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**RESEARCH**

# Serum asprosin and trimethylamine oxide levels in polycystic over syndrome with and without metformin treatment

## Polikistik over sendromunda metformin tedavisi alan ve almayanlarda serum asprosin ve trimetilamin oksit düzeyleri

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**Abstract**

**Purpose:** The presence of a potential relationship between metabolic diseases and hormones and the intestinal flora has recently gained attention. Levels of asprosin and trimethylamine oxide (TMAO) may be associated with polycystic over syndrome (PCOS), which is a metabolic disease. The present study aims to investigate the potential relationship of PCOS with serum asprosin and TMAO levels.

**Materials and Methods:** This cross-sectional study included 30 PCOS patients on metformin, 30 PCOS patients not receiving treatment, and 30 healthy controls. The demographic, glucose, insulin resistance, lipid, and hormone profiles of the participants were analyzed. Serum asprosin and TMAO levels were investigated with the ELISA method.

**Results:** Patients with PCOS had higher BMI, serum glucose, triglyceride, ALT, insulin levels, and HOMA-IR scores compared with controls. The serum testosterone level was 28.1 ng/dl in the control group, 33.3 ng/dl in the PCOS group receiving metformin and 48.0 ng/dl in the untreated PCOS group, and there was a statistically significant difference. Neither serum asprosin nor TMAO levels were significantly different when compared between the three groups.

**Conclusion:** Serum asprosin and TMAO levels of individuals with PCOS and healthy controls were not significantly different. The receipt of metformin treatment by PCOS patients did not have a significant relationship with serum asprosin and TMAO levels.

**Keywords:**. Polycystic ovary syndrome, trimethylamine n-oxide, insulin resistance, gastrointestinal microbiome

**Öz**

**Amaç:** Hormonlar ve bağırsak florası ile metabolik hastalıklar arasında potansiyel bir ilişkinin varlığı son zamanlarda dikkat çekmektedir. Asprosin ve trimetilamin oksit (TMAO) düzeyleri metabolik bir hastalık olan polikistik over sendromu (PKOS) ile ilişkili olabilir. Bu çalışmada PKOS'un serum asprosin ve TMAO seviyeleri ile potansiyel ilişkisini araştırmak amaçlanmıştır.

**Gereç ve Yöntem:** Bu kesitsel çalışmaya metformin kullanan 30 PKOS hastası, tedavi almayan 30 PKOS hastası ve 30 sağlıklı kontrol dahil edildi. Katılımcıların demografik, glukoz, insülin direnci, lipid ve hormon profilleri analiz edildi. Serum asprosin ve TMAO düzeyleri ELISA yöntemi ile araştırıldı.

**Bulgular:** PKOS'lu hastalar, kontrollerle karşılaştırıldığında daha yüksek BMI, serum glukozu, trigliserit, ALT, insülin seviyeleri ve HOMA-IR skorlarına sahipti. Serum testosteron düzeyi kontrol grubunda 28,1 ng/dl, metformin tedavisi alan PCOS grubunda 33,3 ng/dl ve tedavi almayan PCOS grubunda 48,0 ng/dl idi ve istatistiksel olarak anlamlı farklılık vardı. Üç grup arasında serum asprosin ve TMAO seviyeleri arasında anlamlı fark yoktu.

**Sonuç:** PKOS'lu bireyler ile sağlıklı kontrollerin serum asprosin ve TMAO seviyeleri arasında anlamlı fark yoktu. Metformin tedavisi ile PKOS hastalarının serum asprosin ve TMAO seviyeleri ile anlamlı bir ilişkisi yoktu.

**Anahtar kelimeler**: Polikistik over sendromu, trimetilamin n-oksit, insülin direnci, gastrointestinal mikrobiyom

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## INTRODUCTION

Polycystic ovary syndrome (PCOS) is a disorder characterized by excess androgens and ovarian dysfunction. In PCOS, ovarian dysfunction is accompanied by endocrine and metabolic disorders. The diagnosis of PCOS requires oligomenorrhea and hyperandrogenism (clinical or biochemical) in women, as well as the appearance of a polycystic ovary on ultrasound. PCOS is common among adolescent and young females, and its overall prevalence is estimated to be between 6-20%[1].

Women diagnosed with PCOS are predisposed to metabolic diseases and complications in addition to fertility disorders. These women were reported to be under a higher risk for Type 2 diabetes mellitus (t2DM), obesity, endometrial carcinoma, infertility, mood and eating disorders, and cardiovascular diseases[2]. Previous studies in the literature have reported that PCOS was accompanied by obesity, insulin resistance (IR), hyperinsulinemia, and inflammation, resulting in an increase in metabolic complications[3]. Insulin resistance is common among women with PCOS and is implicated in the development of several metabolic complications. Asprosin, which is a recently discovered protein that was described to play a role in the glucose metabolism, is likely to be involved in metabolic disorders. Previous literature data suggest elevated asprosin levels in insulin resistance[4, 5]. In addition, serum asprosin levels were reported to be lower in those with PCOS compared to controls[6, 7]. Current data in the literature are focused on whether asprosin can guide the diagnosis and the elucidation of the pathogenesis of PCOS.

The intestinal flora is defined as the second brain and it is currently reported to play a role in the regulation of the metabolism, along with reports suggesting the involvement of the gut microbiota in metabolic diseases such as obesity and insulin resistance[8]. Recent studies have focused on the potential relationship between the gut microbiota and PCOS. Trimethylamine N-oxide (TMAO) is an organic compound produced by the bowel microbiome. Previous literature data have described a relationship between insulin resistance, T2DM and inflammation[9]. Plasma levels of TMAO were shown to be elevated in women with PCOS[10, 11]. Current data from the literature indicate that the increase in insulin resistance and inflammation induced by PCOS affects TMAO levels, proposing that TMAO can be a marker indicating the severity of PCOS.

Are serum asprosin and TMAO levels altered in PCOS and are they associated with insulin resistance? In light of the literature data summarized above, the hypothesis of the study is that insulin resistance seen in PCOS clinic and an increase in asprosin and TMAO levels are expected. Therefore the current study has focused on asprosin and TMAO levels in individuals with PCOS. The present study aims to investigate serum asprosin and TMAO levels, insulin resistance, certain metabolic parameters, and the association of these with metformin treatment in PCOS.

## MATERIAL AND METHODS

### Study design and population

This cross-sectional study was conducted in the Endocrinology Clinic of Firat University Faculty of Medicine between January - July 2021. The minimum sample size required to detect a significance difference using this test should be at least 17 in each group, (51 in total), considering type I error (alfa) of 0.05, power (1-beta) of 0.8, and effect size of 0.45. This study included 30 PCOS patients on metformin, 30 PCOS patients not on metformin, and 30 healthy controls.

The diagnosis of PCOS was confirmed by gynecologists considering 2003 Rotterdam PCOS diagnostic criteria (Oligo-anovulation or Clinical and/or biochemical findings of hyperandrogenism or Polycystic ovaries). According to the Rotterdam criteria, at least two of three criteria are necessary for the diagnosis of PCOS. Metformin treatment initiation and follow-up were carried out by endocrinologists in the endocrinology clinic, taking into account the adrenal and gonadal diseases guide 2022 of the Turkish Society of Endocrinology and Metabolism.

In order to ensure that all participants were at similar ages, volunteers aged between 20 and 30 years were included. Thus, this study included volunteers diagnosed with PCOS who were aged between 20 and 30 years. Individuals with type 1 and type 2 DM, malignancies, chronic kidney disease, chronic heart failure, rheumatological diseases, acute infections, and individuals receiving hormone therapy due to infertility were excluded from the study. This study was approved by Firat Universtiy Non-Invasional

Research Ethics Committee (date: 05/11/2020, approval number: 2020/15-22). All participants were informed about the study and provided written consent.

## Definitions

The diagnosis of PCOS was confirmed for all patients by gynecologists according to the Rotterdam criteria. For the PCOS diagnosis; at least two of the following criteria were required: presence of a polycystic ovary, presence of oligoovulation and/or anovulation, and hyperandrogenism (high androgen levels and/or presence of hyperandrogenemia symptoms) after the exclusion of other endocrine disorders. The HOMA-IR score was used to determine insulin resistance and the cut-off value was taken as ≥2.5. For the patient group receiving metformin treatment, a minimum dose of 1 mg metformin received regularly over a minimum period of 6 months was defined as a criterion.

## Data collection

Twelve-hour fasting blood samples were obtained from all participants between the 3rd and 5th days of the menstrual cycle for biochemical parameters, asprosin and TMAO levels. Biochemical parameters (glucose, AST, ALT, urea, creatinine, triglyceride, total cholesterol, LDL and HDL cholesterol, HbA1c, fasting insulin level, estradiol, testosteron, CRP, TSH, FSH, LH, DHEA-SO4) and complete blood count were analyzed at Firat University Hospital using an automatic biochemical analysis device according to standard laboratory techniques. In order to measure the asprosin and TMAO levels of the participants, blood was collected into tubes with aprotinin, and stored at -20 $^0$C until the day of the analysis after being centrifuged at 4000 rpm.

## Serum asprosin and TMAO analysis

Analyses of the asprosin (Sunred Biotechnology Company, Catalogue Number:201-12-7691) and TMAO (Sunred Biotechnology Company, Catalogue Number:201-12-7378 ) levels in serum samples were performed using the ELISA method. The ELISA method was applied according to the assay principles specified in the catalogues.

## Statistical analysis

Statistical analysis was conducted using SPSS version 22 (Armonk, NY, USA). The conformance of the variable to the normal distribution was evaluated (Kolmogorov–Smirnov) using both analytical and visual techniques, such as histograms and probability graphs. Categorical variables were expressed as frequencies and percentages. The Pearson chi-square test test was employed to determine whether there was any difference between the groups in terms of categorical variables. Continuous variables were expressed as mean and standard deviation or median and interquartile range depending on the normality of distribution. To determine whether there was any difference between the groups in terms of numerical variables, independent groups were examined using the one-way Anova or the Kruskal-Wallis test. Post-hoc analyses used the Bonferroni test. $p<0.05$ was considered statistically significant.

# RESULTS

Of the total 90 female participants included in the study, 30 were PCOS patients on metformin, 30 were PCOS patients not on metformin, and 30 were healthy controls. The study groups were similar in terms of age ($p=0.556$). Participants with PCOS were determined to have significantly higher weight ($p<0.001$), BMI ($p<0.001$), triglyceride ($p=0.021$), fasting blood sugar ($p=0.022$), and ALT ($p=0.001$) levels compared to the control groups (Table 1).

Participants with PCOS had higher insulin ($p<0.001$), HOMA-IR ($p<0.001$), HOMA-beta ($p=0.008$), HOMA-Q ($p<0.001$), and testosterone levels compared to the control group. PCOS patients on metformin and PCOS patients not on metformin did not have significantly different insulin, HOMA-IR, HOMA-beta, HOMA-Q levels. Meanwhile, participants with PCOS who were not on metformin had significantly higher testoterone levels compared to PCOS patients on metformin (Table 2). There were no statistically significant differences between the three groups included in the study with regard to TMAO ($p=0.589$) or asprosin ($p=0.921$) levels (Table 3). A significant positive correlation was determined between the asprosin and TMAO levels of the PCOS patients on metformin ($r=0.932$, $p<0.001$). Serum TMAO levels showed a positive correlation with systolic blood pressure ($r=0.470$, $p=0.009$) and the HOMA-Q ($r=0.393$, $p=0.032$) score; and a significant negative correlation with insulin ($r= -0.410$, $p=0.024$) and HOMA-IR ($r= -0.407$, $p=0.026$). A significant positive correlation was found between asprosin and systolic blood pressure ($r=0.457$, $p=0.011$) (Table 4).

**Table 1. Comparison of the demographic and biochemical parameters of the participants**

| Variable | On Metformin | Not On Metformin | Control | p |
|---|---|---|---|---|
| Age (years) Median±SD | 24.1±5.6 | 22.8±3.9 | 23.4±4.3 | 0.556* |
| Height (cm) Median±SD | 163.8±5.5 | 163.0±5.8 | 161.6±4.2 | 0.285* |
| Weight (kg) Median±SD | 71.6±11.7[a] | 62.7±10.0[b] | 55.5±8.9[c] | **<0.001*** |
| BMI (kg/m²) Median±SD | 26.7±4.1[a] | 23.9±3.9[b] | 21.3±3.4[c] | **<0.001*** |
| Systolic blood pressure (mmHg) median (IQR) | 120 (110-130) | 120 (110-130) | 120 (110-120) | 0.556*** |
| Diastolic blood pressure (mmHg) median (IQR) | 80 (70-80) | 70 (70-80) | 80 (70-80) | 0.285* |
| T. Cholesterol (mg/dl) | 175.8±35.6 | 163.3±33.0 | 161.4±22.7 | 0.151* |
| LDL (mg/dl) Median±SD | 106.1±30.7 | 96.2±30.3 | 89.1±20.9 | 0.065* |
| LDL-c (mg/dl) Median±SD | 57.3±12.7 | 62.6±15.3 | 62.0±16.0 | 0.322* |
| Triglyceride (mg/dl). median (IQR) | 92.5 (70.0-153.0)[a] | 74.5 (63.0-106.0)[a.b] | 70.5 (61.0-100.0)[b] | **0.021***** |
| Glucose (mg/dl) median (IQR) | 92.5 (88.0-101.0)[a] | 90.0 (86.0-95.0)[a.b] | 88.5 (84.0-91.0)[b] | **0.022***** |
| Urea (mg/dl) median (IQR) | 21.0 (19.0-25.0) | 22.5 (19.0-27.0) | 22.5 (19.0-28.0) | 0.561*** |
| Creatinine (mg/dl). Median±SD | 0.64±0.11 | 0.66±0.09 | 0.63±0.07 | 0.268* |
| ALT (mg/dl) median (IQR) | 16.0 (14.0-21.0)[a] | 14.0 (12.0-20.0)[a] | 12.0 (10.0-13.0)[b] | **0.001***** |
| AST (mg/dl) median (IQR) | 18.0 (15.0-21.0) | 17.0 (14.0-20.0) | 17.0 (15.0-19.0) | 0.860*** |
| Hgb (g/dl) Median±SD | 13.4±1.1 | 13.9±1.0 | 13.7±0.9 | 0.154* |
| Smoking n (%) | | | | |
| Yes | 10 (33.3) | 9 (30.0) | 7 (23.3) | **0.685**** |
| No | 20 (66.7) | 27 (70.0) | 23 (76.7) | |

*One Way ANOVA test; **Chi-square analysis applied; a,b,c: Group causing difference; ***Kruskal Wallis test applied. IQR: Interquartile range. BMI: body mass index; LDL: low-density lipoproteins-cholesterol; AST: Aspartate aminotransferase; ALT: alanine aminotransferase; Hgb: hemoglobin

**Table 2. Comparison of the endocrine and hormonal parameters of the participants**

| Variable | On Metformin | Not On Metformin | Control | p* |
|---|---|---|---|---|
| | Median (IQR) | Median (IQR) | Median (IQR) | |
| Insulin (µIU/ml) | 11.9 (7.5-19.9)[a] | 8.4 (6.1-15.2)[a] | 6.6 (3.9-7.6)[b] | **<0.001**** |
| HbA1c (%). | 5.5±0.4 | 5.2±0.4 | 5.3±0.4 | 0.073* |
| TSH (IU/mL) | 2.2 (1.4-3.0) | 1.5 (1.1-1.9) | 1.4 (1.1-2.7) | 0.079** |
| HOMA-IR | 2.8 (1.6-4.3)[a] | 1.9 (1.3-3.6)[a] | 1.3 (.9-1.6)[b] | **<0.001**** |
| HOMA-Beta | 131.1 (89.3-211.6)[a] | 125.4 (75.2-167.5)[a] | 99.2 (59.6-124.1)[b] | **0.008**** |
| HOMA-Q | 0.33 (0.31-0.36)[a] | 0.35 (0.32-0.37)[a] | 0.37 (0.35-0.39)[b] | **<0.001**** |
| DHEA-SO4 | 285.0 (185.0-417.0) | 277.5 (226.0-349.0) | 219.0 (173.0-302.0) | 0.145** |
| FSH (mIU/mL) | 5.9 (4.8-6.9) | 6.3 (3.3-8.3) | 7.2 (3.5-9.0) | 0.697** |
| LH (mIU/mL) | 6.3 (5.1-11.4) | 7.4 (5.2-11.3) | 5.4 (3.6-8.7) | 0.191** |
| Estradiol (pg/mL) | 51.5 (39.0-81.0) | 71.0 (50.0-134.0) | 75.0 (40.0-183.0) | 0.225** |
| Testosterone (ng/dL) | 33.3 (26.0-48.6)[a] | 48.0 (39.9-64.5)[b] | 28.1 (20.0-29.6)[c] | **<0.001*** |

*One Way ANOVA test; a,b,c: Group causing difference; **Kruskal Wallis test applied. IQR: Interquartile range.
TSH: hyroid-stimulating hormone; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; DHEA-SO4: dehydroepiandrosterone sulfate; FSH: Follicle-stimulating hormone; LH: luteinizing hormone

**Table 3. Comparison of serum asprosin and TMAO levels**

| Variable | On Metformin | Not On Metformin | Control | p* |
|---|---|---|---|---|
| TMAO (ng/ml) | 1.12 (0.75-4.08) | 0.97 (0.64-2.37) | 1.25 (0.69-4.40) | 0.589 |
| Asprosin (ng/ml) | 33.0 (20.4-67.9) | 40.7 (17.8-68.6) | 43.2 (17.0-64.4) | 0.921 |

* Kruskal Wallis test applied; IQR: Interquartile range. TMAO: trimethylamine N-oxide

**Table 4. Correlations between the TMAO and asprosin levels of the group on metformin and various parameters**

| Variables | TMAO (ng/ml) | | Asprosin (ng/ml) | |
|---|---|---|---|---|
| | R | P | R | P |
| Asprosin (ng/ml) | 0.932 | <0.001 | | |
| Age | 0.112 | 0.555 | 0.129 | 0.498 |
| Height (cm) | -0.075 | 0.693 | -0.024 | 0.901 |
| Body weight (kg) | -0.222 | 0.239 | -0.141 | 0.457 |
| BMI (kg/m2) | -0.200 | 0.288 | -0.159 | 0.401 |
| Systolic blood pressure (mmHg) | 0.470 | 0.009 | 0.457 | 0.011 |
| Diastolic blood pressure (mmHg) | 0.175 | 0.363 | 0.225 | 0.240 |
| TG (mg/dL) | -0.239 | 0.202 | -0.216 | 0.251 |
| T. Cholesterol | -0.135 | 0.477 | -0.118 | 0.535 |
| LDL (mg/dL) | -0.209 | 0.267 | -0.184 | 0.330 |
| HDL (mg/dL) | 0.092 | 0.628 | 0.065 | 0.732 |
| Glucose (mg/dL) | -0.107 | 0.575 | -0.100 | 0.599 |
| Urea (mg/dL) | -0.111 | 0.560 | -0.194 | 0.304 |
| Creatinine (mg/dL) | 0.324 | 0.080 | 0.248 | 0.186 |
| ALT (U/L) | -0.247 | 0.188 | -0.252 | 0.180 |
| AST (U/L) | 0.074 | 0.698 | 0.120 | 0.526 |
| Hb (g/dL) | -0.115 | 0.546 | -0.006 | 0.976 |
| Insulin (µIU/ml) | -0.410 | 0.024 | -0.361 | 0.050 |
| HbA1c (%) | -0.017 | 0.929 | -0.022 | 0.912 |
| TSH (IU/mL) | 0.186 | 0.335 | 0.168 | 0.384 |
| DHEA-SO4 | 0.250 | 0.190 | 0.259 | 0.175 |
| FSH (mIU/mL) | 0.087 | 0.649 | 0.086 | 0.653 |
| LH (mIU/mL) | 0.148 | 0.435 | 0.120 | 0.526 |
| Estradiol (pg/mL) | 0.152 | 0.424 | 0.123 | 0.516 |
| Testosteron (ng/dL) | 0.264 | 0.158 | 0.357 | 0.053 |
| HOMA-IR | -0.407 | 0.026 | -0.348 | 0.060 |
| HOMA-BETA | -0.329 | 0.075 | -0.301 | 0.105 |
| HOMA-Q | 0.393 | 0.032 | 0.332 | 0.073 |

BMI: body mass index; LDL: low-density lipoproteins-cholesterol; AST: Aspartate aminotransferase; ALT: alanine aminotransferase; Hgb: hemoglobin; TSH: hyroid-stimulating hormone; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; DHEA-SO4: dehydroepiandrosterone sulfate; FSH: Follicle-stimulating hormone; LH: luteinizing hormone

There was a significant positive correlation between the TMAO and asprosin levels of PCOS patients not on metformin ($r=0.965$, $p<0.001$). Fasting blood sugar had a significant negative correlation with both TMAO ($r= -0.455$, $p=0.012$) and asprosin ($r= -0.501$, $p=0.005$) levels in this group (Table 5).

**Table 5. Correlations between the TMAO and asprosin levels of the group not on metformin and various parameters**

| Variables | TMAO (ng/ml) | | Asprosin (ng/ml) | |
|---|---|---|---|---|
| | r | p | r | p |
| Asprosin (ng/ml) | 0.965 | <0.001 | | |
| Age | 0.150 | 0.428 | 0.206 | 0.275 |
| Height (cm) | 0.087 | 0.646 | 0.118 | 0.536 |
| Body weight (kg) | 0.082 | 0.667 | 0.103 | 0.588 |
| BMI (kg/m2) | -0.091 | 0.632 | -0.083 | 0.661 |
| Systolic blood pressure (mmHg) | -0.056 | 0.768 | -0.030 | 0.876 |
| Diastolic blood pressure (mmHg) | 0.039 | 0.838 | -0.071 | 0.708 |
| Triglyceride (mg/dL) | 0.134 | 0.479 | 0.234 | 0.212 |
| T. Cholesterol | 0.054 | 0.776 | 0.197 | 0.297 |
| LDL (mg/dL) | -0.026 | 0.893 | 0.069 | 0.718 |
| HDL (mg/dL) | 0.026 | 0.890 | 0.059 | 0.759 |
| Glucose (mg/dL) | -0.455 | 0.012 | -0.501 | 0.005 |
| Urea (mg/dL) | 0.025 | 0.895 | -0.083 | 0.664 |
| Creatinine (mg/dL) | 0.256 | 0.172 | 0.233 | 0.214 |
| ALT (U/L) | -0.154 | 0.417 | -0.232 | 0.217 |
| AST (U/L) | -0.203 | 0.282 | -0.321 | 0.084 |
| Hb (g/dL) | 0.079 | 0.678 | 0.157 | 0.408 |
| Insulin (µIU/ml) | -0.166 | 0.380 | -0.059 | 0.757 |
| HbA1c (%) | -0.337 | 0.069 | -0.349 | 0.059 |
| TSH (IU/mL) | 0.017 | 0.929 | -0.010 | 0.959 |
| DHEA-SO4 | -0.028 | 0.882 | -0.046 | 0.807 |
| FSH (mIU/mL) | 0.268 | 0.152 | 0.184 | 0.332 |
| LH (mIU/mL) | -0.250 | 0.182 | -0.312 | 0.093 |
| Estradiol (pg/mL) | -0.164 | 0.386 | -0.159 | 0.402 |
| Testosteron (ng/dL) | -0.092 | 0.629 | -0.167 | 0.379 |
| HOMA-IR | -0.196 | 0.299 | -0.089 | 0.641 |
| HOMA-BETA | -0.200 | 0.290 | -0.057 | 0.766 |
| HOMA-Q | 0.203 | 0.282 | 0.097 | 0.610 |

BMI: body mass index; LDL: low-density lipoproteins-cholesterol; AST: Aspartate aminotransferase; ALT: alanine aminotransferase; Hgb: hemoglobin; TSH: hyroid-stimulating hormone; HOMA-IR: Homeostasis Model Assessment of Insulin Resistance; DHEA-SO4: dehydroepiandrosterone sulfate; FSH: Follicle-stimulating hormone; LH: luteinizing hormone.

## DISCUSSION

The present study investigated serum TMAO and asprosin levels in a patient group with PCOS and the effects of metformin therapy on these two parameters. PCOS is associated with a variety of health problems, the most common of which are metabolic problems. PCOS has been linked to obesity, an elevated risk for cardiovascular diseases, insulin resistance, and a higher predisposition to T2DM[12].

The present study primarily investigated certain biochemical parameters of the participants. It determined higher weight, BMI, triglyceride, fasting blood glucose, and ALT levels in patients with PCOS compared to the control group. As one of the main pathological events in PCOS, hypoandrogenism was reported to induce insulin resistance, which, in turn, can result in a variety of metabolic syndromes. Previous literature data have shown that the insulin resistance seen in PCOS leads to obesity, impaired glucose tolerance, and elevated triglyceride and LDL levels[12, 13]. Results obtained in the present study are consistent with the literature data. As expected, in the present study, insulin and the HOMA-IR score were significantly higher in participants with PCOS compared to the control group. As also mentioned above, insulin resistance is the main factor responsible for the metabolic output in PCOS.

Previous literature data have proven that insulin resistance develops in PCOS[2-5, 12, 13]. Although insulin resistance is an unchangeable reality in PCOS, it has not been clarified if it is caused by PCOS or it is involved in the etiology of PCOS. Goodman et al., reported that insulin resistance increased anovulation and hyperandrogenism by negatively affecting the hypothalamic-pituitary axis[13]. Moreover, reducing insulin resistance and obesity was found to improve PCOS symptoms[14]. It can be said that the relationship between PCOS and insulin resembles a chicken-and-egg relationship. However, it is a known fact that PCOS and insulin resistance are concomitant, which was once again highlighted by the results of the present study.

The present study compared asprosin levels of three participant groups. A statistically significant difference was determined between the groups comprising the study population. Previous literature data have shown that asprosin is released by adipose tissue and primarily causes glucose secretion in the liver. Asprosin is known to be released in fasting conditions to increase blood glucose levels and its levels are expected to increase in conditions of insulin resistance[15]. In the present study, serum asprosin levels were expected to be higher in ovary syndrome with insulin resistance; however, no significant difference was determined between the control group and the patient group. Previous data from the literature have reported elevated asprosin levels in the patient population with PCOS[5-7]. Given the above mentioned mechanism and a multitude of evidence from the literature indicating elevated asprosin levels in PCOS, the absence of a difference in the present study is contradictory to the literature. Chang et al., also reported that asprosin levels and PCOS did not have a significant relationship. The absence of a difference in the asprosin levels of PCOS patients despite the relationship between insulin resistance and asprosin may be attributed to the difference in the insulin resistance mechanisms of PCOS patients. In a study by Yuan et al. that investigated the relationship between asprosin and metabolic diseases, it was stated that the literature did not hold sufficient evidence to explain the relationship between asprosin and PCOS[16]. Although studies reporting higher asprosin levels in PCOS constitute the majority, there are also studies that suggest the opposite, in line with the results of the present study. New studies are needed in order to illuminate the relationship between PCOS and asprosin.

The present study did not determine a statistically significant difference between the TMAO levels of the participants in the three groups. TMAO production is associated with the variety of nutrients obtained through diet and the gut microbiota. It was reported that problems of the gut microbiota induced elevated TMAO production, and that this increased the risk for diseases such as kidney failure, diabetes mellitus, heart failure, atherosclerosis, hypertension, metabolic syndrome, and dyslipidemia[17]. Studies investigating serum TMAO levels in PCOS patients are very scarce. Two previous studies have reported elevated serum TMAO levels in those with PCOS[10, 18]. Annunziata et al. stated that the literature review they conducted to reveal the relationship between PCOS and TMAO did not yield enough data[11]. Serum TMAO levels are directly associated with the gut microbiota. Previous studies and the present study did not take the intestinal flora into consideration in the investigation of the relationship between PCOS and TMAO. As described above, the impairment of the gut microbiota both provides a basis for endocrine diseases and constitutes the primary reason for TMAO elevation. Although two different studies in the literature have demonstrated higher TMAO levels in individuals with PCOS, the literature does not contain a satisfactory level of evidence to elucidate the relationship between TMAO and PCOS. Further studies may shed light on this matter by considering the differences in the intestinal flora in the investigation of TMAO levels in PCOS patients.

The cross-sectional and single-center design of the study constitute its first limitation. The obtained data may not be reflective of the entire population. The number of years with PCOS may have influenced the study results and not involving the duration with PCOS constitutes another limitation of the present study. Again, the absence of an evaluation of the factors influencing the intestinal flora and the existing intestinal flora of the study population may have influenced the TMAO results associated with the intestinal flora. Metformin treatment status was based on patient report and potential drug noncompliance was not taken into account. Despite our many limitations, both PCOS patients on metformin and PCOS patients not on metformin were compared with healthy controls. In the literature, studies investigating asprosin and TMAO levels in PCOS patients are very scarce and the present study has highlighted the need for a more thorough evaluation of the subject matter by

obtaining results that diverged from the previous studies. We think that further studies can obtain stronger evidence by considering the results and limitations of the present study.

In conclusion, results of the current study showed that serum asprosin and TMAO levels were not different from healthy controls in the patient population with PCOS. In addition, it was shown that metformin therapy had no effect on serum asprosin and TMAO levels in PCOS patients. Adequate evidence for the use of serum asprosin and TMAO levels as markers in PCOS has not been gathered. New studies are needed in order to elucidate the relationship between PCOS and serum asprosin and TMAO levels.

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