Are insulin resistance and serum resistin levels increased in women with idiopathic hirsutism?

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Abstract. – OBJECTIVE: To investigate the insulin resistance and serum resistin levels in women with idiopathic hirsutism compared to controls and women with polycystic ovary syndrome (PCOS).

PATIENTS AND METHODS: Three groups of women including 23 women with idiopathic hirsutism, 28 women with PCOS and 28 non-hirsute women serving as controls were included into the study. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), serum fasting insulin and resistin levels were compared between the groups.

RESULTS: There were no statistically significant differences regarding the age, BMI and waist circumferences between the groups. Mean and median fasting blood glucose, fasting insulin, HOMA-IR, serum resistin levels were statistically similar between the groups (p = 0.966, p = 0.378, p = 0.409 and p = 0.784, respectively). There were no correlations between the resistin, HOMA-IR, fasting insulin levels and BMI in any of the three groups.

CONCLUSIONS: Insulin resistance and serum resistin levels do not appear to be increased in women with idiopathic hirsutism compared to controls at similar BMI’s and waist circumferences.

Key Words:
Insulin resistance, Idiopathic hirsutism, Resistin, PCOS.

Introduction

Hirsutism is the excessive growth of terminal hair in androgen sensitive regions of skin in a male-like pattern. Hirsutism is due to either pathologies resulting in increased circulating androgens or it may be due to an increased sensitivity of the pilosebaceous unit (PSU) to androgens or a combination of these factors1-2. Polycystic ovary syndrome (PCOS), the most common cause of hirsutism, is characterized by ovarian and/or adrenal hyperandrogenism, oligo-anovulation, obesity and metabolic disturbances including insulin resistance3,4. Idiopathic hirsutism is the second most common cause of hirsutism and it is considered to be associated with increased peripheral 5α-reductase activity and/or androgen receptor gene polymorphisms with resultant increased activity in PSU5,6. Its diagnosis is based on documentation of normal ovulatory function and normal serum androgen levels with exclusion of other etiologies.

Insulin resistance is well documented in pathologies with increased ovarian and/or adrenal androgen excess7. Insulin resistance and resultant hyperinsulinemia may not only result in long-term metabolic and cardiovascular hazards, but it may also directly or indirectly stimulate hyperandrogenism, hair growth and contribute to the hirsutism8-10. Resistin is a novel adipocyte-derived polypeptide considered to induce insulin resistance and impaired glucose tolerance, and it is thought to play a role in obesity and type 2 diabetes11-13. Numerous studies have reported on the association between the PCOS, obesity, insulin resistance, hirsutism14-20 and serum resistin level was also proposed to be increased and play a role in pathogenesis of PCOS through inducing insulin resistance, although controversial21,22. However, the presence of insulin resistance in idiopathic hirsutism is reported in only few studies with controversial results, and none reported on the serum resistin levels in idiopathic hirsutism so far9,24-25.

The aim of our study is to investigate the insulin resistance and serum resistin levels in women with idiopathic hirsutism and PCOS.
Patients and Methods

This case-control study was carried out in the Departments Obstetrics and Gynecology and Endocrinology outpatient clinics of Duzce University School of Medicine. The study was approved by the Duzce University Non-Invasive Human Research Ethics Committee. Informed consent was obtained from all women.

Population

Three groups of women within their reproductive ages including 23 women with idiopathic hirsutism, 28 women with PCOS and 28 non-hirsute women serving as controls, 79 in total, were sequentially recruited. Hirsutism was diagnosed with a Ferriman Gallway Score of ≥ 8. Women with a history of diabetes (fasting blood glucose ≥ 126 mg/dl), thyroid dysfunction, hyperprolactinemia, hyperandrogenemia, cardiovascular system diseases, kidney or liver dysfunction, malignancy, Cushing syndrome, adrenal or ovarian tumour, autoimmune disease such as asthma and psoriasis and tobacco or any drug use that may have interfered with the resistin level were excluded from the study. Women who have been using hormonal preparations such as oral contraceptive pills in the last 3 months were also excluded.

Idiopathic hirsutism group consisted of hirsute women who were normoovulatory determined by serum progesterone level of 10 ng/ml or higher on the 21st-22nd days of the menstrual cycle and who were normoandrogenic confirmed with normal serum total and free testosteron, androstenedion, 17 hydroxyprogesterone and dihydroepiandrosterone sulphate levels on 2nd or 3rd day of menstrual cycle with exclusion of other causes of hirsutism. The diagnosis of PCOS was based on the revised 2003 Rotterdam diagnostic criteria with the presence of at least two of the three of the following criteria: oligo- or anovulation; clinical and/or biochemical findings of hyperandrogenism; polycystic ovaries, in addition to exclusion of other related disorders. Blood samples of women with PCOS were drawn on 2nd or 3rd day of natural or progesteron induced menstrual cycle. Control group consisted of regularly menstruating women who complained of symptoms unrelated to hirsutism or any other endocrinopathy including hyperandrogenemia and ovarian dysfunction confirmed with normal serum Follicle Stimulating Hormone (FSH), Luteinizing Hormone (LH), Estradiol (E2). Total and free Testosterone, Androstenedione or DHEAS and 17-hydroxyprogesterone levels.

Laboratory

Venous blood samples were drawn from women after 12-hour-night fasting in morning, at 08:00 h-08:30 h in 2nd or 3rd days of the menstrual cycle. Serum specimens were separated from whole blood samples by centrifugation at 5000 rpm for 10 minutes and stored at −20 C in a deep-freeze until assayed. Hemolytic and lipemic serum samples were excluded from the study.

Serum FSH, LH, E2, progesterone, insulin, total testosterone and dehydroepiandrosterone sulphate (DHEAS) levels were analysed by chemiluminescent immunoassay method (IMMULITE 2000, Siemens Healthcare Diagnostics Inc., Flanders, NJ, USA). Serum glucose was measured using the hexokinase method and the lipid panel including total cholesterol, LDL-cholesterol, HDL-cholesterol and triglyceride (TG) levels were measured using the enzymatic colorimetric method (Cobas 6000 C501 Roche Diagnostics GmbH, Mannheim, Germany); Serum LDL-cholesterol level was calculated using the Friedewald formula for those with a TG ≤ 400 mg/dL. The HOMA-IR (Homeostasis Model Assessment of Insulin Resistance) was used for assessing the insulin resistance using the following formula: [Fasting plasma glucose (mg/dl) x Fasting plasma insulin (µIU/ml)]/40527,28.

Serum resistin levels were measured using the commercial ELISA kit (BioVendor, Laboratorni Medicina A.S., Brno, Czech Republic) according to manufacturers instructions. The intra-assay and inter-assay coefficients of variation were 5.2% and 7.0%, respectively with a sensitivity of 0.012 ng/mL.

Statistical Analysis

The PASW program (PASW Inc, Chicago, IL, USA, vers. 18.0 for Windows) was used for the statistical analyses. The normal range of the numeric data was analyzed using the Shapiro Wilk test and the slope histogram. Normally ranged numerical data were expressed as mean ± standard deviation (SD). The Independent Samples t test was used for the comparison of the two groups and the One Way ANOVA test was used for groups of more than two. Aberrantly distributed numerical data were expressed as median (minimum-maximum) and for these, the Mann Whitney U test was used for the comparison of the two groups, and the Kruskal Wallis test was used for the comparison of groups of more than two. The Spearman correlation test was used to investigate the linear correlation between the two numerical
data. The qualitative categorical data were analyzed using the \( \chi^2 \) test, and the results were often expressed as frequency and percentage (%). A \( p \) value of lower than 0.05 was accepted significant.

Statistical software Minitab V.15® was used for calculating sample size. Resistin is accepted as reference value. Mahde et al\(^9\) reported that mean and standard deviation of resistin was 7.66±0.66 in 30 controls. We assumed the percent of larger difference of means among three groups was 10%. When power was 80% and \( \alpha \) was 0.05, the effect size \( f \) was 0.42 and the required sample size for each group was calculated as 19.

## Results

The demographic characteristics and the serum hormone levels of the PCOS, idiopathic hirsutism and control groups are presented in Table I. There were no statistically significant differences regarding the age, BMI and waist circumferences between the groups. Corresponding mean and median fasting blood glucose, fasting insulin, insulin resistance, serum resistin levels and the lipid profiles did not differ statistically between the groups (Table II).

Additionally, the median fasting insulin level [11.4 (7.2-25.6)] of 11 women with PCOS who had BMIs of > 25 kg/m\(^2\) was found to be higher than 21 women in the slim control group [4.7 (2-6.6)] and 16 women in the slim PCOS [4.4 (2-8.4)] group. No differences were observed between the latter two groups (\( P_{\text{overall}} = 0.003 \), Figure 1). The median HOMA-IR [2.6 (1.5-5.9)] of the women with PCOS who had BMI >25 kg/m\(^2\) was found to be higher than that of the women in the slim control group [1 (0.4-1.6)] and the slim PCOS [0.9 (0.4-1.8)] groups. No differences were observed between the latter two groups (\( P_{\text{overall}} = 0.004 \), Figure 1). The median resistin level [8.1 (6.9-9.7)] of the women with PCOS that had BMI >25 kg/m\(^2\) was found to be similar to women

| Table I. Comparison of demographic and hormonal characteristics of groups. |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Control (n = 28) | IH (n = 23) | PCOS (n = 28) | \( p \) |
| Age (years) | 22 (18-33) | 23 (18-41) | 24 (18-34) | 0.770 |
| BMI (kg/m\(^2\)) | 23.6 (20.6-39.6) | 22.8 (17.9-38.5) | 23.4 (17.5-37.2) | 0.624 |
| WC (cm) | 80.7 ± 13.1 | 81.8 ± 12.8 | 83.6 ± 13.4 | 0.711 |
| FGS | 3 (0-6) | 11 (8-20) | 6 (0-30) | \textbf{0.001} |
| LH (mIU/ml) | 5.35 (1.80-13.50) | 5.50 (2.72-31.60) | 7.67 (1.4-34.0) | 0.056 |
| LH/FSH | 0.93 (0.24-5.64) | 1.05 (0.40-4.39) | 1.38 (0.24-5.15) | 0.089 |
| E\(_2\) (pg/ml) | 30.95 (20-243) | 47.2 (21.8-250.0) | 57.4 (19.0-390.0) | \textbf{0.006} |
| Testosterone (ng/dl) | 38.2 (20.0-103.0) | 45.00 (19.0-176.0) | 44.95 (0.55-141.0) | 0.097 |
| E\(_2\)/testosterone | 0.96 (0.19-4.91) | 1.26 (0.18-8.33) | 1.14 (0.16-709) | 0.412 |
| DHEAS (μg/dl) | 228.21±81.01 | 249.91±124.28 | 226.58±112.93 | 0.695 |
| FSH (mIU/ml) | 5.88 ± 2.32 | 5.66 ± 1.88 | 5.82 ± 1.65 | 0.918 |

IH: Idiopathic hirsutism; BMI: Body mass index; WC: Waist circumference; FGS: Ferriman Gallway score.

| Table II. Comparison of metabolic parameters between the groups. |
|----------------------------------|----------------------------------|----------------------------------|----------------------------------|----------------------------------|
| Control (n = 28) | IH (n = 23) | PCOS (n = 28) | \( p \) |
| FBG (mg/dl) | 88.3 ± 11.7 | 87.6 ± 12.1 | 88.5 ± 12.0 | 0.966 |
| Fasting insulin (μIU/ml) | 4.93 (2.00-41.00) | 4.50 (1.00-18.10) | 7.21 (1.00-124.0) | 0.378 |
| HOMA-IR | 1.11 (0.39-12.35) | 0.94 (0.20-3.93) | 1.53 (0.21-23.58) | 0.409 |
| Resistin (ng/ml) | 9.1 (4.4-24.3) | 9.4 (3.6-37.4) | 8.6 (5.3-21.3) | 0.784 |
| TG (mg/dl) | 83 (33-230) | 108 (41-275) | 79 (44-264) | 0.461 |
| HDL (mg/dl) | 52 ± 13 | 55 ± 11 | 52 ± 13 | 0.593 |
| LDL (mg/dl) | 98 ± 31 | 94 ± 19 | 99 ± 37 | 0.884 |

IH: Idiopathic hirsutism; FBG: Fasting blood glucose.
in slim control [8.5 (7.2-11.2)] and slim PCOS [8.9 (7.6-11.2)] groups. No differences were observed between the latter two groups ($P_{overall} = 0.743$, Figure 1).

No correlation was observed between the resistin and HOMA-IR, the fasting insulin and the BMI in groups (Table III).

When all the groups were considered, insulin resistance was observed to be positively correlated with BMI, waist circumference, DHEAS and TG, and negatively correlated with HDL. According to the results of the regression analysis, the BMI (beta = 0.478, $p = 0.023$) and TG (beta = 0.279, $p = 0.014$) were found to be independent predictors for HOMA-IR. There were no correlations between the serum resistin levels and BMI, waist circumference or any other parameters except LDL ($r = 0.305$, $p = 0.006$).

**Discussion**

In this study, we found that the serum resistin, fasting insulin, insulin resistance, fasting blood glucose and lipid profile did not display statistically significant differences among the groups. Additionally, we did not find any correlations between the resistin, HOMA-IR, fasting insulin levels and BMI in any of the three groups. As far as we know, our study is first to report serum resistin levels in women with idiopathic hirsutism in the literature.

Hyperinsulinemia secondary to insulin resistance may play a role and act in concert with circulating androgens in promoting hair growth with hirsutism. Insulin may directly or indirectly stimulate ovarian granulosa or theca cell steroid synthesis resulting in hyperandrogenemia and hirsutism. Since the steroid-secreting cells are present in human pilosebaceous unit; insulin may stimulate local androgen production. It may also directly increase the sensitivity of hair follicles to androgens. Additionally, it has been shown that insulin and insulin-like growth factor-1 can stimulate hair follicle growth in-vitro.

Therefore, elucidation of the presence of insulin resistance in women with idiopathic hirsutism is important for both the possible long term cardiovascular risks and also for the therapeutic utility of interventions directed toward insulin resistance such as insulin sensitizers similar to their use in hirsute women with PCOS.

However, there are only few studies with conflicting results concerning insulin resistance in idiopathic hirsutism. Ünlühzarcı et al compared the insulin resistance of 26 women with idiopathic hirsutism and 17 controls, and they found high HOMA-IR and fasting insulin levels in idiopathic hirsutism group compared to the controls. In contrast, Kuo et al detected insulin resistance in obese women with idiopathic hirsutism and PCOS. However, they determined that the insulin resistance was actually a manifestation of high BMI in idiopathic hirsutism group such that there was no evidence of insulin resistance when BMI’s were one to one matched. In another recent study, increased insulin resistance was observed in BMI-matched non-obese woman with idiopathic hirsutism and PCOS compared to the control groups. However, when they compared the insulin resistance according to the pattern of obesity, all women with insulin resistance were the women with android obesity.
and they concluded that insulin resistance in idiopathic hirsutism appears to be related to android obesity. Our results were in agreement with that of Kuo et al.25 such that the insulin resistance and serum resistin levels did not differ among the idiopathic hirsutism and control groups. Additionally, there were no correlations between resistin and insulin resistance parameters in the idiopathic hirsutism group. We cannot explain the discrepancy between our results and the other studies. However, idiopathic hirsutism may be a heterogeneous syndrome involving different subgroups of women like PCOS since the proposed underlying pathophysiological mechanisms in idiopathic hirsutism differ.

Women with PCOS are affected by a wide range of metabolic risk factors for cardiovascular disease, including metabolic syndrome, obesity, prediabetic states due to insulin resistance including fasting hyperglycaemia, impaired glucose tolerance and type-2 diabetes.32 Insulin resistance with secondary hyperinsulinemia is present in 50-80% of women with PCOS and it may play important role in pathogenesis of PCOS. Resistin as a novel protein secreted from the adipocytes, the primary target of insulin action, have been reported to induce insulin resistance and impaired glucose tolerance35, and thus serve as a potential link between the obesity and insulin resistance.36 However, the evidence for the role of resistin in insulin resistance and pathogenesis of PCOS is much less convincing20,21,37,38. Chu et al.16 reported that the plasma resistin levels in women with PCOS correlated positively with insulin resistance, and that the resistin protein and its mRNA expression were increased. They concluded that resistin may play a role in the pathogenesis of insulin resistance in patients with PCOS. Similarly, Yilmaz et al.19 found increased serum resistin levels in both obese and non-obese women with PCOS compared to obese and non-obese controls, respectively. However, they found no correlations with insulin resistance and BMI. In contrast, most of the studies concerning resistin in PCOS reported no significant increase in serum resistin levels and/or increase only in obese subjects.14,19,21,23. Seow et al.14 demonstrated 2-fold higher expression of resistin mRNA in omental fatty tissue of PCOS cases despite similar plasma resistin levels compared to controls and they suggested that resistin gene over-expression can be a local determinant in the pathogenesis of PCOS. Baba et al.17 demonstrated that the resistin gene (RETN-420 G/G) homozygous protein variant was approximately 2-fold higher than that of the control group, and cases carrying this gene had high BMIs and increased insulin resistances. They also reported that the resistin gene is not a major predisposing factor for PCOS, but that it may be related to the fat deposition pattern in patients with PCOS. Our findings are in line with those studies that reported similar resistin levels in PCOS compared to controls.

Previously, it has been reported that resistin and LDL correlate strongly in women with type 2 diabetes.39. Although our study did not include any women with type 2 diabetes, a weak correlation was observed between resistin and LDL and TG. This may show that resistin is related to dyslipidemia to some extent although it is not directly related to idiopathic hirsutism or PCOS. Our findings also show that insulin resistance (i.e. HOMA-IR) is affected by high BMI. However, there was no correlation between the resistin and high BMI or having PCOS. Additionally, although the insulin resistance was higher, the plasma resistin levels in the PCOS patients with BMI > 25 were similar to those with BMI < 25 and the slim controls. This demonstrates that insulin resistance in PCOS patients is related to a high BMI, independent from the serum resistin levels. Our data agree with the previous reports concluding that resistin levels are related to metabolic parameters such as the fat mass, triglycerides and LDL cholesterol rather than insulin resistance in obese women or women with metabolic syndrome.40,42.
Although we can conclude that serum resistin levels do not appear to be related to insulin resistance in PCOS, we cannot exclude the local role of resistin in inducing insulin resistance as Seow et al.\(^1\) have proposed. Similarly, we cannot exclude the contribution of the local effect of resistin in idiopathic hirsutism despite the similar serum resistin levels in idiopathic hirsutism compared to controls and the absence of any correlation between the plasma resistin and HOMA-IR or BMI in any of the groups. This should especially be considered in the context of the data demonstrating that insulin and insulin-like growth factor-1 can stimulate hair follicle growth \textit{in-vitro}\(^10\) and insulin stimulates local androgen production since the steroid-secreting cells are present in human pilosebaceous unit.\(^30-32\) Additionally, it is observed that increased resistin levels respond to insulin sensitizers such as thiazolidinones.\(^36,43\) In addition, our findings should be discussed with caution because the small sample restricted us in further grouping of women based on their BMI’s since that obesity is a major factor for insulin resistance and resistin level. However, BMI’s were comparable among the groups. We did not exclude the women with impaired glucose tolerance despite excluding the women with diabetes at recruitment. Nevertheless, groups did not differ significantly among the groups for parameters of interest: fasting blood glucose, HOMA-IR.

**Conclusions**

Our results imply that insulin resistance do not appear to be increased in women with idiopathic hirsutism compared to controls at similar BMI’s and waist circumferences. Serum resistin does not seem to be a involved in the pathogenesis of idiopathic hirsutism and PCOS regarding insulin resistance. However, wider scale studies including molecular basis are needed to conclude on this issue.

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**Conflict of interest**

The Authors declare that they have no conflict of interests.

**References**

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18) YILMAZ M, BUKAN N, DEMIRCI H, OZTÜRK C, KANE A, AY-...