Association of Retinol Binding Protein-4 and Microalbuminuria and Blood Glucose Regulation in Type 2 Diabetic Iraqi Patients

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Abstract

Retinol binding protein 4 (RBP-4) has long been known as the carriers for vitamin A, in the plasma it is banded to transthyretin to form too large complex for glomerulus filtration. Discovered as adipokine involved in the pathogenesis of type 2 diabetes induced insulin resistant and linked to obesity. Diabetic nephropathy one of serious microvascular complication that impact large numbers of the patient however, association with RBP-4 is not fully known.

The present study tested this hypothesis by measuring serum RBP4 in samples of Iraqi type 2 diabetic (n=120) with normal albumin excretion (n=57) and microalbuminuria (n=63) of either sex. and Also apparently health subjects (n=30) were involved to serve as control.

Concentrations of the RBP4 were significantly elevated in serum of microalbuminuric diabetic patients as compare with normoalbuminuric patients, ($p=0.000<0.05$). also glycemic index parameters significantly higher (for all $p<0.05$).

In conclusion, the study suggested that serum level of RBP4 are affected by microalbuminuria and the assessment of RBP-4 as controller for insulin resistant in type 2 diabetic in further studies must take into consideration other renal problems.

Key Words: *retinol binding protein 4 (RBP4), type 2 diabetes mellitus, microalbuminuria.*

Introduction

Diabetes is a set of metabolic defects recognizes mainly by hyperglycemia as a result of impairment in insulin sensitivity and/or secretion. Chronically, long-term hyperglycemia causes damage and dysfunction of multiple organs (kidneys, heart, eyes, nerves and blood vessels) [(1)]. The latest approximations show that there was a global prevalence of 463 million people with diabetes (9.3%) in 2019, which is expected to rise to 700 million (10.9%) by 2045, of whom 90% have type 2 diabetes [(2)].

About one third of diabetic patients will finally have progressive deterioration of renal function. The first clinical sign of renal dysfunction is generally microalbuminuria, which is occasionally reversible in type 2 diabetes, but may progresses to obvious proteinuria and then into chronic kidney disease. Most patients who have microalbuminuria are at greater risk to develop cardiovascular diseases (CVD) [(3)], this can be explained by the hypothesis that widespread vascular damage is resulting from renal albumin leakage, in other words, microalbuminuria seems to reflect a state of vascular dysfunction and is associated with changes in vasomotor tone regulation of peripheral vessels [(4)]. Furthermore, damaged endothelial cells secreted cytokines and growth factor and they accumulate into sub endothelial space of the injured region, this promoting atherogenic changes that makes an individual susceptible to CVD and other organ damage [(5)].

Retinol binding protein (RBP4) is a 21,000 Da protein that belongs to the lipocalin family of proteins; it has long been known as the carriers for retinol. Retinol is bound to apo-RBP4 which is attached to transthyretin

(TTR) to form too large complex (retinol-RBP4-TTR). The latter prevents glomerular filtration of RBP4 [6-8].

After delivery of retinol to target tissue, RBP4 is glomerulofiltered and degraded in the proximal tubules; thus, glomerular filtration rate (GFR) determines RBP4 concentration [9]. the impaired catabolism of the RBP4 complex in the kidneys leads to the accumulation of a truncated variant of RBP4 in plasma of patients with chronic renal failure; indicating the central importance of kidney function in the regulation of plasma RBP4 levels [5].

Ziegelmeier et al has correlated RBP4 to clinical and biochemical measures of renal function, glucose and lipid metabolism and inflammation in patients with a mild to moderate decrease in GFR [10]. In this study, higher levels of RBP4 were recorded in plasma of dialysis patients even though they excrete RBP4 in urine. Moreover, serum creatinine had predicted RBP4 concentrations in the control subjects with a GFR >50 ml/min [10].

In addition to its important function as retinol transporter, RBP4 is linked to obesity-induced insulin resistance and type 2 diabetes [11, 12]. The association of microalbuminuria with insulin resistance has been documented in both type 2 diabetic patients as well as in non-diabetic individuals [13, 14]. Serum RBP4 has also shown to be associated with albumin excretion; the relationship was not fully clarified [15, 16].

In the present study, we aimed to investigate the association of serum RBP4 with microalbuminuria levels and blood glycemic indices, fasting blood glucose (FBG) and glycosylated hemoglobin (HbA1c), in sample of type 2 Iraqi diabetic patients.

Subjects and Methods

This cross-sectional study was conducted out in Specialized Center of Endocrinology and Diabetes in AL-Nasiriya city, south of Iraq. A total of 150 subjects were enrolled in the study; 120 of whom were an already diagnosed type 2 diabetes mellitus patients according to the American Diabetes Association Criteria [17], of age ≥ 18years and of either sex, with disease duration since diagnosis of at least 1 year. The remaining 30 were apparently healthy individuals to serve as control group.

Pregnant women, patients with end stage renal disease (ESRD) or patient on renal dialysis and patients with hepatic failure were excluded from the study.

Medical and social history were taken for each participant. Venous blood samples were collected after 12 hours of fasting. Ten milliliters blood samples were taken using 10 ml disposable syringes; about 2 ml of the collected blood was transferred to EDTA tube for analysis of glycosylated hemoglobin (HbA1c) by high performance liquid chromatography [18]. While (8 ml) of the blood was transferred to plane tube and centrifuged at (3000 rpm) for 10 minutes to obtain serum, which is used for the measurement of fasting blood glucose (FSG), blood urea (BU) and serum creatinine (S.cr.) by colorimetric assay [19]. The remaining part of serum was divided in Eppendorf tubes and kept frozen at (–20ºC) for the measurement of serum RBP-4 by enzyme-linked immunosorbent assay (ELISA) [20]. Finally, urine sample was collected from each participant for the measurement of microalbuminuria levels (MU) by NycoCard U-Albumin [21].

Statistical Analysis

The statistical analysis was performed using The statistical package for social science (SPSS), version 25. Data are expressed as mean ± standard error of mean (SEM). Comparison of means of two groups were done by using student t-test. Categorical variables were presented as number and analyzed by the chi-square χ^2- test. Pearson's correlation was used to study the correlation between different parameters and RBP4 Values. $P<0.05$ were considered significant.

Results

The sociodemographic and clinical charecteristics of the participants are selected. Type 2 diabetic patients and control groups were of comparable age, gender and body mass index (BMI) (P-value >0.05). Fifty two of the diabetic subjects were males, and 68 were females. The mean age of them was (50 ±8) ranging from 33 to 69 year; and mean BMI was (29.3 ±4) kg/m^2. Regarding the control group, 13 of the subjects were males and 17 were females. Their mean age was (48.3 ±8.7) ranging from 32 to 65 year, and mean BMI was (27.9 ±4).

Serum RBP4, FBG, HbA1c% and MU levels were significantly higher in the diabetic group as compared with the control group ($P<0.05$). While, there was no significant difference in serum creatinine and blood urea between the diabtic and the control groups.

Diabetic subjects were divided according to urinary albumin excretion into two groups: normoalbuminuric patients with normal urinary albumin excretion (0-18 µg/l), (n=63); and microalbuminuric patients with albumin excretion (> 18 µg/l), (n=57). Sociodemographic and clinical characterizes for each group as choosen. There was no significant difference between the two diabetic groups with regard to gender, BMI, smoking habit and blood urea (P>0.05). Meanwhile, age, disease duration, serum RBP4, FBG, HbA1c and serum creatinine were significantly higher in diabetic patients with microalbuminuria (P<0.05).

Correlation studies of serum RBP4 with the studied variables of the pooled data are shown in Table 1. Serum RBP4 has a positive correlation with BMI, FBG, HbA1c, and microalbuminuria levels (P<0.001), and serum creatinine (P<0.05). While, there was no correlation between serum RBP4 with age, gender and blood urea (P>0.05).

Table 1. Pearson's correlations of serum RBP-4 with the studied variables of the pooled data.

Variable	**r-value**	P-value
Age	0.07	0.36
Gender	-0.07	0.27
BMI	0.38	0.000*
FBG	0.6	0.000*
HbA1c	0.5	0.000*
BU	0.06	0.4
S. cr.	0.17	0.04*
MU	0.35	0.000*

BMI, body mass index, FBS=fasting blood sugar, HbA1c= glycosylated hemoglobin, BU=blood urea, S.cr=serum creatinine, MU=microalbumin in urine**.** * Significant when p<0.05.

Correlation studies of serum RBP4 with the studied variables of the diabetic patients are shown in Table 2. Serum RBP4 has a positive correlation with disease duration, BMI, FBG, HbA1c and microaluminuria levels (P<0.05). While, it was not correlated with age, gender, serum creatinine and blood urea (P>0.05).

Table 2. Pearson's correlations of serum RBP-4 with the studied variables of the diabetic subjects

Variables	**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*
FBG	0.6	0.000*
HBA1c	0.4	0.000*
BU	0.09	0.3
S.cr.	0.14	0.1
MU	0.33	0.000*

BMI= body mass index, FBG=fasting blood glucose, HbA1c= glycosylated hemoglobin, BU=blood urea, S.cr=serum creatinine, MU=microalbumin in urine**.**

* Significant when p<0.05.

Discussion

In the present study serum RBP4 levels were significantly higher in the diabetic study group than in the control group. Serum RBP4 levels were positively correlated with the glycemic indices, FBG and HbA1c, of the total study subjects or of the diabetic group. High plasma levels of RBP4 have been reported in many conditions characterized by insulin resistance such as obesity, polycystic ovarian syndrome, and type 2 DM [22, 23]. Moreover, exercise-induced improvement in insulin sensitivity was shown to be associated with a reduction in serum RBP4 levels [15]. In mice, it was found that RBP4 interferes with expression and phosphorylation of insulin receptor substrates in skeletal muscle. While, in the liver, RBP4 induces expression of phosphoenolpyruvate kinase [22]. Therefore, elevated RBP4 levels in humans might participate in impairment of insulin-induced uptake of glucose by skeletal muscles and elevated fasting hepatic glucose production, which are two important characteristics of type 2 DM. Furthermore, RBP4 may negatively affect pancreatic beta cells function [24]. It is worthy to mention that RBP4 gene is located on human chromosome 10q, a locus near regions that reported to be related with hyperinsulinemia or type 2 DM developed early in life in two populations [25].

Serum RBP4 levels were significantly higher in diabetic patients with microalbuminuria than in those with normoalbuminuric, and there was a significant positive correlation between serum RBP4 levels and microalbuminuria levels. Similar findings were reported in other studies [26]. Microalbuminuria represents an early marker of nephropathy developed in diabetic patients[27] , and it is also reported to be associated with insulin resistance in diabetic patients and in non-diabetic subjects [13]. As mentioned earlier, RBP4 (low molecular weight protein) is mainly cleared by the kidney, and its serum levels are determined by the glomerular filtration rate (GFR) and become greatly elevated in end-stage renal disease [5, 9]. However, in case of microalbuminurea; it is unlikely that pobable variation in GFR was responsible for for the raised RBP4 levels in microalbuminuric diabetic patients [28]. So this elevation of plasma free RBP4 has been recognisesd by the liver as a positive-feedback signal from peripheral tissues for the release of the RBP4-retinol complex [29], whereby RBP4 elevates in plasma of type 2 diabetic subjects with miocroalbuminuria.

In conclusion, our results revealed that serum RBP4 levels are high in type 2 diabetic patients and may be linked to the early manifistations related to the development of nephropathy, specifically, microalbuminuria. In addition, serum RBP4 correlate positively with the FBG and HbA1c. Larger scale study for the assessment of serum RBP4 levels as predictor of diabetic nephropathy is recommended.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

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