Evaluation of the relationship between insulin resistance and different phenotypes of polycystic ovary syndrome

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ABSTRACT

Aims: Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women. Hyperinsulinemia and insulin resistance (IR) are the most important metabolic abnormalities that affect these patients. This study aimed to investigate the variables related to IR in patients with different PCOS phenotypes.

Methods: This retrospective study included 389 women diagnosed with PCOS in Bezmialem Hospital between November 2020 and September 2022. Information about patients was collected through their electronic records. PCOS was diagnosed based on the Rotterdam criteria, and four phenotypes of A (oligoovulation + hyperandrogenism + PCO), B (oligoovulation + hyperandrogenism), C (hyperandrogenism + PCO), and D (oligoovulation + absent PCO) were considered for PCOS. The homeostatic model assessment for insulin resistance (HOMA-IR) was used to evaluate IR. The Mann-Whitney U test was performed to study the difference between the groups.

Results: The highest value of HOMA-IR was for the phenotype B group, and the lowest value was for the phenotype C group. However, the difference between the groups was not significant (p=0.221). Estradiol and free T4 were significantly higher in the phenotype A group (p≤0.001). Thyroid-stimulating hormone (TSH), prolactin, anti-mullerian hormone (AMH), fasting insulin, total testosterone, and red blood cell distribution width (RDW) were significantly higher in the phenotype B group (p≤0.001). Total cholesterol, high density lipoprotein (HDL), leukocyte, basophil, and monocyte were significantly higher in the phenotype C group (p≤0.001). Also, MPV values were significantly higher in the phenotype D group (p≤0.001).

Conclusion: The results showed that the variables related to IR in phenotypes A and B of PCOS are higher than in other phenotypes.

Keywords: Polycystic ovary syndrome, insulin resistance, HOMA-IR, RDW

INTRODUCTION

The absence of ovulation is a common complication with various clinical manifestations, including oligomenorrhea, amenorrhea, hirsutism, and abnormal uterine bleeding.¹ Also, this complication can cause potentially harmful results such as infertility, increased risk of endometrial hyperplasia and neoplasia, and breast cancer.² Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women, and the classic form of this syndrome is amenorrhea or completely irregular menstruation, infertility, hirsutism, obesity, and bilateral enlargement of the ovary full of cysts.³ This complication is seen in reproductive age and affects about 5% of women at this age.⁴ Today, studies have shown that various genetic and environmental factors cause PCOS, all of which are involved in the pathophysiology of this occurrence.⁵ The definition of PCOS includes hyperandrogenism without a specific cause, such as an androgen-producing tumor, congenital adrenal hyperplasia with late-onset, lack of ovulation, the appearance of polycystic ovaries on ultrasound in the form of more than eight follicles with a size of 2 to 8 mm, and the increase of ovarian stroma. On the other hand, the sonographic appearance of polycystic ovaries can be seen in 16% of asymptomatic women.⁶

In addition to these symptoms, the prevalence of obesity, type 2 diabetes mellitus (DM), high blood pressure, and cardiovascular diseases in PCOS patients is higher than in the general population.⁷ Hyperinsulinemia and insulin resistance (IR) are the most important metabolic abnormalities that affect these patients.⁸ IR is when a lower-than-normal glucose decrease is achieved with a certain amount of insulin. First, the beta cells in the pancreas compensate for this resistance by increasing insulin production and keeping the blood glucose level normal. At this time, the patient only has IR with high...
amounts of this hormone. Over time, a person with IR goes from the stage of high levels of this hormone with normal levels of glucose to high and abnormal levels of glucose and finally develops type 2 DM. High amounts of insulin produce large amounts of androgens by stimulating the ovaries. In addition, the high amount of insulin reduces the globulin binding to sex hormones, increasing the strength of androgens. It is possible that high amounts of insulin in the brain also increase the secretion of luteinizing hormone (LH), which stimulates androgen production from the ovary and stimulates appetite. Therefore, factors such as increased secretion of LH, high levels of androgens, and obesity cause ovulation disorders. With evidence of a relationship between PCOS and IR, and since IR is a crucial factor in type 2 DM occurrence, it is suggested that women with this syndrome are at a higher risk of developing type 2 DM. This study aimed to investigate the variables related to IR in patients with PCOS with different phenotypes.

METHODS
In this retrospective study, which was conducted in Bezmialem University Hospital, 389 women in the age range of 18 to 37 years diagnosed with polycystic ovaries in an ultrasound between November 2020 and September 2022 were included. Information about patients was collected through their electronic records. The study was carried out with the permission of Bezmialem University Hospital Ethics Committee (Date: 22.11.2022, Decision No: 2022/321). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Inclusion criteria in the study group; 1) between the ages of 18-37 who cannot have children despite wanting a child for at least one year, 2) diagnosed with PCOS, provided that they have at least two of the 2003 Rotterdam Consensus criteria, 3) who are not diagnosed with DM, impaired glucose tolerance, thyroid dysfunction, hyperprolactinemia and hypercortisolism, 4) who were not given oral contraceptives or any medication known to alter hormone, lipid, or insulin metabolism 3 months prior to the study and non-smokers will be included.

Exclusion criteria in the study group; 1) smokers, 2) who diagnosed with hypertension, DM and any endocrinopathy, 3) who use oral contraceptives in the last 3 months for PCOS, those who use drugs that increase IR or those who use drugs for hyperlipidemia.

Among the patients who applied to our routine obstetrics and gynecology outpatient clinic with menstrual irregularity and desire to have children, we look at the patients on the 3rd day of their menstruation after the examination; Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2), Prolactin, Thyroid Stimulating Hormone (TSH), free T4, Anti-Mullerian Hormone (AMH), Hemogram, biochemistry (total cholesterol, LDL, HDL, Triglyceride), fasting blood glucose, fasting insulin, HOMA-IR values will be examined.

The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) is also used to evaluate IR. HOMA-IR was calculated from the following formula using fasting serum glucose and insulin levels:

\[\text{HOMA-IR} = \frac{\text{Fasting Glucose (mg/dl)} \times \text{Fasting Insulin (uU/ml)}}{22.5}\]

If subjects have a HOMA index greater than or equal to 2.38, they are considered insulin resistant.

In the present study, PCOS was diagnosed based on the Rotterdam criteria. Diagnostic criteria for PCOS is the presence of at least two of the following three symptoms: 1) Menstrual disorders (oligoovulation); 2) Clinical/laboratory hyperandrogenism; 3) Ovaries containing multiple cysts in ultrasound (PCO).

Accordingly, four phenotypes were considered for PCOS: (A) menstrual disorders, clinical/laboratory hyperandrogenism, and ovaries containing multiple cysts on ultrasound; (B) menstrual disorders and clinical/laboratory hyperandrogenism in the absence of ovaries containing multiple cysts on ultrasound; (C) clinical/laboratory hyperandrogenism and ovaries containing multiple cysts on ultrasound in the absence of menstrual disorders; (D) Menstrual disorders and ovaries containing multiple cysts on ultrasound in the absence of clinical/laboratory hyperandrogenism.

Table 1 shown the groups of phenotypes. Clinical hyperandrogenism (CH) was defined as hirsutism (hirsutism score ≤8 using the Freeman Galloway scale) as well as acne or androgenic hair loss. Biochemical hyperandrogenemia (BH) with free thyroxine (FT4), total testosterone (TT), and dehydroepiandrosterone (DHEAS) values above 95% was examined for those women who did not have evidence of Hyperandrogenism at the bedside, menstrual disorders, or taking hormonal drugs. Hyperandrogenism (HA) was diagnosed by the presence of CH and BH. Menstrual disorders were defined as menstrual cycles of more than 35 or less than 26 days or amenorrhea.
Statistical Analysis
In order to examine the normality, the Kolmogorov-Smirnov test was performed. Considering the groups’ non-normality, the nonparametric tests were performed before the statistical analyses. For each continuous variable, mean and standard deviations (SD) were measured. The Mann-Whitney U test was used for statistical analyses. A value of p < 0.05 was accepted as statistically significant. The GPower 3.1 program was used to calculate the sample size. Four groups’ total mean was measured based on the Kruskal Wallis H test with a power of 95%, effect size of 90%, and 0.05 type 1 error for at least 389 patients.

RESULTS
This study included 389 women aged 18 to 37 diagnosed with polycystic ovaries divided into four phenotypes. The descriptive statistics of participants are shown in Table 2.

Table 3 shows the comparison of phenotype groups on the study parameters. As shown in, there is no significant difference between the age and body mass index (BMI) of different phenotypic groups (p=0.981 and 0.963, respectively). The highest value of HOMA-IR was for the second group (phenotype B), and the lowest value was for the third group (phenotype C). However, the difference between the groups was not significant (p=0.221). FSH was significantly different between groups; phenotypes B and C had the highest values and phenotypes A and D had the lowest values (p<0.001).

The values of LH, fasting blood sugar, LDL, triglyceride, DHEAS, Neutrophil, Lymphocyte, Hemoglobin, platelet (PLT), procalcitonin (PCT), and mean corpuscular volume (MCV) did not have any significant differences between different phenotypic groups (p ≥ 0.05).

Estradiol and free thyroxine (FT4) were significantly higher in the first group (phenotype A) (p<0.001). TSH, Prolactin, AMH, Fasting Insulin, total testosterone (TT), and red blood cell distribution width (RDW) were significantly higher in the second group (phenotype B) (p≤ 0.001). Total cholesterol, LDL, leukocyte, basophil, and monocyte were significantly higher in the third group (phenotype C) (p≤ 0.001). Also, mean platelet volume (MPV) values were significantly higher in the fourth group (phenotype D) (p≤ 0.001).

DISCUSSION
The results showed that IR-related variables were more in patients with A (oligoovulation+ hyperandrogenism+ PCO) and B (oligoovulation+hyperandrogenism) phenotypes than in C (hyperandrogenism +PCO) and D (oligoovulation+absent PCO) phenotypes groups. These results were consistent with some previous studies.10-13

In a similar study, Eftekhar et al.14 investigated the prevalence and clinical characteristics of IR in Pakistan's different phenotypic young women with PCOS. This research reported the number of IR-related variables in phenotypes A and B higher than in other phenotypes. The average age of these people was 18 to 39 years old, which is close to the average age of our study. Another study was conducted by Cutler et al.15 to investigate IR and obesity in different PCOS phenotypes. In this study, the variables related to IR in two phenotypes, A and C, were higher than in other phenotypes. In this study, IR was evaluated and calculated with the HOMA-IR, and most women were obese. Researchers found that increased body fat may play a pathogenic role in developing this syndrome in susceptible individuals. The reason for the discrepancy between the results of this study and the previous studies is not clear.
research and the data obtained from our work can be the high weight of the participants in this study. The mean BMI of subjects in our study was 25.5, while it was 27.3 in their study.

The average BMI of the patients in the present study was classified as overweight. The prevalence of obesity in the general population is 30-40%, while this rate is more than 50% in people with PCOS, where fat is usually accumulated centrally. About 50-70% of women with PCOS have varying degrees of IR, a risk factor for developing type 2 DM. Even in non-obese women with PCOS, a slight increase in this risk can be seen.16,17 Kim et al.18 showed a high level of IR in PCOS patients independent of obesity or phenotypes. This study determined lower levels of high molecular weight adiponectin in obese PCOS subjects. Although IR is common in PCOS patients, it is not a universal feature for any particular phenotype. Mumusoglu et al.19 studied the IR prevalence in PCOS of different phenotypes using the HOMA-IR model. According to the model, the IR prevalence in patients with PCOS was 64.4%, and no significant difference was observed between phenotypes, which is inconsistent with our results. They concluded that patients with IR were more clinically affected and showed that race, BMI, and age were determinants, especially when diagnosing IR.

Regardless of the criteria used to diagnose PCOS, phenotypes differences can be caused by different genetic factors, lifestyles, and nutritional habits.20 In addition, the method of using samples can seriously affect the prevalence estimate, which can be affected by selection bias in non-population research. Recent studies have shown that the inclusion of PCO criteria in the definition of PCOS can increase its prevalence to 25,21 According to NIH,22 about 90% of the diagnosis of patients with PCOS are confirmed by the Rotterdam criteria, which indicates that most women with hyperandrogenism also have PCO. Recently, the NIH has recommended using the Rotterdam criteria because it includes all PCOS phenotypes and recommended that more extensive and controlled studies be conducted on the prevalence of PCOS.

Table 3. Comparison of phenotype groups

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Group I (n=106) M±SD</th>
<th>Group II (n=111) M±SD</th>
<th>Group III (n=83) M±SD</th>
<th>Group IV (n=89) M±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Years)</td>
<td>28.37±4.78</td>
<td>28.5±4.3</td>
<td>28.52±4.35</td>
<td>28.51±4.47</td>
<td>0.981</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.95±4.91</td>
<td>25.38±1.93</td>
<td>25.32±1.46</td>
<td>25.35±3.04</td>
<td>0.963</td>
</tr>
<tr>
<td>Insulin resistance (HOMA-IR)</td>
<td>3.6±2.25</td>
<td>4.08±2.27</td>
<td>3.87±2.19</td>
<td>3.84±2.26</td>
<td>0.221</td>
</tr>
<tr>
<td>FSH (mIU/ml)</td>
<td>5.62±1.94</td>
<td>7.06±1.51</td>
<td>7.57±1.42</td>
<td>5.91±1.25</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LH (mIU/ml)</td>
<td>8.59±6.71</td>
<td>6.54±2.36</td>
<td>6.1±1.19</td>
<td>7.73±4.35</td>
<td>0.183</td>
</tr>
<tr>
<td>Estradiol (pg/ml)</td>
<td>69.97±60.54</td>
<td>40.95±8.43</td>
<td>43.59±5.47</td>
<td>52.41±34.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FT4 (ng/dL)</td>
<td>1.26±0.31</td>
<td>1.09±0.31</td>
<td>1.04±0.25</td>
<td>1.21±0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH (μIU/ml)</td>
<td>2.72±1.42</td>
<td>1.71±0.84</td>
<td>1.48±0.52</td>
<td>2.82±4.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prolactin (μg/L)</td>
<td>23.75±17.57</td>
<td>18.01±8.33</td>
<td>16.78±5.51</td>
<td>24.13±15.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AMH (ng/ml)</td>
<td>5.03±3.19</td>
<td>6.12±2.36</td>
<td>6.2±2.65</td>
<td>5.15±0.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fasting blood sugar (mg/dL)</td>
<td>94.77±8.49</td>
<td>95.5±8.98</td>
<td>94.18±9.32</td>
<td>96.24±8.88</td>
<td>0.337</td>
</tr>
<tr>
<td>Fasting insulin (pmol/L)</td>
<td>15.28±9.02</td>
<td>16.67±9.12</td>
<td>10.11±5.08</td>
<td>16.47±11.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>176.35±40.96</td>
<td>171.12±48.04</td>
<td>201.43±49.2</td>
<td>175.87±41.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>102.64±35.88</td>
<td>100.43±39.01</td>
<td>112.11±35.97</td>
<td>102.73±34.46</td>
<td>0.128</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>53.28±15.1</td>
<td>49.52±14.79</td>
<td>63.8±17</td>
<td>52.03±11.78</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>102.48±54.86</td>
<td>110.31±52.28</td>
<td>105.45±45.33</td>
<td>106.67±57.48</td>
<td>0.334</td>
</tr>
<tr>
<td>Total testosterone (ng/dl)</td>
<td>37.5±22.95</td>
<td>72.21±82.95</td>
<td>31.75±14.77</td>
<td>37.2±0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DHEAS (μg/dL)</td>
<td>276.92±122.54</td>
<td>283.65±121.18</td>
<td>289.76±82.24</td>
<td>274.25±122.05</td>
<td>0.158</td>
</tr>
<tr>
<td>Leukocyte (10³/µL)</td>
<td>7.35±1.91</td>
<td>7.44±1.83</td>
<td>8.58±2.27</td>
<td>7.01±2.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Neutrophil (10³/µL)</td>
<td>4.43±1.48</td>
<td>4.94±1.93</td>
<td>5.11±1.71</td>
<td>4.7±1.5</td>
<td>0.048</td>
</tr>
<tr>
<td>Basophil (10³/µL)</td>
<td>0.5±0.24</td>
<td>0.7±0.24</td>
<td>0.1±0.27</td>
<td>0.7±0.19</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Lymphocyte (10³/µL)</td>
<td>2.3±0.74</td>
<td>2.23±0.85</td>
<td>2.45±0.74</td>
<td>2.3±0.66</td>
<td>0.280</td>
</tr>
<tr>
<td>Monocyte (10³/µL)</td>
<td>0.42±0.12</td>
<td>0.43±0.11</td>
<td>0.59±0.19</td>
<td>0.57±0.23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.07±1.18</td>
<td>13.01±1.07</td>
<td>13.36±1.07</td>
<td>13.24±1.6</td>
<td>0.131</td>
</tr>
<tr>
<td>Hematocrit (g/dl)</td>
<td>39.36±2.89</td>
<td>39.01±2.57</td>
<td>39.63±3.18</td>
<td>39.19±3.11</td>
<td>0.254</td>
</tr>
<tr>
<td>PLT (mm³)</td>
<td>271047.17±54068.45</td>
<td>257209.91±49794.29</td>
<td>265079.52±43774.26</td>
<td>252786.52±51574.19</td>
<td>0.160</td>
</tr>
<tr>
<td>PCT (ng/ml)</td>
<td>0.25±0.04</td>
<td>0.23±0.16</td>
<td>0.24±0.11</td>
<td>0.24±0.11</td>
<td>0.241</td>
</tr>
<tr>
<td>RDW (ng/ml)</td>
<td>13.47±1.24</td>
<td>13.33±0.99</td>
<td>12.83±1.32</td>
<td>13.07±1.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MPV (µm³)</td>
<td>9.15±0.92</td>
<td>9.82±0.66</td>
<td>9.8±0.87</td>
<td>9.97±0.79</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV (µm³)</td>
<td>85.82±5.25</td>
<td>84.67±4.76</td>
<td>85.13±4.3</td>
<td>84.6±4.37</td>
<td>0.056</td>
</tr>
</tbody>
</table>

M, Mean; N, number of subjects; BMI, body mass index; HOMA-IR, homeostatic model assessment for insulin resistance; FSH, follicle-stimulating hormone; LH, luteinizing hormone; FT4, Free thyroxine; TSH, thyroid-stimulating hormone; AMH, anti mullerian hormone; LDL, low density lipoprotein; HDL, high density lipoprotein; DHEAS, dehydroepiandrosterone sulfate; PLT, platelet; PCT, procalcitonin; RDW, red cell distribution width; MPV, mean platelet volume; MCV, mean corpuscular volume.
The pathophysiology of anovulation in many women with ovulation disorders and PCOS is IR. Considering that these patients are at increased risk of DM and cardiovascular diseases, infertility, hyperplasia, endometrial cancer, and possibly breast cancer, timely diagnosis of treatment in these people is of particular importance. In addition, according to the studies, the families of people with PCOS constitute a high-risk group in which IR should be examined to prevent the occurrence and development of its complications.

This study’s limitation can be the small population, and it is suggested to study more populations with PCOS of different phenotypes in future studies. Also, using only one method to assess IR can be considered another limitation. It is recommended to improve the interpretation and comparison of existing research based on the non-selective population on the relationship between PCOS phenotypes and IR in different ethnic groups.

CONCLUSION

The results showed that IR-related variables in phenotypes A and B of PCOS are higher than in other phenotypes. Due to the occurrence of ovulation disorders and the risk of diabetes in people with this syndrome, periodic screening and follow-up of these phenotypes regarding risk factors for glucose tolerance and IR are necessary.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Bezmialem University Hospital Ethics Committee (Date: 22.11.2022, Decision No: 2022/321).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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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.

REFERENCES


