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A Comparative Study of Oxidative Stress Indices and Antioxidant Status in Preeclampsia with Normal Pregnancy in a Tertiary Rural Medical College

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Abstract: *Study was carried out at Malda Medical college, Malda amongst 40 patients of pre- eclampsia to find out role of different oxidative radicals & antioxidants and compared with the levels in same number of normal pregnant mothers. Serum uric acid, MDA(malondialdehyde), thiol status and catalase levels were analyzed. Serum uric acid level was found significantly increased in cases of pre-eclampsia in comparison to normal pregnancy. The level of total thiol which is protective against ROS (reactive oxidative stress) molecules was found to be significantly lowered in the cases pre-eclampsia in comparison to control. No significant changes were observed in catalase levels between the two groups. Serum Malondialdehyde (MDA) were found significantly raised in pre-eclampsia patients than in controls. The value of MDA was 5.363µmol/ in pre-eclampsia which showed statistically significant rise in compared to control. In severe Pre-eclampsia the mean value of MDA was further raised to 5.24µmol/l. Pre-eclamptic patients therefore are associated with increased level of oxidative stress factors and decreased level of protective antioxidants which is probably responsible for placental dysfunctions present in these pregnancies.*

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I. Introduction

Pre-eclampsia is an important and potentially dangerous complications of pregnancy. Pre- eclampsia is diagnosed by new development (usually after 20 weeks of gestational age) of hypertension (usually >140/90 mm of Hg), significant proteinuria and remission of this signs after delivery(1). Abnormal placentation is clearly involved in the genesis of pre-eclampsia. Lipid peroxides are formed when poly unsaturated fatty acids interact with free radical . Reactive oxygen species such as super oxides arise from various sources like mitochondria. Super oxide is often the first radical product(5). By itself it is not extremely reactive but in the presence of transaction metals such as iron (FE++, FE+++), super oxide can react directly with H2O2 and lipid peroxide is capable of initiating the chain reaction of lipid peroxidation. Antioxidants oppose the toxic action of lipid peroxides and oxygen radicals and also limit the amount of lipid peroxides formed. Antioxidants are derived from endogenous synthesis (e.g Vitamin C, Vitamin E, Beta-Carotene etc) . The major antioxidant defence of human plasma is to bind transition metal ions, such as iron and copper in such forms that will not stimulate free radical reactions.

This binding is achieved by antioxidants such as transferrin, lactoferrin, ceruloplasmin, albumin and uric acid. Vitamin C is the most important scavenger of water soluble radicals and it is a first line defence. Under condition of oxidative stress, it is the first antioxidant to be consumed. Vitamin C also recycles the vitamin E radical by reducing it. Vitamin E (tocopherol) scavenges lipid soluble peroxide Radicals. Intracellular antioxidant superoxide dismutase (SOD) is the major intracellular antioxidant enzyme that inactivates superoxide. It reduces superoxide radicals to hydrogen peroxide and oxygen. In normal pregnancy women have an increase in oxidative stress & lipid peroxidation when compared with non pregnant women(6). They have also an increase in oxidative stress parameters. Various antioxidants such as vitamin E, ceruloplasmin and erythrocyte thiols and net antioxidant activity (which indicate the iron binding capacity) are increased in the maternal blood in normally pregnant women. Several of the antioxidants increase progressively with advancing gestation and serum iron concentration progressively decreases(7). Thus there is gradual favouring of antioxidant activity over per oxidation as normal pregnancy advances. Therefore in normal pregnancy there is a sufficient increase in anti oxidants to offset the increases in per oxidation. In pre-eclamptic pregnancy there is an imbalance characterized by increase in lipid peroxides and decrease in antioxidant. Lipid peroxides are even further increased in pre-eclampsia as compared with normal pregnancy(8).

In pre-eclamptic woman the levels of vitamin C, Vitamin E, vitamin A, Glutathion and net antioxidant activity (which is iron binding capacity) in blood circulation all are lower than normal(9). In the placenta of pre-eclampsia there is a substantial decrease in the tissue levels of vitamin E and in the activities of SOD and glutathione peroxidase with deficiency of these antioxidants. These women exhibit increased oxidative stress and lipid per oxidation. Since the protective effect of antioxidants is lost due to decline in their levels, this allow oxidative stress factors to worsen in pre eclampsia. Thus the concentration of free radicals and lipid per oxidation products such as malondialdehyde (MDA), lipid peroxides increase in pre-eclampsia. Pregnant woman treated with antioxidants lower the level of MDA due to decreased production of antioxidats. On the basis of the literature support this study evaluates the different oxidative stress parameters in pre-eclamptic pregnancies in comparison with normal pregnancy.

To find out role of different oxidative radicals and antioxidants in control normal antenatal mothers and in patients of pre-eclampsia from the period of 32 weeks of pregnancy to term.

II. Materials And Methods:

All the patients in this study were selected from antenatal clinic in Malda Medical college and Hospital, Malda. The study was carried out from 1st may 2016 to 31st august 2017..

The study included 40 normal mothers as controls , 40 patients of pre-eclampsia. All these patients between the control and cases (pre-eclampsia) were recruited from the similar age group between 18-35 years and similar parity groups. The patients with normal blood pressure and without any medical and obstetrical complications were selected as control.

Pre-eclampsia was diagnosed by :

a) Blood pressure more than or equal to 140/90 mm of Hg after 20 weeks of pregnancy with no previous history of hypertension.
b) Proteinuria more than 300mg per 24hr urine specimen with no previous history of proteinuria.

A proper obstetric history (present and past) , menstrual history, family history, personal history, any medical and surgical history were taken in full details. Physical examination including height, weight, BMI, anemia, pulse, blood pressure, edema, eye examination was done.

Obstetrical examination:

I. Fundal height of uterus was assessed at each antenatal visit by serial measurement of uterine fundus from upper border of symphysis pubis with the help of measuring tape in centimeter.
II. Obstetrical grips like fundal grip, lateral grip, first and second pelvic grip examination were done to assess the presentation, position , presenting part , attitude, engagement, liquor volume and fetal heart sound in every patients.
III. Pelvic examination was done when indicated for assessment of obstetric management.
IV. After delivery the fetal outcome was assessed by noting fetal birth weight, Apgar score and perinatal morbidity and mortality.

Investigations likely to be carried out are :

i. Routine investigations of blood for hemoglobin, blood group and Rh factors, VDRL, Post- prandial blood glucose, hepatitis B surface antigen & routine urine examination were done.
ii. Serial quantitive assessment for 24 hour urine for protein estimation was done to diagnose and monitor pre eclampsia patients.
iii. Serial investigation of blood for platelet count, liver enzymes, urea, creatinine and uric acid was done to assess and monitor the severity of pre-eclamptic disease.
iv. Ophthalmological examination was done in all pre-eclamptic patients for assessment of retinopathy of varying severity.
v. Ultrasonographic-al examination :

Ultrasound biometry of the foetus was done to assess the fetal growth in all patients of my study group. Routine dating and anomaly scan was done at 18 to 20 weeks of gestation . The measurements used were Biparietal diameter(BPD), Head Circumference (HC), Abdominal Circumference (AC), and Femur Length (FL). The ratio of HC/AC between 20 to 36 weeks of gestation was measured to diagnose intra uterine growth restricted babies. Amniotic fluid volume was measured to assess the fetal well being. Amniotic fluid index(AFI) of 5 centimeter or a single vertical pool of 2 centimeter was considered a cut off normal level.

Doppler Velocimetry : Uterine vessels, umbilical artery and middle cerebral artery was examined for systolic(S) and diastolic(D) blood flow (S/D ratio), Pulsatility index (PI) , Resistance index (RI) and cerebro placental ratio (CPR) serially as and when required in all patients in my study. The high risk women by history (previous pre-eclampsia or pre-existing hypertension) shows abnormal uterine Doppler studies at 20 & 24 weeks of gestation. The uterine artery Doppler study has a positive predictive value of 20%, on the basis of the abnormal Doppler study (diastolic notch in uterine artery).

The following oxidative radicals and antioxidants are measured in the department of Biochemistry department of Malda Medical college, Malda and compared between controls and cases of pre-eclampsia:

a) Estimation of Uric Acid - antioxidant
b) Estimation of Total Thiol - antioxidant
c) Estimation of Catalase - antioxidant
d) Estimation of Serum Thiobarbituric Acid Reactive Substances (TBARS), popularly known as Malondialedehyde (MDA), a lipid peroxidation product oxidative stress factor. A critical assessment to find out the positive correlation between the level of oxidative stress factors and antioxidants are analyzed with the severe form of pre-eclampsia.

III. Results:

Total 80 patients were studied and analyzed in reference to various clinical, biochemical and radiological parameters. The main objective of this study was to estimate and analyses the level of oxidative stress factors and anti oxidants between the pre-eclampsia and normal pregnant mothers and severity of the disease as the pregnancy advances. The level of oxidative and antioxidants were estimated twice first in between 32-36 weeks and later in between 36-40 weeks pregnancy.

Table 1: Age wise distribution of control and pre-eclampsia

AGE GROUP (in yrs)	**CONTROL**		**PRE- ECLAMPSIA**	
	NO	%	NO	%
Upto 19 YearS	6	15	2	5
20 – 25 Years	18	45	12	30
26-29 Years	11	27.5	11	27.5
30 and above	5	12.5	15	37.5
TOTAL	40	100	40	100

From the TABLE 1 it is seen that the majority of pre-eclampsia cases belonged to the age group of 30 years and above (37.5%).

TABLE 2 : Parity wise distribution of control and pre-eclampsia

PARITY	**CONTROL**		**PRE-ECLAMPSIA**	
	NO	%	NO	%
P0+0	23	57.5	21	52.5
P1+0	16	40	12	30
P2 OR ABOVE	1	2.5	7	17.5
TOTAL	40	100	40	100

TABLE 2 analyses parity distribution. In the pre-eclampsia group 21 (52.5%) women were primi-gravid and 7 (17.5%) women were multiparous.

TABLE 3 : Distribution of socio economic status

MONTHLY INCOME	**CONTROL**		**PRE-ECLAMPSIA**	
	NO	%	NO	%
LESS THAN RS.500	0	0	0	0
RS. 500/ TO 3000	29	72.5	35	87.5
MORE THAN RS. 3000	11	27.5	5	12.5
TOTAL	40	100	40	100

TABLE 3 Shows distribution of patients according to socio economic status. The socio economic status amongst the patients was distributed in two groups as per family income. Most of the patients belonged to a family with monthly income of RS.500/ - 3000/- per month. 29 (47.5%) patients in the control group & 35(87.5%) pre-eclamptic mothers belong to the middle class. Only 5(12.5%) patients of pre-eclampsia came from good socio economic class.

TABLE 4 : BMI (WT IN KG/ HEIGHT IN M2)

BMI	**CONTROL**		**PRE- ECLAMPSIA**	
	NO	%	NO	%
<19.9	5	12	1	2.5
20 to 24.9	30	75	28	70
25 to 29.9	5	12.5	8	20
MORE THAN 30	0	0	3	7.5
TOTAL	40	100	40	100

TABLE 4 shows 75% of control, 70% each of pre-eclamptic cases had a BMI in the range of 20- 24.9. 20% of pre-eclamptic cases had BMI of 25-29.9. Only 7.5% of pre eclampsia cases had BMI more than 30.

TABLE 5 : Diagnostic criteria for pre-eclampsia cases according to extent of elevated blood pressure and proteinuria.

BP IN MM OF HG	**140/90-160/110 MM OF HG**	**30**	**75%**
	>160/110	10	25%
TOTAL		40	100%
PROTEINURIA	300MG-1GM	36	90%
	>1GM-3GM	2	5%
	>3GM	2	5%
TOTAL		40	100%

Table 5 shows in pre-eclamptic mothers the majority of the patents 75% cases the blood pressure was mildly elevated and 25% cases the blood pressure was severe. Amongst the 40 pre-eclamptic patents, in 90% cases the levels of 24 hours proteinuria were between 300mg - 1gm whereas severe proteinuria more than 3gm were found only in 5% cases.

TABLE 6 : Serum uric acid level in control and pre- eclampsia mothers.

GROUP	NO OF CASES	URIC ACID IN MG/DL MEAN
CONTROL	40	4.588
PRE ECLAMPSIA	40	5.045

P<0.05

TABLE 6 shows that the critical difference is 0.22 after observation of two groups. Here the mean uric acid value in control group is 4.5888 mg/dl. In pre-eclampsia the value is much higher 5.045 and is statistically significant (p<0.05).

Uric acid value from the table clearly indicates higher range in cases of pre-eclampsia with a statistically significant difference from the control.

TABLE 7 : Serum catalase levels in control, pre-eclampsia cases.

GROUP	NO OF CASES	CATALASE IN KU/L MEAN
CONTROL	40	80.125
PRE ECLAMPSIA	40	80.6

TABLE 7 shows there is no difference between control and pre-eclampsia group.

TABLE 8 : Serum total thiol in control, pre eclampsia mothers

GROUP	NO OF CASES	SERUM TOTAL THIOL IN UMOL/L MEAN
CONTROL	40	232.65
PRE ECLAMPSIA	40	92.625

P<0.05

TABLE 8 shows that there is significant difference between control and pre-eclampsia group and which is statistically significant (p<0.05).

TABLE 9: Serum TBARS(thiobarbituric acid, MDA) status in control and pre eclampsia mothers

GROUP	NO OF CASES	TABRS IN µMOL/L (Mean)
CONTROL	40	2.138
PRE ECLAMPSIA	40	3.857

P<0.05

The TABLE 9 shows the mean value shows there is a statistically significant difference of serum TBARS value in pre-eclampsia group with control. In pre-eclampsia the mean value is 3.85µmol/l but in control group the mean value is 2.138. The TBARS value shows elevated levels in pre eclampsia. This reflects to existence of oxidative stressful situation in these different clinical setting.

TABLE 10 : Mean values of variables under study

BIOCHEMICAL PARAMETERS	PRE ECLAMPSIA GROUP N=40	CONTROL GROUP N=40	P VALUE
SERUM URIC ACID MG/DL	5.045	4.588	<0.05
SERUM CATALASE KU/L	80.6	80.125	<0.05
SERUM TOTAL THIOL STATUS µMOL/l	92.625	232.65	<0.05
SERUM TBARS(MDA) µMOL/l	3.857	2.138	<0.05

TABLE 11 : Correlation of stress marker and antioxidant levels in severe Pre-eclampsia patients

PATIENTS		URIC ACID	CATALASE	THIOL	MDA
CONTROL		4.588	80.125	232.65	2.138
PRE ECLAMPSIA	SEVERE	5.67	78.07	100.6	5.24
	TOTAL	5.045	80.6	92.625	3.857

TABLE 11 analyses the level of MD (mean difference) values which was found further increased amongst the 13 severe pre-eclampsia patients. Uric acid level was increased amongst the severe pre- eclampsia patients.

TABLE 12: Birth wt related distribution amongst control and pre-eclampsia patients

BIRTH-WT	CONTROL		PRE- ECLAMPSIA	
	NO	%	NO	%
<2KG	0	0	6	15
2-2.5KG	0	0	16	40
>2.5-3KG	26	65	12	30
>3KG	14	35	6	15

Low birth weight (<2.5kg) was observed amongst 22(55%) of pre-eclamptic babies. All babies of control mothers weighed >2.5kg.

TABLE 13 : Maternal outcome and perinatal outcome

NO. OF BABIES	CONTROL		PRE-ECLAMPSIA	
	NO	%	NO	%
STILLBORN	0	0	2	5
EARLY NEONATAL DEATHS	0	0	1	2.5
LIVING	40	100	37	92.5

TABLE 13 analyses the perinatal outcome of all patients in the study group. There were 3 (7.5%) perinatal deaths in the pre-eclampsia group out of which 2 (5%) were stillborn and 1 (2.5%) died of neonatal sepsis.

IV. Discussion

The study was undertaken to assess the extent of oxidative stress in pregnancies complicated with pre-eclampsia at different gestational period in patients attending OPD of Gynaecology and Obstetrics, Malda Medical college, Malda during the study period of 2016-2017. Out of the total 80 cases investigated equal number of control and pre-eclampsia affected mothers were studied. Apart from epidemiological, clinical and sonological assessment we studied the biochemical markers of oxidative stress events and antioxidant defensive status of all this subjects.

In this study 57.5% of pre-eclamptic mothers were in the age group of 20-30 years whereas 37.5% were elderly (table 1). 75% of women in pre eclampsia had blood pressure of 140/90 to 160/110mm of Hg(table 5). All patients of pre eclampsia in this study were diagnosed between 32-36 weeks of gestation and followed up twice with biochemical analysis of oxidative stress factors and antioxidants.

Table 6 shows serum uric acid level being significantly increased in cases of pre-eclampsia. The rising uric acid in pre-eclampsia is considered to reflect impaired renal function.

Table 7 shows that there is no significant difference in the values of catalase between the two study groups in pregnancy, pointing to no significant importance of this parameter im my present study.

Thiol represents (-SH) group containing molecules mainly cysteine, methionine, peptides, polypeptides, proteins. Albumin in plasma is such an example which contains -SH group due to its amino acid content. Thiols protect our body from toxic ROS molecules by removing them in the form of water which is non toxic. The study of total thiol (Table 8) indicates to the significant lowering in the affected cases with pre-eclampsia compared against control. The critical difference values and the p values are found to be statistically significant.

The reactions are catalyzed by peroxidases and catalases. A positive co-relation between the estimated thiol values were observed in pre-eclampsia subjects where the values are significantly poor than the normal pregnancy control subjects and this reflects the stage of failure of the body to fight against ROS molecules in this complicated setting of pregnancy.

Another parameter which is known as total antioxidant status (TAS) that reflects to the antioxidant machinery potency to fight against ROS in vivo is also found to be significantly poor in similar condition studied by various other investigators at different time(10).

Biochemical investigation revealed as a whole a state of gross oxidative stress as indicated by TBARS (thiobarbiturate) levels in serum, especially in pre-eclampsia compared with control pregnant cases (table 9).

Significantly raised levels of oxidative stress makers (malondialdehyde, glutathione peroxidase and super oxide dismutase) and significantly reduced levels of antioxidant lycopene and vitamin C in women with varying grades of pre-eclampsia in contrast to normal pregnant women and the alteration were higher in more severe disease (13).

TBARS value popularly named as MDA, shows elevated levels in pre-eclamptic mother and the value increases as the gestation advances towards term. The mean value of MDA was 3.857µmol/l in pre-eclampsia (table 10) which showed statistically significant rise in compared to control. In severe pre-eclampsia the mean value of MDA was further raised to 5.24 µmol/l. This reflects to existence of oxidative stress situation in these high risk pregnancies. Disturbance of balance between oxidative process and antioxidant defence causes the oxidative stress to act unopposed which can affect both foetus as well as the mothers these pregnancies.

Table 12 analyses the birth-wt. of 80 babies in this study. 55% of pre-eclamptic babies are low birth weight. Only 7.5% pre-eclamptic babies had perinatal deaths (table 13). Similar observation was made by where perinatal mortality was 5 to 30 times greater amongst the infants with birth-wt less than 2.5kg(14).

Pre-eclamptic pregnancies are associated with gross imbalance of increased oxidative stress and decreased antioxidant which is most likely one of the reasons of placental dysfunctions. This has raised the possibility of oxidative stress markers to have an early prediction value for diagnosis of these conditions and probable pharmacological intervention with antioxidants to improve the pregnancy states which however are yet to make a major breakthrough.

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