(International Print/Online Journal)

SJIF IMPACT FACTOR: 5.565
PUBMED-National Library of Medicine ID-101739732

ISSN (Print): 2209-2870
ISSN (Online): 2209-2862

International Journal of Medical Science and Current Research (IJMSCR)
Available online at: www.ijmscr.com
Volume 5, Issue 4 , Page No: 375-385
July-August 2022

IJMSCR

Comparison of MDA levels As A Measure Of Lipid Peroxidation in Normal Pregnancy and Hypertensive Disorders of Pregnancy

Renu Chane[1],Udaybir Singh Bhullar[2], Raghav Mittal[3], Gurdeep Kaur Bedi[4]
[1]Department of Biochemistry,
[1]Noida International Institute of Medical Sciences, Greater Noida
[2]DMC&H, Ludhiana
[3,4]Rajindra Medical College, Patiala

***Corresponding Author:**
Renu Chane
Department of Biochemistry, Noida International Institute of Medical Sciences, Greater Noida

Type of Publication: Original Research Paper
Conflicts of Interest: Nil

Abstract

Background: Pregnancy because of the mitochondrial-rich placenta, is a condition that favors oxidative stress. Protective mechanism against free radical generation and damage increases throughout pregnancy.Oxidative stress peaks by the second trimester of pregnancy. Conditions associated with pregnancy such as gestational hypertension exhibit exaggerated indications of free radical damage.[1] Lipid peroxidation occurs when free radicals are generated adjacent to polyunsaturated fatty acids in membrane lipids. The net result of one very reactive radical species attack upon membrane is to convert polyunsaturated fatty acids into lipid hydroperoxides. Lipid hydroperoxides decompose in the presence of ions and copper ions to form a wide range of cytotoxic aldehydes, such as malondialdehyde (MDA) and hydroxynonenal.[2]

Objectives: The present study was undertaken to estimate malonaldehyde levels as a measure of lipid peroxidation in normal pregnancy and hypertensive disorders of pregnancy. These levels were then compared with controls comprising non-pregnant women.

Subject and Methods: The study was conducted on 30 healthy non-pregnant women and 60 pregnant women attending the Antenatal clinic of Rajindra Hospital, Patiala. They were divided into 3 Study groups I, II, and III. Group I (study group) consisted of 30 pregnant women with PIH/ pre-eclampsia/ eclampsia. Group II (study group) consisted of 30 normotensive pregnant women. Group III (control group) had 30 healthy non-pregnant women. The subjects were recruited according to the inclusion and exclusion criteria. MDA levels were estimated in the serum collected from the patients.

Results: The mean MDA levels in group I and II was 5.26±1.57 μmol/L and 4.75±1.61 μmol/L, while in the control group was 4.62±1.68 μmol/L. On statistical analysis between all the groups at 24 weeks the difference in MDA level was found to be non-significant ($p > 0.05$). At 34 weeks, MDA levels in both study group (I and II) was 7.95±1.47 μmol/L and 6.08±1.49 μmol/L respectively. On statistical analysis at 34 weeks between all the 3 groups the difference in MDA level was found to be highly significant ($p<0.01$).

Conclusion: MDA which served as an index of lipid peroxidation, increases throughout normal pregnancy but the increase is more marked in patients with hypertensive disorders of pregnancy.

Keywords: MDA, Pregnancy/eclampsia/preeclampsia, oxidative stress

Introduction

Pregnancy is a period of physiological stress in which many changes occur in the 'milieu interior'. There are

marked changes in the functioning of all the vital organs. The placenta develops and it establishes a connection between mother and fetus through the umbilical cord.

Hypertensive disorders of pregnancy include a heterogeneous collection of diseases, of which, pre-eclampsia is the commonest form i.e. hypertension associated with proteinuria.[3] . It is a common complication of pregnancy. The incidence of PIH in India varies from 7-to 10%. Severe pre-eclampsia is diagnosed when blood pressure is > 160/110mmHg, proteinuria >5g/24 hours, oliguria, cerebral disturbances, and pulmonary edema. The disease is a complex clinical entity involving all organic systems like CVS, hepatic, renal, and coagulation abnormalities with hypertension as one of the manifestations.[4]

PIH is well known for its contradictory and controversial clinicopathological findings. Variable involvement of different key organs of the body, with the variable presentation of disorders of pregnancy, makes it a very controversial subject making its exact recognition or exclusion at times very difficult.

Studies have shown that lipid peroxidation assessed by malondialdehyde and conjugated dienes was significantly increased in hypertensive patients as compared with control patients.[5]

Free radicals and lipid peroxidation

Pregnancy, mostly because of the mitochondrial - rich placenta, is a condition that favors oxidative stress. Protective mechanism against free radical generation and damage increases throughout pregnancy. Oxidative stress peaks by the second trimester of pregnancy. Conditions restricted to pregnancy such as gestational hypertension exhibit exaggerated indications of free radical damage.[1]

Lipid peroxidation occurs when free radicals are generated adjacent to polyunsaturated fatty acids in membrane lipids. The net result of one very reactive radical species attack upon membrane is to convert polyunsaturated fatty acids into lipid hydroperoxides. Lipid hydroperoxides decompose in the presence of ions and copper ions to form a wide range of cytotoxic aldehydes, such as malondialdehyde (MDA) and hydroxynonenal.[2]

The tendency of free radicals to cause lipid peroxidation has been used as an indirect measure for oxidative stress. Markers of Lipid peroxidation like MDA have been found to arise in association with pre-eclampsia.[6]

As there is a great potential for clinical confusion and for the appalling problems associated with hypertensive disorder of pregnancy and its complications the accurate identification of this condition assumes immense importance. Hypertension, edema, and proteinuria, all have caused clinical confusion in the diagnosis of PIH, as none of these findings alone or in combination are specific for PIH.[7]

Having encountered such problems with the clinical criteria, clinicians have long sought help from the laboratory parameters. Unfortunately, most of these available parameters to date are neither specific nor always sensitive. So, the present study was undertaken to assess malondialdehyde levels in normal pregnancy and hypertensive disorders of pregnancy at >20 weeks of gestation and 34 weeks.

Material and methods

The present study was conducted on 30 healthy non-pregnant women and 60 pregnant women attending the Antenatal Clinic of Rajindra Hospital, Patiala.

Group I - (Study group) consisted of 30 pregnant women with PIH/pre-eclampsia/eclampsia (n-30).

Group II - (Study group) consisted of 30 normotensive pregnant women (n=30).

Group III - (Control group) consisted of 30 healthy non-pregnant women (n=30).

Inclusion criteria

1. Normotensive Pregnant Women At More Than 20 Weeks Of Gestation.
2. Pregnant Women, With PIH/Pre-Eclampsia At More Than 20 Weeks Of Gestation Who Were Normotensive Before 20 Weeks.
3. Pregnant Women With Antepartum Eclampsia At 20 Weeks Or More Of Gestation.
4. Control Group Consisted Of Non-Pregnant Healthy Women.

Exclusion criteria

1. All cases of essential hypertension or chronic hypertension due to any other cause.

Volume 5, Issue 4; July-August 2022; Page No 375-385

2. Any associated pulmonary/renal/hepatic/neurologic etc. or systemic disorder causing oxidative stress.
3. Associated hydramnios, molar pregnancy, diabetes mellitus & Rh-isoimmunization.
4. Associated hematological disorders in pregnancy
5. Haemoglobin less than 10gm%. •

PIH/pre-eclampsia was diagnosed using the criteria laid down by the American College of Obstetrician & Gynaecologists, 1996 [8] which are as follows:

Development of hypertension to the extent of 140/90 mmHg or more recorded on two different occasions at least 6 hours apart, after 20 weeks of pregnancy.

Proteinuria more than 300 mg in 24 hours of urine or more than l00 mg/dL in two random urine samples.

Pregnant women with hypertension and proteinuria after 20 weeks of gestation were labeled as having pre-eclampsia.

Pregnant women with hypertension to the extent of 140/ 90mmHg or more after 20 weeks of pregnancy were labeled as having gestational hypertension.

Collection and treatment of the sample

Under aseptic conditions, 5ml of venous blood from human subjects was taken into the plain vial to collect serum. The blood in a plain vial was centrifuged at 3000 r.p.m for 10 minutes to remove serum. Serum was used for the estimation of MDA levels.

Estimation of MDA levels(MALONDIALDEHYDE)

Malondialdehyde was taken as a marker of lipid peroxidation and its levels were estimated in serum using the method of Ohkawa et al.[9]

Principle - Malondialdehyde forms a colored complex with thiobarbituric acid and its optical density is measured colorimetrically at 532 nm. The values are expressed as micromoles of MDA per liter of serum.

Procedure - 0.5 ml of serum was taken in a screw-capped bottle and the following reagents were added sequentially.

1. 0.2 ml of 8.1% sodium dodecyl sulfate
2. 1.5 ml of 20% acetic acid
3. 1.5 ml of 0.8% thiobarbituric acid
4. 0.3 ml of distilled water

The whole combination was heated for 60 minutes at 95°C in a water bath and was cooled at room temperature.

1ml of distilled water was added followed by 5 ml of n- butanol pyridine mixture (15:1 ratio).

Then the solution was centrifuged at 4000 rpm for 10 minutes. The upper orange-colored layer was read colorimetrically at 532 nm and the optical density was noted.

Calculation - MDA was calculated in (µmol/L) of serum by the following formula :

$$\frac{\text{Optical density (O.D.)}}{\text{Extraction Coefficient}} \times \frac{\text{1000 x Final volume (ml)}}{\text{Effective Volume of Serum (ml)}}$$

$$\frac{\text{O.D.}}{0.152} \times \frac{5}{0.5} = \times 65.79\mu\text{mol/L}$$

Results

The present study was conducted on 90 subjects to compare lipid peroxidation in normal pregnancy and hypertensive disorders of pregnancy. For this malondialdehyde (MDA) levels were taken as a marker of lipid peroxidation.

For this MDA levels were measured in the following three groups:

Volume 5, Issue 4; July-August 2022; Page No 375-385

Group I - (Study group) consisted of 30 pregnant women with PIH/pre-eclampsia/eclampsia (n=30).

Group II - (Study group) consisted of 30 normotensive pregnant women (n=30).

Group III - (Control group) consisted of 30 healthy non-pregnant women (n=30).

In both, the study groups serial maternal venous MDA levels were done at 24 weeks and 34 weeks of pregnancy. In the control group, MDA levels were measured at the time of recruitment in the study. The various observations noted in the study groups and control groups were tabulated as follows :

Table 1 Distribution Of Cases According To Age

Group	No.	Range (in yrs.)	Mean±SD (yrs.)
I (PIH)	30	19-42	28.20±6.53
II (Normotensive)	30	18-44	27.30±6.26
III (Control)	30	18-42	27.86±5.67

Table 2 Distribution Of Cases According To Gestational Age At 24 Weeks

Group	No.	Range (weeks)	Mean±SD (weeks)
I (PIH)	30	22-26	23.63±1.18
II (Normotensive)	30	22-25	23.80±0.96
III (Control) (Non-pregnant)	30	Not applicable	-

Table 3 Distribution Of Cases According To *Gestational Age At 34 Weeks*

Group	No.	Range (weeks)	Mean±SD (weeks)
I (PIH)	30	33-36	34.56±0.93
II (Normotensive)	30	33-36	34.80±0.88
III (Control) (Non-pregnant)	30	Not applicable	-

Volume 5, Issue 4; July-August 2022; Page No 375-385

Table 4 Comparison of Systolic Blood Pressure of control group and the study groups at 24 and 34 weeks of gestation

Study Group	No.	24 weeks		34 weeks	
		Range (mmHg)	Mean±SD (mmHg)	Range (mmHg)	Mean±SD (mmHg)
I	30	90-132	118.20±10.78	140-164	150.60±8.43
II	30	90-100	95.66±3.28	114-126	122.0±3.10

Group III	No.	Range (mmHg)	Mean±SD (mmHg)
Control	30	110-124	120.80±2.65

Statistical Analysis

Comparison	24 weeks			34 weeks		
	't'	'p'	Sig.	't'	'p'	Sig.
I and II	10.95	<0.001	HS	17.41	<0.001	HS
I and III	0.93	>0.05	NS	18.21	<0.001	HS
II and III	27.67	<0.001	HS	2.16	<0.05	S

Volume 5, Issue 4; July-August 2022; Page No 375-385

Comparison of SBP of the control group and the study groups at 24 and 34 weeks of gestation

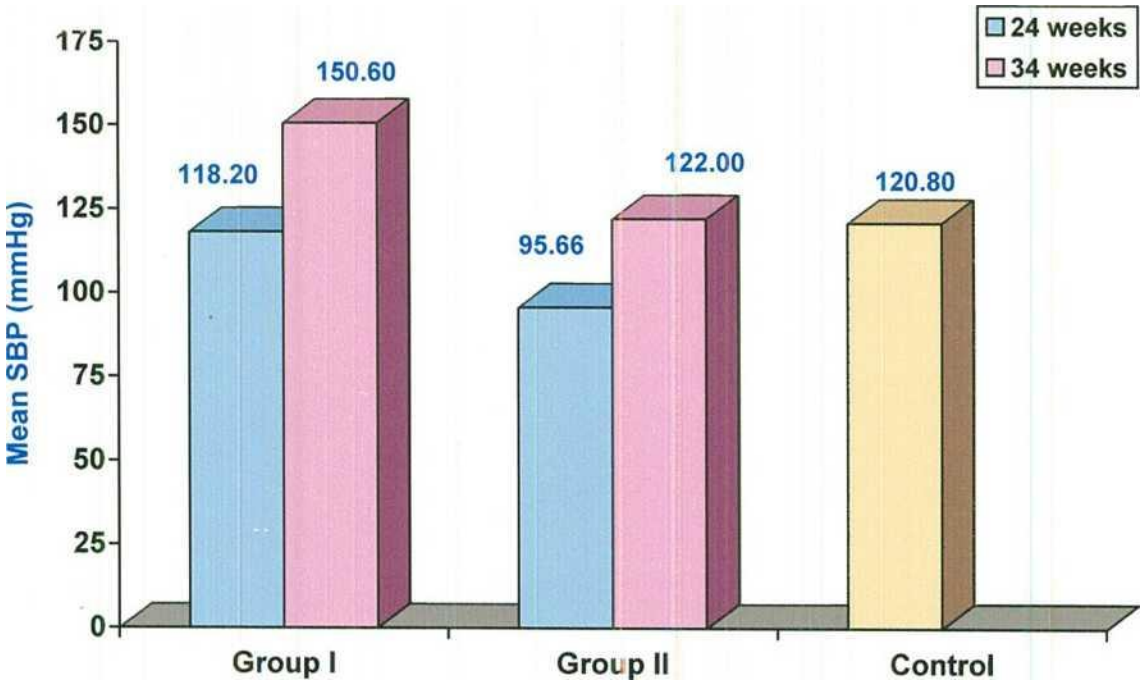


Table 5 Comparison Of Diastolic Blood Pressure Of The Control Group And The Study Groups At 24 And 34 Weeks Of Gestation

Study Group	No.	24 weeks		34 weeks	
		Range (mmHg)	Mean±SD (mmHg)	Range (mmHg)	Mean±SD (mmHg)
I	30	60-90	80.23±6.86	90-112	97-13±7.67
II	30	60-70	65.60±3.25	74-86	81.66±2.63

Group HI	No.	Range (mmHg)	Mean±SD (mmHg)
Control	30	78-84	80.73±1.61

Statistical Analysis

Comparison	24 weeks			34 weeks		
	't'	'p'	Sig.	't'	'p'	Sig.

Volume 5, Issue 4; July-August 2022; Page No 375-385

I and II	10.54	<0.001	HS	10.58	<0.001	HS
I and III	0.12	>0.05	NS	11.70	<0.001	HS
II and III	19.17	<0.001	HS	2.39	<0.05	S

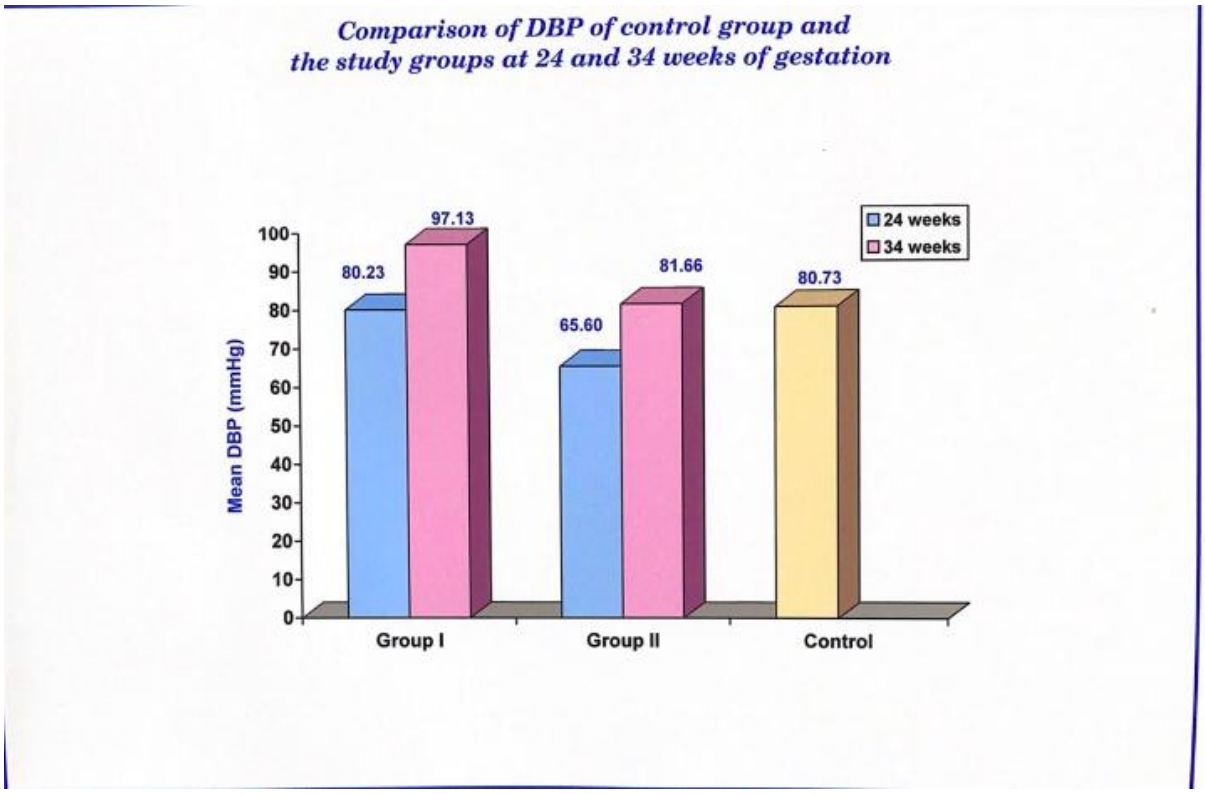


Table 6 Comparison Of Serum Mda Levels Of The Control Group And The Study Groups At 24 And 34 Weeks Of Gestation

Study Group	No.	24 weeks		34 weeks	
		Range (μmol/L)	Mean±SD (μmol/L)	Range (μmol/L)	Mean±SD (μmol/L)
I	30	2.85-8.1	5.26±1.57	6.25-12.15	7.95±1.47
II	30	2.63-7.89	4.75±1.61	4.15-9.85	6.08±1.49

Group III	No.	Range (μmol/L)	Mean±SD μmol/L)
Control	30	2.63-7.89	4.62±1.68

Volume 5, Issue 4; July-August 2022; Page No 375-385

Statistical Analysis

Comparison	24 weeks			34 weeks		
	't'	'p'	Sig.	't'	'p'	Sig.
I and II	1.23	>0.05	NS	4.85	<0.001	HS
I and III	1.53	>0.05	NS	8.16	<0.001	HS
II and III	0.32	>0.05	NS	3.56	<0.01	HS

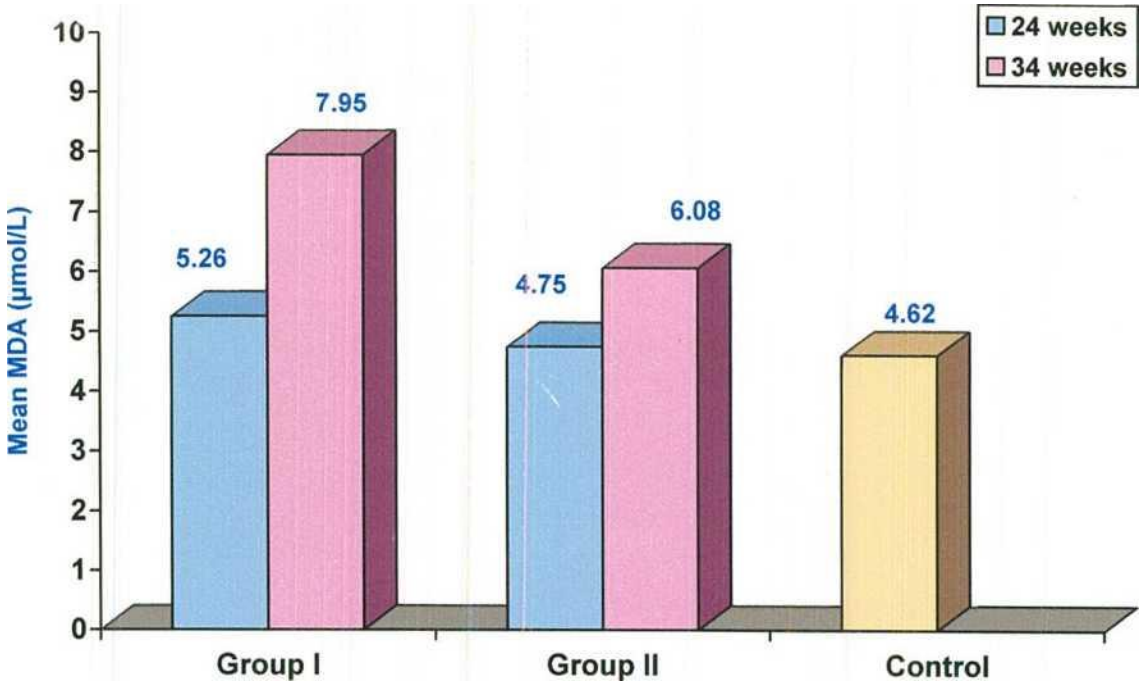
The above table shows MDA levels at 24 weeks in both the study group (I and II) and the control group (III). The mean in groups I and II was 5.26±1.57 μmol mol/L and 4.75±1.61μmol/L, while that in the control group was 4.62±1.68 μmol mol/L.

On statistical analysis between all the groups at 24 weeks the difference in MDA level was found to be non-significant (p >0.05).

At 34 weeks, MDA levels in both study group (I and II) was 7.95±1.47 μmol mol/L and 6.08±1.49 / μmol mol/L respectively.

On statistical analysis between all the three groups the difference in MDA level was found to be highly significant (p <0.01).

Comparison of MDA of the control group and the study groups at 24 and 34 weeks of gestation



Volume 5, Issue 4; July-August 2022; Page No 375-385

Table 7 Comparison of MDA levels in study and control groups by various authors

Author (year)	PIH	Normotensive Pregnancy	Control group (Non-pregnant Women)
Kabi et al (1994)"*	382.1±91 nm/dl erythrocytic	190.1+50 nm/dl Erythrocytic	152.7+58 nm/dl erythrocytic
Desai et al (2003)**	7.53±0.48 nm/1	6.82+0.35 nm/1	6.14+0.27 nm/1
Shah A et al (2019)***	9.28±0.99 nmol/ml	3.65±1.52 nmol/ml	2.98±0.82 nmol/ml
The present study (2022)***	7.95±1.47 $\mu mol/l$	6.08+1.49 $\mu mol/l$	4.62±1.68 $\mu mol/l$

* MDA level measured in erythrocytes.

** MDA level measured in plasma.

*** MDA level in serum.

As seen above, the levels of MDA levels are higher in the PIH group in all the studies as compared to those in normotensive pregnant women and non-pregnant women.

Discussion

MDA was taken as a marker of lipid peroxidation as it gives an indirect assessment of free radical activity. At 24 weeks, the levels of MDA were raised in women with PIH as compared to those in normotensive pregnancy and non-pregnant healthy women. But on statistical analysis, this increase was found to be non-significant. This observation is per Loverro et al[10] who concluded that as the pregnancy progressed, there was an increase in MDA levels from the 2nd trimester to the 3rd trimester.

At 34 weeks mean MDA levels in groups I and II were 7.95±1.47 μmol/L and 6.08±1.49 μmol/L respectively. In the control group mean MDA levels were 4.62±1.68 μmol/L (Table 6). Statistical analysis showed that levels of MDA were significantly higher in PIH patients as compared to those in normotensive pregnancy (group-II) and non-pregnant healthy women. It can be seen from Table 7 that the results of the present study were comparable with various studies conducted previously.

Kabi et al[11] estimated MDA levels in erythrocytes and concluded that PIH is associated with markedly raised MDA levels ($p < 0.001$) as compared to the levels in healthy non-pregnant women and normotensive pregnant women. The same trends were seen in the present study. The mean levels in PIH patients when compared with normotensive pregnant women were statistically significant ($p < 0.001$) in all the tabulated studies including the present study. However, the different values in different studies may be due to the estimation of MDA levels in different specimens (e.g. serum/ plasma/erythrocytes by different authors) and calculation in different units.

Desai et al[12] evaluated pro-oxidants and antioxidants in pre-eclampsia and suggested that there was a progressive increase in free radical formation during normal pregnancy and this increase was even higher in hypertensive disorders of pregnancy

Results from the study of Shah A et al[13] showed a substantial decline in endogenous antioxidant status and rise in oxidative stress markers by pregnancy-induced hypertension pointing to the key role of hypertension in initiating stress-induced oxidative damage.

It was observed that although in normal pregnancy an increase in malondialdehyde level was seen

Volume 5, Issue 4; July-August 2022; Page No 375-385

malondialdehyde levels in patients with preeclampsia increased beyond normal pregnancy levels by the second trimester. According to Hubei et al[14], vascular contact with the circulating peroxidation products directly causes dysfunction of the vascular endothelium thereby promoting peroxidative damage of endothelial cell membranes.

As is evident from the above hypothesis, which causes increased lipid peroxidation, the levels of MDA were higher in PIH patients as compared to non-pregnant women and normal pregnancy subjects.

Conclusion

The present study was undertaken to evaluate lipid peroxidation and antioxidant levels in normal pregnancy and hypertensive disorders of pregnancy.

30 diagnosed cases of PIH and 30 normotensive pregnant women attending the Antenatal Clinic of Rajindra Hospital, Patiala were studied. The Control group consisted of 30 healthy non-pregnant women. The majority of patients were in the range of 18-42 years of age.

In the present study, the amount of lipid peroxidation was compared in normal pregnancy and hypertensive disorders of pregnancy, first at 24 weeks of gestation and then at 34 weeks of gestation.

The malondialdehyde served as an index of lipid peroxidation in serum in both the control group and the study groups at 24 weeks of gestation and 34 weeks of gestation. The increase in MDA in PIH patients compared to normotensive pregnancy and control group was statistically significant at 34 weeks but, was statistically non-significant at 24 weeks.

So, it can be safely concluded that Lipid peroxidation increases throughout normal pregnancy, but the increase is more marked in patients with hypertensive disorders of pregnancy.

References

1. Casanueva E and Viteri FE. Iron and oxidative stress in pregnancy. J Nutr 2003; 133(5 Suppl 2): 1700-1708.
2. Ward RJ and Peters TJ. Free Radicals. In: Clinical Biochemistry. Metabolic and Clinical Aspects. Churchill Livingston 1995; 1:765.
3. Chesley LC. Diagnosis of preeclampsia. Obstet Gynecol. 1985 Mar;65(3):423-5. PMID: 3883267.
4. Friedman SA, Taylor RN, and Robert TH. Pathophysiology of preeclampsia. Clin Prenatal 1991; 18:661.
5. Uotila JT, Tuimala RJ, Aarnio TM, et al. Findings on lipid peroxidation and antioxidation function in hypertensive complications of pregnancy. Br J Obstet Gynaecol 1993;100(3):270-276.
6. Shaarawy M, Aref A, Emad Salem M, et al. radical-scavenging antioxidants in pre-eclampsia. Int J Gynec Obstet 1998;60:123-128.
7. Das SS, Dhall GI, Dhall K, et al. Significance of serum iron levels as a biochemical marker in pregnancy-induced hypertension Int J Gynecol Obstet 1994;45:3-9.
8. American College of Obstetricians and Gynaecologists: ACOG Technical Bulletin No: 219. Washington DC: The College, 1996.
9. Ohkawa H, Ohishi N, and Yagi K. Assay for lipid peroxides in animal tissue by the thiobarbituric acid reaction. Analytical Biochemistry 1979;95:351-358.
10. Loverro G, Greco P, Capuano F, et al. Lipoperoxidation and antioxidant enzymes activity in pregnancy complicated with hypertension. Eur J Obstet Gynecol Reprod Biol 1996;70(2):123-7.
11. Kabi BC, Goel N, Rao YN, et al. Levels of erythrocyte malondialdehyde, vitamin E, reduced glutathione, G6PD activity and plasma urate in patients of pregnancy-induced hypertension. Indian J Med Res 1994;100:23-25.
12. Desai P, Rathod SP, Vaibhavi G, et al. Evaluation of prooxidants and antioxidants in pre-eclampsia. J Obstet Gynecol Ina. 2003;55(5):445-448.
13. Shah A, Rashid A, Khan MS, Parvez T, Kaisar M, Mudassar S. Pregnancy-induced hypertension: lipid peroxidation and

Volume 5, Issue 4; July-August 2022; Page No 375-385

antioxidant status. Int J Res Med Sci 2019;7:xxx-xx.

14. Carl A. Hubel, James M. Roberts, Robert N. Taylor, Thomas J. Musci, George M. Rogers, Margaret K. Mclaughlin, Lipid peroxidation in pregnancy: New perspectives on preeclampsia, American Journal of Obstetrics and Gynecology, 1989; Volume 161, Issue 4: 1025-1034.

Volume 5, Issue 4; July-August 2022; Page No 375-385