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"Study of serum antioxidants and C-reactive protein level in preeclampsia."

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Abstract

Present study was conducted in Department of Biochemistry Dr. SCGMC Nanded over the period of 18 months. Well informed written consent was taken from every study subject and it was ethically cleared from the institutional ethical committee. The study population consisted of 120 pregnant women, of whom 60 were normal pregnant healthy women taken as control and 60 pregnant women with diagnosis of Preeclampsia were taken as cases. The cases were divided in two groups as mild and severe cases of preeclampsia. Measurement of inflammatory marker like C-reactive protein, measurement of lipid peroxidation markers like serum malondialdehyde. And for oxidative stress serum superoxide dismutase, serum uric acid and Vitamin C were measured among the study subject.

Serum CRP was found to be significantly increased with the severity of preeclampsia in mild and severe cases when compared with normal pregnant women. High levels of CRP in preeclampsia highlight the underlying pathogenesis of endothelial inflammation and disease progression.

Serum MDA levels was also found to be significantly increased with the severity of preeclampsia in mild and severe cases when compared to controls. This rise was because of the increased oxidative stress in preeclampsia resulting in higher lipid peroxidation. Also, antioxidants like serum SOD and Vit C were decreased with the severity of preeclampsia as compared to controls indicating their increased turnover for the prevention of oxidative damage and lipid per-oxidation.

. In contrast serum uric acid was shown to be increased with the severity of pre-eclampsia in mild and severe cases when compared to controls. Increase in the levels of serum uric acid was mainly because of decreased urate excretion commonly found in preeclamptic women.

Key words:

Preeclampsia, C-reactive protein(CRP), Lipid peroxidation, Malondialdehyde(MDA), Oxidative stress, Superoxide dismutase(SOD), Uric acid, Vitamin C, Pregnency induced hypertention (PIH).

Introduction:

Pregnancy is a physiological stress in which many changes occur in the milieu interior of the body. Preeclampsia is one specific change which occurs only in pregnant women. Preeclampsia is an abnormality usually occurring during second and third trimesters of pregnancy and it is more common in nulliparous women. Maternal health is especially affected when preeclampsia or more severe complications such as eclampsia or HELLP (Hemolysis, elevated liver enzymes and low platelet count) syndrome develops. These syndromes

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substantially contribute to maternal morbidity and mortality as well as perinatal morbidity and mortality worldwide[1] . The symptoms of preeclampsia include hypertension and proteinuria. It is associated with general endothelial dysfunction[2].It's etiology has been postulated as a part of an exaggerated maternal inflammatory response to pregnancy[3]. Activated circulating leukocytes[4,5]; increased production of reactive oxygen species[6] and increased release of inflammatory cytokines, such as Tumor necrosis factor α (TNF-α) and Interleukin-6 (IL-6) [7,8], as well as abnormal activation of the clotting system[9] in women with preeclampsia compared with normotensive women.

C - reactive protein (CRP) is an objective and sensitive index of overall inflammatory activity in the body[10]. Plasma CRP levels rise in cases of acute infection, malignancy & inflammatory diseases. CRP can bind to chromatin, released from apoptotic or necrotic cells and to small nuclear ribonucleoprotein particles. It has been proposed that CRP acts as a scavenger and is responsible for the clearance of membranes and nuclear antigens[11,12]. It has been suggested that CRP, in accordance with its proposed function, may play a role in eliciting the inflammatory response characteristics of preeclampsia[3]. CRP is thought to be elevated in women with overt preeclampsia[13].

It is well known that oxidative stress increases during normal pregnancy. In healthy pregnancy it has been reported that plasma lipid hydroperoxide levels are increased and total antioxidant capacity is decreased[14].

More oxidative stress in preeclampsia results in lipid peroxides, reactive oxygen species and super oxide anion radicals to cause endothelial injury and dysfunction, platelet and neutrophil activation, increased cytokines, superoxide radical production and endothelial damage in a vicious cycle[15]. These observations on the effects of oxidative stress in preeclampsia have given rise to increased interest in antioxidants such as vitamin C (Ascorbic acid), superoxide dismutase (SOD), Uric acid etc.

Thus this study was undertaken to study the role of CRP in inflammatory response and to find out the antioxidant status and free radical damage (in the form of lipid perixidation product MDA) in preeclampsia.

Aims and objectives:

To determine the C-reactive protein level and extent of free radical damage (in the form of lipid peroxidation product malondialdehyde) and antioxidant status (in the form of superoxide dismutase, Uric acid and Vit C) in preeclampsia in comparison with normal pregnancy.

Material and methods:

This study was conducted in Department of Biochemistry Dr. SCGMC Nanded over the period of 18 months. Each study subject gave well informed written consent and was approved by an institutional ethical committee. The study population consist of 120 pregnant women, of whom 60 normal pregnant healthy women were taken as control and 60 pregnant women with diagnosis of preeclampsia were taken as cases. All subjects of study population, selected for present study were attending and admitted to our medical college and hospital, were pregnant women (primigravidae and multigravidae) with gestational age above 20 weeks.

The diagnosis of preeclampsia was done by Obstetrics and Gynaecology Department based on the definition of textbook of obstetrics by D.C.Dutta sixth edition i.e; Systolic blood pressure ≥ 140 mm Hg or diastolic blood pressure ≥90 mm Hg if the previous blood pressure is not known or a rise in systolic pressure of at atleast 30 mmHg , or a rise of atleast 15 mmHg over

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the previously known blood pressure and Proteinuria ≥300 mg in 24 hr urine collection or dipstick protein ≥ 1 + (on two occasion at least 6 hrs apart) after the 20 weeks of gestation is defined as preeclampsia[15].

The study subjects were divided into two groups- Group A and Group B

Group A: Control subjects

60 women having normal uncomplicated pregnancy without hypertension were taken as control

Group B: Cases

Preeclampsia cases were again divided into two groups, mild and severe.

Mild cases: preeclamptic cases having systolic blood pressure ≥140 mmHg and < 160 mmHg or diastolic blood pressure having ≥90 mmHg and <110 mmHg were considered as mild cases[15].

Severe cases: preeclamptic cases having systolic blood pressure ≥160 mm Hg or diastolic blood pressure ≥110 mm Hg constituted severe cases[15]

Inclusion Criteria

- Pre-eclamptic pregnant women above 20 weeks of gestation
- BP → ≥140/90 mm Hg constituted cases and <140/90 mm Hg constituted controls.
- Urine albumin ≥ 1 + dipstick or 300 mg per 24 hour urine
- Normal pregnant women above 20 weeks of gestation were taken as controls.

Exclusion Criteria

- Previous history of hypertension, DM, thyroid disorder, dyslipidemia, preeclampsia or renal disease
- Other medication except for vitamins, iron & calcium.

Specimen collection:

10 ml of blood was collected in clean plain bulb after an overnight fast (after 10 to 12 hours) by venepuncture. Samples were collected between 7am to 9 am. The serum was separated by centrifugation. Serum CRP, MDA, SOD, uric acid, Vit C were measured on the same day. In this study serum CRP and uric Acid were measured using accustar semi-autoanalyzer, while serum MDA, SOD and Vit C were measured by colorimetric method.

Table 1: Biochemical parameters with Method of estimation.

Sr. No.	Biochemical parameter	Methods of estimation
1.	C Reactive Protein	Turbilatex kit
2.	Serum malondialdehyde	Kei- Satoh method
3.	Serum Superoxide	Marklund S , Marklund G

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	dismutase	
4.	Serum Uric acid	Enzymatic Uricase method
5.	Serum Vit C	Caraway method

Observations:

Table 1: CRP level among study group

Parameter	Cases (mg/L)		Control (mg/L)	One way ANOVA (kruskal wallis test)
	mild	severe		
1. Mean CRP with S.D	19.4±6.6	38.28±9.4	7.1±3.8	p<0.0001*
Lower 95% C.I of mean CRP	17.30	33.92	6.1	--
Upper 95% C.I of mean CRP	21.59	42.54	8.1	--

*Statistically highly significant

Table 2: Parameters to assess lipid peroxidation and anti-oxidant status among study group

Parameter	Cases		control	One way ANOVA (kruskal wallis test)
	mild	severe		
1. Mean MDA with S.D (nmol/ml)	6.1±1.18	7.8±1.0	2.4±0.89	p<0.0001*
Lower 95% C.I of mean MDA(nmol/ml)	5.7	7.4	2.17	--

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Upper 95% C.I of mean MDA(nmol/ml)	6.5	8.3	2.63	--
2. Mean SOD with S.D(units/ml)	2.65±0.30	2±0.30	3.75±0.37	p<0.0001*
Lower 95% C.I of mean SOD(units/ml)	2.55	1.84	3.66	--
Upper 95% C.I of mean SOD(units/ml)	2.75	2.11	3.85	--
3. Mean uric acid with S.D(mg/dl)	5.1±0.81	7±0.63	3.9±0.79	p<0.0001*
Lower 95% C.I of mean uric acid (mg/dl)	4.84	6.73	3.74	--
Upper 95% C.I of mean uric acid (mg/dl)	5.37	7.31	4.16	--
4.Mean Vit C with S.D(mg%)	0.66±0.15	0.52±0.16	1.07±0.22	p<0.0001*
Lower 95% C.I of mean Vit C (mg%)	0.61	0.44	1.01	--
Upper 95% C.I of mean Vit C (mg%)	0.71	0.59	1.13	--

*Statistically highly significant

Discussion:

This study had been done to determine the C-reactive protein level and extent of free radical damage (in the form of lipid peroxidation product malondialdehyde) and antioxidant status (in the form of superoxide dismutase, Uric acid and Vit C) in preeclampsia in comparison with normal pregnancy.

C Reactive Protein (CRP)

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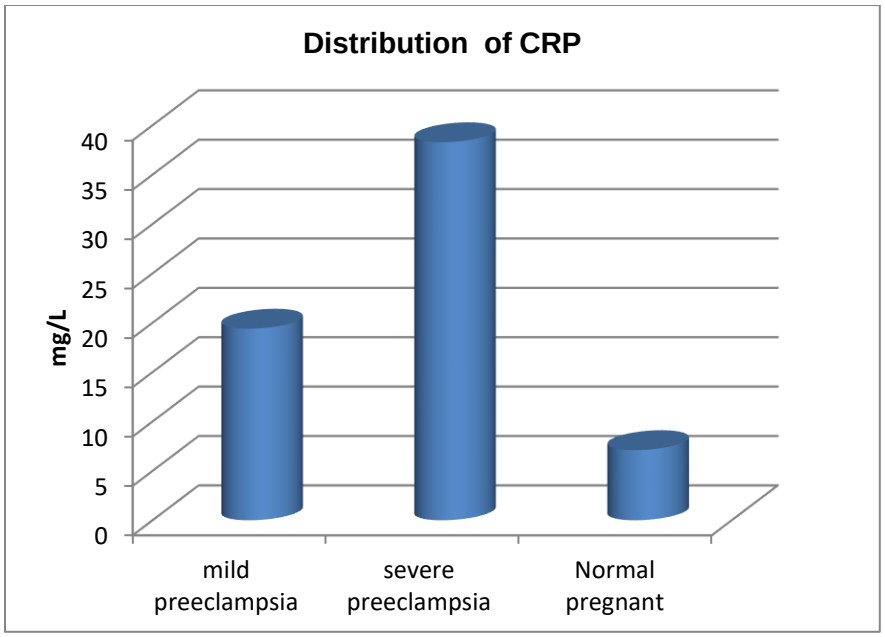

Graph no.1: Association of CRP among study group.

The mean CRP levels in mild, severe and control were 19.4±10.6 mg/L, 38.28 ±14.6 mg/L and 7.1±6 mg/L respectively. There was significant increase in CRP levels in mild and severe cases as compared to control ($p<0.0001$). Also about 95% cases of severe preeclampsia was above 33.92 mg/L.

CRP, a sensitive marker of tissue damage and inflammation, is proposed to play a role in eliciting the inflammatory response characteristics of preeclampsia[16].

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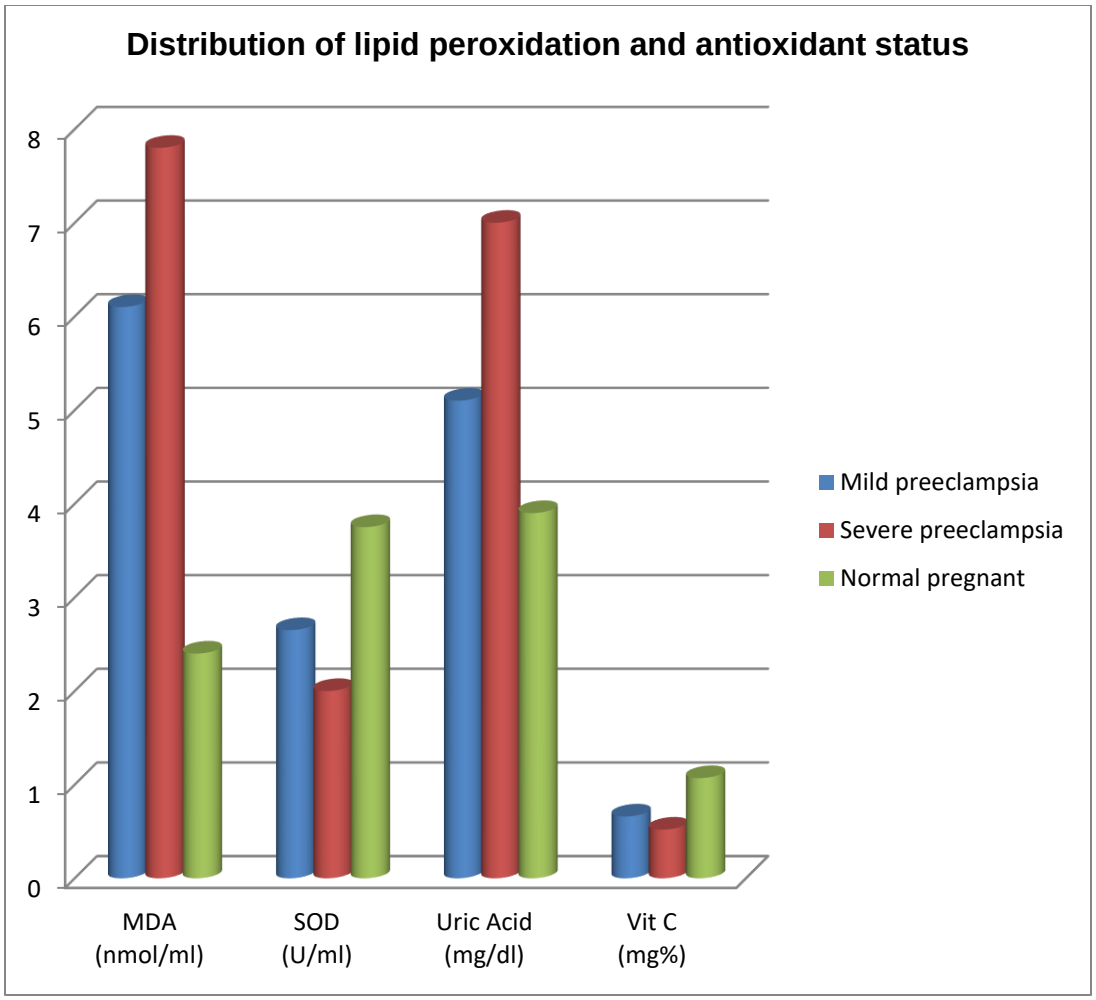

Graph no.2: Measurement of biochemical parameters to assess lipid peroxidation and anti-oxidant status of cases and controls

The mean MDA levels in mild, severe and control were 6.1±1.18 nmol/ml, 7.8±1.0 nmol/ml and 2.4±0.89 nmol/ml respectively. There was significant increase in MDA levels in mild and severe cases as compared to control ($p<0.0001$).

The mean SOD levels in mild, severe and control were 2.65±0.30 units/ml, 2±0.30 units/ml and 3.75±0.37 units/ml respectively. There was significant decrease in SOD in mild and severe cases as compared to control ($p<0.0001$). Also about 95% cases of severe preeclampsia had values less than 2.11 units/ml.

The mean uric acid levels in mild and severe cases and control were 5.1±0.81 mg/dl, 7±0.63 mg/dl and 3.9±0.79 mg/dl respectively. There is significant increase in uric acid in mild and severe cases as compared to control ($p<0.0001$). About 95% cases of severe preeclampsia had values more than 6.73 mg/dl.

The mean Vit C levels in mild and severe cases and control were 0.66±0.15 mg%, 0.52±0.16 mg% and 1.07±0.22 mg% respectively. There was significant decrease in Vit C in mild and severe cases as compared to control ($p<0.0001$). Also 95% cases of severe cases of preeclampsia have values less than 0.59 mg%.

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PIH associated with endothelial dysfunction could be caused by oxidative stress. The unsaturated lipids and thiol containing proteins of the cell membranes are susceptible to free radical attack. Lipid peroxidation mediated by free radicals is considered to be the major mechanism of cell membrane destruction and cell damage and is a key contributing factor to pathophysiologic condition of preeclampsia. Alteration in the oxidant – antioxidant profile is known to occur in PIH. In our study we found that MDA increases significantly in mild and severe cases as compared to controls ($p<0.0001$).

The antioxidant status in our study was measured by serum SOD, uric acid and Vit C. The mean serum SOD levels in our study decreases significantly with the severity of preeclampsia in mild and severe cases as compared to normal pregnant women.

The mean uric acid levels were significantly higher in mild and severe cases as compared to controls ($p<0.0001$). Also the mean level of uric acid increased according to the severity of the disease in mild and severe cases. Elevated serum uric acid levels mainly due to decreased renal urate excretion are frequently found in women with preeclampsia. Soluble uric acid impairs nitric oxide generation in endothelial cells[17]. Thus, hyperuricemia can induce endothelial dysfunction and disease progression.

Another anti-oxidant Vit C which was measured in our study was found to be decreased in mild and severe cases of preeclampsia as compared to controls($p<0.0001$). Also it was found that Vit C decreased more in severe cases as compared to mild cases of preeclampsia.

Conclusion:

Preeclampsia is a multisystem disease of the pregnancy in which many factors come into play. This study suggests that inflammatory marker like C-reactive protein increases more with the severity of preeclampsia. Lipid peroxidation marker like malondialdehyde increases and antioxidant markers like superoxide dismutase and Vitamin C decreases and uric acid increases in preeclampsia according to its severity. Thus we can conclude that markers like C-reactive protein, superoxide dismutase and uric acid may be used as a marker for indicating the severity of preeclampsia.

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