Role of Retinol-binding Protein 4 as an Early Biomarker for Diabetic Nephropathy in Type 2 Diabetic Patients

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Abstract

Assess the relationship and diagnostic performance of retinol-binding protein 4 (RBP-4) as a biomarker of diabetic nephropathy (DN) at different levels of albuminuria (normo-, micro- and macro-albuminuria). A case-control study, that involved 185 participants, 47 were healthy control, and 138 diabetic patients (46 were normoalbuminuric patients [NA], 48 patients were microalbuminuria [MiA], and 44 patients were macroalbuminuria [MaA]). All diabetic patients were treated with antidiabetic medication (metformin and/or oral sulfonylureas), all the participants were interviewed by the researcher, and full medical (history of liver, kidney, lung, and other diseases), and sociodemographic data taken from them and recorded in the questionnaire. In the present study, RBP-4 was 14.08±1.82 ng/ml in the control group, 15.98±3.74 ng/ml in the normoalbuminuria (NA) group, 30.38±6.02 ng/ml in the micro-albuminuria (MiA), and 41.01±5.78 ng/ml for macro-albuminuria (MaA), there was a significant difference between all their groups compared to control (p-value <0.001), and between each other (p-value <0.001) except that no significant difference found between control and normoalbuminuria. In conclusion, RBP4 is an excellent predictor of both microalbuminuria and microalbuminuria, there was an inverse relationship between RBP4 with GFR, which indicate it is a good predictor for the progression of diabetic kidney impairment.

Keywords: *Albuminuria, renal impairment, RBP4, microalbuminuria, macroalbuminuria, diabetic nephropathy.*

Introduction

The term diabetes mellitus describes diseases of abnormal carbohydrate metabolism that are characterized by hyperglycemia. It is associated with a relative or absolute impairment in insulin secretion, along with varying degrees of peripheral resistance to the action of insulin. Type 2 diabetes accounts for over 90 percent of cases of diabetes in the United States, Canada, and Europe; type 1 diabetes accounts for another 5 to 10 percent, with the remainder due to other causes[1].

The term "diabetic nephropathy" was historically defined by the presence of albuminuria accompanied by retinopathy in patients with type 1 diabetes[2]. The presence of albuminuria was considered to be an early sign of classical diabetic glomerulopathy, which is characterized by glomerular basement membrane thickening, endothelial damage, mesangial expansion and nodules, and podocytes loss. Diabetic nephropathy was further subdivided into "overt nephropathy" by "macroalbuminuria" and "incipient nephropathy" by "microalbuminuria." These albuminuria distinctions were proposed to reflect a disease spectrum from mild to severe [2].Retinol binding protein 4 (RBP4) It is a specific transporter for vitamin A in the blood, and it is produced from either the liver or adipocytes, which was later proven to be associated with insulin resistance in type II DM. It was shown that increased of RBP-4 will cause reduction in insulin-dependent glucose uptake in muscular tissues[3].

The current work aimed to assess the relationship between the studied biomarker and the different levels of albuminuria (normo, micro and macroalbuminuria), and

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the relationship between the serum levels of the RBP-4 with glomerular filtration rate (calculated GFR).

Method

Study Design: A case control study (a diagnostic accuracy study [67]), that involved 185 participants, 47 were healthy control, and 138 diabetic patients (46 were normoalbuminic patients [NA], 48 patients were microalbuminuria [MiA], and 44 patients were macroalbuminuria [MaA]).

Study Settings: The study carried out in privet laboratory in Al-Nasiriyah, Iraq, the participants were recruited from the Diabetes Mellitus at Diabetic and Endocrine Center in Al-. Nasiriyah City. The recruitment started in October 2019 and ended in February 2020.

Participants: All diabetic patients were treated with antidiabetic medication (metformin and/or oral sulfonylureas), all the participants were interviewed by the researcher and full medical (history of liver, kidney, lung, and other diseases), and sociodemographic data taken from them and recorded in the questionnaire.

Inclusion criteria: Type II diabetic patients diagnosed by physician based on the 2010 American Diabetes Association guidelines (which is briefly as fasting blood sugar [FBS] above 126 mg/dL, or random blood sugar above 200 mg/dL) [4], or HbA1c above 6.5% [5].

Exclusion criteria: pregnant women., Patients on insulin therapy, Patients with other metabolic, disorders, Patients with cardiovascular disease, Patients providing incomplete information during completion of the questionnaire also will be excluded from the study, End stage kidney and liver disease, and eGFR below 60 ml/min (stage III or higher).

Statistical Analysis: Chi square test used for assessing the statistical significance between categorical variables, while independent t-test used for assessing the degree of significance between 2 continues variables, in case of assessing the difference between 4 continues variables one way ANOVA was used.

Linear regression analysis was used to assess the relationship between eGFR and various markers, negative sign indicate inverse relationship while positive sign indicate direct relationship. Receiver operator curve (ROC) used to assess the diagnostic performance of various biomarkers for predicting nephropathies. All analysis carried out using SPSS 24.1 (Chicago, IL), MedCalc Statistical Software version 14.9.2 software package, p-value is significant if less than 0.05.

Results

Table 1: Assessment of sociodemographic variables

Variables	Control	DM	p-value
Number	48	138	-
Age (years), mean ± SD	43.7 ± 6.3	43.9 ± 7.8	0.826
Gender, n (%)			0.003
Female	9 (19.1 %)	60 (43.5 %)	
Male	38 (80.9 %)	78 (56.5 %)	
Occupation, n (%)			0.363
Unemployed	36 (76.6 %)	114 (82.6 %)	
Employed	11 (23.4 %)	24 (17.4 %)	
Education level, n (%)			0.646
Primary	13 (27.7 %)	47 (34.1 %)	
High school	18 (38.3 %)	44 (31.9 %)	
College	16 (34.0 %)	47 (34.1 %)	
Marital status			0.840
Single	21 (44.7 %)	64 (46.4 %)	
Married	26 (55.3 %)	74 (53.6 %)	

Mean RBP-4 was higher in 4 in comparison to group 3, 2, and 1, also it was higher in group 3 in comparison to group 2 and 1, while no significant difference between group 1 and 2 was found see table 2 and figure 1.

Table 2: Assessment of RBP and various factors according to study groups

Variables	Group 1	Group 2	Group 3	Group 4
Number	47	46	48	44
eGFR (ml/min), mean ± SD	100.66±10.83	98.59±11.46	83.85±9.11	72.64±5.64
Duration of DM (y), mean ± SD	-	10.22±4.36	9.54±3.94	13.20±2.06
RBP4 (ng/mL)	14.08±1.82	15.98±3.74	30.38±6.02	41.01±5.78
Group 1: control, group 2: Normo-albuminuria, group 3: Microalbuminuria, group 4: Macroalbuminuria				

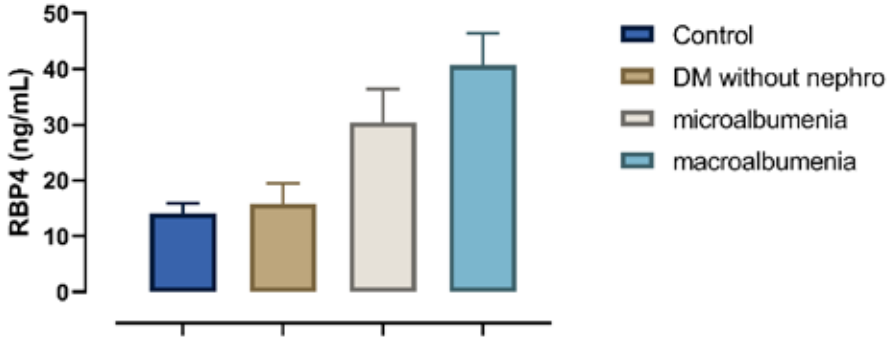

Figure 1: Assessment of serum RBP4 (ng/mL) according to study groups

There was inverse moderate relationship between eGFR with RBP-4 as illustrated in figure 2.

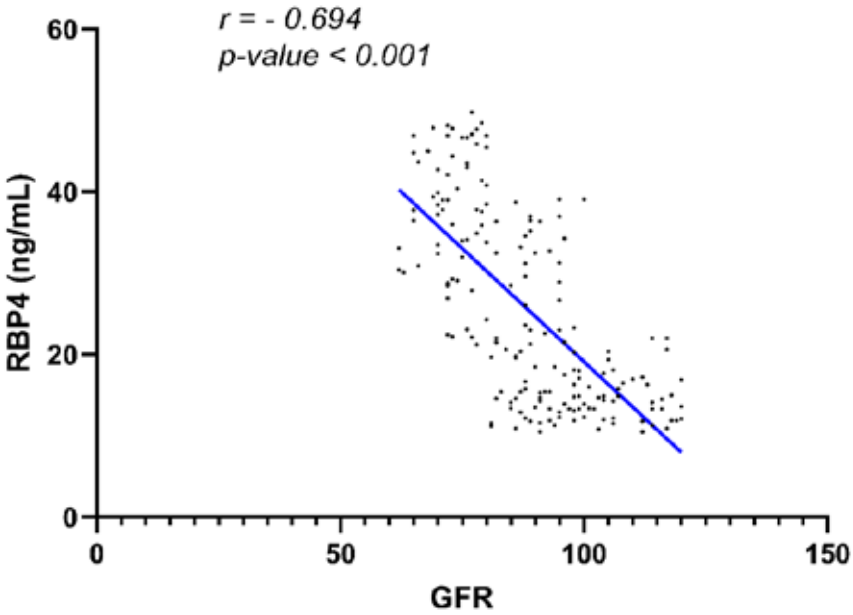

Figure 2: Scatter plot describes the relationship between eGFR with RBP-4

RBP-4 showed excellent sensitivity and specificity to similar extent, with 96.3% accuracy, with an optimal cut–off above 22 ng/ml, as illustrated in table 3 and figure 3.

Table 3: diagnostic performance of various markers for the prediction of microalbuminuria from normo-albuminuria

Variables	AUC	p-value	Cut-off	SN	SP	AC	PPV	NPV
RBP4 (ng/mL)	0.993	<0.001	>22	93.8	99	96.3	99	93.9
AUC: Area under the curve, CI: confidence interval, LH: likelihood ratio, SN: sensitivity, SP: specificity, AC: accuracy, PPV: positive predictive value, NPV: negative predictive value								

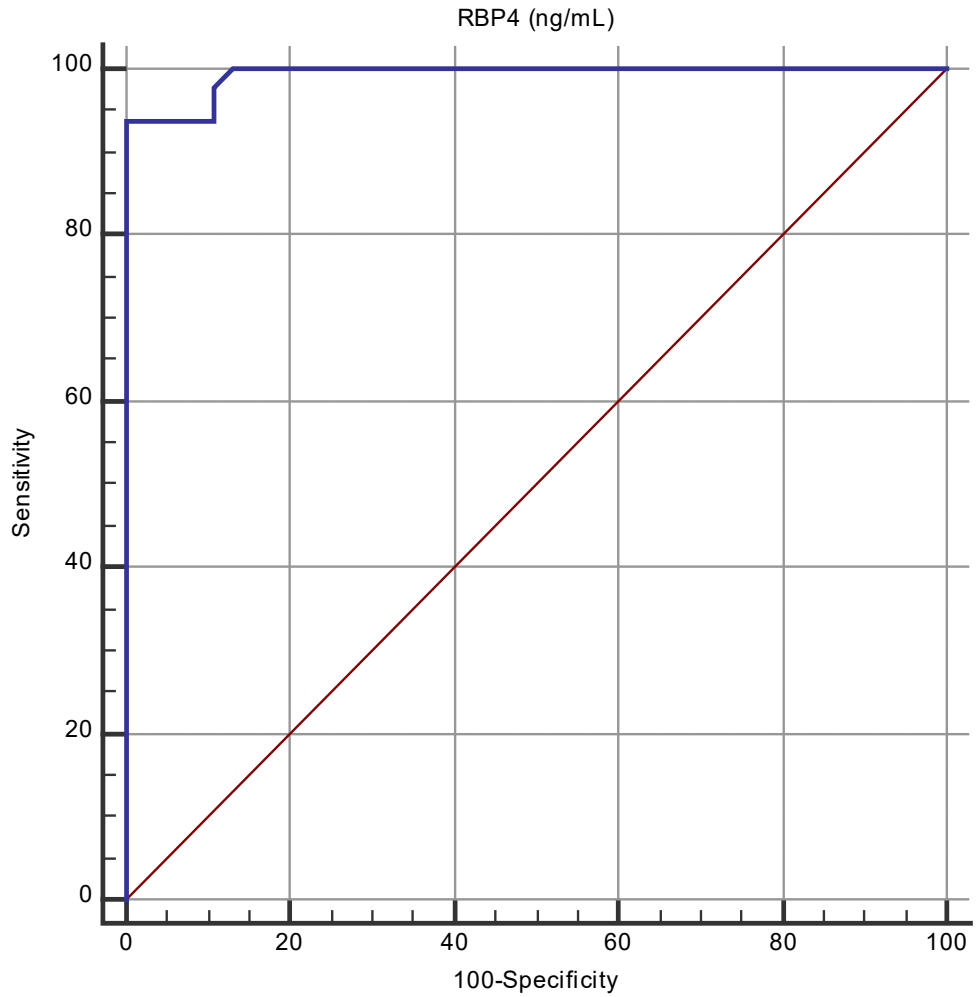

Figure 3: ROC curve of RBP-4 for the prediction of microalbuminuria from normo-albuminuria

Discussion

It is an adipokine marker that was initially examined as a marker for vitamin A (since its bind to vitamin A) [3], the primary production site is the liver and adipocyte [6]. Many studies had correlated RBP-4 to some of the aspects of diabetic including obesity and insulin resistance [7].

In the present study RBP-4 was 14.08±1.82 ng/ml in the control group, 15.98±3.74 ng/ml in the normo-albuminuria (NA) group, 30.38±6.02 ng/ml in the micro-albuminuria (MiA), and 41.01±5.78 ng/ml for macro-albuminuria (MaA), there was significant difference between all there groups compared to control (p-value <0.001), and between each other (p-value <0.001) except that no significant difference found between control and normo-albuminuria.

Our findings was in agreement with previous studies; in a Saudi study Mahfouz et al 2016 examined RBP-4 in the same setting like our study (200 participants divided into four groups each with 50 patients), they found that like us serum RBP-4 was significantly higher MaA

compared to MiA, NA and healthy control (39.4 ± 7.7, 28.9 ± 8.26, 17.5 ± 4.2, and 14.4 ± 1.95 ng/mL) [8].

In Raila et al study 2007, was in agreement with our work in which RBP-4 was significantly higher in the MiA compared to both NA and control (2.22, 1.75, and 1.59 µmmol/l, p-value <0.05) [9].

In Chang et al 2008 study similar findings to our study was observed in which was signifincalty higher in MaA, MiA compared to both NA diabetic and healthy control (64.7 ± 27.6, 57.3 ± 24.2, 43.4 ± 14.9, and 32.6 ± 10.0 respectively), while there was significant difference between MiA and MaA [10]. All these studies showed an important observation as the diabetic nephropathy progress it is directly associated with increase in RBP-4 levels, since there was inverse relationship between RBP-4 with eGFR (*r = - 0.694, p-value <0.001*).

The kidneys play central role in the control of normal homeostasis of the body [11], since GFR give us an estimation about the functionality of the kidneys so any deterioration will be associated with decline in GFR, and inability to perfume the reabsorption process of many materials including RBP-4 in the PCT [11].

Our observation of the inverse relationship was also noted in other studies like Mahfouz et al 2016, (*r = - 0.306, p-value = 0.002*) [8], thus increase the accumulation of RBP-4 in the body.

Several other factor can also increase RBP-4 which included increase excretion of transthyretin (the protein that bound and transport RBP-4 in the blood) thus increase the accumulation of RBP-4 [12]. In addition elevation in RBP-4 is associated with increased risk of hypertensive, abnormal lipid profile, and deterioration in GFR [13].

In the present study RBP-4 showed excellent ability to predict MiA from NA diabetic patient since the area under the curve (AUC) = 0.993, in addition the positive likelihood ratio was 58.0 which indicate it had 45% increased posterior probability to confirm the MiA when used as conformation test, while its negative likelihood was 0.001 which indicate it increase the exclusion of disease by 45%. In term of diagnostic performance RBP-4 value above 22 ng/ml has 99.0% specificity and 99.0% sensitivity and 99% accuracy. This indicate the overall value of this marker is to both confirm and exclude the disease MiA to similar degree.

In the present study RBP-4 showed excellent ability to predict MaA from NA diabetic patient since the area under the curve (AUC) = 0.999, in addition the positive likelihood ratio was 58.0 which indicate it had 45% increased posterior probability to confirm the MaA when used as conformation test, while its negative likelihood was 0.001 which indicate it increase the exclusion of disease by 45%. In term of diagnostic performance RBP-4 value above 22 ng/ml has 99.0% specificity and 99.0% sensitivity and 99% accuracy. This indicate the overall value of this marker is to both confirm and exclude the disease MaA to similar degree.

Our findings were in agreement with Mahfouz et al 2016 in which RBP-4 showed excellent ability (AUC = 0.912) to predict nephropathy from those diabetic patients without nephropathy in which they found that the optimal cut – off was >24.5 ng/ml with 84% sensitivity, 90% specificity and 86% accuracy which comparable but somewhat lower than our findings, this could be attributed to differences in studied population since Mahfouz et al involved Saudi subjects while in ours involved Iraq patients [8].

The kidney play central role for RBP-4 homeostasis, since its metabolic degradation is dysregulated in chronic renal impairment (CRI) patients. A possible explanation that diabetic patients are exposed to increased risk of oxidative stress which in turn lead to endothelial damage. In addition RBP-4 enhance oxidative stress, so its accumulation will lead to further enhancement of kidney impairment in diabetic patients [14]. RBB-4 elevation thus is most likely to be related to kidney dysfunction rather than diabetic itself [15], which will lead to MiA and MaA. Which indicate its usefulness for predicting diabetic nephropathy rather than diabetic.

These observations is interesting since it suggest we could use RBP-4 as target for treatment by reducing its circulatory level will be proven to reduce the risk of kidney impairment progression, especially those related PCT damage [8]. In adipose-Glut4-knockout mice, therapy with anti-diabetic (rosiglitazone) will invert insulin resistance and stop RBP-4 elevation [16]. While treating obese mice with fenretinide will enhanced RBP4 renal elimination thus return RBP-4 to its normal values [16].

Conclusion

Retinol binding protein – 4 is an excellent predictor of both microalbuminuria and microalbuminuria, and there was inverse relationship between Retinol binding

protein – 4 with GFR, which indicate it is good predictor for the progression of diabetic kidney impairment.

Ethical Clearance: The approval taken from ethical committee of College of Pharmacy, Baghdad University and was done in accordance with Declaration of Helsinki1975 and its later amendments.

Source of Funding: Self

Conflict of Interest: Nil

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