

Relationship of Adipocyte binding protein with insulin resistance in Type II Diabetes mellitus

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Abstract

Background: Adipocyte binding protein (ABP) is an adipokine hormone, although its association with insulin resistance remains elusive, and the underlying mechanism are also unknown. The aim of the present study is to investigate the possible association between RBP4 with blood glucose, glycated hemoglobin (HbA1C) in diabetes mellitus (DM) with insulin resistance. **Methods:** in this study 45 apparently healthy subjects in both sexes their ages were 50-70 years and 44 subjects with DM type II were taken. Anthropometric parameters (weight, height, body mass index (BMI) and waist circumference (WC)) were measured. Furthermore, serum RBP4, homeostatic association model of insulin resistance (HOMA IR), homeostatic association model of b-cell function (HOMA B), blood glucose, HbA1C, and liver function tests were measured. **Results:** serum RBP4 was significantly reduced in type II DM compared with the control group, and according to the receiver operating characteristic (ROC) curve, the RBP4 could be representing as biomarker for DM type II. The WC in DM type II was lower than non-diabetic subjects. On the other hand, the BMI were not differ between two studied groups. Interestingly, the result showed that HOMA IR and HbA1C were markedly increased in diabetic subjects as compared to the non-diabetic group, and their ROC were 0.8 and 0.96, respectively, which means a very good indicator marker for severity of DM. In addition, serum alanine aminotransferase (ALT) and alkaline phosphates (ALP) significantly increased in type II DM as compared with the control group. However, aspartate aminotransferase (AST) represented as an excellent biomarker for DM complications but with no change. Statistical analysis showed that RBP4 was negatively correlated with the blood glucose, HbA1C, HOMA IR and HOMA B. **Conclusions:** The current study, and for the first time the obtained result suggested that serum RBP4 could be represented as a new biomarker for DM type II because it is negatively associated with the insulin resistance markers.

Keyword: ABP, diabetes mellitus, insulin resistance, HbA1C.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic condition caused by a reduction in insulin action and/or secretion in the body. With the evolution of the disease, pathological alterations such as nephropathy, retinopathy, and cardiovascular problems eventually emerge in the body (Padhi et al., 2020). Dyslipidemia is common in diabetes mellitus because both insulin deficiency and resistance affect lipid metabolism enzymes and pathways. Lipid abnormalities in DM type II include elevated triglyceride (TG) levels, decreased athero-protective high density lipoprotein-cholesterol (HDL) levels, and increased levels of low density lipoprotein-cholesterol (LDL) (Gordon et al., 2010).

Because the liver is important in the metabolism of glucose in our bodies, DM type II may be an epiphenomenon of hepatic diseases such as liver cirrhosis, liver failure, fatty liver, chronic hepatitis, and hepatocellular carcinoma. According to some reports, extensive changes in enzyme structures at the molecular level in diabetic patients may cause liver function damage and thus accelerate hepatic cancer (Mukherjee et al., 2015).

DM is a metabolic disease that causes renal failure, and renal failure increases diabetic patients' requirement for insulin. In patients with chronic renal failure (CRF), the accumulation of uremic toxins and increased parathyroid hormone levels cause insulin resistance in tissues, particularly skeletal muscle tissues. This has been linked to damage caused by insulin binding to its receptors, which disrupts glucose metabolism and glycogen production (Nasri and Rafieian-Kopaei, 2015).

Obesity, particularly abdominal obesity, has been linked to insulin resistance and DM type II. The Body Mass Index (BMI),

defined as the weight in kilograms divided by the height in meters squared, the Waist to Hip Ratio (WHR), and the Waist Circumference (WC) are the three main anthropometrics parameters used to assess body fat and fat distribution in adults. And these parameters are ethnically susceptible. BMI and WHR were found to be predictors of DM type II outcome by some researchers. On the other hand, WC was a better predictor of DM type II and was more strongly correlated with intra-abdominal fat than WHR (Lotfi et al., 2014)

The main retinol transporter in plasma is retinol binding protein 4 (RBP4), a 21-kDa lipocalin protein that belongs to the lipocalin family. It is encoded by the RBP4 gene, which is found on chromosome 10q23-q24 and has been linked to an increased risk of type II diabetes (Yokomizo et al., 2019).

Circulating RBP4 levels were significantly raised in obese and diabetic murine models, as well as in people, according to cross-sectional investigations. Furthermore, genetic studies show that RBP4 has an inductive role in the development of DM type II, with a gain-of function human nucleotide polymorphism in the RBP4 promoter linked to an elevated risk of the disease (Perduca et al., 2018). A new prospective cohort study is being conducted. Insulin resistance and pancreatic b-cell dysfunction are two characteristics of type II diabetes. Previous research indicated that raising RBP4 levels in mice via genetic or pharmacologic means resulted in insulin resistance, whereas reducing RBP4 levels significantly enhanced insulin sensitivity. These connections, however, are not corroborated by all human investigations, as several studies revealed no correlation (Wessel et al., 2019). Despite the fact that insulin resistance is a well-known pathophysiological hallmark of type II diabetes, pancreatic b-cell failure appears to be the most common cause of the shift from a specific metabolic dysfunctional state to DM type II. However, little is currently known about the association of RBP4 levels with pancreatic b-cell function (Huang et al., 2020).

The aim of the present study is to find out the association between RBP4 with insulin resistance in type II diabetes mellitus, to be a key driver in enhancing the lives of people living with diabetes.

STUDY DESIGN AND SUBJECTS

The study was performed in 90 subjects, 43 subjects of them represent as non-diabetes, obtained from local community considered as control group, other 47 subjects whom with DM type II disease were visited Nafie Akray clinical center for diabetic consultation, Erbil, Iraq. The subjects were 30 men and 60 women whose mean age was 65 years ranged between (52-73 years). Those with the medical illness such as cancer, hormonal and infectious diseases were excluded from the enrollment. Also, serum did not obtain from smokers and non-fasting subjects.

Height, weight, BMI, waist circumference, systolic and diastolic blood pressure were measured for all subjects.

Blood sample collection

Five milliliters (ml) of venous blood from the subjects were drawn by using disposable plastic syringe in the morning and transferred into gel and clot activator test tube, then the blood was allowed to clot and centrifuged at 1000g for 15 minutes. The sera were stored at -80°C until analysis.

Biochemical analyses

The fasting blood sampling was done. Fasting blood sugar, HbA1C and liver function test were measured in serum by using Cobas E411.

Hormonal assay

Also, the hormonal assay RBP-4 and insulin were performed by ELISA. Insulin resistance was evaluated by the homeostatic model assessment of insulin resistance (HOMA-IR).

$HOMA-IR = (\text{fasting insulin } [\mu\text{IU/mL}] \times \text{fasting glucose } [\text{mg/dL}]) / 405$.

Statistical analysis

The data of the obtained results were expressed as a median (interquartile range, IQR). By using GraphPad Prism 7 (San Diego, CA, USA), a Mann-Whitney non-parametric t-test was applied to compare all the studied variables between non-DM controls and T2DM patients. The receiver operating characteristic (ROC) curve was created to compare the sensitivity and specificity of variables in both control and patient groups.

Non-parametric Spearman correlations were used to analyze the correlation coefficient of between RBP-4 hormone as dependent variable and blood glucose, HbA1C and HOMA-IR as independent variables. A p-value <0.05 was considered statistically significant.

RESULTS AND DISCUSSIONS

The RBP-4 now has been considered as a new adipocytokine involved in carbohydrate and lipid metabolism. As shown in the table (1) RBP-4 was markedly increased in DM type II group as compared with the control group. Little studies are present till now to explain how RBP-4 levels changes in DM type II (Larsson et al., 2017). However, previous study concluded that from the beginning of DM both increased and decreased levels of RBP4 occur (Kwanbunjan et al., 2018), one possible reason for elevation of RBP-4 levels is that this hormone release from adipocyte, and hence the DM change the fat cells because of reduce insulin receptor activity. Furthermore, RBP-4 represent as an important adipocyte-derived cytokine which has a role in the energy metabolism regulation and insulin sensitivity. (Huang et al., 2015) recorded that RBP4 levels in the maternal circulation were markedly higher in gestational diabetes than in the control subjects. Therefore, further studies are needed to explain exact mechanism by which RBP-4 level tended to rise in DM type II.

As there is evidence that the fibrosis of the hepatic is the crucial for hepatitis C patients when liver disease progression monitored and the hormone RBP4 is secreted by the hepatocytes which is strongly may deteriorated in health and diseases (Fayed et al., 2020). They concluded that RBP4 to be in performance diagnostic in predicting liver fibrosis. Hence, RBP4 secreted by the hepatocytes and adipose tissue which associated with non-alcoholic fatty liver disease.

As seen in the table (1) Serum ALT and ALP activities were markedly elevated in DM type II group compared with non-diabetic group. These two enzymes represent as biomarkers for liver injury (McGill, 2016). Hyperglycemia and HbA1C rises in DM type II (Forbes et al., 2018), interestingly the level of oxidative stress was increases in diabetic group due to the high glucose level. An increase in reactive oxygen species (ROS) is the most causes of tissue destruction especially the liver (Qiu et al., 2019). On the other hand, the AST activity was also increases and the value of ROC curve was elevated as a marker of tissue injuries (Prieto and Monsalve, 2017).

In the current study, the HOMA-IR value was significantly increased in DM type II as compared with the control group. An increase in both HOMA-IR and WC were representing as risk factor for DM type II. Frequently, the insulin receptor reduces its sensitivity or increase in its resistance which mostly can be detected by HOMA-IR level and ROC curves as shown in table (3). Furthermore, there is no evidence that WC is representing a powerful risk factor for DM type II rather than BMI (Larsson et al., 2017).

Table (1): Metabolic characteristics of the study population

	All		
	Non-DM	DM	P-value
ABP	22.95 (15.11-26.51)	28.29(26.13-34.21)	< 0.0001
Cholesterol	183.0 (162.3-206.8)	175.2(150.0- 216.5)	0.7777
Triglyceride	163.5 (94.00-195.5)	199.0(153.0-255.0)	0.0010

HDL-cholesterol	42.00(35.50-47.00)	46.75(40.45-55.00)	0.0716
LDL-cholesterol	114.3(96.00-129.8)	120.00(79.50-150.5)	0.6740
Creatinine	0.9000(0.7000-1.000)	0.8100(0.6575-1.093)	0.3239
Urea	29.00(26.00-34.00)	26.70 (21.75-35.25)	0.2615

Table (2): Anthropometric characteristics of the study population

	All subjects (n=)		
	Non-DM (n=)	DM (n=) Median (IQR)	P-value
SBP	122.0(114.3-127.8)	130.0(120-140)	0.0180
DBP	78.0(72.00-82.00)	80.00(80.00-80.00)	0.2164
MAP	99.67(87.33-116.9)	95.00(93.33-93.33)	0.3213

IQR: interquartile range

Table (3): The ROC curves for the anthropometrics, hormones and biochemical parameters of the study population.

	Area Under the ROC Curve	S.E.	95% Confidence Interval	P- Value
ABP	0.8067	0.05261	0.7036 to 0.9098	<0.0001
Cholesterol	0.6249	0.06098	0.5053 to 0.7444	<0.0475
Triglyceride	0.7064	0.05626	0.5962 to 0.8167	<0.0012
HDL-cholesterol	0.9638	0.01879	0.9269 to 1.000	<0.0001
LDL-cholesterol	0.9638	0.01879	0.9269 to 1.000	<0.0001
Creatinine	0.9638	0.01879	0.9269 to 1.000	<0.0001
Urea	0.5686	0.06216	0.4468 to 0.6904	<0.2597

SBP	0.6483	0.06007	0.5305 to 0.7660	<0.0190
DBP	0.6483	0.06007	0.5305 to 0.7660	<0.0190
MAP	0.6483	0.06007	0.5305 to 0.7660	0.0190

Table (4): Correlation coefficient of ABP and insulin hormones in non-diabetics and diabetic patients.

	ABP (n= 84)		Insulin (n=41)	
	r	P	r	P
Age	0.2394	0.0373	-0.2644	0.0210
ABP				
BMI	- 0.1874	0.1005		
Height	- 0.4708	< 0.0001		
Weight	- 0.2480	0.0266		
Waist circumference	- 0.2344	0.0389	-0.2344	0.0389
SBP	- 0.1096	0.4788	-0.292	0.009
DBP				
MAP				
Weight	- 0.2480	0.0266		
Glucose	0.4154	0.0002	-0.4529	<0.0001
Insulin				
HOMA-IR			0.5259	<0.0001
HOMA-B	0.4036	0.0006		
HbA1c	0.162	0.001	-0.4837	<0.0001

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Cholesterol				
Triglyceride			-0.2876	0.0107
HDL-cholesterol	0.331	0.004		
LDL-cholesterol				
Creatinine			0.325	0.0035
Urea	- 0.1612	0.1531	0.2340	0.0392
ALP				
AST				
ALT				

As seen in the figure (1), there was a negative association between serum RBP4 and glucose level ($r = -0.299$ $P < 0.0155$). Also, RBP-4 was negatively correlated with HbA1C level ($r = -0.199$ $P < 0.099$), figure (2). Few reports still now are studied such association. However, there is evidence that hyperglycemia and hence high HbA1C reduce RBP4 (Ram et al., 2015), but the exact mechanism till now is not fully understood. On the other hand, (Lewandowski et al., 2008b) showed that short-term glucose-induced hyperglycaemia and glucocorticosteroid-induced raise insulin resistance might be correlated with serum RBP-4 levels.

Interestingly, the present result indicated that RBP4 tended to reduce and negatively associated with HOMA-IR value ($r = -0.201$ $P < 0.075$). The products of the adipocytes may contribute to the deterioration in carbohydrate control and elevation of insulin resistance levels. RBP4 may also raise IR in mice, with increased levels in insulin-resistant with obesity and diabetes. However, the mechanisms regulating RBP4 synthesis still now is not fully understood. (Lewandowski et al., 2008a) concluded that association between RBP-4 and insulin resistance indices could not be not exclude the fact that RBP4 might be factors that can affect insulin resistance in humans. Furthermore, and interestingly (Bose et al., 2012) recorded that the elevated RBP4 in the serum and its association with Insulin, glucose, and HOMA-IR in the control subjects strongly indicated that this hormone has a role as a predictor for the onset of diabetes in humans.

Figure 1: Linear regression presenting the relationships of RBP-4 with glucose

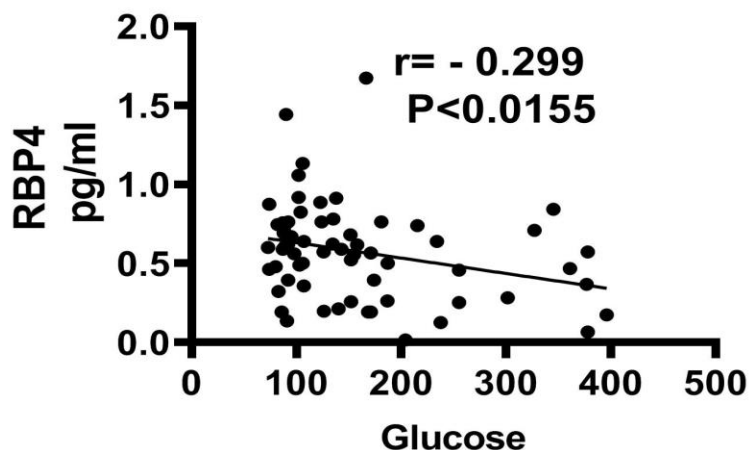


Figure 2: Linear regression presenting the relationships of RBP-4 with HbA1c

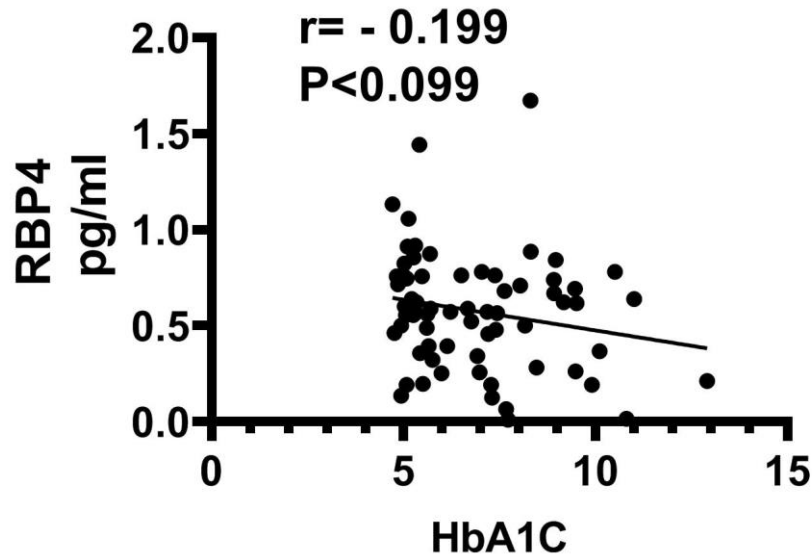
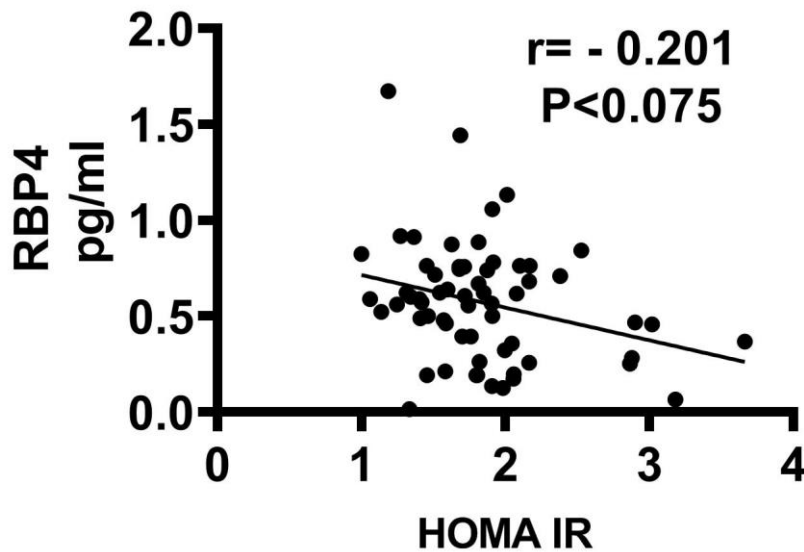


Figure 3: Linear regression presenting the relationships of RBP-4 with HOMA IR



CONCLUSIONS:

The current study, and for the first time, the obtained results suggested that serum RBP4 could be represented as a new biomarker for DM type II. Interestingly, this hormone negatively associated with the insulin resistance markers.

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