

Comparative Study of Retinol Binding Protein 4 Between Septic and non Septic Diabetic Patients

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Abstract: Retinol-binding protein 4 (RBP4) is a small visceral protein, mainly synthesized in the liver and catabolized in the kidneys. Recently discussed as a new adipokine that is possibly linked to insulin resistance and type 2 diabetes. The aim of this work was to investigate serum levels of RBP4 in patients with type 2 diabetes with and without sepsis and to determine its relation with insulin resistance. This study included 50 patients had Type 2 Diabetes mellitus, 25 diabetic patients without sepsis (group I) and 25 diabetic patients with sepsis (group II) and 25 healthy control subjects (group III). All patients were subjected to clinical examination and laboratory investigation including fasting blood glucose, HbA1C, lipid profile, urea, creatinine, insulin level and serum level of RBP4. There was significant increase in RBP4, in diabetic patients without sepsis and significant decrease in RBP4, in diabetic patients with sepsis, compared to control group ($p < 0.05$) and highly significant increase in RBP4, in diabetic patients without sepsis, compared to diabetic patients with sepsis ($p < 0.001$). Also there was significant increase in serum insulin levels in diabetic patients without sepsis and in diabetic patients with sepsis, compared to control group ($p < 0.05$). Regression analysis was done and showed negative significant correlation between RBP4 and insulin level in diabetic patients without sepsis ($r = -0.56$, $p < 0.05$) and no correlation between RBP4 and blood glucose, BMI and HOMA-IR. But in diabetic patients with sepsis there was negative significant correlation between RBP4 and fasting blood glucose ($r = -0.59$, $p < 0.05$) and no correlation with insulin level, BMI and HOMA-IR. Among all diabetic patients there was negative significant correlation between RBP4 and insulin level ($r = -0.40$, $p < 0.05$). Our finding suggests that increase RBP4 in diabetic patients without infection and decrease in diabetic with sepsis can be used as a biomarker to monitor infection, as its increase in diabetic patients without sepsis and decrease in diabetic patients with sepsis, but may not play a role in insulin resistance in T2DM.

Key words: Retinol-Binding Protein 4(RBP4) • Type 2 Diabetes with Sepsis and non sepsis • BMI

INTRODUCTION

Retinol binding protein 4 (RBP 4) is regarded as a novel cardiometabolic risk factor, which is secreted mainly by the hepatocytes and also by the adipose tissue. RBP 4 has been shown to induce insulin resistance and plasma RBP 4 values are increased in type 2 diabetes mellitus, obesity, metabolic syndrome and cardiovascular disease [1]. Moreover, it has been found that circulating RBP 4 decreases during medical interventions that result in amelioration of the metabolic profile, such as diet, exercise, oral antidiabetic drugs and hypolipidemic agents. Importantly, circulating RBP 4 is influenced by some nonmetabolic conditions, such as renal failure, acute

illness, injury and liver failure [2]. Type 2 diabetes is caused by resistance to insulin action in multiple tissues, accompanied by failure of the pancreatic beta cells to compensate sufficiently by increased insulin secretion. Measurement of insulin resistance provides an early and strong predictor of type 2 diabetes. Even in the absence of hyperglycemia or diabetes, insulin resistance constitutes an important risk factor for cardiovascular disease and early death [3]. Obesity, which has reached epidemic proportions worldwide, is a major cause of insulin resistance [4]. However, insulin resistance does not develop in all obese persons and genetic background contributes strongly to insulin resistance, even in non obese persons [5]. Retinol-binding protein-4 (RBP4) is an

adipokine that may play a role in regulating glucose metabolism and insulin sensitivity. Over expression of RBP4 induces systemic insulin resistance, whereas genetic disruption of RBP4 increases insulin sensitivity [6]. Several studies have demonstrated that circulating RBP4 concentration is elevated in humans with insulin resistance [7, 8]. In contrast, other studies have shown normal serum RBP4 concentration in obese menopausal women and low concentration in individuals with type 2 diabetes mellitus and that RBP4 concentration are unrelated to insulin sensitivity in calorie- restricted obese individuals [9].

The aim of the work was to investigate serum levels of RBP4 in patients with type 2 diabetes with and without sepsis and determine its relation with insulin resistance.

Patients and Methods: The present work was carried out on Type 2 diabetic patients attending to Internal Medicine Department, Al-zahra University Hospital, Egypt, they were 30 female and 20 males, their mean age was (47.9 ±8.6 years). Subjects were divided into 3 groups:

Group I: Included 25 diabetic patients without sepsis, 16 females and 9 males. Their age ranged from (33-57years) and mean of (44.4 ±6.4) and their mean BMI was (30.1±3.8)

Group II: Included 25 diabetic patients with sepsis (pulmonary infection, diabetic foot and skin infection), 14 females and 11 males. Their age ranged from (42-68years) and mean of (50.5 ±9), their mean BMI was (33.6±3)

Group III: Control group: 25 healthy control subject 15 females and 10 males. Their age ranged from (22-50years) and mean of (38.9 ±7), their mean BMI was (23.4±1.3). All participant gave their approval to participate in the study and informed consent was obtained from each subject in the study. The study was approved by medical the ethical committee.

All Patients and Controls Were Submitted to the Following:

- A-Full history and physical examination, fundus examination, BMI calculated by dividing the subject weight by square height (BMI=kg/m²)
- Routine laboratory investigations including :
 - Complete Kx-21-Japan) blood counts (CBC) using fully automated cell counter (Sysmex

- FBS, Lipid profile, AST, ALT, protein, albumine, total Bil. and ALP, blood urea, serum creatinine. All of them were measured on Hitachi 911 autoanalyzer using Rouche reagent kits

- HbA1C

- Abdominal ultrasonography
- Fasting serum insulin was determined using chemiluminescent immunoassay an immulite2000 analyzer (Siemens Health Diagnostic Inc, west sacramento, USA). Insulin resistance was calculated as HOMA-IR using the following equation: HOMA-IR=fasting blood glucose (mmol/l) x fasting serum insulin(mIU/ml) (Wallace, 2004)
- Fasting serum RBP4 was determined using ELISA a complete set of ELISAreader model SLT Spectra, Human RBP4 ELISA kit supplied by RandD Systems (USA). Patients who were renal failure, cardiac failure and carcinoma elsewhere were excluded from the study.

Statistical Analysis: Results were tabulated and statistically analyzed through Acer computer. The value were given as mean±SD. Comparison of the value were performed using the unpaired student T test and on tailed p value is considered significant when it is <0.05. Pearson correlates different quantitative variables.

RESULTS

Baseline clinical and laboratory data of the studied three groups were illustrated in Tables 1 and 2 and Fig. 1 and 2. There was statistically significant increase in RBP4, in diabetic patients without sepsis, compared to control group (P<0.05) and statistically significant decrease in RBP4, in diabetic patients with sepsis when compared to control group (p<0.05) and highly significant increase in RBP4, in diabetic patients without sepsis compared to diabetic patients with sepsis (p<0.001). Also there was significant increase in serum insulin levels in diabetic patients without sepsis and in diabetic patients with sepsis, compared to control group (p<0.05) with no significant difference relation among diabetic groups. Regression analysis was done and showed negative significant correlation between RBP4 and insulin level in diabetic patients without sepsis (r=-0.56, p<0.05) (Fig. 3) and no correlation between RBP4 and blood glucose, BMI and HOMA-IR. But in diabetic patients with sepsis there was negative significant correlation between RBP4 and fasting blood glucose (r=-0.59, p<0.05) (Fig 4) and no

Table 1: Clinical and laboratory data of the studied groups

Parameter	Group I DM patients without sepsis N=25 mean±SD	Group II DM patients with sepsis N=25 mean±SD	Group III control N=25 mean±SD
Age (years)	44.4±6.4	51.5±9	38.9±7.5
Sex (m/f)	16/9	14/11	15/10
BMI(Kg/m ²)	30.1±3.8	33.6±3	23.4±1.3
cholesterol (mg/dl)	190.6±23	204.9±33.7	120.9±26.5
triglycerides (mg/dl)	137.9±38.9	153.7±48.5	113.4±27.2
LDL(mg/dl)	110.5±20.2	115.6±22.9	92.5±12.3
HDL(mg/dl)	39.37±4.4	36.6±4.4	47.3±5.7
urea(mg/dl)	48.5±14.5	54.8±10.1	30.4±6.9
creatinin(mg/dl)	0.86±0.19	1±0.15	0.8±0.1
FBS(mg/dl)	104.8±6.7	299.3±42.6	87.5±7.7
Insulin (mIU/ml)	28.4±5.8	32.3±5.1	11.4±1.3
HbA1C	6.5±0.4	9±1.3	4±0.6
HOMA-IR	163.6±39.8	533.8±105.4	55.2±8.4
RBP4 (ng/ml)	136.5±34.6	41.8±4.4	78.8±38

Table 2: Comparison of (mean±SD) value level of blood Insulin and RBP4 in different studied groups

Parameter	Group I mean±SD	Group II mean±SD	Group III mean±SD	Group I vs Group III (P value)	Group II vs Group III (P value)	Group I vs Group II (P value)
Insulin	28.4±5.8	32.3±5.1	11.4±1.3	P<0.05	P <0.05	NS
RBP4	136.5±34.6	41.8±4.4	78.8±38	P <0.05	P <0.05	P <0.001

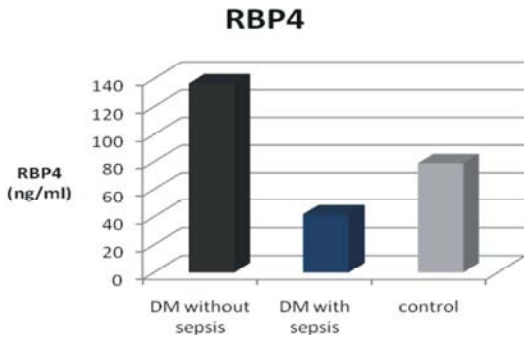


Fig. 1: Mean value of RBP4 among studied groups

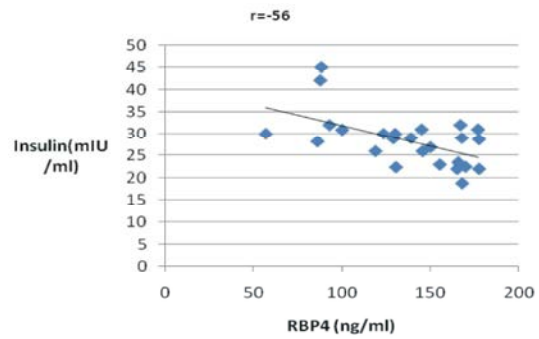


Fig. 3: Correlation between RBP4 and insulin levels in diabetic patients without sepsis (group I)

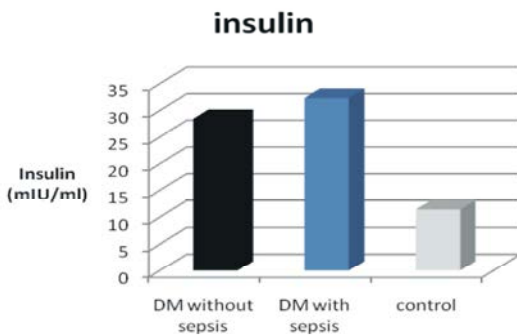


Fig. 2: Mean value of Insulin level among studied groups

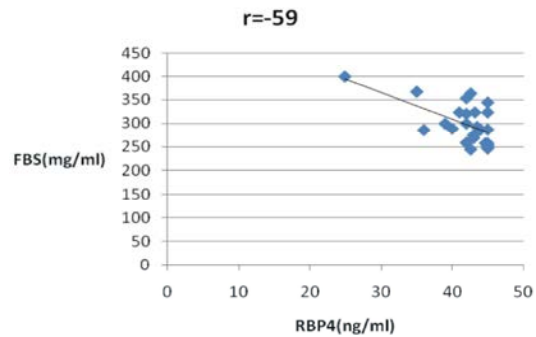


Fig. 4: Correlation between RBP4 and FBS among diabetic patients with sepsis (group II)

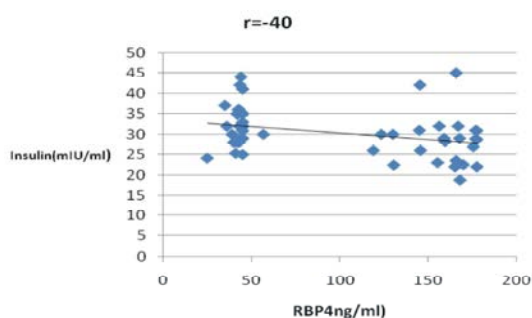


Fig. 5: Correlation between RBP4 and insulin levels among all diabetic patients

correlation with insulin level, BMI and HOMA-IR. Among all diabetic patients there was negative significant correlation between RBP4 and insulin level ($r=-0.40$, $p<0.05$) (Fig. 5).

DISCUSSION

Insulin resistance and beta cell failure are major components of the pathogenesis of type 2 diabetes. In the past years, it has become apparent that adipose tissue is an endocrine organ that secretes adipokines affecting insulin sensitivity [10]. In patients with obesity, metabolic syndrome and type 2 diabetes, several adipocytokines have been identified that mediate agonist and antagonist effects on insulin resistance. In these patients, chronic inflammatory conditions apparently promote adipocytic secretion of mediators of insulin resistance into the circulation, thereby providing a link between adipocytokines, inflammation and systemic insulin resistance [11]. Retinol-binding protein 4 (RBP4) was identified as a new adipokine that links glucose uptake in adipocytes to systemic insulin resistance [6]. Several studies demonstrated elevated RBP4 serum levels in subjects with obesity, insulin resistance, or type 2 diabetes [6, 7, 12]. In the present study serum level of RBP4 was measured in 25 diabetic patients without sepsis, 25 diabetic patients with sepsis in comparison to 25 healthy controls. Our results showed statistically significant increase in RBP4, in diabetic patients without sepsis, compared to control group. Our results are in agreement with those obtained by Cho *et al.* [13], who reported that plasma RBP4 concentrations were higher in the (Impaired glucose tolerance test) IGT and type 2 diabetic groups than in the normal glucose tolerance (NGT) group. Also our results are in agreement with those findings of Nehal *et al.* [14], Alberto *et al.* [15] and Graham *et al.* [7] that demonstrated elevated serum RBP4 levels in type 2 diabetic patients.

Also our result showed no correlation between serum RBP4 and blood glucose, BMI and in diabetic patients without sepsis. These results are in agreement with Stefan *et al.* [16], who reported that plasma RBP4 levels were not significantly associated with weight, total body fat, abdominal or visceral fat and FBS and 2-h glycemia. Also Nehal *et al.* [14] reported that no correlation between RBP4 and BMI, waist/hip ratio, but observed that serum RBP4 was significant correlation with FBS. Graham *et al.* [7] demonstrated that RBP4 levels correlated with total adiposity (body mass index, BMI), abdominal obesity and fasting insulin levels and were inversely proportionate to glucose disposal using euglycemic clamp studies, also RBP4 levels were increased in subjects with impaired glucose tolerance or diabetes relative to euglycemic controls and RBP4 serum levels fell in individuals responding to exercise training with improved insulin sensitivity. A recent study demonstrated an association of circulating RBP4 levels in serum with insulin resistance, however the mechanisms by which RBP4 induces insulin resistance are not well understood [14]. Our data demonstrated that significant increase in serum insulin levels in diabetic patients with and without sepsis, when compared to control group, but no statistically significant relation among diabetic groups. On the other hand, no significant correlation between RBP4 and HOMA-IR in diabetic patients without sepsis. Also in diabetic patients with sepsis no significant correlation with insulin level, BMI and HOMA-IR. But among all diabetic patients there was negative significant correlation between RBP4 and insulin level. Our study coincide with the study of Nehal *et al.* [14] who did not find any correlation between insulin sensitivity and serum RBP4 in obese diabetic patients. Consistent with previous studies which have shown that RBP4 concentration does not correlate with insulin resistance in individuals with (normal glucose tolerance test) NGT, under calorie-restricted diets, or with type 2 diabetes mellitus [17, 18]. In contrary, to Cho *et al.* [19], who reported that RBP4 concentration was positively correlated with systolic blood pressure, fasting insulin concentration, abdominal fat and HOMA-IR, which are markers of insulin resistance. Langouche *et al.* [20] showed that in obesity, even in the absence of diabetes, serum RBP4 was increased and in even slightly obese (overweight) subjects, serum RBP4 was proportional to BMI and insulin resistance. Also, Graham *et al.* [7] and Cho *et al.* [13] reported that serum RBP4 correlate positively with the presence of insulin resistance in individuals with obesity, impaired glucose tolerance or type 2 diabetes. Elevated serum RBP4 were an independent predictive biomarker at early stage of insulin resistance.

Recently, low level of serum RBP4 have been reported in critical ill patients with sepsis of pulmonary origin compared to non septic patients. However, remind unclear whether this observation was related to sepsis or whether it could be extrapolated to all critically ill patients. The present study showed statistically significant decrease in RBP4, in diabetic patients with sepsis when compared to control group and highly significant increase in RBP4, in diabetic patients without sepsis when compared to diabetic patients with sepsis. Langouche *et al.* [20] observed low circulating levels of RBP4 in critically ill patients in ICU with low values in patients with sepsis, but also in patients not critically ill under acute surgical stress. These observations would suggest an acute stress response. Also, Koch *et al.* [11] reported low circulating levels of RBP4 in patients with acute illness of respiratory etiology compared to healthy controls and hypothesized that acute decrease of RBP4 concentration could be explained by reduced synthesis or increased removal by extravasations due to capillary leakage. Also, reported that serum RBP4 levels were negatively correlated with markers of inflammation. This rise the possibility that RBP4 could be an important component of so called acute phase protein at least in patients with sepsis

CONCLUSION

Our finding suggests that increased in RBP4 in diabetic patients without sepsis and decrease in diabetic with sepsis may be used as biomarker to monitor of infection, but may not play a role in insulin resistance in T2DM.

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