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Comparison of Maternal Serum Adiponectin and Leptin Measurements in Screening and Diagnosis of Gestational Diabetes Mellitus

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Abstract

Objective: To compare and to evaluate the measurements of maternal serum adiponectin and leptin levels in screening and diagnosis of gestational diabetes mellitus (GDM).

Methods: Two hundred and twelve pregnant women who were between 24-28 gestational weeks followed in our clinic and investigated concomitantly in two different studies ("Role of Serum Leptin Levels and Oxidative Stress Test in Screening and Diagnosis of Gestational Diabetes Mellitus" and "Role of Serum Adiponectin Levels in Screening and Diagnosis of Gestational Diabetes Mellitus") were included in our study. Single step 75 g oral glucose tolerance test (OGTT) was performed in 96 cases whereas two steps 50/100 g OGTT was performed in 116 cases and leptin and adiponectin levels have been measured in all women. The cut-off value has been accepted as 10.3 µg/ml for adiponectin and 43 ng/ml for leptin. Sensitivity and specificity of leptin and adiponectin are compared statistically.

Results: GDM has been diagnosed in 31 (26.7%) of 116 cases who underwent two steps OGTT and 23 (24.0%) of 96 cases who underwent single step OGTT. GDM was detected in 54 (25.5%) in total. Adiponectin levels were significantly low in patients with GDM compared to women without GDM in both 75 g and 50/100 g OGTT groups (p:0.001 and p: 0.007 respectively). Leptin levels were significantly high in patients with GDM compared to women without GDM in 75 g OGTT group (p: 0.021), but there was no significant difference in cases and controls in 50/100 g OGTT group. (p: 0.08) In 75 g OGTT group sensitivity, specificity and positive predictive value (PPV) of adiponectin is 85%, 53% and 65.3% respectively whereas in 50/100 g OGTT group sensitivity, specificity and PPV of adiponectin was 82%, 40% and 57.7% respectively. In 75 g OGTT group sensitivity, specificity and PPV of leptin was 70%, 55% and 60.8% respectively.

Conclusion: If these cut-off values are taken, in 75 g OGTT group adiponectin is more sensitive than leptin, and adiponectin is as equally specific as leptin. In 50/100 g OGTT group adiponectin is significantly lower in patients with GDM compared to women without GDM and leptin levels are not significantly different in two subgroups.

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

Gestasyonel diabetes mellitus tanı ve taramasında maternal serum adiponektin ile leptin ölçümlerinin karşılaştırılması

Amaç: Gestasyonel diabetes mellitus (GDM) tanı ve taramasında maternal serum adiponektin ve leptin ölçümlerinin karşılaştırmalı olarak irdelenmesi.

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Yöntem: "Gestasyonel Diabetes Mellitus Tanı ve Taramasında Serum Leptin Seviyesi, Oksidatif Stres Testin Önemi" başlıklı uzmanlık tezi ile eş zamanlı olarak yürütülen "Gestasyonel Diabetes Mellitus Tanı ve Taramasında Serum Adiponektin Önemi" başlıklı uzmanlık tezinde incelenen ve kliniğimizde takipleri yapılan 24-28 gebelik haftaları arasındaki 212 gebenin 96'sına tek aşamalı 75 gr OGTT ve 116'sına iki aşamalı 50/100 gr OGTT gebelik diyabeti tarama testi uygulanarak, tüm gebelerde aynı anda leptin ve adiponektin değerleri ölçüldü. Eşik değer adiponektin için 10.3 µg/ml, leptin için 43 ng/ml olarak alındı. İstatistiksel değerlendirmeler sonucunda leptin ve adiponektinin GDM tanı ve taramasındaki duyarlılık ve özgüllükleri 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. Toplam 212 hastanın 54'ünde (%25.5) GDM olduğu görüldü. Adiponektin düzeylerini incelediğimizde 75 gr ve 50/100g OGTT ile GDM saptanan gebelerde anlamlı derecede düşük olduğu görüldü (sırasıyla p: 0.001 ve p: 0.007). Leptin düzeylerine baktığımızda 75 gr OGTT ile GDM saptanan gebelerde leptin düzeyi anlamlı derecede yüksek (p: 0.021) bulunurken, iki aşamalı test ile GDM saptanan gebeler ile normal gebeler arasındaki fark anlamlı değildi (p: 0.08). Tek aşamalı 75 gr OGTT uygulanan grupta adiponektinin GDM tanı ve taramasındaki duyarlılığı %85, özgüllüğü %55, pozitif prediktif değeri (PPV) 65.3%; iki aşamalı 50/100 gr OGTT uygulanan grupta adiponektinin duyarlılığı %82, özgüllüğü %40, PPV %57.7 olarak hesaplandı. Tek aşamalı 75 gr OGTT uygulanan grupta leptinin GDM tanı ve taramasındaki duyarlılığı %70, özgüllüğü %55, PPV %60.8 olarak hesaplandı.

Sonuç: Gestasyonel diyabetin tanı ve taramasında uygulanan 75 g OGTT uygulanan grupta belirtilen eşik değerler kabul edildiğinde adiponektin, leptine kıyasla daha duyarlı; leptin ise adiponektinle eşit özgüllükte bulunmuştur. Buna karşılık 50/100 g OGTT uygulanan grupta adiponektin anlamlı derecede düşük bulunmuş, leptin değerlerindeki yüksekliğin istatistiksel olarak anlamlı olmadığı gösterilmiştir.

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

Introduction

Adiponectin is secreted from the adipose tissue and is the most abundant adipokine in circulation and it plays a key role in metabolic syndrome.[1] The plasma level is 2-30 µg/ml. Adiponectin has anti-inflammatory, anti-atherosclerotic and anti-diabetogenic effects. The most well-known effect of adiponectin is regulation of insulin sensitivity. Leptin is a hormone which is coded on the ob/ob gene, in the long arm of the 7th chromosome (7q31) mainly secreted from adipose tissue. It's first detected as a mutagenic gene product in ob/ob mutant rats.[2,3] In addition to adipose tissue, it's also shown to be secreted from placenta, gastric epithelium, skeletal muscle, pituitary gland and mammary glands.[4] Various methods have been suggested to be performed in screening and diagnosis of gestational diabetes mellitus (GDM). In some countries 75 g oral glucose tolerance test (OGTT) is used whereas in some others 100 g following 50 g OGTT are used. The populations screened for GDM are also different in different regions of the world, in some countries routine screening in all pregnant women is performed and in some countries only high risk women are screened. Leptin levels are shown to decrease and adiponectin levels are shown to increase significantly in GDM. According to the aim of the test, the cut-off values can be changed and the sensitivity and specificity may be adjusted. Our objective is to discuss if adiponectin and leptin can replace OGTT in screening and diagnosis of GDM as well as to determine which one of these markers is more sensitive or specific.

Methods

Two hundred and twelve pregnant women between 24-28 gestational weeks who were investigated in two concomittant studies performed in University of Istanbul, Cerrahpasa Medical Faculty, Department of Obstetrics and Gynecology: "Role of Serum Leptin Levels In

Screening and Diagnosis of Gestational Diabetes Mellitus and Importance of Oxidative Stress Test" and "Role of Adiponectin In Screening and Diagnosis of Gestational Diabetes Mellitus" and were followed in our clinic have been included. Our study is designed as a comperative study. All 212 pregnant women included in the study "Role of Serum Leptin Levels in Screening and Diagnosis of Gestational Diabetes Mellitus and Importance of Oxidative Stress Test" and the same 212 pregnant women which are a part of the 274 women included in the study "Role of Adiponectin In Screening and Diagnosis of Gestational Diabetes Mellitus" were enrolled into the comperative study. Single and two steps OGTTs were performed and presence of GDM was investigated. Blood samples were collected from all women and adiponectin and leptin levels were measured and compared. Gestational ages of the women were calculated according to the last menstrual 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 till target patient population is reached to be evaluated at once. Leptin and adiponectin 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 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 ACOG criteria. The diagnostic test was performed in screening test positive patients after a 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. 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. Serum adiponectin levels were measured by a kit which is based on ELISA (Human adiponectin assaypro catalog no: EA2500-1). Adiponectin levels are expressed in microgram/ml (μg/ml). Cut off values were taken as 10.3 μg/ml for adiponectin and 43 ng/ml for leptin. Sensitivity and specificity rates of leptin and adiponectin in screening and diagnosis of GDM were compared statistically. 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, negative predictive value (NPV), positive predictive value (PPV) and area under curve values were calculated ROC (Receiver operating characteristic) curves.

Results

GDM was detected in 54 (25.4%) of 212 pregnant women. 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. Mean leptin concentration was 49.36±14.5 ng/ml in women with GDM compared to 40.10±17.12 ng/ml in women without GDM in 75 g OGTT group. This difference is found to be significant. (p:0.021) However the difference between two groups was not significant in 50/100 g OGTT group (44.41±15.22 ng/ml vs 38.29±17.02 ng/ml, p: 0.08). Area under curve (AUC) values were calculated from the ROC curves drown which was based on the leptin levels of the OGTT results of 212 women. (Graphic 1 and 2) AUC values were 0,653 for 75 g OGTT and 0.613 for 50/100 g OGTT. The treshold for leptin was set as 43 ng/ml. In 75 g group sensitivity, specificity and PPV are found to be 70%, 55% and 60.8% respectively. In 50/100 g OGTT group sensitivity and specificity are found to be 48% and 63% respectively, but since there's no statistical difference between cases and controls, these sensitivity and specificity values have no clinical benefit. Adiponectin levels of GDM patients diagnosed by 75 g OGTT had a median of 11.7±6.4 µg/ml, and normal patients had a median of 17±6.5 µg/ml, this difference was statistically significant. (p:0.001) Adiponectin levels of GDM patients diagnosed by 50/100 g OGTT had a median of 12.8±5.3 µg/ml, and normal patients had a median of 16.2±6.7 µg/ml. (p: 0.007) AUC was detected to be 0.734 for 75 g OGTT [0.73, Confidence interval (CI) %95, 0.60-0.76] and

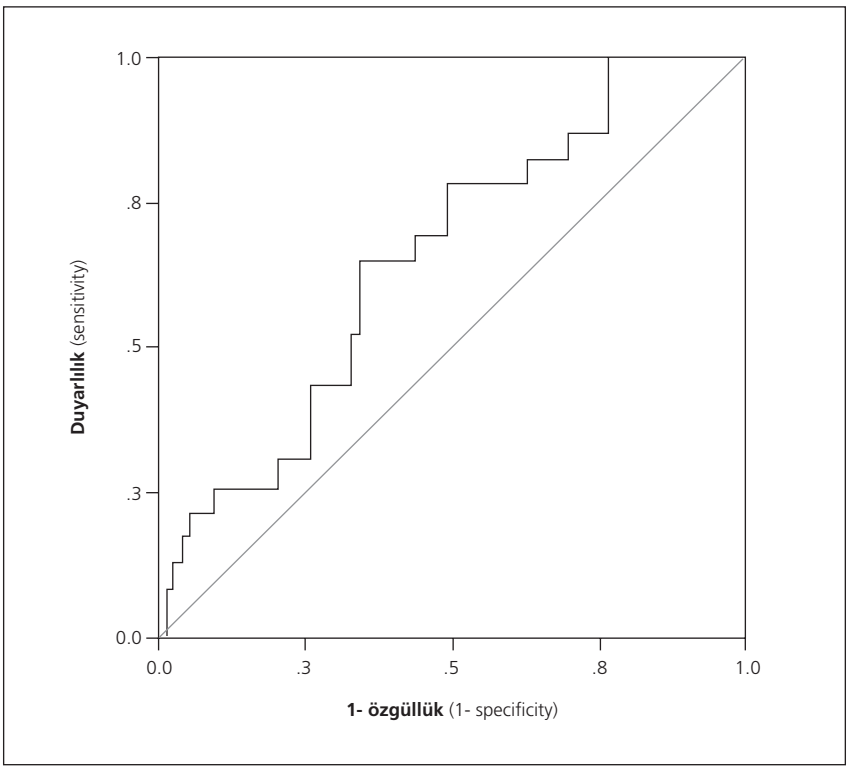

Figure 1. ROC curve for serum leptin levels in 75 g OGTT group.

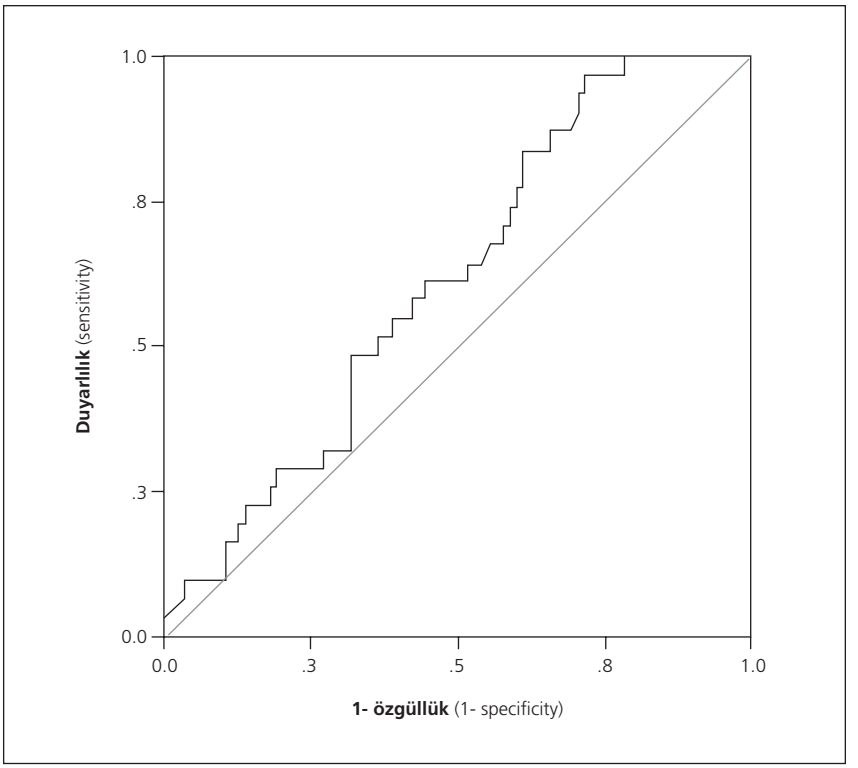

Figure 2. ROC curve for serum leptin levels in 50/100 g OGTT group.

0,617 for 50/100 g OGTT [0.61, Confidence interval (CI) %95, 0.49-0.73]. If we accept the treshold as 10.3 μg/ml, the sensitivity, specificity and PPV were 85%, 55% and 65.3% for 75 g OGTT and 82%, 40% and 57.7% for 50/100 g OGTT respectively (Figures 3 and 4) If cut-off value of adiponectin was taken as 5.3 μg/ml, sensitivity was 100% and specificity was 18% in 75 g OGTT group. If cut-off value was taken as 27 μg/ml, sensitivity was 11%, specificity is 100%. In our study, there are 4 and 8 patients whose adiponectin level was ≤5.3 μg/ml and ≥27 μg/ml respectively. If cut-off value of adiponectin was taken as 7 μg/ml, sensitivity was 100% and specificity was 23% in 50/100 g OGTT group. If cut-off value was taken as 20,5 μg/ml, sensitivity was 25%, specificity was 100%. In our study, there are 7 and 27 patients whose adiponectin level was ≤7 μg/ml and ≥20.5 μg/ml respectively. In conclusion if these cut-off values are taken, in 75 g OGTT group adiponectin is more sensitive than leptin, and adiponectin is as equally specific as leptin. In 50/100 g OGTT group adiponectin is significantly lower in patients with GDM compared to women without GDM and leptin levels are not significantly different in two subgroups.

Discussion

Gestational diabetes mellitus (GDM) is the most common complication seen during pregnancy. It threatens both mother and her fetus, hopefully pregnancy outcomes are much better if glycemic control is achieved. That's why today, GDM is a disorder which must not remain unnoticed. Different screening methods are used in different countries. In our clinic, we

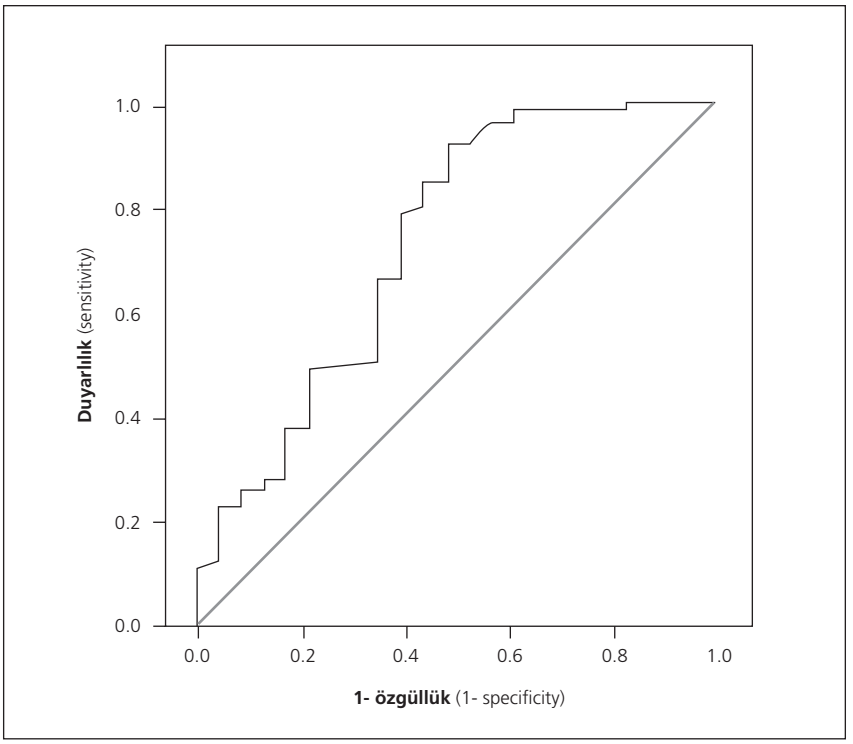

Figure 3. ROC curve for serum adiponectin levels in 75 g OGTT group.

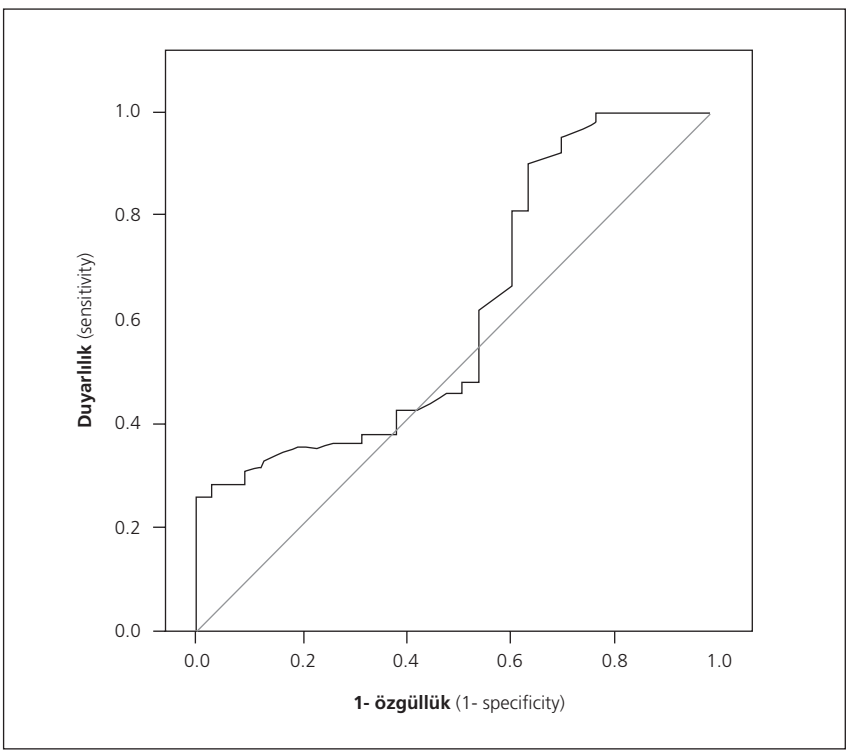

Figure 4. ROC curve for serum adiponectin levels in 50/100 g OGTT group.

Table 1. Sensitivity and specificity rates for 50, 75 ve 100 gr OGTTs.[8]

	Sensitivity (%)	Specificity (%)
50 g (cut off: 140 mg/dl)	58.3	67.8
50 g (cut off: 137 mg/dl)	66.7	63.2
75 g (cut off: 140 mg/dl)	41.7	90.8
75 g (cut off: 117 mg/dl)	66.7	64.4
100 gr	27.3	96.5

perform 50 g OGTT between 24-28 gestational weeks in all pregnant women. If 50 g OGTT is positive then 100 g OGTT is performed and diagnosis of GDM is established according to ACOG criteria. Management of GDM includes diet and exercise primarily to maintain glycemic control and insulin if necessary. There have been many studies till present to understand the pathophysiology of GDM, to diagnose GDM at an early stage and to improve maternal and fetal outcomes in women with fully established GDM. Some cytokines such as adiponectin and leptin have been investigated considering the relationship between GDM and other conditions associated with insulin resistance. Adiponectin (Acrp 30, AdipoQ, apM-1 veya GBP28) is a protein hormone which plays a role in a series of metabolic reactions including glucose regulation and fatty acid catabolism. Adiponectin is secreted into the blood manly from adipose tissue. The blood level of adiponectin is inversely proportional with the fat ratio. Maternal serum adiponectin levels are not correlated with maternal weight and BMI. Total liquid amount is increased during pregnancy and that's why body weight and BMI are not weak parameters to assess adiposity in early postpartum period. Maternal serum adiponectin concentrations do not correlate with serum glucose and insulin levels. However, the negative correlation between the maternal serum adiponectin levels and maternal fasting glucose/insulin ratio may indicate that adiponectin has a role in glucose regulation. It's possible that adiponectin levels change as a result of effective glucose management and this may make it a beneficial marker of insulin sensitivity. Altinova et al.[5] have shown that decreased adiponectin levels are associated with insulin resistance and GDM pathogenesis. In our study, we aimed to measure leptin and adiponectin levels in the same patients, and also to classify GDM patients acoording to the OGTT method preferred. Median leptin concentration was 49.36±14.5 ng/ml in women with GDM compared to 40.10±17.12 ng/ml in women without GDM. This difference is found to be significant. (p:0.021) However the difference between two groups was not significant in 75 g OGTT group (44.41±15.22 ng/ml vs 38.29±17.02 ng/ml, p:0,08). We do not know why the difference between leptin levels of case and control groups is not significant in the 50/100 g OGTT group, although in 75 g OGTT group leptin is significantly higher in case group compared with the controls. Kautzky-Willer et al.[6] 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.

Conclusion

In conclusion, if these cut-off values are taken, in 75 g OGTT group adiponectin is more sensitive than leptin, and adiponectin is as equally specific as leptin. In 50/100 g OGTT group adiponectin is significantly lower in patients with GDM compared to women without GDM and leptin levels are not significantly different in two subgroups. Considering that sensitivity and specificity rates of OGTT methods are not 100%, therefore the sensitivity and specificity rates of adiponectin interpreted according to the diagnosis of GDM which is established by OGTTs are not 100% precise.

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