DOI: https://doi.org/10.31351/vol29iss2pp263-270

Association of Retinol Binding Protein- 4 (RBP4) with Glycemia, Dyslipidemia, Hypertension, and Obesity in Type 2 Diabetic Iraqi Patients

Thair L. Jabbar[*,1] and Ali A. Kasim [**]

[*] Ministry of Health and Environment ,Thiqar Health Directorate, Nasiriya , Iraq.
[**]Department of Clinical Laboratory Sciences, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract

Retinol binding protein 4 (RBP4), an adipokine that participate in a lipid metabolism or insulin resistance through a complex regulatory network. Recently, RBP4 was reported to be associated with many cardiovascular diseases (CVDs) risk factors in type 2 diabetes mellitus (T2DM) patients. This study aims to study the correlation of serum RBP4 with some markers of glycemic control, dyslipidemia, hypertension, and obesity in T2DM Iraqi patients. One hundred twenty (120) T2DM patients were enrolled in this cross-sectional study.

Serum RBP4 levels are higher in T2DM patients with dyslipidemia, hypertension, or obesity. The difference was statistically non significant between hypertensive and normotensive T2DM patients. Serum RBP4 is positively correlated with body mass index, fasting blood glucose, glycosylated hemoglobin (HbA1c), systolic blood pressure ($P<0.001$), and low density lipoprotein cholesterol ($P<0.05$), and negatively correlated with high density lipoprotein cholesterol ($P<0.001$).

Serum RBP4 is correlated with many risk factors of CVD including, poor glycemic indices, dyslipidemia, hypertension, and obesity, in T2DM Iraqi patients.

keywords: RBP4, Type 2 diabetes mellitus, Dyslipidemia, Hypertension, Obesity.

ارتباط البروتين الرابط للريتينول – ٤ مع جلوكوز الدم، شحوم الدم، ارتفاع ضغط الدم، والسمنة في مرضى السكري النوع الثاني في العراق

ثائر لطيف جبار*[،١] و علي عبد الحسين قاسم**

* وزارة الصحة والبيئة ، دائرة صحة ذي قار، الناصرية،العراق
** فرع العلوم المختبرية السريرية، كلية الصيدلة، جامعة بغداد ، بغداد ، العراق .

الخلاصة

يشارك بروتين ربط الريتينول ٤ ((RBP4)بشكل كبير في استقلاب الدهون أو مقاومة الأنسولين من خلال شبكة تنظيمية معقدة. حديثا، التقارير العلمية تتحدث عن ارتباط RBP4 بالعديد من عوامل الخطر القلبية الوعائية لدى مرضى السكري من النوع ٢. تهدف هذه الدراسة إلى دراسة ارتباط المصل RBP4 مع بعض مؤشرات السيطرة على نسبة السكر في الدم، اضطراب نسبة الشحوم، وارتفاع ضغط الدم والسمنة لدى مرضى السكري العراقيين من النوع ٢.

تم تسجيل ما مجموعه ١٢٠ مشاركًا كانوا من مرضى السكري النوع ٢ في هذه الدراسة المستعرضة.مستويات RBP4 في الدم كانت عالية لدى مرضى السكري من النوع ٢ الذين يعانون اضطراب شحوم الدم، أو ارتفاع ضغط الدم، أو السمنة. الاختلاف لم يكن ذو دلالة إحصائية بين مرضى السكري المصابين ارتفاع ضغط الدم عن اولائك ذوو الضغط المنتظم. أظهر المصل RBP4 ارتباطًا إيجابيًا بمؤشر كتلة الجسم، وجلوكوز الدم في الصيام، والهيموغلوبين السكري المتراكم، وضغط الدم الانقباضي ($P <0.001$)، وكولسترول البروتين الدهني ذو الكثافة المنخفضة ($P <0.05$)وارتباطا سلبًيا مع كوليسترول البروتين الدهني ذو الكثافة العالية ($P<0.001$).

يرتبط المصل RBP4 بالعديد من عوامل الخطر لأمراض القلب والأوعية الدموية كضعف مؤشرات تنظيم سكر الدم او اضطراب الشحوم او ارتفاع ضغط الدم او السمنة في عينة من مرضى السكري من النوع ٢ في العراق.

الكلمات المفتاحية: RBP4، داء السكري من النوع ٢، اضطراب شحوم الدم، ارتفاع ضغط الدم، السمنة .

Introduction

Diabetes mellitus (DM) is a common endocrinopathy which is usually presented as a set of metabolic defects characterized mainly by hyperglycemia as a result of impairment in insulin sensitivity and/or secretion [(1)]. Long-term

[1]Corresponding author E-mail**:** thairjabbar9@gmail.com
Received: 6/7 /2020
Accepted: 31/8 /2020

hyperglycemia causes damage and dysfunction of multiple organs; including kidneys, heart, eyes, nerves, and blood vessels. The cardiovascular diseases (CVDs) are the main cause of morbidity and mortality in DM patients [(2)], as indicated by the intensive regulation of glucose and lipid levels, in addition to blood pressure and body weight are essential to reduce the hazard of cardiovascular complications and diabetes advancement [(3)].

Type 2 diabetes (T2DM), hypertension, and dyslipidemia can *occur comorbidly* and all participate in the development of atherosclerosis [(4)], an important pathological hallmark in the cardiovascular diseases (CVDs). Obesity is common in about 90–95% of adult type 2 DM patients [(5)]. It is manifested by low-grade chronic inflammation with increased systemic adrenergic activity, and perturbed metabolism of lipid and glucose [(6)].

Retinol binding protein 4 (RBP4) is an adipokine, synthesized primarily by the liver; a smaller amount is synthesized in the adipose tissue [(7)]. Increased systemic RBP4 concentrations can be seen in conditions of impaired glucose tolerance, T2DM, and obesity. The link of RBP4 with insulin resistance (IR) **shows** certain degree of controversy [(8, 9)]. In the liver, RBP4 increases fasting blood glucose by augmenting the action of phosphoenolpyruvate carboxykinase (PEPCK) enzyme increasing hepatic glucose liberation. In skeletal muscle and white adipose tissue, RBP4 decreases the insulin receptors, as well as the alterations in phosphorylation of some signal proteins, such as insulin receptor substrate (IRS), PDK1 and Akt [(10, 11)].

Apart from its effects on glucose metabolism, RBP4 also affects lipid metabolism. Graham *et al.* described that high RBP4 levels were associated with high serum triacylglycerol (TG) and low high-density lipoprotein cholesterol (HDL-C) levels in lean, obese, and in diabetic subjects [(12)]. In a study conducted on Chinese obese subjects, Wang *et al.* showed that RBP4 affects lipid metabolism in gender dependent manner. He also reported that high serum RBP4 correlates with high total cholesterol (TC) and TG levels in Chinese obese female subjects. Also, females with high serum TG or TC levels have higher serum RBP4 levels [(13)]. Moreover, genetic variant of Apolipoprotein A5 (APOA5 gene) enhances the association of serum levels of RBP4 and TG in T2DM patients [(14)].

The aim of this work was to study the associations between circulatory RBP4 and different risk factors for cardiovascular diseases in a sample of Iraqi T2DM patients.

Subjects and Methods

One hundred twenty (120) T2DM patients were enrolled in this cross sectional study, which was conducted in The Specialized Center of Endocrinology and Diabetes in AL-Nasiriya city, south of Iraq from 4th October 2019 to 30th March 2020. T2DM patients were already diagnosed according to the American Diabetes Association Criteria [(15)]; of age ≥ 18years with mean age of them was (50 ±8) years, ranging from 33 to 69 years, and fifty-two of the diabetic subjects were males and 68 were females. with disease duration since diagnosis of at least 1 year.

Pregnant women, patients with chronic renal failure and hepatic failure were excluded in this study.

This work was performed according to the Helsinki II Declaration [(16)] and approved by the Ethics Committee of the College of Pharmacy, University of Baghdad. All participants were informed about the purpose and the expected benefits of the study before agreement of participation was documented.

Sociodemographic and medical histories were taken for each participant; venous blood samples were collected after fasting for 12 hours. Ten milliliters blood samples were withdrawn, about (2 ml) of each sample was transferred into ethylene diamine tetracetic acid (EDTA) tubes to be stored at (+2 to +8 C) for analysis of HbA1c within 1 week by high-performance liquid chromatography [(17)]. The remaining (8 ml) samples were transferred into plane tubes and centrifuged for 10 minutes to obtain serum. The collected sera were stored frozen at (–20ºC) as aliquots in Eppendorf tubes until analysis. Fasting blood glucose (FBG) and lipid profile were analyzed using suitable colorimetric assays [(18-22)]. The assay of serum RBP4 was performed by commercially available human enzyme-linked immunosorbent assay (ELISA) kit [(23)].

Statistical analysis

The statistical analysis was achieved using SPSS® v.25. Data are presented as mean ± standard error. Student t-test was used to compare means of two groups; while, analysis of variance (ANOVA) test was used to compare means of more than two groups, which is followed by post hoc analysis using Tukey's test to describe differences among different groups. Categorical variables were expressed as number and tested using chi-square (χ^2) test. The correlation between different parameters and RBP4 was tested using Pearson's correlation. Level of significance is set to $P<0.05$.

Results

Based on the dyslipidemia status, T2DM patients were grouped as dyslipidemic (n=66), and normolipidemic (n=54); according to "The American Association of Clinical Endocrinologists' (AACE)" 2017[(24)]. Sociodemographic and clinical characterizes for each group are presented in table-1. There difference between the dyslipidemic and normolipidemic T2DM patients concerning gender, body mass index (BMI), smoking habit, diastolic blood pressure (DBP), and high density lipoproteins cholesterol (HDL-C) was non-significant ($P>0.05$). Meanwhile, age, disease duration, systolic blood

pressure (SBP), serum RBP4, fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), and low density lipoproteins cholesterol (LDL-C) were significantly higher in diabetic patients with dyslipidemia (P<0.05).

Table- 1. Characteristics of dyslipidemic and normolipidemic T2DM patients

Variable	T2DM		P-value
	Dyslipidemic (n=66)	Normolipidemic (n=54)	
Age(years)	51.6±0.9	48±1	0.01*
Gender (M/F)	30/36	22/32	0.6
BMI(kg/m^2)	29.8±0.4	28.9±0.6	0.2
Disease Duration (years)	11±0.5	6±0.6	0.0001*
Smokers number	23	18	0.8
SBP (mmHg)	135.8±2	129±2	0.04*
DBP (mmHg)	78.7±1	77.9±1	0.7
S.RBP4(pg/ml)	519.9±23	377.5±19	0.0001*
FBG (mg/dl)	218.3±7	172.2±7	0.0001*
HbA1c%	9.6±0.2	8.2±0.2	0.0001*
VLDL-C(mg/dl)	43.4±2.6	224.8±1	0.0001*
LDL-C (mg/dl)	108.7±6	90.4±4	0.018
HDL-C (mg/dl)	42.6±1	46.1±1	0.06

* Significant when p<0.05

In table-2, T2DM patients were grouped according to the prevalence of hypertension, diagnosed according to "The World Health Organization International Society of Hypertension Guidelines" [25]. There difference between the hypertensive and normotensive T2DM patients concerning gender, smoking habit, serum RBP4, FBG, HbA1c%, and lipid profile parameters was nonsignificant (P>0.05). Meanwhile, age, disease duration, and BMI were significantly higher in hypertensive T2DM patients (P<0.05).

Table- 2. Characteristics of hypertensive and normotensive T2DM patients

Variable	**T2DM**		**P-value**
	Hypertensive (BP>140/90 mmHg) (n=30)	**Normotensive (BP≤140/90 mmHg) (n=90)**	
Age (years)	54±1.3	48±0.8	0.001*
Gender (M/F)	15/15	37/53	0.4
BMI (kg/m^2)	30.7±0.7	28.9±0.4	0.03*
Disease Duration (years)	11±1	8±0.4	0.001*
Smokers number	13	28	0.2
S.RBP4 (pg/ml)	449±28	413.7±18	0.12
FBG (mg/dl)	204±11	195.2±6	0.5
HbA1c%	9.3±0.2	8.9±0.19	0.3
TG (mg/dl)	165±19	178±9	0.5
TC (mg/dl)	169±9	183±4	0.19
VLDL-C (mg/dl)	33±3	35±2	0.5
LDL-C (mg/dl)	93±8	102±4	0.3
HDL-C (mg/dl)	43.6±1.6	44.1±1	0.4

In table-3, T2DM patients were categorized into three groups, depending on BMI, into: normal weight, overweight and obese; non of the participant was underweight [26]. Serum RBP4 levels were significantly higher in obese T2DM patients than in normal weight patients (p=0.0001). Similarly, diabetes duration, systolic BP, and the diastolic BP were significantly higher and serum HDL-C levels were significantly lower in obese T2DM patients compared to the normal weight patients (P<0.05). HbA1c was significantly higher in obese T2DM patients compared to normal weight and overweight patients (P<0.05). There was no significant difference in age, gender, smoking habit,

FBG, total cholesterol (TC), triglyceride (TG), very low density lipoprotein (VLDL-C) and LDL-C among obese, overweight and normal weight T2DM patients.

Table-3. Characteristics of obese, overweight, and normoweight T2DM patients

Variable	T2DM			p-value
	Obese BMI>30 n=54	Overweight BMI(25-30) n=46	Normoweight BMI(18.5-24.9) n=20	
Age (years)	49±1	51±1	49±2	0.5
Gender (M/F)	25/29	21/25	6/14	0.4
Disease Duration (years)	10±0.7[a]	8.9±0.6[ab]	5±0.8[b]	0.001*
Smokers number	20	17	4	0.3
SBP (mmHg)	135±2[a]	134±2[ab]	121±2[b]	0.01*
DBP (mmHg)	80±1[a]	77±1[ab]	74±2[b]	0.03*
S.RBP4 (pg/ml)	499.5±21[a]	453.8±21[ab]	311.3±29[b]	0.0001*
FBS (mg/dl)	196±5	196±8	171±15	0.5
HbA1c%	9.5±0.2[a]	8.7±0.2[b]	8±0.4[c]	0.007*
TG (mg/dl)	184±14	178±12	142±15	0.2
TC (mg/dl)	185±7	178±6	167±10	0.3
VLDL-C (mg/dl)	36±2	35±2	28±3	0.24
LDL-C (mg/dl)	106±6	98±5	89±9	0.3
HDL-C (mg/dl)	42±1[b]	44±1[ab]	49±2[a]	0.02*

superscripts (**a,b,c**) among different groups are considered significantly different (P<0.05)

Correlation studies of serum RBP4 with the studied variables of T2DM patients are presented in table- 4. Serum RBP4 was positively correlated with BMI, FBG, HbA1c, and SBP (P<0.001), and LDL-C (P<0.05) and negatively correlated with HDL-C (P<0.001). While, there was no significant correlation between serum RBP4 levels and age, gender, DBP, or TG, VLDL-c, and TC levels (P>0.05). Strong positive correlation between serum RBP4 levels and diabetes duration in years was also recorded (P < 0.001).

Table- 4. Pearson's correlations of serum RBP4 levels with the studied variables

Variable	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*
SBP	0.2	0.02*
DBP	012	0.17
FBG	0.6	0.000*
HBA1c	0.4	0.000*
TC	0.17	0.06
TG	0.12	0.17
VLDL-C	0.12	0.17
LDL-C	0.25	0.008*
HDL-C	-0.45	0.000*

Discussion

In the present study, serum RBP4 has significant positive correlation with markers of glycemic control, FBG and HbA1c (table 4). Large mass of evidence suggest an important role played by RBP4 in the development of T2DM and its chronic complications, as well as, with biochemical markers of carbohydrate metabolism. Elevated serum RBP4 levels associate with an augmented risk of transferring impaired glucose tolerance into overt T2DM [27]. In addition, it is associated with IR in T2DM patients [28, 29]. Moreover, RBP4 expression is elevated in T2DM patients with retinopathy [30], nephropathy [31] and CVD [32]. Graham *et al.* reported positive correlation of serum RBP4 levels with FBG and with HbA1c levels [12]. However, serum RBP4 level and RBP4 synthesis speed were reported to be diminished in type 1 DM patients

compared to control individuals [33, 34]. As stated earlier, RBP4 participates in IR by increasing the hepatic liberation of glucose, an action mediated by augmenting PEPCK, and by decreasing the insulin receptors expression and interfering with intracellular insulin signaling in muscle and adipose tissue [10, 11].

Unique pattern of dyslipidemia occurs in T2DM patients, characterized by hypertriglyceridemia, high blood levels of Apolipoprotein B and of small dense LDL (sdLDL), with low HDL-C levels. Serum TC levels are high but not to the levels of TG [35]. Alterations in lipid profile in DM are attributed to increased lipolysis secondary to insulin resistance, and hence, there is an inappropriate spillover of TG and free fatty acids (FFAs) [36].

In the present study, Serum RBP4 levels in dyslipidemic diabetic patients were higher than in normolipidemic patients. Dyslipidemic T2DM patients were older with longer diabetes duration, higher systolic blood pressure and poorer glycemic control as compared with normolipidemic T2DM patients (Table-1). Moreover, serum RBP4 positively correlated with LDL-c level and negatively correlated with HDL-C levels, but there was no significant correlation with TG and VLDL-C levels (Table- 4). This may be explained by the use of antilipidemic medicines by most of T2DM patients that may disguised the actual relationship between serum RBP4 and lipid profile.

Mounting data proposes that RBP4 contributes in lipid metabolism by means other than its role in IR. Many clinical studies showed that serum RBP4 levels correlate positively with blood TG levels, but not with IR [37-39]. However, some studies that examined the link between serum levels of RBP4 and TG have showed an association with IR [40, 41]. A positive association between serum levels of RBP4 and atherogenic lipoproteins in T2DM patients has been reported previously [37, 42, 43]. Yamasaki *et al.* proposed that RBP4 may interfere with lipoprotein remnant metabolism [42]. While, Vergès *et al.* showed that RBP4 may reduce the catabolic rate of VLDL [37].

Many studies showed a close association between serum RBP4 levels and blood pressure in prehypertensive or in untreated essential hypertension patients [44, 45]. In the present study, the difference in serum RBP4 levels between hypertensive and normotensive T2DM patients was statistically nonsignificant (table-3). Yet, serum RBP levels correlate positively with the SBP but not the DBP levels (table -4).

High prevalence of hypertension in type 1 or 2 DM patients, is well established [46, 47]. Actually, about 40% of T2DM patients are already hypertensive when diagnosed as having DM [48]. Ephraim *et al.* reported that 37.4% of T2DM patients have systolic, but not diastolic, hypertension; and hyperglycemia in those patients was worse than in normotensive T2DM patients [49]. Hyperglycemia aids in vascular stiffness and raised vascular tonicity resulting in increased SBP [50]. Arterial stiffness develops mainly as a consequence of aging [51]. Pinto *et al.* has reported an increase in SBP in hypertensive patients, aged 50 years or older, arising either as primary event or developed after prolonged period of systolic-diastolic hypertension whether on antihypertensive treatment or not [46]. Still, progression of arterial stiffness can be hastened if it is co-occupied by additional risk factors, particularly elevated blood glucose levels, [52, 53]. Hyperglycemia, enhance development of arterial stiffness via several mechanisms that lead to endothelial dysfunction along with structural modifications of vascular extracellular matrix [54, 55].

In the present study, obese T2DM patients have elevated serum levels of RBP4 when compared to normoweight T2DM patients. Furthermore, obese T2DM patients have longer diabetes duration, and higher SBP, DBP and HbA1c levels, when compared to normoweight T2DM patients. On the contrary, HDL-C levels in obese T2DM patients are lower than that of normoweight T2DM patients (Table- 3). Additionally, serum RBP4 levels show significant positive correlation with BMI (tTable-4).As RBP4 is an adipokine that is likely relating obesity, IR and T2DM [12, 56-58]. Graham *et al.* reported a positive correlation between serum RBP4 and BMI or waist-to-hip ratio; and normoweight individuals with IR also showed elevated levels of serum RBP4 indicating that IR was independent of obesity [12]. In severely obese patients, expression of RBP4 is enhanced in the omental visceral fat [59]. Significant reduction in body weight, by dietary restrictions, physical workout, or by surgical interventions, leads to a reduction in RBP4 in blood and/or in adipose tissue [12, 60-62]. The decrease in RBP4 in non-diabetic individuals, accompanying bodyweight reduction, positively associate with the reduction of fat content in the omental or mesenteric regions and not with the reduction in the whole body fat [61].

Several gene variants of *RBP4* were associated with the level of adiposity and central obesity characterized by elevated BMI and waist-to-hip ratio [63]. A regulatory single-nucleotide polymorphism (SNP) *in its* gene enhanced RBP4 expression with predisposition for obesity, by increasing lipogenesis [56]. In addition, diabetes associated RBP4 haplotype carriers have been reported to overexpress visceral *RBP4* [63].

Conclusion

Serum RBP4 correlates with different risk factors of cardiovascular diseases; including glycemic control, dyslipidemia, hypertension and obesity, in T2DM Iraqi patients

References

1. American Diabetic Association. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020;43(Suppl 1):S14.
2. Kishimoto S, Kinoshita Y, Matsumoto T, Maruhashi T, Kajikawa M, Matsui S, et al. Effects of the Dipeptidyl Peptidase 4 Inhibitor Alogliptin on Blood Pressure in Hypertensive Patients with Type 2 Diabetes Mellitus. American journal of hypertension. 2019;32(7):695-702.
3. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. The Lancet. 2017;389(10085):2239-51.
4. Van den Berg E, Kloppenborg RP, Kessels RP, Kappelle LJ, Biessels GJ. Type 2 diabetes mellitus, hypertension, dyslipidemia and obesity: a systematic comparison of their impact on cognition. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 2009;1792(5):470-81.
5. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, et al. Heart disease and stroke statistics--2015 update: a report from the American Heart Association. Circulation. 2015;131(4):e29-322.
6. Scherer PE, Hill JA. Obesity, Diabetes, and Cardiovascular Diseases: A Compendium. Circulation research. 2016;118(11):1703-5.
7. Perduca M, Nicolis S, Mannucci B, Galliano M, Monaco HL. Human plasma retinol-binding protein (RBP4) is also a fatty acid-binding protein. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2018;1863(4):458-66.
8. Kotnik P, Fischer-Posovszky P, Wabitsch M. RBP4: a controversial adipokine. European journal of endocrinology. 2011;165(5):703-11.
9. Esteve E, Ricart W, Fernández-Real JM. Adipocytokines and insulin resistance: the possible role of lipocalin-2, retinol binding protein-4, and adiponectin. Diabetes care. 2009;32(suppl 2):S362-S7.
10. Aigner E, Datz C. Retinol-Binding Protein 4 as an Adipokine. In: Hunter iRPRJ, editor. Adipokines. London: CRC press; 2016. p. 110.
11. Muniyappa R, Montagnani M, Koh KK, Quon MJ. Cardiovascular actions of insulin. Endocrine reviews. 2007;28(5):463-91.
12. Graham TE, Yang Q, Bluher M, Hammarstedt A, Ciaraldi TP, Henry RR, et al. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. The New England journal of medicine. 2006;354(24):2552-63.
13. Wang X, Huang Y, Gao J, Sun H, Jayachandran M, Qu S. Changes of serum retinol-binding protein 4 associated with improved insulin resistance after laparoscopic sleeve gastrectomy in Chinese obese patients. Diabetology & metabolic syndrome. 2020;12(1):7.
14. Cabré A, Lázaro I, Girona J, Manzanares Errazu J-M, Marimon F, Plana N, et al. The APOA5-1131 T>C variant enhances the association between RBP4 and hypertriglyceridemia in diabetes. Nutr Metab Cardiovasc Dis. 2009;20:243-8.
15. Association AD. Standards of medical care in diabetes—2014. Diabetes care. 2014;37(Supplement 1):S14-S80.
16. PP R. Human experimentation. Code of ethics of the world medical association. Declaration of Helsinki. British medical journal. 1964;2(5402):177-.
17. Davis JE, McDonald JM, Jarett L. A high-performance liquid chromatography method for hemoglobin A1c. Diabetes. 1978;27(2):102-107.
18. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by the oxidase system. Analyst. 1972;97(1151):142-5.
19. Naito H.K. Cholesterol. Kaplan A *et al*. *Clin Chem.* The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1194-11206 and 437.
20. Bucolo G, David H. Quantitative determination of serum triglycerides by the use of enzymes. Clinical chemistry. 1973;19(5):476-82.
21. National Institute of Health Consensus Development Conference Statement: Triglyceride, High Density Lipoprotein and Coronary Heart Disease. *NIH Consens Statement* 1992; 26-28; 10(2):1-28.
22. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clinical chemistry. 1972;18(6):499-502.
23. Aydin S. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. Peptides. 2015;72:4-15.
24. Jellinger PS, Handelsman Y, Rosenblit PD, Bloomgarden ZT, Fonseca VA, Garber AJ, et al. American Association of Clinical Endocrinologists and American College of Endocrinology Guidelines for Management of Dyslipidemia and Prevention of Cardiovascular Disease. Endocrine practice : official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2017;23(Suppl 2):1-87.
25. Beaney T, Burrell LM, Castillo RR, Charchar FJ, Cro S, Damasceno A, et al. May Measurement Month 2018: a pragmatic global screening campaign to raise awareness of blood pressure by the International Society of Hypertension. European heart journal. 2019;40(25):2006-17.

26. Consultation WHOE. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004;363(9403):157-63.
27. 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.
28. Cheng X, Zhang H. Serum retinal-binding protein 4 is positively related to insulin resistance in Chinese subjects with type 2 diabetes. Diabetes research and clinical practice. 2009;84(1):58-60.
29. Huang R, Bai X, Li X, Zhao L, Xia MIN. Retinol Binding Protein 4 Impairs Pancreatic Beta-Cell Function, Leading to the Development of Type 2 Diabetes. Diabetes. 2018;67(Supplement 1):1826-P.
30. Li J-Y, Chen X-X, Lu X-H, Zhang C-B, Shi Q-P, Feng L. Elevated RBP4 plasma levels were associated with diabetic retinopathy in type 2 diabetes. Bioscience reports. 2018;38(5):BSR20181100.
31. Klisic A, Kavaric N, Ninic A. Retinol-binding protein 4 versus albuminuria as predictors of estimated glomerular filtration rate decline in patients with type 2 diabetes. J Res Med Sci. 2018;23:44-.
32. Liu G, Ding M, Chiuve SE, Rimm EB, Franks PW, Meigs JB, et al. Plasma Levels of Fatty Acid-Binding Protein 4, Retinol-Binding Protein 4, High-Molecular-Weight Adiponectin, and Cardiovascular Mortality Among Men With Type 2 Diabetes: A 22-Year Prospective Study. Arteriosclerosis, thrombosis, and vascular biology. 2016;36(11):2259-67.
33. Krill D, O'Leary L, Koehler AN, Kramer MK, Warty V, Wagner MA, et al. Association of retinol binding protein in multiple-case families with insulin-dependent diabetes. Human biology. 1997;69(1):89-96.
34. Jourdan M, Jaleel A, Karakelides H, Ford GC, Kahn BB, Nair KS. Impact of type 1 diabetes and insulin treatment on plasma levels and fractional synthesis rate of retinol-binding protein 4. The Journal of clinical endocrinology and metabolism. 2009;94(12):5125-30.
35. Brunzell JD, Ayyobi AF. Dyslipidemia in the metabolic syndrome and type 2 diabetes mellitus. The American journal of medicine. 2003;115(8):24-8.
36. Mooradian AD. Dyslipidemia in type 2 diabetes mellitus. Nature Reviews Endocrinology. 2009;5(3):150.
37. Vergès B, Guiu B, Cercueil JP, Duvillard L, Robin I, Buffier P, et al. Retinol-binding protein 4 is an independent factor associated with triglycerides and a determinant of very low-density lipoprotein-apolipoprotein B100 catabolism in type 2 diabetes mellitus. Arteriosclerosis, thrombosis, and vascular biology. 2012;32(12):3050-7.
38. Makino S, Fujiwara M, Suzukawa K, Handa H, Fujie T, Ohtaka Y, et al. Visceral obesity is associated with the metabolic syndrome and elevated plasma retinol binding protein-4 level in obstructive sleep apnea syndrome. Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme. 2009;41(3):221-6.
39. Rocha M, Bañuls C, Bellod L, Rovira-Llopis S, Morillas C, Solá E, et al. Association of serum retinol binding protein 4 with atherogenic dyslipidemia in morbid obese patients. PloS one. 2013;8(11):e78670-e.
40. Takebayashi K, Suetsugu M, Wakabayashi S, Aso Y, Inukai T. Retinol binding protein-4 levels and clinical features of type 2 diabetes patients. The Journal of clinical endocrinology and metabolism. 2007;92(7):2712-9.
41. Qi Q, Yu Z, Ye X, Zhao F, Huang P, Hu FB, et al. Elevated retinol-binding protein 4 levels are associated with metabolic syndrome in Chinese people. The Journal of clinical endocrinology and metabolism. 2007;92(12):4827-34.
42. Yamaaki N, Yagi K, Kobayashi J, Nohara A, Ito N, Asano A, et al. Impact of Serum Retinol-Binding Protein 4 Levels on Regulation of Remnant-Like Particles Triglyceride in Type 2 Diabetes Mellitus. Journal of Diabetes Research. 2013;2013:1-5.
43. Kwanbunjan K, Panprathip P, Phosat C, Chumpathat N, Wechjakwen N, Puduang S, et al. Association of retinol binding protein 4 and transthyretin with triglyceride levels and insulin resistance in rural thais with high type 2 diabetes risk. BMC endocrine disorders. 2018;18(1):26.
44. Zhang JX, Zhu GP, Zhang BL, Cheng YY. Elevated serum retinol-binding protein 4 levels are correlated with blood pressure in prehypertensive Chinese. Journal of Human Hypertension. 2017;31(10):611-5.
45. Li X, Zhu S, Song G, Zhang K, Gao W, Huang J, et al. Retinol-binding protein 4 is closely correlated to blood pressure level and E/A in untreated essential hypertension patients. Annals of palliative medicine. 2019;8(5):645.
46. Pinto E. Blood pressure and ageing. Postgraduate medical journal. 2007;83(976):109-14.
47. Cheung BM, Li C. Diabetes and hypertension: is there a common metabolic pathway? Current atherosclerosis reports. 2012;14(2):160-6.
48. Hypertension in Diabetes Study (HDS): I. Prevalence of hypertension in newly presenting type 2 diabetic patients and the association with risk factors for cardiovascular and diabetic

complications. Journal of hypertension. 1993;11(3):309-17.
49. Ephraim RK, Saasi A-R, Anto EO, Adoba P. Determinants of isolated systolic hypertension among diabetic patients visiting the diabetic clinic at the Tamale Teaching Hospital, Northern Ghana. Afr Health Sci. 2016;16(4):1151-6.
50. Rubin J, Nambi V, Chambless LE, Steffes MW, Juraschek SP, Coresh J, et al. Hyperglycemia and arterial stiffness: the atherosclerosis risk in the communities study. Atherosclerosis. 2012;225(1):246-51.
51. Benetos A, Waeber B, Izzo J, Mitchell G, Resnick L, Asmar R, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. American journal of hypertension. 2002;15(12):1101-8.
52. Schram MT, Henry RM, van Dijk RA, Kostense PJ, Dekker JM, Nijpels G, et al. Increased central artery stiffness in impaired glucose metabolism and type 2 diabetes: the Hoorn Study. Hypertension. 2004;43(2):176-81.
53. Henry RM, Kostense PJ, Spijkerman AM, Dekker JM, Nijpels G, Heine RJ, et al. Arterial stiffness increases with deteriorating glucose tolerance status: the Hoorn Study. Circulation. 2003;107(16):2089-95.
54. Potenza MA, Gagliardi S, Nacci C, Carratu MR, Montagnani M. Endothelial dysfunction in diabetes: from mechanisms to therapeutic targets. Current medicinal chemistry. 2009;16(1):94-112.
55. Watanabe K, Suzuki T, Ouchi M, Suzuki K, Ohara M, Hashimoto M, et al. Relationship between postprandial glucose level and carotid artery stiffness in patients without diabetes or cardiovascular disease. BMC cardiovascular disorders. 2013;13:11.
56. Munkhtulga L, Nagashima S, Nakayama K, Utsumi N, Yanagisawa Y, Gotoh T, et al. Regulatory SNP in the RBP4 gene modified the expression in adipocytes and associated with BMI. Obesity. 2010;18:1006-14.
57. Jia W, Wu H, Bao Y, Wang C, Lu J, Zhu J, et al. Association of serum retinol-binding protein 4 and visceral adiposity in Chinese subjects with and without type 2 diabetes. Journal of Clinical Endocrinology and Metabolism. 2007;92:3224-9.
58. Kloting N, Fasshauer M, Dietrich A, Kovacs P, Schon M, Kern M, et al. Insulin-sensitive obesity. American journal of physiology Endocrinology and metabolism. 2010;299:E506-E15.
59. Kelly K, Kashyap S, O'Leary V, Major J, Schauer P, Kirwan J. Retinol-binding protein 4 (RBP4) protein expression is increased in omental adipose tissue of severely obese patients. Obesity. 2010;18:663-6.
60. Janke J, Engeli S, Boschmann M, Adams F, Bohnke J, Luft F, et al. Retinol-binding protein 4 in human obesity. Diabetes. 2006;55:2805-10.
61. Lee J, Lee H, Shim J, Im J, Lee D. Abdominal visceral fat reduction is associated with favorable changes of serum retinol binding protein-4 in nondiabetic subjects. Endocrine journal. 2008;55:811-8.
62. Vitkova M, Klimcakova E, Kovacikova M, Valle C, Moro C, Polak J, et al. Plasma levels and adipose tissue messenger ribonucleic acid expression of retinol-binding protein 4 are reduced during calorie restriction in obese subjects but are not related to diet-induced changes in insulin sensitivity. Journal of Clinical Endocrinology and Metabolism. 2007;92:2330-5.
63. Kovacs P, Geyer M, Berndt J, Kloting N, Graham T, Bottcher Y, et al. Effects of genetic variation in the human retinol binding protein-4 gene (RBP4) on insulin resistance and fat depot-specific mRNA expression. Diabetes. 2007;56:3095-100.