Original article

Evaluation of oxidative stress in pathogenesis of pre eclampsia by studying plasma malondialdehyde and ceruloplasmin level

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ABSTRACT :

BACKGROUND: Preeclampsia is a human pregnancy specific, multi system disorder that is characterized by hypertension and proteinuria[1]Pre-eclampsia is one of the leading causes of high rates of maternal and perinatal mortality and morbidity. Currently, there are no screening tests for pre-eclampsia that are reliable, valid, and economical. Parameters of oxidative stress could be early markers of endothelial dysfunction that predates clinical pre-eclampsia.

MATERIAL & METHODS: The study population includes 3 groups 1 group consists of 10 non pregnant, normotensive females 2nd group consistsof 10 normotensive pregnancies,3rd group consists of 10 pre-eclamptic pregnancies.group 1 served as control for group 2,group 2 served as control for group 3. The diagnosis of pre eclampsia was based on elevated blood pressure & proteinuria. 5 ml of blood was collected & the plasma was separated and MDA and CP analysis were performed immediately.Random mid-stream urine was collected in a test tube and the degree of proteinuria was estimated immediately.

RESULTS : The present study was designed to find out the role of oxidative stress in pathogenesis of preeclampsia. Increased MDA and CP activity were found in uncomplicated normotensive pregnancy group compared to normotensive non pregnant control group ($p < 0.001$).Increased MDA levels and decreased CP activity in preeclampsic pregancies compared to normotensive pregnancies ($p < 0.001$). No correlation was observed between MDA & CP in group 1 and group 2. There was correlation between MDA and CP in preeclampsic group ($p < 0.001$).

CONCLUSION : The present study has found an increased oxidative stress as shown by increased plasma MDA, decreased plasma CP activity and increased MDA / CP ratio in patient having preeclampsia.

From this study it is concluded that oxidative stress is one of the major factors in the pathogenesis of preeclampsia

KEY WORDS: MDA-Malondialdehyde, CP-Ceruloplasmin,Pre-eclampsia,Oxidative stress

INTRODUCTION

Preeclampsia is a human pregnancy specific, multi system disorder that is characterized by hypertension and proteinuria[1]. Inspite of intensive effort to identify the pathophysiological mechanism neither a specific cause nor a pathogenesis has been identified. It has been hypothesized that endothelial dysfunction oxidative stress and increased level of lipid peroxidation products plays a major role in preeclampsia[2]. Activity of malon dialdehyde is found to be increased. Ceruloplasmin a major plasma antioxidant decreased in preeclampsia[3].

The present study was carried out to determine the level of plasma malondialdehyde [MDA] as a marker of lipid peroxidation and ceruloplasmin as an antioxidant against lipid peroxidation. The term hypertensive disorders complicating pregnancy includes a heterogenous collection of hypertensive disorders which are peculiar to pregnancy. Nearly 18% of maternal deaths are related to these hypertensive disorders.The working group of National High Blood Pressure Education Programme proposed a classification for the hypertensive disorders complicating pregnancy[4].

There are five types of hypertensive disorders complicating pregnancy. They are

1) Gestational hypertension
2) Preeclampsia
3) Eclampsia
4) Preeclampsia super imposed on chronic hypertension
5) Chronic hypertension

Minimum criteria for diagnosing preeclampsia are

- BP ≥ 140 / 90 mmHg after 20 weeks of gestation.
- Proteinuria ≥ 300 mg / 24 hours

other symptoms increasing the certainty of preeclampsia

- BP ≥ 160 / 110 mm Hg
- Proteinuria 2.0 g / 24 hours
- S. creatinine > 1.2 mg / dl
- Microangiopathic haemolysis as evidenced by increased LDH.
- Elevated Alanine & Aspartate transaminase.
- Persistent headache or other cerebral or visual disturbance
- Persistent epigastric pain

It occurs chiefly in primigravidae[5],more common in multiple pregnancy, hydramnios and vesicular moles. It may occur in association with maternal disease like diabetes mellitus, hypertension and renal disorder[5].Preeclampsia adversely affects the mother and fetus.

Changes in the maternal organs and functions

1) Cardiovascular Changes

High systemic vascular resistance, hyper dynamic ventricular function & reduced blood volume [6].

2) Hematological Changes

Thrombocytopenia which is likely due to platelet activation and consumption is most common preeclampsia[7]. Reticulocytosis is also seen.

3) Endocrine Changes

There is sodium retention and hypertension both of which leads to decrease secretion of rennin by juxtaglomerular apparatus. Due to decrease rennin secretion the aldosterone secretion also impaired[8].

4) Fluid Changes

In preeclampsia renal endothelial injury leads to proteinuria.

5) Renal Changes

Glomeruli are enlarged with glomerular capillary endothelial swelling. The swollen endothelial cells block the capillary lumen[9].

Renal perfusion and GFR are reduced. Preeclampsia is associated with increased concentration of serum creatinine and uric acid and diminished urinary calcium excretion[10].

6) Liver

Due to numerous scattered areas of sub scapular hemorrhage its appearance is mottled. Increased liver enzymes are due to periportal hemorrhagic necrosis in the periphery of liver lobules[11].

7) Placenta

Placenta plays a major role in maternal endothelial dysfunction in preeclampsia[12]. This is evidenced by the disappearance or remission of preeclampsia after termination of pregnancy. The genesis of preeclampsia is clearly related to deficient trophoblast invasion and failure of uterine artery remodeling[13]. Preeclampsia placentas show abnormal expression of integrin molecules. Integrin molecules regulate cell to cell and cell to matrix interactions. As a result trophoblastic invasion is inhibited and spiral artery remodeling is often limited to the decidual portions. So the myometrial segments do not widen and remain contractile. This defective spiral artery remodeling results in reduced utero placental perfusion and foci of placental hypoxia or ischemia.

Numerous markers of oxidative stress are altered in placenta of preeclampsia.

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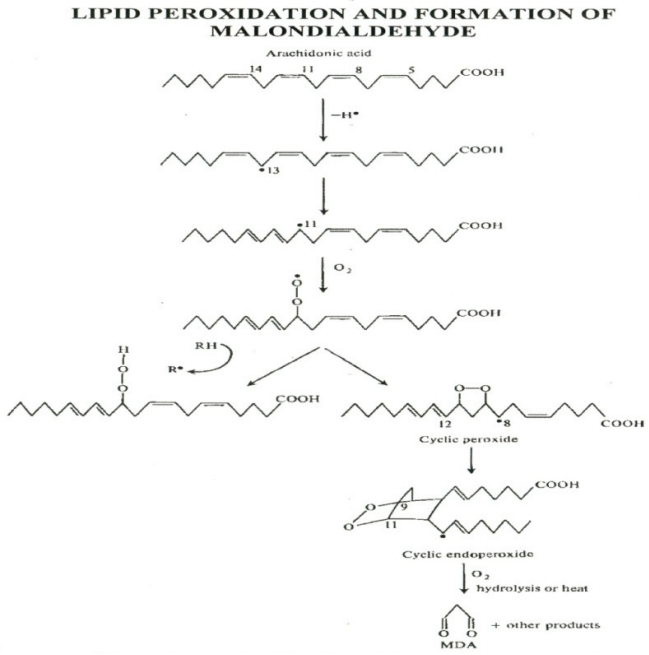


BIOLOGICAL EFFECTS OF THE PRODUCTS OF LIPID PEROXIDATION:

During the propagation of lipid peroxidation the peroxyl and alkoxyl radicals are generated. The end product lipid hydroperoxide undergoes decomposition and produces malondialdehyde, hydroxy nonenals (HNE) and isoprostanes. These products serve as a marker for lipid peroxidation and are implicated in damages caused to various cellular structure and function.

MALONDIALDEHYDE

Malondialdehyde [MDA] is one of the low molecular weight end products of lipid hydroperoxide decomposition and most often measured as an index of lipid peroxidation. MDA can also be formed during eicosanoid metabolism.

Acting as ferroxidase, ceruloplasmin is vitally important in regulating the ionic state of iron in particular oxidizing $Fe3^{+}$ to $Fe2^{+}$. It thus permits the incorporation of iron into transferrin without the formation of toxic iron products[64].

Under physiological condition ceruloplasmin is also important in the control of membrane lipid oxidation – probably by direct oxidation of cations thus preventing their catalysis of lipid peroxidation.

Ceruloplasmin inhibits the decomposition of lipid hydroperoxides exhibited by copper ions by binding to the metal.

AIM OF THE STUDY

The aim of the study was to evaluate the role of oxidative stress in the pathogenesis of preeclampsia by measuring the plasma MDA and CP as a markers for oxidative stress in preeclampsia. The study includes the measurement of ceruloplasmin activity to assess the decreased antioxidant capacity in preeclampsia. The relationship between MDA and CP were statistically analysed to assess the oxidative stress in preeclampsia.

MATERIALS & METHODS

STUDY DESIGN & INCLUSION CRITERIA

The study population includes three different groups.

Group 1 served as control for group 2. Group 2 served as control for group 3.

Normal pregnant and preeclampsic women were chosen from K.M. Hospital chingleput.. They were included into the study after obtaining informed consent. Healthy unpregnant subjects were chosen from general population and after obtaining informed consent they were included into the study.

Diagnosis of Preeclampsia

The diagnosis of preeclampsia was made on the basis of elevated blood pressure and proteinuria.

Hypertension was diagnosed when blood pressure was > 140 / 90 mmHg, in a pregnant woman after 20 weeks of gestation.A dipstick reading >1+ in a random sample was considered as proteinuria.

Case Selection

Inclusion Criteria: Group 1 : N=10

Non pregnant, normotensive women with age between 21 - 32 years, without hyper tension and proteinuria.

Group 2: N=10

Pregnant women with age group 21 - 32 years without hypertension and proteinuria with gestational age 20 - 35 weeks.

Group 3: N=10

Proven cases of preeclampsia with age group of 21 - 32 years with gestational age of 20 - 35 weeks.

Exclusion Criteria

1) History of hypertension, diabetes mellitus, tuberculosis, cardio vascular disorder.
2) Bad obstetric history.
3) Primary lipid disorder.
4) Any infection.
5) Anemia.

Sample Collection

After obtaining written and informed consent urine and blood samples were collected.

Collection of Blood Samples

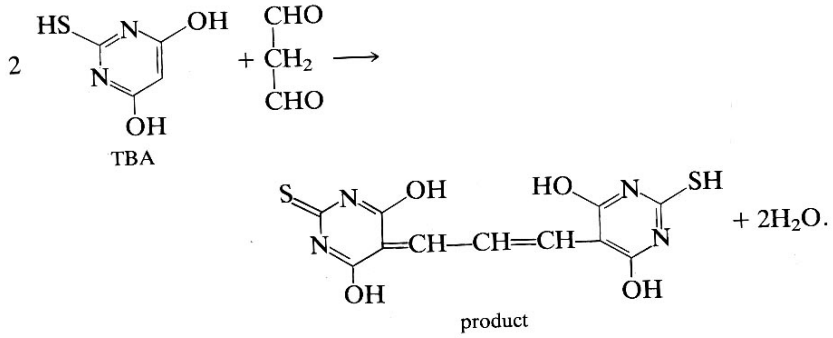
5 ml of blood was collected from each subject participating in this study. The samples were collected in a EDTA tubes. The tubes were allowed to stand for 15 minutes and then centrifuged. The plasma was separated and MDA and CP analysis were performed immediately.

Collection of Urine Sample

Random mid-stream urine was collected in a test tube and the degree of proteinuria was estimated immediately.

MALONDIALDEHYDE ESTIMATED BY BEUJE JA & AUSTIN JD METHOD[14].

Malondialdehyde reacts with thiobarbituric acid forming MDA - TBA2 adduct with pale pink colour that absorbs light strongly at 532 nm.



CERULOPLASMIN OXIDASE ACTIVITY ESTIMATED BY SCHOSINKY ET AL METHOD[15]

In this method O dianisidine dihydrochloride was used as substrate. This substrate was converted to yellowish brown product by ceruloplasmin oxidase and oxygen at pH 5. The pH was provided by acetate buffer. Acidification by sulfuric acid stops the enzymatic reaction and forms a purplish red solution that can be measured at 540 nm.

PROTEINURIA

In the dipstix method when protein is added to the dipstix, the affinity of the anionic form of the indicator dye for protein cause a shift of equilibrium between anionic and protonated forms of the indicator towards the formation of blue anionic species. The intensity of the blue colour produced is proportional to the concentration of protein present in the urine.

RESULTS

A total number of 30 subjects were included in the study. They were divided into three groups.

Group 1: Unpregnant normotensive women N = 10

Group 2: Uncomplicated normotensive pregnancies N = 10.

Group 3: Preeclamptic pregnancies N = 10

Statistical Analysis

In all the groups one way ANOVA was used to calculate the P valve.

Multiple range test by Tukey - HSD procedure was employed to identify the significant groups at 5% level.

Table 1

Comparison of mean levels of plasma MDA between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA levels Mean ± SD in µ mol / L	p	Significant groups at 5% Level
1	10	2.5 ± 0.3	< 0.001	
2	10	3.9 ± 0.3		2 vs 1
3	10	4.7 ± 0.3		3 vs 2

The mean level of plasma MDA of group 2 was compared with the mean level of plasma MDA of group 1. The mean level of plasma MDA was significantly higher in group 2 than in group 1 ($p<0.001$)

The mean level of plasma MDA of group 3 was compared with the mean level of plasma MDA of group 2. The mean level of plasma MDA was significantly higher in group 3 than in group 2 ($p<0.001$).

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Table 2

Comparison of mean levels of plasma CP activity between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	CP activity Levels Mean ± SD (U/L)	P	Significant groups at 5% Level
1	10	156.9 ± 12.6	< 0.001	
2	10	336.6 ± 26.5		2 vs 1
3	10	268.9 ± 22.8		3 vs 2

The mean level of plasma CP activity of group 2 was compared with the mean level of plasma CP activity of group 1. The mean level of plasma CP activity was significantly higher in group 2 than in group 1 ($p<0.001$)

The mean level of plasma CP activity of group 3 was compared with the mean level of plasma CP activity of group 2. The mean level of plasma CP activity was significantly lower in group 3 than in group 2 ($p<0.001$)

Table 3

Comparison of mean levels of MDA / CP ratios between non pregnant (group 1), un complicated pregnancy (group 2),and preeclampsia(group 3)

Group	N	MDA / CP ratio Mean ± SD	P	Significant groups at 5% Level
1	10	0.016 ± 0.002	< 0.001	
2	10	0.011 ± 0.001		2 vs 1
3	10	0.020 ± 0.001		3 vs 2

The mean ratio of MDA / CP of group 2 was compared with the mean ratio of MDA / CP of group 1. The mean ratio of MDA / CP in group 2 was significantly lower in group 2 than in group 1 ($p<0.001$)

The mean ratio of MDA / CP of group 3 was compared with the mean ratio of MDA / CP of group 2. The mean ratio of MDA / CP is significantly higher in group 3 than in group 2 ($p<0.001$)

DISCUSSION

The etiology and pathogenesis of pregnancy syndrome preeclampsia remain poorly understood. The diverse manifestation of preeclampsia including altered vascular reactivity, vasospasm are derived from pathological changes within the maternal vascular endothelium.

The present study was designed to find out the role of oxidative stress in pathogenesis of preeclampsia. Oxidative stress is an imbalance favouring oxidant over antioxidant system. In this study increased MDA and CP activity were found in uncomplicated normotensive pregnancy group compared to normotensive non pregnant control group ($p < 0.001$) as found in certain other studies done by Atamer et al[16]. The

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ratio of MDA/ CP was decreased in normotensive pregnancy group compared to normotensive nonpregnant group ($p < 0.001$). This indicates that oxidative stress does not occur in group 2. Results obtained indicate increased ceruloplasmin level of maternal plasma is possibly due to a compensatory response to increased lipid peroxidation in normal pregnancy. This antioxidant defence protects the cells against oxidative damage.

The present study shows increased MDA levels and decreased CP activity in preeclampsic pregancies compared to normotensive pregnancies ($p < 0.001$) as similar to studies done by Akshoy, H et al[17].Increased MDA/ CP ratio was observed in preeclampsic group compared to normotensive pregnancies.($p<0.001$) This increased MDA/ CP ratio ($p<0.001$) in preeclampsic group implies an impaired oxidant and antioxidant balance in favour of oxidants and can be defined as oxidative stress. In the present study no correlation was observed between MDA & CP in group 1 and group 2. There was correlation between MDA and CP in preeclampsic group ($p < 0.001$) which proves the presence of protection against oxidative stress which is as similar to the study done by H. Gurer Orhan et al[18].

CONCLUSION

Hydroxyl and alkoxyl radicals are generated during lipid peroxidation. MDA, HNE and Isoprostanes are produced during the decomposition of lipid hydroperoxides. All these products are probably the cause of the pathogenesis of damage to vascular endothelium.

The increased prooxidant activity is augmented by decreased levels of many intra and extra cellular antioxidants. Such a role of oxidative stress as a factor for pathogenesis of preeclampsia can be postulated as there are reports of decreased level of many anti-oxidants like superoxide dismutase, glutathione, vitamin C, vitamin E and ceruloplasmin in preeclampsia.

The present study has found an increased oxidative stress as shown by increased plasma MDA, decreased plasma CP activity and increased MDA / CP ratio in patient having preeclampsia.

Oxidative stress may be the point at which multiple factors converge resulting in endothelial dysfunction and consequent clinical manifestation of preeclampsia. From this study it is concluded that oxidative stress is one of the major factors in the pathogenesis of preeclampsia.

LIST OF ABBREVIATIONS USED :

MDA : Malondialdehyde,
CP :Ceruloplasmin,
BP :Blood Pressure,mm
Hg: Millimeter mercury,
LDH :Lactate dehydrogenase,
GFR : Glomerular filtration rate,
FFA :Free fatty acid,
TGL :Triglycerides,
LDL :Low density lipoprotein,
HDL :High density lipoprotein,
$OH^{\bullet}$: Hydroxyl radical,

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$RO_2^{\bullet}$ Peroxyl radical,

$RO^{\bullet}$: Alkoxyl radical,

$O_2^{\bullet-}$: Superoxide radical,

Fe^{2+}: Ferrous ion,

Fe^{3+} : Ferric ion,

H_2O_2: Hydrogen peroxide,

ROOH : Lipid hydroperoxide,

PUFA : Polyunsaturated Fatty acid,

$R^{\bullet}$: Carbon centered radical,

Cu^{+} : Cuprous ion,

CU^{2+} : Cupric ion,

DNA : De oxy ribo nucleic acid,

GS – SG : Oxidized Glutathione,

GSH: Reduced glutathione,

NADPH: Nicotinamide adenine di nucleotide phosphate reduced

NADP : Nicotinamide adenine di nucleotide phosphate,

HMP : Hexose Mono Phosphate, NADH: Nicotinamide adenine di nucleotide reduced,

NAD : Nicotinamide adenine di nucleotide, Vit.

E - $O^{\bullet}$ Tocopheroxyl rdical,

EDTA : Ethylene diamine tetra acetic acid,

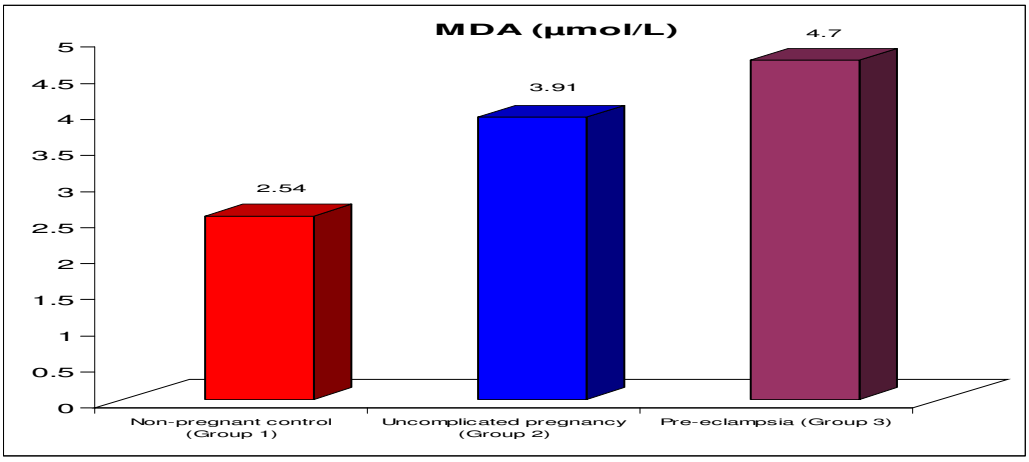
TBA :Thiobarbituric acid,

TCA : Trichloroacetic acid, Hcl : Hydrochloric acid,

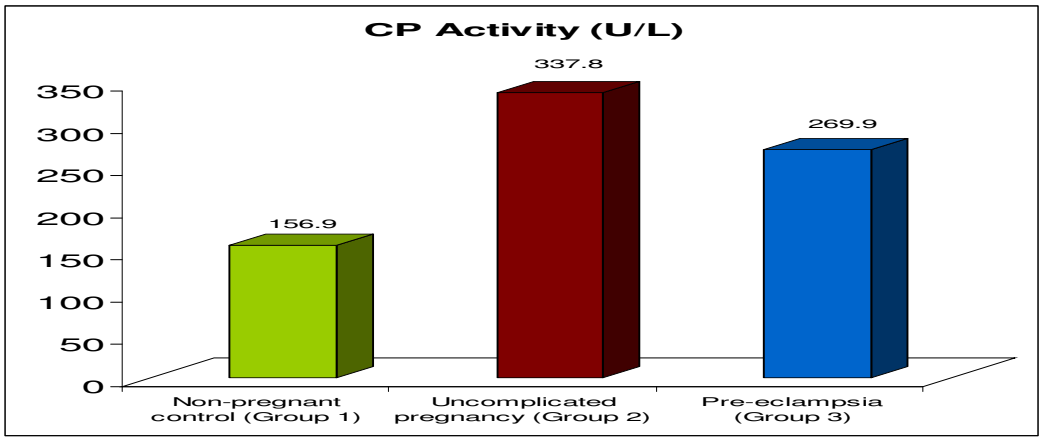
O.D. :Optical density,

H_2SO_4: Sulfuric acid, □mol : Micromole, mmol :millimole,

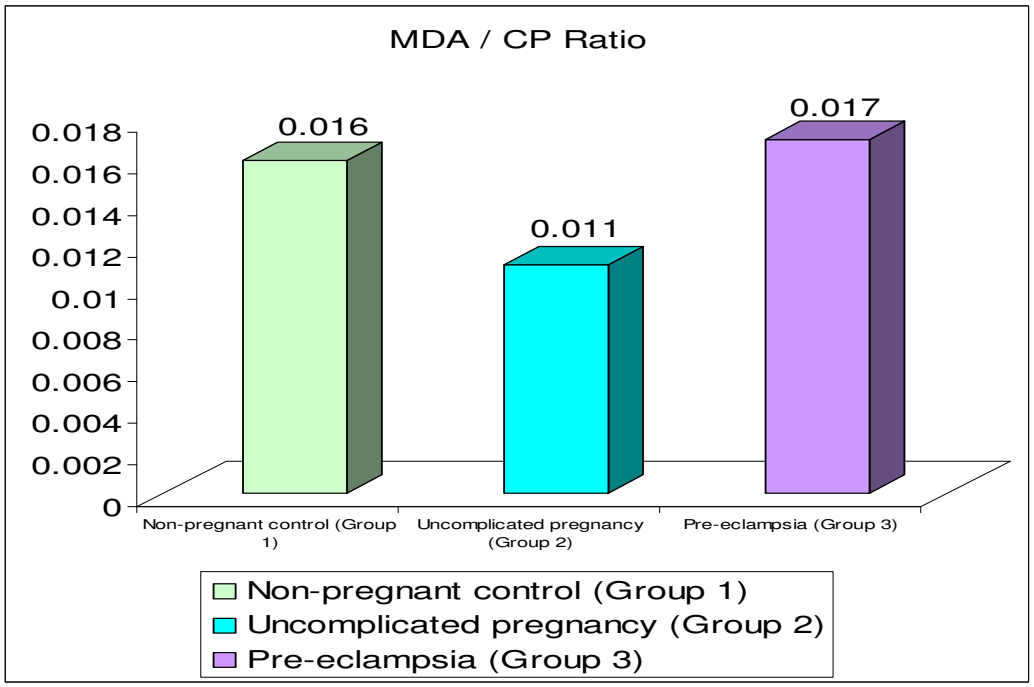
U/L: Units per litre



The mean level of plasma MDA is found to increase in preeclampsia (Group 3) when compared to uncomplicated pregnancy (Group 2), showing the presence of more oxidative stress in preeclampsia.



Group 2 shows increased mean level of plasma ceruloplasmin activity proving the protective function of ceruloplasmin on oxidative stress.



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