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ORIGINAL RESEARCH PERIOPERATIVE MEDICINE

The association between retinol binding protein 4 and peripheral neuropathy in type 2 diabetic patient

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ABSTRACT

Background & Objective: The most common microvascular consequence of diabetes is peripheral neuropathy. A very common ailment that has a significant impact on a patient's quality of life is peripheral neuropathy (PN). The purpose of this study was to look into the possible link between retinol binding protein 4 (RBP4) and peripheral neuropathy in individuals with type 2 diabetes mellitus (T2D), as the relationship between RBP4 and PN is still unclear.

Methodology: We enrolled 152 participants who were matched for age and sex in a case control study during August and December 2023. There were 50 healthy controls, 50 diabetic patients without PN, and 52 diabetic patients with PN. ELISA was used to measure serum RBP4.

Results: Diabetic individuals with PN had significantly greater serum levels of RBP4 than diabetic patients without PN ($P < 0.001$). Increased age ($r = 0.262$, $P < 0.01$), DM duration ($r = 0.565$, $P < 0.01$), and BMI ($r = 0.183$, $P < 0.05$) were all substantially linked with RBP4 levels. RBP4 levels exhibited a negative correlation with high-density lipoproteins (HDL) and a positive correlation with total cholesterol, triglycerides, low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL).

Conclusion: Elevated RBP4 level is strongly and independently linked with PN in type 2 diabetic patients, and may play an important role in PN progression.

Abbreviations: DM - Diabetes mellitus; PN - peripheral neuropathy; PBP4 - retinol binding protein 4; T2D - type 2 diabetes mellitus

Keywords: diabetes mellitus; peripheral neuropathy; retinol binding protein 4.

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1. INTRODUCTION

Diabetes mellitus (DM) is a chronic, complicated metabolic disease that affects a large portion of the world's population. Hyperglycemia, the term for elevated blood glucose levels, is a hallmark of the illness. This occurs when the body fails to utilize the insulin that is produced or fails to produce enough of it.[1] Globally, the number of people with diabetes increased by 6.4 percent from 171 million in 2006 to 285 million in 2010. This is predicted to affect 439 million people by 2030, or a prevalence of 7.7%. From 2010 to 2030, While the amount of DM in developing countries would increase by 69%, the proportion of DM in developed nations will rise by 20%.[2]

"DM may be associated with extensive biochemical, functional and morphological defects that might be lead to many complications like nephropathy, neuropathy, retinopathy and to some extent, cardiovascular diseases and diabetic foot"[3] Hemoglobin A1C (HbA1c) levels and the length of DM duration are the primary predictors of neuropathy.[4]), with DM being the primary risk factor involved in its origin.

Diabetes-related neuropathy's etiology is still not fully understood. A number of factors, including metabolic ones (prolonged hyperglycemia, elevated blood fat, and decreased insulin levels), neurovascular ones (compromise of blood vessels supplying nutrients to nerves), autoimmune ones (inflammatory microenvironment surrounding nerves), mechanical injury to nerves, inherited traits that may increase susceptibility to nerve disease, and lifestyle factors, are likely responsible for nerve damage.[5]

RBP4 is the main vitamin A transport protein that makes it easier for retinol to be transported from the liver to peripheral tissues. Recently, RBP4 has been identified as an adipokine, several epidemiological studies have indicated that high serum RBP4 levels are crucial for the emergence of metabolic disorders such as type 2 diabetes and insulin resistance.[6] RBP4 is linked to several comorbidities of type 2 diabetes. For example, people with diabetic retinopathy (DR) had considerably higher plasma RBP4 levels. The RBP4/DR axis may be explained by a relationship between plasma RBP4 levels and oxidative stress, which is linked to the advancement of DR, and an unfavorable profile of inflammatory markers.[7] Significantly higher RBP4 levels were seen in T2DM participants with reduced GFR and chronic renal disease in diabetic nephropathy.[8]

2. METHODOLOGY

From August to December 2023, a case control study was carried out at the diabetes and endocrine center at Al-Sader Teaching Hospital in Najaf, Iraq. There were 152 participants in this study who were matched for age and sex. There were 50 healthy controls, 50 diabetic patients without PN, and 52 diabetic patients with PN. The patients are type 2 diabetics who come to the hospital on a regular basis for follow-up care. Insulin and/or oral hypoglycemic medications are used to treat their condition. Prior to the screening tests being conducted, the patients who were enrolled in this study signed an informed consent form. Every patient receives a thorough evaluation that includes a biochemical study, physical examination, and a thorough history. This study excluded females who were pregnant or nursing, patients with type I diabetes mellitus, females with gestational diabetes, patients with chronic renal disease, patients with chronic hepatic disease, and patients with peripheral neuropathy secondary to causes other than diabetes, such as chemotherapy-induced peripheral neuropathy and spinal cord disorders.

The Michigan Neuropathy Screening Instrument (MNSI), which consists of a lower extremity examination and a 15-item questionnaire, was used to determine the presence of PN. A score of more than 2.0 on the MNSI examination or seven or more positive responses on the MNSI questionnaire were considered indicators of PN.[9]

Each participant had a total of five milliliters of blood drawn in the morning. The blood was centrifuged at 3000 rpm for ten minutes to produce serum, which was then kept in Eppendorf tubes at -20 °C until analysis. Using an applied blood biochemical detector, the levels of triglycerides (TG), total cholesterol, and low-density lipoprotein cholesterol (LDL) were measured (Hitachi 7600, Hitachi, Japan). Ion exchange high-performance liquid chromatography (HPLC) methods are used by the D-10 Hemoglobin A1c Testing System to measure HbA1c. Using the Elabscience® Human RBP4 (Retinol Binding Protein 4) ELISA Kit (Elabscience Biotechnology Co., Ltd, USA) and the manufacturer's instructions, the concentration of serum RBP4 in µg/mL was determined.

2.1. Statistical analysis

Using the Lilliefors-corrected Kolmogorov-Smirnov test, the results group's distribution types were investigated. The normal distribution of the variable's data was expressed as (mean ± standard deviation). Conversely, the non-normally distributed variables' values were reported as medians and the interquartile range of 25% to 75%. Analysis of variance (ANOVA) was used to compare three or more groups, and the LSD (Least Significant Difference) test was used as a post-hoc analysis to evaluate each pair of groups. The Kruskal-Wallis test was used to compare three non-normally distributed variables. By computing the correlation Spearman's coefficients (ρ, rho), one can estimate the correlation between parameters. When $P < 0.05$, the difference between the groups is regarded as statistically significant. To further investigate the correlation between the two variables, Pearson's product-moment correlation coefficients were used. Receiver operating characteristics (ROC) curves were used to assess the RBP4's diagnostic efficacy in identifying neuropathy in patients with type 2 diabetes. The concentrations that result in the best sensitivity and specificity are known as the cut-off values. All statistical analyzes were conducted using IBM-USA and SPSS Statistics version 26. Plotting of the figures was done concurrently with Excel Microsoft Office 2021.

3. RESULTS

3.1. Clinical, socio-demographic, and hematology values

Table I displays the clinical and socio-demographic information for both the diabetic patients and the healthy control groups. According to the data, no significant difference in age, sex, and BMI among the three groups

Table 1: Comparative demographic and clinical characteristics of three groups

Parameter	Control [A]	T2DM [B]	T2DM+Neuropathy [C]	F/χ^2	P
Age (y)	47.16 ± 8.377	49.7 ± 6.894	50.808 ± 8.151	2.886	0.059
Gender Female/Male	21/29	20/30	19/33	2.016	0.362
BMI (kg/m^2)	27.682 ± 1.838	27.761 ± 3.549	27.775 ± 4.348	0.011	0.989
Body fat (%)	32.389 ± 6.483	32.864 ± 7.082	32.762 ± 7.099	0.066	0.936
Smoker	0	2	2	0.327	0.849
HbA1c (%)	4.77 ± 0.512	8.408 ± 1.168	9.508 ± 1.523	233.875	< 0.001
Metformin Yes	-	30 [C]	41 [B]	4.28	0.039
Insulin Yes	-	6 [C]	25 [B]	15.682	< 0.001
DPP4 Yes	-	6	7	0.049	0.825
TG (mM)	1.302 ± 0.179 [B,C]	1.779 ± 0.403 [A,C]	2.021 ± 0.306 [A,B]	70.582	< 0.001
Cholesterol (mM)	4.631 ± 0.537 [C]	4.807 ± 0.809 C	5.807 ± 0.595 [A,B]	47.862	< 0.001
HDL (mM)	1.256 ± 0.139 [B,C]	1.105 ± 0.229 [A,C]	0.941 ± 0.175 [A,B]	37.152	< 0.001
VLDL (mM)	0.595 ± 0.082 [B,C]	0.812 ± 0.184 [A,C]	0.923 ± 0.14 [A,B]	70.582	< 0.001
LDL (mM)	2.78 ± 0.584 C	2.89 ± 0.853 C	3.943 ± 0.601 [A,B]	44.540	< 0.001

[A,B,C]: Pairwise comparison. Data given as mean ± SD. F/χ^2: F-statistics value for continuous variables or Chi-square statistic value for binominal variables, *KWT: Kruskal-Wallis test,* P < *0.05 considered significant; BMI: body mass index, DPP4: dipeptidyl peptidase inhibitors.*

that were compared. When compared to diabetic patients without PN, diabetic patients with PN had significantly higher HbA1c values, longer durations of diabetes, and higher insulin usage.

3.2. Comparative RBP4 between patients and controls

The results displayed a significant decrease (P < 0.001) in RBP4 in the study groups with the highest value in the T2DM+Neuropathy group [91.075 (69.900-107.500) µg/mL], followed by T2DM [69.865 (37.300-95.943) µg/mL], to the lowest value in the control group [36.615 (26.900-49.775) µg/mL] as shown in Figure I.

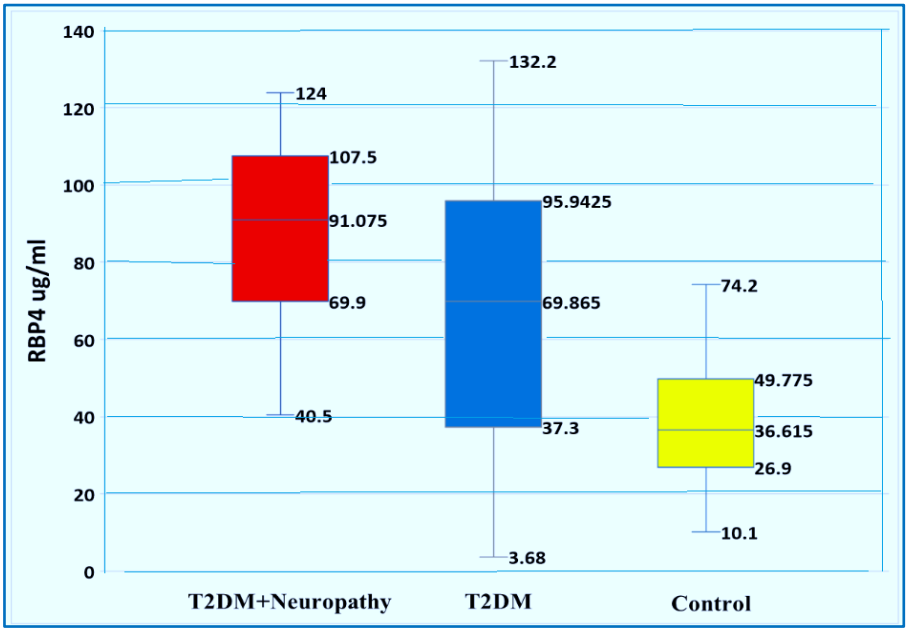

Figure I: Serum RBP4 in T2DM+Neuropathy, T2DM, and control groups. Error bars represent the 95% confidence intervals (CI).

3.3. Correlation between clinical characteristics and RBP4

The results in Table 2 indicate that RBP4 was positively correlated with age, BMI, and DM duration.

3.4. Correlation between lipid profile and RBP4

According to the findings of the correlation analysis between RBP4 and lipid profile, RBP4 had a negative correlation with HDL and a positive correlation with cholesterol, TG, VLDL, and LDL.

Table 2: Correlation between characteristics and clinical and RBP4

	Sex	Age	BMI	BFP	Smoker	DM duration
RBP4	0.025	0.262**	0.183*	0.149	0.064	0.565**

**: Significant correlation (P < 0.05), **: Highly significant correlation (P < 0.01)*

Table 3: Correlation of lipid profile with RBP4

	TG	cholesterol	HDL	VLDL	LDL
RBP4	0.576**	0.469**	-0.367**	0.576**	0.418**

**: Significant correlation (P < 0.05), **: Highly significant correlation (P < 0.01)*

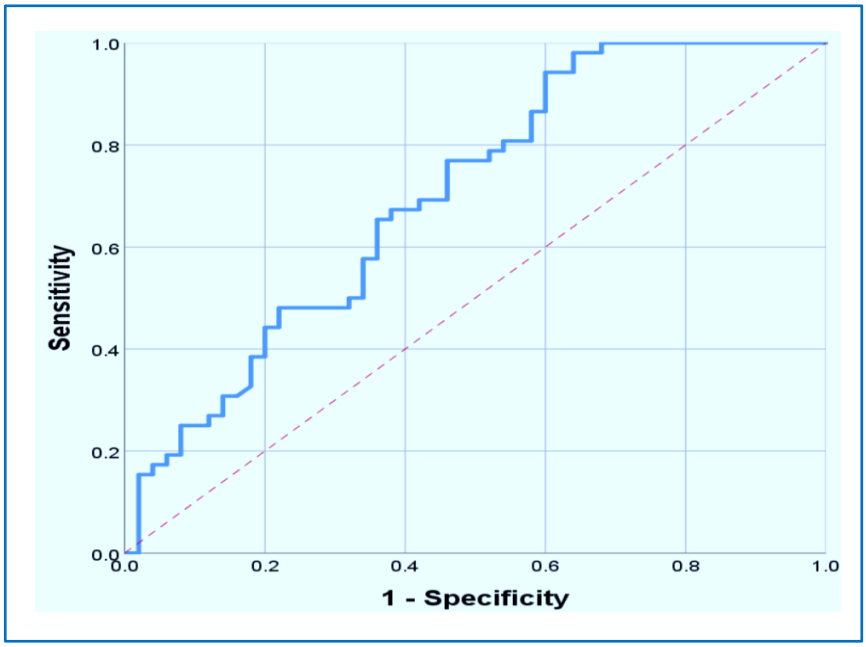

Figure 2: ROC curves of the RBP4 in prediction of PN in T2DM patients.

3.5. Receiver operating characteristics (ROC) study

Study of diagnostic ability of RBP4 for prediction of Neuropathy in T2DM patients. The receiver operating characteristics (ROC) analysis was conducted to evaluate the sensitivity and specificity at each concentration in order to assess biomarkers' diagnostic potential for identifying neuropathy in people with T2DM. Figure 2 displays the ROC curves for the measured parameters, and Table 4 presents the analysis's findings.

According to Table 4 data, there was a substantial rise in RBP4 levels (63.5%) and specificity (64.0%) in participants who had levels higher than the cut-off value (81.95 µg/mL), suggesting that they may have PN.

4. DISCUSSION

To the best of our knowledge, this is the first study investigating the association between RBP4 levels and diabetic PN. The major result of this study is that diabetic patients with PN had significantly and independently higher RBP4 levels than diabetic patients without PN. PN has been increasingly linked to oxidative stress in recent years. When the ratio of beneficial antioxidants to harmful free radicals is unbalanced and tilts toward oxidation, oxidative stress results.[10] Numerous investigations demonstrated the connection between oxidative stress indicators and RBP4 levels.

RBP4 reduces endothelial mitochondrial function, which increases oxidative stress.[11] According to Chevalier et al., there is a marked increase in RBP4 levels with aging. This could be because the general turnover of hepatic and whole-body proteins decreases with age.[12] There was no discernible impact of sex on RBP4 levels in this investigation. This finding is consistent with that of Bose et al., who reported no sex-related differences in RBP4 gene expression and no association between sex and RBP4.[13]

Regarding dyslipidemia, there is a strong positive association between serum total cholesterol, TG, LDL, VLDL, and RBP4 levels. Von Eynatten et al. demonstrated a substantial positive correlation between

Table 4: ROC-area under curve (AUC) analysis of RBP4 in prediction of PN in T2DM patients.

Test	Cut-off	Sensitivity %	Specificity %	Youden's statistic J	AUC (95% CI)	p-value
RBP4 µg/mL	81.95	63.5	64.0	0.275	0.701(0.599-0.802)	<0.001

**The decrease in these biomarkers is a predictor of Neuropathy; CI: Confidence interval.*

serum total cholesterol, TG, LDL, VLDL, and RBP4.[14] The reason for this could be that RBP4 increases lipid concentrations via controlling the expression of genes related to lipid metabolism and its impact on the metabolism of fatty acids in the liver.[15] Furthermore, there was a substantial negative association between HDL and RBP4 in the current study. Similar findings were made by Majerczyk et al. and Elsherbeny et al., who found a substantial negative association between RBP4 and HDL in the studied group.[16,17] Because of the decreased lipoprotein lipase activity and triglyceride enrichment, HDL declined in patients with diabetes, obesity, or metabolic syndrome.[18] It is possible that the negative link between RBP4 and HDL can be explained by the positive correlation that RBP4 has with diabetes, insulin resistance, and hypertriglyceridemia. Within the ROC area under the curve, RBP4 demonstrated a high degree of sensitivity (63.5%) and specificity (64.0%) in its diagnostic capacity for PN prediction in patients with diabetes. Thus, in diabetic individuals, RBP4 can be utilized to predict PN.

5. LIMITATION

The primary limitation of this research was the absence of analysis of other inflammatory biomarkers. Future studies may offer us comparative results of these biomarkers in this cohort of patients.

6. CONCLUSION

Elevated RBP4 level is strongly and independently linked with PN in type 2 diabetic patients, and may play an important role in PN progression.

7. Data availability

The numerical data generated during this research is available with the authors.

8. Acknowledgement

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9. Conflict of interest

The authors declare that there was no conflict if interest involved.

10. Funding

The study utilized the hospital resources only, and no external or industry funding was involved in the conduct of this study.

11. Authors' contribution

Both authors contributed equally in the conduct of this study and the data analysis as well as the manuscript preparation.

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