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ORIGINAL ARTICLE

Insulin resistance assessment in patients with polycystic ovary syndrome using different diagnostic criteria – Impact of metformin treatment

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Abstract

Introduction and objective: Polycystic ovary syndrome (PCOS) is one of the most frequent reasons for anovulation in infertile women. It can affect 5% – 10% of women of reproductive age. One of the important factors associated with the typical clinical signs and hormonal disorders could be insulin resistance and hyperinsulinaemia. The primary objective of this study was to assess the prevalence of insulin resistance in PCOS women. The secondary objective was to evaluate changes in body mass index (BMI), waist-to-hip ratio (WHR), and insulin sensitivity after 3 months of metformin therapy

Materials and methods: 68 patients were enrolled in the study. In all participants fasting and 2-h post-load glucose and insulin levels, WHR and BMI were evaluated before and after metformin (2 x 850 mg) therapy. Insulin resistance was assessed using G0/I0, G120/I120, and HOMA-IR indexes

Results: Before the treatment, insulin resistance was observed in 26% patients according to HOMA-IR, and in 16% or 28% according to G_0/I_0 or G_{120}/I_{120}, respectively. Metformin therapy was associated with improvement in insulin sensitivity in HOMA-IR and G120/I120 defined insulin resistant patients.

Conclusions: The percentage of insulin resistant PCOS patients differed depending on the method applied. It is necessary to find a single most useful method to measure insulin resistance. Metformin treatment significantly improves insulin sensitivity in insulin resistant patients.

Key words

HOMA-IR, insulin resistance, metformin, OGTT, polycystic ovary syndrome

INTRODUCTION

Polycystic ovary syndrome (PCOS) is one of the most frequent causes of functional female infertility. It may affect approximately 5% – 10% of females of reproductive age [1, 2]. The etiology of the syndrome is not fully understood, and recently numerous hypotheses have attempted to elucidate the causes and disturbances characteristic of PCOS. One of these hypotheses pointed towards genetic abnormalities [3].

The idea of abnormal gonadotropin-induced regulation of ovarian hormone release has been studied for many years. It had been found that overproduction of stromal androgens in the ovary was caused by constant, acyclic LH stimulation, and chronic anovulation was the result of the relative FSH deficiency [4].

Currently, it is believed that insulin resistance and hyperinsulinemia cause characteristic clinical symptoms and hormonal abnormalities in polycystic ovary syndrome. It has been proved that hyperinsulinemia aggravated ovarian production of androgens [5, 6]. Although the disease was first described by Stein and Leventhal in 1935, the criteria for diagnosis of the syndrome has changed across the decades [7, 8].

In 2003, a consensus workshop at a conference of the European Society for Human Reproduction and Embryology and American Society for Reproductive Medicine determined polycystic ovary syndrome to be present if two out of the three following criteria are met: anovulation or oligo-ovulation, clinical or laboratory excess androgen activity, or an ultrasound image of polycystic ovaries (if other endocrine disorders are excluded) [9, 10].

The clinical picture may include various degrees of hirsutism and acne [11], and the abdominal type of obesity is present in 50% of patients [12]. Ultrasound imagery of polycystic ovaries shows increased ovarian volume (more than 10 cm^3) and the presence of numerous (>10) small follicles (5–8 mm in diameter) that are oriented in the ovarian periphery or located within the stroma. Laboratory studies most frequently reveal increased levels of androstenedione and testosterone [9].

Hormonal abnormalities may be associated with carbohydrate imbalance; hyperinsulinaemia and insulin resistance may, in consequence, lead to the development of type 2 diabetes. Insulin resistance associated with hyperinsulinaemia affects approximately 50% of PCOS patients, both obese and slim [13, 14, 15]. This results from the resistance of peripheral tissues to insulin and from decreased

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hepatic degradation of insulin. Resistance to insulin is most likely caused by tissue insulin receptor defects [16, 17, 18].

Increased insulin concentrations cause hyperandrogenism. Insulin directly promotes ovarian steroidogenesis, and inhibits liver release of the sex hormone binding globulin (SHBG) and production of insulin-like growth factor binding protein 1 (IGFBP-1). Increased concentrations of IGF-1 additionally promote ovarian release of androgens [16, 19].

To date, a single, adequate marker of insulin resistance that would be both highly sensitive and specific is lacking. The gold standard worldwide is the hyperinsulinemic-euglicemic clamp technique; however, this method is time-consuming and thus more applicable to research than to daily clinical practice. Measurement of the fasting insulin concentration (I_0) is an easy marker to obtain, and values equal to 20 or higher indicate the presence of insulin resistance.

However, it should be borne in mind that the aforementioned marker must not be used in patients with impaired glucose tolerance and diabetes. The fasting glucose to insulin ratio is another highly sensitive and specific marker. Results equal to 4.5 or higher are regarded as characteristic for insulin resistance. Another marker, based on measurements of fasting glucose and insulin levels, is the homeostatic model assessment (HOMA-IR). Resistance to insulin is diagnosed at HOMA-IR levels ≥3.8. The glucose to insulin ratio, measured 2 hours after ingestion of 75 g oral glucose in the oral glucose tolerance test (OGTT), is another marker. Resistance to insulin is recognized for values ≤1 [20].

Recent Androgen Excess Society guidelines indicate the need for OGTT testing in all PCOS patients, regardless of their body mass. The OGTT should be repeated every two years, or more frequently if the patient presents with other risk factors for diabetes, or every year if glucose values obtained by OGTT indicate impaired glucose tolerance [21].

Metformin plays a role in the treatment of the PCOS where obesity and hormonal and metabolic abnormalities coexist. Metformin belongs to the class of biguanides and acts by decreasing the tissue resistance to insulin through increased glucose uptake in skeletal muscle and fatty tissue, decreasing hepatic gluconeogenesis, and decreasing intestinal glucose uptake. As a result, the body mass decreases. Furthermore, metformin therapy decreases androgen concentrations, increases SHBG release, and induces spontaneous ovulation and pregnancy in some patients [17, 22, 23]. Recent studies confirmed the usefulness of metformin in the treatment of PCOS-related infertility [2].

OBJECTIVES

The aim of the study was to evaluate the frequency of female patients with PCOS and insulin resistance diagnosed by means of various markers: G_0/I_0, G_{120}/I_{120} and HOMA-IR. The influence of metformin therapy on resistance to insulin, BMI, and WHR was also analyzed.

MATERIALS AND METHOD

The study enrolled 68 female patients treated in the Department of Reproductive Medicine and Gynecology at the Pomeranian Medical University in Szczecin, Poland, due to PCOS recognized based on the Rotterdam criteria. The subjects gave their informed consent before the start of any procedure. The exclusion criteria were as follows: hypersensitivity to metformin, heart disease, arterial hypertension, anaemia, increased serum aminotransferase concentrations (AST>31 U/l, ALT>36.4 U/l), increased serum urea or creatinine concentrations (urea>34 mg/dl, creatinine>1.09 mg/dl), alcoholism, and type 1 and 2 diabetes. All procedures were approved by the Local Ethics Committee of the Pomeranian Medical University in Szczecin (Approval No. BN-001/33/03).

A detailed history regarding abnormal menstrual cycles and fertility was taken in all patients. Clinical examination included measurements of body mass, waist and hip circumference, and the presence of hirsutism or acne. Based on the obtained results, body mass index (BMI, body weight divided by the patient's height squared) and waist-to-hip ratio (WHR) were calculated. WHR allowed the recognition of abdominal type obesity, which is characteristic for PCOS patients.

The reproductive organs were examined by transvaginal ultrasound, performed using the Voluson 730 Pro (Korea) apparatus with a 6.5 MHz head.

A 75 g OGTT was performed in all patients, with measurements of fasting and 120 minute glucose and insulin concentrations. Based on the obtained results, the following markers of resistance to insulin were calculated: G_0/I_0 (fasting glucose to insulin ratio; cut-off value 4.5), G_{120}/I_{120} (glucose to insulin ratio 120 minutes after oral glucose administration; cut- off value 1), and HOMA-IR (cut-off value 3.8). All measurements were performed using venous blood. Fasting blood samples were collected in the morning. Insulin concentrations were measured by means of immunoenzymatic assay using the Immulite apparatus with DPC reagents (Diagnostic Products Corporation, Los Angeles, CA, USA), while glucose concentrations were checked by means of the hexokinase test using an Olympus AU 400 Chemistry Immuno Analyzer (Olympus America, Center Valley, PA, USA).

According to American Diabetes Association guidelines the following venous blood glucose cut-offs were considered:

1. normal glucose tolerance – fasting plasma glucose <100 mg/dl or a 75 g 2-hours postload glucose below 140 mg/dl;
2. impaired glucose tolerance – fasting plasma glucose from 100 mg/dl to 125 mg/dl or a 75 g 2-hours post-load glucose from 140 mg/dl to 199 mg/dl;
3. diabetes – fasting plasma glucose ≥126 mg/dl or a 75 g 2-hours post-load glucose ≥200 mg/dl.

The patients were divided into 2 groups with regard to the 3 above-mentioned indexes: 1) an insulin resistant group, and 2) a normal insulin sensitivity group.

After evaluation of liver and kidney function (serum levels of AST, ALT, urea and creatinine), the study subjects were initiated on a 3-month course of metformin therapy at a dose of 850 mg, twice daily. To avoid any adverse reactions, patients were initially administered only one tablet with the morning meal, and a second tablet was introduced later with the evening meal. After 3 months of therapy, all examinations were repeated.

The normal distribution of variables analyzed was tested by Shapiro-Wilk test. Arithmetic means of baseline and post-treatment parameters were compared with the t-test for the dependent data or the Wilcoxon signed-rank test. Calculations were performed using Statistica 7 (StatSoft®,

Poland) software, and statistical significance was defined as $p \leq 0.05$.

RESULTS

The study enrolled 68 patients, all of whom completed the study. Their baseline characteristics are summarized in Table 1. Insulin resistant groups were identified based on G_0/I_0, G_{120}/I_{120}, and HOMA-IR values, and comprised of 11 (16%), 19 (28%) and 18 (26%) subjects, respectively.

Table 1. Baseline characteristics of PCOS patients (n=68).

		G_0/I_0	G_{120}/I_{120}	HOMA-IR
BMI	19–25	30%	50%	30%
	26–30	30%	20%	30%
	>30	40%	30%	40%
WHR	<0,8	35%	40%	40%
	≥0,8	65%	60%	60%

G_0 – fasting glucose; G_{120} – glucose in 120-min. OGTT; I_0 – fasting insulin; I_{120} – insulin in 120-min. OGTT; HOMA-IR – homeostatic model assessment; BMI – body mass index; WHR – waist-to-hip ratio.

In the group of patients with resistance to insulin, diagnosed by means of G_0/I_0, the only significant change after the treatment with metformin referred to a drop in fasting insulin. Amongst patients identified as sensitive to insulin by means of the index discussed, significant post-treatment decrease was observed in WHR and BMI (Tab. 2).

Table 2. Characteristics of PCOS patients (n=68) at baseline and following metformin treatment, stratified based on insulin resistance/sensitivity diagnosed by G_0/I_0 index.

Parameter	Insulin resistant (n=11)			Insulin sensitivity (n=57)		
	baseline	post-therapy	p	Baseline	post-therapy	p
G_0/I_0	3.6±0.86	6.61±5.83	0.11	12.2±8.4	13.85±9.7	0.1
WHR	0.88±0.07	0.86±0.07	0.12	0.83±0.07	0.82±0.08	0.09
BMI	32.4±5.6	31.4±5.4	0.07	25.9±5.7	25.25±5.9	0.05
I_0	28.18±7.84	19.27±8.11	0.007	9.9±5.6	10.9±16.75	0.4

Following metformin therapy, significant increase in G_{120}/I_{120} value, together with a significant decrease in fasting insulin and WHR, were observed in the group of patients resistant to insulin according to the G_{120}/I_{120} index. In patients who were diagnosed as sensitive to insulin based on their G_{120}/I_{120} values, the treatment was reflected in a significant decrease in WHR and BMI (Tab. 3).

Table 3. Characteristics of PCOS patients (n=68) at baseline and following metformin treatment, stratified based on insulin resistance/sensitivity diagnosed by G_{120}/I_{120} index.

Parameter	Insulin resistance (n=19)			Insulin sensitivity (n=49)		
	baseline	post-therapy	p	baseline	post-therapy	p
G_{120}/I_{120}	0.7±0.15	1.4±0.88	0.002	3.2±3.2	3.3±2.1	0.6
WHR	0.85±0.08	0.84±0.08	0.012	0.83±0.07	0.81±0.08	0.05
BMI	29.45±7.0	29.0±6.83	0.3	25.8±5.7	25.1±5.7	<0.001
I_0	21.38±9.76	14.54±7.56	0.004	9.5±6.2	11.3±18.2	0.4

In the group of patients resistant to insulin according to the HOMA-IR, asignificant decrease in HOMA, BMI and fasting insulin was noted after metformin therapy. In the group of patients identified as sensitive to insulin based on the HOMA-IR, the treatment was reflected in a significant decrease in WHR and BMI (Tab. 4).

Table 4. Characteristics of PCOS patients (n=68) at baseline and following metformin treatment, stratified based on insulin resistance/sensitivity diagnosed by HOMA index.

Parameter	Insulin resistance (n=18)			Insulin sensitivity (n=50)		
	baseline	post-therapy	p	baseline	post-therapy	p
HOMA	5.9±1.69	3.7±1.97	<0.001	1.79±0.83	2.5±5.3	0.35
WHR	0.87±0.07	0.86±0.08	0.43	0.82±0.07	0.81±0.08	0.025
BMI	30.63±6.44	29.44±6.04	0.006	25.5±5.6	24.99±5.96	0.05
I_0	25.1±7.7	16.4±8.09	<0.001	8.39±3.87	10.68±17.8	0.35

DISCUSSION

Insulin resistance is common in PCOS patients and accompanied by hyperinsulinaemia [24]. It is recognized that insulin resistance may affect up to 50% of patients with PCOS [25, 26, 27]. In the presented study, insulin resistance was found in 16% of patients according to G0/I0, in 28% of patients according to G120/I120, and in 18% of patients according to HOMA-IR. The results obtained may be different from the results of other authors, because of the too small number of patients in the study group. Insulin resistance with compensatory hyperinsulinaemia is considered to be one of the possible factors of pathogenesis of PCOS, and the cause of hyperandrogenism in this disease. It is believed that obesity, hiperinsulinaemia and insulin resistance are closely related and each of these pathologies affects the other [28, 29]. Numerous reports have proved the presence of insulin resistance in a substantial percentage of PCOS patients [18, 24]. Resistance to insulin is diagnosed based on various indexes since there is a lack of any single, generally accepted diagnostic measure. Due to the lack of uniform methodology to assess insulin resistance, the actual incidence of this disorder in PCOS is difficult to estimate [20, 31, 32, 33]. This may result in discrepancies in insulin resistance rates; thus leading to inadequate therapy and misleading conclusions from the results of treatment. In the group of patients in the presented study, the resistance to insulin was diagnosed based on the HOMA-IR, G_0/I_0, and G_{120}/I_{120} indexes. The percentage of patients resistant to insulin differed with regard to the type of index applied, being markedly higher in the G_{120}/I_{120} and HOMA-IR groups, compared to the G_0/I_0 group.

The rates of patients resistant to insulin were similar for the HOMA and G_{120}/I_{120} indexes. So far there is no clear opinion which of these indexes are the most useful, and the best way to indicate the percentage of patients with insulin resistance. Some authors suggest HOMA-IR and G_0/I_0 to be useful in diagnostics, others indicate the usefulness of the G_{120}/I_{120} index. Saxena et al. [31] suggested '2-hour post-glucose insulin levels' as a good indicator of IR. Santana et al. [33] favoured the QUICKI, HOMA-IR, and fasting insulin to fasting glucose ratio as the most useful indexes in the evaluation of resistance to insulin. However, DeUgarte et al believe that the greatest predictive value is characterized by

HOMA-IR. At the same time, these authors point out that insulin resistance may depend on various factors, such as not only body weight and BMI, but also on race and age. Hence, the big discrepancies obtained in the results in different populations, and the need to adjust the value of insulin resistance standards for study groups of patients [34].

According to Legro et al. [35], the G_0/I_0 index shows high specificity and sensitivity (84% and 95%, respectively) when compared to the hyperinsulinaemic-euglycaemic clamp technique, which is recognized as the gold standard. The G_{120}/I_{120} index, however, revealed 95% sensitivity and a lower specificity of 63% [35].

In the studied group of patients, insulin resistance was evaluated based on the fasting glucose to fasting insulin ratio, HOMA-IR, and a 75 g 2-hours post-load glucose to insulin ratio.

The markers chosen for the purpose of the presented study differ from one another since the methods of evaluation are different: the first 2 methods incorporate fasting glucose and insulin, while the third one uses 75 g 2-hours post-load glucose and insulin levels. On the other hand, these methods are easier to apply in the clinical and ambulatory settings when compared to the hyperinsulinaemic-euglycaemic clamp technique.

In the group of insulin resistant patients diagnosed by both the HOMA-IR (n=18) and the G_{120}/I_{120} index (n=19), the level of insulin resistance decreased after metformin therapy. Moreover, in all studied groups with insulin resistance (diagnosed by any of the methods) a significant decrease in fasting insulin was found. Due to the coexistence of glucose metabolism disorders in PCOS and the possible role of insulin resistance in the pathogenesis of this disease, it is reasonable to use metformin in the therapy of such patients [36]. These results acknowledge the reports by other authors on the metformin-induced insulin sensitizing effect in PCOS patients [37, 38].

In the group of insulin resistant patients, a significant decrease in BMI was achieved only in the group evaluated with the HOMA-IR. Significant decrease in BMI was observed, however, in all the subgroups diagnosed with normal insulin sensitivity. These aforementioned differences in results may be caused by the heterogeneity of PCOS and the heterogeneity of clinical symptoms found in patients in the presented study. These results confirm the need for insulin sensitizing treatment, especially in patients who are insulin resistance. The fact of its presence, rather than excess body weight, should be a major prerequisite for metformin therapy.

Furthermore, insignificant weight loss and WHR decrease were also observed in the patients in the presented study who were evaluated with the G120/I120 index and G0/I0 index. According to other authors, even a small weight loss can improve menstrual function and result in spontaneous ovulation [36, 38].

CONCLUSIONS

In conclusion, the presented study reveals the following:

1. depending on type of marker, different percentages of patients with insulin resistance are found;
2. there is a need for elaboration of a universal marker in the diagnosis of insulin resistance that could be applied in both clinical and ambulatory settings;
3. metformin therapy improves insulin sensitivity in patients with diagnosed insulin resistance.

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