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RESEARCH ARTICLE
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INFLAMMATORY MARKERS CRP, FERRITIN, TUMOUR NECROSIS FACTOR ALPHA IN PREECLAMPSIA.

Ms. Shubhangi H Gawade[1], Dr. Pooja N Joshi[2], Dr. Gouri A Gulavani[3]*, Ms. Farheen Mujawar[4]

[1]Tutor, Department of Biochemistry, SKNMCGH, PUNE
[2]Lecturer, Department of Biochemistry, BJGMC, PUNE
[3]*Associate professor, Department of Biochemistry, SKNMCGH, PUNE
[4]Tutor, Department of Biochemistry, SKNMCGH, PUNE

***Corresponding Author:** Dr. Gouri A Gulavani
*Associate professor, Department of Biochemistry, SKNMCGH, PUNE

Introduction:
Preeclampsia (PE) is one of the most serious pregnancy complications. The worldwide prevalence of PE ranges from 3 to 7% of pregnancies, affecting a total of 8.5 million women worldwide.PE is responsible for about 18% of maternal deaths and up to 40% of fetal mortality. At this time, PE still lacks a safe and effective therapy, as well as a reliable, early means of diagnosis or prediction.(3)
Being considered as extreme maternal response to pregnancy, complicated by multiparity, kidney disease, obesity, autoimmune conditions and advanced maternal age, pre-eclampsia and eclampsia both are intravascular inflammatory reactions with complement system and clotting factors involvement.(9) During inflammation, ferritin is a positive acute phase protein, along with another acute phase protein CRP. CRP is an inflammatory marker which plays role in phagocytosis of apoptotic cells and small nuclear RNA protein molecules. Once necrosis sets in role of CRP becomes uncertain. Hence inflammatory response is characterised by raised CRP levels.(4)The pathophysiology of ferritin as indicator of preeclampsia is oxidative stress caused by free radical injury, initiating lipid peroxidation causing endothelial dysfunction. Fenton reaction, involving ion, which comes from ischaemic placenta, generates highly reactive hydroxyl radical.(5)

Total body iron status is indicated by serum ferritin levels but high ferritin doesn't always mean iron excess in the body. It is basically iron storage protein in spleen, bone marrow, liver, placenta and muscle. In pregnancy, serum ferritin is maximum at 12-16 weeks. While low levels at 3rd trimester rules out risk of PROM and eclampsia, which is a good sign for foetus.(5)Pre-eclampsia and eclampsia associated with local ischemia, thrombosis of spiral arteries, in turn leads to oxidative stress. Incomplete invasion of spiral arterioles by trophoblasts results in oxidative stress in the placenta . This placental Oxidative stress leads to oxidative stress in maternal circulation resulting in activation of maternal neutrophils, these release TNF –α and IL-6 causing preeclampsia. The non pregnant state is characterised by balance of Th1 and Th2 response. In normal pregnancy , Type 2 bias exists, when there is shift of maternal immune response. While in preeclampsia Th1 response predominates.(7)Present study was conducted to assess the role of Ferritin , CRP and TNF alpha as pre-eclampsia indicators.

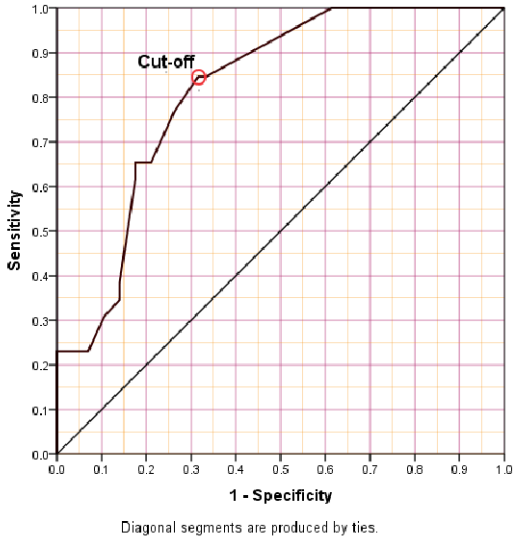
Materials and Methods:
This case control study was conducted at BJMC and SGH. After approval IEC, the sample size was calculated. The total
No. of pregnant women is 100 from OPD of Obstetrics and Gynaecology Department. Aged 18-40 years having viable singleton pregnancy with gestational age >18 weeks were included in this study 100 mothers with no previous history of medical conditions; medications were included in the study. The diagnosis of preeclampsia was based on the definition of preeclampsia by WHO i.e. systolic blood pressure of more than 140 mm Hg, diastolic pressure higher than 90 mm Hg on two different occasions after the 20th week of pregnancy and proteinuria.
Exclusion criteria was Pregnant women with a history of hypertension before pregnancy and Pregnant women with a history of diabetes mellitus, cardiovascular disease, autoimmune disease, kidney disorders, uterine disorders, active smokers, and currently experiencing infections.
5 ml of venous blood was collected from the patients under all aseptic precautions. Serum CRP, ferritin, were done on EM 360 fully autoanalyser. Serum TNF alpha was estimated on ELISA machine.

Data Analysis:
Data was analysed using SPSS windows version 22.0. with $p<0.001$ considered as the threshold for statistical significance. The cut off value for CRP TNF alpha, ferritin was estimated by ROC curve for their cut off values.

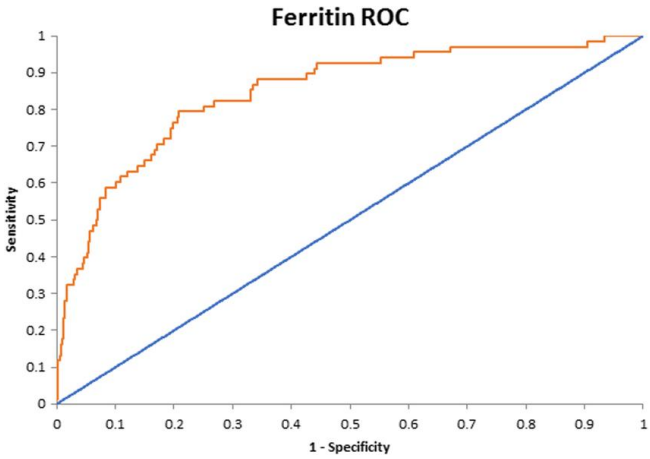
Result: The study involved 100 pregnant women with 50 normotensive pregnant and 50 preeclamptic pregnant women.

Variables	Pre-eclampsia (n=50)	Normotensive pregnant (n = 50)	P Value
Parity (n)	0.46 (0- 3)	1.1 (0- 3)	0.76
Age (Years)	28.5 (18- 40)	28 (18-40)	0.73
BMI	22.7 (18.6- 34)	23.1 (18.6 - 40)	0.8
CRP	35.8±6.5	4.1±1.2	< 0.001
Serum ferritin	124.6 ± 32.5	20.8 ± 1.4	< 0.001
Serum TNF alpha	94.32 ± 16.21	17.32 ± 5.8	< 0.001

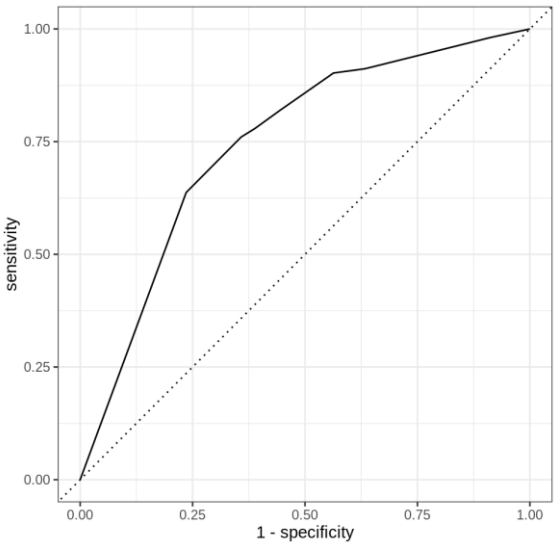
The median age of the case group was 28.5 years with an age range of 18–40 years, while the median age of the control group was 28.0 years with an age range of 18–40 years. The median BMI both the case and control groups, showed similar results, namely 22.7 kg/m2 and 23.10 kg/m2, respectively. Most of the patients in the group of pregnant women with preeclampsia were nulliparous (50.0%).
Mann Whitney test was used to calculate level of significance . based on above results, there was no significant difference in these two groups based on age, BMI and parity.
CRP , ferritin and TNF alpha were expressed as mean ± SD . In this study, there was significant difference in cases and controls as far as CRP, ferritin TNF alpha are concerned. (p value < 0.001)
ROC curve for serum TNF alpha cut off values



ROC curve for serum ferritin cut off values



ROC curve for serum CRP cut off values



In this study, serum ferritin level was found to be 4 to 5 fold higher in cases (124.6 ± 32.5) than controls (20.8 ± 1.4) .This is statistically significant (P value <0.001) . Being an acute phase reactant, serum ferritin stimulates lipid peroxidation leading to endothelial cell damage (7).
In our study, serum TNF–α levels were 5 fold higher in cases (94.32 ± 16.21) than controls (17.32 ± 5.8). This is statistically significant (P value <0.001)

Gestational Age Distribution:

Gestational Age	Cases (n=50)		Controls (n=50)		P value
	N	%	N	%	
<37 weeks	42	84	32	64	0.281
≥37 weeks	8	16	18	36	

In this study, gestational age of <37 weeks was there in 84% of cases and 64% of controls.
Gestational age of ≥ 37 weeks was there in 16% of cases and 36 % of controls.

Discussion:
This study has objective of finding the correlation between maternal serum CRP, Ferritin and TNF – α in normotensive pregnancy and preeclampsia. The mean age was 28 years in normotensive pregnancy while 28.5 was in preeclampsia patients. This is reported to be similar by study done by M. Adenkekan et. al in 2012 [6]
This study has found statistically significant difference in the levels of TNF –α , CRP and Ferritin in normotensive pregnancy and preeclampsia.
The findings were similar to the metanalysis done by Lau et.al.(2) while Afshari et. al could not find similar results in 2013 [3]
TNF –α is derived from lymphocytes, vascular epithelium, and placenta. It function is upregulation of endothelial PLGF, Endothelin – 1 and plasminogen activator inhibitor-1 [4]
Normotensive pregnancy can have TNF –α production, while in preeclampsia , immunological mechanism , inflammatory mechanism can be the cause of increased TNF –α production.
Studies have shown three fold increase TNF –α levels in preeclampsia than normotensive pregnancy is contributed to implantation, production of IL-6 from decidual cells, increased estrogen levels and apoptic changes of decidua.
Many studies have shown increased CRP levels in Preeclampsia than in normotensive pregnancy. The study by D. Mithu ea. Al shows CRP > 3000ng/ml proved specific for Preeclampsia group than normotensive pregnancy.[4]
Parchim et. Al suggested local production sites in the placenta are responsible for production of CRP and activates complement cascade.[8] In an experimental trial , infusion of CRP in mice at the levels comparable to those in preeclamptic women had lead to hypertension , glomerular damage and proteinuria. .[8]
Lasowska et.al indicated TNF –α is increased in Preeclampsia patients and suggested this cytokine plays crucial role in the pathogenesis of preeclampsia. ([5] Trophoblastic apoptosis, incomplete invasion of trophoblasts to spiral arteries are the roles played by this inflammatory cytokine.
Taheripanah et .al found no abnormality in the serum ferritin levels in normotensive pregnant women.
Another study indicated difference in serum ferritin levels in cases and control groups.[7] Free radical mediated injury , releasing transition metals like iron originating from ischaemic placenta leading to thrombotic and hemorrhagic events. This pathway, fenton reaction are responsible for raised ferritin levels in preeclamptic women.[9]

Conclusion:
Upraised levels of CRP, TNF –α and Ferritin may be pathogenic etiology of preeclampsia. It will be of great use for prediction and monitoring in patients with preeclampsia.
Further research may illuminate whether monitoring serum CRP, ferritin, TNF alpha levels have a positive impact on predicting the severity of preeclampsia.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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