ABSTRACT

The present study was carried out on two groups of pregnant Iraqi women. Group one consisted of 45 preeclampsia; 23 mild and 22 severe preeclampsia compared to second group consisted of 25 normotensive pregnant women. The two groups were with same age, body mass indices and gestational age. Serum lipid peroxidation marker malondialdehyde (MDA), lipid profile, leptin, total antioxidant capacity (TAO) and paraoxanese activity (PON) were measured. Mean systolic and diastolic blood pressure between preeclamptic and control group showed a marked difference \( (p<0.01) \) ranging from 154.67 ± 4.2 and 118.73 ± 3.7mmHg in severe preeclampsia, 135.3 ± 4.8 and 95.45 ± 4.2 mmHg in mild preeclampsia compared to 120.2 ± 1.88 and 70.50 ± 2.2 mmHg in control respectively. Serum MDA level was significantly higher in women with severe preeclampsia (3.51 ± 0.45 µmoles /dl) than mild preeclampsia (2.52 ± 0.14 µmoles /dl) compared with (0.92 ± 0.12 µmoles /dl) in control group, when adjusting to cholesterol, the peroxidation remained significantly increased MDA/Cholesterol ratio: 1.41 vs. 1.18 pmol/L. The serum triglycerides and cholesterol were higher in women with preeclampsia especially in the severe preeclampsia. Mean serum leptin level was significantly high \( (p<0.01) \) in all preeclamptic (76 ±5.3ng/ml), when compared with a control group (26 ± 3.3ng/ml) and the value of serum leptin level was much higher in severe preeclamptics (76 ±5.3ng/ml) than in women with mild preeclampsia (42±4.1ng/ml). All the variables of the lipid profile of preeclamptic patients were found to be significantly elevated as compared to controls. The total lipid profile was also compared to the severity of preeclampsia and total cholesterol was found to be significantly raised \( (p <0.01) \) in severe preeclampsia when compared to mild. On correlating serum leptin with lipid profile again total cholesterol was found to be significantly high \( (p <0.05) \) in preeclamptic group compared to controls.

A significant reduce in TAC status and in paraoxanese activity (PON) was noticed in severe and mild preeclampsia than control group. We conclude that hypercholesterolemia leads to excessive lipid peroxidation. Coexistent diminution in antioxidant activity leads to an imbalance between prooxidants and antioxidants, resulting in oxidative stress. Oxidative stress and elevated atherogenic index may contribute to atherogenicity in preeclampsia.
INTRODUCTION

Preeclampsia is a pregnancy-specific condition characterized by hypertension and proteinuria that remits after delivery [1]. Although its exact etiology is unknown, maternal symptoms are thought to be secondary to endothelial cell dysfunction [2]. It has been suggested that free radicals are likely promoters of maternal vascular malfunction, as reactive oxygen species, particularly superoxide anions, evoke endothelial cell activation [3]. Markers of lipid peroxidation have been noted to be increased in the plasma of women with preeclampsia [4]. Preeclampsia is associated with elevated lipid peroxidation and reduced antioxidant status. Preeclampsia is an endothelial disease with a major involvement of lipid mediated oxidative damage. A consistent positive association between maternal dyslipidemia and the risk of preeclampsia has already been found. Leptin is a peptide hormone of 16 kDa molecular weight comprising...
167 amino acids. Leptin is a product of (obese) ob/ob gene produced by adipose tissue and is synthesized by placenta during pregnancy resulting in increased serum leptin level with increasing gestational age particularly in preeclampsia and level declines in post partum period [5]. The major source of leptin is the adipose tissue, but it can also be produced by other organs, including the placenta [6]. This anti-obesity hormone decreases food intake and increases energy expenditure, thereby reducing body weight and adiposity [7]. It also modulates glucose metabolism by increasing insulin sensitivity [8] and activates the sympathetic nervous system [9, 10]. Furthermore, leptin has been implicated in the control of the reproductive function, including embryonic development and implantation [11]. Leptin can also be considered as a pro-inflammatory cytokine that belongs to the type I cytokine super family and has structural similarity with interleukin-6 [12]. Increasing evidence suggests that leptin is involved in the regulation of innate and adaptive immune responses and inflammation [13]. Anim-Nyame [14] in his longitudinal study had shown that plasma leptin level in early gestational age precedes the significant risk of preeclampsia with rise in maternal serum leptin concentration and can be used as a marker of preeclampsia, while Ning Y [15] observed a strong linear component of trend in preeclampsia with increased maternal plasma leptin concentration, as per him each 10 ng/ml increase in leptin concentration was associated with 30% increase in preeclampsia risk.

Circulating leptin levels are significantly higher in pregnant than in non-pregnant women [17] and there is a further increase in complicated pregnancies, such as gestational diabetes mellitus, preeclampsia and intrauterine growth restriction [18-21]. Preeclampsia is a pregnancy specific disorder and its etiopathogenesis is complex and incompletely understood. Preeclampsia is associated with elevated lipid peroxidation and reduced antioxidant status. Preeclampsia is an endothelial disease with a major involvement of lipid mediated oxidative damage [22]. A consistent positive association between maternal dyslipidemia and the risk of preeclampsia have already been found. Preeclampsia is a syndrome defined as the onset of hypertension and proteinuria after 20 weeks of gestation in previously normotensive and non proteinuria women. Although the precise mechanism of disorder remained elusive but according to new emerging consensus it is a complex polygenic trait in which maternal and fetal genes as well as environmental factors are involved [23].

PON1 (E.C 3.1.1.2) an esterase enzyme associated with HDL exerts a protective effect against oxidative damage of circulating cells and lipoproteins[24]. The hydrolysis of the oxidized phospholipids by PON destroys the biologically active lipids in mildly oxidized LDL(25) . Thereby, HDL and its associated PON interrupt a process that would otherwise lead to oxidative damage. PON thus can be an indicator of antioxidant status in preeclampsia. Oxidative stress is known to be increased in preeclampsia. Hence this study was aimed to assess the PON activity and MDA levels in preeclampsia along with the lipid profile and to find their correlation.

The aim of our study is to detect and compare lipid profile, leptin, lipid peroxidation marker malondialdehyde, total antioxidant capacity and paraoxanse activity between pregnant women with normal blood pressure compared with, mild and severe
preeclampsia pregnant patients, and to investigate the correlation between them and the roles of these parameters in aetio-pathogenesis of preeclampsia.

SUBJECTS AND METHODS
The study included 70 pregnant women of age ranging between 16-32 years and gestational age between 28-38 weeks. All the subjects were briefed about the nature of the study and an informed consent was taken. **Inclusion Criteria:** Twenty five normotensive women without any previous history of hospitalization or any medical complication were taken as control. Forty five obstetric patients with singleton pregnancies, diagnosed as having preeclampsia according to ISSHP (International society for the study of hypertension) when they presented with blood pressure > 140/90 mmHg on 2 separate occasions 4 hours apart or a single recording of a diastolic blood pressure of 110 mmHg in association with proteinuria > 2+ on dipstick testing, formed the study group. **Exclusion Criteria:** Pre-existing chronic hypertension; Pre-existing diabetes; Gestational diabetes; Diseases involving kidneys; Diseases involving liver; Known history of any peripheral vascular disease; Twin pregnancy and Smoking or any drug addiction.

All the subjects included in the study were primigravida with same maternal age, gestational age, height and weight. A detailed general physical examination was done and history was taken. The arterial blood pressure in the brachial artery was measured by using a simple mercury sphygmomanometer on right arm in a comfortable sitting position after 10 minutes of rest. Blood pressure was measured using both palpatory and auscultatory methods. The reported values represent the mean of two readings taken at 5 minutes interval. The Fasting blood samples were collected under strict aseptic measures. Samples were analyzed in one run at the end of the study. The blood samples centrifuged at 1500xg for 10 minutes and serum was separated as early as possible and stored at 0 (-20°C) for analysis. Paraoxanase activity (PON) was determined by measuring the rate of hydrolysis of paraoxon by monitoring the increase of absorbance at 412 nm at 37°C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at a pH of 8.5, which was 18290 mol$^{-1}$ cm$^{-1}$ (26). PON activity was expressed as U/L serum. One Unit (U) of PON activity was defined as one micro mol of released p-nitrophenol per liter of serum per minute and expressed in U/L (27). Serum MDA was estimated using 40% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA) and absorbance was recorded at 530 nm. The MDA content was calculated using the molar extinction coefficient 1.56 x 10$^5$ and expressed as nmole/dl(28). Serum total cholesterol, triglyceride and HDL-C were assayed spectrophotometrically by using commercially available kits (Human diagnostics). The concentration of LDL was calculated using commercially available kits (Human diagnostics). Serum leptin was determined by human ELISA kit (Organmetric company, Germany). The total antioxidant capacity of the serum of preeclamptic cases was determined by Koracevic et al’s method (29). The assay measures the capacity of the serum to inhibit the production of TBA reactive substances (TBARS) from sodium benzoate, under the influence of the oxygen free radicals derived from Fenton’s reaction. The reaction was measured...
spectrophotometrically at 532 nm. Antioxidants from the added sample cause suppression of the production of TBARS, and the inhibition of color development is defined as TAC.

Mild and severe preeclampsia was categorized according to the recommendations of the American College of Obstetrics and Gynecology (30). Preeclampsia is considered mild when the blood pressure is > 140/90 mmHg and proteinuria is 300 mg in 24 hours or 1+ or 2+ on dipstick analysis. Severe preeclampsia includes anyone of the following: BP > 160/110 mmHg on two occasions, 6 hours apart, 5 gm proteins in 24 hours urine or 3+ or 4+ on semi quantitative assay, oliguria or urine less than 500 ml in 24 hours, cerebral or visual disturbances, pulmonary oedema or cyanosis, right upper quadrant tenderness, fetal growth restriction, thrombocytopenia and impaired liver function tests.

Data analysis was done on computer package SPSS (Statistical Package for Social Sciences) version 10. The Statistical significance of difference between the mean values of two groups was evaluated by the student's "t" test. The difference in the mean values of the two groups was regarded as statistically significant, if the P-Value was less than 0.05 and it was taken as highly significant, if P-Value was less than 0.001. Correlation Coefficient was detected using Pearson Coefficient of Correlation.

RESULTS AND DISCUSSION

A total of 70 pregnant women were included in this study, 25 women had uncomplicated pregnancies and 45 were diagnosed as preeclampsia (PE). The preeclamptic group was again divided according to the severity of the disease into patients with mild PE (n=23) and patients with severe PE (n=22). The demographic features and clinical data of pregnant women with and without preeclampsia are summarized in table 1. The mean value of systolic blood pressure (SBP) in severe and mild preeclamptic cases were 154.67 ± 7.5 mmHg and 135.3 ± 4.8 mmHg respectively and there was a significant difference (P<0.01) between two cases vs. control. The Diastolic Blood Pressure (DBP) mean in severe and mild preeclamptic cases and control were 118.73 ± 3.7 mmHg and 95.45 ± 4.2 mmHg respectively, and there was a significant difference (P<0.01) between cases vs. control. The mean BMI, in normotensive pregnant group is 25.3 ± 2.22 and 25.53 ± 3.08 in mild preeclamptic cases compared with 26.83 ± 3.28 in severe preeclamptic cases group. P value is more than 0.05, which is not significant. No significant difference was found between clinical features of the preeclamptic and the normotensive pregnant controls other than hypertension, proteinuria, and pretibial edema (p>0.05).

Table (1) Mean ±SD values of Blood Pressure and Body Mass Index in severe, mild preeclampsia Cases and healthy pregnant groups.

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>Severe preeclampsia</th>
<th>Mild preeclampsia</th>
<th>Healthy Pregnant</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>27.7 ± 0.9</td>
<td>26±0.8</td>
<td>27±0.77</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age (W)</td>
<td>34 ± 0.6</td>
<td>33±0.7</td>
<td>33 ±0.67</td>
<td>NS</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.55±0.33</td>
<td>1.58±0.43</td>
<td>1. ± 0.34</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table (2) shows the comparison of the lipid profile variables between preeclamptic group of patients along with the severe and mild PE cases vs. control group. The total cholesterol (p<0.01) and triglycerides (p<0.01) were significantly higher in mild and severe preeclampsia women as compared to the control group Table (2). The LDL-c level was also significantly higher in women with each of mild and severe preeclampsia than in normotensive pregnant women without preeclampsia (p<0.01) while the HDL level was significantly lower in women with severe preeclampsia than mild preeclampsia in comparison with pregnant women without preeclampsia (p<0.05). MDA level and LDL/HDL ratio were significantly higher (p<0.01, p < 0.05) respectively in severe more than mild preeclampsia cases compared with control Table (2). Total antioxidant capacity (TAC) and paraoxasne (PON) activity was significantly lower in women with severe preeclampsia than in mild preeclampsia compared with pregnant women without preeclampsia (p<0.01). Paraoxasne (PON) activity was significantly negative correlated with MDA levels in preeclampsia women (r= -0.63), while a significant positive association of MDA with both systolic and diastolic blood pressures was found in preeclampsia (r = 0.657 for systolic and r = 0.583 for diastolic blood pressure. One of the first biomarkers of lipid peroxidation found to be elevated in the...
plasma of women with preeclampsia was MDA, a major metabolite of lipid peroxide breakdown. MDA is significantly elevated in preeclampsia compared to healthy pregnant women ($p < 0.01$). The increased MDA levels in preeclampsia is known to be due to increased generation of reactive oxygen species and increased oxygen demand along with reduction in activities of enzymes like superoxide dismutase, glutathione peroxidase and decrease in concentration of antioxidants like Vitamin C and Vitamin E. Reactive oxygen species can cause enhanced lipid peroxidation. The values obtained are in close agreement with those reported by Sharma (36). Oxidative stress is a normal phenomenon in normotensive pregnancy; however, in preeclampsia, oxidative stress is exaggerated and may result in a greater potential for endothelial oxidative damage. It has been seen in our study that higher MDA/total antioxidant capacity (TAC) ratio is indicative of oxidative stress in women with preeclampsia. It has been suggested that uncontrolled lipid peroxidation may play a role in the etiology of the preeclampsia; therefore, in preeclampsia can increase oxidative stress and potential free radical damage increases the vasospasm which in turn increases the peripheral resistance, hence DBP increases.

**Table (2) Mean ± SD values of serum lipid profile, Leptin, MDA, PON and TAC in healthy pregnant women (control), mild, severe preeclampsia patients**

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Control(n=25)</th>
<th>MPE(n=23)</th>
<th>SPE(n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>144±13</td>
<td>200±12**</td>
<td>240±14**</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>200±13</td>
<td>228±22*</td>
<td>254±31**</td>
</tr>
<tr>
<td>HDL-c (mg/dl)</td>
<td>46.4±2.7</td>
<td>36.2±3.8*</td>
<td>33.3±2.4</td>
</tr>
<tr>
<td>LDL-c (mg/dl)</td>
<td>128±13</td>
<td>156±21*</td>
<td>172±27**</td>
</tr>
<tr>
<td>VLDL-c (mg/dl)</td>
<td>32±4.8</td>
<td>50±12*</td>
<td>51±8.8*</td>
</tr>
<tr>
<td>PON U/L</td>
<td>144±13</td>
<td>120±11*</td>
<td>100±14**</td>
</tr>
<tr>
<td>MDA (µmoles /dl)</td>
<td>0.92±0.12</td>
<td>2.52±0.14*</td>
<td>3.50±0.26**</td>
</tr>
<tr>
<td>LDL/HDL ratio</td>
<td>3.76±0.62</td>
<td>4.32±0.46</td>
<td>5.34±0.76*</td>
</tr>
<tr>
<td>PON /HDL ratio</td>
<td>3.16±0.67</td>
<td>3.30±0.26</td>
<td>3.09±0.46</td>
</tr>
<tr>
<td>Total antioxidant capacity (µmole/L)</td>
<td>956±66</td>
<td>733±46**</td>
<td>618±53**</td>
</tr>
<tr>
<td>Leptin ng/ml</td>
<td>26±3.3</td>
<td>42±4.1**</td>
<td>76±5.3**</td>
</tr>
</tbody>
</table>

n= Number of subjects. *P < 0.05 when compared to control. **P < 0.01. ***P < 0.001

**Table (3) Correlation between lipid profile and paraoxanse activity vs. serum leptin in healthy pregnant women (control) and in severe preeclampsia patients.**

<table>
<thead>
<tr>
<th>Lipid profile</th>
<th>Control Group Serum leptin (ng/ml)</th>
<th>SPE Serum leptin (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>r = 0.25</td>
<td>r = 0.61</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>r = 0.17</td>
<td>r = 0.68</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>r = 0.32</td>
<td>r = -0.74</td>
</tr>
</tbody>
</table>
Excessive lipid peroxidation occurring in preeclampsia can be attributed to hypercholesterolemia. Hypercholesterolemia promotes the formation of free radicals. Increased oxygen demand to meet the bodily functions in pregnancy is also a contributory factor for the oxidative stress that results in the formation of free radicals. Thus, lipid alterations observed may promote oxidative stress, leading to endothelial dysfunction in preeclampsia. Highly significant reduction in TAC was observed in the severe preeclamptic patients in this study. We have not encountered any study concerning TAC and preeclampsia. There are several proposed mechanisms for explaining the antiatherogenic properties of HDL. PON is calcium dependent esterase, is exclusively bound to the HDL fraction of serum. [37]. PON prevents the oxidative modification of LDL and is responsible for the antioxidant activity of HDL [38]. Preeclampsia has been associated with atherogenic wall changes in the uteroplacental bed [39]. These changes consequently results in necrosis of vessel wall and accumulation of lipid laden foam cells with oxidized LDL. In preeclampsia, the placental damage is progressive and can be compensated for sometime depending on the severity of initial placental defect and intrinsic placental antioxidant capacity [40].

Mean serum leptin level was significantly high in preeclamptic (76±5.3ng/ml) when compared with a control group (26. ±3.3ng/ml).It has been observed that Mean value of serum leptin level was much higher in severe preeclampsics (76 ±5.3) than in women with mild preeclampsia (42±4.1ng/ml). All the parameters correlated positively and significantly with increased blood pressure. Elevated plasma leptin concentration appears to be a marker of preeclampsia independently or along with other parameters of preeclampsia could be used to reduce the severity of preeclampsia thus avoiding risk effects of preeclampsia to mother and fetus. The association of serum leptin concentration with serum lipids has been inconsistent and in a study conducted by Hallikainen [41] who was found that high serum leptin concentration associated with high cholesterol synthesis and low cholesterol absorption but not with serum lipids. Leptin has been associated with atherosclerosis and has been shown to interfere with lipoprotein profiles [42].Leptin levels during PE were associated with atherogenic lipid profiles which may contribute to increased risk of cardiovascular disease that has been linked to hyperleptinaemia.

These alterations may be the result of oxidative stress, thus affecting abnormal lipid profile and atherogenesis. Serum leptin has been suggested to be involved in the proatherogenic process by increasing oxidative stress [43] and leptin has been reported to induce oxidative stress in cultured endothelial cells [44].Our results positively showed that changes in serum leptin level could contribute to lipid metabolism alterations in patients with PE which might be related to Proatherogenic process and increases the cardiovascular risk.
Decreased TAC is indicative of a disturbance in the antioxidant system which could be due to diminished individual antioxidants. A decrease in essential antioxidants, vitamins A and E and carotene, have been reported in preeclampsia[45-46]. Thus, we conclude that the hypercholesterolemia observed in our study resulted in excessive lipid peroxidation and generation of free radicals. Diminution in TAC adds to the imbalance between prooxidants and antioxidants, resulting in oxidative stress, which in turn may cause endothelial damage. Raised atherogenic index (AI) suggests increased susceptibility to atherogenicity in preeclamptics. Dyslipidemia appears to be the starting point of this chain of events. Further studies on the role of genetic factors (apoE polymorphism) in causing dyslipidemia may contribute to the understanding of the mechanism underlying endothelial dysfunction in preeclampsia.

Maternal plasma lipids are significantly elevated during pregnancy. In preeclampsia dyslipidemia pattern of increased concentration of triglyceride, cholesterol and LDL and decreased concentration of HDL have been noticed. Peroxidation of lipids brings about changes in its molecular structure and these changes becomes more marked when the damaged lipids are the constituents of biological membrane. Low antioxidant levels may aggravate prooxidants injury on endothelial cells, altering prostacycline / thromboxane balance and culminating in preeclampsia. Hence mechanisms that prevent oxidation of LDL have received increased attention in recent years. One such mechanism is prevention of LDL oxidation by PON.

Preeclampsia is characterized by profound lipid abnormalities [47]. Preeclampsia and atherosclerosis are both endothelial diseases with an important involvement of lipid-mediated oxidative damage, and their lipid profiles are remarkably similar. [48]. Our findings revealed that there was a significant increase in serum levels of triglycerides, total cholesterol, LDL-cholesterol, VLDL-cholesterol in preeclamptic women when compared to controls. Serum levels of HDL-cholesterol were significantly decreased when compared to normotensive pregnant women. These findings are in agreement with the previous studies [22, 49, and 50]. On comparing lipid variables according to the severity of preeclampsia, a positive statistical significant difference was found between mild and severe group. Hypertensive subjects frequently have higher cholesterol levels than normotensive subjects. A positive relationship between serum cholesterol level and blood pressure has been reported in many epidemiological studies [50]. Information concerning relations between serum leptin levels and the levels of serum lipids are limited [51-52], although the hormone is produced by adipocytes and in most cases reflect the fat content. To evaluate this, we calculated the correlation between serum leptin and lipid profile.

A significant positive correlation between serum leptin and total cholesterol in our population we found but the correlation with other lipid fractions was found to be non significant. The association of serum leptin concentration with serum lipids has been inconsistent and in a study conducted by Hallikainen, [41] was found that high serum leptin concentration was associated with high cholesterol synthesis and low cholesterol absorption but not with serum lipids. Leptin has been associated with atherosclerosis and has been shown to interfere with lipoprotein profiles [42]. Leptin levels during preeclampsia were associated with atherogenic lipid profiles which may contribute to
increased risk of cardiovascular disease that has been linked to hyperleptinaemia. These alterations may be the result of oxidative stress, thus affecting abnormal lipid profile and atherogenesis (53-54). Serum leptin has been suggested to be involved in the proatherogenic process by increasing oxidative stress [43] and leptin has been reported to induce oxidative stress in cultured endothelial cells [44]. Our results positively showed that changes in serum leptin level could contribute to lipid metabolism alterations in patients with preeclampsia which might be related to proatherogenic process and increases the cardiovascular risk.

From this comparative cross-sectional study, it is concluded that Leptin levels during preeclampsia are strongly associated with total serum cholesterol and triglyceride whereas relation with other lipid variables is significant. On comparing different lipid variables according to severity of preeclampsia, a relationship was found between severe preeclampsia, leptin and total cholesterol. During preeclampsia when leptin level rises with severity, total cholesterol also rises which may contribute to the elevated cardiovascular risk that has been linked to hyperleptinaemia.

REFERENCES


