pre-eclamptic patients. Among the pre-eclamptic patients, serum CRP concentration was found to be significantly higher (p<0.001) in severe pre-eclampsia as compared to mild pre-eclampsia.

Similar type of study done by Ghazavi A et al.[17] found that serum CRP concentration was higher in pre-eclampsia group as compared to normotensive pregnant group. CRP levels were also significantly elevated in women with severe pre-eclampsia as compared to those patients with mild pre-eclampsia.

In the present study, a significant positive correlation was found between serum CRP concentration and blood pressure (systolic and diastolic) in both mild and severe pre-eclamptic patients (p <0.01).

Ahmed K et al.[18] also found significant positive correlation between serum CRP levels in both systolic (p<0.05) and diastolic blood pressure(p<0.05) among 30 pre-eclamptic patients admitted in Aim Shams University Maternity Hospital, Egypt.

Fatemeh M et al.[19] conducted a study in Afzalipour Hospital, Iran consisting of 43 mild and 43 severe pre-eclamptic patients in their third trimester of pregnancy. They found that in mild to severe pre-eclampsia groups, there was a positive correlation between serum CRP levels and systolic and diastolic blood pressure.

In our study, a significant positive correlation was found between albuminuria and blood pressure (mmHg) (systolic and diastolic) in both mild and severe pre-eclamptic patients. The p value <0.01 in each case indicating that there is a significant relationship between blood pressure and proteinuria. As the blood pressure increases, proteinuria increases similarly.

In our study, we could also establish a significant positive correlation between serum CRP and the level of albuminuria. (p value <0.01). Rani Sauriasari et al. in their study demonstrated a significant association among the high sensitivity CRP, a high e-GFR and high urinary protein in current smokers.[20]

Microalbuminuria might be used as an early marker of endothelial dysfunction in pre-eclampsia, before the onset of the overt syndrome, as it is likely that overt proteinuria is preceded by a microalbuminuric phase.

6. Conclusions

This is a hospital based cross sectional study which was conducted on 100 pre-eclamptic women with gestational age >20weeks with BP≥140/90mmHg and proteinuria ≥+1, in the Department of Obstetrics & Gynaecology, RNT Medical College, Udaipur. The main aim of this study was to establish a correlation between serum CRP level and albuminuria in pre-eclampsia.

There was statistically significant increase in the levels of serum CRP and Albumin excretion in urine,

and also there was a significant positive correlation between CRP and Albuminuria (p<0.01). This suggests the immune activation related to endothelial dysfunction, the inflammatory mechanisms that lead to vasospasm, and the inflammatory response associated with the presence of necrotic placental cells in the uterine and placental bed. Serum CRP and Albuminuria are positive markers of inflammation and can be used as utility parameters for the assessment of pre-eclampsia.

7. Source of Funding

None.

8. Conflict of Interest

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

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Author biography

Mitasha Nagori, Senior Resident

Madhubala Chauhan, Senior Professor & Head