ABSTRACT

Objective: The aim of study is to investigate the relationship between neuropeptide Y (NPY) and insulin resistance which is important in the pathogenesis of polycystic ovary syndrome (PCOS).

Materials and Methods: This study was conducted between May 2012 and May 2013. The study included 45 patients with PCOS and 44 healthy controls at productive age. Insulin, fasting blood sugar, follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin, testosterone, dehydroepiandrosterone sulfate (DHEA-S), thyroid stimulating hormone (TSH), cortisol, estradiol, and NPY levels were measured at early follicular phase in patients with PCOS, while, insulin, fasting blood sugar, prolactin, DHEA-S, TSH, cortisol, and 17-OH progesterone levels were measured in control group. Homeostatic model assessment for insulin resistance (HOMA-IR) scores were calculated and antropometric measures were recorded. Pelvic ultrasonography was performed.

Results: Fasting insulin levels and HOMA-IR scores showed insulin resistance to be higher in obese patients with PCOS when compared with healthy controls and patients with normal weight PCOS. NPY levels were found to be higher in obese-overweight patients with PCOS than the values observed in healthy controls and in patients with normal weight but they were not statistically significant (P>0.05). NPY levels did not differ in patients with and without insulin resistance.

Conclusion: No correlation was detected between insulin resistance and NPY levels but NPY levels were higher in overweight PCOS patients.

Keywords: Polycystic ovary syndrome, Insulin resistance, Neuropeptide Y

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder in women during reproductive period and characterized by hyperandrogenism, chronic anovulation and polycystic ovary appearance on ultrasonography (US).
Insulin resistance and hyperinsulinemia can be observed in both weak and overweight/obese PCOS patients, as an important feature [2]. However, insulin resistance is not a necessary parameter for the diagnosis of PCOS. The effect of insulin resistance in PCOS has not yet been completely elucidated, but insulin resistance is thought to play an important role in the pathogenesis of PCOS. It has been shown that insulin resistance in PCOS is caused by some molecular disorders that occur in the post-receptor insulin signalling pathway.

Neuropeptide Y (NPY) is an appetite enhancing peptide and its effect on appetite was first shown in 1984. It is structurally and immunologically similar to the pancreatic polypeptide, belongs to the pancreatic polypeptide family, and consists of 36 amino acids [3]. NPY is commonly found in the central and peripheral nervous system. In many studies, in which NPY has been applied to the central nervous system, it has been shown that NPY regulates nutrient intake and body weight. In addition, it has been demonstrated in studies that NPY has an inhibitor effect on the hypothalamo-hypophyseal-ovarian axis [4]. This study aims to determine the relationship between insulin resistance and NPY levels in patients with PCOS for the etiopathogenesis.

Material and Methods

This study was carried out prospectively and controlled between May 2012-May 2013. A total of 45 women, 25 normal weight (Body mass index (BMI) <25kg / m2) and 20 overweight or obese (BMI> 25kg / m2) with PCOS who in reproductive period were included in the study. For the control group, 44 healthy women without any chronic disease, menstrual irregularity and hyperandrogenemia were included. Ethical committee approval has been obtained before commencement of the study (No: 2012/202). The participants were informed about the purpose of the study and the procedures. “The Androgen Excess and PCOS Society Criteria” were considered as a diagnostic criteria in the selection of PCOS patients [5]:

1. Hirsutism and / or hyperandrogenemia,
2. Oligo/anovulation and / or polycystic ovaries,
3. Exclusion of other causes.

“Ferriman-Gallwey Score” was used for hirsutism scoring. Patients with a score of 8 and above were included into the study group. Patients with any systemic disease, androgen-releasing tumor or hyperprolactinemia, drug users (oral contraceptives, metformin, glitazones, NSAID) that affect insulin resistance, and smokers were not included into the study.

Patients BMI and waist / hip ratios were calculated. Serum levels of blood glucose, insulin, NPY, total testosterone, follicular stimulating hormone (FSH), luteinizing hormone (LH), 17-OH progesterone, prolectin (PRL), dehydroepiandrosterone sulfate (DHEA-S) and thyroid stimulating hormone (TSH) were measured in all patients at the early follicular phase (2-5 days of menstruation) after 12-hour fasting.

NPY levels were studied using enzyme-linked immunosorbent assay (ELISA) method using USCN brand (USCN Life Science Inc., E90879Hu, 96 Tests, China) kits. The measurement range of the method was 2.47-200 pg / mL. Glucose level was studied in the Cobas Integra 800 autoanalyser (Roche Diagnostics, Manheim, Germany). Insulin, cortisol, testosterone, FSH, LH, estrogen, prolactin, DHEAS, TSH, free T4 and T3 levels were studied by the electrochemiluminescent method in the Modular E170 autoanalyser (Advia Gentaur XP Siemens). 17-OH Progesterone levels were determined by ELISA method using the 17-OH Progesterone ELISA (DSX automated ELISA SYSTEM) commercial kit. HOMA-IR was calculated by [Fasting Insulin (mIU / ml)xFasting blood glucose (mg/dl)]/405 fasting glucose (mg / dl) formula. For insulin resistance, HOMA-IR values of 2.5 and above were accepted.

Ultrasonography measurements of the patients were made by using transabdominal US. According to the 2003 Rotterdam consensus, a polycystic over-view was defined when there were 12 or more follicles 2-9 mm in diameter and / or the ovary volume was above 10cm [3].

Statistical Analysis

The normal distribution suitability of the variables was examined by the Shapiro Wilk test. Variables that provided normal distribution assumption were summarized in terms of mean ± SD, while variables that did not provide assumption were summarized as median [min-max]. Categorical variables were expressed in numbers and percentages. The independent sample t test was used when the distribution assumption was provided in the comparison of the two groups, whereas the Mann Whitney U test was used when the assumption is not provided. ANOVA was used when the distributional assumption was provided in more than two groups comparisons and Tukey was used as a post hoc test. In case of not provided, it was analyzed by Kruskal Wallis test and Dunn test was applied as post hoc test. The Spearman correlation coefficient
was calculated to determine the relationship between the two continuous variables. Statistical significance was P<0.05. The SPSS 11.5 program was used in the data analysis.

Results

Forty-five female patients and 44 healthy volunteer women were included in the study. Demographic and biochemical characteristics of the patient and control groups are shown in Table I.

Hirsutism scores, insulin levels and homeostatic model assessment for insulin resistance (HOMA-IR) levels in patients with PCOS were higher and statistically significant than control group (P<0.05). No statistically significant difference was found between the PCOS group and the control group in terms of other parameters (P>0.05).

Table I. Demographic and biochemical characteristics of the patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>24.89±5.17</td>
<td>26.02±3.41</td>
<td>0.051</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.09±6.81</td>
<td>161.66±4.30</td>
<td>0.239</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.97±18.85</td>
<td>62.67±11.26</td>
<td>0.139</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.71±6.07</td>
<td>23.90±3.98</td>
<td>0.205</td>
</tr>
<tr>
<td>WS (cm)</td>
<td>79.93±13.66</td>
<td>76.22±9.49</td>
<td>0.216</td>
</tr>
<tr>
<td>HS (cm)</td>
<td>101.69±15.02</td>
<td>98.53±11.42</td>
<td>0.406</td>
</tr>
<tr>
<td>W/H (cm)</td>
<td>0.78±0.04</td>
<td>0.77±0.04</td>
<td>0.222</td>
</tr>
<tr>
<td>HS</td>
<td>12.24±1.88</td>
<td>3.66±0.80</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>91.21±6.89</td>
<td>87.58±5.76</td>
<td>0.214</td>
</tr>
<tr>
<td>IL (mIU/ml)</td>
<td>9.26±6.10</td>
<td>4.68±4.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.13±1.46</td>
<td>0.98±0.88</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NPY (pg/mL)</td>
<td>82.38±74.71</td>
<td>59.25±55.03</td>
<td>0.080</td>
</tr>
</tbody>
</table>


Patient and control groups were divided into two subgroups with normal BMI and high BMI. The demographic characteristics, HOMA-IR, insulin and NPY levels of the PCOS and control subgroups are shown in Table II. All findings in the overweight-obese PCOS subgroup were higher and statistically significant than the normal weight PCOS group (P<0.001). In control subgroups, waist and hip ratios, insulin levels and HOMA-IR values were found to be higher and statistically significant in overweight - obese subgroups when compared to the normal weight control group (P<0.05). No statistically significant difference was found in the comparison of the other data in the control group.

There was a statistically significant difference between the overweight PCOS group and the control group for all three findings (fasting blood glucose (FBG), insulin, HOMA-IR) when compared with the findings of PCOS subgroups and control group. There was a significant difference for FBG between overweight PCOS and normal PCOS groups but there is no significant difference in comparison of other findings. The difference between the insulin level and insulin resistance of the patient and control groups was also statistically significant (P<0.001).

No statistically significant results were found in the patient and control groups when the relationship was evaluated between insulin and NPY levels, BMI and NPY levels, waist size and NPY levels (Table III).

Values above 2.5 of HOMA-IR indicate insulin resistance at varying degrees and insulin resistance was found in 48.8% of the patients in the subgroup of PCOS, and 9% of the control group. The insulin level of patient group was found to be statistically significantly higher than the control group (P<0.05). In PCOS patients with insulin resistance, NPY

Table II. Demographic characteristics, HOMA-IR, Insulin and NPY values of PCOS and control subgroups

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &lt;25</td>
<td>22.92±4.07</td>
<td>26.85±5.43</td>
<td>0.142</td>
</tr>
<tr>
<td>BMI &gt;25</td>
<td>25.00±2.60</td>
<td>27.00±3.98</td>
<td>0.213</td>
</tr>
<tr>
<td>Age (year)</td>
<td>22.92±4.07</td>
<td>26.85±5.43</td>
<td>0.142</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>162.56±7.50</td>
<td>163.75±5.95</td>
<td>0.566</td>
</tr>
<tr>
<td>WS (cm)</td>
<td>71.56±7.75</td>
<td>90.40±12.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HS (cm)</td>
<td>93.04±8.28</td>
<td>112.50±14.64</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>W/H (cm)</td>
<td>0.77±0.04</td>
<td>0.80±0.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IL (mIU/L)</td>
<td>6.88±6.44</td>
<td>12.25±4.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.54±1.50</td>
<td>2.86±1.05</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NPY (pg/mL)</td>
<td>71.08±60.66</td>
<td>96.50±88.89</td>
<td>0.451</td>
</tr>
</tbody>
</table>

levels were found to be higher than patients without insulin resistance but it was not statistically significant (P: 0.595). There was no statistically significant difference when NPY levels were compared between obese and normal weight PCOS patients and control group (Overweight PCOS-control, normal weight PCOS-control and overweight PCOS-normal weight PCOS P values were 0.094, 0.751, 0.451, respectively).

Tablo III. The relationship between insulin and NPY levels, BMI and NPY levels, waist size and NPY levels in the patient and control groups

<table>
<thead>
<tr>
<th></th>
<th>PCOS (n:45)</th>
<th>PCOS (n:44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin and NPY levels – r</td>
<td>0.003</td>
<td>0.086</td>
</tr>
<tr>
<td>Insulin and NPY levels – P</td>
<td>0.983</td>
<td>0.581</td>
</tr>
<tr>
<td>BMI and NPY levels – r</td>
<td>0.031</td>
<td>0.119</td>
</tr>
<tr>
<td>BMI and NPY levels – P</td>
<td>0.838</td>
<td>0.440</td>
</tr>
<tr>
<td>Waist size and NPY levels – r</td>
<td>0.148</td>
<td>0.164</td>
</tr>
<tr>
<td>Waist size and NPY levels – P</td>
<td>0.332</td>
<td>0.292</td>
</tr>
</tbody>
</table>

Discussion

Polycystic ovary syndrome is the most common endocrine disorder in women during reproductive age [1]. The disease presents with chronic anovulation and hyperandrogenism findings [6]. Despite many studies, the pathogenesis of PCOS is still unknown completely, but it is a fact that insulin resistance and hyperinsulinemia play an important role in the pathogenesis of the disease [7-11]. It has been shown that women with PCOS have more insulin resistance and hyperinsulinemia than normal women who are in similar age and weight [12-14]. The relationship between glucose intolerance and hyperandrogenemia was first described by Achard and Thiers in 1921 and is called “diabetes in bearded women” [2-15]. The incidence of insulin resistance in patients with PCOS ranges from 53% to 75% [13,16,17]. Various methods can be used to demonstrate insulin resistance, one of which is the HOMA-IR method [15]. Values 2.5 and above according to the HOMA-IR method reflect insulin resistance. In our study, insulin resistance in patients with PCOS was statistically significantly higher than the control group. When the PCOS group was divided into two groups as normal weight and overweight-obese, the HOMA-IR level of overweight – obese PCOS group was statistically higher than normal weight PCOS group. On the other hand, some studies have shown that PCOS patients with normal weight have insulin resistance, while some of them show that insulin resistance is absent [13,14,17,19]. There are studies showing that insulin resistance is more severe in overweight – obese PCOS patients [20]. In our study, the mean insulin resistance in the overweight-obese group was also higher than the normal weight PCOS group.

Patients with PCOS have a 50-60% obesity rate [21]. Clinical and hormonal disorders become more prominent in PCOS patients with obesity. Metabolic and endocrine parameters are improved in these patients with weight loss [22,23]. Patients with overweight-obese PCOS usually have android type fat distribution [24,25]. In our study, the waist size and waist / hip ratio in overweight-obese PCOS patients were also significantly higher than normal women. This findings suggest that our overweight-obese PCOS patients have an android-like body fat distribution. On the other hand, there are studies in the literature that have different results regarding the occurrence of android type fat distribution in PCOS patients with normal weight [24-27]. The presence of android type fat distribution in patients with PCOS has been reported as a good indicator of insulin resistance and metabolic disorders [28,29].

Neuropeptide Y is an appetite enhancing peptide and its effect on appetite was first shown in 1984 [27]. Insulin and leptin cause a decrease in NPY levels, while ghrelin and glucocorticoid lead to an increase [30,31]. NPY also has an inhibitor effect on the hypothalamo-hypophyseal-ovarian axis [32]. Studies have shown that NPY neurons in the pancreas regulate insulin secretion, and also hyperinsulinemia and insulin resistance develop after prolonged exposure to NPY [32-35]. In our study, no statistically significant relationship was found between insulin resistance and NPY levels in patients with PCOS. However, in the group of PCOS patients with insulin resistance, the NPY level was found to be higher than the group with non-insulin resistant PCOS.

Neuropeptide Y values were found to be high in patients with obese and non-obese PCOS in a study conducted by Baranowska et al. [36]. On the other hand, it was shown that, NPY values increased in obese women without PCOS as BMI values increased. In the study of Baranowska et al., there was no relationship between NPY and insulin levels. But, in our study, the NPY levels in PCOS patients were higher than the NPY levels of the control group, but these findings were not statistically significant. The NPY level in the overweight-obese PCOS group was higher than the NPY level of the normal weight PCOS group, but this finding was not statistically significant. Furthermore, contrary to the study of Baranowska et al., in our study, no statistically
significant finding was found between BMI and NPY level in the control group. This difference may be due to the low number of overweight – obese patients in the control group of our study. Also, similar to this study, there was no relation between insulin and NPY level in our study.

In the study of Gunes and Bukan, the patients were divided into three groups as obese PCOS, normal weight PCOS and control groups. According to this study, in obese PCOS patients, NPY levels were higher than other groups and they were statistically significant [37]. In our study, NPY levels in obese PCOS patients were higher than normal weight PCOS patients and control group, but it was not statistically significant.

In a study by Orbetzova et al., that included non-PCOS overweight-obese and normal weight patients, NPY levels were found to be higher in normal weight patients [38]. Orbetzova et al., declared that the lower NPY levels of the obese patients comparing to control group, may be due to increased levels of leptin. In our study, although, the NPY levels of overweight – obese control group were higher than the NPY levels of normal weighted group, there was no statistical difference. This result was found to be opposite to the findings of Orbetzova et al. [38].

The most common sign of hyperandrogenism in PCOS is hirsutism and is evaluated with modified Ferriman-Gallwey method [39]. In our study, we used the Ferriman Gallwey scoring method and hirsutism score was found to be higher in PCOS patients compared to the control group and it was statistically significant.

Some studies on the relationship between NPY levels and BMI in PCOS patients have shown different results. Baranowska et al. detected that the NPY level in overweight-obese PCOS patients was lower than the overweight obese – control group [36]. In the study of Gennarelli et al., NPY levels were close to each other in the overweight-obese PCOS and overweight-obese control group, but the impaired response of NPY with hypoglycemia was observed [40]. In our study, NPY levels were found to be higher in the overweight-obese PCOS group than the NPY levels of the overweight-obese control group, in contrast to the findings of both two studies. This difference is thought to be due to the numbers of patients taken into the study are different.

In conclusion, NPY level was higher in obese and normal weight PCOS patients than control group but it was not statistically significant. In addition, NPY levels in patients with insulin resistance were higher than PCOS patients without insulin resistance, but it was not statistically significant. We think that multicenter studies with more patients are needed to reveal these relationships.

References


