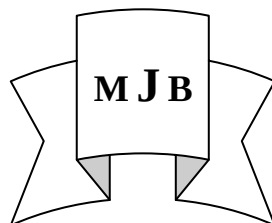


The Relationship between Insulin Resistance and Oxidative Stress in Pre-eclamptic Women in Babylon Governorate

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Abstract

Background: Pre-eclampsia is a medical condition characterized by a BP of 140/90 mm Hg or greater after 20 weeks' gestation in a woman with previously normal BP and who has proteinuria (≥ 0.3 g protein in 24-h urine specimen).

Aim of the study: In this study, we tried to establish the relationship between insulin resistance and oxidative stress in sera of pre-eclamptic women and their effect on the clinical severity of disease.

Patients and Methods: The study was conducted in Babylon Maternity and Pediatric Teaching Hospital for the period starting from April 2011 to the end of August 2011 on the fifty pre-eclamptic women [fifteen in the second trimester (G1), twenty in the third trimester (G2) with mild pre-eclampsia and fifteen patients in the third trimester with severe pre-eclampsia (G3)]. Fasting serum insulin, Fasting blood glucose, Total antioxidant level, Lipid profile, Malondialdehyde and uric acid were determined. Insulin resistance was calculated according to HOMA score. The results were compared with results obtained from age and BMI-matched control groups which consisted of forty apparently healthy pregnant women [fifteen in the second trimester (G4) and twenty five women in the third trimester (G5)].

Results: The results of the present study revealed a significant increment in insulin resistance (IR-HOMA) in sera of (G1) compared with (G4) ($P < 0.05$), also there was a significant increment in IR-HOMA in sera of (G2&G3) compared with control group (G5) ($P < 0.01$), and also there was a significant increment in IR-HOMA in (G3) group compared with (G2) ($P < 0.01$). Serum total antioxidant status (TAS) was significantly declined in (G1, G2&G3) compared with (G4&G5) ($p < 0.01$) and there was a significant decline in TAS seen in sera of (G3) compared with (G2) ($P < 0.05$). Serum MDA concentration was significantly increased in (G1, G2 and G3) compared to (G4 and G5) ($P < 0.01$). Also serum MDA concentration was significantly higher in (G3) compared with (G2) ($p < 0.05$). S. uric acid concentration was significantly higher in (G1&G2) compared to (G4 and G5) ($P < 0.05$). Also S. uric acid concentration was significantly increased in (G3) compared with (G5) ($p < 0.01$), and also S. uric acid was significantly higher in (G3) compared with (G2) ($p < 0.05$). Serum TG & VLDL-c were significantly higher in (G1&G2) compared with (G4, G5) ($p < 0.05$). Also a significant increment in TG & VLDL-c were seen in (G3) compared with (G5) ($p < 0.01$). There was a significant increment in previous parameters in (G3) compared with (G2) ($p < 0.05$). No significant difference was seen in the serum levels of TC, LDL-c, HDL-c in sera of all groups. Finally no significant correlation between IR-HOMA and TAS in sera of all groups ($p > 0.05$), No significant correlation between IR-HOMA and S. MDA in sera of different groups $p > 0.05$ except in G4 there was a significant positive correlation between IR-HOMA and MDA ($p < 0.01$) and also no significant correlation between TAS and MDA in different groups ($P > 0.05$) except in (G1) a significant positive correlation was seen between TAS and MDA ($p < 0.05$).

العلاقة بين مقاومة الأنسولين و جهد التأكسد في النساء الحوامل المصابات بمرض ما قبل الشنج
في محافظة بابل

الخلاصة

مرض ما قبل الشنج هو حالة طبية تتميز بارتفاع ضغط الدم بما يساوي (140/90) ملم زئبق أو أكثر بعد الأسبوع العشرين من الحمل عند النساء طبيعيات ضغط الدم سابقاً. ويتميز المرض بوجود البروتين في الأدرار بما يساوي (0.3) غرام أو أكثر عند جمع الأدرار لمدة أربعة وعشرين ساعة .

الهدف من الدراسة هو محاولة ايجاد العلاقة بين مقاومة الأنسولين و جبهة التأكد في مصول مريضات ما قبل الشنج وتأثير تلك المتغيرات على شدة ظهور المرض.

المرضى والطرائق: أجريت الدراسة في مستشفى بابل للنسائية والأطفال للفترة من نيسان ٢٠١١ ولغاية آب ٢٠١١ على خمسين امرأة مصابة بمرض ما قبل الشنج الحلمي [خمس عشر امرأة في الفصل الثاني من الحمل (مجموعة ١) ، عشرون امرأة في الفصل الثالث من الحمل مصابة بدرجة خفيفة بالمرض (مجموعة ٢) وخمس عشر مريضة في الفصل الثالث مصابة بالمرض بدرجة حادة (مجموعة ٣)] . وقد تم قياس كل من [الأنسولين ، كلوكوز الدم وكلاهما في حالة الصيام ، مستوى مضادات الأكسدة الكلية ونطاق الدهون ، مالونداي أدهايد وحامض اليوريك] . مقاومة الأنسولين تم حسابها وفقا لمقياس هوما (HOMA score) . النتائج تم مقارنتها مع النتائج المستحصلة لمجاميع السيطرة والتي تتقارب في الأعمار ومقياس كتلة الجسم مع المرضى و تتضمن اربعين من الحوامل السليمات [خمس عشر في الفصل الثاني من الحمل (مجموعة ٤) و خمسة وعشرين امرأة في الفصل الثالث (مجموعة ٥)] .

النتائج: أظهرت نتائج هذه الدراسة زيادة معنوية في مقاومة الأنسولين في مصل المجموعة (١) مقارنة بالمجموعة (٤) ($p < 0.05$) . كذلك هنالك زيادة معنوية في مقاومة الأنسولين في مصول المجاميع (٢،٣) مقارنة بمجموعة السيطرة (٥) ($p < 0.01$) . وكذلك وجدت زيادة معنوية في مقاومة الأنسولين عند المجموعة (٣) الشديدة المرض مقارنة بالمجموعة (٢) الخفيفة المرض ($p < 0.01$) . نقصان معنوي في مضادات الأكسدة الكلية عند المجاميع (١،٢،٣) مقارنة بالمجاميع (٤،٥) ($p < 0.01$) . ولوحظ كذلك نقصان معنوي في مضادات الأكسدة في مصل المجموعة (٣) مقارنة بالمجموعة (٢) ($p < 0.05$) . لوحظ ايضا أن تركيز المالونداي أدهايد أعلى بدرجة ملحوظة عند المجاميع (١،٢،٣) مقارنة بالمجاميع (٤،٥) ($p < 0.01$) مع زيادة ملحوظة في تركيز المالونداي أدهايد في المجموعة (٣) مقارنة بالمجموعة (٢) ($p < 0.05$) . زيادة معنوية في تركيز حامض اليوريك عند المجاميع (١،٢) مقارنة بالمجاميع (٤،٥) ($p < 0.05$) . وحصول زيادة معنوية في تركيز حامض اليوريك في المجموعة (٣) مقارنة بالمجموعة (٥) ($p < 0.01$) مع زيادة معنوية في المجموعة (٣) مقارنة بالمجموعة (٢) ($p < 0.05$) . اضافة الى حصول زيادة ملحوظة في الكليسيريدات الثلاثية والبروتينات الدهنية الواطئة الكثافة جدا في المجاميع (١،٢) مقارنة بالمجاميع (٤،٥) ($p < 0.05$) . كذلك وجد ان هنالك زيادة معنوية في الكليسيريدات الثلاثية والبروتينات الدهنية الواطئة الكثافة جدا عند المجموعة (٣) مقارنة بالمجموعة (٥) ($p < 0.01$) وكذلك زيادة معنوية في الكليسيريدات الثلاثية والبروتينات الدهنية الواطئة الكثافة جدا عند المجموعة (٣) مقارنة بالمجموعة (٢) ($p < 0.05$) . عند قياس الكوليسترول الكلي و البروتينات الدهنية واطئة الكثافة والبروتينات الدهنية عالية الكثافة لوحظ انه لا يوجد أي فرق معنوي بين المجاميع ($p > 0.05$) . وأخيرا يوجد ارتباط لكنه غير معنوي بين مقاومة الانسولين و مضادات الاكسدة في مصول المجاميع كافة ($p > 0.05$) . ارتباط غير معنوي بين مقاومة الانسولين والمالونداي ادهايد في مصول المجاميع المختلفة ($p > 0.05$) . ماعدا المجموعة (٤) حيث وجد ان هنالك ارتباط ايجابي معنوي بين مقاومة الانسولين والمالونداي ادهايد ($p < 0.01$) . كذلك يوجد ارتباط غير معنوي بين مضادات الاكسدة والمالونداي ادهايد بين المجاميع المختلفة ($p > 0.05$) ماعدا ارتباط ايجابي معنوي بين مضادات الاكسدة و المالونداي ادهايد عند المجموعة (١) ($p < 0.05$) .

Introduction

Pre-eclampsia occurs in about 3-5% of pregnancies and is an important cause of fetal and maternal morbidity and mortality world wide [1]. Pre-eclampsia occurs during second and third trimester of pregnancy and it is more common in nulliparous women [2]. It is characterized by blood pressure of 140/90 mm Hg or rise in systolic blood pressure of more than 30 mmHg or diastolic blood pressure of more than 15 mmHg after 20 weeks of gestation, in conjugation with proteinuria ≥ 300

mg/24 hours or greater or equal to 1+ or 100 mg/dl by dipstick response [3].

Pre-eclampsia refers to a set of symptoms rather than any causative factor, and there are many different causes for the condition. It appears likely that there are substances from the placenta that can cause endothelial dysfunction in the maternal blood vessels of susceptible women. While blood pressure elevation is the most visible sign of the disease. It involves generalized damage to the maternal endothelium, kidneys, and liver, with the release of

vasoconstrictive factors being secondary to the original damage[4].

Pre-eclampsia is mild in 75% of cases and severe in 25% of them. In its extreme, the disease may lead to liver and renal failure, disseminated intravascular coagulopathy (DIC), and central nervous system (CNS) abnormalities. If pre-eclampsia-associated seizures develop, the disorder has developed into the condition called eclampsia [5]. Mild pre-eclampsia is defined as the presence of hypertension (BP $\geq$ 140/90 mm Hg) on 2 occasions, at least 4 hours apart, but without evidence of end-organ damage in the patient[5]. Severe pre-eclampsia is defined as the presence of one of the following symptoms or signs in the presence of pre-eclampsia: (1) Systolic blood pressure (SBP) of 160 mm Hg or higher and diastolic blood pressure (DBP) of 110 mm Hg or higher on 2 occasions at least 4 hours apart. (2) Proteinuria of more than 5 g in a 24-hour collection or more than 3+ on 2 random urine samples collected at least 4 hours apart.(3) Pulmonary edema or cyanosis.(4) Oliguria (< 400 mL in 24 h).(5) Persistent headaches.(6) Epigastric pain and/or impaired liver function.(7) Thrombocytopenia.(8) Oligohydramnios, decreased fetal growth, or placental abruption [5].

Insulin resistance is decreased ability of target tissues, such as liver, adipose, and muscle, to respond properly to normal circulating concentration of insulin. For example, insulin resistance is characterized by uncontrolled hepatic glucose production, and decreased uptake by muscle and adipose tissue[6]. Insulin resistance and resultant hyperinsulinemia are characteristic of normal pregnancy (increased insulin secretion by the pancreatic β cells, and following initially increased insulin sensitivity,

there follows a progressive increase in insulin resistance throughout the second and third trimesters [7]. Pre-eclampsia may be associated with greater degrees of insulin resistance than characteristic of normal pregnancy [8]. A time when the insulin resistance characteristic of pregnancy is maximal, supports a possible association. Postulated mechanisms through which insulin resistance might increase blood pressure in pregnancy, as in essential hypertensives, include sympathetic nervous system activation [9], renal sodium retention, increased cation transport [10], and associated endothelial dysfunction [11].

Oxidative stress represents an imbalance between the production and manifestation of reactive oxygen species and a biological systems ability to readily detoxify the reactive intermediates or to repair the resulting damage [12]. Oxidative stress may cause endothelial cell dysfunction in pre-eclampsia [13]. Due to the abnormal placentation leads to placental ischemia [14]. The ischemia reperfusion injury to the placenta leads to generation of placental oxidative stress [15]. Oxidative stress may play a role in the pathophysiology of pre-eclampsia [15]. Generation of free radicals increases during pregnancy, and placental mitochondria are the major sources of ROS production [16]. Lipid peroxides are formed and bind to the lipoproteins and are then transported to distant sites in the body [16]. In pre-eclamptic women, lipoperoxidation products, especially malondialdehyde (MDA) increase [17]. In contrast to normal pregnancy, pre-eclampsia is characterized by increased oxidative stress and decreased antioxidants [18]. Cumulative evidence in recent years has shown that a biochemical imbalance in pre-eclampsia occurs with an increase of oxidative stress and

lipoperoxidation and at the same time, a deficient antioxidant protection [19]. Several important non-enzymatic antioxidants are significantly decreased (vitamin C, vitamin A, vitamin E, β -carotene, glutathione levels, and iron-binding capacity are lower in the maternal circulation of women with pre-eclampsia than in women with a normal pregnancy) [20], and enzymatic antioxidants (superoxide dismutase SOD, and glutathione peroxidase GPx) are decreased [21]. In the presence of deficiency of superoxide dismutase activity, nitric oxide (NO) reacts with superoxide to form peroxynitrite (ONOO-) which is a strong oxidizing agent capable of initiating lipid peroxidation. Since NO is a potent vasodilator, at times when SOD is deficient, not only the vasodilating action of nitric oxide is impaired but also a strong oxidizing agent is produced [22].

In the course of normal gestation, serum lipid and lipoprotein levels undergo variations, and triglycerides (TG), cholesterol and phospholipids are elevated. These changes are considered a reflection of increased metabolic demands by the mother's organism [23]. Serum low-density lipoprotein cholesterol (LDL-c) levels increase as gestation progresses, probably due to the hepatic effects of estradiol and progesterone [24]. High-density lipoprotein cholesterol (HDL-c) levels increase. It is thought that estrogen is responsible for elevating HDL-c levels during the first half of gestation [24]. In PE, TG levels are raised above those seen in normal pregnancy, especially in the third trimester [25]. In combination with the other metabolic changes, the abnormal lipoprotein metabolism in PE is considered to be a maternal adaptive response to placental insufficiency [25].

Patients and Methods

This study was conducted in Babylon Maternity and Pediatric Teaching Hospital for the period starting from April 2011 to the end of August 2011. Fifty pregnant women with pre-eclampsia (fifteen of them in the second trimester of pregnancy while thirty five of them were in the third trimester of pregnancy). Those patients were admitted to hospital for further investigations, monitoring, and/or delivery. These selected pre-eclamptic pregnant women were divided into three groups according to gestational age and severity of disease:-

- Group (G1) includes 15 pre-eclamptics in the second trimester.
- Group (G2) includes 20 pre-eclamptics in the third trimester with mild pre-eclampsia.
- Group (G3) includes 15 pre-eclamptics in the third trimester with severe pre-eclampsia.

Control groups include forty apparently healthy pregnant women which attended the primary antenatal care center which were also divided into two groups according to gestational age:-

- Group (G4) includes 15 normotensives in the second trimester.
- Group (G5) includes 25 normotensives in the third trimester.

After an overnight fasting (14 hours) five milliliters of venous blood were obtained from pre-eclamptic and healthy pregnant women, then collected in tubes without anticoagulants and were left for 15 minutes at room temperature to clot. The serum was separated by centrifugation at 2000 xg for approximately 10 minutes. Fasting blood glucose was assessed by enzymatic method using a kit supplied by Biomeghreb company (Tunis) [26]. Insulin level was measured by enzyme linked immunosorbent assay (ELISA) using a kit supplied by DRG Instruments company (Germany)

[27].An indirect method used for the assessment of insulin resistance IR-HOMA (Homeostasis model assessment HOMA) was calculated using the equation mentioned below[28].

$$\text{HOMA} = [\text{glucose (in mmol/L)} \times \text{insulin (in } \mu\text{IU/mL)}] / 22.5$$

Patients were considered as insulin resistant when $\text{HOMA} \geq 2.6$ [28]. Total antioxidants status (TAS) measured by colorimetric method using a kit supplied by RANDOX Company (United Kingdom) [29].Uric acid was assessed by enzymatic method using a kit supplied by Biomeghreb company (Tunis) [30]. Serum MDA assay was based on the colorimetric reaction of MDA with thiobarbituric acid (TBA) forming an MDA-TBA2 adduct that absorbs strongly at 532 nm [31].Serum total cholesterol TC[32], triglyceride TG[33], high density lipoprotein-cholesterol HDL-C [34] were measured using kits provided by Biolabo,SA company (France).VLDL-cholesterol concentration was calculated by dividing triglycerides value by 2.22. LDL-cholesterol concentration was calculated by using Friedewald equation [35].

Statistical analysis

Analysis of the data was carried out using SPSS 17 software for Windows. Data were expressed as mean $\pm$ SD.The statistical analysis based on ANOVA test to determine the differences between groups and within groups. Correlation, regression and correlation coefficient (r).P value of < 0.05 was considered to be statistically significant.

Results

The studied groups had approximately similar ages, BMI, and gestational ages, where as systolic (SBP) and diastolic blood pressure (DBP) values were significantly

different in the pre-eclamptic groups than in healthy pregnant $p < 0.001$ as in tables (1) and (3). In this study it has been found a significant increment in insulin resistance in pre-eclamptic group in 2nd trimester (G1) compared with normal pregnant women in same trimester (G4) $p < 0.05$, also there was a highly significant increment in insulin resistance in pre-eclamptic groups (G2&G3) in 3rd trimester compared with control group in the same trimester (G5) $p < 0.01$, and also there was a highly significant increment in severe PE (G3) compared with mild PE (G2) $p < 0.01$. Serum total antioxidants revealed a highly significant decline in pre-eclamptic groups (G1, G2 & G3) compared with normal pregnant women (G4 & G5) $p < 0.01$, and there was a significant decline in TAS in sera of severe (G3) compared with mild (G2) $p < 0.05$. S. MDA concentration showed a highly significant increment in the pre-eclamptic groups (G1, G2 and G3) compared to normal pregnant groups (G4 and G5) $P < 0.01$. Also S. MDA concentration was significantly higher in severe pre-eclampsics (G3) compared with mild pre-eclampsics (G2) $p < 0.05$. S.uric acid concentration was significantly higher in the pre-eclamptic groups (G1&G2) compared to normal pregnant groups (G4 and G5) $P < 0.05$. Also s.uric acid concentration was a highly significant increase in severe pre-eclampsics (G3) compared with normal pregnant women (G5) $p < 0.01$. The results showed that s.uric acid was significantly higher in severe PE (G3) compared with mild PE (G2) $p < 0.05$. Serum TG & VLDL-c were significantly higher in pre-eclamptic group (G1& G2) compared with normal pregnant groups (G4& G5) $P < 0.05$. Also, a highly significant increase in TG & VLDL-c were seen in sera of severe PE (G3) compared with

control (G5) $P < 0.01$. Serum levels of TG & VLDL-c were significantly higher in severe (G3) compared with mild (G2) $P < 0.05$. No significant difference were seen in TC, LDL-c, HDL-c in sera of pre-eclamptic groups (G1, G2 & G3) compared with control groups (G4 & G5) $P > 0.05$, with no significant difference between severe (G3) and mild (G2) $P > 0.05$, as shown in table (2). Beside these results, it was found no significant negative correlation between IR-HOMA and TAS in (G1 and G4), Also no significant positive correlation between IR-HOMA and TAS in (G2, G3 and G5) $p > 0.05$ as in figs (1,2,3,4,5). No significant positive

correlation between insulin resistance (IR-HOMA) and Malonyldialdehyde (MDA) was seen in (G1 & G5) and also no significant negative correlation was seen in (G2 & G3) $p > 0.05$, except in control group G4 there was a significant positive correlation between IR-HOMA and MDA $p < 0.01$ as in figs (6,7,8,9,10). A significant positive correlation between TAS and MDA was noticed in pre-eclamptic group in 2nd trimester G1 $p < 0.05$. While there was no significant positive correlation seen in (G2 and G3), also no significant negative correlation between previous parameters was seen in (G4 and G5) $p > 0.05$ as in figs (11,12,13,14,15).

Table 1 Clinical characteristics of the studied groups

Characters	G1	G2	G3	G4	G5
Number of subjects	15	20	15	15	25
Age/years Mean±SD	28.4±6.8	27.9±6.7	27.9±5.2	28.6±6.6	28.8±7.5
Range	19-41	19-45	19-36	18-40	17-41
BMI Kg/m ² Mean ±SD	34.4±4.0	33.5±4.5	33.5±5.8	32.2±6.0	32.3±5.2
Range	27.7-39.1	27-46.7	24.4-45.6	22.5-45.6	22.9-45.6
Gestational age weeks) (Mean±SD)	24.6±3.1	33.1±3.3	33.5±3.3	23.5±3.1	33.6±3.5
Range	20-28	29-39	29-39	20-28	29-39
SBP mmHg Mean±SD	144.6±6.3	146±5.0	168±8.6	111.3±8.3	114.8±6.5
Range	140-160	140-150	160-180	100-120	100-120
DBP mmHg Mean±SD	92.6±4.5	93.5±4.8	114.6±5.1	71.3±6.3	71.6±6.8
Range	90-100	90-100	110-120	60-80	60-80

Table 2 Biochemical parameters of pre-eclamptic and control groups

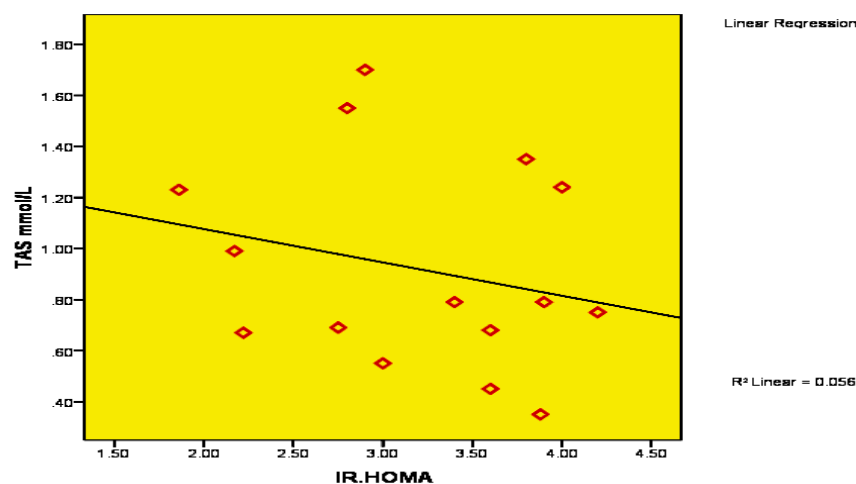
Parameters	G1	G2	G3	G4	G5
FBS mmol/L Mean±SD	4.9±0.56	5.4±1.0	6.0±0.82	4.0±0.51	4.7±1.0
Fasting insulin μIU/ml Mean±SD	14. 8±3.1	17. 5±4.7	21. 2±6.0	10.9±4.5	13.0±5.0
IR-HOMA Mean ±SD	3.2±0.73	4.2±1.44	5.7±2.4	1.92±0.87	2.5±0.86
TAS mmol/L Mean±SD	0.91±0.4	0.76±0.38	0.49±0.19	1.34±0.29	1.1±0.36
MDA μmol/L Mean±SD	4.4±1.2	4.9±1.0	5.8±1.2	3.4±0.72	3.6±0.79
Uric Acid μmol/L Mean±SD	308.7±79.6	322.5±79.2	396.2±144.8	235.3±45.9	267.3±46.1
TG mmol/L Mean±SD	2.34±0.6	2.43±0.52	2.93±1.17	1.83±0.48	1.9±0.54
VLDL-C mmol/L Mean±SD	1.0±0.27	1.0±0.24	1.3±0.51	0.86± 0.21	0.88±0.24
TC mmol/L Mean±SD	5.4±0.89	5.8±1.0	5.9±0.92	4.9±0.9	5.3±0.85
HDL-C mmol/L Mean±SD	1.59±0.47	1.55±0.31	1.55±0.51	1.63±0.43	1.66±0.4
LDL-C mmol/L Mean±SD	2.8±0.99	3.2±1.0	3.4±1.3	2.4±1.0	2.8±0.85

Table 3 Significance of the results among different groups

Parameters	G1 vs G4	G2 vs G3	G2 vs G5	G3 vs G5
Age years P- value	NS	NS	NS	NS
BMI kg/m ² P- value	NS	NS	NS	NS
Gestational age weeks P- value	NS	NS	NS	NS
SBP mmHg P- value	p< 0.001	p<0.001	p< 0.001	p< 0.001
DBP mmHg P- value	p< 0.001	p< 0.001	p< 0.001	p< 0.001

NS : No significant difference between the mean values of the corresponding groups $p > 0.05$.

$p < 0.001$: very highly significant difference.

**Figure 1** Correlation between insulin resistance (IR-HOMA) and total antioxidant status(TAS) in G1 ($r = -0.23$)

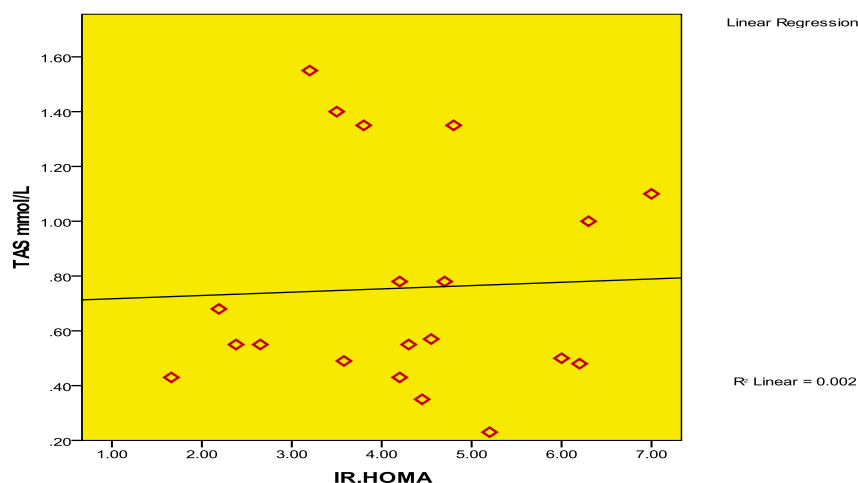


Figure 2 Correlation between IR-HOMA and TAS in G2 ($r=0.044$)

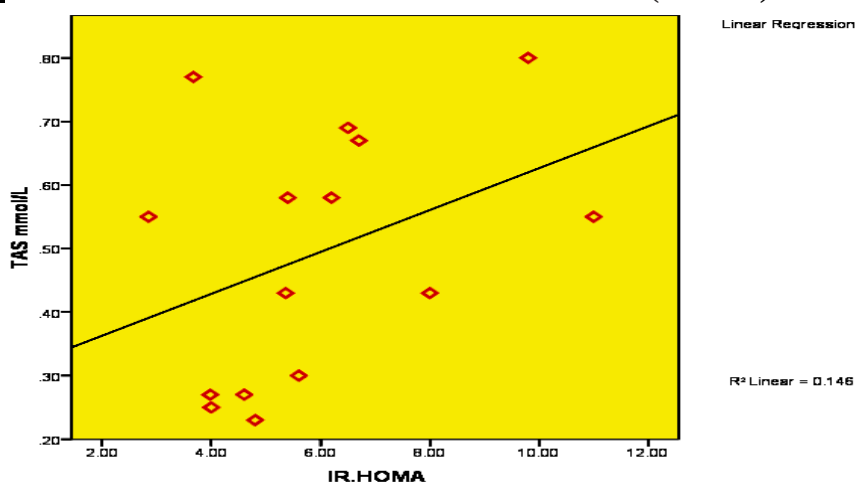


Figure 3 Correlation between IR-HOMA and TAS in G3($r=0.38$)

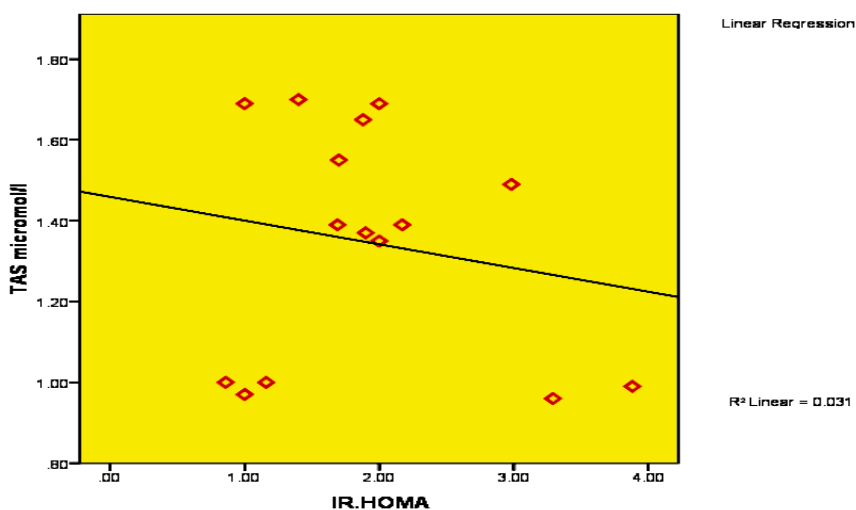


Figure 4 Correlation between IR-HOMA and TAS in G4($r= - 0.17$)

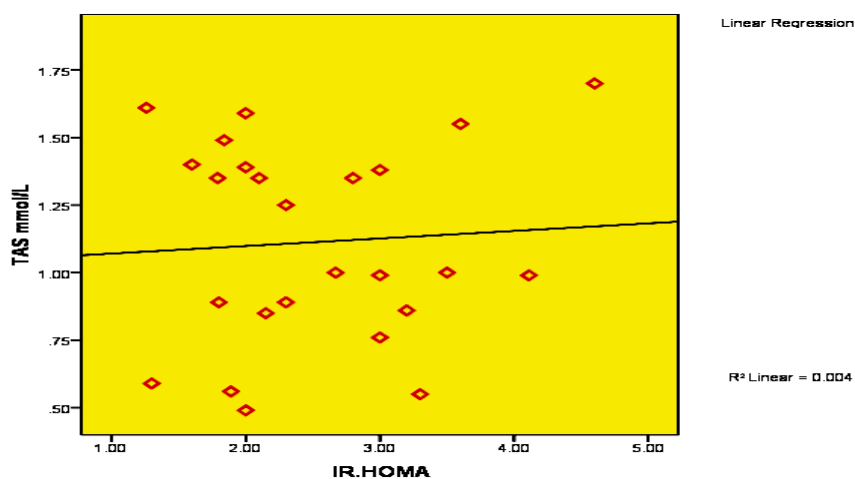


Figure 5 Correlation between IR-HOMA and TAS in G5($r= 0.065$)

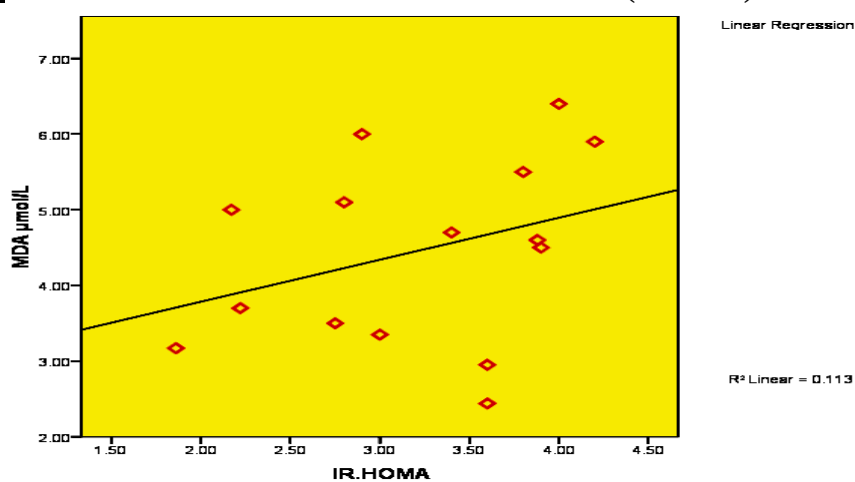


Figure 6 Correlation between IR-HOMA and MDA in G1($r=0.33$)

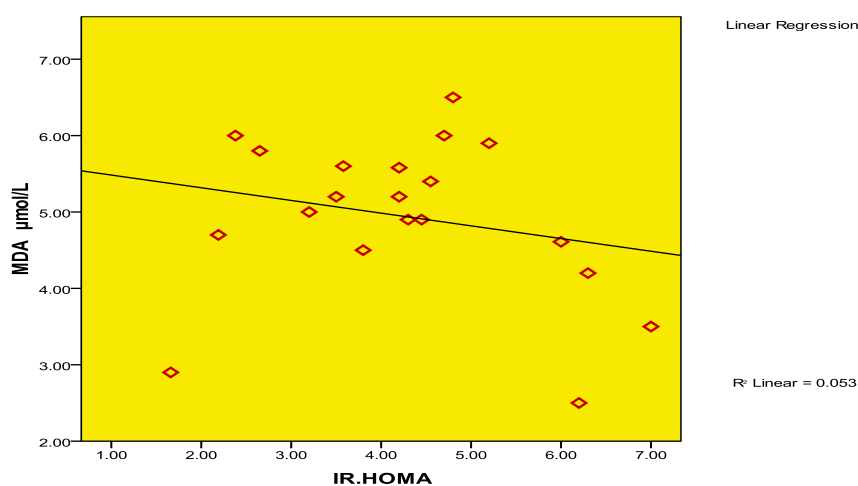


Figure 7 Correlation between IR-HOMA and MDA in G2 ($r= -0.23$)

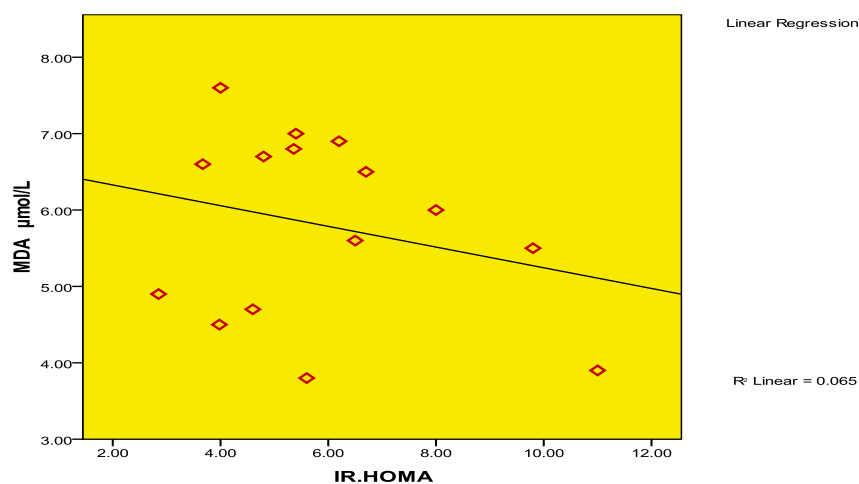


Figure 8 Correlation between IR-HOMA and MDA in G3 ($r = -0.25$)

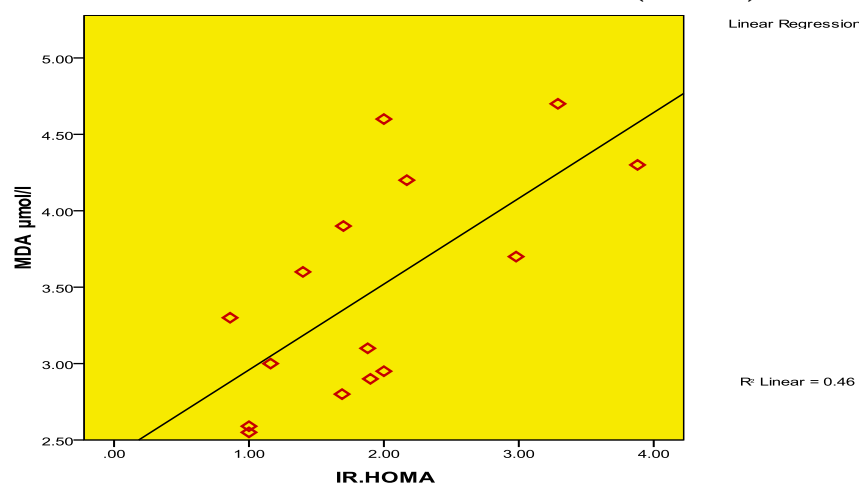


Figure 9 Correlation between IR-HOMA and MDA in G4 ($r = 0.67$)

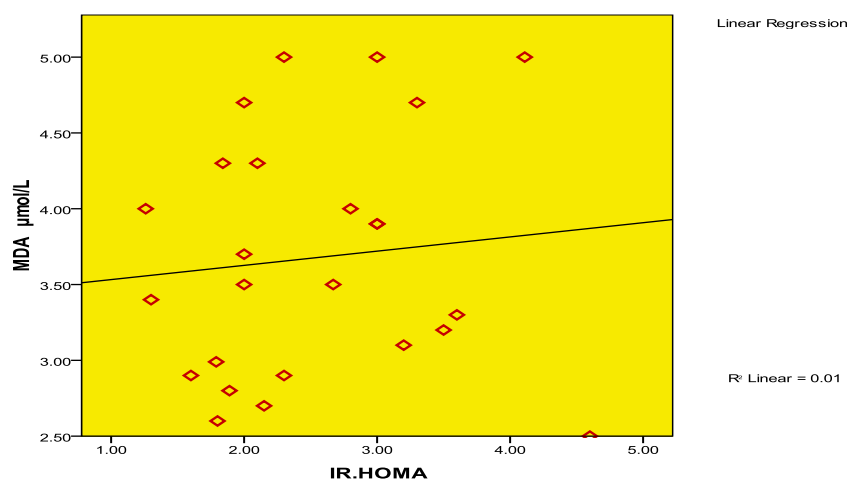


Figure 10 Correlation between IR-HOMA and MDA in G5 ($r = 0.1$)

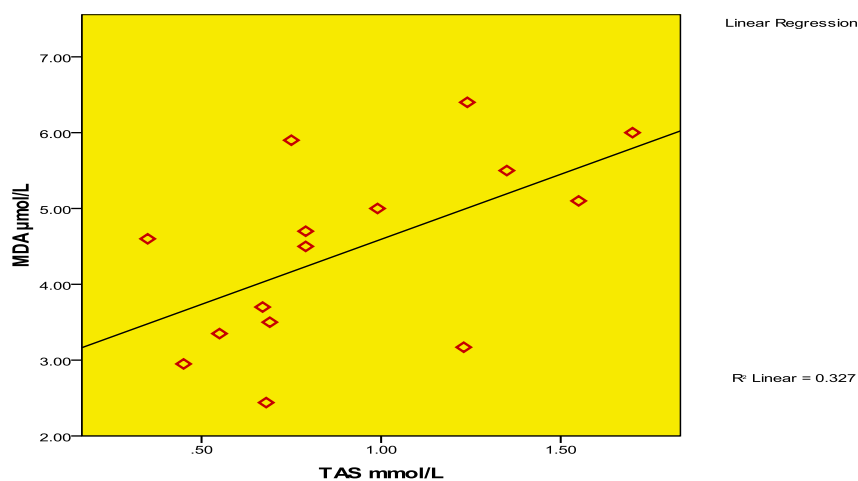


Figure 11 Correlation between TAS and MDA in G1 ($r=0.57$)

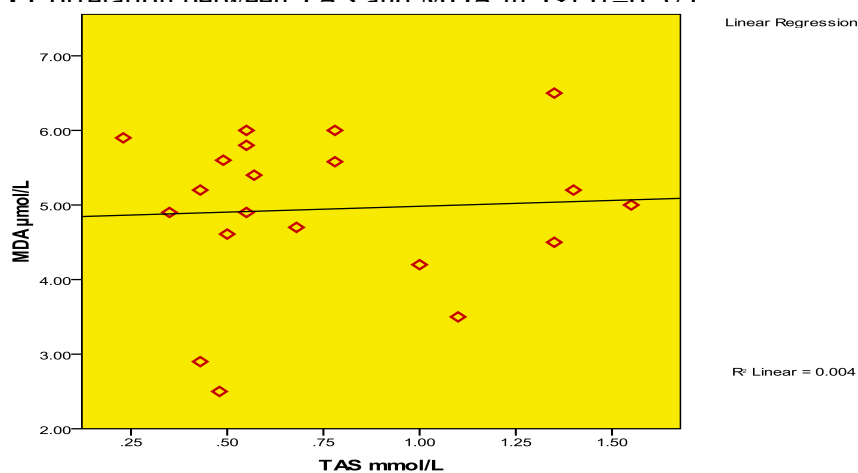


Figure 12 Correlation between TAS and MDA in G2 ($r=0.059$)

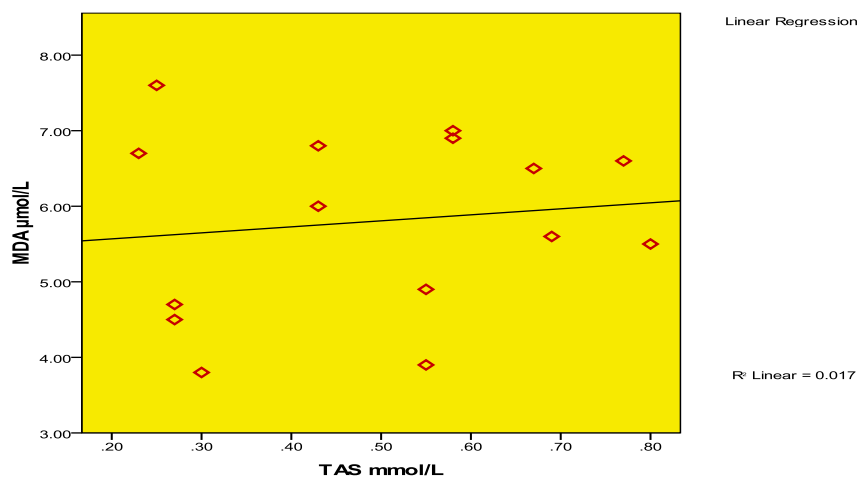


Figure 13 Correlation between TAS and MDA in G3 ($r=0.12$)

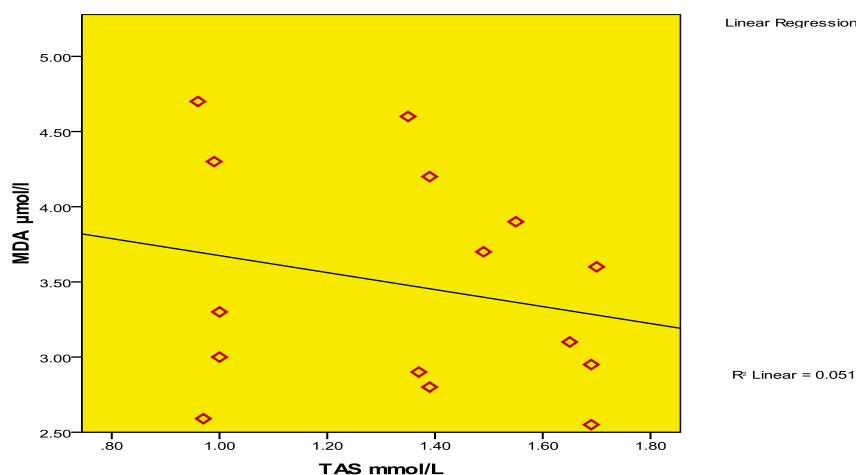


Figure 14 Correlation between TAS and MDA in G4 ($r = -0.22$)

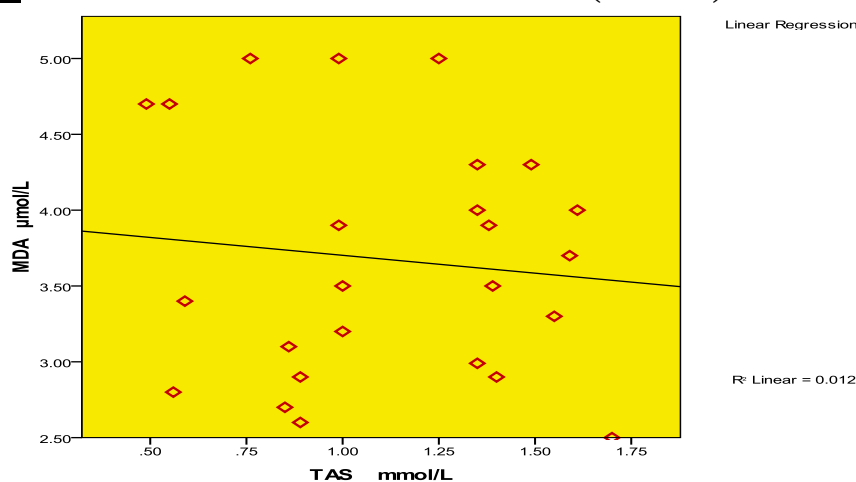


Figure 15 Correlation between TAS and MDA in G5 ($r = -0.1$)

Discussion

The mechanisms by which hyperinsulinaemia/insulin resistance may predispose to pre-eclampsia are not well defined, although there are several plausible hypotheses. (1) DeFronzo *et al.* [36]. found that hyperinsulinaemia inhibited renal sodium transport in the proximal tubule, reducing sodium excretion. Sodium retention could then result in hypertension. (2) Wu *et al.* [37]. reported that insulin enhanced vasoconstriction. Alternatively, hyperinsulinaemia/insulin resistance might affect angiogenic pathways. Increased concentrations of the anti-angiogenic protein sFlt-1 and decreased concentrations of the proangiogenic proteins VEGF and

PlGF are thought to damage vascular endothelium and result in clinical pre-eclampsia. (3) Anderson *et al.* [38]. noted that hyperinsulinaemia was associated with increased plasma norepinephrine levels and increased muscle sympathetic nerve activity, which could elevate blood pressure. Insulin resistance is only one of the components of the metabolic syndrome that is present in pre-eclamptic women. Whether other components are the important factors is an open question. Altered lipids, especially increased free fatty acids, triglycerides and perhaps even increased uric acid, may have relevant pathophysiological effects [39]. Increased insulin resistance also associated with increased oxidative stress, and prostaglandin imbalance (an

increased ratio of the thromboxane levels to prostacyclin levels)[40] . The results of present study were in agreement with result reported by Stefanović- M *et al.* [41] who observed that insulin resistance significantly higher in pre-eclampsics than normotensive women and these increment in insulin resistance was closely related to clinical severity of disease. These results in disagreement with results reported by Teimoori- B *et al.* [42] ,who stated that pathophysiology of pre-eclampsia has no relationship to insulin resistance and insulin resistance did not have any statistical significance in pre-eclamptic and normotensive women at term gestational age. This discrepancy can be attributed to dietary and environmental changes .

Pre-eclampsia is characterized as a state of oxidative stress resulting from increased generation of free radicals and decreased levels of antioxidants, which scavenge free radicals [43]. In patients with pre-eclampsia, antioxidants scavenge the increased free radicals, resulting in lowered antioxidant levels [43]. In our study we found that total antioxidants level was lower in pre-eclamptic women than healthy pregnant women and these results were similar to the results of some studies .such as those done by Rukmini MS *et al.* [44]. Who found that impaired antioxidant activity and the reduction of antioxidants could be the possible cause for the increased lipid peroxidation observed which may cause damage to vascular endothelium resulting in clinical symptoms of pre-eclampsia. In addition our study also demonstrates significantly lower levels of total antioxidant capacity in severe PE compared to mild PE, confirming that the degree of biochemical disorder is closely related to clinical severity .This result is in a good agreement with result reported by Chamy VM *et*

al. [45] who found that reduction in TAS was closely related to clinical severity of pre-eclampsia. Also as a result of oxidative stress was an increased production of lipid peroxides. Placental oxidative stress has been proposed as a promoter of lipid peroxidation, and endothelial cell dysfunction associated with pre-eclampsia[46]. The increased MDA levels in pre-eclampsia 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 [47]. The results of these study was in agreement with results reported by Kashinakunti SV *et al.* [48] who found increase in level of MDA in patients with PE. In addition CHAMY VM *et al.* [45] demonstrated a close relationship between the degree of oxidative stress and the clinical severity of this disease, since levels of lipid damage in the severe PE group was much higher than the mild PE group, suggesting that toxic effects of lipoperoxide lead to hypertension.

The increment in serum uric acid in pre-eclamptic compared with normal pregnant women can be attributed to: (1)Reduction in the clearance of uric acid secondary to the reduction in the glomerular filtration rate, increased absorption and a decrease in the secretion may be the cause for the rise in the level of serum uric acid in Pre-eclamptic women[49]. (2)There may be increased placental production of uric acid secondary to placental ischemia and increased trophoblast shedding that lead to further purine availability for breakdown[50]. In this study the results is in a good agreement with result reported by Kashinakunti SV *et al.* [48]. Who found that the uric acid play an

important role in the pathophysiology of pre-eclampsia. Also Lim K-H *et al.* [51] found an elevated level of uric acid reflects the degree of placental cell destruction as well as severity of disease.

The present study showed significantly increase in TG levels in sera of pre-eclampsics compared with control and may be attributed to the principle modulator of hypertriglyceridemia oestrogen as pregnancy is associated with hyperoestrogenaemia. Oestrogen induces hepatic biosynthesis of endogenous triglycerides, which is carried by VLDL-C [52]. Increased TG levels results in endothelial cell dysfunction and in pre-eclampsia gets deposited in predisposed vessels [53], causes generation of small dense LDL [25] and hypercoaguability [53]. Kornacki J *et al.* [54] who found an elevated serum triglycerides as an important contributing factor of pre-eclampsia. Also the present study showed insignificant difference in TC, LDL-c, HDL-c in sera of pre-eclamptic groups compared with control groups. These results were consistent with the results reported by KASHINAKUNTI SV *et al.* [55] Who observed an insignificant difference in TC, LDL-c, HDL-c parameters of cases and controls. Many of the variations in the lipid profile values between different populations can be attributed to disparate environmental factors and dietary habits. Thus, these variations may contributed to the pathogenesis of pre-eclampsia [55].

Insignificant correlation was seen between IR- HOMA and TAS in all groups. Also the correlation between IR-HOMA and MDA in different groups was insignificant except in (G4) (control group in 2nd trimester) IR- HOMA was significantly positive correlated with serum levels of MDA and that can be attributed to: 1) increased oxidative stress together

with the decreased antioxidative defence seems to contribute to decreased insulin sensitivity with the impaired insulin secretory response and that lead to increase of insulin resistance [56]. 2) Also insulin resistance encourages oxidative stress because hyperglycemia and higher levels of free fatty acids lead to increase ROS production as consequence that lead to increase production of lipid peroxides especially MDA [56]. We found also insignificant correlation between MDA and TAS in different groups except in (G1) (pre-eclamptic group in 2nd trimester) there was positive significant correlation between MDA and TAS and we can attributed that to the increase of free radicals that released from the poorly perfused fetoplacental unit. These free radicals initiate lipid peroxidation by attacking polyunsaturated fatty acids in cell membranes, converting them to lipid peroxides and that lead to increased activity of antioxidant defenses as a compensatory regulation in response to increased MDA production [57].

Conclusions and Recommendations

Insulin resistance is an important part of metabolic syndrome and may be a contributor factor in pre-eclampsia. Our finding suggested presence of insulin resistance in pre-eclamptics and normotensives pregnant but the insulin resistance higher in PE than normal pregnant women. The diminution in TAS in sera of PE added to imbalance between prooxidants and antioxidants would result in oxidative stress, which in turn may cause oxidative stress in pre-eclampsia. Also The present study is consistent with previous studies suggesting that plasma lipid appears to be of immense value in understanding the pathogenesis and elevated serum TG as an important contributing factor of pre-elampsia and these changes in

TG was related with pre-eclampsia especially severe pre-eclampsia.

Finally, we strongly recommend the estimation of antioxidant status and insulin resistance in pre-eclamptic patients, especially in severe cases. Besides, administration of antioxidants is quite necessary to modulate and hence relieve oxidative stress in the corresponding patients.

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