Al-Azhar University Journal
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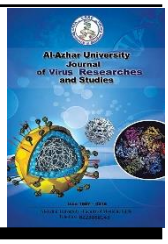

Retinol Binding Protein 4 as a Recent Biomarker for Prediction of Diabetic Nephropathy in Type 2 Diabetic Patients

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Abstract

Diabetic Nephropathy, which is one of the most common and severe microvascular complications of diabetes, accounts for not only the leading cause of end-stage renal disease but also causes major morbidity and mortality in patients with type 2 diabetes. However, DN often occurs with no obvious symptoms in the early stage. We aim to evaluate the role of RBP4 as a recent biomarker for the prediction of diabetic nephropathy in type 2 diabetic patients. This study was conducted on 60 patients with type Π diabetes mellitus considered as Group A which was subdivided into 2 subgroups, Group A1: 30 type Π diabetic patients without albuminuria, Group A2: 30 type Π diabetic patients with albuminuria and 30 age and sex matched healthy individuals Group B considered as control group. There is statistically significant increase of serum RBP4 level in diabetic patients when compared to control group. There is statistically significant increase of serum RBP4 level in diabetic patient groupA2 when compared to diabetic patients group A1 and between each subgroup when compared with control. There is also significant difference between patient and control as regard to the inflammatory marker Hs CRP with also significant difference in patients' subgroups. Even though that tubular damage marker such as serum RBP4 as single diagnostic parameter of renal impairment showed good clinical accuracy, we aim to clarify that combination of markers of tubular damage (RBP4), inflammation markers (HsCRP) and traditional markers has the higher sensitivity and specificity than each single marker alone in prediction of DN. There is a strong positive correlation between serum level of RBP4 and serum urea, creatinine and Alb/creat ratio while negative correlation with eGFR and serum albumin level. There is also a positive correlation between serum level of RBP4 and HsCRP level. Serum level of RBP4 was higher in type Π diabetic patients compared to control group and it increased in diabetic patients with micro albuminuria more than diabetic patients without micro albuminuria.

Keywords: Retinol Binding Protein 4, Diabetic Nephropathy, Type 2 Diabetic.

1. Introduction

Diabetic Nephropathy (DN), which is one of the most common and severe microvascular complications of diabetes, accounts for not only the leading cause of end-stage renal disease but also causes major morbidity and mortality in patients with type 2 diabetes mellitus (T2 DM). However, DN often occurs with no obvious

symptoms in the early stage [1]. Many pre-inflammatory cells, growth regulators, and adhesion factors interact with each other and cross-link, resulting in an expansion of the corresponding cascade of inflammation. Common diagnostic indicators for diabetic nephropathy include 24-hour urine microalbumin, urea nitrogen, and serum creatinine. However, they can be affected by many factors, such as urinary tract or systemic infections, strenuous exercise, bleeding, or drugs that affect the kidneys. The accuracy and specificity of these indicators are not high, and they have limitations; thus, more research is needed to identify newer, more accurate, and specific early diagnostic markers of diabetic nephropathy. In recent years, the rapid development of proteomics technology has provided us with new methods and ideas for identifying early diagnostic markers of DN [2]. Retinol-binding protein 4 (RBP4) has been widely explored as adipokine, closely related to cardiometabolic indices. RBP4 plasma levels are also associated with the development of endothelial dysfunction and clinical atherosclerosis through the induction of vascular inflammation and endothelial oxidative stress [3]. Furthermore, due to its low molecular weight, it is freely filtered through the glomeruli and then almost completely reabsorbed in the proximal tubules, Therefore, RBP-4 has been identified as a very sensitive biomarker for proximal tubular cells dysfunction [4]. Recent studies showed that serum RBP4 levels were associated with decline in estimated glomerular filtration rate (eGFR), as well as positively correlated with changes in serum creatinine, confirming its association with renal function [4]. In order to get better insight into the pathophysiological mechanisms of renal function decline, we need to examine markers of glomerular damage (i.e., urinary albumin), markers of tubular damage (i.e., serum RBP4), and inflammation markers (i.e., serum high sensitivity C-reactive protein level [hsCRP]) in patients with T2 DM. In this study, we aimed to evaluate the role of Retinol Binding Protein 4 as a recent biomarker for the prediction of diabetic nephropathy in type 2 diabetic patients.

2. Patients and Methods

This study was conducted on 60 patients with type Π diabetes mellitus diagnosed from history, fasting and 2hour post prandial blood glucose and according to ADA criteria considered as (Group A) and 30 age and sex matched healthy individuals considered as control group (Group B). Group A was subdivided into 2 subgroups: *Group A1*: 30 type Π diabetic patients without microalbuminuria, from them 17 were females and 13 were males, their age ranged between (40 - 67) years with Mean ± SDE (53.63 ± 8.85) and duration of diabetes was 7-11 years, *Group A2*: 30 type Π diabetic patients with microalbuminuria (>30 µg/gm.creat), from them 14 were females and 16 were males, their age ranged between (45 – 67) years with Mean ± SDE (56.32 ± 8.93). and duration of DM was (8-15) years, group B: included 30 age and sex matched healthy individuals their age ranged between (32 – 67) years with Mean ± SDE (53.8±9.15) considered as control group. Diabetic patients were recruited from Internal medicine departments in Al-Zahraa University Hospital after oral consents and after the approval of the ethical committee of the university during the period from December 2020 to August 2022.

2.1 Exclusion Criteria

From our work we excluded patients with chronic liver disease, chronic inflammatory diseases, morbid obesity, patients with any malignancy, end-stage renal disease and Type -1 diabetic patients. All patients and control groups were subjected to the following: full medical history including history of Hypertension and ischemic heart disease, full clinical examination with

special emphasis on blood pressure and body mass index.

All Patients and control groups were subjected to measurement of the Following:

- Fasting and post-prandial blood glucose, glycosylated hemoglobin (HbA1c), highly sensitive C Reactive Protein (HsCRP) and serum albumin.
- Serum urea, serum creatinine, serum uric acid and calculation of Estimated GFR by MDRD formula. (GFR (ml / min-1 / 1.73 m-2) = 186 × [serum creatinine (mg/dl)-1.154 × age (years)-0.203 × (0.742 if female) × (1.210 if African American)]. **(KIDIGO,2012)**
- Serum cholesterol, triglyceride, HDL, LDL.
- Serum Retinol Binding Protein 4 was determined using immunoassay (ELISA).

2.2. Statistical Analysis

Data were collected, revised, coded and entered into the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also, qualitative variables were presented as numbers and percentages. The p-value was considered significant as the following: P-value > 0.05: Non-significant (NS), P-value < 0.05: Significant (S), P-value < 0.01: Highly significant (HS).

3 .Results

Table.1 showed that there is non-significant difference between patients' subgroups and control as regard to demographic data. The previous table showed that there is highly significant difference between patients' subgroups A1, A2 as regard to serum urea, creatinine, uric acid, ACR as all of which increased in A2 while albumin and eGFR decreased in A2 and there is also significant difference between albuminuric group A2 and control as regard to previous parameters. There is non-significant difference between groups A1 and B as regard to ACR. There is highly significant difference between group A1 and A2 as regard to HDL increased in A1 with non-significant difference as regard to other parameters of lipid profile between patients' subgroups. There is highly significant difference between A1and B groups as regard to serum HDL, LDL and triglecride increased in A1 and significant increase of serum LDL and triglecride in group A2 in comparison with group B. There is highly significant difference between group A1 and A2 as regard to HsCRP which increased in group A2 with significant increase in each group (A1, A2) compared with control group (B). The previous table showed that there is highly significant difference between group A1 and A2 as regard to serum RBP4 which increased in group A2, with also significant increase in each group (A1, A2) compared with control group (B).

Table (1): Demographic data of the studied groups.

		Group A1	Group A2	Group B	Test value	P-value	Sig.
		No. = 30	No. = 30	No. = 30			
Sex	Female	17 (56.7%)	14 (46.7%)	17 (56.7%)	0.804*	0.669	**NS**
	Male	13 (43.3%)	16 (53.3%)	13 (43.3%)			
Age	Mean ± SD	53.63 ± 8.85	56.23 ± 8.93	53.8 ± 9.15	0.788•	0.458	**NS**
	Range (year)	40 – 67	45 – 67	32 – 67			
BMI	Mean ± SD	27.71 ± 2.13	27.25 ± 1.79	26.94 ± 1.58	1.329	0.270	**NS**
	Range (kg/m2)	22.4 – 30.6	23.4 – 30.7	23.4 – 29.5			
Duration of DM (year)	Mean ± SD	8.60 ± 1.33	10.63 ± 2.70	--	--	--	--
	Range (year)	7 – 11	8 – 15	--			

Table (2): Comparison between group A1, A2 and group B as regard, renal function tests, lipid profile, HsCRP, serum RPB4 of the studied subjects.

		Group A1 No. = 30	Group A2 No. = 30	Group B No. = 30	Test value	P-value	Sig.
S.creatinine	Mean ± SD	0.65 ± 0.11	0.94 ± 0.15	0.65 ± 0.10	53.416•	0.000	**HS**
	Range (mg/dl)	0.5 – 0.9	0.7 – 1.2	0.5 – 0.8			
S. UREA	Mean ± SD	24.90 ± 6.92	33.67 ± 10.13	22.07 ± 4.47	19.311•	0.000	**HS**
	Range (mg/dl)	12 – 37	12 – 52	12 – 33			
S.Uric acid	Mean ± SD	5.05 ± 1.23	5.66 ± 1.11	4.39 ± 0.67	11.475•	0.000	**HS**
	Range (mg/dl)	2.7 – 8.1	3.6 – 7.6	36.1			
S.Albumin	Mean ± SD	4.22 ± 0.35	3.93 ± 0.29	4.39 ± 0.51	10.215•	0.000	**HS**
	Range (gm/dl)	3.5 – 4.9	3.5 – 4.6	3.5 – 5.3			
E. GFR	Mean ± SD	123.36 ± 11.54	80.06 ± 7.48	126.41 ± 6.61	259.870•	0.000	**HS**
	Range (ml/m/1.73m2)	89.2 – 139.9	66.7 – 100.3	116.8 – 145			
A/C ratio	Mean ± SD	19.90 ± 4.01	187.24 ± 73.78	6.37 ± 1.88	79.129≠	0.000	**HS**
	Range (mcg/gm cr)	12.4 – 28	62 – 291.7	3.5 – 11			
S. cholesterol	Mean ± SD	186.70 ± 45.31	179.97 ± 51.99	163.07 ± 22.65	2.532•	0.085	**NS**
	Range (mg/dl)	94 – 256	68 – 287	112 – 220			
HDL	Mean ± SD	44.18 ± 12.08	34.85 ± 12.98	38.30 ± 7.75	5.344•	0.006	**HS**
	Range (mg/dl)	25.4 – 85	12 – 73	27 – 60.7			
LDL	Mean ± SD	109.94 ± 25.09	115.81 ± 39.11	85.35 ± 23.51	8.666•	0.000	**HS**
	Range (mg/dl)	69 – 174	28 – 196	30.5 – 140			
Triglyceride	Mean ± SD	163.97 ± 55.53	196.83 ± 111.73	114.30 ± 44.46	8.857•	0.000	**HS**
	Range (mg/dl)	86 – 288	68 – 499	65 – 286			
HsCRP	Mean ± SD	5.39 ± 1.13	10.42 ± 2.30	3.26 ± 0.97	72.528≠	0.000	**HS**
	Range (mg/L)	3.7 – 9.1	6.1 – 14	1.5 – 4.9			
RBP4	**Mean ± SD**	17.77 ± 6.10	57.55 ± 32.47	9.94 ± 3.95	63.058≠	<0.001	**HS**
	Range (ng/ml)	7.4 – 33.9	11.2 – 155	3.3 – 22.1			

Table (2): Comparison between group A1, A2 and group B as regard, renal function tests, lipid profile, HsCRP, serum RPB4 of the studied subjects.

	Post Hoc Analysis by LSD		
	Group A1 Vs Group A2	Group A1 Vs Group B	Group A2 Vs Group B
S. Creatinine	0.000	0.836	0.000
S. UREA	0.000	0.149	0.000
S. Uric acid	0.024	0.014	0.000
Albumin	0.006	0.107	0.000
E. GFR	0.000	0.182	0.000
A/C ratio	0.000	0.223	0.000
HDL	0.002	0.040	0.236
Triglyceride	0.100	0.014	0.000
LDL	0.451	0.002	0.000
HsCRP	0.000	0.000	0.000
RBP4	0.000	0.000	0.000

Table (3): Correlation of RBP4 with other studied parameters in all patients group.

	RBP4	
	R	P-value
S. Creatinine	0.625**	0.000
S. UREA	0.362**	0.004
A/C ratio	0.840**	0.000
S. Albumin	-0.272*	0.035
E. GFR	-0.728**	0.000
HsCRP	0.837**	0.000

Table. 3 showed that there is significant positive correlation of RBP4 with serum urea, creatinine, ACR and HsCRP in studied groups and significant negative correlation with serum albumin and estimated GFR. Figure. 4 ROC curve showed that the best cut off point for RBP4 to detect cases with DM was found >12.5 with sensitivity of 90%, specificity of 93.33% and area under curve (AUC) of 94.1%. Figure. 5 ROC curve shows that the best cut off point for RBP4 to differentiate diabetic patients with and without albuminurea was found >24.8 with sensitivity of 86.67%, specificity of 93.33% and area under curve (AUC) of 91.7%.

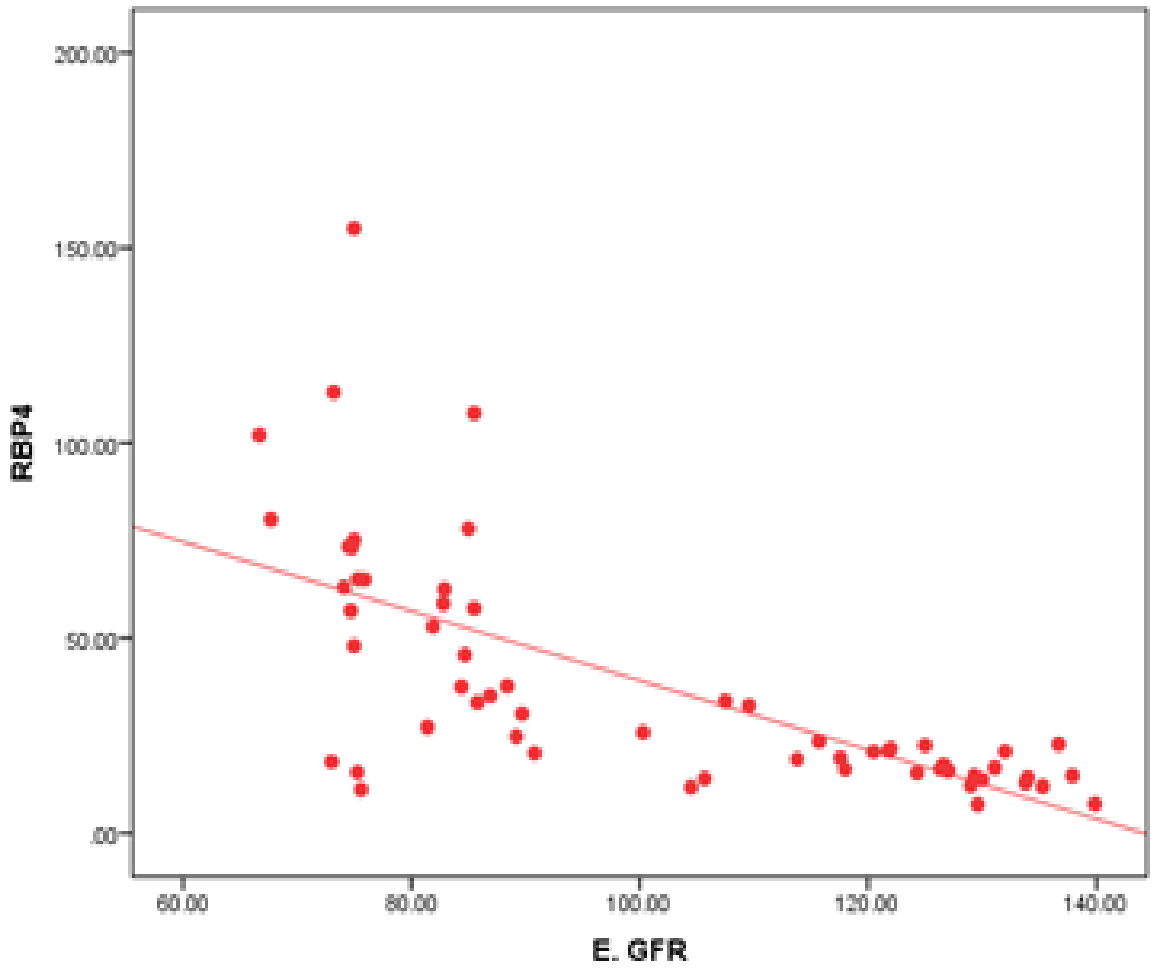

Figure (1): Correlation of RBP4 with GFR.

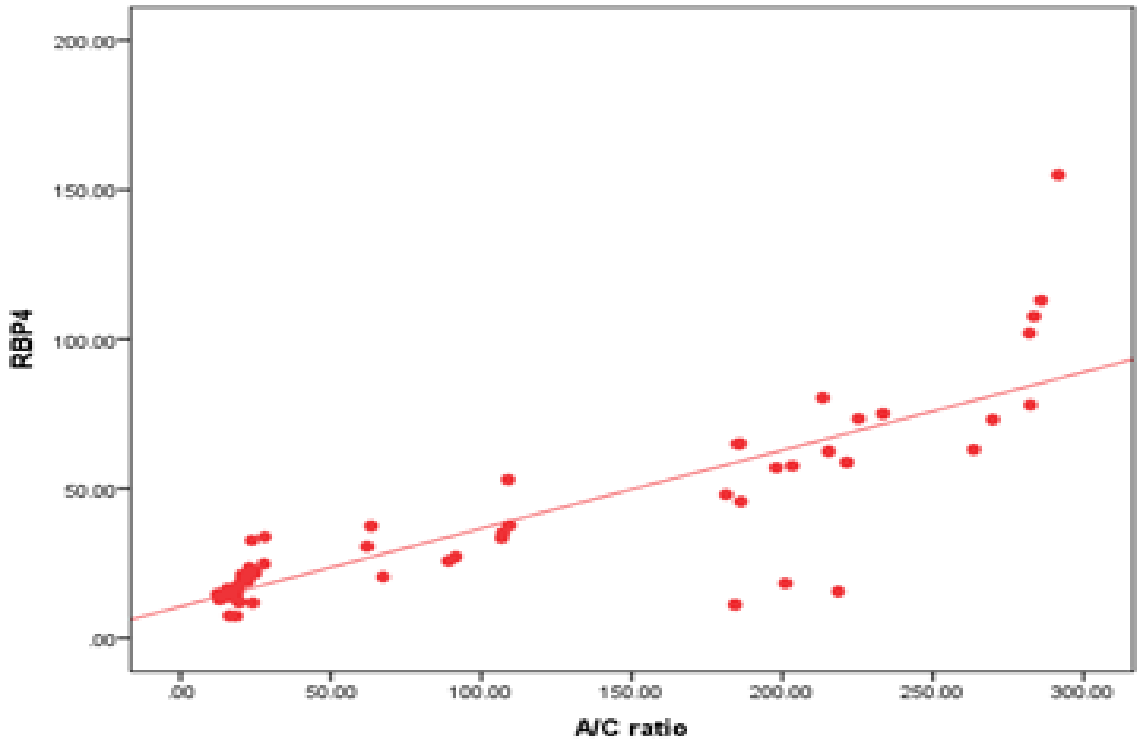

Figure (2): Correlation of RBP4 with ACR.

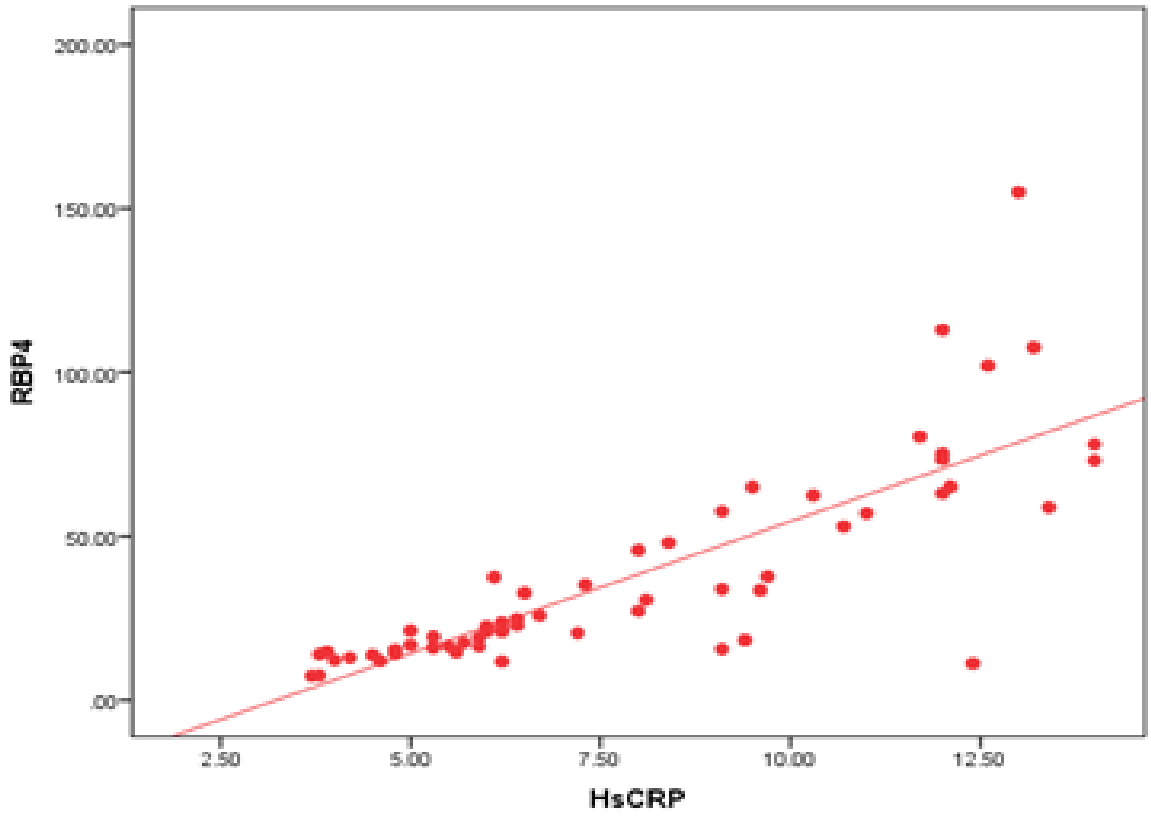

Figure (3): Correlation of RBP4 with HsCRP.

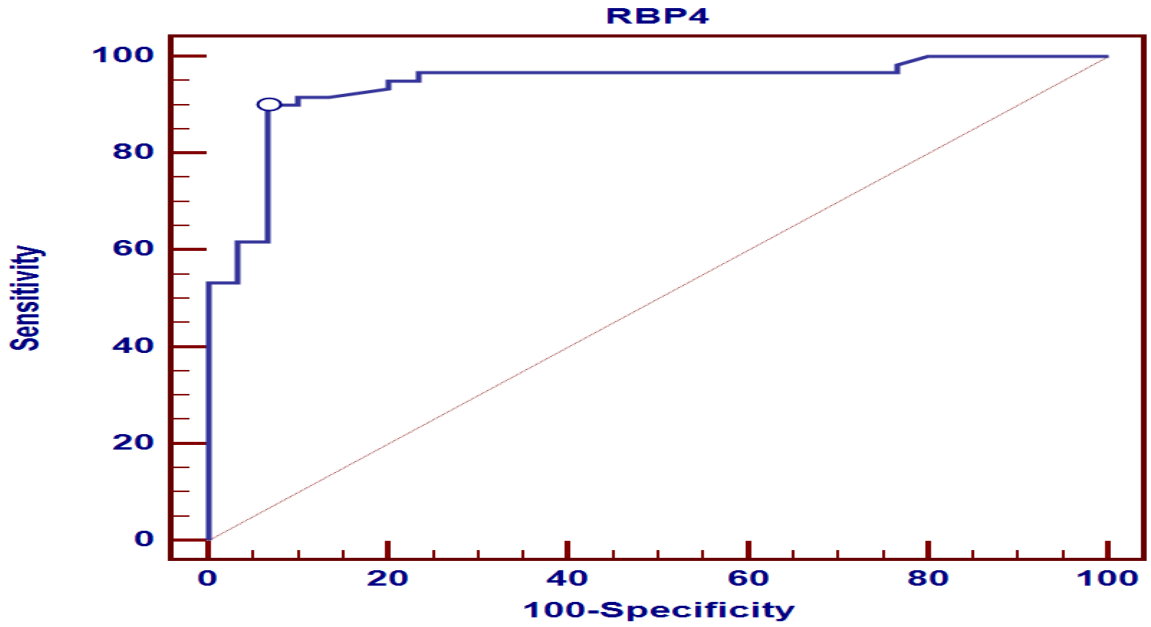

Figure (4): ROC curve of RBP4 as a predictor of diabetic cases.

Table (4): Sensitivity and specificity of RBP4 in all studied groups.

Parameter	AUC	Cut off Point	Sensitivity	Specificity	PPV	NPV
RBP4(ng/dl)	0.941	>12.5	90.00	93.33	96.4	82.4

Table (5): Sensitivity and specificity of RBP4 differentiating between group A1 and group A2.

Variables	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
RBP4 (ng/dl)	>24.8	0.917	86.67	93.33	92.9	87.5

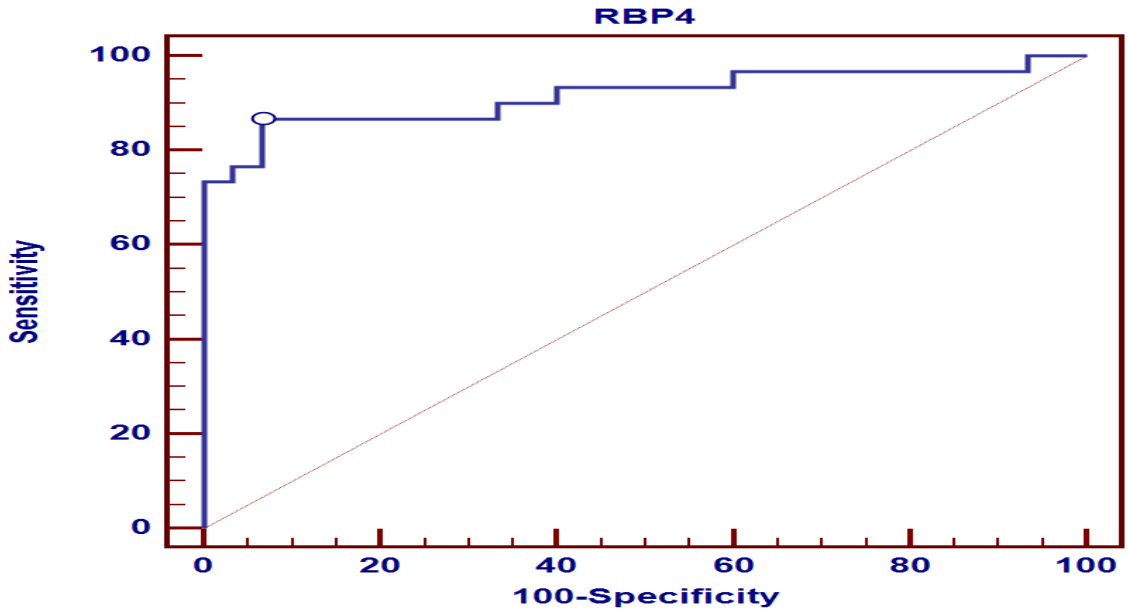

Figure (5): ROC curve of RBP4 as a predictor of diabetic cases.

4. Discussion

Diabetic nephropathy is a common and serious complication of DM and is one of the leading causes of end-stage renal disease worldwide [5]. It is also associated with cardiovascular causes of mortality. Therefore, accurate identification of DN is critically important to improve clinical prognosis and reduce the economic burden. Although there have been many investigations on biomarkers for DN, there is no consistent conclusion about reliable biomarkers [6].

Albuminuria is widely used to indicate early phases of diabetic nephropathy although it is limited by the fact that structural damage might precede albumin excretion [7].

Studies have also stated that micro albuminuria may develop in non-diabetic patients with progressive chronic kidney disease so micro albuminuria is not specific for patients with DN alone, also not all diabetic patients with micro albuminuria progress to end stage renal disease. Therefore, sensitive and specific biomarkers that can early predict susceptibility to diabetic nephropathy is needed [8].

Thus, the use of a panel with a combination of biomarkers instead of urinary albumin alone seems to be an interesting approach for early detection of DN, including markers of glomerular damage (e.g. albumin), tubular damage (e.g. RBP), inflammation (e.g. HsCRP) and oxidative stress because these mechanisms contribute to the development and outcomes of this disease [9]. RBP4 was identified in 2005 and is mainly synthesized in adipose tissues and hepatocytes. It is a circulating transport protein of retinol [10] which delivers it to tissues as a retinol-RBP complex in circulation. RBP4 plasma levels are also associated with the development of endothelial dysfunction and clinical atherosclerosis through the induction of vascular inflammation and endothelial oxidative stress [11].

Furthermore, due to its low molecular weight, it is freely filtered through the glomeruli and then almost completely reabsorbed in the proximal tubules, Therefore, RBP-4 has been identified as a very sensitive biomarker for proximal tubular cells dysfunction [4].

In our study, we aim to evaluate the role of RBP 4 as a recent biomarker for the prediction of diabetic nephropathy in type 2 diabetic patients.

Our study showed that the serum level of RBP4 is significantly higher in diabetic group than control group and higher in diabetic patients with albuminurea (A2) than non albuminuric group (A1), and higher in each subgroup in comparison with control, denoting that RBP4 may play a role in the pathogenesis of DN.

These results agree with [12] who found that serum RBP4 levels were significantly higher in the diabetic group when compared with the control group and significantly higher in diabetic patients with microalbuminuria in comparison with non albuminuric group.

There may be two reasons to explain these differences in circulating RBP4 levels in diabetic subjects with and without kidney diseases. First, hepatocytes and adipocytes are important sites of synthesis of RBP4, whereas the kidneys are important sites of catabolism of circulating RBP4 [13]. Maintenance of retinol homeostasis throughout the body is mediated by filtration through the glomeruli and subsequent reabsorption of RBP4 in the proximal tubule tissues. Thus, reduced catabolism resulting from microvascular damage in the kidney leads to a gradual elevation in the plasma RBP4 concentration and hence to higher levels in subjects with DN than in T2DM patients without DN, second, RBP4 is an adipokine whose increased circulating levels are linked to IR in patients with diabetic kidney diseases [14]. This result differs from a meta-analysis done by [6] who found that

concentrations of RBP4 were similar in the normal albuminuria group in comparison with control but increased in the albuminuric group when compared with the control. This could be due to the fact that subjects in both the diabetic and control groups in this meta-analysis were obese. However, [14] and [15] had previously reported that the mean circulating RBP4 concentrations were comparable in the non-DM obese and DM obese subjects. Also, [2] found that the level of RBP4 showed non-significant difference between control and diabetic non albumiuric group which may be attributed to the small sample size in their study and that all the participants were Chinese. Kidney plays a pivotal role in control of homeostasis, so that the decline in GFR will be associated with inability to perform reabsorbtion of many materials including RBP4 in the proximal convoluted tubules. In our study there is a negative correlation between serum level of RBP4 with eGFR denoting that RBP4 increases with increasing the severity of renal damage. We also found a strong positive correlation between plasma level of RBP4 and urinary ACR, which showed that as microalbumin excretion increases, serum RBP4 increases pointing to a contributory role of serum RBP4 toward renal damage. These findings indicate that serum RBP4 increases with the severity of diabetic renal complications. This is correlated with the study of [2] who found that RBP4 had a significant positive correlation with microalbumin and a significant negative correlation with eGFR. To some extent our results differ from [6] who showed a poor correlation between RBP4 concentrations and ACR. Better correlation was observed between circulating RBP4 levels and eGFR than with ACR.

This difference may be attributed to several reasons, firstly, their sample size was small, and some critical data had not been presented in the publications. For example, only one study performed ROC analysis of prediction for eGFR and another one for albuminuria. Hence, they could not perform a pooled analysis for sensitivity and specificity in the diagnosis of eGFR and albuminuria. Secondly, the heterogeneity in this meta-analysis was obvious, although the sensitivity analysis indicated that the results were stable. The serum or plasma RBP4 concentrations had been measured by using different reagent kits, and the diagnostic thresholds of the various studies were not consistent. Additionally, there were some differences in the inclusion criteria of each study. All of these aspects may contribute to the heterogeneity in their results.

Our study showed significant differences between patient and control as regards the inflammatory marker HsCRP with also significant difference in patients' subgroups. This result illustrates that the serum hs-CRP concentration is strongly related to Type 2 and diabetic nephropathy. This finding was consistent with a known mechanism of CRP as a strong pro inflammatory agent that act to increase release of other inflammatory mediator as well as activate migration of macrophage and monocyte to the kidney which secret free radical and release of inflammatory agents these chemicals promote cells injury by amplifying inflammatory response.

In agreement with our study CRP plays an important role in mesenchymal cell proliferation by release of proinflammatory cytokines in addition to increase glomerular albumin permeability which finally lead to albuminuria [16].

This result agrees with [17] who found that there was a significant higher CRP level in patients' group than that of healthy control group and the concentration of CRP in serum samples was higher in diabetic patients with nephropathy than those without nephropathy. [18] reported that microalbuminuria was accompanied by elevated HsCRP, suggesting activation of inflammatory pathways in the progression of renal disease in Type 2 diabetic patients which is in agreement with our findings. In our study there is also positive correlation

between serum level of RBP4 level and HsCRP. The positive correlation between RBP4 and ACR and HsCRP with each other's illustrates that if we use a combination panel of plasma level of RBP4 as a tubular marker and ACR as a glomerular damage marker and HsCRP as inflammatory marker can promote the early prediction of DN and enhance its accuracy. These results agree with [19] who found that even though that tubular damage marker such as serum RBP4 as single diagnostic parameter of renal impairment showed excellent clinical accuracy a combination of markers of tubular damage, inflammation markers, and traditional markers has the higher sensitivity and specificity than urinary albumin alone.

From our results we found that RBP4 showed good accuracy in predicting diabetic nephropathy. Our study showed that it could detect cases with DM with cutoff point >12.5 ng/ml, AUC: 0.941 with sensitivity of 90%, specificity of 93.3%. We also found that RBP4 could predict diabetic nephropathy with cutoff point >24.8 ng/ml, AUC:0.917 with sensitivity of 86.67%, specificity of 93.33%.

5. Conclusion

Our study concluded that serum level of RBP4 was higher in type Π diabetic patients compared to control group and it increased in diabetic patients with micro albuminuria more than diabetic patients without micro albuminuria, increased level of RBP4 was negatively correlated with estimated GFR which denotes decline in renal function, these results may predict RBP4 as a good biomarker of detection of early DN.

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