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A Study To Compare The Parameters Of Oxidative Stress Indices And Antioxidant Status In Intra- Uterine Growth Restriction With Normal Pregnancy In A Rural Medical College & Hospital, West Bengal, India.

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Abstract: *Study was carried out at Malda Medical college & Hospital, Malda amongst 40 patients of IUGR (Intrauterine Growth Restriction) patients to find out the role of different oxidative radicals and antioxidants and compared with the levels in same number of normal pregnancy patients (i.e. control group). Serum uric acid, MDA (Malondialdehyde), thiol status and catalase levels were analyzed. The level of total thiol which is protective against ROS (reactive oxidative stress), molecules were found to be significantly lowered in the cases with IUGR in comparison to control (i.e. normal pregnancy). Significant changes were observed in catalase levels between the two groups. There is no significant difference in uric acid level between control and IUGR mothers. Serum Malondialdehyde (MDA) were found significantly raised in IUGR patients than in controls. The mean value of MDA was raised to 6.3µmol/l in complicated IUGR. Pregnancies with IUGR 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.*

Keywords: *Thiobarbiturate, Malondialdehyde, Intrauterine growth restriction.*

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I. Introduction:

Intrauterine growth restriction is one important and potentially dangerous complication of pregnancy. Abnormal placentation is clearly involved in the genesis of fetal intrauterine growth restriction (IUGR). Lipid peroxides are formed when poly unsaturated fatty acids interact with free radical. Reactive oxygen species (ROS) 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. The major antioxidant defense 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, lactoflavin, ceruloplasmin, albumin and uric acid. Vitamin c is the most important scavenger of water-soluble radicals and it is a `first line defense. 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 antioxidants 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 they have also an increase in oxidative stress parameters. Various antioxidants such as Vitamin E, Ceruloplasmin and Erythrocyte Thiols and net antioxidant activity 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. Thus, there is gradual favouring of antioxidant activity over peroxidation as normal pregnancy advances. Therefore, in normal pregnancy there is a sufficient increase in anti-oxidants to offset the increases in peroxidation. In IUGR pregnancy there is an imbalance characterized by increase in lipid peroxides and decrease in antioxidant. Lipid peroxides are even further increased in IUGR pregnancy as compared with normal pregnancy.

In the placenta of IUGR pregnancy 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 peroxidation. Since the protective effect of antioxidants is lost due to decline in their levels, this allow oxidative stress factors to worsen in IUGR. Thus, the concentration of free radicals on the basis of the literature support this study. It evaluates the different oxidative stress parameters in IUGR pregnancies in comparison with normal pregnancy to find out role of different oxidative radicals and antioxidants in control normal antenatal mothers and intra uterine growth restriction 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 and 40 patients with intrauterine growth restriction pregnancies. All these patients amongst the control and cases 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.

IUGR was diagnosed by:

a) Clinical: Symphysio-fundal height deficit of 4 cm or more by serial measurement with the help of measuring tape and expressed in cm.

b) Serial ultrasonography to confirm biometric growth deficit, presence of oligohydramnios and Doppler velocimetric assessment of fetal wellbeing.

c) Birth weight of the babies <2.5 kg at term.

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, anaemia, pulse, blood pressure, edema, eye examination were 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 (cm). Period of pregnancy was accurately assessed to confirm the exact period of gestation a fundal height which lags by 4 centimeter or more than estimated period of gestation suggests growth restriction.

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 patient.

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 was done.

ii. Serial investigation of blood for platelet count, liver enzymes, urea, creatinine and uric acid were done in IUGR patients.

iii. Ultrasonographical examination: Ultrasound biometry of the fetus was done to assess the fetal growth in all patients of my study group. Routine dating and anomaly scan were done at 18 to 22 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 wellbeing. 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 and diastolic blood flow S/D (systolic/diastolic) 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 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 (diastolic notch in uterine artery).

The following oxidative radicals and antioxidants are measured in the department of biochemistry department of Malda Medical college & Hospital and compared between controls and cases of intrauterine growth restriction

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 malondialdehyde (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 intra uterine growth restriction pregnancies.

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 analyze the level of oxidative stress factors and anti-oxidants amongst IUGR patients and its correlations with 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.
Majority of the IUGR pregnancy in this study occurred in between the age group 20-25 years (60%).

Table 1: Age wise distribution of control and IUGR mothers

AGE GROUP (in yrs)	**CONTROL**		**IUGR**	
	NO	%	NO	%
UPTO 19 YEARS	6	15	4	10
20 - 25	18	45	24	60
26-29	11	27.5	9	22.5
30 AND ABOVE	5	12.5	3	7.5
TOTAL	40	100	40	100

From the TABLE 1, it is seen that the majority (60%) of the IUGR pregnancy in this study occurred in between the age group 20-25 years.

TABLE 2: Parity wise distribution of control and IUGR pregnancy

PARITY	**CONTROL**		**IUGR**	
	NO	%	NO	%
P0+0	23	57.5	28	70
P1+0	16	40	10	25
P2 OR ABOVE	1	2.5	2	5
TOTAL	40	100	40	100

TABLE 2 analyses parity distribution. In case of pregnancies with intra uterine growth restriction it is observed that 28 (70%) mothers were primi-gravida and only 5% of mothers caring IUGR babies were multiparous.

TABLE 3: Distribution of socio-economic status

MONTHLY INCOME	**CONTROL**		**IUGR**	
	NO	%	NO	%
LESS THAN RS.500	0	0	0	0
RS. 500/ TO 3000	29	72.5	31	77.5
MORE THAN RS. 3000	11	27.5	9	22.5
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 three groups as per family income. Most of the patients belonged to a family with monthly income of Rs.500/ - Rs.3000/ - per month. 29 (72.5%) patients in the control group, and 31 (77.5%) mothers in the IUGR group belonged to the middle class. Only 11 (27.5%) patients of control and 9 (22.5%) cases of IUGR patients came from good socio-economic class.

TABLE 4: BMI (WT IN KG/ HEIGHT IN M^2)

BMI	**CONTROL**		**IUGR**	
	NO	%	NO	%
<19.9	5	12.5	9	22.5
20 to 24.9	30	75	28	70
25 to 29.9	5	12.5	1	2.5
MORE THAN 30	0	0	2	5
TOTAL	40	100	40	100

TABLE 4 shows 75% of control and 70% IUGR cases had a BMI in the range of 20- 24.9. 2.5% IUGR cases had BMI of 25-29.9. But 22.5% of IUGR group had BMI<19.9 which proves the fact that there is more prevalence of underweight in IUGR group.

TABLE 5: Diagnostic criteria of IUGR mothers

SYMPHYSIO-FUNDAL HEIGHT DEFICIT IN CM	4-6 CM		>6CM	
	NO	%	NO	%
	33	82.5	7	17.5
AMNITIC FLUID INDEX IN CM (AFI) ON USG	<5CM		>5CM	
	NO	%	NO	%
	7	17.5	33	82.5
CEREBROPLACENTAL RATIO ON DOPPLER STUDY	<1		>1	
	NO	%	NO	%
	7	17.5	33	82.5

TABLE 5 shows that deficit of symphysio-fundal height of 4-6 cms were noticed in 82.5 % of IUGR cases and only in 17.5% cases the deficit was found greater than 6 cm. Following the USG criteria for the diagnosis of IUGR it has been found that in 82.5% cases the liquor volume was more than 5 to 10 cms and only in 17.5% cases AFI was below 5 cm.

TABLE 6: Serum uric acid level in control and IUGR mothers.

GROUP	NO OF CASES	URIC ACID IN MG/DL MEAN
CONTROL	40	4.588
IUGR	40	4.73

TABLE 6 shows that here the mean uric acid value in control group is 4.5888 mg/dl and in IUGR cases the value is 4.73. In comparison the IUGR mothers did not exhibit any significant statistical difference from control mothers.

TABLE 7: Serum catalase levels in control and IUGR mothers

GROUP	NO OF CASES	CATALASE IN KU/L (MEAN)
CONTROL	40	80.125
IUGR	40	73.175

TABLE 7: There is a little significant difference between control and IUGR group and which is not statistically significant.

TABLE 8: Serum total Thiol in control and IUGR mothers

GROUP	No of cases	Serum total Thiol in µmol/lit mean
CONTROL	40	232.65
IUGR	40	108.35

TABLE 8 shows that there is significant difference between control and IUGR group which is statistically significant ($p<0.05$). The study of total Thiol indicates significant lowering in affected cases with IUGR compared against control.

TABLE 9: Serum TBARS (Thiobarbituric Acid Reactive Substances or MDA) status in control and IUGR mothers.

GROUP	**NO OF CASES**	**TABRS IN µMOL/L (MEAN)**
CONTROL	40	2.138
IUGR	40	5.363

The TABLE 9 shows the mean value shows there is a statistically significant difference of serum TBARS value in IUGR group with control. The mean value in IUGR group is 5.363.but in control group the mean value is 2.138. Here the IUGR group shows much higher levels of TBARS in serum.

The TBARS value shows elevated levels in IUGR mothers but the value is more elevated in IUGR as the pregnancy advances towards term. This reflects to existence of oxidative stressful situation in these different clinical setting.

TABLE 10: Mean values of variables under study

BIOCHEMICAL PARAMETERS	IUGR GROUP N=40	CONTROL GROUP N=40	CRITICAL DIFFERENCE	P VALUE
SERUM URIC ACID MG/DL	4.73	4.58	0.22	<0.05
SERUM CATALASE KU/L	71.175	80.125	7.04	<0.05
SERUM TOTAL THIOL STATUS µMOL/l	108.35	232.65	34.06	<0.05
SERUM TBARS(MDA) µMOL/l	5.363	2.138	0.874	<0.05

TABLE 11: Correlation of stress marker and antioxidant levels in control and IUGR patients

PATIENTS		URIC ACID	CATALASE	THIOL	MDA
CONTROL		4.568	80.125	232.65	2.138
IUGR	SEVERE	4.94	82.3	100.01	6.3
	TOTAL	4.73	73.175	108.35	5.363

TABLE 11 analyses the level of MD (mean difference) values which was found further increased amongst the 7 critical IUGR mothers with severe oligohydramnios. Similar change was found in thiol levels which was very much reduced in complicated IUGR pregnancies.

TABLE 12: Birth wt. related distribution between control and IUGR patients

BIRTH-WT	CONTROL		IUGR	
	NO	%	NO	%
<2KG	0	0	7	17.5
2-2.5KG	0	0	25	62.5
>2.5-3KG	26	65	8	20
>3KG	14	35	0	0

Table 12 shows that low birth weight (<2.5kg) was observed in 32 (80%) of IUGR babies. IUGR babies with oligohydramnios and uteroplacental insufficiency however weighed >2.5 kg. All babies of control mothers weighed >2.5kg.

TABLE 13: Maternal outcome and perinatal outcome

NO. OF BABIES	CONTROL		IUGR	
	NO	%	NO	%
STILLBORN	0	0	3	7.5
EARLY NEONATAL DEATHS	0	0	1	2.5
LIVING	40	100	36	90

TABLE 13 analyses the perinatal outcome of all patients in the study group. Amongst the IUGR babies 3 (7.5%) babies were stillborn and 1 (2.5%) baby died of multiple congenital disorder.

IV. Discussion:

The study was undertaken to assess the extent of oxidative stress in pregnancies complicated with IUGR at different gestational period in patients attending OPD of Gynaecology and Obstetrics Department, Malda Medical College and Hospital, Malda during the study period of May'2016 – August'2017. Out of the total 80 cases investigated, equal number of control and IUGR 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 these subjects.

In this present study, IUGR pregnancies were also diagnosed around 32 weeks of pregnancy by serial symphysio-fundal height and sonological assessment. Oxidative and antioxidant parameters were estimated twice and compared with control in all of them.

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 nontoxic. The study of total Thiol (Table 7) indicates to the significant lowering in the affected cases with IUGR 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 correlation between the estimated thiol values were observed in IUGR 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.

Biochemical investigation revealed as a whole a state of gross oxidative stress as indicated by TBARS (thiobarbiturate) levels in serum in IUGR compared with control pregnant cases. However, in IUGR affected mothers the levels were in higher range than in normal pregnant mothers in this study (table 9).

Oxidative stress is a damaging event resulting from multiple etiology in pregnancy. We observed the outcome of pregnancy in terms of the fetal well-being is poor in IUGR cases where the TBARS values were higher.

In one similar series, malondialdehyde (MDA) concentration measured in the group with normal pregnancy without symptoms of IUGR and intrauterine hypoxia was found significantly lower than in group of women with the diagnosed IUGR. The MDA value in IUGR patients changed during the treatment period. After five days of treatment a decrease in MDA level was observed lowered down to a mean value of 2.14±1.27, and after ten days of treatment down to 1.71±0.65 (11). In another study the erythrocyte MDA levels were found significantly elevated in mothers of IUGR babies when compared to controls (12).

Significantly raised levels of oxidative stress makers (malondialdehyde, glutathione peroxidase and super oxide diastase) and significantly reduced levels of antioxidant lycopene and vitamin C in women with varying grades of IUGR 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 IUGR mothers and the value increases as the gestation advances towards term. The mean value of MDA was 5.363umol/l in IUGR and in complicated IUGR was 6.3umol/l (table 11). This reflects to existence of oxidative stress situation in these high-risk pregnancies. Disturbance of balance between oxidative process and antioxidant defense cause the oxidative stress to act unopposed which can affect both fetus as well as the mothers of these pregnancies.

Table 11 analyses the birth-wt. of 80 babies in this study 80% of IUGR babies are low birth weight. 10% IUGR babies had perinatal deaths (table 13). Similar observation was made by one study (14) where perinatal mortality was 5 to 30 times greater amongst the infants with birth-wt. less than 2.5kg.

IUGR pregnancies are associated with decreased antioxidant which 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 is yet to make a major breakthrough.

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