Serum retinol-binding protein-4 levels in polycystic ovary syndrome patients undergoing controlled ovarian hyperstimulation for *in-vitro* fertilization cycle

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Summary

Aims: To determine serum retinol-binding rotein 4 (RBP-4) levels in polycystic ovary syndrome (PCOS) patients undergoing controlled ovarian hyperstimulation (COH) for an in vitro fertilization-embryo transfer (IVF-ET) cycle and the possible correlation to COH variables. *Patients and Methods:* 11 consecutive PCOS patients undergoing our routine IVF flexible multidose gonadotropin-releasing hormone (GnRH)-antagonist protocol. Blood was drawn three times during the COH cycle: (1) day 1 or 2 of menstruation, and prior to gonadotropin administration (Day-S) (Day-S); (2) day of or prior to human chorionic gonadotropin (hCG) administration (Day-hCG); and (3) day of ovum pick-up (Day-OPU). Levels of estradiol and serum RBP-4 were compared among the three time points. Serum RBP-4 was measured with a commercial immunoassay. *Results:* Results showed significantly lower levels of serum RBP-4 on Day-OPU and Day-hCG than on Day-S. Though significant correlations were observed between serum RBP-4 and body mass index, fasting glucose or glucose to insulin ratio, no correlations were found between serum RBP-4 and IVF treatment variables or pregnancy rate. *Conclusion:* While serum RBP-4 decreases during COH for IVF, there is apparently no correlation of serum RBP-4 levels with IVF treatment variables or outcome.

Key words: Retinol-binding protein-4; PCOS; Insulin resistance; Ovulation induction; Sex steroids; BMI.

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinopathy among women of reproductive age, associated with reproductive and metabolic dysfunction [1]. The clinical presentation varies from eumenorrhea and a sonographic picture of polycystic ovaries but with subtle phenotypic abnormalities or signs of hyperandrogenism, to advanced Stein-Leventhal syndrome [2] and its associated long- term sequelae. Moreover, most women with PCOS also exhibit features of the metabolic syndrome, including insulin resistance, obesity and dyslipidemia [3-5]. Of mention, insulin resistance in women with PCOS appears both in obese and non-obese women [6].

PCOS women with insulin resistance undergoing ovulation induction with gonadotropins have a longer duration of treatment, use a higher total FSH dose, have an elevated cancellation rate and a lower conception rate [7, 8]. Improving insulin sensitivity – through both lifestyle and pharmacological intervention – was suggested to ameliorate the aforementioned abnormalities, restore ovulation and enhance pregnancy in women with PCOS. However, a systematic review by Costello *et al*. [9] demonstrated that while the co-administration of metformin, the most widely studied insulin-sensitizing drug in PCOS patients, to gonadotropin ovulation induction and IVF does not improve ovulation, pregnancy or live birth rates, it does consistently affect ovarian response during ovulation induction, with variable effects on the length of ovarian stimulation, total dose of FSH used, peak serum E_2 level or the number of oocytes collected. Moreover, a recently published ESHRE/ASRM-Consensus [10] that addressed the therapeutic challenges raised in women with infertility and PCOS has concluded that metformin should be restricted only to those patients with glucose intolerance.

Several dynamic invasive tests and calculated indices are currently available for detecting insulin resistance. While the euglycemic clamp technique is considered the most accurate test for the assessment of insulin resistance, this cumbersome test is frequently replaced by the simple measurement of the ratio of fasting glucose to fasting insulin. The later test has been advocated to inversely correlate with the degree of insulin resistance [11], and since, has been used worldwide.

Insulin resistance in adipose tissue is associated with reduced levels of glucose transporter 4, which in turn, causes an increase in retinol-binding protein-4 (RBP-4) production [12]. Retinol-binding protein-4 (RBP-4), a molecule secreted by adipocytes and liver, was recently demonstrated to contribute to [13], and to correlate with the magnitude of insulin resistance [14]. Moreover, elevated serum RBP4 levels were associated with the components of the metabolic syndrome, including increased body-mass index (BMI) [14].

This project was supported by a grant from the Goldman Faculty Fund for Medical Research of the Faculty of Health Sciences, Ben Gurion University of the Negev, Beer Sheva, Israel.

Revised manuscript accepted for publication October 1, 2009

Table 1. — *Hormone profile and RBP-4 levels of the study patients.*

	Day-S	Day-hCG	Day-OPU
Estradiol (pg/ml)	41.4 ± 13.5[a,b]	1650 ± 713[a,c]	1016 ± 503[b,c]
RBP-4 (ng/ml)	37260 ± 10063[d,e]	32459 ± 8311[d]	32719 ± 7488[e]
Glucose (mg/dl)			87 ± 8
Insulin (IU/ml)			10.3 ± 3.8
Glucose/insulin ratio			9.5 ± 3.4

[a,b,d] p <0.001 between the subgroups; [c] $p < 0.03$ between the subgroups; [e] $p < 0.02$ between the subgroups.
All value are mean ± SD.
Day-S = day 1 or 2 of menstruation, and prior to gonadotropin administration; Day-hCG = day of or day prior to human chorionic gonadotropin administration; Day-OPU = day of oocyte pick-up.

Table 2. — *Correlations between serum RBP-4 levels and other patients variables.*

	Serum RBP-4 on Day-S	Serum RBP-4 on Day-hCG	Serum RBP-4 on Day-OPU	Serum RBP-4 All
Serum estradiol				r = 0.14
				$p = 0.4$
BMI	r = 0.73	r = 0.76	r = 0.86	
	$p < 0.01$	$p < 0.01$	$p < 0.001$	
Fasting insulin			r = 0.74	
			$p < 0.01$	
Fasting glucose/insulin r			r = 0.61	
			$p < 0.045$	

Day-S = day 1 or 2 of menstruation, and prior to gonadotropin administration; Day-hCG = day of or day prior to human chorionic gonadotropin administration; Day-OPU = day of oocyte pick-up.

Recently, several studies have measured RBP-4 levels in PCOS patients and their relationship to various endocrine variables, indices of insulin resistance or metabolic syndrome [15-21]. Most of these studies have demonstrated an increase in RBP-4 levels in PCOS patients as compared to healthy controls that correlated with patients BMI and insulin levels.

Prompted by these findings, in the present prospective preliminary study, we sought to longitudinally investigate serum RBP-4 levels in PCOS patients undergoing controlled ovarian hyperstimulation (COH) using GnRH-antagonists for IVF, and to examine whether it correlates with serum estradiol or other COH variables.

Patients and Methods

The study population consisted of 11 consecutive PCOS patients attending the in vitro fertilization (IVF) unit of our department for treatment of infertility. All patients met the PCOS criteria of the recent ESHRE/ASRM consensus (1) and underwent COH using the flexible multidose GnRH-antagonist protocol. The study required no modification of our routine IVF protocols. We included only the GnRH-antagonist protocol due to our program policy [22], which offers high-responder patients the use of GnRH-antagonist during their first IVF attempt. With this strategy we are able to substitute hCG with GnRH agonist to trigger ovulation, with the consequent elimination of severe OHSS.

For the purpose of the study, in addition to the routine monitoring during the COH cycle, blood samples were drawn to determine the hormonal profile (E2, progesterone) and serum RBP-4 levels at three time points: (1) day 1 or 2 of menstruation, and prior to gonadotropin administration (Day-S); (2) day of or prior to hCG administration (Day-hCG); and (3) day of ovum pick-up (Day-OPU).

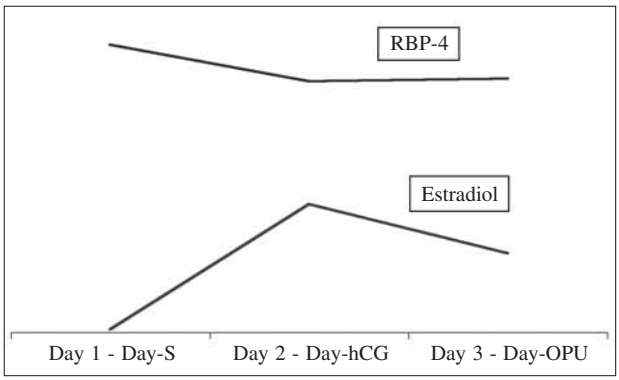

Figure 1. — Correlation between serum RBP-4 and estradiol levels during COH.

Data on patients' age, BMI and infertility-treatment-related variables were collected from the files. Ovarian stimulation characteristics, number of oocytes retrieved, and number of embryos transferred per cycle were recorded. Clinical pregnancy was defined as visualization of a gestational sac and fetal cardiac activity on transvaginal ultrasound.

For serum RBP-4 determination, blood samples were centrifuged for 10 min at 1000 g, and the plasma was stored in aliquots at –70°C until assayed. Serum RBP-4 was measured in duplicate with a commercial immunoassay which employs the quantitative sandwitch enzyme immunoassay technique (R&D Systems, Inc. Minneapolis, USA). All samples were assayed at one time to avoid inter-assay variations. The minimal sensitivity of the assay was 0.224 ng/ml and the intra- and interassay variability were 6.9% and 7.2%, respectively. Blanks and controls were included in all experiments.

Moreover, on Day-OPU and prior to OPU, fasting blood samples were drawn for glucose and insulin levels. While serum glucose was tested by the enzymatic UV test for the quantitative determination of glucose on Olympus analyzers, serum insulin was determined by microparticle enzyme immunoassay technology (AxSym Insulin Abbott, Germany).

Informed consent was obtained from all patients before participation in the study, and the study was approved by the Clinical Research Committee.

The results are expressed as means ± standard deviations or rates. Findings were analyzed statistically with the nonparametric Wilcoxon signed rank test and correlation analysis; p values of 0.05 or less were considered significant.

Results

Mean age of the 11 patients was 30.7 ± 6.3 years, and mean BMI was 28.5 ± 5.6 (range 20.7-35.5). Mean number of gonadotropin ampoules used during the COH cycle was 37 ± 21, mean number of oocytes retrieved 12.1 ± 3.9 and mean number of embryos transferred 2.6 ± 1. Pregnancy rate was 36.3%.

Mean serum E2 and RBP-4 levels on Day-S, Day-hCG and Day-OPU, and glucose and insulin level on Day-OPU are presented in Table 1. As expected, serum E2

Fig. 2a

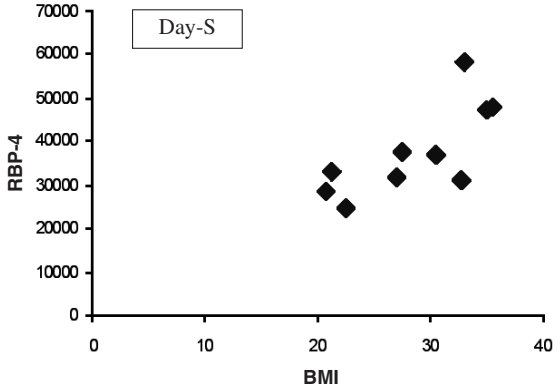

Fig. 2b

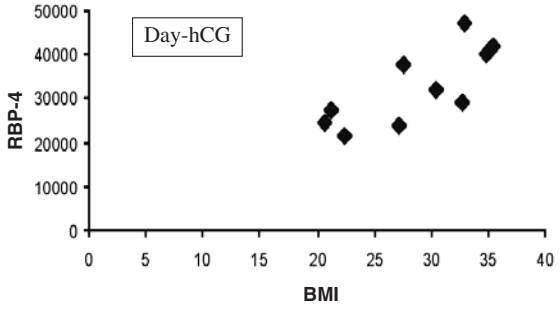

Fig. 2c

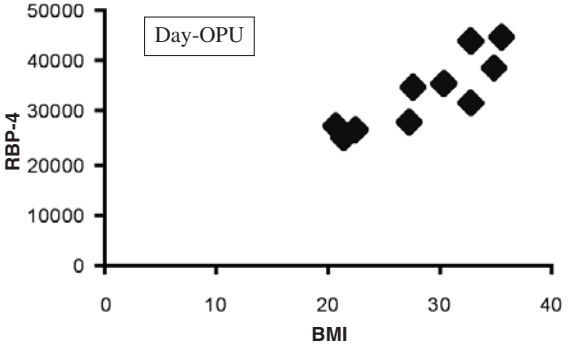

Figure 2. — Correlations between patient BMI and serum RBP-4 levels on Day-S, Day-hCG and Day-OPU.

levels were significantly higher on Day-hCG than Day-S and Day-OPU ($p < 0.001$ and $p < 0.03$, repectively) and significantly higher on Day-OPU than Day-S ($p < 0.001$).

While serum RBP-4 levels were significantly higher on Day-S than Day-hCG and Day-OPU ($p < 0.001$ and $p < 0.02$, respectively) (Table 1), no statistically significant difference was observed in serum RBP-4 levels between Day-hCG and Day-OPU ($p = 0.8$). The correlations between serum RBP-4 levels and several patient variables are presented in Table 2. Figure 1 presents the E2 and serum RBP-4 levels during the cycle. No significant correlations were observed between serum RBP-4 and E2 levels (R = 0.14, $p = 0.4$).

BMI significantly correlated with serum RBP-4 levels on Day-S (R = 0.73, $p < 0.01$), Day-hCG (R = 0.76, $p < 0.01$), and Day-OPU (R = 0.86, $p < 0.001$) (Figure 2).

Fig. 3a

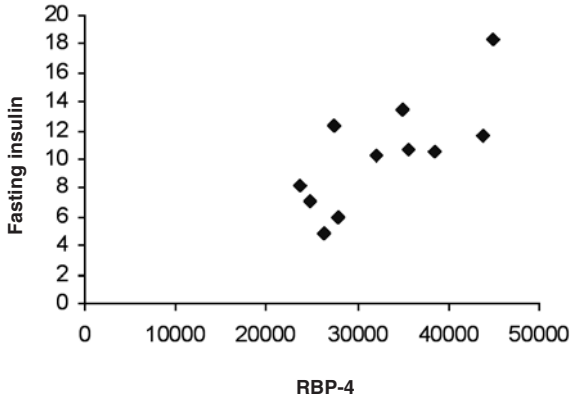

Fig. 3b

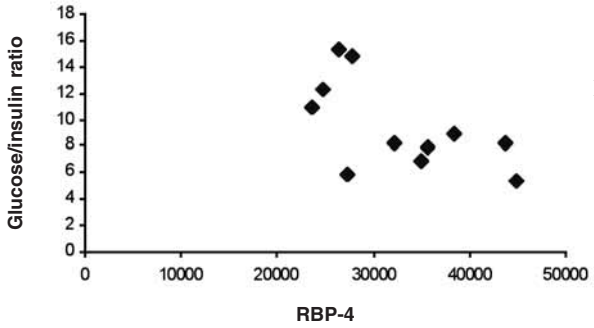

Figure 3. — Correlations between serum RBP-4 levels on Day-OPU and fasting insulin and fasting glucose/insulin ratio.

Moreover, serum RBP-4 levels on Day-OPU significantly correlated with fasting insulin and the fasting glucose/insulin ratio (R = 0.74, $p < 0.01$ and R = 0.61, $p < 0.045$, respectively) (Figure 3).

There were no significant correlations of serum RBP-4 level with patient age, duration of stimulation, amount of gonadotropins used, number of oocytes retrieved, fertilization rate, or pregnancy rates.

Discussion

The present preliminary study shows that RBP-4 level is significantly decreased during COH until peak E2 is reached, with no significant difference after hCG administration. Furthermore, while serum RBP-4 level significantly correlated with patients' BMI, fasting glucose or glucose to insulin ratio, there was no correlation between serum RBP-4 and patients' age, IVF treatment variables or pregnancy rate.

The statistically significant correlation between serum RBP-4 and BMI demonstrated in our study, was also reported by others. Graham *et al*. [14] studied lean and obese, diabetic and non-diabetic subjects and found an association between RBP-4 and components of the metabolic syndrome, including increased BMI. Hahn *et al*. [19] assessed the correlation between metabolic and endocrine parameters with RBP4 levels in PCOS and

healthy controls. They found RBP4 levels to positively correlate with BMI, body fat, and waist circumference. Moreover, while Barber *et al*. [23] observed a positive association between RBP-4 and visceral fat area in PCOS patients, Mohlig *et al*. [17] and Weiping *et al*. [20] demonstrated significant correlations between RBP-4 and lean body mass or waist-to-hip ratio (respectively), but not with BMI. Lack of correlation between RBP-4 and BMI in PCOS patients was also observed by Chan *et al*. [15].

In the present study we also observed significant correlations between RBP-4 levels and fasting insulin or glucose to insulin ratio - the latter reflects and inversely correlates with the degree of insulin resistance. These observations are consistent with previous reports by other groups [14, 17-20] that used several other measures to assess insulin resistance and glucose metabolism, including fasting glucose and area under the curve for glucose, oral or intravenous glucose tolerance test and the euglycemic-hyperinsulinemic clamp test. On the other hand, those who estimated insulin resistance by calculating the total insulin area under the curve on an oral glucose tolerance test [16], or by the homeostasis model assessment of insulin resistance using the formula: {[fasting insulin (mIU/ml) X fasting glucose (mg/dl)/18]/22.5} [15] could not confirm this correlation, and therefore suggested that RBP4 might not be a useful marker of insulin resistance and glucose metabolism.

The lack of association between serum RBP-4 and estradiol levels during ovarian hyperstimulation noted here, is consistent with previous reports by other groups [15, 17]. While Chan *et al*. [15] investigated the correlation of E2 and T with RBP-4 levels in PCOS patients and found that RPB-4 levels were not directly affected by T and estradiol, Mohlig *et al*. [17] could not demonstrate any correlation between RBP-4 and free testosterone, DHEAS, androstenedione, and estradiol. On the other hand, Tan *et al*. [21], while studying RBP4 expression from adipose tissue of overweight PCOS women, observed a significant increase in RBP-4 mRNA and protein expressions following exposure to estradiol. The solution to this discrepancy may lie in the known negative impact of obesity on patient response to COH or pregnancy outcome [24]. That is, the RBP-4 level may act as a confounding factor which positively correlates with obesity, rather than an independent variable affecting ovarian response to COH. We therefore conclude that in PCOS patients, while RBP-4 significantly correlates with BMI and the degree of insulin resistance and decreases during COH, it probably has no direct effect on follicular growth and maturation or ovarian response to COH.

To the best of our knowledge, the present preliminary study is the first to provide information on RBP-4 levels during COH. We observed a significant decrease in RBP-4 levels from Day-S to Day-hCG, with a non-significant increase after hCG administration. This trend is exactly opposite to that shown for serum E2 levels. Moreover, our study yielded no correlation of serum RBP-4 levels with serum estradiol level nor other IVF treatment variables (duration of stimulation, amount of gonadotropins used, number of oocytes retrieved, or fertilization rate) or pregnancy outcome. We therefore conclude that serum RBP-4 might not help in choosing the proper approach to COH nor aid fertility specialists and their PCOS patients in the decision-making process.

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