

A study of malondialdehyde and lipid profile in pregnancy induced hypertension

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Abstract

Pregnancy induced hypertensive disorders are one of the commonest complication of the pregnancy which accounts for most of the maternal and peri-natal mortality and morbidity.

Keywords: Hypertension, Preeclampsia, Malondialdehyde

Introduction

Pregnancy induced hypertensive disorder is a pregnancy specific syndrome complex occurring after 20th week of gestation, which subsides post partum. It is the term encompassing preeclampsia, eclampsia or previously the toxemia of pregnancy. Pregnancies occurring in patients with essential hypertension, renal hypertension and other causes of hypertension do not come under PIH. PIH is pregnancy specific and resolves after delivery.

Definition: Hypertension that develops as a consequence of pregnancy and regress Post-partum.

Classification: The following definitions are from National High Blood Pressure Education Program classification.

Preeclampsia is defined as a pregnancy-specific syndrome observed after the 20th week of pregnancy with systolic blood pressure of ≥ 140 mm Hg or diastolic blood pressure of ≥ 90 mm Hg accompanied by significant proteinuria (i.e., urinary excretion

of ≥ 0.3 g protein in a 24-h specimen). In women with preeclampsia, blood pressure usually returns to baseline within days to weeks after delivery.

Eclampsia is the occurrence, of seizures, in a woman with preeclampsia that cannot be attributed to other causes.

Gestational hypertension is defined as a blood pressure elevation detected for the first time after midpregnancy and is distinguished from preeclampsia by the absence of proteinuria. Gestational hypertension is a working diagnosis only during pregnancy. If proteinuria develops and the hypertension resolves after the pregnancy, the diagnosis is changed to preeclampsia. If elevated blood pressure persists, chronic hypertension is diagnosed. In the absence of other factors, the diagnosis is termed transient hypertension of pregnancy.

Chronic hypertension refers to an elevated blood pressure in the mother that predated the pregnancy. It can also be diagnosed in

retrospect when preeclampsia or gestational hypertension fails to normalize after delivery. Thus, hypertension that has not normalized by 12 wk postpartum is considered to be chronic hypertension.

Complications: Antepartum - abruption, eclampsia (2%), renal failure, visual disturbances, pregnancy loss, coagulation disorders such as disseminated intra vascular coagulation and HELLP (hemolytic anemia, elevated liver enzymes, low platelet count.

Intrapartum: Convulsions, haemorrhage.

Postpartum: Circulatory collapse, sepsis due to operative interference.

Fetal outcomes include stillbirth, intrauterine growth restriction, low birth weight, and prematurity.

Materials and methods

The study was performed in the department of General medicine and Biochemistry RIMS, KADAPA, in collaboration with its obstetrics department.

The subjects were ranging in age group of 20-32 years. Pregnant cases were in their third trimester. The subjects selected were stratified into 3 groups.

- Group-NN: Non pregnant Normal women (n=16).
- Group-NP: Normal uncomplicated Pregnancy (n=14).
- Group-PIH: Women with Pregnancy induced hypertension (n=17). (Preeclampsia).

Pregnant controls were selected from out patients who are healthy and without complications of pregnancy and other medical disorders.

PIH cases were from ante natal ward and labour room admitted for moderate to severe hypertension. The cases are without history of essential hypertension and other medical complications. Pregnancies with history of Eclampsia, essential hypertension and with other diseases are excluded from the study.

After obtaining an oral informed consent for the investigations, a fasting sample was collected from the subjects.

Fasting venous samples were collected into clean, dry sterile bottle and allowed to clot. Serum was separated after centrifugation. The serum samples were estimated on the same day for the following parameters: Malondialdehyde, Total cholesterol, triglycerides, HDL, VLDL and LDL.

Malondialdehyde (MDA) estimation:

(Bird et al, 1983; Nair et al, 1985).

Principle:

The Malondialdehyde in the serum reacts with Thiobarbituric acid in acidic medium and give rise to pink colored complex, which is measured at 532 nm against distilled water in a spectrophotometer (6, 40).

Reagents:

1. Thiobarbituric acid (TBA) (0.67 %): Prepared freshly every day. It is prepared by weighing 200 mg of Thiobarbituric acid and dissolved in 30 ml of double distilled water and mixed stirrer. 30 ml glacial acetic acid was then added.
2. 0.9% saline.
3. 10% TCA.

S. NO.	Reagent	Test
1.	serum	0.5 ml
2.	0.9 % saline	0.5 ml
3.	10 % TCA	1.0 ml

Mixed and centrifuged at 3000 rpm for ½ hour.

S. NO.	Reagent	Test
1.	Supernatant	1.00 ml
2.	0.67 % TBA (freshly prepared)	0.25 ml

Kept in boiling water bath for ½ hour.

Cooled immediately in ice-cold water. Absorbance taken at 532 nm against distilled water using a 1 cm. Cuvette in a spectrophotometer.

Calculations:

Malondialdehyde is calculated from its molar extinction coefficient (Molar absorptivity) (Mahfouz et al, 1986).

C = Concentration of the substance in mol/L
 L = Length of the light path (1 cm). Taken as constant.

In test 0.5 ml of serum was taken, diluted to 2ml. Out of this 1.0 ml was taken. So 0.25 ml of serum was used in the test. Concentration of malondialdehyde in nmoles/100ml of serum.

MDA in test sample =

$$\text{O.D. of test X } \frac{4 \times 100}{0.15} \text{ n. mole \%}$$

Precautions:

1. See that there is no hemolysis as intracellular Malondialdehyde is very high.
2. Perform the experiment within 24 hrs as Malondialdehyde in serum may disintegrate.
3. The reading of colour is stable.
4. Heating should be done precisely.
5. Use clean and sterile glass-ware normal range.
6. 247 ± 35 n mol /100 ml.

Lipid profile

Estimation of Total Triglycerides:

Method:

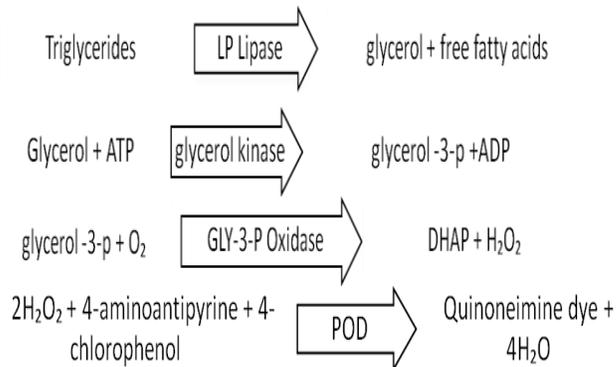
Enzymatic colorimetric method using triglyceride kit.

Principle:

Triglycerides incubated with lipoprotein lipase are hydrolyzed to free fatty acids and glycerol. Glycerol kinase catalyzes the conversion of glycerol and ATP to glycerol-3-phosphate and ADP. The glycerol-3-phosphate is further oxidized by glycerol 3 phosphate oxidase (GPO) to dihydroxy acetone phosphate and Hydrogen peroxide (H₂O₂). In presence of Peroxidase (POD),

Hydrogen peroxide couples with 4-aminoantipyrine (4-AAP) and 4-Chlorophenol to produce red Quinoneimine dye. Absorbance of colored dye is measured at 505 nm and is proportional to triglycerides concentration in the sample.

Reaction:



Procedure:

Bring all the reagents of assay to room temperature.

Working assay table

	Procedure for 1ml		
	B.	S.	T.
Enzyme reagent	1ml	1ml	1ml
Standard	-	10µl	-
Sample	-	-	10µl

Mix well. Incubate at 37°C for 10 minutes or at room temperature (15-30)°C for 30minutes.

Measure the absorbance of standard and sample against the reagent blank at 546 nm (500-546 nm).

Calculations:

Conc. Of triglycerides (mg/dl) =

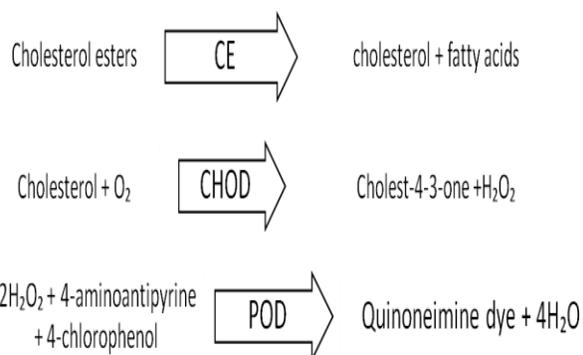
$$\frac{\text{O.D.}(sample)}{\text{O.D.}(standard)} \times 200$$

Normal value:

Females: 40-150mg%.
 Males : 50-200mg%.

Estimation of Total cholesterol:**Assay principle:**

Cholesterol esters are hydrolyzed by cholesterol Esterase (CE) to give free cholesterol and fatty acids. In subsequent reaction, cholesterol oxidase (CHOD) oxidizes the 3-OH group of free cholesterol to liberate cholest-4-en-3-one and hydrogen peroxide. In presence of peroxidase (POD), hydrogen peroxide couples with 4-aminoantipyrine (4-AAP) and phenol to produce Red quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of total cholesterol concentration in the sample.

**Procedure:**

	B.	S.	T.
Serum/ plasma	-	-	10 μ l
Reagent 2	-	10 μ l	-
Reagent 1	1000 μ l	1000 μ l	1000 μ l

Mix well. Incubate at 37°C for 10 minutes or at room temperature (15-30)°C for 30minutes.

Measure the absorbance of standard and sample against the reagent blank at 505 nm (490-530nm).

Calculations:

$$\text{Conc. Of Cholesterol (mg/dl)} = \frac{\text{O.D.}(sample)}{\text{O.D.}(standard)} \times 200$$

Normal values: 150-200mg%.

Estimation of HDL Cholesterol:**Principle:**

Chylomicrons, VLDL and LDL fraction in serum or plasma are separated from HDL by precipitating by polyethylglycol. After centrifugation cholesterol in the HDL fraction, which remains supernatant assayed with enzymatic cholesterol method.

Reagents:

Precipitating reagent – PEG 6000-200mmol/L

HDL cholesterol standard – 50mg/100ml

Procedure: Precipitation:

	Test
Serum/ plasma	200 μ l
Precipitating Reagent	200 μ l

Mix well. Keep at room temperature (15-30)°C for 10 min. Centrifuge for 15 min. at 2000 rpm and separate clear supernatant. Use the supernatant for HDL cholesterol estimation.

HDL – Cholesterol estimation:

	B.	S.	T.
Supernatant step 1	-	-	100 μ l
HDL standard Reagent	-	100 μ l	-
Cholesterol enzyme Reagent	1000 μ l	1000 μ l	1000 μ l

Mix well. Incubate at 37°C for 10 minutes or at Room temperature (15-30°) for 30 minutes.

Measure the absorbance of standard and sample against the reagent blank at 505 nm (490-530nm).

Calculations:

$$\text{Conc. Of HDL Cholesterol (mg/dl)} = \frac{\text{O.D.}(sample)}{\text{O.D.}(standard)} \times 2 \times 50. \text{ (2 is the dilution factor)}$$

Normal values:

Females: 35-75mg%

Males : 30-60mg%.

Calculation of VLDL Cholesterol:

This is calculated by using the formula $VLDL-C = \text{triglycerides} / 5$. This factor is based on the average ratio of triglycerides to cholesterol in VLDL.

Calculation of LDL Cholesterol:

LDL cholesterol is calculated based on Friedewald's equation:

$$LDL -C \text{ (mg/dl)} = \text{total cholesterol} - \text{triglyceride} / 5 - HDL \text{ cholesterol}$$

Where all concentration is given in mg/dl.

Friedewald's equation cannot be used in samples that have triglycerides concentrations above 400mg/dl or in a non fasting specimen. At high triglycerides concentration, the factor $[\text{triglyceride}]/5$ cannot be applied as an estimate of VLDL cholesterol concentration. These samples can also contain chylomicrons, chylomicron remnants, or VLDL remnants, all of which have higher triglycerides: cholesterol ratios than normal VLDL. Under such circumstances, the use of factor $[\text{triglycerides}]/5$ would overestimate VLDL

cholesterol and therefore underestimate LDL cholesterol.

Normal Values:

VLDL: < 40mg%.

LDL : 60-140mg%.

Results

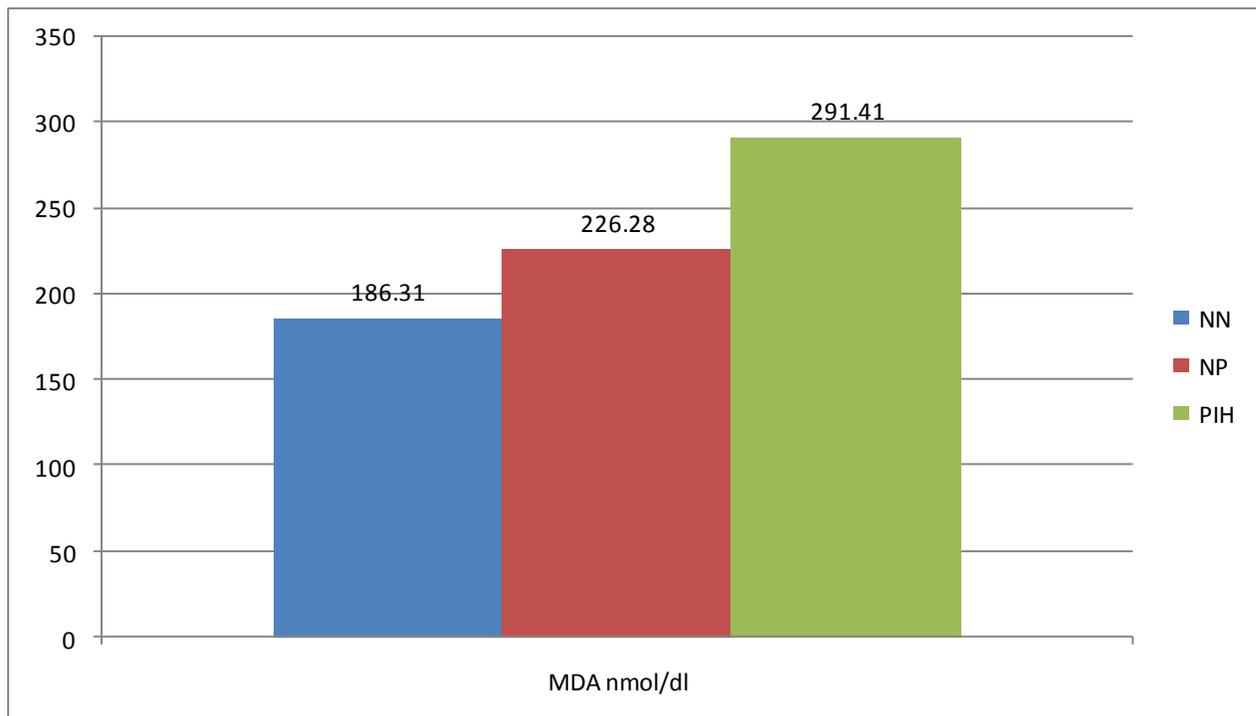
The study was done in a total group of 47 subjects, who are stratified under three groups.

Non pregnant Normal women (NN)	n =16
Normal Pregnant women (NP)	n=14
PIH (Only preeclampsia)	n=17

Pregnant subjects were in the third trimester. Malondialdehyde and lipid profile were estimated in the serum samples of the three groups.

The results are evaluated for Mean, Standard deviation and Probability was assessed by subjecting the results to T- test, 95% confidence limits were taken as level of significance ($P < 0.05$).

NN are taken as controls for NP, and NP as controls for PIH.



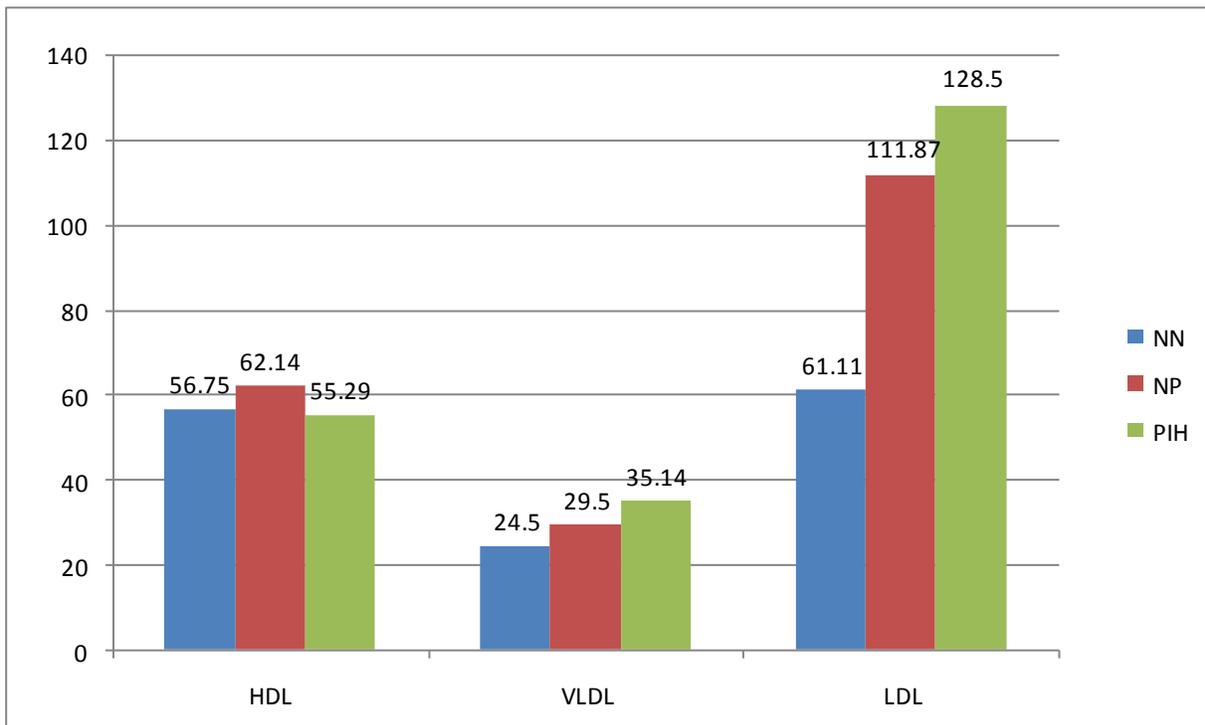
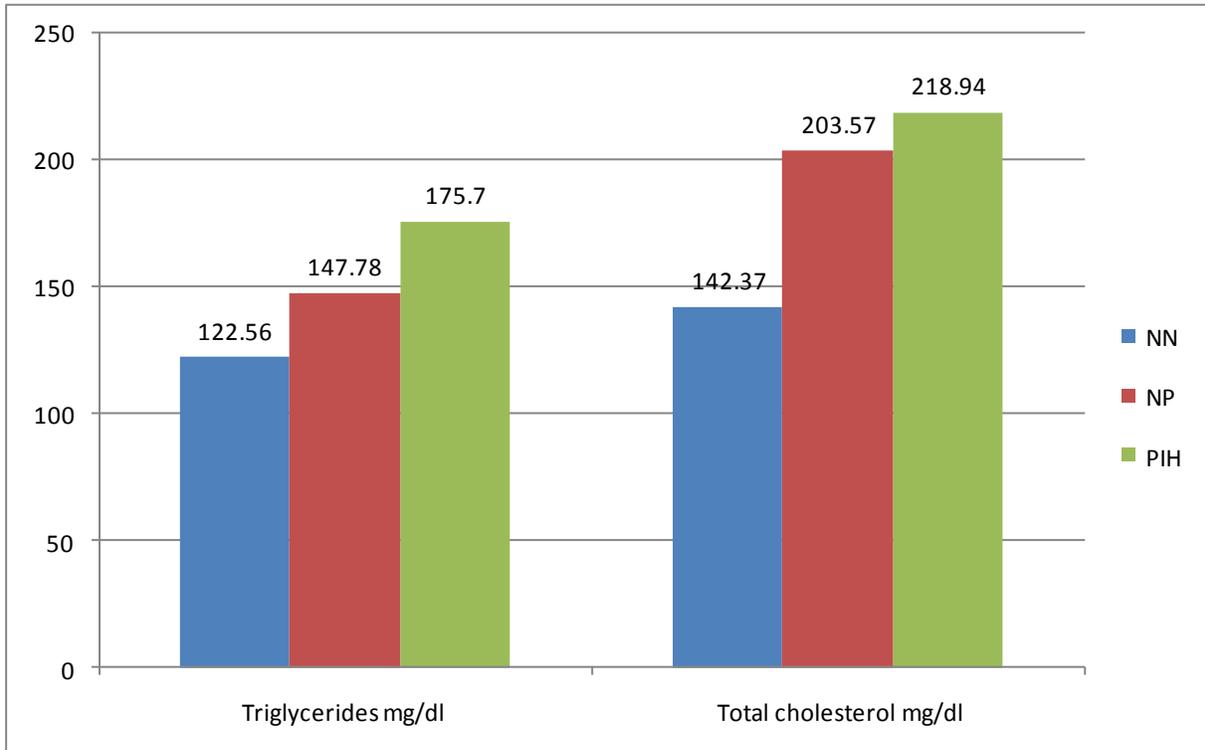


Table: Over view of estimated parameters and their significance in three groups

Parameters Mean \pm S.D.	NN	NP	Statistical Significance (NP vs. NN)	PIH	Statistical Significance (PIH vs. NP)
MDA nmols/dl	186.31 \pm 38.88	226.28 \pm 48.86	<0.05	291.41 \pm 66.38	<0.005
Triglycerides mg/dl	122.56 \pm 16.00	147.78 \pm 24.58	<0.005	175.70 \pm 19.84	<0.005
Total Cholesterol mg/dl	142.37 \pm 20.06	203.57 \pm 54.12	<0.005	218.94 \pm 47.37	NS
HDL-C mg/dl	56.75 \pm 9.64	62.14 \pm 7.40	NS	55.29 \pm 9.62	<0.05
VLDL-C mg/dl	24.51 \pm 3.20	29.55 \pm 4.91	<0.005	35.14 \pm 3.99	<0.005
LDL-C mg/dl	61.11 \pm 21.18	111.87 \pm 51.68	<0.005	128.50 \pm 49.01	NS

Discussion

Oxidative stress has been implicated in many diseases. Lipid peroxidation i.e., oxidation of the PUFA along with the augmented hyperlipidemic conditions in pregnancy is being much supported by studies in etiopathogenesis of PIH.

In the present study Malondialdehyde a lipid peroxidation product was significantly increased in Normal pregnant (NP) in comparison with the Non-pregnant normal controls (NN) (P<0.05)., also there is significant rise between normal pregnancy and PIH.(Preeclampsia).(P<0.005).

A Study by (Hubel, Carl A, 1996) (27) showed ante partum concentrations of malondialdehyde were 50% higher in women with preeclampsia (p < 0.01).

Serum MDA levels has showed no significant difference between NP and NN(P>0.05), in the study by Nguyễn Thị Thu Uyên, Nguyễn Nghiêm Luật /2007,(50) but the study has shown a significant increase of MDA level in the PIH compared in NP subjects.(P<0.005).

Hubel et al, and Kharb et al, (26,34) have also shown that serum lipid peroxides are known to increase in pregnancy and this increase was exaggerated in pre-eclampsia.

This increased lipid peroxides level can increase the susceptibility of polyunsaturated fatty acid to peroxidative damage, presumably by free radicals that may lead to the formation of malondialdehyde.

Llurba et al. (2004) (38) reported that there is no evidence for enhanced lipid peroxidation in PE patients. Contrary to the above studies, our results showed significantly increased plasma lipid hydroperoxides, in PIH women. This is consistent with other previous reports (32,50,42).

Lipid peroxides damage endothelial cells, inhibit the synthesis of prostacyclin (PGI₂) and increase the production of thromboxane (TxA₂), which is a potent vasoconstrictor and stimulator of platelet aggregation.(Walsh 1998)(49)

Normal pregnancy is characterized by progressive increase in body fat and in serum lipid levels reaching a peak at 32-36 weeks of gestation.(13). This increase is of approximately 40% rise of previous levels. (8) This atherogenic profile well tolerated during normal pregnancy is disrupted in PIH. Free fatty acids rise during pregnancy (Hubel .A.Carl). FFA induces formation of

ROS which could destroy Nitric oxide (NO) and thus attenuate NO- dependant vasodilation.

In present study lipid profile parameters are estimated to elucidate the role of dyslipidemia in pre-eclampsia.

An elevated triglyceride in the major lipoprotein fractions in pregnancy is confirmed. (Hubel A. Carl 1996.).(27)

In the present study statistically significant rises are seen in serum Triglyceride in NP compared NN controls ($p < 0.005$), and in PIH in relation to pregnant normal ,showed significance of ($P < 0.005$), which is consistent with the findings of previous researches (Sattar et al ., 1997, Wakatsuki et al., 2000)(46,48)

Antepartum serum triglyceride concentrations were increased approximately twofold in women with pre-eclampsia relative to uncomplicated pregnancies ($p < 0.02$) (27).

The triglyceride rises in VLDL and IDL are associated with proportional rises in cholesterol and phospholipid.

The Total Cholesterol levels in the present study revealed significant rise in normal pregnant compared to non pregnant controls ($P < 0.05$), though the Mean values have risen in PIH in relation to NP they are not statistically significant (218mg% and 203mg% respectively).Much of the literature is in support of this finding.(29,42)

The HDL mean values have increased in NP over NN, though it is not statistically significant, this rise shows a maintenance of the HDL cholesterol level in the pregnancy, distinguishes the pregnancy from other endogenous hypertriglyceridemias where HDL cholesterol is reduced. In the present study HDL cholesterol has significantly decreased in PIH cases in relation to Normal Pregnant ($P < 0.05$).

A study by Jayant De, Rubina A.(29,49) showed a decrease HDL-C significantly ($P < 0.05$), which are consistent with our study.

Similar results were already given in studies of (Sattar et al., 1997, Cekman et al., 2003,

L.Belo)(46,9,30). In pre-eclampsia the reduced levels HDL-C reveal the failure of HDL to rise during gestation. The low level of HDL -C is also due to the increased insulin resistance in preeclampsia.

The results of VLDL in the present study showed significant change in mean values in NP in relation to NN ($P < 0.005$). PIH has also shown similar significantly raised values in relation to NP (< 0.005). A 2-4 fold rise in triglycerides in pregnancy and preeclampsia, and the increased insulin resistance in PIH are responsible. Hypertriglyceridemia leads to enhanced biosynthesis of triglycerides, which is carried by VLDL into the circulation.

LDL values significantly increased in NP in relation to NN ($P < 0.005$), PIH showed a increase in mean value which were not significant in relation to NP(128.50 mg%,111.87mg% resp.).

In LDL, and HDL fractions, triglyceride content rises more than cholesterol and phospholipid. As a result, an increase in triglyceride on a percentage basis tends to reduce the contribution of the other two lipids.

Also LDL increased significantly in Normal pregnant cases due to significant rise in Total cholesterol and Triglycerides . In pre-eclampsia, the mean values of LDL have increased, but not significantly, because the levels of total cholesterol in normal pregnant and PIH are not significantly increased .

Studies showed elevated levels of LDL-C (Sattar et al., 1997, Belo et al., 2002, Ogura et al., 2002, Lorentzen & Henrikson, 1998) (46,3,47,39) in PIH compared to NP. Wakat suki et al., 2000(48) reported that there was no difference in the levels of LDL-C between PIH and NP women, which is consistent with our study.

Conclusion

Triglycerides and free fatty acids, but not cholesterol, are increased in pre-eclampsia and correlate with the lipid peroxidation

metabolite malondialdehyde. These interactions may contribute to endothelial cell dysfunction in preeclampsia

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