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The Role of Maternal Serum Leptin and Malondialdehyde Levels in Screening and Diagnosis of Gestational Diabetes Mellitus

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Abstract

Objective: To evaluate the role of maternal serum leptin and malondialdehyde (MDA) levels in screening and diagnosis of gestational diabetes mellitus (GDM).

Methods: Two hundred and twelve pregnant patients which were followed-up in our clinic were enrolled. Between the 24th and 28th gestational weeks we performed single step (75 g) OGTT in 96 pregnant patients and two steps (50/100 g) OGTT in 116 pregnant patients. We measured maternal leptin, MDA and HbA1c levels in all patients and compared the results of the GDM group and the control group.

Results: In two steps OGTT we detected 31 (26,7%) GDM cases out of 116 patients. In the single step OGTT we detected 23 (24,0%) GDM cases out of 96 patients (p>0.05). GDM was detected in 54 of 212 patients (25,5%). Pregnant women with GDM had significantly higher levels of leptin (46,52±14,99 ng/ml vs. 39,13±17,04 ng/ml, p: 0,005), MDA (3,83±0,91 nmol/L vs. 2,57±0,76 nmol/L, p<0,001) and HbA1c (5,33±0,47 vs. 5.12±0,47, p:0,001) compared to pregnant women without GDM.

Conclusion: Leptin, MDA and HbA1c levels are significantly elevated in GDM patients and these are found to improve the specificity of GDM screening tests.

Keywords: Gestational diabetes mellitus, leptin, malondialdehyde, oral glucose tolerance test, HbA1c.

Gestasyonel diabetes mellitus tanı ve taramasında maternal serum leptin ve malondialdehitin yeri

Amaç: Gestasyonel diabetes mellitus (GDM) tanı ve taramasında maternal serum leptin ve malondialdehit (MDA) seviyesinin öneminin irdelenmesi.

Yöntem: Çalışmaya kliniğimizde takipleri yapılan 212 gebe dahil edildi. 24-28 gebelik haftasında 96 gebeye tek aşamalı 75 gr OGTT ve 116 gebeye de iki aşamalı gebelik diyabeti tarama testi uygulandı. Tüm gebelerin aynı zamanda maternal leptin, MDA ve HbA1c düzeylerine bakıldı. Uygulanan testler sonucu GDM tanısı koyulan gebelerle kontrol grubunun verileri karşılaştırıldı.

Bulgular: Yüz on altı hastadan oluşan iki aşamalı test grubunun 31'inde (%26,7) GDM tespit edilirken, 96 gebeden oluşan tek aşamalı test grubunun 23'ünde (%24,0) GDM tespit edildi (p>0.05). Toplam 212 hastanın 54'ünde (%25,5) GDM olduğu görüldü. Yapılan testler sonucunda GDM tespit edilen gebelerde, GDM görülmeyen gebelere kıyasla, serum leptin (46,52±14,99 ng/ml'ye karşı 39,13±17,04 ng/ml, p: 0,007), MDA (3,83±0,91 nmol/L'ye karşılık 2,57±0,76 nmol/L, p<0,001) ve HbA1c (5,33±0,47'ye karşılık 5.12±0,47, p:0,001) değerlerinin anlamlı derecede yüksek olduğu tespit edildi.

Sonuç: Gestasyonel diyabetli gebelerde Leptin, MDA ve HbA1c düzeyleri anlamlı olarak artmış olup, saptanan bulgular GDM taramasında yapılmakta olan testlerin özgüllüğünü artırıcı nitelikte olacağı bulunmuştur.

Anahtar Sözcükler: Gestasyonel diabetes mellitus, leptin, MDA, oral glukoz tolerans testi, HbA1c.

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Introduction

Gestational diabetes mellitus (GDM) is defined as hyperglycemia seen after the 20th gestational week or a carbohydrate intolerance which either begins or is diagnosed during pregnancy.[1] Leptin (greek: leptos=thin) is a protein hormone which contains 167 aminoacids and resembles cytokines, is discovered by Zhang et al.[2] in 1994. It has a molecular weight of 16 kDA.[3] It's shown to have many functions in the body.

Leptin is coded on the ob/ob gene, in the long arm of the 7th chromosome (7q31) and is mainly secreted from adipose tissue. It's first detected as a mutagenic gene product in ob/ob mutant rats.[4,5] In addition to adipose tissue, it's also shown to be secreted from placenta, gastric epithelium, skeletal muscle, pituitary gland and mammary glands.[6] It's found in free and protein-bound forms in blood. Free form is thought to be responsible from its activity. Free form of leptin is shown to be the major form of leptin in obese patients.[7] Therefore increased concentrations of leptin in obese patients indicate that the main problem in development of obesity is leptin resistance, not leptin deficiency.

Many factors play role in regulation of leptin, however the main regulators of leptin levels are amount of body fat and body mass index (BMI).[8,9] Insulin, glucocorticoids and prolactin stimulate; whereas thyroid hormone, growth hormone, somatostatin, free fatty acids, cold exposure and catecholamines inhibit leptin synthesis. In pregnant women plasma leptin concentrations are shown to be increased compared to nonpregnant women at the same age group.[10-12] Increased level of plasma leptin is correlated with free plasma leptin concentrations and alterations in leptin binding proteins.[12] Although placenta is the major source of leptin during pregnancy, the reason and function of leptin secretion is not clear yet.[11] Gestational hormones, most of the estrogens and cortisol also stimulate leptin secretion from adipose tissue.[10] Though the accumulation of fats and increased body mass in first two trimesters may be responsible for secretion of leptin, however it can also be secondary to hyperinsulinemia, considering that late pregnacy is characterized by physiologic insulin resistance and compansatory increase in insulin secretion.[10]

Maternal leptin concentrations increase 2-3 folds during pregnancy and peak at the 28th gestational week.[13] It's also shown that leptin correlates with systemic blood pressure, triglycerides and covariants of metabolic syndrome such as postpartum waist-hip ratio.

Pregnancy is known to induce gestational diabetes in women with previous latent metabolic syndrome. This may imply that hyperleptinemia may be a marker for latent metabolic syndrome which reversibly proceeds to diabetes during pregnancy.[10] It's not clear if changes in leptin concentrations are the cause or the result of gestational diabetes.[10,14] The correlation between increased serum leptin concentrations and glucose intolerance has been shown in women with normal body weight and it's associated with increased risk of development of type II DM.[15]

Leptin levels are found to increase due to increased fasting insulin levels in pregnant women with GDM and impaired glucose tolerance compared to healthy pregnant women.[16,17]

Oxidative stress and malondialdehyde is that cell membrane is prone to oxidative damage because of the presence of polyunsaturated fatty acids. Lipid peroxidation is a chemical reaction induced by free radicals and progresses by oxidation of polyunsaturated fatty acids in cell membrane. A strong oxygen derivative free radical which is present in organism separates the hydrogen atom from the _-methylen groups of polyunsaturated fatty acid chains in cell membrane, starting the reaction.[18]

The fatty acid chain gains features of a radical. The lipid radical produced is a labile compound, it undergoes several reactions. "-dien" compounds are formed by intramolecular double-bond transfer, followed by production of lipid peroxide radical which is formed as lipid radical reacts with molecular oxygen.[18]

Lipid peroxide radicals react with polyunsaturated fatty acids in the membrane producing new lipid radicals and collect new hydrogen atoms to transform into lipid peroxides. So the reaction continues in an autocatalytic way. Lipid peroxidation ends when lipid hydroperoxides are transformed into aldeyhde and carbonyl compounds. These compounds include malondialdehyde (MDA), 4-hydroxynoneal (HNA), alcohols, ethan and penthan. MDA and HNA can be used to detect lipid peroxide levels. MDA can lead to cross-binding and polymerization of the membrane components which effects functions of the internal membrane such as flexibility, ion transfer, enzyme activity and it may also react with the DNA bases causing damage. It has mutagenic, carcinogenic and genotoxic effects.[19,20] Thiobarbituric acid (TBA) test is the most important method to detect the hydroxyl radical damage in the cells, which detects the presence of MDA.[21]

Fetal hyperglycemia, maternal hyperlipidemia, hyperinsulinemia, placental endothelial dysfunction and also oxidative stress are responsible in the pathogenesis of maternal and fetal complications seen in GDM.[22] A product of lipid peroxydation, MDA, can be measured as an indicator of oxidative stress.[21,23] HbA1c, measaurement of glycated hemoglobin is the standard method for assessing long term glycemic control. When plasma glucose is consistently elevated there is an increase in nonenzymatic glycation of hemoglobin; this alteration reflects the glycemic history over the previous 2 to 3 months, since the red blood cells have a life span of 120 days. It's a highly specific and reliable marker HbA1c should be measured in all individuals with DM during their initial evaluation and to predict long term complications of DM. Screening and diagnosis of gestational diabetes mellitus Either 75 g OGTT or 50/100 g OGTT is used in screening or diagnosis of gestational diabetes mellitus. World Health Organisation (WHO) recommends the use of 75 g OGTT in diagnosis of GDM whereas in many countries including Turkey 50/100 g OGTT is used for screening and diagnosis of GDM.

Methods

Two hundred and seventy pregnant women who had been followed in pregnancy outpatient clinic and division of perinatology in Cerrahpasa Medical Faculty, Department of Obstetrics and Gynecology, March 2005- February 2006 inclusive, were enrolled to our study. Our study is designed as a descriptive study. Fifty eight patients were excluded due to inadequacy of their data, and remaining 212 women have been investigated.

Gestational ages of the women were calculated according to the last menstral period, and early pregnancy ultrasound measurements if in doubt. 10 cc of venous blood samples from all patients in the study group were collected in dry tubes before performing the diabetes screening tests between 24-28 GWs. Serum parts were separated and preserved in -80°C to be evaluated at once, when target patient population is reached. Leptin and malondialdehyde levels were measured in biochemistry laboratory. GDM screening and diagnosis tests were performed between 24-28 GWs in all 212 patients. Single step 2 hours 75 g OGTT was performed in 125 patients. The test results were interpreted according to the American Diabetes Association (ADA) criteria (≥2 values above treshold, fasting glucose levels: 95 mg/dl, 1 hour: 180 mg/dl, 2 hours 155 mg/dl). Two steps 50 g OGTT was performed in 149 patients. The patients with 1 hour blood glucose levels of ≥140 mg/dl were

accepted as screening test positive according to ADA and American College of Obstetricians and Gynecologists (ACOG) criteria.

The diagnostic test was performed in screening test positive patients after 3 days standard diet (at least 250 g of daily carbonhydrate). After a fasting period of 12-16 hours, the blood samples were collected at 8 am and the 1st, 2nd and 3rd hours. Carpenter and Coustan's criteria were considered in the interpretation of 100 g OGTT and ≥2 levels above treshold (fasting: 95 mg/dl, 1. hour 180 mg/dl, 2. hour 155 mg/dl, 3. hour 155 mg/dl) were accepted to have GDM. HbA1c levels were measured in all patients at the time of screening. Serum MDA level measurement: The absorbance of the complex which is produced by the reaction between MDA and thiobarbituric acid is measured spectrophotometrically. MDA level for the measured absorbance was calculated from the curve. Serum MDA levels are given in nmol/L. Serum leptin levels were measured by a kit which is based on ELISA (Human Leptin Elisa DSL-10-23100i, Texas, USA). Leptin levels are expressed in ng/ml. Statistical Package for Social Sciences (SPSS Release 11,5, SPSS inc., Chicago, IL, USA) was used during statistical calculations. Student's t test was used for parametric variables and chi-square test was used for comparing qualitative data. 0.05 was accepted as treshold for statistical significance. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and area under curve values were calculated ROC (Receiver operating characteristic) curves.

Results

GDM was detected in 54 (25.4%) of 212 pregnant women enrolled in our study. Two steps OGTT was performed in 116 cases and single step OGTT was performed in 96 women. These two groups were similar in age, gravidity, parity, maternal weight, body mass index and gestational weeks at the time of test. GDM was diagnosed in 31 (26.7%) of 116 pregnant women who underwent 50/100 g OGTT and 23 (24%) of 96 pregnant women who underwent 75 g OGTT.

Serum leptin, MDA and HbA1c levels measured between 24-28 gestational weeks were statistically different in GDM and non-GDM groups. Leptin levels of women with GDM and without GDM were 46.52±14.99 ng/ml and 39.13±17.04 ng/ml, respectively (p:0.007, AUC: 0.623, %95 CI: 0.542-0,703). Malondialdehyde levels of women with GDM and without GDM were 3.83±0.91 nmol/L and 2.57±0.76 nmol/L, respectively (p<0.001, AUC: 0.856, %95 CI: 0.800-0.912). HbA1c levels of women with GDM and without GDM were 5.33±0.47 and 5.12±0.37, respectively (p:0.001, AUC: 0.655, %95 CI: 0.565-0,744). (Table 1) Thirty four pregnant women who were 50 g OGTT positive and 100 g OGTT negative, and 178 women without GDM were compared; no significant difference between two groups in terms of mean serum leptin (38.38±17.62 ng/ml vs 39.36±16.94 ng/ml respectively, p >0.05) (Table 2), MDA (2.59±0.77 nmol/L vs 2.57±0.71 nmol/L respectively, p>0.05) (Table 3) and HbA1c (5.24±0.41 vs 5.08±0.36 respectively, p >0.05) levels (Table 4).

Discussion

It's not only controversial whether screening for GDM in all patients or only the risk groups, but also the method of choice. Screening for GDM in most population groups may seem unnecessary regarding that its prevalence is below 5%, but if the 4 folds increase in perinatal mortality is considered, it's a reasonable effort.[24]

Although the average incidence of GDM is 3-5%, it may vary between 1-14% depending on the method used. GDM incidence in Turkey is reported as 1.23-6,6%. In our study, GDM incidence was found as 21.1% and this high ratio is attributed to the fact that our clinic is a tertiary (reference) cen-

ter. Recently it's emphasized that leptin is effective not only in obesity but also in glucose regulation.[28,29] Weight loss and fasting is known to decrease leptin concentration whereas weight gain and hyperinsulinemia increase it.[30,31] Increased leptin concentration in pregnant women is attributed to the changes in maternal fat and glucose metabolism.[13]

Maternal leptin levels increase 2-3 folds during pregnancy and peak at the 28th gestational week.[13] Clinical studies suggest that increased maternal leptin concentrations are associated with insulin resistance and hyperinsulinemia which occurs in the second trimester.[31] Qiu et al.[14] enrolled 823 pregnant women and investigated the effects of increased leptin levels in early pregnancy. They observed that increased leptin concentrations in early pregnancy are associated with 4.7 folds increase in GDM risk, compared to women with leptin levels below 14.3 ng/ml. It's estimated that every 10 ng/ml increase in leptin concentration leads to 20% increase in GDM risk.[14] Serum leptin levels measured between 24-28 gestational weeks were statistically different in GDM and non-GDM groups. Leptin levels of women with GDM and without GDM were 46.52±14.99 ng/ml and 39.13±17.04 ng/ml, respectively (p:0.007). Kautzky-Willer et al.[10] investigated leptin levels in 25 healthy pregnant women, 55 women with GDM, 10 type I DM and 10 healthy nonpregnant women. Leptin levels are shown to be increased in all pregnant women compared with nonpregnant women at the same age group. (p<0.0005) Increased level of plasma leptin is correlated with free plasma leptin concentrations and alterations in leptin binding proteins.[12] Although placenta is the major source of leptin during pregnancy, the reason and function of leptin secretion is not clear yet.[11] Gestational hormones, most of the estrogens and cortisol also stimulate leptin secretion from adipose tissue.[10] Though the accumulation of fats and increased body mass in first two trimesters may be responsible for secretion of leptin, however this can also be secondary to hyperinsulinemia.

Leptin levels are shown to be significantly high

Table 1. Leptin, MDA and HbA1c levels of GDM and non-GDM groups.

	Normal	GDM	p
Number of patients (n)	158	54	
Leptin (ng/ml)	39.13±17.04	46.52±14.99	0.007
MDA (nmol/L)	2.57±0.76	3.83±0.91	<0.001
HbA1c (%)	5.12±0.37	5.33±0.47	0.001

p< 0,05: Significant; **MDA:** Malondialdehyde, **GDM:** Gestational diabetes mellitus

Table 2. Sensitivity, specificity, PPV and NPV for various leptin levels of women with GDM.

Leptin level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
23,06 ng/ml	100	22,5	30	100
30,21 ng/ml	83	45	34	88
40,08 ng/ml	64	54	31	80
50,09 ng/ml	42	68	30	76
60,00 ng/ml	20	88	36	76
70,1 ng/ml	7	97,5	42	75

GDM: Gestational diabetes mellitus, **PPV:** positive predictive value, **NPV:** negative predictive value

Table 3. Sensitivity, specificity, PPV and NPV for various MDA levels of women with GDM.

MDA level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%))
1.79 nmol/L	100	11	27	100
2.53 nmol/L	92	66	52	96
3.50 nmol/L	55	88	61	85
4.50 nmol/L	31	98	84	80
5.00 nmol/L	5	99	60	75

GDM: Gestational diabetes mellitus, **MDA:** Malondialdehyde **PPV:** positive predictive value, **NPV:** negative predictive value

Table 4. Sensitivity, specificity, PPV and NPV for various HbA1c levels of women with GDM.

HbA1c level	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
5.0%	76	47,5	34	87
5.5%	26	87	4	77
5.9%	7	98	50	75
6.3%	2	99	50	75

GDM: Gestational diabetes mellitus, **PPV:** positive predictive value, **NPV:** negative predictive value

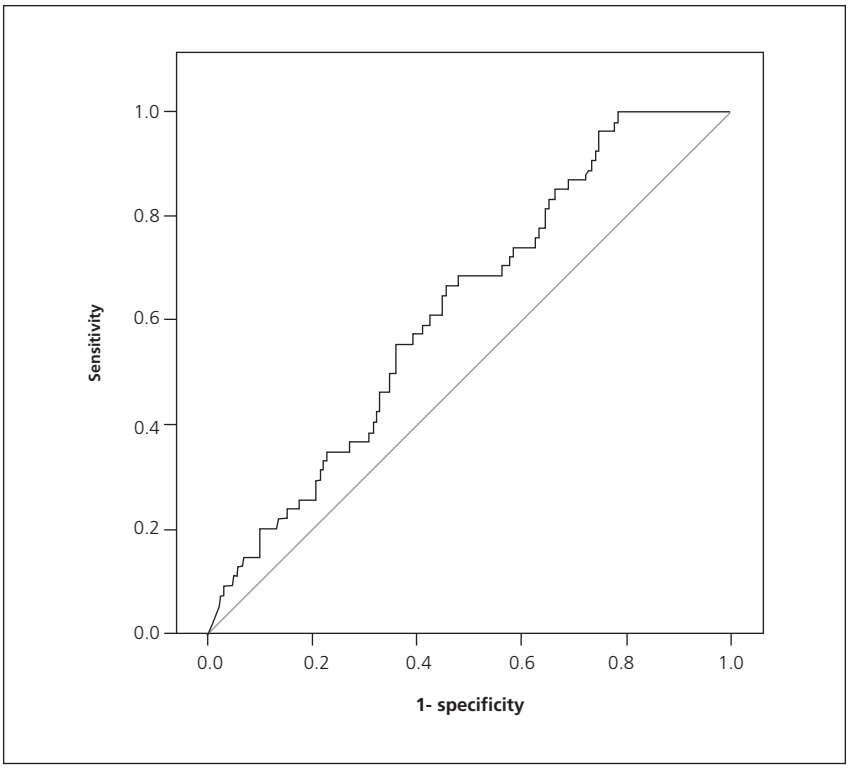

Figure 1. ROC curve for serum leptin levels in patients with GDM.

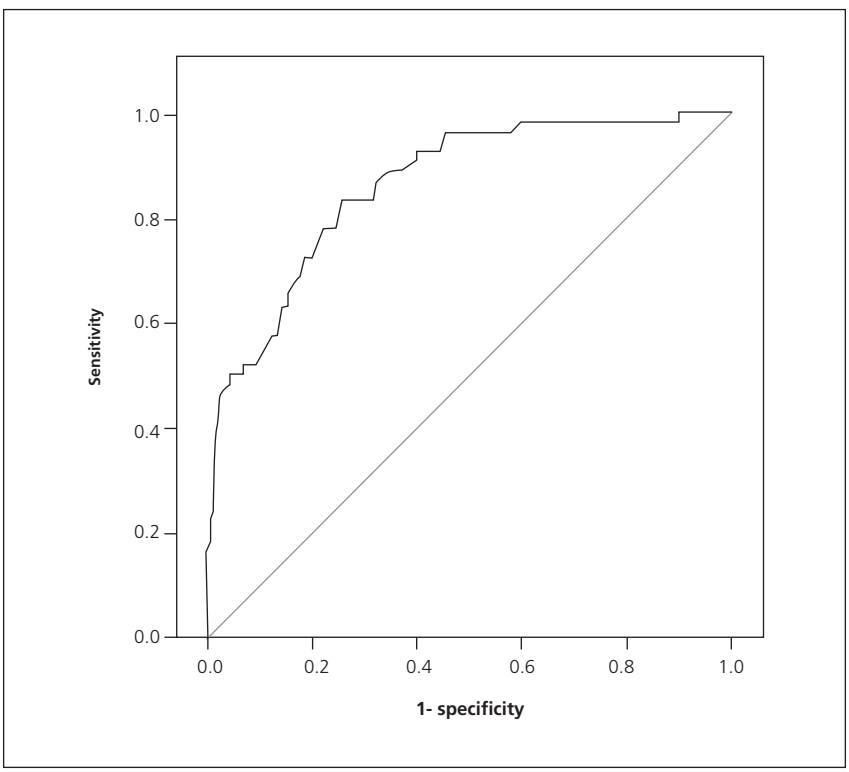

Figure 2. ROC curve for serum MDA levels in patients with GDM.

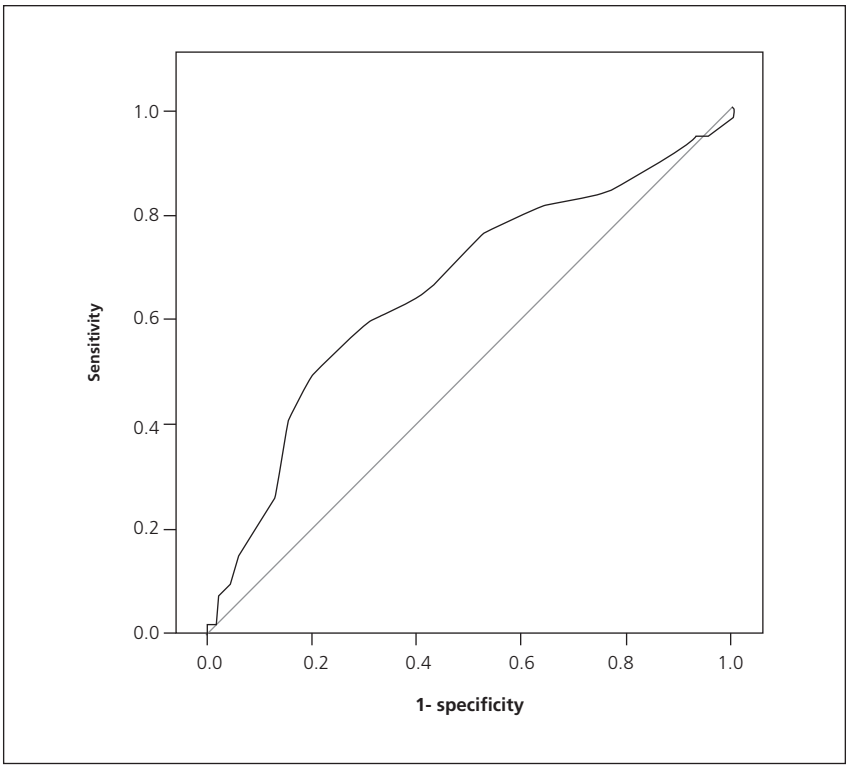

Figure 3. ROC curve for serum HbA1c levels in patients with GDM.

in women with GDM compared to pregnant women with type I DM and pregnant women with normal glucose tolerance. ($p<0.008$).[10] Moriya et al.[32] have shown that hyperglycemia has an inhibitory effect on leptin secretion in patients with non-insulin dependent DM by showing that HbA1c is negatively correlated with leptin concentrations. Previous studies implied that insulin stimulates leptin secretion from adipocytes. In patients with uncontrolled diabetes it's observed that longstanding hyperglycemia has a suppressive effect on insulin. In these patients decreased insulin levels may be accompanied by decreased leptin.

In contrast leptin levels are expected to rise in hyperinsulinemia which is seen in the 2nd trimester in patients with GDM.[32] Results of our investigation support the hitherto publications, leptin levels are significantly high in women with GDM. Therefore leptin can be used as a marker in screening and diagnosis of GDM. Fetal hyperglycemia, maternal hyperlipidemia, hyperinsulinemia, placental endothelial dysfunction and also oxidative stress is responsible in the pathogenesis of maternal and fetal complications seen in GDM.[22] Oxidative stress in diabetes may be associated with increased reactive oxygen radicals such as O2-, OH- and H2O2 or deficiency in antioxidant defence mechanisms. Increase in oxygen radicals can be attributed to protein glycosylation or autooxidation of glucose in hyperglycemic media.[33-35]

Superoxide dismutase (SOD) activity is shown to decrease in red blood cells in studies on diabetic rats.[36] and humans.[37] Glutathione peroxidase and catalase enzyme activities are decreased in chronic diabetes.[38] Vitamin E is one of the major intracellular nonenzymatic antioxidants and it's known to be decreased in patients with diabetic

patients.[39] In an experimental study free oxygen radical activity is observed in embryos of diabetic rats and is thought to be the underlying mechanism in teratogenicity in diabetic pregnant women.[40] Besides antioxidant therapy is shown to prevent in vivo and in vitro embryonic dysmorphogenesis associated with diabetes.[41] On the other hand, glucose regulation alone can maintain better perinatal outcomes in these patients.[42] Kharb et al.[43] studied on 25 healthy pregnant women and 25 women with GDM and they have shown that maternal MDA and SOD levels are increased whereas vitamin C and E levels are decreased in women with GDM. Peuchant et al.[22] also observed high plasma and free erythrocyte MDA levels in women with GDM, this may be a proof for oxidative stress. In the same study significant decrease in glutathione peroxidase and low vitamin E and erythrocyte vitamin A levels imply deficiency in antioxidant mechanisms.[22]

In our study, significantly low MDA levels seen in women with GDM indicate that oxidative stress begins to be effective since very early stages of pregnancy in these patients. This finding may imply that oxidative stress may play a role in pathogenesis of maternal and fetal complications. Few studies have been made so far on this issue, but all support our study. A potential bias in our study is that MDA levels were measured indirectly, and our results should be tested by MDA spesific kits.

Conclusion

In conclusion MDA levels are shown to increase in women with GDM and it may increase the specificity of the other tests used in screening of GDM.

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