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STUDY OF SERUM LIPID PROFILE LEVEL IN PRE-ECLAMPSIA

BHAGYASHREE K BHUYAR*[1] AND MOHAMMED SHAMSUDDIN[2]

1. Department of Biochemistry D Y Patil Medical College Kolhapur.
2. Department of Biochemistry Al-Ameen Medical College Bijapur.

ABSTRACT

The current theory suggests that in preeclampsia there is an increase in the lipid peroxidation products and leads to a decrease in the plasma antioxidants except uric acid.Along with this change in the lipid profile level leads to the pathogenesis of preeclampsia.In this context, this study was undertaken to determine the changes in plasma levels of lipid peroxide, antioxidant levels in women with preeclampsia and to investigate the effect on lipid profile. To measure the levels of serum lipid profile in pre-eclampsia in comparison with normal pregnancy. Cross sectional study consisting of 30 preeclamptic and 30 healthy pregnant women. Fasting venous blood samples were collected during antepartum period and plasma levels of TG, TC, HDL-C, VLDL-C, and LDL-C were measured.: In the preeclamptic group, there was a significant increase in the triglyceride levels, but there were no significant changes in other lipid profile parameters. The findings of the present study are consistent with previous studies, lipid profile levels are important factors in the pathogenesis of preeclampsia. In pre-eclampsia plasma antioxidants are excessively utilized to counteract the cellular changes mediated by free radicals.

KEYWORDS: **Preeclampsia, Lipid peroxidation, Lipid profile, Antioxidants.**

BHAGYASHREE K BHUYAR
Department of Biochemistry D Y Patil Medical College Kolhapur.

INTRODUCTION

Pregnancy is a physiological stress in which many changes occur in the milieu interior of the body, more and more stress is being laid on the biochemical changes, which occur in the blood during the normal pregnancy and becomes exaggerated in complications of pregnancy like pre-eclampsia[1]. Pre-eclampsia is defined as a pregnancy-specific syndrome observed after the 20th week of pregnancy with systolic blood pressure of (140 mm of Hg or diastolic blood pressure of ≥90 mm of Hg accompanied by significant proteinuria (i.e., urinary excretion of (0.3 g protein in a 24-h specimen). In women with pre-eclampsia, blood pressure usually returns to baseline within days to weeks after delivery [2]. Pre-eclampsia is a complex multisystem disorder seen exclusively in the human species. Worldwide, it is a leading cause of maternal and fetal morbidity and mortality [3]. Pre-eclampsia is a hypertensive disorder which develops in late pregnancy and is usually associated with placental hypoxia and dysfunction [6]. Various factors are implicated in the pathogenesis of pre-eclampsia, including genetic, immune, vascular and oxidative stress [7]. Pre-eclampsia occurs during second and third trimester of pregnancy and is more common in nulliparous women. Proteinuria is an important sign of pre-eclampsia and Chesley (1985) rightfully concluded that the diagnosis is questionable in its absence[1]. More oxidative stress in pre-eclampsia results in lipid peroxides, reactive oxygen species and super oxide anion radicals to cause endothelial injury and dysfunction, platelet and neutrophil activation, increased cytokines, superoxide radical production and endothelial damage in a vicious cycle.[4] Lipid peroxides are generated when free radicals interact with polyunsaturated fatty acids in the cell membrane and in plasma lipoproteins. This process can become self-perpetuating, leading to a cascade of lipid oxidation[3]. The increased lipid peroxidation leads to the consumption of antioxidants. This leads to reduction in levels of nonenzymatic antioxidants such as Vitamins A, C, and E, erythrocyte thiol, and glutathione as well as enzymatic antioxidants such as glutathione peroxidase and superoxide dismutase[3]. Uric acid is water soluble and a weak serum antioxidant. Nevertheless, the patients with pre-eclampsia show hyperuricemia, which mean that the serum levels doesn't protect the pre-eclamptic patients against free radical activity [1]. An increase in resistance to angiotensin, a predominance of lipid metabolism over glucose utilization and an increased synthesis by the liver of thyroid and steroid-binding proteins, fibrinogen and other proteins are characteristic of pregnancy. Plasma lipids and lipoproteins undergo both quantitative and qualitative changes during pregnancy. There is a gradual 2-3 fold increase in triglyceride levels and these levels reach their peak (200-300mg/dl) at term and gradually fall thereafter approaching pre-pregnancy levels in 6 weeks postpartum. By the 36th week of gestation, VLDL and other lipoprotein particles increase their triglyceride content proportionately. Total cholesterol levels at term change less dramatically with only a 50-60% rise above pre-pregnancy levels. The cholesterol increase in LDL is proportional to that of total cholesterol. Striking changes occur in circulating lipids in normal pregnancy and these are accentuated in pre-eclampsia[5]. Triglycerides and Fatty acid levels are elevated and these changes antedate clinically evident disease by weeks to months. Levels of cardio protective, HDL-C are reduced, whereas levels of a variant of LDL, small dense LDL are strongly increased. All these change revert towards normal shortly after delivery[5]. The present study has been undertaken to determine the change in serum levels of lipid profile, i.e. Triglyceride and other parameters in women with pre-eclampsia.

MATERIALS AND METHODS

The study was carried out in 30 pre-eclampsia primi patient and 30 normotensive primi pregnant controls who attended the outpatient and inpatient departments of Kempegowda Institute of Medical Sciences, Bangalore during the year 2011-12. The institutional ethical committee approved the study protocol.

SOURCES OF DATA

Inclusion criteria

Cases of pre-eclampsia primi patients in the age group of 18 to 30 years and with gestation age more than 20 weeks with hypertension and proteinuria.

Controls on normotensive primi pregnant women in the age group of 18 to 30 years and more than 20 weeks of gestation.

Exclusion criteria

Elderly primi gravida subjects, gestational diabetes, chronic hypertension, multiple gestation, those with family history of pre-eclampsia, acute and chronic infections, renal diseases, liver diseases, endocrine disorders, smokers, alcoholics and with history of multivitamin intake.

Method of collection of data

Informed consent was taken from patients and controls. A pre-structured and pre-tested proforma was used to collect the data. Baseline data, including age and BMI, detailed medical history, clinical examinations and relevant investigations were included as part of the methodology.

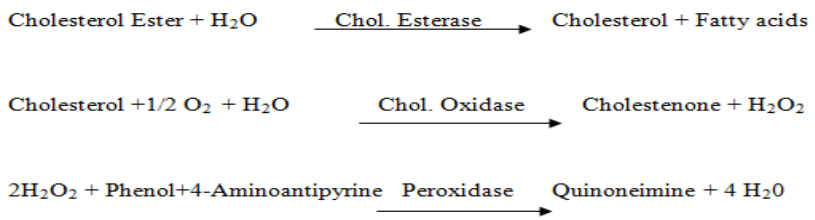
Specimen collection

Blood: 5 ml plain venous blood sample after overnight fasting was obtained by venepuncture from both cases and controls. This was followed by centrifugation and then sample was processed immediately. Estimations of fasting blood glucose, blood urea, serum creatinine, serum total cholesterol, serum triglycerides, HDL cholesterol, were performed using the serum. Serum VLDL-Cholesterol was calculated using the formula, VLDL=S.TG/5. LDL cholesterol was calculated from the values of total cholesterol, triglycerides and HDL cholesterol by applying Friedewald's equation. LDL-C was estimated by direct method where TG values were more than 400mg/dl. TC/ HDL and LDL/HDL ratio are determined.

Urine: Overnight fasting urine sample was collected in a clean, dry container and was tested for sugar and albumin immediately.

1. Estimation of Total Cholesterol in Serum. (Enzymatic method- Cholesterol oxidase/ Peroxidase) [19]

Principle: Free and esterified cholesterol in the sample originates, by the coupled reactions described below, produces a coloured complex which is measured at 510 nm. The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (quinoneimine).



Reagents

- Pipes 35 mmol/l
- Cholesterol Esterase ≥ 0.2 U/mL
- Cholesterol Oxidase ≥ 0.1 U/mL
- Peroxidase ≥ 0.8 U/mL
- 4-Aminoantipyrine 0.5 mmol/L
- Phenol 28 mmol/L
- Sodium cholate 0.5 mmol/L
- Cholesterol standard 200 mg/dl

***Reagent preparation*:** Reagent and standard are provided ready to use.

Procedure: The assay was carried out using A25 biosystem auto analyzer.

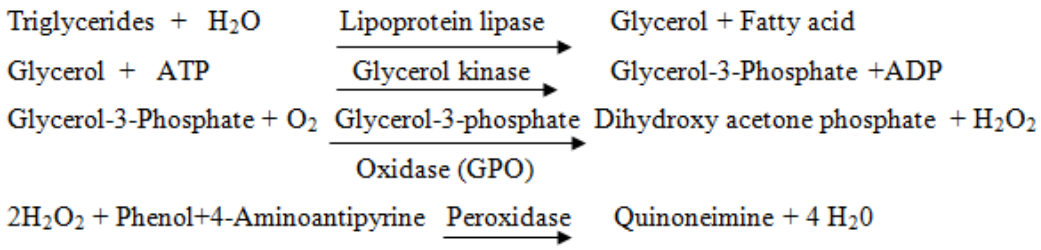
Reference range

Normal serum level

- Up to 200 mg/dl : Desirable
- 220-239 mg/dl : Borderline High
- > 240 mg/dl : High

2. Estimation of Serum Triglycerides (Enzymatic method – Glycerol phosphate / peroxidase) [20]

Principle: Glycerol released from hydrolysis of triglycerides by lipoprotein lipase is converted by glycerol kinase into glycerol –3-phosphate, which is oxidized by glycerol phosphate oxidase to dihydroxy acetone phosphate and hydrogen peroxide. In presence of peroxidase, hydrogen peroxide oxidizes phenolic chromogen to a red coloured compound.



The intensity of colour developed for triglycerides is measured at 510 nm.

Reagents

- Pipes 45 mmol/ L
- Lipoprotein lipase ≥ 100 U/ml
- Glycerol kinase ≥ 1.5 U/ml
- Glycerol phosphate oxidase ≥ 4U/ml
- Peroxidase ≥ 0.8 U/ml
- 4-aminoantipyrine 0.75 mmol/L
- Magnesium chloride 5 mmol/L
- 4-Chlorophenol 6 mmol/ L
- ATP ≥ 0.9mmol/ L
- Triglyceride standard (Glycerol equivalent) 200mg/dl

Reagent preparation: Reagent and standard are provided ready to use.

Procedure: The assay was carried out using A 25 biosystem auto analyzer.

Reference range

Normal serum levels

- Up to 150 mg/dl : Normal
- 150-199 mg/dl : Borderline-high
- 200-499 mg/dl : High
- > 500 mg/dl : Very high

3. Estimation of Serum High-Density Lipoprotein – Cholesterol (Direct detergent method) [21, 22]

Principle: The cholesterol from LDL, VLDL, and chylomicrons, are broken down by the cholesterol oxidase in an enzymatic accelerated non – color forming reaction. The detergent present in the reagent B, solubilises cholesterol from HDL in the sample. The HDL–C is then spectrophotometrically measured at 610 nm by means of the coupled reactions described below.

Reagents

Reagent A

Good's buffer, Cholesterol Oxidase < 1 U/mL, Peroxidase < 1 U/mL, N,N-bis(4-sulfobutyl)-m-toluidine (DSBmT) 1 mmol/L, Accelerator 1 mmol/L

Reagent B

Good's buffer, Cholesterol Esterase <1.5 U/mL, 4-Aminoantipyrne 1 mmol/L, Ascorbate oxidase < 3 KU/ L, Detergent

HDL calibrator (Human serum). Reconstitute with 1 ml of distilled water.

***Reagent prepar*ation:** Reagent are provided ready to use

***Procedure*:** The assay was carried out using A25 biosystem auto analyzer.

Reference Range

Normal serum level

< 40mg/dl: Low (High risk for cardiovascular disease)

≥ 60mg/dl: High (Low risk for cardiovascular disease)

***4. Estimation of Low-Density Lipoprotein Cholesterol.*[8, 23]**

Serum LDL cholesterol is calculated by Friedwald's equation

$$\text{LDL cholesterol} = \text{Total cholesterol} - \left(\text{HDL cholesterol} + \frac{\text{triglycerides}}{5}\right)$$

LDL was estimated by direct method when TG values were > 400 mg/dl

Reference range

- Up to 100mg/dl Optimal
- 100-129mg/dl Near optimal/above optimal
- 130-159mg/dl Borderline high
- 160-189mg/dl High
- >190mg/dl Very High

***5. Estimation of Very Low-Density Lipoprotein Cholesterol.*[15]**

Serum VLDL-Cholesterol (S.VLDL-C) is calculated using the formula:

$$\text{VLDL Cholesterol} = \frac{\text{Triglycerides (mg/dl)}}{5}$$

Reference Range

Normal serum level**:** 06-40 mg/dl

6. TC / HDL and LDL/HDL ratio are determined

RESULTS AND OBSERVATIONS

The present study is undertaken to evaluate the significance of serum triglyceride and other parameter level in pre-eclampsia.30 pre-eclampsia cases were considered for the study. 30 ages matched normotensive primi pregnant were chosen as controls.

Statistical test used: arithmetic mean, standard deviation, student paired t test

Distribution of study sample according to age group

The distribution of the study samples according to the age is given in Table 1 and graphically represented in fig.1. The cases and controls are

divided into 3 groups (≤ 20years, 21-24yrs, ≥ 25yrs). Maximum numbers of cases are in the age group of ≥ 25yrs (53.34%) and maximum numbers of controls are in the age group of 21-24yrs (63.33%).

Distribution of study sample according to gestational age group

The distribution of the study samples according to the gestational age is given in Table 2 and graphically represented in fig.2. The cases and controls are divided into 2 groups (22-28 weeks and 29-34 weeks). Maximum numbers of cases are in the gestational age group of 29-34 weeks (56.67%) and maximum numbers of controls are in the gestational age group of 22-28 weeks (53.33%).

Comparison of Blood Pressure between cases and controls

Comparison of Blood Pressure between cases and controls are shown in the Table 3 and graphically represented in fig.3, respectively. The mean value of systolic blood pressure among cases as compared to controls was statistically significant, (p value < 0.001,t test value 17.02) and mean value of diastolic blood pressure among cases as compared to controls was statistically significant,(p value < 0.001,t test value 28.31).

Comparison of lipid profile between cases and controls

Comparison of lipid profile between cases and controls are shown in the Table 4.The mean serum total cholesterol levels are higher among pre-eclampsia cases as compared to controls. The difference is statistically not significant (p value > 0.05, t test value 1.11).Distribution of controls and cases, according to serum total cholesterol levels are graphically represented in fig.4. The mean serum triglyceride levels are higher among pre-eclampsia cases as compared to controls. The difference is statistically significant (p value < 0.001, t test value 3.83). Distribution of controls and cases, according to serum Triglyceride level is graphically represented in Fig.5. The mean serum HDL-C levels are lower among pre-eclampsia cases as compared to controls. The difference is statistically not significant (p value > 0.05, t test value 0.88). The mean serum LDL levels are higher among pre-eclampsia cases as compared to controls. The difference is statistically not significant (p value > 0.05, t test value 1.24). The mean serum VLDL levels are higher among pre-eclampsia cases as compared to controls. The difference is statistically not significant (p value > 0.05, t test value 1.83). Distribution of controls and cases, according to serum HDL, LDL, VLDL levels are graphically represented in Fig. 6. The mean serum total cholesterol/ HDL-C ratio levels are higher among pre-eclampsia cases as compared to controls. The difference is not statistically significant (p value 0.09, t test value 1.69). Distribution of controls and cases, according to serum total cholesterol/ HDL-C ratio levels are graphically represented in Fig. 7. The mean serum LDL-C / HDL-C ratio levels are higher among pre-eclampsia cases as compared to controls. The difference is not statistically significant (p value 0.07, t test value 1.79). Distribution of controls and cases according to serum total cholesterol/ HDL-C ratio levels are graphically represented in Fig. 8.

Table 1
Age distribution of cases and controls

Age in years	Cases		Controls	
	Number	%	Number	%
≤ 20	4	13.33	2	6.67
21-24	10	33.33	19	63.33
≥25	16	53.34	9	30.00
Total	30	100.00	30	100

Figure 1
Bar diagram showing age distribution of cases and controls

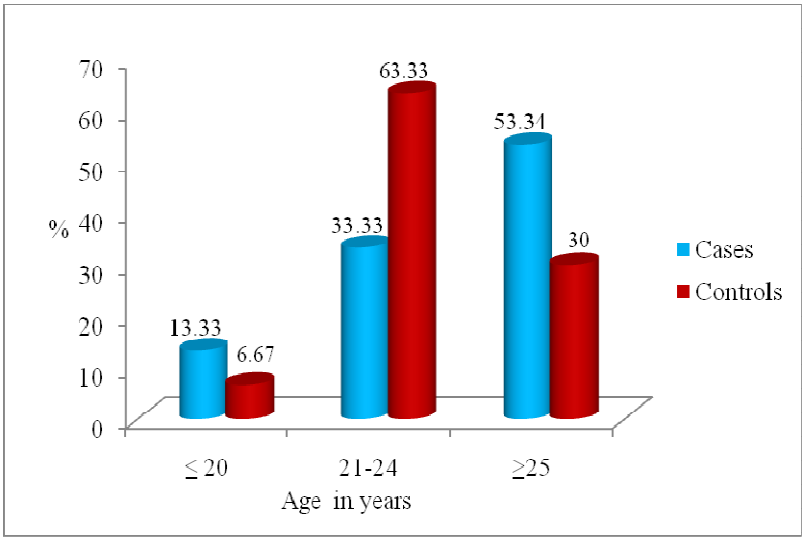


Table 2
Distribution of gestational age in cases and controls

Gestational age in weeks	Cases		Controls	
	Number	%	Number	%
22 - 28	13	43.33	16	53.33
29 - 34	17	56.67	14	46.67
Total	30	100	30	100

Figure 2
Bar diagram showing distribution of gestational age in cases and controls

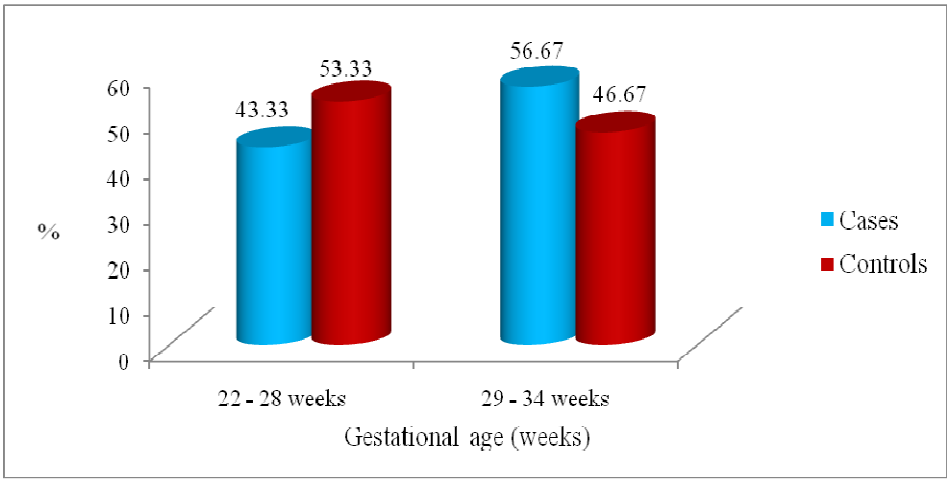


Table 3
Blood Pressure Levels in cases and controls

		Cases	Controls	t test values	P values
BP (mm Hg)	SBP	167.07 ± 12.82	123.93 ± 5.32	17.02	0.001
	DBP	98.67 ± 2.43	78.4 ± 3.08	28.31	0.001

Figure 3
Bar diagram showing Blood pressure ranges in cases and Controls

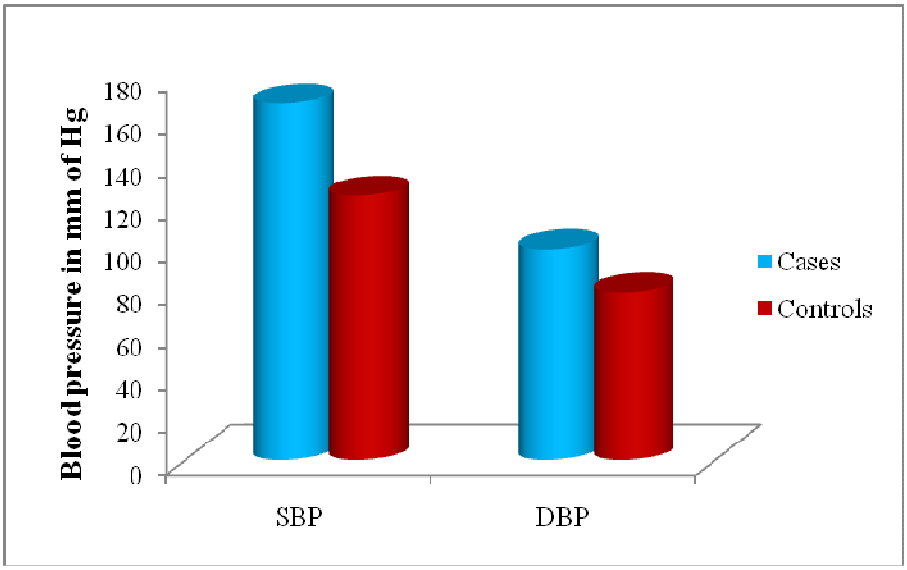


Table 4
Lipid profile of cases and controls

Lipid Profile	Cases	Controls	t test value	p values
TC (mg/dl)	164.1 ± 31.04	155.2 ± 30.99	1.11	0.05
TG (mg/dl)	174.98±69.32	120.2±36.50	3.83	0.001
HDL (mg/dl)	37.62 ± 9.58	39.92 ± 10.70	0.88	0.05
LDL (mg/dl)	98.63 ± 19.7	91.13 ± 26.50	1.24	0.05
VLDL (mg/dl)	31.95 ± 15.45	25.15 ± 13.18	1.83	0.05
TC / HDL	4.61 ± 1.38	4.08 ± 0.99	1.69	0.09
LDL / HDL	2.77 ± 0.85	2.4 ± 0.79	1.79	0.07

Figure 4
Bar diagram showing total cholesterol level in cases and controls

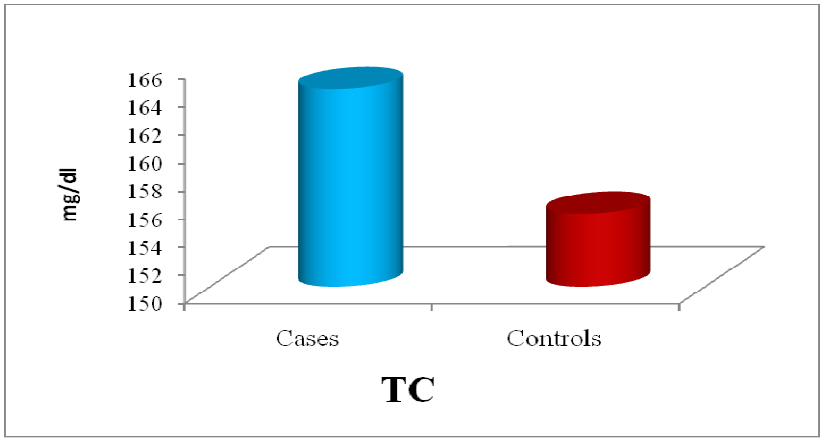


Figure 5
Bar diagram showing triglyceride level in cases and controls

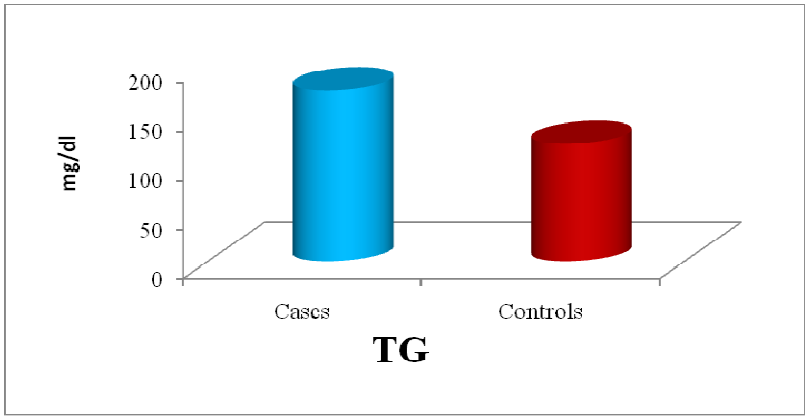


Figure 6
Bar diagram showing HDL, LDL and VLDL levels in cases and Controls

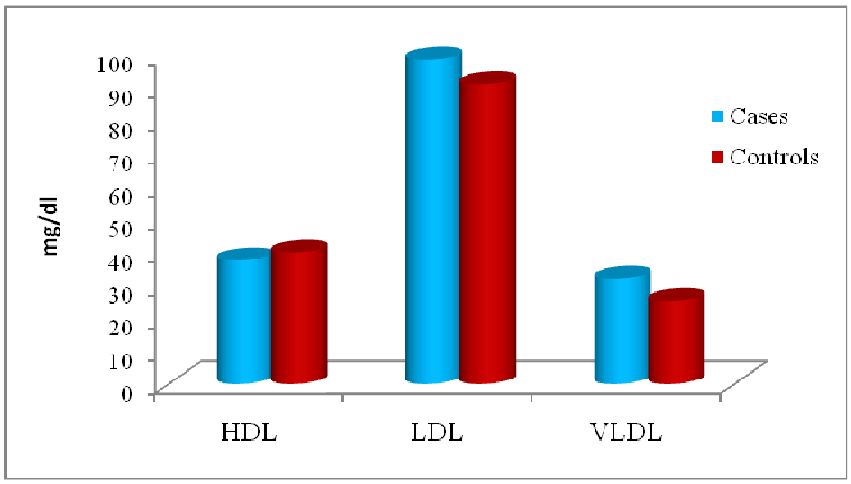


Figure 7
Bar diagram showing TC/ HDL ratio of cases and controls

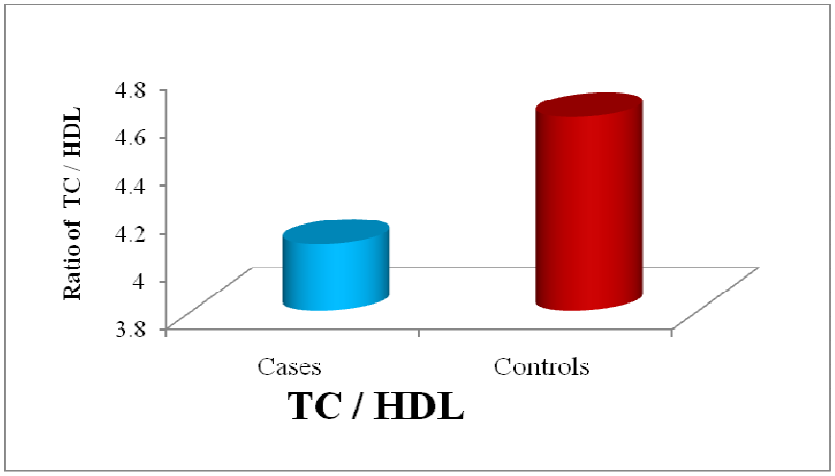
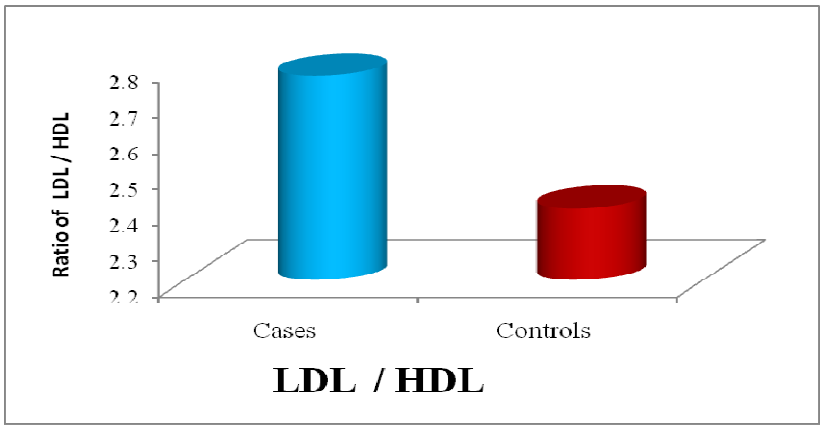


Figure 8
Bar diagram showing LDL/ HDL ratio of cases and controls



DISCUSSION

Preeclampsia remains one of the most serious complications of pregnancy. The pathophysiology of the disease remains poorly understood. The exact cause of preeclampsia remains elusive; placental ischemia, immune maladaptation, genetic factor is probably all involved to some extent. In normal pregnancy the diameter of the spiral arteries increases greatly due to trophoblastic invasion of the spiral arteries in the decidual and myometrial segments of the placental bed, whereas in preeclampsia such physiological adaptation does not occur[24]. Abundant evidence indicates reduced placental perfusion in preeclampsia[25]. Implantation is superficial in preeclampsia. In particular, cytotrophoblasts fail to invade the spiral arterioles. As a result, these vessels do not enlarge, severely compromising their ability to deliver maternal blood in the intervillous space. Predisposing to the medical condition[26]. Recent investigations suggest that endothelial cell injury may be the initiator of the pathophysiological events of preeclampsia[13]. Free radicals and other damaging reactive oxygen species, such as the superoxide anion, are produced in oxidative metabolic and physiological processes. Their activity is thought to increase during pregnancy and especially during preeclampsia [14]. Feto-placental unit may be the origin of oxygen free radicals and lipid peroxides [27,28]. Reactive oxygen species can cause cellular damage by oxidizing nucleic

acids, proteins and membrane lipids[9]. They may also influence vascular tonicity; either indirectly by inactivating the endothelium derived relaxing factor, which is nitric oxide, and reducing the release of prostacyclin or directly by contracting smooth muscles [29]. Such events establish a cycle ultimately leading to manifestations of preeclampsia [12]. Thus uncontrolled lipid peroxidation may play an important role in the pathophysiology of preeclampsia. Preeclampsia is associated with an imbalance between the oxidant and antioxidant status. Preeclamptic patients are exposed to increased oxidative stress. Either placental hyper secretion of lipid peroxides or decreased placental antioxidant enzyme activity can lead to endothelial dysfunction. Insufficient antioxidant capacity leads to oxidative stress, and subsequently, oxidative injury may occur in both the maternal and placental compartment[14]. Uncontrolled lipid peroxidation may contribute to various disease processes via disruption of membrane lipids and cell components [12]. Lipid peroxidation of membrane associated fatty acids and cholesterol may alter cell membrane fluidity and permeability, causing cell membrane damage [10]. The byproducts of tissue lipid peroxidation propagate further lipid peroxidation in the same tissue and at sites distal to areas of initial damage [29]. In a study by J.T.Uotila et al and Hideaki, a number of reports indicate that blood levels of lipid peroxidation products are elevated in women with preeclampsia relative to normal pregnancy [30]. A.K.Poranen et al., demonstrated that, more placental production of lipid peroxides has been to be abnormally increased in preeclampsia [27]. Preeclampsia is associated with increased utilization of antioxidants. In study by Y. Wang et al, their studies have demonstrated decreased plasma levels of ascorbic acid compared to normal pregnant women [28]. Riza Madazli et al., shows that decrease in plasma antioxidant levels seen in preeclampsia is most probably due to the increased lipid peroxidation [12].

Vascular contact with placenta originated circulating peroxidation products may cause dysfunction of the vascular endothelium by promoting peroxidative damage of endothelial cell membranes. Since antioxidant deficiency is a cause of lipid peroxide accumulation, ascorbic acid therapy may alter the disease process if initiated in early gestation in patients at risk. Further studies are needed to clarify the effectiveness of prophylactic antioxidant therapy in preeclampsia [12]. Uric acid is another well known low molecular weight water soluble plasma antioxidant. Although an effective scavenger of aqueous peroxide radicals, its antioxidant power against potent oxidative reactions is weak. Mehmet Hama et al., shoes that the patients with preeclampsia show hyperuricemia, which means that the plasma samples does not protect the pre-eclamptic patients against free radical activity [14]. A significant positive correlation between serum uric acid and E-GSHPx again suggests the possible role for uric acid as an important antioxidant (Ames et al. 1981). Perhaps the rise of uric acid in pre-eclampsia is not merely a nonspecific reflection of kidney damage, but a sign of antioxidative response together with the rise in GSHPx, possibly related to the pathogenesis of pre-eclampsia [32]. Thus, it is important to emphasize that in pregnant women serum creatinine levels of 88µmol per liter (1mg per deciliter) may indicate, substantial kidney involvement. Urate clearance also decreases, and often to a greater degree than does glomerular filtration; thus, hyperuricemia can be an early indicator of preeclampsia [31]. Thus, in preeclampsia, placental abnormality and the associated metabolic changes cause increased oxidative stress [14]. Normal pregnancy is characterized by gestational increase in total and LDL cholesterol followed by a progressive decrease during the puerperium. There is a general consensus that these lipids are not further increased in preeclampsia [11]. In the present study an attempt has been made to assess the plasma levels of lipid parameters such as TC, TG, HDL– C, LDL-C and VLDL-C. The lipid profile, in the present study is characterized by an increase in TG concentration ($P < 0.001$). Total-C, HDL – C & LDL-C, VLDL-C levels remain unchanged. This observation is similar to that of Farah Kahioliz et al 2000[18]. Elevation in plasma triglyceride in PIH has been reported in several studies. According to Sattar et al 1997[16], raised plasma TG may be

a potential contributor to endothelial dysfunction. Several studies have shown that endothelial dysfunction is related to hyperlipidemia.Roberts et al 1989, Kokia et al 1990[16, 17]. The mechanisms driving the abnormal elevation in triglycerides and in preeclampsia are unclear. Metabolic patterns resembling “Syndrome X” or “Insulin resistance syndrome” are more common in pre-eclampsia”. In one possible scenario, heightened insulin resistance occurring in preeclampsia would increase fatty acid mobilization from visceral fat, promote overproduction of VLDL by the liver, and suppress activity of post heparin lipoprotein lipase, resulting in elevated serum free fatty acids and triglycerides [11]. PIH and related disorders are known to affect the functions of various organs involved in lipid and lipoprotein metabolism. The vascular lesions of PIH and arterial lesions of atherosclerosis share a common pathophysiological pathway which involves lipid metabolism[33]. The interaction of plasma lipids, free radicals, and endothelial cells is hypothesized to be of major importance in the early development of vascular dysfunction in diabetes [11]. Whether such interactions contribute to the pathophysiological mechanisms of preeclampsia warrants further analysis.

CONCLUSION

Serum triglyceride and other parameter levels in pre-eclampsia cases have been evaluated with age and BMI matched controls.

- Serum Triglycerides levels are significantly higher in pre-eclampsia patients compared with controls.
- Serum TC, HDL, LDL, VLDL, TC/ HDL ratio and LDL/ HDL-C ratio levels were not significantly higher in pre-eclampsia compared with controls.

The present study is consistent with previous studies suggesting that lipid peroxidation and lipid profile appears to be of immense value in understanding the pathogenesis of preeclampsia. In preeclamptic patients antioxidants may be utilized to a greater extent to counteract free radical mediated cellular changes, resulting in the reduction of plasma antioxidant levels.

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