TITLE PAGE

RETINOL-BINDING PROTEIN 4, AS A NEGATIVE ACUTE-PHASE REACTANT IN POLYCYSTIC OVARY SYNDROME

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ABSTRACT

Objective: The aim of our study was to compare serum levels of RBP4 in women with PCOS to the control group and to understand the relationship among RBP4 and biochemical and hormonal parameters related to disease process, especially gonadal steroids and markers of inflammation.

Materials-Methods: 28 women with PCOS (18 normal weight and 10 obese) and 27 normally menstruating healthy women (20 normal weight and 7 obese) were included.

Results: Women with PCOS had higher RBP4 concentrations. RBP4 levels correlated negatively with LDL, hsCRP and LH in women with PCOS and positively with BMI in the control group. When obese PCOS were compared to normal weight PCOS, increased CRP levels correlated negatively with RBP4 only in the normal weight PCOS group (normal PCOS $r=-0.465$, $p=0.042$; obese PCOS $r=-0.505$, $p=0.137$). Regression analysis of the effects of CRP and BMI on RBP4 levels revealed a statistically significant relationship between CRP and RBP4 independent of BMI.

Conclusions: Serum RBP4 levels increased in women with PCOS and correlated negatively with CRP, LH and LDL. RBP4 probably acts as a negative acute phase reactant in normal weight PCOS. It cannot be used as a markaer of chronic low grade inflammation in women with PCOS.

Keywords: Retinol-binding protein 4, polycystic ovary syndrome, CRP, inflammation, acute-phase reactant

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a frequent endocrine disease characterized by hyperandrogenism and chronic anovulation. Whether obese or nonobese many PCOS women have to put up with insulin resistance (IR) and its consequences, and weight gain increases IR in women with PCOS more than age and body mass index (BMI) matched healthy women (1,2). Retinol-binding protein 4 (RBP4) is the specific transport protein for retinol, but it is also secreted by the adipocytes. As an adipokine it was suggested to cause IR (3) and yet others related it only to adiposity (4). Recent studies related it to gonadotropins and markers of inflammation (5,6).

Previous studies that have searched the role of RBP4 in the pathogenesis of PCOS have given conflicting results (7-12). The aim of our study was to compare serum levels of RBP4 in women with PCOS to the control group and to understand the relationship among RBP4 and biochemical and hormonal parameters related to disease process, especially gonadal steroids and markers of inflammation.

MATERIALS-METHODS

This is a retrospective study achieved with unused data and stored blood of PCOS patients preserved for a previously published study () . Twenty-eight women with PCOS (18 nonobese and 10 obese) and 27 normally menstruating, age and BMI matched healthy women (20 nonobese and 7 obese) were included. All PCOS patients were diagnosed according to 2003 Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group Criteria (13). All participants in the control group were menstruating normally, had a normal pelvic ultrasound, and had no clinical/biochemical hyperandrogenism. Exclusion criteria were the presence of systemic diseases such as diabetes mellitus, cardiovascular diseases, hypertension, thyroid diseases, chronic renal failure, malignancy, Cushing syndrome, congenital adrenal

hyperplasia, hyperprolactinemia use of medications for at least 3 months before the study including oral contraceptives, glucocorticoids, lipid-lowering, antiobesity, antidiabetes, antiandrogenic, antihypertensive or ovulation-inducing agents. The study protocol was in confirmation with the ethical guidelines of Declaration of Helsinki. All of the subjects gave written informed consent.

Before the study all of the participants underwent a physical examination and appropriate laboratory tests were performed and anthropometric measurements were obtained. BMI was calculated as body weight in kilograms divided by height in metre squared (kg/m^2). Those with BMI <25 were classified as nonobese, others were considered as obese. Weight, height and waist and hip circumferences were measured. Waist circumference (WC) was obtained as the smallest circumference at the level of umbilicus. Hip circumference (HC) was obtained as the widest circumference at the level of the buttocks. Serum samples were obtained from all women in the early follicular phase, during the 3rd-4th days of the cycle after an overnight fasting. The levels of fasting plasma glucose, insulin, total cholesterol (TC), high-density lipoprotein (HDL), low density lipoprotein (LDL), triglycerides (TG), LH, FSH, prolactin, TSH, C-reactive protein (CRP), dehydroepiandosterone sulfate (DHEAS), free testosterone, cortisol, free T4, 17-OH progesterone, estradiol (E2), sex-hormone binding globulin (SHBG), lipoprotein-a, interleukin-6 (IL-6), interleukin-1beta (IL-1beta) and RBP4 were measured. Plasma RBP4 levels were measured using a commercially available ELISA (Assaypro,Belgium) according to the manufacturer's protocol. All parameters except RBP4, IL-1beta and IL-6 were measured immediately. Blood samples for RBP4, IL-1beta and IL-6 were centrifuged and stored at -80°C until analysis.

Insulin resistance was determined by homeostasis model assessment (HOMA) index with the formula: HOMA-IR= fasting insulin (μU/ml)x fasting glucose (mg/dl)/405.

Statistical analysis were performed using the NCSS 2007 and PASS 2008 statistical software (Utah, USA). The data showing normal distribution of parameters were compared with Student's t-test, the data showing non-normal distribution of parameters were compared with Mann Whitney U test. Pearson and Spearman's correlation analysis and lineer regression anaysis were used. p values<0.05 were considered as statistically significant.

RESULTS

The results about patient characteristics and biochemical and hormonal parameters were shown in Table 1. As expected PCOS patients had higher LH, free testosterone, FAI and lower FSH levels when compared to the control group, also their RBP4 levels were higher.

Correlation of anthropometric, biochemical and hormonal parameters with RBP4 were shown in Table 2. RBP4 levels correlated negatively with LDL, hsCRP and LH in women with PCOS and positively with BMI in the control group. When obese PCOS were compared to nonobese PCOS, increased CRP levels correlated negatively with RBP4 only in the normal weight PCOS group (nonobese PCOS r=-0.465, p=0.042; obese PCOS r=-0.505, p=0.137). Regression analysis of the effects of CRP and BMI on RBP4 revealed a statistically significant relationship between CRP and RBP4 independent of BMI (Table3).

DISCUSSION

Previous studies searching for the role of RBP4 in women with PCOS unveiled controversial results, some detected no difference in RBP4 levels between women with PCOS and the control group (7,12,14), while others detected both higher levels (10,11,15-17) and lower levels in women with PCOS (18). In our study we found increased RBP4 levels in women with PCOS when compared to the control group, which correlated negatively with LDL, LH and especially with CRP levels.

Obesity is an increasing health problem of the developed world and is associated with a chronic low-grade inflammation mediated by activation of adipose tissue macrophages (19).

RBP4 was shown to be related to the markers of inflammation and it was suggested to play a role in atherogenesis (20). The connection between increased RBP4 and CRP was also confirmed by other studies (21,22). In contrast to these previous studies, RBP4 levels of our nonobese PCOS patients correlated negatively with LDL and CRP levels, markers of cardiovascular disease. Broch et al failed to demonstrate an association between RBP4 and CRP in obese women undergoing bariatric surgery (23). This was also in line with our study, we failed to detect an association between RBP4 and CRP in the control group and also in obese PCOS patients. Two previous studies checked the association between serum RBP4 levels and CRP in women with PCOS, and did not detect a relationship between them (9,18). To the best of our knowledge no other studies have searched for this relationship in women with PCOS later. A recent experimental study has determined the role of RBP4 in the pathway activating proinflammatory cytokines, TNF-α and IL-6 (24). We did not detect a relationship between RBP4 and IL-6 levels. RBP4 was also reported to be expressed by macrophages and to be regulated by inflammatory stimuli (6). Previously RBP4 was reported as a negative acute-phase reactant together with albumin, they decreased while CRP increased (25). One previous study also reported RBP4 as a negative acute phase reactant in patients with sepsis (26).

Adipose tissue is certainly involved in reproduction by converting androgens to estrogens. Previous studies investigated the role of adipokines in the regulation of pituitary-ovarian reproductive axis. Presence of receptors for gonadotropins in adipocytes (27) and the response of adipocytes to gonadotropins with growth and diferentiation (28) suggested regulation of RBP4 by gonadotropins (5). Our study might contribute to this hypothesis by showing the negative relationship between serum RBP4 and LH levels, as shown in a recently published study (29). Other studies demonstrated no relationship between gonadotropins and RBP4 in women with PCOS (8,9,18) and one study reported a positive correlation (17). A

decrease in serum LH levels as a response to decreasing insulin levels was observed in vitro (30). We did not observe a relationship between LH and RBP4 levels in the control group and Makimura et al observed higher RBP4 levels in healthy pre- and postmenopausal women and detected a correlation between RBP4 and gonadotropins (5). Women with PCOS and postmenopausal women have similarities, a preponderance to visceral obesity and metabolic syndrome, therefore it is logical to expect the same mediator to play a role in the pathogenesis.

Adipocytes express both estrogen and androgen receptors (31). Increased production of RBP4 after treatment with 17-betaestradiol was reported in adipose tissue of women with PCOS; testosterone, androstenedione and DHEAS failed to show this effect (10). Most of the data in the literature have noted no association among RBP4 and serum estrogens and androgens (7-9,11,12,18). Mellati and Aigner et al reported a positive correlation between RBP4 and serum androgens (11,32). We didnot find a correlation between RBP4 and sex steroids.

The increase in visceral adipose tissue was related to the metabolic disturbances associated with IR (33). The experimental study of Yang et al detected an increased RBP4 expression in adipose tissue and increased plasma levels of RBP4 in GLUT4 knockout mice (3). Following this, studies relating RBP4 to increased IR were published (34). Increased levels of RBP4 have also been reported to be associated with IR in women with PCOS (7-9,12). We did not detect such a relationship in this study, as some other studies did (11,14,15). Studies with patients other than PCOS also failed to detect a relationship between RBP4 and IR (35-37). In another study RBP4 levels were lower in women with PCOS and IR (29).

Production of RBP4 was reported to increase several folds during differentiation of preadipocytes to adipocytes (37). We detected an association between RBP4 and BMI in the

control group, similar to Olscnecka et al. RBP4 levels were related to BMI both in women with PCOS and in the control group in some previous studies (7,16), and were not related in others (8-10). In adolescents RBP4 levels have been postulated to be associated with BMI (36). Increased RBP4 levels were reported in obese subjects (3,34). Weight loss was associated with a decrease in serum RBP4 levels (38) but this was mostly associated with visceral fat loss (39) .

In conclusion serum RBP4 levels increased in women with PCOS and correlated negatively with CRP, LH and LDL. RBP4 probably acts as a negative acute phase reactant in normal weight PCOS. The significance of these findings can be elucidated with future studies. Based on the findings of this study we cannot suggest RBP4 to be used as a marker of chronic low grade inflammation in women with PCOS.

Declaration of interest: Authors declare no conflicts of interest

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