Relationship between insulin-like growth factor 2 and metabolic profile in women with polycystic ovary syndrome: A pilot study

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Abstract

Purpose: Insulin–like growth factor 2 (IGF2) is a widely expressed mitogenic peptide that may be involved in the pathogenesis of metabolic syndrome and polycystic ovary syndrome (PCOS). The aim of this pilot study was to compare serum IGF2 levels and IGF2 gene expression in non-obese PCOS women and those with higher body mass index (BMI) and to assess their relationship with metabolic and hormonal variables.

Methods: The study included 64 Caucasian women diagnosed with PCOS (ages 28.9 ± 5 years), 34 with BMI <27 and 30 with BMI >27 kg/m². Parameters of glucose and lipid metabolism, steroid hormones, and serum IGF2 levels were recorded. Polymerase chain reaction (PCR) was used to assess mRNA IGF2 expression in 28 patients.

Results: No significant differences in serum IGF2 levels (P = 0.56) and mRNA IGF2 expression (P = 0.675) were found between non-obese PCOS women and those with higher BMI. There was a significant correlation between IGF2 and dihydrotestosterone (DHT) (R² = 0.08, R² adjusted = 0.07, P = 0.0222). There was no correlation between serum IGF2 and metabolic variables nor between IGF2 and other steroid hormones, but log mRNA IGF2 positively correlated with BMI (R² = 0.14, R² adjusted = 0.11, P = 0.0482), serum glucose (R² = 0.23, R² adjusted = 0.2, P = 0.0131), cholesterol (R² = 0.29, R² adjusted = 0.26, P = 0.0069), insulin (R² = 0.48, R² adjusted = 0.45, P = 0.0005), homeostasis model assessment insulin resistance (R² = 0.46, R² adjusted = 0.43, P = 0.0009), and total testosterone [TT] (R² = 0.18, R² adjusted = 0.15, P = 0.0257).

Conclusions: Serum IGF2 levels and IGF2 gene expression are not increased in obese PCOS women as compared to those with lower BMI. IGF2 positively correlated with DHT and log mRNA IGF2 positively correlated with serum glucose, insulin, cholesterol, and TT in PCOS women.

Key words: Polycystic ovary syndrome; body mass index; metabolic syndrome; insulin–like growth factor 2
1. Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder with a wide phenotypic variability, affecting approximately 5–15% of reproductive-aged women [1]. Based on the Rotterdam criteria, it is defined by two of the following features: Chronic oligoovulation or anovulation, hyperandrogenic state (clinical and/or biochemical), and polycystic ovarian morphology (PCOM) detected by ultrasound [2]. There is evidence that PCOS is also commonly associated with the metabolic syndrome (MS) or its components, and an increased prevalence of cardiometabolic risk factors has been consistently reported in the literature [3,4]. Data suggest an 80–100% increased risk of MS in PCOS women [5]. It is well known that dyslipidemia, insulin resistance or type 2 diabetes mellitus (T2DM), and obesity are more prevalent in PCOS women with clinical or biochemical hyperandrogenism [6,7]; however, the exact molecular or genetic mechanisms of this relationship are still not fully understood.

Several biomarkers have been identified that might be involved in the pathogenesis of obesity and adiposity, and subsequently, insulin resistance in humans and also in patients with PCOS. Identifying the presence of these hormones and other biomarkers can serve as a platform for the prevention of cardiometabolic consequences in obese subjects.

Insulin-like growth factor 2 (IGF2) is a widely expressed mitogenic peptide. It regulates fetal development and differentiation; however, its role in adults is less understood [8,9]. Evidence suggests the potential role of IGF2 in the regulation of numerous organs and tissues including adipose tissue, skeletal muscle, bone, and the ovaries [10]. In some studies, IGF2 levels or IGF2 gene expression has been found to be associated with metabolic parameters such as insulin resistance or body weight [11]. However, the possible role of IGF2 and its dysregulation in the pathogenesis of PCOS is unrecognized, and data from the literature are scarce.

The primary aim of this pilot study was to compare metabolic parameters and IGF2 levels between non-obese PCOS women and overweight or obese PCOS women. The secondary aim of this study was to assess the relationship between serum IGF2 or IGF2 gene expression and metabolic variables in PCOS patients.

2. Subjects and Methods

2.1. Subjects

The study included 64 Caucasian women diagnosed with PCOS. The mean age of the patients was 28.9 ± 5 years (range 22–43, median 28 years). The diagnosis of PCOS was based on the Rotterdam criteria [2]. Clinical hyperandrogenism was defined as the presence of hirsutism (modified Ferriman-Gallwey score >6), acne or androgenic alopecia, and biochemical hyperandrogenemia was defined as serum free testosterone (FT) levels ≥1.1 ng/ml, or free androgen index (FAI) higher than 8%. Chronic anovulation was defined by a menstrual cycle of <21 or more than 35 days, simultaneously with progesterone levels ≤6 ng/ml on days 20–23 of the menstrual cycle in two consecutive cycles. PCOM was defined as the presence of 12 or more ovarian follicles on ultrasound or ovarian volume larger than 10 ml.

Exclusion criteria were congenital adrenal hyperplasia, Cushing’s syndrome, prolactinomas, androgen-secreting tumors, and T2DM. All patients had normal thyroid, liver and kidney function, and they did not take any medication that could influence the hypothalamic-pituitary-ovarian axis during the past 6 months.

The most common manifestations of PCOS were as follows: Menstrual cycle irregularities (49, i.e., 76.6%), PCOM (44, i.e. 74.6%), hirsutism (41, i.e., 64%), and acne (18, i.e., 28%). All patients were divided according to body mass index (BMI) into two groups. The first group consisted of 34 patients with BMI <27 kg/m², and the second group consisted of 30 patients with BMI >27 kg/m².

All subjects signed a written informed consent, and the study was approved by the Ethical Committee of the University Hospital in Košice, Slovakia.

3. Methods

Weight, height, and waist circumference (WC) were measured in all subjects. Weight and height were used to calculate BMI, which was assessed by dividing weight
by height squared (kg/m²). WC was measured as the smallest circumference at the level of the umbilicus. Fasting blood samples were collected in the early follicular phase, i.e., between days 3 and 7 of the menstrual cycle, or whenever in those with amenorea. Anthropometric data were obtained on the same day. Blood samples were taken, and serum glucose, insulin, total cholesterol, low-density lipoprotein-cholesterol (LDL-C), high-density lipoproteins-cholesterol (HDL-C), and triglycerides (TG) were determined. Circulating levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), sex hormone-binding globulin (SHBG), total testosterone (TT), dihydrotestosterone (DHT), dehydroepiandrosterone sulfate (DHEAS), estrone (E1), and estradiol (E2) were evaluated from the same blood sample. FAI was calculated as TTx100/SHBG. The homeostasis model assessment insulin resistance (HOMA-IR) was assessed by the equation: HOMA-IR= (glucose × insulin)/22.5.

Serum glucose and lipids were analyzed routinely using an autoanalyzer (Roche Diagnostics GmbH). Hormonal levels of SHBG, LH, FSH, DHEAS, and E2 were determined using chemiluminescent immunoassay (analyzer Architect, module C, Abbott, USA). TT and FT were detected by radioimmunoassay using commercially available kits of DIA Source (Belgium). Serum E1 and DHT were measured by enzyme-linked immunosorbent assay (ELISA) (Demeditec Diagnostics GmbH). The serum IGF2 was measured by a commercially available ELISA kit with the catalog number E30 from Medagnost (Germany). The analytical sensitivity of the ELISA E30 yields 0.02 ng/ml. The inter- and intra-assay variation coefficients are <7.2% and 6.6%, respectively.

3.1. Real-time polymerase chain reaction (RT-PCR) analysis
To find the evidence of changes in mRNA IGF2 levels (IGF2 messenger RNA expression), RT-PCR was used. We performed four analyses for IGF2 gene expression in 28 subjects with PCOS. For the diagnostic procedure, 1 ml of peripheral blood was taken, and RNA was isolated from the blood of each patient, washed in RNAse free water, and weighed and stored at −80°C. Total RNA was isolated using a diagnostic isolation kit (Qiagen). The reverse transcription from mRNA to complementary DNA (cDNA) was made using superscript II (Invitrogen). Amplification of the specific gene IGF2 and β-actin like a housekeeping gene (HKG) ran for 33 cycles (94°C 5 min, 94°C 15 s and 61°C 20 s and 72°C 25 s), using appropriate primer sequences (Forward: ACACC CTCCAGTTTGTTCTGT, Reverse: GGGGTATCTTTGGG GAAGTTGT) in the thermocycler LightCycler® 480 Instrument II (Roche Life Science). Normalization of the results was performed using the housekeeping gene β-actin. Numerical quantification of changes in expression levels was evaluated using the LightCycler® 480 Software, Version 1.5. All values were expressed as the increase relative to the expression of HKG. The median value of the replicates for each sample was calculated and expressed as the cycle threshold (CT); cycle number at which each PCR reaches a predetermined fluorescence threshold, set within the linear range of all reactions): ΔCT was calculated as CT of HKG minus CT of target gene in each sample. The relative amount of target gene expression in each sample was then calculated as 2ΔCT.

3.2. Gene copies analysis
Gene copy analysis was made after DNA isolation using specific primers for IGF2 in DNA opposite with beta-actin like HKG for normalization. Amplification of the specific gene IGF2 and β-actin in DNA samples ran for 33 cycles (94°C 5 min, 94°C 15 s and 61°C 20 s and 72°C 25 s), using appropriate primer sequences in the thermocycler LightCycler® 480 Instrument II (Roche Life Science).

The gene copy number (also “copy number variants” or CNVs) is the number of copies of a particular gene in the genotype of an individual. Recent evidence shows that the gene copy number can be elevated in cells. It was generally thought that genes were almost always present in two copies in a genome. For example, genes that were thought to always occur in two copies per genome have now been found to be present in one, three, or more than three copies. In a few rare instances, the genes are missing altogether. That is why we also analyzed CNV in the DNA of patients with PCOS using RT-PCR with specific primers of IGF2 opposite with beta-actin like HKG. The analysis of gene copies for IGF2 showed that CNV in samples of patients were presented in one copy number.

3.3. Statistical analysis
SAS JMP version 13.0.0 (USA) software was used for statistical analysis. Data were presented as mean ±
standard deviation regardless of its distribution. Data with non-normal distribution (mRNA IGF2) were initially logarithmically transformed for further evaluation. For normally distributed variables, Student’s t-test was used to compare means between groups, whereas for non-normally distributed data, the non-parametric Mann–Whitney test was used to compare means among two groups. Linear regression analysis was used to assess correlations between variables. Values were considered to be statistically significant at $P \leq 0.05$.

4. Results

Anthropometric data, biochemical and hormonal variables, IGF2 levels, and IGF2 gene expression in PCOS women and subgroups according to BMI are presented in Table 1.

Table 1: Mean values of measured anthropometric, biochemical, and hormonal variables in PCOS patients and divided according to BMI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PCOS all group n=64</th>
<th>PCOS BMI&lt;27 n=34</th>
<th>PCOS BMI&gt;27 n=30</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>28.9±5</td>
<td>27±5</td>
<td>30.7±5.2</td>
<td>0.011</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.7±7</td>
<td>22.3±2.3</td>
<td>34.2±5.4</td>
<td>0.00001</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>84±18</td>
<td>72±7</td>
<td>100±15</td>
<td>0.00001</td>
</tr>
<tr>
<td>Glycemia (mmol/l)</td>
<td>5.1±1.5</td>
<td>4.7±0.4</td>
<td>5.5±2.2</td>
<td>0.0731</td>
</tr>
<tr>
<td>Insulin (mIU/l)</td>
<td>14.4±9.7</td>
<td>9.1±5.1</td>
<td>21.5±9</td>
<td>0.00001</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.2±2.5</td>
<td>1.9±1.1</td>
<td>4.9±2.7</td>
<td>0.00026</td>
</tr>
<tr>
<td>T-C (mmol/l)</td>
<td>4.9±1.1</td>
<td>4.6±1</td>
<td>5.3±1.26</td>
<td>0.03</td>
</tr>
<tr>
<td>TG (mmol/l)</td>
<td>2.1±4.8</td>
<td>1.02±0.5</td>
<td>3.3±6.9</td>
<td>0.09</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.5±0.4</td>
<td>1.7±0.3</td>
<td>1.23±0.3</td>
<td>0.00001</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>2.7±0.8</td>
<td>2.5±0.7</td>
<td>2.9±0.77</td>
<td>0.03</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>4.2±1.6</td>
<td>4.5±1.6</td>
<td>3.8±1.4</td>
<td>0.11</td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>8.6±7.1</td>
<td>10.0±8</td>
<td>7.0±4.7</td>
<td>0.121</td>
</tr>
<tr>
<td>TT (ng/ml)</td>
<td>1.1±0.38</td>
<td>1.03±0.35</td>
<td>1.1±0.3</td>
<td>0.321</td>
</tr>
<tr>
<td>FT (pg/ml)</td>
<td>4.3±1.2</td>
<td>3.9±1.1</td>
<td>4.7±1.2</td>
<td>0.014</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td>58.7±65</td>
<td>79.7±80</td>
<td>36.7±36.5</td>
<td>0.016</td>
</tr>
<tr>
<td>FAI</td>
<td>11.3±11.1</td>
<td>7.6±7.8</td>
<td>14.8±12.9</td>
<td>0.02</td>
</tr>
<tr>
<td>DHEAS (ug/100ml)</td>
<td>322±164</td>
<td>306±137</td>
<td>332±194</td>
<td>0.619</td>
</tr>
<tr>
<td>DHT (pg/ml)</td>
<td>683±449</td>
<td>587±267</td>
<td>808±596</td>
<td>0.06</td>
</tr>
<tr>
<td>E2 (pg/ml)</td>
<td>64.5±42.1</td>
<td>75.12±43.7</td>
<td>58.5±39.7</td>
<td>0.09</td>
</tr>
<tr>
<td>E1 (pg/ml)</td>
<td>114.2±129</td>
<td>78.3±75</td>
<td>161±165</td>
<td>0.01</td>
</tr>
<tr>
<td>IGF2 (ng/ml)</td>
<td>697.7±283</td>
<td>678.5±283</td>
<td>721.8±291</td>
<td>0.56</td>
</tr>
<tr>
<td>Log mRNA IGF2 (n=28)</td>
<td>6.75.10⁻⁴ ± 21.10⁻¹</td>
<td>5.54.10⁻⁴ ± 22.7.10⁻¹</td>
<td>9.31.10⁻⁴ ± 20.35.10⁻¹</td>
<td>0.675</td>
</tr>
</tbody>
</table>

As expected, PCOS women with BMI >27 kg/m² had significantly higher insulin levels and HOMA-IR (P = 0.00001, P = 0.00026), respectively, and a tendency to higher fasting glycermia (P = 0.0731). Moreover, obese patients had worse lipid profiles, i.e. higher serum total and LDL-cholesterol levels (P = 0.03 for both) and slightly higher TG (borderline significance). There was significantly higher FT (P = 0.014), FAI (P = 0.02), estrone (P = 0.01), lower SHBG (P = 0.016), and moderately higher serum DHT levels (P = 0.06) with borderline significance in PCOS women. There were no significant differences in DHEAS (P = 0.619) and TT (P = 0.321) levels between both groups [Table 1].

There was no significant difference in serum IGF2 (P = 0.56) and mRNA IGF2 expression (P = 0.675) in non-obese PCOS women and those with higher BMI. Using linear regression, there was a significant correlation between IGF2 and DHT (R² = 0.08, R² adjusted = 0.07, P = 0.0222) [Figure 1]. However, no correlation between serum IGF2 and metabolic variables or between IGF2 and other steroid hormones was demonstrated. On the other hand, IGF2 gene expression (log mRNA IGF2) positively correlated with BMI (R² = 0.14, R² adjusted = 0.11, P = 0.0482) [Figure 2], serum glucose (R² = 0.23, R² adjusted = 0.2, P = 0.0131), cholesterol (R² = 0.29, R² adjusted = 0.26, P = 0.0069), serum insulin (R² = 0.48, R² adjusted = 0.45, P = 0.0005), HOMA-IR (R² = 0.46, R² adjusted = 0.43, P = 0.0009), and testosterone (R² = 0.18, R² adjusted = 0.15, P = 0.0257) [Figure 3].

5. Discussion

IGF2 is a widely expressed polypeptide hormone primarily secreted by the liver but also in utero by the placenta. It has approximately 67% homology with IGF1. Its serum concentration in adults is approximately 700 ng/ml making it more abundant than IGF1 [10]. Despite this fact, understanding the role of IGF2 in adults is markedly limited as compared with IGF1. In the ovary, IGF2 appears to be more important than IGF1. In the prepubertal ovary, the peptide is absent, and its expression increases during puberty. This increase appears to be under the influence of gonadotrophins on granulosa cells. IGF2 acts on granulosa cells to stimulate their proliferation and secretion of estradiol and progesterone, as well as on theca cells to increase androgen production [12-14].
Altered IGFL2 gene expression has been observed in metabolic disorders, notably obesity and T2DM, but also PCOS [15,16]. Because PCOS is highly associated with obesity and other metabolic disturbances, this study focused on the relationship between IGFL2 and metabolic parameters in PCOS women. Growing evidence points to an association between type 2 diabetes and IGFL2, and a higher risk of breast, colon, endometrial, and ovarian cancer, which are also more common in PCOS women [17-19]. In the study by Espelund et al., women with early-stage breast cancer showed elevated circulating free IGFL2 [20]. Due to the higher risk of cancer in obese PCOS women, it is important to understand the role of IGFL2 in PCOS.

The primary aim of this pilot study was to compare serum IGFL2 levels as well as IGFL2 gene expression (mRNA IGFL2) in the group of PCOS women in relation to BMI. The current study did not demonstrate significant differences in serum IGFL2 levels and mRNA IGFL2 between non-obese and obese PCOS women (cut off BMI 27 kg/m²).

The role of IGFL2 in the pathogenesis of PCOS, especially in patients with MS is still not completely understood. Tian et al. reported that oocytes from women with hyperandrogenism showed increased IGFL2 expression, and lymphocytes of their children showed increased IGFL2 expression as well. The study showed that pregestational hyperandrogenism may predispose offspring to disorders of glucose metabolism [21]. Su et al. demonstrated that placental IGFL2 expression was significantly higher in women with gestational diabetes than in those without hyperglycemia [22]. Other studies showed that IGFL2 levels were related to insulin resistance and increased susceptibility to diabetes mellitus [16]. Fowke et al. found that serum IGFL2 tended to rise with BMI but without statistical significance. However, the lowest IGFL2 levels were observed in subjects with BMI <20 kg/m² [23]. Unfortunately, no other study comparing IGFL2 levels or mRNA IGFL2 in PCOS women has been conducted, and we are unable to compare our results.

The second aim of this study was to assess the relationship between IGFL2, mRNA IGFL2 and metabolic and hormonal variables in PCOS women. We were not able to find a significant correlation between serum IGFL2 levels and parameters of glucose and lipid metabolism. Moreover, we did not find any relationship between anthropometric parameters (BMI and WC) and IGFL2 levels in these patients. Among steroid hormones, IGFL2 was in borderline positive correlation with DHT. On the other hand, mRNA IGFL2 positively correlated with BMI, WC, glycemia, serum cholesterol, insulin, HOMA-IR, and testosterone levels. This pilot study is the first to evaluate serum IGFL2 levels and IGFL2 gene expression in PCOS women in relation to metabolic and hormonal parameters. Our results are in agreement with previous data on the role of IGFL2 in metabolic disturbances and obesity, although some studies did not confirm associations between IGFL2 gene expression and BMI [24].

One of the strengths of this study is that we measured both IGFL2 and mRNA IGFL2 in the serum of patients with PCOS, and simultaneously measured steroid hormones and metabolic parameters. However, the study has several limitations. The main limitation is a small sample size, especially for mRNA IGFL2 evaluation. Nevertheless, the results are noteworthy considering this is the first study to examine the relationship between mRNA IGFL2 expression and metabolic parameters in women with PCOS. Further studies are required to confirm this finding.

In conclusion, serum IGFL2 levels and mRNA IGFL2 are not increased in obese PCOS women as compared with those with lower BMI. IGFL2 positively correlated with DHT and log mRNA IGFL2 positively correlated with anthropometric data (BMI and WC), parameters of glycemia, lipid metabolism, and TT in PCOS women. Further studies in this field are required.

Authors contribution
IL - conception and design of the study, drafting the article and revising it critically for important intellectual content, and final approval of the version to be submitted. ZL - conception, design of the study, acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content. JF - acquisition of data, analysis and interpretation of data, drafting the article and revising it critically for important intellectual content. ST - conception and design of the study, acquisition and interpretation of data. ID - drafting the article and revising it critically for important intellectual content, final approval of the version to be submitted. MR - acquisition of data, analysis and interpretation of data. JM -
analysis, acquisition and interpretation of data. All authors have approved the final version of the article.

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