

Association of Retinol Binding Protein- 4 (RBP4) with Glycemia, Dyslipidemia, Hypertension, and Obesity in Type 2 Diabetic Iraqi Patients

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Abstract

Retinol binding protein 4 (RBP4), an adipokine that participate in a lipid metabolism or insulin resistance through a complex regulatory network. Recently, RBP4 was reported to be associated with many cardiovascular diseases (CVDs) risk factors in type 2 diabetes mellitus (T2DM) patients. This study aims to study the correlation of serum RBP4 with some markers of glycemic control, dyslipidemia, hypertension, and obesity in T2DM Iraqi patients. One hundred twenty (120) T2DM patients were enrolled in this cross-sectional study.

Serum RBP4 levels are higher in T2DM patients with dyslipidemia, hypertension, or obesity. The difference was statistically non significant between hypertensive and normotensive T2DM patients. Serum RBP4 is positively correlated with body mass index, fasting blood glucose, glycosylated hemoglobin (HbA1c), systolic blood pressure ($P < 0.001$), and low density lipoprotein cholesterol ($P < 0.05$), and negatively correlated with high density lipoprotein cholesterol ($P < 0.001$).

Serum RBP4 is correlated with many risk factors of CVD including, poor glycemic indices, dyslipidemia, hypertension, and obesity, in T2DM Iraqi patients.

keywords: RBP4, Type 2 diabetes mellitus, Dyslipidemia, Hypertension, Obesity.

ارتباط البروتين الرابط للريتينول – ٤ مع جلوكوز الدم، شحوم الدم، ارتفاع ضغط الدم، والسمنة في مرضى السكري النوع الثاني في العراق
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الخلاصة

يشترك بروتين ربط الريتينول ٤ (RBP4) بشكل كبير في استقلاب الدهون أو مقاومة الأنسولين من خلال شبكة تنظيمية معقدة. حديثاً، التقارير العلمية تتحدث عن ارتباط RBP4 بالعديد من عوامل الخطر القلبية الوعائية لدى مرضى السكري من النوع ٢. تهدف هذه الدراسة إلى دراسة ارتباط المصل RBP4 مع بعض مؤشرات السيطرة على نسبة السكر في الدم، اضطراب نسبة الشحوم، وارتفاع ضغط الدم والسمنة لدى مرضى السكري العراقيين من النوع ٢.

تم تسجيل ما مجموعه ١٢٠ مشاركاً كانوا من مرضى السكري النوع ٢ في هذه الدراسة المستعرضة. مستويات RBP4 في الدم كانت عالية لدى مرضى السكري من النوع ٢ الذين يعانون اضطراب شحوم الدم، أو ارتفاع ضغط الدم، أو السمنة. الاختلاف لم يكن ذو دلالة إحصائية بين مرضى السكري المصابين ارتفاع ضغط الدم عن أولئك ذوو الضغط المنتظم. أظهر المصل RBP4 ارتباطاً إيجابياً بمؤشر كتلة الجسم، وجلوكوز الدم في الصيام، والهيموغلوبين السكري المتراكم، وضغط الدم الانقباضي ($P < 0.001$)، وكوليسترول البروتين الدهني ذو الكثافة المنخفضة ($P < 0.05$) وارتباطاً سلبياً مع كوليسترول البروتين الدهني ذو الكثافة العالية ($P < 0.001$).

يرتبط المصل RBP4 بالعديد من عوامل الخطر لأمراض القلب والأوعية الدموية كضعف مؤشرات تنظيم سكر الدم أو اضطراب الشحوم أو ارتفاع ضغط الدم أو السمنة في عينة من مرضى السكري من النوع ٢ في العراق.

الكلمات المفتاحية: RBP4، داء السكري من النوع ٢، اضطراب شحوم الدم، ارتفاع ضغط الدم، السمنة .

Introduction

Diabetes mellitus (DM) is a common endocrinopathy which is usually presented as a set

of metabolic defects characterized mainly by hyperglycemia as a result of impairment in insulin sensitivity and/or secretion⁽¹⁾. Long-term

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hyperglycemia causes damage and dysfunction of multiple organs; including kidneys, heart, eyes, nerves, and blood vessels. The cardiovascular diseases (CVDs) are the main cause of morbidity and mortality in DM patients ⁽²⁾, as indicated by the intensive regulation of glucose and lipid levels, in addition to blood pressure and body weight are essential to reduce the hazard of cardiovascular complications and diabetes advancement ⁽³⁾.

Type 2 diabetes (T2DM), hypertension, and dyslipidemia can occur comorbidly and all participate in the development of atherosclerosis ⁽⁴⁾, an important pathological hallmark in the cardiovascular diseases (CVDs). Obesity is common in about 90–95% of adult type 2 DM patients ⁽⁵⁾. It is manifested by low-grade chronic inflammation with increased systemic adrenergic activity, and perturbed metabolism of lipid and glucose ⁽⁶⁾.

Retinol binding protein 4 (RBP4) is an adipokine, synthesized primarily by the liver; a smaller amount is synthesized in the adipose tissue ⁽⁷⁾. Increased systemic RBP4 concentrations can be seen in conditions of impaired glucose tolerance, T2DM, and obesity. The link of RBP4 with insulin resistance (IR) shows certain degree of controversy ^(8, 9). In the liver, RBP4 increases fasting blood glucose by augmenting the action of phosphoenolpyruvate carboxykinase (PEPCK) enzyme increasing hepatic glucose liberation. In skeletal muscle and white adipose tissue, RBP4 decreases the insulin receptors, as well as the alterations in phosphorylation of some signal proteins, such as insulin receptor substrate (IRS), PDK1 and Akt ^(10, 11).

Apart from its effects on glucose metabolism, RBP4 also affects lipid metabolism. Graham *et al.* described that high RBP4 levels were associated with high serum triacylglycerol (TG) and low high-density lipoprotein cholesterol (HDL-C) levels in lean, obese, and in diabetic subjects ⁽¹²⁾. In a study conducted on Chinese obese subjects, Wang *et al.* showed that RBP4 affects lipid metabolism in gender dependent manner. He also reported that high serum RBP4 correlates with high total cholesterol (TC) and TG levels in Chinese obese female subjects. Also, females with high serum TG or TC levels have higher serum RBP4 levels ⁽¹³⁾. Moreover, genetic variant of Apolipoprotein A5 (APOA5 gene) enhances the association of serum levels of RBP4 and TG in T2DM patients ⁽¹⁴⁾.

The aim of this work was to study the associations between circulatory RBP4 and different risk factors for cardiovascular diseases in a sample of Iraqi T2DM patients.

Subjects and Methods

One hundred twenty (120) T2DM patients were enrolled in this cross sectional study, which was conducted in The Specialized Center of Endocrinology and Diabetes in AL-Nasiriya city, south of Iraq from 4th October 2019 to 30th March

2020. T2DM patients were already diagnosed according to the American Diabetes Association Criteria ⁽¹⁵⁾; of age ≥ 18 years with mean age of them was (50 ± 8) years, ranging from 33 to 69 years, and fifty-two of the diabetic subjects were males and 68 were females. with disease duration since diagnosis of at least 1 year.

Pregnant women, patients with chronic renal failure and hepatic failure were excluded in this study.

This work was performed according to the Helsinki II Declaration ⁽¹⁶⁾ and approved by the Ethics Committee of the College of Pharmacy, University of Baghdad. All participants were informed about the purpose and the expected benefits of the study before agreement of participation was documented.

Sociodemographic and medical histories were taken for each participant; venous blood samples were collected after fasting for 12 hours. Ten milliliters blood samples were withdrawn, about (2 ml) of each sample was transferred into ethylene diamine tetracetic acid (EDTA) tubes to be stored at $(+2$ to $+8^\circ\text{C})$ for analysis of HbA1c within 1 week by high-performance liquid chromatography ⁽¹⁷⁾. The remaining (8 ml) samples were transferred into plane tubes and centrifuged for 10 minutes to obtain serum. The collected sera were stored frozen at (-20°C) as aliquots in Eppendorf tubes until analysis. Fasting blood glucose (FBG) and lipid profile were analyzed using suitable colorimetric assays ⁽¹⁸⁻²²⁾. The assay of serum RBP4 was performed by commercially available human enzyme-linked immunosorbent assay (ELISA) kit ⁽²³⁾.

Statistical analysis

The statistical analysis was achieved using SPSS® v.25. Data are presented as mean \pm standard error. Student t-test was used to compare means of two groups; while, analysis of variance (ANOVA) test was used to compare means of more than two groups, which is followed by post hoc analysis using Tukey's test to describe differences among different groups. Categorical variables were expressed as number and tested using chi-square (χ^2) test. The correlation between different parameters and RBP4 was tested using Pearson's correlation. Level of significance is set to $P < 0.05$.

Results

Based on the dyslipidemia status, T2DM patients were grouped as dyslipidemic ($n=66$), and normolipidemic ($n=54$); according to "The American Association of Clinical Endocrinologists' (AACE)" 2017⁽²⁴⁾. Sociodemographic and clinical characterizes for each group are presented in table-1. There difference between the dyslipidemic and normolipidemic T2DM patients concerning gender, body mass index (BMI), smoking habit, diastolic blood pressure (DBP), and high density lipoproteins cholesterol (HDL-C) was non-significant ($P > 0.05$). Meanwhile, age, disease duration, systolic blood

pressure (SBP), serum RBP4, fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), and low density lipoproteins cholesterol (LDL-C) were

significantly higher in diabetic patients with dyslipidemia ($P<0.05$).

Table- 1. Characteristics of dyslipidemic and normolipidemic T2DM patients

Variable	T2DM		P-value
	Dyslipidemic (n=66)	Normolipidemic (n=54)	
Age(years)	51.6±0.9	48±1	0.01*
Gender (M/F)	30/36	22/32	0.6
BMI(kg/m ²)	29.8±0.4	28.9±0.6	0.2
Disease Duration (years)	11±0.5	6±0.6	0.0001*
Smokers number	23	18	0.8
SBP (mmHg)	135.8±2	129±2	0.04*
DBP (mmHg)	78.7±1	77.9±1	0.7
S.RBP4(pg/ml)	519.9±23	377.5±19	0.0001*
FBG (mg/dl)	218.3±7	172.2±7	0.0001*
HbA1c%	9.6±0.2	8.2±0.2	0.0001*
VLDL-C(mg/dl)	43.4±2.6	224.8±1	0.0001*
LDL-C (mg/dl)	108.7±6	90.4±4	0.018
HDL-C (mg/dl)	42.6±1	46.1±1	0.06

* Significant when $p<0.05$

In table-2, T2DM patients were grouped according to the prevalence of hypertension, diagnosed according to “The World Health Organization International Society of Hypertension Guidelines”⁽²⁵⁾. There difference between the hypertensive and normotensive T2DM patients

concerning gender, smoking habit, serum RBP4, FBG, HbA1c%, and lipid profile parameters was nonsignificant ($P>0.05$). Meanwhile, age, disease duration, and BMI were significantly higher in hypertensive T2DM patients ($P<0.05$).

Table- 2. Characteristics of hypertensive and normotensive T2DM patients

Variable	T2DM		P-value
	Hypertensive (BP>140/90 mmHg) (n=30)	Normotensive (BP≤140/90 mmHg) (n=90)	
Age (years)	54±1.3	48±0.8	0.001*
Gender (M/F)	15/15	37/53	0.4
BMI (kg/m ²)	30.7±0.7	28.9±0.4	0.03*
Disease Duration (years)	11±1	8±0.4	0.001*
Smokers number	13	28	0.2
S.RBP4 (pg/ml)	449±28	413.7±18	0.12
FBG (mg/dl)	204±11	195.2±6	0.5
HbA1c%	9.3±0.2	8.9±0.19	0.3
TG (mg/dl)	165±19	178±9	0.5
TC (mg/dl)	169±9	183±4	0.19
VLDL-C (mg/dl)	33±3	35±2	0.5
LDL-C (mg/dl)	93±8	102±4	0.3
HDL-C (mg/dl)	43.6±1.6	44.1±1	0.4

In table-3, T2DM patients were categorized into three groups, depending on BMI, into: normal weight, overweight and obese; non of the participant was underweight⁽²⁶⁾. Serum RBP4 levels were significantly higher in obese T2DM patients than in normal weight patients ($p=0.0001$). Similarly, diabetes duration, systolic BP, and the

diastolic BP were significantly higher and serum HDL-C levels were significantly lower in obese T2DM patients compared to the normal weight patients ($P<0.05$). HbA1c was significantly higher in obese T2DM patients compared to normal weight and overweight patients ($P<0.05$). There was no significant difference in age, gender, smoking habit,

FBG, total cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL-C) and LDL-C

among obese, overweight and normal weight T2DM patients.

Table-3. Characteristics of obese, overweight, and normoweight T2DM patients

Variable	T2DM			p-value
	Obese BMI>30 n=54	Overweight BMI(25-30) n=46	Normoweight BMI(18.5-24.9) n=20	
Age (years)	49±1	51±1	49±2	0.5
Gender (M/F)	25/29	21/25	6/14	0.4
Disease Duration (years)	10±0.7 ^a	8.9±0.6 ^{ab}	5±0.8 ^b	0.001*
Smokers number	20	17	4	0.3
SBP (mmHg)	135±2 ^a	134±2 ^{ab}	121±2 ^b	0.01*
DBP (mmHg)	80±1 ^a	77±1 ^{ab}	74±2 ^b	0.03*
S.RBP4 (pg/ml)	499.5±21 ^a	453.8±21 ^{ab}	311.3±29 ^b	0.0001*
FBS (mg/dl)	196±5	196±8	171±15	0.5
HbA1c%	9.5±0.2 ^a	8.7±0.2 ^b	8±0.4 ^c	0.007*
TG (mg/dl)	184±14	178±12	142±15	0.2
TC (mg/dl)	185±7	178±6	167±10	0.3
VLDL-C (mg/dl)	36±2	35±2	28±3	0.24
LDL-C (mg/dl)	106±6	98±5	89±9	0.3
HDL-C (mg/dl)	42±1 ^b	44±1 ^{ab}	49±2 ^a	0.02*

superscripts (a,b,c) among different groups are considered significantly different ($P<0.05$)

Correlation studies of serum RBP4 with the studied variables of T2DM patients are presented in table- 4. Serum RBP4 was positively correlated with BMI, FBG, HbA1c, and SBP ($P<0.001$), and LDL-C ($P<0.05$) and negatively correlated with HDL-C

($P<0.001$). While, there was no significant correlation between serum RBP4 levels and age, gender, DBP, or TG, VLDL-c, and TC levels ($P>0.05$). Strong positive correlation between serum RBP4 levels and diabetes duration in years was also recorded ($P < 0.001$).

Table- 4. Pearson's correlations of serum RBP4 levels with the studied variables

Variable	r-value	P-value
Age	0.14	0.13
Gender	-0.1	0.15
Disease duration	0.5	0.000*
BMI	0.31	0.001*
SBP	0.2	0.02*
DBP	0.12	0.17
FBG	0.6	0.000*
HbA1c	0.4	0.000*
TC	0.17	0.06
TG	0.12	0.17
VLDL-C	0.12	0.17
LDL-C	0.25	0.008*
HDL-C	-0.45	0.000*

Discussion

In the present study, serum RBP4 has significant positive correlation with markers of glycemic control, FBG and HbA1c (table 4). Large mass of evidence suggest an important role played by RBP4 in the development of T2DM and its chronic complications, as well as, with biochemical markers of carbohydrate metabolism. Elevated serum RBP4 levels associate with an augmented risk

of transferring impaired glucose tolerance into overt T2DM⁽²⁷⁾. In addition, it is associated with IR in T2DM patients^(28, 29). Moreover, RBP4 expression is elevated in T2DM patients with retinopathy⁽³⁰⁾, nephropathy⁽³¹⁾ and CVD⁽³²⁾. Graham *et al.* reported positive correlation of serum RBP4 levels with FBG and with HbA1c levels⁽¹²⁾. However, serum RBP4 level and RBP4 synthesis speed were reported to be diminished in type 1 DM patients

compared to control individuals^(33, 34). As stated earlier, RBP4 participates in IR by increasing the hepatic liberation of glucose, an action mediated by augmenting PEPCK, and by decreasing the insulin receptors expression and interfering with intracellular insulin signaling in muscle and adipose tissue^(10, 11).

Unique pattern of dyslipidemia occurs in T2DM patients, characterized by hypertriglyceridemia, high blood levels of Apolipoprotein B and of small dense LDL (sdLDL), with low HDL-C levels. Serum TC levels are high but not to the levels of TG⁽³⁵⁾. Alterations in lipid profile in DM are attributed to increased lipolysis secondary to insulin resistance, and hence, there is an inappropriate spillover of TG and free fatty acids (FFAs)⁽³⁶⁾.

In the present study, Serum RBP4 levels in dyslipidemic diabetic patients were higher than in normolipidemic patients. Dyslipidemic T2DM patients were older with longer diabetes duration, higher systolic blood pressure and poorer glycemic control as compared with normolipidemic T2DM patients (Table-1). Moreover, serum RBP4 positively correlated with LDL-c level and negatively correlated with HDL-C levels, but there was no significant correlation with TG and VLDL-C levels (Table- 4). This may be explained by the use of antilipidemic medicines by most of T2DM patients that may disguised the actual relationship between serum RBP4 and lipid profile.

Mounting data proposes that RBP4 contributes in lipid metabolism by means other than its role in IR. Many clinical studies showed that serum RBP4 levels correlate positively with blood TG levels, but not with IR

⁽³⁷⁻³⁹⁾. However, some studies that examined the link between serum levels of RBP4 and TG have showed an association with IR^(40, 41). A positive association between serum levels of RBP4 and atherogenic lipoproteins in T2DM patients has been reported previously^(37, 42, 43). Yamasaki *et al.* proposed that RBP4 may interfere with lipoprotein remnant metabolism⁽⁴²⁾. While, Vergès *et al.* showed that RBP4 may reduce the catabolic rate of VLDL⁽³⁷⁾.

Many studies showed a close association between serum RBP4 levels and blood pressure in prehypertensive or in untreated essential hypertension patients^(44, 45). In the present study, the difference in serum RBP4 levels between hypertensive and normotensive T2DM patients was statistically nonsignificant (table-3). Yet, serum RBP levels correlate positively with the SBP but not the DBP levels (table -4).

High prevalence of hypertension in type 1 or 2 DM patients, is well established^(46, 47). Actually, about 40% of T2DM patients are already hypertensive when diagnosed as having DM⁽⁴⁸⁾. Ephraim *et al.* reported that 37.4% of T2DM patients have systolic, but not diastolic, hypertension; and hyperglycemia in those patients was worse than in normotensive

T2DM patients⁽⁴⁹⁾. Hyperglycemia aids in vascular stiffness and raised vascular tonicity resulting in increased SBP⁽⁵⁰⁾. Arterial stiffness develops mainly as a consequence of aging⁽⁵¹⁾. Pinto *et al.* has reported an increase in SBP in hypertensive patients, aged 50 years or older, arising either as primary event or developed after prolonged period of systolic-diastolic hypertension whether on antihypertensive treatment or not⁽⁴⁶⁾. Still, progression of arterial stiffness can be hastened if it is co-occupied by additional risk factors, particularly elevated blood glucose levels,^(52, 53). Hyperglycemia, enhance development of arterial stiffness via several mechanisms that lead to endothelial dysfunction along with structural modifications of vascular extracellular matrix^(54, 55).

In the present study, obese T2DM patients have elevated serum levels of RBP4 when compared to normoweight T2DM patients. Furthermore, obese T2DM patients have longer diabetes duration, and higher SBP, DBP and HbA1c levels, when compared to normoweight T2DM patients. On the contrary, HDL-C levels in obese T2DM patients are lower than that of normoweight T2DM patients (Table- 3). Additionally, serum RBP4 levels show significant positive correlation with BMI (tTable-4). As RBP4 is an adipokine that is likely relating obesity, IR and T2DM^(12, 56-58). Graham *et al.* reported a positive correlation between serum RBP4 and BMI or waist-to-hip ratio; and normoweight individuals with IR also showed elevated levels of serum RBP4 indicating that IR was independent of obesity⁽¹²⁾. In severely obese patients, expression of RBP4 is enhanced in the omental visceral fat⁽⁵⁹⁾. Significant reduction in body weight, by dietary restrictions, physical workout, or by surgical interventions, leads to a reduction in RBP4 in blood and/or in adipose tissue^(12, 60-62). The decrease in RBP4 in non-diabetic individuals, accompanying bodyweight reduction, positively associate with the reduction of fat content in the omental or mesenteric regions and not with the reduction in the whole body fat⁽⁶¹⁾.

Several gene variants of *RBP4* were associated with the level of adiposity and central obesity characterized by elevated BMI and waist-to-hip ratio⁽⁶³⁾. A regulatory single-nucleotide polymorphism (SNP) in *its* gene enhanced RBP4 expression with predisposition for obesity, by increasing lipogenesis⁽⁵⁶⁾. In addition, diabetes associated RBP4 haplotype carriers have been reported to overexpress visceral *RBP4*⁽⁶³⁾.

Conclusion

Serum RBP4 correlates with different risk factors of cardiovascular diseases; including glycemic control, dyslipidemia, hypertension and obesity, in T2DM Iraqi patients

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