Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

The Role of RBP4 in Insulin Resistance and Type 2 Diabetes Mellitus

Iman Ali Nafakhi [1], Nibras Hussein Abdulsada[2]

[1] Pharmacist, Al-Nuaman teaching Hospital, Al-Rusafa Health Directorate, Baghdad, Ministry of Health, Iraq.

Email: imana.nafakhi@student.uokufa.edu.iq

[2] Assistant professor, Department of Pharmacology, College of Pharmacy, University of Kufa, Iraq.

Email: nibrash.abdalsada@uokufa.edu.iq

Abstract :

Retinol is transported from the liver to peripheral tissues via the vitamin A transport protein which known as a retinol binding protein 4 (RBP4). According to several epidemiological studies, the high concentrations of serum RBP4 are related to the risk of type 2 diabetes (T2DM) and insulin resistance. Therefore, the aim of the current study was to assess the role of RBP4 in the insulin resistance and type 2 diabetes. From August 2023 to December 2023, 100 participants who were matched for age (30-70) years and sex (59 female- 41 male) participated in a case control study. They divided into two groups: 50 patients with type 2 diabetes and 50 healthy individuals as a control group. Enzyme-Linked Immunosorbent Assay was used to measure the serum RBP4. According to results, RBP4 was considerably higher in patients with statistical significant (p value<0.001) compared to control group. This study was concluded that T2DM and insulin resistance were substantially correlated to levels of serum RBP4.

Keywords: insulin resistance, retinol binding protein 4 (RBP4), type 2 diabetes mellitus (T2DM).

Abbreviations: P stands for probability value. Body mass index (BMI), fasting blood glucose (FBG), insulin resistance (HOMA2IR), insulin sensitivity (HOMA2%S), and beta cell function percentage (HOMA2%B) are all terms related to homeostasis model assessment 2 (HOMA2). HbA1c is glycated hemoglobin, while I/G stands for insulin to glucose ratio.

Introduction : The condition known as diabetes mellitus (DM) is a complex and persistent metabolic illness that affects a substantial portion of the global population. High blood glucose levels are the primary manifestation of DM, a systemic metabolic disease caused by a partial or total inability to produce insulin and a deficient insulin action (1). With a frequency of 463 millions and a predicted 548 millions by 2045, DM ranked as the fifth most common cause of death worldwide in 2019. More than 90% of cases of diabetes are T2DM, making it the most prevalent form of the disease (2). DM affects around 1.4 million individuals in Iraq. The frequency of Type

II diabetes mellitus (T2DM) in Iraq varies between 8.5% and 13.9% (3). The hormone insulin, generate by pancreas, is responsible for regulating blood glucose levels and enhancing the uptake of glucose into body cells for energy production (4). Reduced sensitivity and response to insulin-mediated glucose clearance, and suppression of hepatic glucose synthesis are the standard definitions of insulin resistance (5). The homeostasis model assessment of insulin resistance (HOMA-IR) offers a rapid and cost-effective method for estimating the insulin resistance, which is regarded as a define feature of T2DM (6).

According to Tan et al. (2023), RBP4 is formerly thought to be a hormone generated by the hepatocytes and a carrier that moved retinol from the hepatocytes to the peripheral tissues (7). Hepatocytes are the main source for circulation of RBP4, with adipocytes and other cell types which are less. It has recently been established that RBP4 is an adipokine with endocrine, autocrine, or paracrine effects onto several body cells and organs, involved each of muscles, pancreas, brain, hepatocytes and adipocytes (8). Numerous epidemiological studies have found a correlation between the emergence of metabolic diseases like insulin resistance and T2DM and high serum RBP4 levels (9), therefore the aim of this study was to investigate the role of RBP4 in the development of insulin resistance and T2DM.

Materials and Methods

1) Collection of Samples

From August 2023 to December 2023, a case control study was carried out at the diabetic and endocrine center at Al-Sader Teaching Hospital in Najaf, Iraq. There were 100 age (30-70) years and sex (59 females-41 males) matched volunteers in this study. Two groups were formed: 50 patients with T2DM and 50 healthy individuals as control. Each patient was examined through laboratory tests, physical examination and taking the medical history.
Five milliliters of blood was drawn from patients and control in the fasting state. Blood samples then centrifuged at 3000 rpm for 10 minutes to get serum, which then kept in Eppendorf tubes at -20 °C until analysis.

2) The HOMA2 calculator program was used to compute the insulin resistance parameters, such as insulin resistance (HOMA2IR), beta-cell activity (HOMA2%B), and insulin sensitivity (HOMA2%S), using fasting insulin and fasting blood glucose (FBG). Using the Elabscience® Human RBP4 (Retinol Binding Protein 4) ELISA Kit (Elabscience Biotechnology Co.,Ltd, USA), the concentration of serum RBP4 in µg/ml was determined.

3) Inclusion criteria for case group involved patients with T2DM and exclude patients with T1DM and females who are pregnant or breast feeding or have gestational diabetes. Inclusion criteria for control group involved any healthy participants and exclude children, participants with chronic disease and smokers

4) Ethical approval: This study was conducted under approval by the medical ethics committee for the clinical studies at the faculty of medicine/University of Kufa (Ref#: MEC-32). Prior to the screening tests being conducted, the patients who were participated in our study signed an informed consent form.

5) Statistical analysis

All statistical analyzes were conducted using IBM-USA and SPSS Statistics version 26. 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%. ANOVA, or analysis of variance, was employed to compare the groups under study. The comparison of the non-normally distributed variables was computed using the Mann-Whitney U test.

By computing the correlation Spearman's coefficients (ρ, rho), one can estimate the correlation between parameters. When $p<0.05$, It seems that there is a statistically significant difference between the groups. To further investigate the correlation between the two variables, Pearson's product-moment correlation coefficients were used. Receiver operating characteristics (ROC) curves were made to assess the RBP4's diagnostic capacity in T2DM patient diagnosis. The concentrations that result in the best sensitivity and specificity are known as the cut-off values.

Results :(A) Comparison in sociodemographic parameters

The findings of the clinical and demographic parameters for T2DM patients and control group presented that there was no significant difference in patients and controls sex, age, body fat percentage and body mass index as shown in Table 1.

Table (1): Features of T2DM in terms of demographics compared to control groups.

Parameter	Control	T2DM	P
Age Yrs	47.16±8.377	49.7±6.894	0.059
Sex Female/Male	21/29	20/30	0.362
BMI Kg/M^2	27.682±1.838	27.761±3.549	0.989
Body Fat Percentage %	32.389±6.483	32.864±7.082	0.936

B) Comparison in insulin resistance parameters between patients and control

Between the T2DM and control group, there is a substantial difference in HbA1c, FBG, fasting insulin, HOMA2IR, HOMA2%B, HOMA2%S and I/G ratio as shown in Table 2.

Thi-Qar Medical Journal (TQMJ): Vol. (28), No. (2), 2024
Web Site: https://jmed.utq.edu Email: utjmed@utq.edu.iq
ISSN (Print):1992-9218 ISSN (Online): 3006-4791

Table (2): Comparison in insulin resistance parameters between patients and control

Parameter	Control	T2DM	P
FBG Mg/Dl	5.231±0.473	9.164±1.99	<0.001
Insulin Miu/Ml	58.873±8.948	94.112±25.979	<0.001
HOMA2%B	94.05 (79.325-110.95)	45.9 (34.525-60.875)	<0.001
HOMA2%S	91.798±11.889	54.962±18.25	<0.001
HOMA2IR	1.106 (0.973-1.202)	1.98 (1.574-2.354)	<0.001
I/G Nm	11.216 (9.491-12.879)	10.337 (8.761-12.493)	<0.001
HbA1c %	4.77±0.512	8.408±1.168	<0.001

C) Comparison in RBP4 between patients and controls

There was a noticeable variation in the outcomes (significant difference) ($p<0.001$) in RBP4 in the studied groups with the highest value in the 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 displayed in Figure 1.

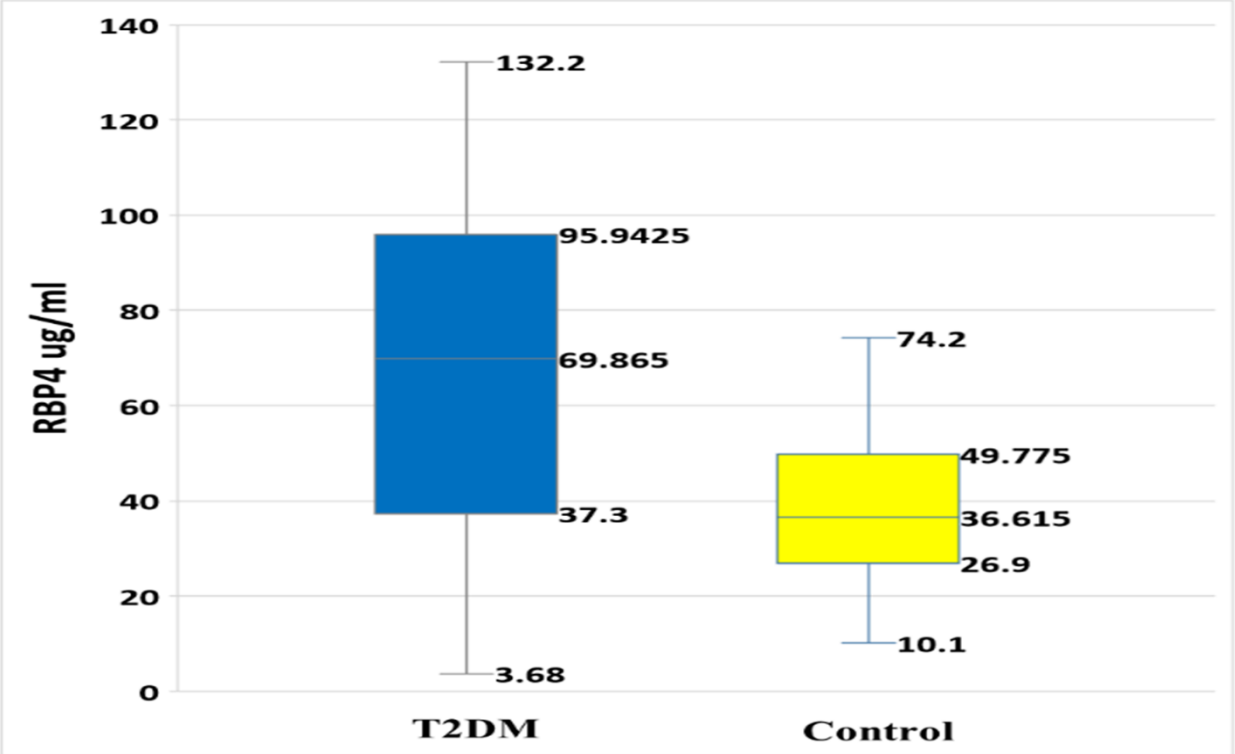

Figure (1). Serum RBP4 in T2DM and control group. Error bars represent the 95% Confidence-Intervals (CI).

D) Correlation of insulin resistance parameters and RBP4

There is a strong positive correlation between RBP4 and HbA1c, FBG, HOMA2IR and strong negative correlation between RBP4 and HOMA2%B, HOMA2%S and I/G ratio, there is no correlation between RBP4 and insulin serum levels as shown in Table 3.

Table (3): Correlation of insulin resistance parameters and RBP4

	HbA1c	FBG	Insulin	HOMA2 %B	HOMA2 %S	HOMA2 IR	I/G
RBP4	**0.494****	**0.555****	**0.093**	**-0.507****	**-0.231****	**0.231****	**-0.344****

*: High-Correlation (P<0.05), **: Very High-Correlation (P<0.01)

E) Receiver operating characteristics (ROC) study

Study of diagnostic ability of RBP4 for prediction of T2DM:

An analysis of receiver operating characteristics (ROC) was done to quantify the sensitivity and specificity at each concentration in order to assess the measured RBP4's diagnostic potential for T2DM diagnosis as shown in Figure 2 which display the plotted ROC curves for the measured RBP4. However, Table 4 presents the analysis's findings.

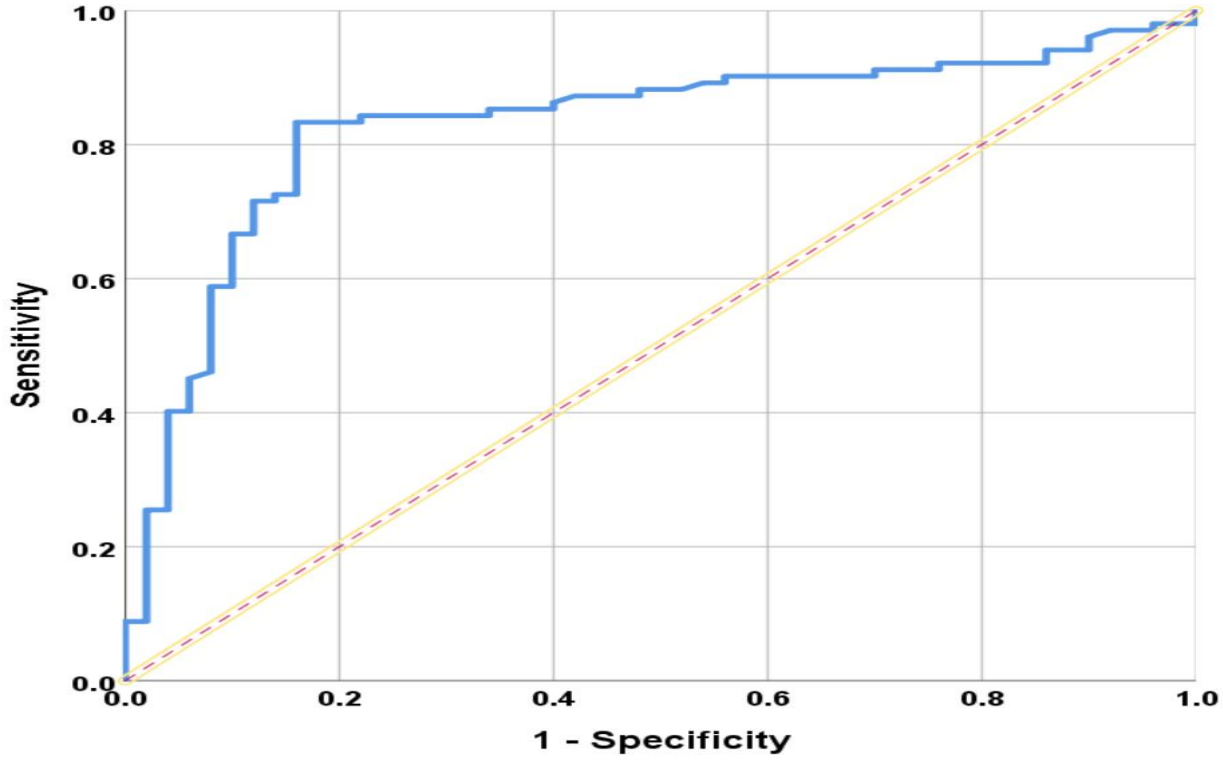

Figure (2). Serum RBP4 receiver operating characteristic curves for T2DM prediction.

With a significant sensitivity (83.3%) and specificity (84.0%), the results in Table 4 indicated that the participants may have T2DM if their RBP4 level increased beyond the cut-off value (55.45 µg/ml).

Table (4): Features of the RBP4 by ROC-AUC study for T2DM prediction.

Parameters	Cut-Off Value	Sensitivity %	Specificity%	Youden's J Statistic	AUC (95% CI)	P
RBP4 µg/Ml	**55.45**	**83.3**	**84.0**	**0.677**	**0.83(0.76-0.90)**	**<0.001**

CI: Confidence Intervals

Discussion : RBP4 is a type of the adipokines which assists in the evolution of insulin resistance by stimulating hepatic gluconeogenesis and disrupting insulin signaling both at the receptor level and post-receptor level. Liver and adipocytes are the main in vivo sources of RBP4. Insulin-resistant people and mice had higher plasma RBP4 levels (10). Elevated blood RBP4 concentrations have been noted in people with lean insulin resistance, demonstrating that the association between RBP4 and insulin resistance is not only related with obesity. Regardless of BMI, Serum RBP4 and insulin resistance are correlated, independent of BMI (11). By triggering JAK2/STAT5 signaling, which in turn causes SOCS3 activation, RBP4 directly suppresses insulin signaling in adipocytes. This pathway involves the membrane protein STRA6, and it is dependent on retinol. Through JNK- and TLR4-dependent pathways, RBP4 causes macrophages to become proinflammatory, which leads to the generation of cytokines that cause adipocytes to become insulin resistant. RBP4's proinflammatory effects on macrophages are independent of STRA6. Crucially, RBP4 may influence adipocyte insulin signaling directly or indirectly, which could lead to insulin resistance (12).

According to Jiménez-Martínez et al. (2023), RBP4 is essential for maintaining glucose homeostasis and GLUT transporter efficiency, which relates to changed metabolic states and the risk of T2DM (13). The mechanisms underlying the affirmative relation between RBP4 grade and the risk of T2DM include increased hepatic glucose production, decreased efficiency of the insulin receptor PI3K, inhibition of insulin signaling via promotion of the inflammatory status in adipocytes through the induction of cytokines in macrophages, and activation of the JAK2/STAT5 signal to prevent the transition of insulin signals in adipose tissue (7).

In agree with Elsherbeny et al. (2019), there was a positive correlation found between FBG, HbA1c and RBP4 in the current study. This finding may be explained by RBP4's involvement in high levels of insulin resistance and impaired glucose metabolism (14).

By agreement with our finding, Montserrat Broch et al. (2007), revealed that RBP4 had a negative correlation with β-cell function in their study. Chen et al. (2021), examined the effects of long-noncoding RNA (lncRNA) PTGS2 on pancreatic beta cell function. They discovered that via upregulating RBP4 and regulating miR-146a-5p, PTGS2 can negatively affect islet β-cell activity. This finding lends credence to the theory linking pancreatic beta cell function and RBP4 levels, Furthermore, it's noteworthy that studies have shown diabetic lowering drugs could enhance beta cell activity and lower circulation RBP4 levels (1). Along with the findings of Ülgen et al. (2010), who also found no link between fasting insulin and RBP4, the current investigation did not observe any significant relationship between the two variables (15). RBP4 demonstrated good sensitivity (83.3%) and specificity (84.0%) in the AUC of the ROC analysis evaluating its diagnostic abilities for T2DM prediction. RBP4 can therefore be used to prognose T2DM. The primary constraint of this research is the absence of examination of other inflammatory indicators.

Conclusion: Depending on this study, it is recommended to use the RBP4 level as a predictor of T2DM because of its significant correlation with both insulin resistance and T2DM.

References

1. Fan J, Hu J. Retinol binding protein 4 and type 2 diabetes: from insulin resistance to pancreatic β-cell function. Endocrine. 2024;

2. Wu Y, Fu R, Lei C, Deng Y, Lou W, Wang L, et al. Estimates of Type 2 Diabetes Mellitus Burden Attributable to Particulate Matter Pollution and Its 30-Year Change Patterns: A Systematic Analysis of Data From the Global Burden of Disease Study 2019. Front Endocrinol (Lausanne). 2021;12(August).

3. Abusaib M, Ahmed M, Nwayyir HA, Alidrisi HA, Al-Abbood M, Al-Bayati A, et al. Iraqi Experts Consensus on the Management of Type 2 Diabetes/Prediabetes in Adults. Clin Med Insights Endocrinol Diabetes. 2020;13.

4. Yun JS, Ko SH. Current trends in epidemiology of cardiovascular disease and cardiovascular risk management in type 2 diabetes. Metabolism [Internet].

2021;123:154838. Available from: https://doi.org/10.1016/j.metabol.2021.154838

5. Sampath Kumar A, Maiya AG, Shastry BA, Vaishali K, Ravishankar N, Hazari A, et al. Exercise and insulin resistance in type 2 diabetes mellitus: A systematic review and meta-analysis. Ann Phys Rehabil Med [Internet]. 2019;62(2):98–103. Available from: https://doi.org/10.1016/j.rehab.2018.11.001

6. Narayanan S, Gopikuttan R. The correlation of vitamin D with HOMA-IR and glycated hemoglobin in type 2 diabetes mellitus patients. Baghdad J Biochem Appl Biol Sci. 2022;3(04):273–85.

7. Tan X, Zhang H, Liu L, Yu Z, Liu X, Cui L, et al. The association between retinol-binding protein 4 and risk of type 2 diabetes: A systematic review and meta-analysis. International Journal of Environmental Health Research. 2023.

8. Weschenfelder C, De Quadros AS, Dos Santos JL, Garofallo SB, Marcadenti A. Adipokines and adipose tissue-related metabolites, nuts and cardiovascular disease. Metabolites. 2020;10(1).

9. Fan J, Yin S, Lin D, Liu Y, Chen N, Bai X, et al. Association of serum retinol-binding protein 4 levels and the risk of incident type 2 diabetes in subjects with prediabetes. Diabetes Care. 2019;42(8):1574–81.

10. Majerczyk M, Kocełak P, Choręza P, Arabzada H, Owczarek AJ, Bożentowicz-Wikarek M, et al. Components of metabolic syndrome in relation to plasma levels of retinol binding protein 4 (RBP4) in a cohort of people aged 65 years and older. J Endocrinol Invest [Internet]. 2018;41(10):1211–9. Available from: https://doi.org/10.1007/s40618-018-0856-6

11. Graham TE, Yang Q, Blüher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-Binding Protein 4 and Insulin Resistance in Lean, Obese, and Diabetic

Subjects. N Engl J Med. 2006;354(24):2552–63.

12. Norseen J, Hosooka T, Hammarstedt A, Yore MM, Kant S, Aryal P, et al. Retinol-Binding Protein 4 Inhibits Insulin Signaling in Adipocytes by Inducing Proinflammatory Cytokines in Macrophages through a c-Jun N-Terminal Kinase- and Toll-Like Receptor 4-Dependent and Retinol-Independent Mechanism. Mol Cell Biol. 2012;32(10):2010–9.

13. Jiménez-Martínez P, Ramirez-Campillo R, Alix-Fages C, Gene-Morales J, García-Ramos A, Colado JC. Chronic Resistance Training Effects on Serum Adipokines in Type 2 Diabetes Mellitus: A Systematic Review. Vol. 11, Healthcare (Switzerland). 2023.

14. Elsherbeny AA, Abdel E, Abdel M, Elmahdi AR. Journal of Metabolic Syndrome Retinol Binding Protein 4 : Possible Relation between Insulin Resistance in Type 2 Diabetes and Visceral Obesity. 2019;8(2):2–6.

15. Ülgen F, Herder C, Kühn MC, Willenberg HS, Schott M, Scherbaum WA, et al. Association of serum levels of retinol-binding protein 4 with male sex but not with insulin resistance in obese patients. Arch Physiol Biochem. 2010;116(2):5762.