Elevated Serum Retinol Binding Protein 4 Level in Non-Obese Women with Polycystic Ovary Syndrome

Obez Olmayan Polikistik Over Sendromlu Kadınlarda Yükselmiş Serum Retinol Bağlayıcı Protein 4 Düzeyleri

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ABSTRACT Objective: It has been shown that insulin resistance in women with polycystic ovary syndrome (PCOS) is independent of obesity and body composition. Retinol binding protein 4 (RBP 4) is an adipokine which plays a role in insulin resistance in association with glucose transporter 4 (GLUT 4). In this study, we aimed to evaluate the relationship of RBP4 with hormonal, metabolic parameters and interleukin-6 (IL-6) as a marker of subclinical inflammation in non-obese patients with PCOS. **Material and Methods:** The study groups were composed of 21 non-obese women with PCOS and 15 healthy, normally menstruating women. Serum concentrations of RBP4 and IL-6 were determined along with metabolic and hormonal parameters. **Results:** HOMA-IR values and serum RBP4 levels in PCOS group were significantly increased compared to the control group. RBP4 levels were showing positive correlation with IL-6 and 17-HP in the PCOS group. However, there was no correlation between serum RBP4 levels and metabolic and hormonal parameters. Patients with serum RBP4 concentrations higher than the median level were more insulin resistant than patients with serum RBP concentrations lower than the median level, and they had higher insulin and 17-hydroxyprogesterone values. **Conclusion:** Our data indicate that serum RBP4 levels increase in lean insulin resistant women with PCOS. Eventhough this increase was correlated with insulin resistance, patients with significantly high levels of RBP4 had more insulin resistance. Elevated serum RBP4 levels in non-obese patients with PCOS was related to IL-6, whereas there was no relationship with other metabolic parameters and testosterone levels.

Key Words: RBP4 protein, human; polycystic ovary syndrome; interleukin-6; insulin resistance

ÖZET Amaç: Polikistik over sendromlu (PKOS) kadınlarda insülin direncinin obezite ve vücut kompozisyonundan bağımsız olduğu gösterilmiştir. Retinol bağlayıcı protein 4 (RBP 4) glukoz taşıyıcısı 4 (GLUT 4) ile ilişkili olarak insülin direncinde rol oynayan bir adipokindir. Bu çalışmada, RBP4'ün obez olmayan PKOS'lu hastalarda hormonal, metabolik parametreler ve subklinik inflamasyonun bir göstergesi olan interlökin-6 (IL-6) ile olan ilişkisinin değerlendirilmesi amaçlandı. **Gereç ve Yöntemler:** Normal kilolu 21 PCOS'lu hasta ve normal menstruasyonu olan 15 sağlıklı kadın kontrol grubu olarak çalışmaya alındı. RBP4 ve IL-6 düzeyleri metabolik ve hormonal parametrelerle birlikte değerlendirildi. **Bulgular:** PKOS grubunda HOMA-IR değerleri ve serum RBP4 düzeyleri kontrol grubuna göre anlamlı derecede yüksek bulundu. RBP4 düzeyleri PKOS grubunda IL-6 ve 17-hidroksiprogesteron ile pozitif korelasyon gösterdi. Ancak, serum RBP4 düzeyleri ile metabolik ve hormonal parametreler arasında korelasyon saptanmadı. Serum RBP4 konsantrasyonları median değerin üstünde olan hastalarda insülin direnci, insülin ve 17-hidroksiprogesteron değerleri RBP4 konsantrasyonları median değerin altında olan hastalara göre daha yüksekti. **Sonuç:** Verilerimiz insülin direnci olan normal kilolu PKOS'lu kadınlarda serum RBP4 düzeylerinin arttığını göstermektedir. Bu artışın insülin direnci ile korelasyonu olmamasına rağmen, RBP4 düzeyleri anlamlı derecede yüksek olan hastalarda daha fazla insülin direnci vardı. Normal kilolu PCOS'lu kadınlarda RBP4 artışı IL-6 ile ilişkiliyken diğer metabolik parametrelerle ve testosteron düzeyi ile ilişki göstermiyordu.

Anahtar Kelimeler: RBP4, protein, insan; polikistik over sendromu; interlökin-6; insülin direnci

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Except oligo-anovulation, hyperandrogenism, polycystic ovary morphology and insulin resistance have been found mostly associated with obesity in patients with polycystic ovary syndrome (PCOS). Insulin resistance and hyperinsulinemia are present up to 70% in women with PCOS.[1] Non-obese subjects with PCOS who composes about 40 to 45% of all PCOS patients also have insulin resistance.[2] Post-receptor intrinsic defects in metabolic signaling of insulin in combination with yet undefined in vivo acquired defects developed due to environmental factors play an important role in the insulin resistance pathogenesis of patients with PCOS.[3-5] These in vivo factors may be adipokines and free fatty acids.[5] Moreover, in women with PCOS, glucose transporter 4 (GLUT4) expression in the adipose tissue is decreased independent of obesity.[6] The alteration in the expression of GLUT4 in mice either in adipose or muscular tissue affect the insulin-stimulated secondary intake of glucose to the liver or other tissues including adipose and muscular tissues, thus affects the insulin sensivity.[7,8] It has been suggested that this interaction between adipose tissue, muscle and liver is provided by some adipose tissue-based proteins including retinol binding protein 4 (RBP4).[9] RBP4 is the specific transport protein for retinol in the blood. RBP4 has been considered as an adipokine -which takes role in the insuline resistance in association with GLUT4- after it has been found to be secreted from the adipose tissue.[10] Yang et al.[11] reported that RBP4 mRNA expression in adipocytes and serum RBP4 levels were increased in adipose-specific GLUT4 knockout mice.[11] RBP4 concentrations and the magnitude of insulin resistance were correlated with each other in patients with obesity and impaired glucose tolerance, as well as in patients with type 2 diabetes.[12,13]

The results of the investigations evaluating the relationship between PCOS and RBP4 are conflicting. There are some studies stating no difference between the control group and obese PCOS subjects in terms of RBP4 levels.[14,15] Investigations which found higher levels of RBP4 in circulation in PCOS patients when compared to controls, also report conflicting results about the relationship of this increase with insulin resistance, body mass index (BMI), fat mass and androgenemia.[16-19] Furthermore, these studies were mostly performed on overweight and obese patients with PCOS. In our study, since we consider that non-obese and obese patients with PCOS have different metabolic profiles, we aimed to investigate RPB4 in patients having PCOS without obesity.

Since cardiovascular risk is higher in patients with PCOS and RBP4 is considered as a cardiometabolic risk factor, we also evaluated the relation with interleukin-6 (IL-6), which is an another inflammatory marker, and a cardiometabolic risk factor.[20,21]

MATERIAL AND METHODS

The groups studied composed of 21 lean women with PCOS and 15 healthy normally menstruating women. Local Research Ethics Committee approved the study, informed consents were obtained from all patients in accordance with the guidelines in the declaration of Helsinki, 2000. All PCOS patients met two out of three criteria of the revised 2003 Rotterdam ESHRE/ ASRM PCOS Consensus Workshop Group diagnostic criteria.[22] All subjects in the control group had normal findings on pelvic ultrasound, regular periods and no hirsutism or acne. Exclusion criteria were known cardiovascular and thyroid disease, neoplasms, current smoking, diabetes mellitus, hypertension and renal impairment. None of the participants were on any medications for at least three months prior to the study, including oral contraceptives, glucocorticoids, ovulation induction agents, antidiabetic and antiobesity drugs, or estrogenic, antiandrogenic medications. Other endocrinopathies were ruled out at the time of recruitment by measuring basal serum 17-hydroxyprogesterone, prolactin and morning cortisol levels after 1.0 mg overnight dexamethasone suppression (A value of <50 nmol/L was considered to rule out Cushing's syndrome) also. All participants' cortisol levels were suppresed to values less than 50 nmol/L. Physical examinations of patients and appropriate laboratory tests were performed before the study. Severity of hirsutism was assessed according the modified Ferriman-Gallwey sco-

ring system. Tests were performed in regularly cycling women during early follicular phase (3-5 days) of their menstrual cycle and in the PCOS group, 3 to 5 days after a spontaneous menstruation or independent of cycle phase in the presence of amenorrhea. The BMI was calculated as body weight in kilograms divided to height in metre squares (kg/m^2). Weight, height, waist and hip circumferences were measured. While waist circumference was obtained as the smallest circumference at the level of the umbilicus, hip circumference was measured as the widest circumference at the level of the buttocks.

BIOCHEMICAL AND HORMONAL ANALYSES

Assays for glucose, total cholesterol, HDL-C and triglyceride were performed by using a Cobas Integra 800 automated analyser (Roche Diagnostics, Manheim, Germany). The serum LDL-C levels were calculated according to the Friedewald's formula. Insulin, cortisol, testosteron, FSH, LH, estradiol, prolactin, TSH, FT4 levels were measured with Modular E170 automated analyser (Roche Diagnostics, Manheim, Germany). 17-OH progesterone level measurement was done by enzyme-linked immunosorbent assay (17-OH Progesterone ELISACat No. 063608, Biosource International, Inc., Belgium). Serum RBP4 levels were measured with human Competitive ELİSA-kit (Biosource International, Inc. Camarillo, California, USA). The intra-assay and interassay coefficients of variation (CV) were 9.2% and 10.3%, respectively. Sex hormone- binding globulin (SHBG) and IL-6 levels were measured with chemiluminescence method by using Immulite One analyser (Diagnostics Products Corporation, Los Angeles, USA) with a coefficient of variation of less than 4%.

Free androgen index (FAI) was calculated as serum testosterone (nmol/L) X 100/SHBG (nmol/L) ratio.[23] The insulin sensitivity was determined by homeostasis model assessment model (HOMA) index with a formula of HOMA-IR= [fasting insulin (mIU/mL) x fasting glucose (mg/dL)/18]/22.5.[24]

STATISTICAL ANALYSIS

SPSS package program (version 11.0, Chicago, IL) was used for statistical analysis. Descriptive statistics were performed for all parameters.

Variables were presented as means ± standart deviations (SD). Statistical analyses were performed by using independent Student's t-tests. Simple correlation analysis was performed between serum RBP4 and various parameters by using Pearson's correlation analysis. The median value of RBP4 was calculated in the lean group of PCOS patients and the patients were divided into two groups according to the values above and below of the median value. Statistical significance was defined as $P< 0.05$.

MedCalc version 9.5.2.0 was used for power analysis. The difference between the patients and control subjects in terms of RBP4 means was estimated as 18 μg/ml after reviewing related literature.[16,17] Power analysis, assuming a standard deviation of 20 μg/ml for RBP4, a type I error rate of 5% and a type II error rate of 10%, revealed a sample size of 26 subjects in both groups together.

RESULTS

Table 1 shows the characteristics of the subjects participated in the study. Age, BMI, waist circumference, fasting plasma glucose, insulin, total cholesterol, HDL, LDL and triglyceride levels were similar in both groups. HOMA-IR values in PCOS group were found to be increased significantly compared to the control group ($P< 0.05$). Hirsutism score, total testosteron, free androgen index and LH values in the PCOS group were significantly higher than the control group ($P< 0.05$) as expected. SHBG levels in the PCOS group were significantly lower than the control group ($P< 0.05$), while IL-6 levels were similar in both groups ($P> 0.05$). Serum RBP4 levels in the PCOS group were higher than the control group ($P< 0.05$). Table 2 shows Pearson's correlation analysis between serum RPB4 and various parameters in women with PCOS. RBP4 showed positive correlations with IL-6 and 17-HP in the PCOS group and r values were $r= 0.646$ and $r= 0.446$, respectively ($P< 0.05$).

However, we could not find any correlation of RBP4 either with metabolic parameters such as BMI, waist circumference, HOMA-IR, insulin le-

TABLE 1: Clinical, metabolic, and hormonal parameters of women with PCOS and control subjects.

	Lean PCOS (n= 21)	Control (n= 15)	p
Age (year)	21.85 ± 4.06	23.46 ± 5.15	NS
Waist circumference (cm)	73.45 ± 7.42	78.40 ± 15.63	NS
BMI (kg/ m^2)	20.74 ± 1.75	20.85 ± 2.08	NS
Hirsutism score	17.57 ± 5.79	4.86 ± 1.40	0.000
Fasting glucose (mg/dl)	85.80 ± 6.40	85.60 ± 5.87	NS
Fasting insulin (mU/ml)	13.42 ± 9.6	9.24 ± 3.59	NS
HOMA-IR	3.40 ± 2.59	1.96 ± 0.81	0.025
T. cholesterol (mg/dl)	175.14 ± 55.09	152.06 ± 25.50	NS
HDL-C (mg/dl)	61.35 ±13.20	54.85 ± 7.87	NS
Triglyceride (mg/dl)	85.38 ± 35.43	73.93 ± 19.74	NS
LDL-cholesterol (mg/dl)	96.23 ± 49.71	82.00 ± 26.64	NS
LH (mIU/ml)	9.47 ± 6.08	5.60 ± 3.16	0.018
FSH (mIU/ml)	5.69 ± 1.36	6.04 ± 1.72	NS
LH/FSH	1.81 ± 1.32	0.63 ± 0.16	0.019
Estradiol (pg/m)l	72.35 ± 47.26	90.51 ± 49.32	NS
T. testosterone (ng/dl)	0.92 ± 0.43	0.46 ± 0.18	0.000
17-OH progesterone (ng/dl)	1.38 ± 0.61	1.27 ± 0.41	NS
SHBG (nmol/l)	30.93 ± 17.03	60.52 ± 14.71	0.000
Free androgen index	3.83 ± 2.60	0.83 ± 0.59	0.000
IL-6 (pg/m)l	2.11 ± 0.50	2.13 ± 0.63	NS
RBP4 (µg/ml)	49.92 ± 19.38	28.83 ± 16.46	0.002

All values are means ± SD. NS, not significant.
PCOS: Polycystic ovary syndrome.

TABLE 2: Pearson's correlation analysis between plasma RPB4 and various parameters in women with polycystic ovary syndrome (PCOS).

Variables	r	p
Waist circumference (cm)	-0.32	0.891
BMI (kg m^{72})	0.058	0.802
Estradiol (pg/m)l	0.327	0.148
Serum LH (mIU/ml)	-0.042	0.856
Serum FSH (mIU/ml)	-0.400	0.072
17-OH progesterone(ng/dl)	0.446	0.043
Serum testosterone (ng/dl)	-0.128	0.581
Serum SHBG (nmol/l)	-0.209	0.363
Free androgen index	-0.035	0.881
Hirsutism score	-1.176	0.446
Total cholesterol (mg/dl)	0.009	0.969
HDL-C (mg/dl)	0.314	0.166
Triglyceride (mg/dl)	-0.244	0.286
LDL-cholesterol (mg/dl)	-0.038	0.870
Fasting glucose (mg/dl)	-0.128	0.581
Fasting insulin (mU/ml)	0.343	0.128
HOMA-IR	0.330	0.145
IL-6(pg/ml)	0.646	0.002

vel and lipid profile, or with other endocrine parameters including hirsutism score, testosterone, estradiol, LH, SHBG levels, and FAI. Subjects with PCOS were divided into two groups by using the median level of 42.75 µg/ml as a cutoff value. The mean HOMA-IR value of eleven subjects with levels of RBP4 higher than the median value was higher than the mean HOMA-IR value of ten patients with levels of RBP4 lower than the median value (3.74 ± 2.3 vs 1.91 ± 1.15, p= 0.037).

DISCUSSION

In this study,non-obese women with PCOS were found to be more insulin resistant compared to the controls. However other metabolic parameters were similar in each group. In the previous studies it has been shown that insulin resistance in PCOS women is independent of obesity and body composition.[25] The most important defect responsible for the insulin resistance in PCOS is the excessive phosphorylation of the serine residues of the insulin receptor.[2] Another defect which plays role in insulin resistance in PCOS is the decreased expression of GLUT 4 in the adipose tissue independent of the obesity.[6] Previously, relationship between decrease in GLUT4 expression and increase in RBP4 levels has been shown in mice.[11] In our study, we have found significantly higher levels of RBP4 in non-obese and insulin resistant women with PCOS compared to normal women. Some of the other previous investigations also revealed similar results.[16,26]

In certain studies that reported an increase in levels of RBP4 in subjects with PCOS a correlation has been shown between RBP4 and insulin resistance .[16,19] On the contrary, some other investigations did not find any correlation between increased RBP4 level and insulin resistance.[17,18] Although we could not show any correlation between the increased RBP4 levels and insulin resistance, we observed that patients having RBP4 levels above median values were more insulin resistant compared to ones having levels under the median value. Intrinsic variability in insulin resistance measured with the HOMA method detected higher variability in patients with PCOS com-

pareked to normal women.[27] Since we have not performed repetetive measurements for the calculation of HOMA-IR, we might have not been able to show the correlation between HOMA-IR and RBP4. Moreover, similar to our findings, insulin resistance might be increasing when RBP4 levels barely increase to higher values in non obese patients with PCOS. Previously, Möhling et al.[28] in one of their studies in which 110 PCOS patients were divided into tertiles according to HOMA %S, have represented that the RBP4 levels were elevated the most in the tertile where the insulin resistance was the highest.

In our study, we did not find any correlation between the increase of RBP4 and other metabolic parameters. Previously, Yıldız et al.[29] showed that the levels of RBP4 in lean, normal glucose- tolerant women with PCOS were not different than the control group and RBP4 levels were not correlated with the clinical, hormonal or biochemical variables. They have suggested that the conflicting results observed in different studies may be associated with geographical divergence of PCOS patients or commercial immunoassay variations.[29]

Retinol is essential for female reproduction, and retinoids have been suggested to play a role in ovarian steroidogenesis, oocyte maturation, and formation of the corpus luteum. The enzymes responsible for retinol metabolism are present in theca cells and may be altered in PCOS.[30] Changes in the synthesis and action of retinoic acid increase the production of androgens in PCOS.[31] This effect could be mediated by enhanced delivery of the retinol ligand by RBP4. In other words, RBP4 may be increasing the androgen production with its retinol dependent effect. In our study, like we have expected, testosterone was significantly increased in PCOS patients when compared to normal women. Despite its linear correlation with 17-hydroxyprogesterone, RBP4 did not have any relation with testosterone. In PCOS, the disregulation of CYP 17 enzyme activity which is a key enzyme in the production of 17-hydroxyprogesterone has been shown.[31] Moreover it has been reported that retinol stimulates the accumulation of CYP17 mRNA.[32] RBP4, with its retinol dependent effect, may increase this accumulation.

Recently, it has been shown that inflammation markers increase the atherosclerosis risk in insulin-resistant situations including PCOS. It has been reported in patients with PCOS, particularly in obese women the most, that inflammatory mediators including IL-6 are higher compared to weight-mathced controls.[33] However, there are some studies stating that there is no increase in IL-6 levels either in obese or non-obese patients with PCOS.[34] In our study, IL-6 levels in non-obese women with PCOS were not different compared to the healthy women. The potential relationship of RBP4 with cardiometabolic risk factors like inflammatory markers is unclear. It has been shown that reduction in levels of RBP4 with lifestyle intervention in obese children is correlated with the magnitude of decrease in inflammatory factors.[35] In our PCOS group, there was a significant positive correlation between IL-6 and RBP4 levels which shows that the increase of the IL-6 out of the normal ranges is related with the RBP4 increase. IL-6 is a cytokine which has paracrine and autocrine effects even not synthesized in high levels. Therefore, we suppose that investigation of the relationship between IL-6 and RBP4 in tissue level would provide valvable information for investigators.

On conclusion, we showed that RBP4 levels were increased in insulin-resistant lean patients with PCOS. Even though this increase was not correlated with insulin resistance, the resistance was higher in patients who had significantly high levels of RBP4. The increase in RBP4 levels in lean women with PCOS, was not associated wih other metabolic parameters or testosterone levels. IL-6 which is an inflammatory marker, and considered as a cardiometabolic risk factor was associated with RBP4 increase.

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