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Study of Levels of Oxidative Stress Markers in Nonpregnancy, Normal Pregnancy, and Preeclampsia Women and Their Follow-Up After Delivery Within 48 Hours.

Pradeep Kumar Vegi[1], G Rajeswari[2], S Lavanya Kumari[3]

[1]Ph.D. Scholar in Biochemistry, Rangaraya Medical College, Kakinada and Assistant Professor in Biochemistry, Great Eastern Medical School and Hospital, Srikakulam, Andhra Pradesh.

[2]Professor and Head, Department of Biochemistry, Rangaraya Medical College, Kakinada and Principal/ ADME, ACSR Medical College, Nellore, Andhra Pradesh

[3]Medical Superintendent /Professor and Head, Department of Obstetrics and Gynecology, Rangaraya Medical College, Kakinada.

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ABSTRACT

Background: Preeclampsia is a pregnancy-specific disease process that affects approximately 3-5% of all births. Present study, focuses on to know the oxidative stress markers in terms of macromolecules like carbohydrates, lipids, DNA and proteins. Present study was aimed to study levels of oxidative stress markers in nonpregnancy, normal pregnancy, and preeclampsia women and their follow-up after delivery within 48 hours. **Material and Methods:** Present study was a prospective observational study performed in pregnant women after the 20th week of gestation with preeclampsia. **Results:** All oxidative stress markers were significantly increased in the preeclampsia group than in the normotensive pregnant and non-pregnant women. There was a decrease in the levels of malondialdehyde, protein carbonyl, and DNA damage in patients after 48 hours of delivery but the significance was noted only in malondialdehyde parameter. It was noted that there was significant reduction in malondialdehyde, protein carbonyl and DNA damage post-delivery with $P<0.05$. MDA levels were elevated in preeclamptic pregnant women and therefore, increased lipid peroxidation is considered to be a causative factor for preeclampsia. Increased protein carbonyl content and decreased antioxidant capacity indicate high levels of oxidative stress in women with preeclampsia which plays a crucial role in the endothelial dysfunction initiation and expression of preeclampsia. DNA damage is elevated in preeclamptic pregnant women which can be considered as a marker in preeclampsia. Decreased oxidative stress post-delivery within 48 hours in preeclampsia indicate placental abnormality. The levels of E and P selectins were remarkably more in Preeclampsia pregnant women and post-delivery P selectin levels were decreased in normal pregnancy and preeclampsia but are not significant. **Conclusion:** Increased protein carbonyl content and decreased antioxidant capacity indicate high levels of oxidative stress in women with preeclampsia which plays a crucial role in the endothelial dysfunction initiation and expression of preeclampsia.

INTRODUCTION:

Preeclampsia is a pregnancy-specific disease process that affects approximately 3-5% of all births.[1] Symptoms include abdominal pain, blurred vision, headache, and vomiting. It is the chief source of maternal, fetal, and neonatal mortality, especially in low socioeconomic settings and third-world countries. The complications involve seizures, pulmonary edema, or kidney failure. Preeclampsia affects the growth of the baby by uterine perfusion.[2]

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Due to hypertension, the flow of blood to the fetus is changed leading to growth restriction in the fetus followed by preterm birth either spontaneous or iatrogenic delivery.[3]

Two potentially interrelated events appear to underlie the clinical features of pre-eclampsia: placental hypoxia/ischemia and dilated maternal endothelial cell activation. Local damage in the placenta in pre-eclampsia in the form of impaired production of angiogenic and anti-angiogenic factors results in systemic inflammation, endothelial activation, systemic oxidative stress, and altered endothelial no radical dot generation. When this vascular endothelial activation and dysfunction occurs at the level of the liver, kidney, brain, and placenta, the clinical presentation of preeclampsia worsens.[4]

It is also reported that in preeclampsia, oxidative stress and inflammatory condition play a significant role in relation to involvement of NADPH oxidase, maternal, endothelial and leukocyte activation. Present study, focuses on to know the oxidative stress markers in terms of macromolecules like carbohydrates, lipids, DNA and proteins. Present study was aimed to study levels of oxidative stress markers in nonpregnancy, normal pregnancy, and preeclampsia women and their follow-up after delivery within 48 hours.

MATERIAL AND METHODS:

Present study was a prospective observational study performed in the RMC Govt. General Hospital, Kakinada, East Godavari District from August 2019 to August 2023. Ethical clearance was obtained from the Institutional Ethical Committee of RMC Govt. General Hospital. Written informed consent was obtained from all the patients. The study was conducted in the Department of Biochemistry in collaboration with the Department of Obstetrics and Gynaecology.

Inclusion criteria:

- All pregnant women after the 20th week of gestation with preeclampsia diagnosed as per the National High Blood Pressure Education Programme working group (NHBPEP) classification admitted at our Hospital, willing to participate in present study

Exclusion criteria:

- Pregnant women with History of renal disease, thyroid disorder, chronic hypertension, gestational diabetes, epilepsy, hypertensive encephalopathy, cardiovascular disease, and high risk of anaemia.

Preeclampsia was diagnosed as blood pressure of ≥ 140/90 mm of Hg noted for the first-time during pregnancy on ≥ 2 occasions at least 6 hours apart, after 20 weeks of gestation with proteinuria of ≥ 300 mg/ 24 hours or ≥ 1 + by dipstick method in a random urine sample (NHBPEP criteria).

A sample size of 135 in each group (A total of 3 groups, one group is controlled, 2 groups will be followed up thrice) was calculated. Accordingly, out of 405 subjects it will be classified as Group 1(n=135) non-hypertensive non-pregnant under the control group, Group 2 (n=135) non-hypertensive normal pregnant after 20th-week follow-up at successive intervals of 24 , 28th, 30th and 34th weeks before delivery and within 48 hours of delivery. Group 3 (n=135) pregnant with preeclampsia after 20th-week follow-up at successive intervals 24th, 28th, 30th and 34th weeks before delivery and within 48 hours of delivery depending on the progression of the disease. Groups 2 and 3 will undergo regular follow-up for sample collection.

Three ml of heparinized and 2 ml of EDTA blood was collected, heparinised blood samples were transferred into clean dry sterile centrifuge tubes, and centrifuged at 3000 rpm for 10 minutes to obtain the clear plasma. The plasma was separated and stored at -80^{o}C until analysis. The remaining packed cell is used to prepare the hemolysate and stored in triplicates at -80^{O}C to use in the future for any other investigations. Whole blood was used for DNA extraction and PCR along with the genetically obtained informed consent form.

In present study, methods for detecting oxidative stress markers were damage of lipid by malondialdehyde (determined as a thiobarbituric acid reactive substance), protein damage by protein carbonyl level & DNA damage by 8 hydroxy 2 deoxyguanosine ELISA Kit. For Endothelial Dysfunction Markers we studied Nitric oxide (determination by Griess Reagent Kit), Human Endothelial Selectin (E- selectin) (by Elisa Kit) & P-Selectin (by ELISA Kit -Abcam). For antioxidants determination as non-enzymatic antioxidants, we measured Vitamin C (L-Ascorbic acid), Vitamin E & Uric Acid. For enzyme antioxidants levels we measured Superoxide Dismutase, Glutathione Peroxidase, Glutathione Reductase, Catalase &Ferric reducing ability of plasma (FRAP).

Results were expressed as mean ± standard error mean and analyzed using a one-way ANOVA test with posthoc Bonferroni analysis to compare the values between the three groups. A paired t-test was used to compare before and after delivery status between the groups. Pearson correlation was used to analyze the correlation between the various parameters. The difference between the groups was

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determined by using Fisher's exact test. P-values of 0.05 were considered statistically significant. All statistical tests were two-tailed. The study population was tested for conformity to Hardy-Weinberg equilibrium using a web program.

RESULTS:

The study subjects were divided into 3 groups of which 135 each. Group 1 (n=135) comprising of non-pregnant as the control population; Group 2 (n= 135) non-hypertensive normal pregnant after 20th-week follow-up at successive intervals of 24th, 28th, 30th and 34 weeks before delivery and within 48 hours of delivery. Group 3 (n=135) pregnant with preeclampsia after 20th-week follow-up at successive intervals 24th, 28th, 30th and 34th weeks before delivery and within 48 hours of delivery depending on the progression of the disease.

There was no significance between age groups among both cases and controls (P- value 0.192). The systolic and diastolic blood pressures were significantly higher in PE group. A significant p-value was observed in systolic (< 0.0002) and diastolic (<0.0008) blood pressure. However, gestational age and birth weight of neonatal were significantly higher in PE women. The chi-square p value was statistically significant in gestational age, total WBC (cells/cumm), and platelet count. There is not much difference between blood parameters like hemoglobin (HB), RBC, packed cell volume (PCV), mean cell volume (MCV fL), mean cell hemoglobin (MCH pg), mean cell hemoglobin concentration (MCHC gms%), lymphocytes, monocytes, eosinophils, basophils in three groups. The random blood sugar (RBS) was also higher in PE groups and the chi-square p-value was also statistically significant.

Table 1: Baseline characteristics of study participants

Parameters	Preeclampsia (n=135)	Normal Pregnant Women (n=135)	Non-pregnant (n=135)	P-value
Age	23.25 ± 3.07	22.49±2.93	23.33±2.87	0.192
Systolic BP	146.19 ±17.99	109.68±8.22	110.47±8.43	< 0.0002
Diastolic BP	96.03 ±12.64	70.95 ± 9.10	71.74±8.07	<0.0008
Weight (Kg)	69.01 ± 18.88	61.90±9.75	62.38 ± 10.01	0.015
Height(cm)	4.90 ± 0.50	5.04 ±0.39	5.09±0.40	0.65
Gestational age	35.51± 0.50	40.20±1.07	NA	<0.0000001
HB%(gm/dL)	11.03 ± 1.83	11.01 ± 1.65	11.43±1.21	0.426
T. RBC mil/cumm	4.23 ± 0.54	4.08±0.53	4.25±0.40	0.683
T.WBC (cells/cumm	12055.56 ± 3378.527	11450.91 ± 3022.623	11467.86 ± 2678.693	<0.0004
PCV%	32.74 ± 4.47	33.56 ± 8.86	34.26±7.2714	0.138
MCV (fL)	78.33 ± 7.68	80.36± 10.63	78.37 ± 8.86	0.022
MCH pg	25.87±3.49	27.70±3.65	26.69±2.77	0.150
MCHC gms%	32.17 ± 3.15	33.78±4.71	33.96±5.03	0.139
PLT count	3398.84 ± 16277.94	2.379434 ± 0.756877	1675.083±12291.66	<0.0000001
P%	74.53 ± 8.79	70.76±8.03	71.75 ±9.40	0.022
L%	23.41 ±9.63	23.54±5.83	23.03 ± 7.64	0.156
M%	2.58 ± 4.30	2 ± 0	2 ± 0	0.662
E%	1.98± 0.12	2.38 ± 1.50	1.96±0.26	0.753
B%	0.031± 0.25	0±0	0.03±0.26	0.984
RBS	94.27 ± 21.45	87.00 ±21.87	84.26±12.83	0.006

All oxidative stress markers were significantly increased in the preeclampsia group than in the normotensive pregnant and non-pregnant women.

Table 2: Comparison of oxidative stress markers between the groups

Parameters	Non-pregnant women (mean ± SEM)	Normotensive pregnant (mean ± SEM)	Preeclampsia (mean ± SEM)
Malondialdehyde (µmoles/L)	5.09 ± 0.32	7.2 ± 0.21	17.3 ± 1.8
Protein carbonyl (nmol/L)	97.89 ± 4.3	141.28 ± 5.2	162.86 ± 17.9
DNA damage (Arbitrary units)	1.23± 2.9	15.43 ± 2.2	45.76 ± 4.2

It was found that there was a decrease in the levels of malondialdehyde, protein carbonyl, and DNA damage in patients after 48 hours of delivery but the significance was noted only in malondialdehyde parameter.

Table 3: Oxidative stress parameters in Normal pregnant women before and after delivery

Parameters	Pre-delivery	Post delivery	P value
Malondialdehyde (µmoles/ml)	7.2 ± 0.21	3.28 ± 0.01	0.000*
Protein carbonyl (nmoles/L)	141.28 ± 5.2	123.31 ± 2.5	0.154
DNA damage (Arbitrary units)	15.43 ± 2.2	14.24 ± 1.9	0.876

It was noted that there was significant reduction in malondialdehyde, protein carbonyl and DNA damage post-delivery with P<0.05.

Table 4: Oxidative stress parameters in Preeclamptic women before and after delivery

Parameters	Pre-delivery	Post delivery	P value
Malondialdehyde	17.3 ± 1.8	11.23 ±	0.001*

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(µmoles/ml)		3.2	
Protein carbonyl (nmoles/L)	162.86 ± 17.9	132.18 ± 2.4	0.001*
DNA damage (Arbitrary units)	45.76 ± 4.2	23.56± 1.3	0.001*

The nitric oxide levels were significantly reduced between normal pregnant and preeclampsia pregnant women. The levels of E selectin and P selectin were markedly increased in Preeclamptic women than in normal pregnant women.

There was a significant reduction in the non-enzyme antioxidant vitamin C levels among non-pregnant, normotensive women than preeclampsia women. Whereas Vitamin E levels were found to be higher in normotensive women than in preeclampsia. Uric acid levels were found to be increased in normotensive and preeclamptic women than in normal pregnant women.

Glutathione reductase and catalase were found to be remarkedly higher in the preeclampsia group than in normal pregnant women. Whereas superoxide dismutase and glutathione peroxidase were significantly reduced in preeclamptic women than in normal pregnant women. Total antioxidant status was significantly reduced in preeclampsia women than in normotensive pregnant women. All the results were found to have an inverse relation with oxidative stress parameters.

Table 5: Comparison of various markers between the groups

Parameters	Non-Pregnant women	Normal Pregnant women	Preeclampsia pregnant women
Endothelial markers			
Nitric oxide (µmoles/L)	7.34 ± 3.4	6.52 ± 7.6	5.91 ± 6.2
E selectin (pg/ml)	07	11	23
P selectin (ng/ml)	30	20	152
Non-enzyme antioxidants			
Vitamin C (mg/dl)	1.01 ± 0.02	0.7 ± 0.03	0.5 ± 0.04
Vitamin E (mg/L)	9.6 ± 0.43	12.8 ± 1.8	9.2 ± 0.42
Uric Acid (mg/dl)	2.4 ± 0.12	3. 83 ± 0.21	7.5 ± 0.23
Enzyme antioxidants			
Superoxide dismutase (SOD) (U/ml)	6.7 ± 0.5	12.5 ± 0.45	7.5 ± 0.73
Glutathione peroxidase (U/L)	534.6 ± 52.4	601.23 ± 32.1	435 ± 54.89
Glutathione reductase (U/L)	28.7 ± 4.2	10.23 ± 2.3	20.3 ± 6.85
Catalase (U/ml)	112.5 ± 9.2	35. 6 ± 6.5	65.5± 3.2
FRAP (µmoles/ml)	1786 ± 78.96	1021 ± 13.5	786 .4 ± 76.4

We studied correlation of oxidative stress parameters and probability values with non-enzyme antioxidants. The results in all the parameters were not statistically significant.

Table 6: Correlation results of oxidative stress parameters with non-enzyme antioxidants in preeclampsia

Parameters		Vitamin C	Vitamin E	Uric Acid
Malondialdehyde (µmoles/L)	R	-0.15	0.023	0.12
	p	0.213	0.562	0.211
Protein carbonyl (nmol/L)	r	-0.045	-0.019	0.187
	p	0.567	0.856	0. 286
DNA damage	r	0.217	0.104	0.143
	p	0.476	0.324	0.123

We studied correlation of oxidative stress parameters and probability values with enzyme antioxidants. The results in all the parameters were not statistically significant.

Table 7: Correlation of oxidative stress parameters with enzyme antioxidants in preeclampsia women

Parameters		Superoxide dismutase	Glutathione peroxidase	Glutathione reductase	Catalase	FRAP
Malondialdehyde (µmoles/L)	r	0.080	-0.019	-0.018	-0.054	0.152
	p	0.654	0.865	0.198	0.514	0.231
Protein carbonyl (nmol/L)	r	0.016	-0.150	-0.190	0.100	0.165
	p	0.864	0. 156	0.169	0.432	0.186
DNA damage	r	-0.412	-0.021	0. 065	0.083	0. 164
	p	0.003	0.882	0. 567	0.435	0. 261

We studied correlation of oxidative stress parameters and probability values with endothelial dysfunction markers. The results were not statistically significant.

Table 8: Correlation of oxidative stress parameters with endothelial dysfunction markers in preeclampsia

Parameters		Nitric oxide	E selectin	P selectin
Malondialdehyde (µmoles/L)	R	-0.189	0. 041	0. 101
	P	0.093	0. 654	0. 421
Protein carbonyl (nmol/L)	r	-0.212	0. 184	0. 043
	P	0.034	0. 165	0. 975
DNA damage	R	-0.142	0. 038	0. 087
	p	0.278	0. 654	0. 478

DISCUSSION:

This four-year study was conducted at RMC Government General Hospital, Kakinada. The study mainly focused on evaluating the oxidative stress parameters with special emphasis on endothelial dysfunction in association with hypoxia-inducible

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factor 1 α polymorphisms in preeclampsia. In view of hypertensive disorders in pregnancy, there is an increased incidence of maternal and fetal mortality in India.

The pathogenesis of preeclampsia is still not clear which has impeded the development of clinically useful diagnostic biomarkers, particularly those of evolving preeclampsia. Several researchers have proposed that preeclampsia is developed because of cell injury which produces oxidative species that results in uncontrolled lipid peroxidation. The cause of impairment of nitric oxide is due to endothelial dysfunction which reduces blood flow and poor placentation. Oxidative stress is mainly due to an imbalance between the production of free radicals and antioxidants that leads to a cause of potential damage to the cell as in normal conditions free radicals and antioxidants are in balance.[6]

The studies proved that oxidative stress is increased in preeclampsia which results in enhanced production of lipid peroxidases. Malondialdehyde is an indicator of lipid peroxidation.[7] This is highly increased in various disease conditions and in particular pregnancy-induced hypertension. As Malondialdehyde is a specific marker of preeclampsia, various studies have reported its increased levels in preeclampsia subjects. In the present study, the malondialdehyde levels were significantly increased in preeclamptic women than in normotensive pregnant and nonpregnant.

The levels of MDA were also compared between pre- and post-delivery in normal and preeclamptic pregnant women. The levels of MDA were reduced post-delivery in both normal pregnant and preeclamptic women which is significant at $P<0.05$. The significant decrease of MDA levels after delivery in normotensive pregnant and preeclampsia indicated a downtrend of MDA values after placental removal within 48 hours.

Protein carbonyl is an indicator of the damage of protein in the biological system. Reactive oxygen species oxidize amino acid residues such as glutamate, histidine, and tryptophan in proteins and form end products with carbonyl groups. In the present study, the content of protein carbonyl was increased in normotensive pregnant and preeclamptic women than in non-pregnant. The levels of protein content were compared between pre and post-delivery in normal and preeclamptic pregnant women. In normal pregnant women, though the protein carbonyl content was decreased in a preeclamptic group than in non-pregnant and normotensive pregnant, the results are not statistically significant. Whereas the same observation was observed in preeclamptic women and the results are statistically significant. These results indicate that decreased protein damage maker indicates revocable changes after delivery with preeclampsia. These results are in coordination with the results of other previous studies.

DNA damage can be seen in preeclampsia due to increased oxidative stress and oxygen free radicals. Many pathological conditions like cancers, and cardiovascular and neurodegenerative diseases were associated with DNA damage.[8] In the present study, DNA damage was noted in preeclamptic women more than in normotensive and non-pregnant women. The amount of DNA damage in preeclamptic women post-delivery is reduced with statistical significance. These observations are inconsistent with the Nasi Hilali et al where pregnancies were found complicated by mild preeclampsia with increased levels of mononuclear DNA damage.[9]

Nitric oxide is synthesized from L-arginine under the action of Nitric Oxide Synthetase (NOS) which catalyzes L-arginine oxidation into NO and L-citrulline.[10] Reports have suggested that reduced production of vasodilator agent nitric oxide could lead to preeclampsia. These reports are coordinated with our study observations where NO is reduced in normotensive and preeclamptic pregnant women than in nonpregnant women. The levels of NO were also measured in pre and post-delivery in normal pregnant and preeclamptic women and the results revealed decreased NO but are not statistically significant.

Selectins are type 1 transmembrane glycoproteins. E- selectin is expressed on leukocytes and endothelial cells with the help of interleukin-1 and tumor necrosis factor-alpha.[11] P selectin is a cellular adhesion molecule that mediates the interaction of activated endothelial cells with leukocytes. Both these have key players in implantation and are in charge of immune acceptance along with trophoblast migration in spiral arterioles. The endothelium has crucial functions such as organizing vascular tone and vascular permeability and balancing coagulation and fibrinolysis. As PE is associated with platelet activation and endothelial dysfunction, the amount of E selectin and P selectin is thought to play an important role in the pathophysiology of PE. In the present study, the levels of E selectin and P selectin were higher in Preeclampsia pregnant than in normotensive and non-pregnant women. Post-delivery these high levels were decreased in both the groups but are not significant.

Superoxide dismutase (SOD) stands as the first line of defence against free radical scavenging.[12] SOD was significantly reduced in preeclamptic pregnant women than in normal pregnant women whereas the

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levels of SOD were significantly increased in normotensive pregnant when compared to non-pregnant. With respect to pre and post-delivery, SOD was reduced in normotensive and preeclamptic women post-delivery significantly. Studies by Bakacak and his coinvestigators proved that reduced SOD activity may be due to increased Cu/Zn ratio. Another study reported that decreased SOD activity might be due to overproduction of lipid peroxidation in preeclampsia.[13]

Glutathione is a tripeptide produced by human cells. Glutathione plays an important role in antioxidant defence, nutrient metabolism, and regulation of cellular events.[91] Low levels of glutathione in pre-eclampsia are reported in the literature which is consistent with the present study results. Glutathione peroxidase was reduced in preeclamptic women than in normotensive pregnant women. Glutathione reductase was found to be increased in preeclamptic women than in normotensive pregnant women. With respect to pre and post-delivery status in normotensive and preeclamptic women; the levels of glutathione peroxidase and reductase were reduced which is statistically significant in normotensive pregnant women and glutathione peroxidase was increased post-delivery followed by a decrease of glutathione reductase.

Catalase functions by cleaving hydrogen peroxide into water and oxygen and protects the cell from oxidative damage.[14] Catalase enzyme activity was found to be increased in preeclamptic women than in normotensive and normal pregnant women. The catalase activity post-delivery was increased post-delivery than pre-delivery in preeclampsia and decreased in normal pregnant women. Ferric reducing ability of plasma (FRAP) is a novel method for assessing anti-oxidant power. In the present study, FRAP was reduced in post-delivery in normotensive women and increased in preeclamptic pregnant women.

CONCLUSION:

MDA levels were elevated in preeclamptic pregnant women and therefore, increased lipid peroxidation is considered to be a causative factor for preeclampsia. Increased protein carbonyl content and decreased antioxidant capacity indicate high levels of oxidative stress in women with preeclampsia which plays a crucial role in the endothelial dysfunction initiation and expression of preeclampsia. Therefore, intake of adjuvant antioxidants supplementation can minimize the progression of preeclampsia.

CONFLICT OF INTEREST: None to declare

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