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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

# Ability of Metabolic Score for Insulin Resistance to Detect Insulin Resistance

## İnsülin Direnci için Metabolik Skorun İnsülin Direncini Tespit Edebilme Yeteneği

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## ABSTRACT

**Aim**: To evaluate the usability of metabolic score for insulin resistance (METS-IR), a novel insulin resistance index, in our country and to determine the optimal cut-off value of this index for detecting insulin resistance.

**Material and Method**: One thousand five hundred sixty seven individuals who participated in our check-up program between 2020 and 2021 were retrospectively evaluated with the patient files for inclusion in the study. Insulin resistance was accepted when HOMA-IR≥2.7. Subjects were divided into 4 quartiles according to their METS-IR levels. Receiver-operating characteristic curve was used to determine the indices' predictive performance and the optimal cut-off value of METS-IR to identify insulin resistance. Binary logistic regression model was used to associate insulin resistance with the varying indexes.

**Results**: Among the 494 participants, 294 (59.5%) were women and the mean age of the subjects was 48.61±12.90 years. As the quartile of METS-IR increased, prevalence of male gender, metabolic syndrome, fatty liver, and levels of age, blood pressure, cigarette smoking, obesity, and insulin resistance indexes, HbA1c increased (all, p<0.001). METS-IR had the highest predictive value for the presence of insulin resistance (AUC=0.813, p<0.001). The highest sensitivity and specificity were achieved at METS-IR between 39–42. The increase in METS-IR is more significant when compared to other indexes for the prediction of insulin resistance (OR=1.332, p<0.001).

**Conclusions**: METS-IR can be used as a screening test for insulin resistance in settings such as primary care centers where insulin levels cannot be measured.

**Keywords**: insulin resistance, insulin resistance indexes, metabolic score for insulin resistance, primary health care.

## ÖZ

**Amaç**: Yeni bir insülin direnci indeksi olan insülin direnci için metabolik skorun (METS-IR) ülkemizdeki kullanılabilirliğini ve bu indeksin insülin direncini tespit etmek için kullanılabilecek optimal kesme değerini belirlemektir.

**Gereç ve Yöntem:** 2020-2021 yılları arasında check-up programımıza katılmış olan 1567 kişi hasta dosyalarından geriye dönük olarak çalışmaya dahil edilmek üzere değerlendirildi. İnsülin direnci varlığı HOMA-IR≥2.7 kabul edildi. Bireyler METS-IR seviyelerine göre 4 çeyreğe ayrıldı. İndekslerin öngörücü performansını ve insulin direncini öngören METS-IR'in optimal kesme değerini belirlemek için ROC eğrisi kullanıldı. İnsülin direncini indekslerle ilişkilendirmek için ikili lojistik regresyon modeli kullanıldı.

**Bulgular**: Çalışmaya dahil edilen 494 katılımcının 294'ü (%59.5) kadındı ve olguların yaş ortalaması 48.61±12.90 yıldı. METS-IR çeyreği arttıkça, erkek cinsiyet, metabolik sendrom, yağlı karaciğer prevalansları ve yaş, kan basıncı, sigara içme miktarı, obezite ve insülin direnci indekslerinin ve HbA1c'nin seviyelerinin arttığı saptandı (tümü, p<0.001). METS-IR, insülin direnci varlığı için en yüksek öngörücü değere sahipti (AUC=0.813, p<0.001). En yüksek duyarlılık ve özgüllük METS-IR'in 39–42 değerleri arasında gözlemlendi. METS-IR'deki artış, insülin direncinin öngörülmesi için diğer indekslerle karşılaştırıldığında daha anlamlıdır (OR=1.332, p<0.001).

**Sonuç**: METS-IR, birinci basamak sağlık merkezleri gibi insülin düzeylerinin ölçülemediği ortamlarda insülin direnci için bir tarama testi olarak kullanılabilir.

**Anahtar Kelimeler:** insülin direnci, insülin direnci indeksleri, insülin direnci için metabolik skor, birinci basamak sağlık hizmetleri.

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## INTRODUCTION

Insulin resistance (IR) leads to impaired glucose disposal by disrupting the biological response of tissues such as liver, muscle, adipose tissue to insulin, and causes metabolic changes secondary to compensatory hyperinsulinemia. It is generally considered to be a root causative factor for obesity-related type 2 diabetes, metabolic syndrome (MetS), non-alcoholic fatty liver disease (NAFLD), polycystic ovary syndrome, and atherosclerotic cardiovascular disease (1).

The hyperinsulinemic-euglycemic clamp test, is the gold standard method for measuring insulin sensitivity. However, it is costly, requires trained personnel, and is invasive (2). Homeostasis model assessment for insulin resistance (HOMA-IR), the most common index used to evaluate IR, is limited by the requirements for insulin measurement, which is not readily available everywhere. In addition, since the half-life of insulin is short, its basal level fluctuates, although it is considered significant in studies using large numbers of patients, a one-time measurement is not considered very reliable for the individual, this situation increases the use of c-peptide, which is produced from proinsulin together with insulin (3). Unfortunately, c-peptide measurement cannot be performed in every center either. Therefore, simpler methods for detecting IR have been sought and the need to develop non-insulin-based IR indices such as triglyceride-glucose index (TyG), triglyceride-high-density lipoprotein cholesterol ratio (TG/HDL), metabolic score for insulin resistance (METS-IR) has emerged (4).

In our country, insulin levels cannot be measured in family health centers that provide primary care. When the fasting blood glucose, which can be measured in these centers, begins to increase, the initial stage of insulin resistance has already passed. To the best of our knowledge, the cut-off values of METS-IR, which may be population specific, have not been studied before in the Turkish population. In this study, we aimed to investigate whether METS-IR is a useful tool for assessing insulin resistance in our population.

## MATERIAL AND METHOD

This study included data from individuals who participated in a check-up program between 2020 and 2021 at our tertiary university hospital. The files of 1567 patients were retrospectively evaluated for inclusion in the study and the following parameters were noted: patients' height, weight, waist and hip circumferences, blood pressure values, smoking status, chronic diseases, medications, alcohol consumption, complete blood count, fasting glucose, fasting insulin, lipid values, uric acid, HbA1c, TSH and abdominal ultrasonography results. Patients with the following were excluded from the study: missing data, age <18 years, those with insulin-dependent diabetes mellitus, malignancies, hepatitis, HIV, use of antidiabetic drugs other than metformin, corticosteroids, use of parenteral nutrition, and those consuming alcohol (>20 g/day for women and >30 g/day for men). After consideration of exclusion criteria, four hundred ninety-four subjects were included in the study. MetS was diagnosed using IDF-2006 guidelines (5). According to the results of abdominal ultrasonography, patients with grade one or more adiposity were accepted as having NAFLD. Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as follows: Fasting Glucose (mg/dL) x Fasting Insulin (uIU/mL)/405 (6). Those with HOMA-IR≥2.7 were considered as having insulin resistance.

The following parameters and indexes were calculated: Body mass index (BMI): Weight in kilograms divided by height in meters squared, Waist/hip ratio (WHR): Waist circumference divided by hip circumference, Waist/height ratio (WHtR): Waist circumference divided by height, TG/HDL: TG (mg/dL) / HDL-C (mg/dL) (7), TyG: Ln (TG (mg/dL) × Fasting Glucose (mg/dL)/2) (8), VAI (women): WC/(36.58 + (1.89 × BMI))) × (TG (mmol/L)/0.81) × (1.52/HDL-C (mmol/L), VAI (men): WC/ (39.68 + (1.88 × BMI))) × (TG (mmol/L)/1.03) × (1.31/HDL-C (mmol/L) (9), METS-IR: Ln ((2 × Fasting Glucose (mg/dL)) + TG (mg/dL)) × BMI)/ (Ln (HDL-C (mg/dL)) (10).

Maltepe University Clinical Research Ethics Committee approved the study (Approval Date: 20.10.2021, Approval Number: 2021/900/105), which was carried out in adherence to the Declaration of Helsinki II.

IBM SPSS Statistics 25.0 software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) was used for statistical analysis. Normality was tested using the Kolmogorov-Smirnov test. Continuous variables with a normal distribution are expressed as the mean±standard deviation, and those without a normal distribution are expressed as the median (min-max). Comparison between the continuous variables in the studied groups was achieved using student t-test, one-way ANOVA test, Mann–Whitney U-test or Kruskal–Wallis tests as appropriate and Chi-square test was used to compare categorical data. The ability of indices to detect IR and the optimal cut-off value of METS-IR to identify IR were determined using receiver operating characteristic (ROC) curve analysis. Binary logistic regression models were used to associate IR and MetS, using IR and MetS as the dependent and the indexes as the independent variables. A p-value ≤ 0.05 was taken as statistically significant.

## RESULTS

Among the 494 participants, 294 (59.5%) were women and 200 (40.5 %) were men. The mean age of the subjects was 48.61±12.90 years. The prevalence of IR was 30.57% (n=151). Table 1 shows a comparative analysis of anthropometric, clinical, and biochemical characteristics between IR and non-IR groups. In subjects with IR, a higher

prevalence of male gender, MetS, NAFLD were observed as well as higher levels of blood pressure, WC, WHR, WHtR, BMI, triglyceride, uric acid, HbA1c, HOMA-IR, Tg/HDL, TyG, VAI, METS-IR and lower levels of HDL-C (all p<0.001). There was no statistically significant difference between the groups in terms of age, cigarette smoking, TSH or LDL-C values (**Table 1**).

The participants were categorized into four quartiles according to METS-IR, as shown in **Table 2**. As the quartile of METS-IR increased, prevalence of male gender, MetS, NAFLD and levels of age, blood pressure, cigarette smoking, WC, WHR, WHtR, BMI, HbA1c, HOMA-IR, Tg/HDL, TyG, and VAI increased (all, p<0.001).

**Table 1: Comparison of Anthropometric, Clinical and Biochemical Characteristics of Patients with and without Insulin Resistance**

| | HOMA-IR <2.7 (n=343) | HOMA-IR ≥ 2.7 (n=151) | P value |
|---|---|---|---|
| Gender (F/M) (n) | 229/114 | 65/86 | <0.001 |
| Age (years) | 48.30±13.21 | 49.33±12.24 | 0.416 |
| MetS (-/+) | 267/76 | 56/95 | <0.001 |
| NAFLD(-/+) | 156/173 | 33/113 | <0.001 |
| Smoking(pack-years) | 8 (0-100) | 10 (0-105) | 0.110 |
| SBP (mm Hg) | 120.0 (90.0-220.0) | 129.5 (90.0-161.0) | <0.001 |
| DBP (mm Hg) | 78.5 (58.0-110.0) | 80.0 (60.0-110.0) | <0.001 |
| WC (cm) | 88 (61-130) | 101 (66-160) | <0.001 |
| WHR | 0.86 (0.44-1.00) | 0.94 (0.66-1.07) | <0.001 |
| WHtR | 0.52 (0.37-0.81) | 0.59(0.40-0.94) | <0.001 |
| BMI (kg/m2 ) | 25.92±4.26 | 29.96±4.30 | <0.001 |
| Total Cholesterol (mg/dL) | 223.44±47.87 | 218.84±40.83 | 0.305 |
| HDL-C (mg/dL) | 59 (11-126) | 45 (25-87) | <0.001 |
| LDL-C (mg/dL) | 140.50±42.73 | 139.77±37.29 | 0.857 |
| Triglyceride (mg/dL) | 97 (13-835) | 143 (31-531) | <0.001 |
| Uric Acid (mg/dL) | 4.52±1.27 | 5.66±1.42 | <0.001 |
| TSH (uIU/ml) | 1.76 (0.01-24.00) | 1.59 (0.05-13.20) | 0.108 |
| HbA1c (%) | 5.5 (4.4-7.0) | 5.6 (4.6-6.9) | 0.001 |
| HOMA-IR | 1.63 (0.39-2.69) | 3.49 (2.70-13.80) | <0.001 |
| Tg/HDL | 1.60 (0.19-28.79) | 2.95 (0.44-20.42) | <0.001 |
| TyG | 8.46 (6.38-11.45) | 8.91(7.35-10.47) | <0.001 |
| VAI | 2.79 (0.36-40.34) | 4.90 (0.72-30.72) | <0.001 |
| METS-IR | 36.71±7.77 | 46.43±8.22 | <0.001 |

Abbreviations: MetS: metabolic syndrome, NAFLD: non-alcoholic fatty liver disease, SBP: systolic blood pressure, DBP: diastolic blood pressure, WC: waist circumference, WHR: waist hip ratio, WHtR: waist-to-height ratio, BMI: body mass index, HOMA-IR: homeostatic model assessment for insulin resistance, Tg/HDL: triglyceride to HDL-C ratio, TyG: triglyceride to glucose ratio, VAI: visceral adiposity index, METS-IR: metabolic score for insulin resistance

**Table 2: Characteristics of Study Participants According to METS-IR Index Quartiles.**

| | Q1 | Q2 | Q3 | Q4 | P value |
|---|---|---|---|---|---|
| METS-IR | (21.99-32.6) | (32.71-39.26) | (39.27-45.60) | (45.66-72.82) | |
| Gender (F/M) (n) | 98/25 | 84/39 | 58/66 | 54/70 | <0.001 |
| Age (years) | 42.46±12.35 | 49.61±12.37 | 51.72±13.44 | 50.67±11.47 | <0.001 |
| MetS (-/+) | 119/4 | 95/28 | 72/52 | 37/87 | <0.001 |
| NAFLD(-/+) | 97/22 | 49/68 | 34/86 | 9/110 | <0.001 |
| Smoking(pack-years) | 5 (0-60) | 8 (0-55) | 7 (0-100) | 15 (0-105) | 0.012 |
| SBP (mmHg) | 110 (90-160) | 120 (90-180) | 120 (90-220) | 126 (90-160) | <0.001 |
| DBP (mmHg) | 70 (58-100) | 80 (60-102) | 80 (60-110) | 80 (60-110) | <0.001 |
| WC (cm) | 76 (61-102) | 88 (70-113) | 97 (69-130) | 109 (72-160) | <0.001 |
| WHR | 0.78 (0.44-0.95) | 0.86(0.75-1.00) | 0.91(0.68-1.07) | 0.97 (0.66-1.06) | <0.001 |
| WHtR | 0.45 (0.37-0.55) | 0.53 (0.44-0.66) | 0.56(0.42-0.77) | 0.64(0.41-0.94) | <0.001 |
| BMI (kg/m2 ) | 21.81±2.07 | 25.74±1.74 | 28.30±2.19 | 32.70±3.48 | <0.001 |
| HbA1c (%) | 5.3(4.5-6.5) | 5.5(4.5-6.7) | 5.6 (4.4-6.8) | 5.6(4.8-7.0) | <0.001 |
| HOMA-IR | 1.38(0.39-3.01) | 1.77 (0.50-5.16) | 2.24 (1.03-6.98) | 3.22(0.96-13.80) | <0.001 |
| Tg/HDL | 1.03(0.19-4.55) | 1.69 (0.53-10.90) | 2.49(0.79-13.32) | 3.62(0.71-28.79) | <0.001 |
| TyG | 8.06(6.38-9.41) | 8.49(7.20-9.86) | 8.73 (7.76-10.34) | 9.08(7.76-11.45) | <0.001 |
| VAI | 1.80(0.36-6.85) | 2.95(0.79-20.31) | 4.09(1.26-17.38) | 5.85(1.45-40.34) | <0.001 |
| METS-IR | 28.70±2.60 | 35.92±1.77 | 42.30±1.84 | 51.67±5.62 | <0.001 |

Abbreviations: METS-IR: metabolic score for insulin resistance, MetS: metabolic syndrome, NAFLD: non-alcoholic fatty liver disease, SBP: systolic blood pressure, DBP: diastolic blood pressure, WC: waist circumference, WHR: waist hip ratio, WHtR: waist-to-height ratio, BMI: body mass index, Tg/HDL: triglyceride to HDL-C ratio, TyG: triglyceride to glucose ratio, VAI: visceral adiposity index.

The highest predictive value of IR amongst the indexes was found to be for METS-IR (AUC=0.813, p<0.001). The remaining obesity and insulin resistance indexes also showed a significant predictive value for the presence of IR with AUC between 0.771 for WC and 0.725 for TyG (**Figure 1**, **Table 3**).

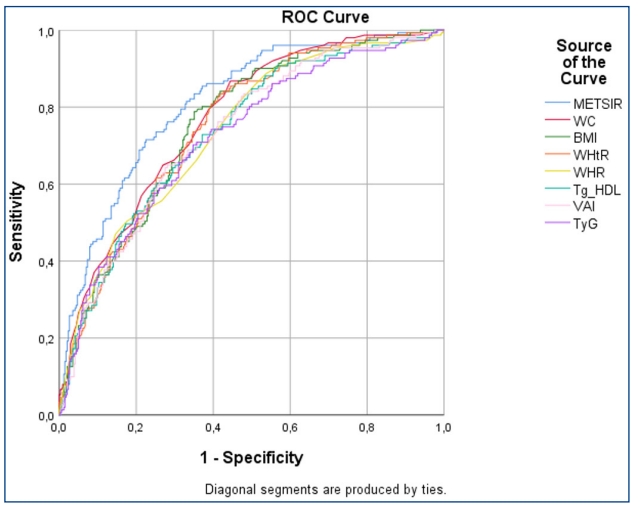


**Figure 1:** ROC curve of indexes to identify insulin resistance.

Abbreviations: ROC: receiver operating characteristic, METS-IR: metabolic score for insulin resistance, WC: waist circumference, BMI: body mass index, WHtR: waist-to-height ratio, WHR: waist hip ratio, Tg/HDL: triglyceride to HDL-C ratio, TyG: triglycerides–glucose index, VAI: visceral adiposity index.

**Table 3: AUC comparison of indexes to identify insulin resistance.**

| INDEX | AUC (95% CI) | P value |
|---|---|---|
| METS-IR | 0.813(0.774-0.853) | <0.001 |
| WC (cm) | 0.771(0.728-0.813) | <0.001 |
| BMI (kg/m2 ) | 0.757(0.713-0.801) | <0.001 |
| WHtR | 0.753(0.709-0.797) | <0.001 |
| WHR | 0.740(0.693-0.786) | <0.001 |
| Tg/HDL | 0.736(0.689-0.783) | <0.001 |
| VAI | 0.733 (0.687-0.780) | <0.001 |
| TyG | 0.725(0.676-0.773) | <0.001 |

A p-value ≤ 0.05 was taken as statistically significant. Abbreviations: AUC: area under the curve, CI: confidence interval, METS-IR: metabolic score for insulin resistance, WC: waist circumference, BMI: body mass index, WHtR: waist-to-height ratio, WHR: waist hip ratio, Tg/HDL: triglyceride to HDL-C ratio, TyG: triglycerides–glucose index, VAI: visceral adiposity index.

ROC curve was used to detect the optimum cut-off values for the highest sensitivity and specificity of METS-IR in predicting IR. At a cut-off value of 39.27, METS-IR had a sensitivity of 83.4% and a specificity of 65%; cut-off value of 39.69, METS-IR had a sensitivity of 81.5% and a specificity of 67%; cut-off value of 42.2, METS-IR had a sensitivity of 71.5% and a specificity of 77.5% (**Table 4**).

When the binary logistic regression model was used to associate MetS and IR as the dependent variables with indexes in **Figure 1** as the independent variables, although TyG and VAI creates a higher risk of MetS than METS-IR, the increase in METS-IR creates the highest risk of IR than the increase in other indexes (**Table 5**).

**Table 5: Logistic Regression Models to Identify MetS and IR.**

| | MetS | | IR | |
|---|---|---|---|---|
| | p | OR %95 CI | p | OR %95 CI |
| METS-IR | <0.001 | 1.332 (1.182-1.502) | <0.001 | 1.332 (1.189-1.492) |
| BMI (kg/m2 ) | <0.001 | 0.596 (0.480-0.741) | <0.001 | 0.695 (0.570-0.847) |
| WC | <0.001 | 1.080 (1.040-1.122) | 0.006 | 1.046 (1.013-1.080) |
| TyG | <0.001 | 12.933 (5.108-32.747) | 0.075 | 1.964 (0.934-4.133) |
| VAI | 0.001 | 1.743 (1.252-2.427) | 0.281 | 0.873 (0.683-1.117) |
| Tg/HDL | <0.001 | 0.293 (0.176-0.487) | 0.564 | 0.888 (0.594-1.328) |
| HOMA-IR | 0.045 | 1.291 (1.006-1.656) | | |

Binary logistic regression models were used to associate MetS and IR diagnosis as the dependent variables with insulin resistance and adiposity indexes as the independent variables. A p-value ≤ 0.05 was taken as statistically significant. Abbreviations: 95% CI = 95% confidence interval, BMI: body mass index, IR: Insulin resistance, MetS: metabolic syndrome, METS-IR: metabolic score for insulin resistance, OR: odds ratio, Tg/HDL: triglyceride to HDL-C ratio, TyG: triglycerides–glucose index, VAI: visceral adiposity index, WC: waist circumference.

## DISCUSSION

METS-IR, a new insulin resistance index developed by Bello-Chavolla et al., is calculated using BMI, fasting glucose, triglyceride, and HDL-C measurements. It has been verified using the hyperinsulinaemic-euglycaemic clamp test, which is the gold standard method for measuring insulin resistance (10). As it does not require insulin measurement, which is not readily available in primary care centers, METS-IR may have an important role for primary prevention of metabolic diseases such as diabetes mellitus, through screening of IR. We evaluated the use of METS-IR for detecting IR and its optimal cut-off value in the Turkish population as METS-IR has been shown to be associated with IR in other ethnic groups and has been proven by several studies to predict metabolic disorders. METS-IR was found to have a better diagnostic performance than WC, BMI, WHtR, WHR, Tg/HDL, VAI and TyG indexes. The cut-off values of 39.27, 39.69 and 42.20 were found to be more associated with the IR. We observed that the increase in METS-IR created a higher risk of IR than the increase in other indices.

**Table 4: The Cut-off Values of METS-IR to Identify Insulin Resistance.**

| | Cut-Off | Sensitivity (%) | Specificity (%) | AUC | 95% CI | P |
|---|---|---|---|---|---|---|
| METS-IR | 39.27 | 83.4 | 65 | 0.813 | 0.774-0.853 | <0.001 |
| METS-IR | 39.69 | 81.5 | 67 | | | |
| METS-IR | 42.20 | 71.5 | 77.5 | | | |

The optimal cut-off value was obtained as the maximum sensitivity and specificity. A p-value ≤ 0.05 was taken as statistically significant. METS-IR: metabolic score for insulin resistance and AUC: area under the curve. 95% CI = 95% confidence interval.

At a study which included 12290 non-obese Japanese participants that were followed up for 5.5 years, investigated associations between the METS-IR and the risk of type 2 diabetes mellitus. Diabetes occurred in 176 participants and the risk of developing diabetes was reported to increase with the quartile of change in the METS-IR index, even after adjustment for multiple potential confounding factors. The HRs for the Q4 group versus the Q1 group was found 4.01 (11).

TyG, Tg/HDL and the METS-IR indexes were compared for the evaluation of metabolic status in 30291 individuals in China. Although the TyG index was more significant, all three indices were found to have high sensitivity and specificity for the identification of metabolic pathologies. Similar to this study, in the regression analysis, in which the relationship between TyG, Tg/HDL, METS-IR, BMI, WC, VAI and HOMA-IR with the presence of metabolic syndrome was examined, the highest significant value was observed with the TyG index (12).

Zhang et al. investigated the association of METS-IR and its six-year change with risk of incident diabetes mellitus in a Chinese population. They include 12107 participants with the mean age of 50.48 years. After six years of follow-up, type 2 diabetes mellitus developed in 758 participants. An increasing risk of incident type 2 diabetes mellitus with increasing METS-IR levels and six-years METS-IR changes by age, sex, and basal fasting glucose levels were found. The mean of baseline METS-IR levels were 42.19 in participants with diabetes, while it was 37.05 in those without diabetes. In our study, the METS-IR level of the group with insulin resistance was 46.43, while that of the group without insulin resistance was 36.71, in line with this study (13).

In a study of 142005 patients in which the relationship between TG/HDL, TyG, METS-IR indices and hypertension was investigated, and only METS-IR showed a significant correlation with blood pressure level. In our study, increased blood pressure levels were observed as the METS-IR quartiles increased (14).

### Limitations of the Study

Our study had some limitations: First, the diagnosis of IR was made with HOMA-IR and not with the euglycemic–hyperinsulinemic clamp test, which is the gold standard. Secondly, our study is cross-sectional. However, we plan to follow the patient population with further studies to observe the diabetes incidence. Thirdly, the number of patients in our study is low because we do not measure insulin levels in every patient, therefore, we may not be able to generalize our findings to the entire population. However we believe that our study has given important and valuable preliminary data.

## CONCLUSION

Inability to measure insulin levels in family health centers, which is the main center of preventive medicine, can hinder the early diagnosis of IR. Detection of IR, which as being a leading cause of future metabolic disorders, with simple anthropometric and biochemical methods can enable early precautions. Although there is a need for future studies on this subject, it has been revealed that METS-IR values between 39 – 42 may be associated with IR, especially in cases where insulin measurement cannot be performed, the use of this index can provide us with early diagnosis.

## ETHICAL DECLARATIONS

**Ethics Committee Approval:** Maltepe University Clinical Research Ethics Committee approved the study (Approval Date: 20.10.2021, Approval Number: 2021/900/105).

**Informed Consent:** Because the study was retrospective, written informed consent wasnot obtained from patients.

**Referee Evaluation Process:** Externally peer-reviewed.

**Conflict of Interest Statement:** The authors have no conflicts of interest to declare.

**Financial Disclosure:** The authors declared that this study has received no financial support.

**Author Contributions:** All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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