Role of Retinol Binding Protein-4 in Obesity and Type 2 Diabetes Mellitus

Mostafa M. El-Shafie[1], Ahmed Mohammed M. El-Sisy[1],*, Ahmed A. Ali[1], Shawkey S. Ali[1], Mohammed M. El-Zahabi[1] and Ashraf I. Amin[2]

[1] Faculty of Pharmacy, Biochemistry Department, Al-Azhar University, Nasr city, 11566 Cairo, Egypt
[2] Clinical Pathology and Laboratory Department, National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt
ahmedelsisy@gmail.com

Abstract: Currently retinol binding protein-4 (RBP-4) has been identified as interesting novel adipokine that suggested to link obesity with its complication, especially insulin resistance, type 2 diabetes (T2D), and certain components of the metabolic syndrome (MS). However, the relationship between them has not been elucidated; and their circulating levels in obesity and type 2 diabetes mellitus (T2DM) have not been adequately studied. Therefore, this study was designed to investigate whether its level was altered in Egyptian obese and T2DM patients and to study the correlation of this novel adipokine with insulin resistance, and other biochemical parameters. The levels of RBP-4, insulin, leptin, CRP and TNF-α were measured in healthy obese, non-obese T2DM and obese T2DM patients together with matched healthy nondiabetic control subjects. RBP-4, insulin, leptin, CRP and TNF-α levels were measured by enzyme-linked immunosorbent assay. RBP-4 level was found to be significantly elevated in obese (13.9 ± 0.99), obese T2DM patients (25.1 ± 2.05) and non obese T2DM (13.9 ± 1.10) compared with control subjects (4.84 ± 0.51) at $P < 0.05$. As well as, it was significantly higher in obese T2DM (25.1 ± 2.05) when compared to obese (13.9 ± 0.99) and Non-obese T2DM (13.9 ± 1.10) groups. In addition, RBP-4 level was found to be significantly positively correlated with other biochemical parameters. In conclusion, RBP-4 might play an important role in the pathogenesis of T2DM. In addition, the RBP-4, leptin, TNF-α and CRP are significantly interrelated with each other.

1. Introduction

Obesity, the excessive accumulation of fat, is a risk factor for the metabolic syndrome (i.e. diabetes, dyslipidemia and cardiovascular complications). However, not every form of obesity poses a similar clinical threat. Strong epidemiological evidence indicates that the preferential accumulation of intra-abdominal (visceral) fat, surrounding the gastrointestinal organs, poses a greater cardiovascular risk than in other forms of obesity, in which fat is preferentially accumulated under the skin (subcutaneous) in the gluteal region [1].

Type 2 diabetes mellitus (T2DM) is a complex metabolic disorder that affects more than 150 million people worldwide and is projected to increase to 439 million worldwide in 2030. Its prevalence is expected to increase exponentially around the world particularly in developing countries [2,3]. Insulin resistance and inflammation play a major role in the development of T2DM [4]. In addition, increased abdominal/ visceral fat is associated with insulin resistance and T2DM [5]. Vigorous efforts have been made to delineate the relationship between increased adiposity and insulin resistance. However, the molecular mechanisms that lead to the development of insulin resistance and T2DM are far from complete elucidation. The realization that adipose tissue acts as an endocrine gland affecting whole-body energy homeostasis was a major breakthrough toward a better molecular understanding of T2DM [6,7], and growing evidence implicates adipocyte-derived factors (adipokines) as major regulators of insulin resistance[8].

RBP-4 belongs to the lipocalin family of proteins that transport small hydrophobic molecules [9]. RBP-4 is a transport protein for retinol (vitamin A) in the circulation. It transports retinol from the liver to the peripheral tissues [10].

RBP-4 does not interact only with retinol. Formation of a complex with transthyretin – a carrier of thyroid hormone and retinol – prevents glomerular filtration of RBP-4 and its subsequent excretion through the kidney [11]. It is debatable whether increased transthyretin levels and/or enhanced RBP-4 to transthyretin interaction can result in decreased renal clearance of RBP-4 and consequently its increased circulating levels [12]. Circulating RBP-4 levels are also influenced by iron and ferritin status [13].

In obesity and type 2 diabetes, expression of the GLUT4 glucose transporter is decreased

selectively in adipocytes. Adipose-specific Glut4 (also known as Slc2a4) knockout (adipose-Glut4 (-/-)) mice show insulin resistance secondarily in muscle and liver. The expression of retinol binding protein-4 (RBP4) is elevated in adipose tissue of adipose-Glut4 (-/-) mice. Serum RBP4 levels were elevated in insulin-resistant mice and humans with obesity and T2D. RBP4 levels are normalized by rosiglitazone, an insulin-sensitizing drug. Transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice causes insulin resistance. Conversely, genetic deletion of Rbp4 enhances insulin sensitivity. Fenretinide, a synthetic retinoid that increases urinary excretion of RBP4, normalizes serum RBP4 levels and improves insulin resistance and glucose intolerance in mice with obesity induced by a high-fat diet. Increasing serum RBP4 induces hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and impairs insulin signalling in muscle. Thus, RBP4 is an adipocyte-derived 'signal' that may contribute to the pathogenesis of T2D. Lowering RBP4 could be a new strategy for treating T2D [14].

In normal individuals, binding of insulin to its receptor on the cell membrane stimulates glucose uptake into muscle and fat cells through the GLUT4 transporter. It also inhibits glucose production in liver, thereby maintaining normal glucose levels in the blood. In adipose tissue, glucose provides fuel for the synthesis of fat stores, which serve as the body's main energy reservoir. Yang *et al.* [15] found that the decrease in GLUT4 expression that occurs in the fatty tissue of obese animals is accompanied by increased expression and secretion of the fat-derived factor RBP4. This factor, possibly working in concert with retinol (vitamin A), impairs insulin signalling in muscle, inhibiting glucose uptake, and interferes with insulin-mediated suppression of glucose production in the liver, causing blood glucose levels to rise. [14, 15].

Another adipiokine is leptin, one major breakthrough in the understanding of energy balance regulation and adipose tissue biology was the description of leptin, a product of the ob gene Leptin, from the Greek leptos means lean and is a protein with a molecular mass of 16 kDa, constituted by 167-amino acids. It regulates energy metabolism, increasing energy expenditure and decreasing energy consumption. It is now considered that leptin is a metabolic signal for energy sufficiency [16]. Leptin improves insulin sensitivity through activation of adenosine monophosphate-activated protein kinase (AMPK), which controls cellular concentrations of malonyl-CoA, thereby inhibiting acetyl-CoA carboxylase [17]. As a result, there is a decrease of intracellular malonyl-CoA and a decline of lipogenesis associated with increased fatty acid β-oxidation. In

fact, in generalized lipodystrophy, where adipose tissue is nearly absent, leptin administration improves insulin sensitivity [18]. This highlights the influence of this adipocyte hormone on whole-body glucose homoeostasis. However, in common human obesity, there are high circulating leptin levels, suggesting leptin resistance, and leptin administration has little or no effect on IR. In fact, the leptin-signaling pathway activates suppressor of cytokine signaling-3 (SOCS-3), which might inhibit insulin signaling [19].Therefore, while leptin deficiency very likely contributes to IR resistance when adipose tissue is lacking, leptin resistance is a main feature of human obesity. So far, the precise role of leptin in IR remains unclear [20]

All these data indicate that RBP-4 to play a role in the pathogenesis of T2DM. However, its circulating level in T2DM has not been studied; and it's correlation with insulin resistance or obesity is still controversial. Therefore, the current study was designed to determine the circulating levels of RBP-4 in Egyptian T2DM patients compared with healthy control subjects and to study the correlation of this interesting adipokines with one another and between each of them with other parameters like TNF-α, insulin resistance, as well as other biochemical and anthropometric parameters. According to our knowledge, the interrelation between RBP-4, CRP, and TNF-α and leptin have not been elucidated before our current work.

2. Subjects and methods:
2.1. Study population and anthropometric measurements

This study was approved by the ethical committee of National Institute of Diabetes and Endocrinology, Cairo, Egypt; and informed consent was obtained from every subject before participating in the study. A total of 120 male subjects were enrolled in the study: 62 patients with T2DM, 33 age- and sex-matched non-diabetic healthy obese subjects and 25 age- and sex-matched non-diabetic healthy control subjects. The definition of a non-diabetic is a subject who has a fasting plasma glucose (FPG) level lower than 110 mg/dL and has no family history of T2DM. The 62 patients with T2DM were recruited from the outpatient clinic of the National Institute of Diabetes and Endocrinology and were further classified into non-obese group (N: 25; body mass index [BMI] ≤ 25 kg/m^2) and obese group (N: 37; BMI ≥ 30 kg/m^2). Both the non-obese diabetic group and the control non-diabetic group were selected to have matching BMI. The characteristics of the subjects are summarized in Table 1. The exclusion criteria were age less than 25 or more than 63 years, type 1 DM, insulin treatment, renal or hepatic disease,

acute or chronic inflammatory disease, thyroid dysfunction, ischemic cardiovascular disease, retinopathy, alcohol or drug abuse, cancer, acute or chronic infections, any hematologic disorder (assessed by making complete blood count for every subject), and smoking. Subjects taking thiazolidinediones, fenofibrate, statins, spironolactone [21], or hormonal therapy were also excluded. The control and obese subjects were not suffering any health problems and were not receiving any medications or dietary supplements. The diabetic subjects were selected not to have long duration of T2DM to help avoid the presence of diabetic complications, which was further confirmed by physical examination and laboratory investigations.

Before inclusion, all the study subjects underwent careful physical examination, detailed history, and laboratory investigations to exclude any condition that may interfere with glucose tolerance. Anthropometric parameters measured included BMI. Standing height and body weight were measured in normal clothing without shoes. The BMI was calculated as weight divided by squared height (in kilograms per square meter). Waist and hip circumferences were measured to the nearest 0.1 cm at the narrowest point between the lowest rib and the uppermost lateral border of the iliac crest. The hips were measured at their widest point.

2.2 Blood sampling

All of the blood samples were drawn after overnight fasting, and the samples were divided into 3 aliquots. The first aliquot of blood was collected on Vacutainer tubes (BD, Franklin Lakes, NJ) containing sodium fluoride for plasma preparation used for the assay of FPG. The second aliquot of blood was collected on Vacutainer tubes containing sodium EDTA for complete blood count and for the assay of glycosylated hemoglobin (HbA$_{1c}$ %). The third aliquot of blood was collected on plain Vaccutainer tubes for serum preparation used for the assay of lipids profile, routine parameters (creatinine, urea, and alanine aminotransferase [ALT]), RBP-4, insulin, TNF-α, leptin and CRP levels. Serum samples were divided into aliquots and kept at −80°C for subsequent assay.

2.3. Laboratory analyses

Fasting plasma glucose and serum biochemical parameters including triacylgycerol (TAG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), creatinine, urea, and ALT were measured using Jenway 6305 spectrophotometer automated biochemistry analyzer. Low-density lipoprotein cholesterol (LDL-C) level was calculated by the formula of Friedewald *et al.* [22]. The HbA$_{1c}$ % was measured in whole blood with ion-exchange high-performance liquid chromatography using the Bio-Rad D-10 system (Bio-Rad Laboratories, Hercules, CA).

The concentration of serum insulin, RBP-4, TNF-α, CRP and leptin were determined by enzyme-linked immunosorbent assay (ELISA) using commercially available kits: (Human insulin ELIZA kit, Calbiotec, USA), (Human RBP-4 ELIZA kit, RayBio Pharmaceuticals, California; USA), (Human TNF- alpha ELIZA kit, RayBio Pharmaceuticals, California, USA), (Human CRP ELIZA kit, Diagnostic biochem Canada, Canada) and (Human leptin ELIZA kit, Diagnostic biochem Canada) respectively. The homeostasis model assessment of insulin resistance index (HOMA$_{IR}$) was calculated from fasting insulin and glucose by the following equation: HOMA$_{IR}$= fasting insulin (in microunits per milliliter) × fasting glucose (in milligrams per deciliter)/405 [23]. All ELISA procedures were done by (TECAN, SUNRISE) according to the manufacturer's instructions.

2.4. Statistical analysis

All data were expressed as mean ± standard error of mean ($\bar{\chi}$ ± SEM). Descriptive statistics were performed using Microsoft Excel 2007. All analysis and graphics were performed using Graph pad prism (windows version 5; Graph pad software 2007). Difference between means was assessed by unpaired student t test. Differences were considered statistically significant at $P < 0.05$. Correlation between different parameters was carried out using Graph pad prism (windows version 5; Graph pad software 2007) and considered statistically significant at $P < 0.001$.

All statistical analyses were done under supervision of faculty of pharmacy (boys), Al-Azhar University, Cairo, Egypt and Institute of Statistical Studies and Research, Cairo University, Egypt.

3. Results

The clinical characteristics as well as the levels of circulating insulin, HOMA-IR, RBP-4, leptin, CRP and TNF-α of the studied subjects are shown in Tables (1, 2). Concerning serum insulin as shown in table (2) was significantly higher in obese, obese T2DM and non-obese T2DM groups as compared to normal group. Also, it was significantly higher in obese T2DM group when compared to both obese and non-obese T2DM groups.

Additionally, the HOMA-IR index as shown in table (2) was significantly higher in obese, obese T2DM and non-obese T2DM groups as compared to normal group. As well as, it was significantly higher in obese T2DM and non-obese T2DM groups when compared to obese group. In addition, it was significantly higher in obese T2DM comparing with non-obese T2DM one.

Concerning the serum level of RBP-4 as shown in table (2) and figure (1), this was significantly higher in obese, obese T2DM and Non-obese T2DM groups comparing with normal group. As well as, it was significantly higher in obese T2DM when compared to obese and Non-obese T2DM groups.

Concerning for serum leptin as shown in table (2) and figure (2), this was significantly higher in obese and obese T2DM groups as compared to both normal and Non-obese T2DM groups. As well as, it was significantly higher in obese T2DM groups when compared to obese group.

Concerning for serum CRP as shown in table (2) and figure (3), this was significantly higher in obese, obese T2DM and Non-obese T2DM groups as compared to normal group, and it was significantly higher in obese T2DM comparing with obese and Non-obese T2DM groups.

Concerning for serum TNF-α as shown in table (2) and figure (4), TNF-α was significantly higher in obese T2DM and Non-obese T2DM groups as compared to both normal and obese groups.

We next analyzed the correlation between each of insulin RBP-4, leptin, CRP and TNF-α level with each other and with other parameters. Concerning with serum insulin levels as shown in Table 3, simple linear regression analysis revealed that they were found to be significantly positively correlated with age, BMI, BF%, FPG, HbA1c %, HOMA-IR, RBP-4, leptin, CRP and TNF-α levels.

Furthermore, as shown in Table 3, simple linear regression analysis revealed that serum RBP-4 levels were found to be significantly positively correlated with BMI, BF%, FPG, HbA1c %, HOMA-IR, insulin, leptin, CRP and TNF-α levels.

With respect to serum leptin levels as shown in Table 3 simple linear regression analysis revealed that they significantly positively correlated with BMI, BF%, FPG, HbA1c %, HOMA-IR, insulin, RBP-4, CRP and TNF-α levels.

Furthermore, as shown in Table 3, simple linear regression analysis revealed that serum CRP levels were found to be significantly positively correlated with BMI, BF%, FPG, HbA1c %, HOMA-IR, insulin, leptin, RBP-4 and TNF-α levels.

With respect to serum TNF-α levels as shown in Table 3 simple linear regression analysis revealed that they significantly positively correlated with FPG, HbA1c %, HOMA-IR, insulin, RBP-4, CRP and leptin levels.

Table (1): Anthropometric parameters in all studied groups ($\bar{\chi}$ ±SEM)

Factor	Controls	Obese	Obese T2DM	Non-Obese T2DM
Number	25	33	37	25
Age	34.6 ± 1.74	36.5 ± 1.96	43.9 ± 1.51	40.2 ± 1.86
Diabetes duration	----------	----------	3.69 ± 0.18	4.62 ± 0.23
Weight (kg)	73.7 ± 0.84	99.9 ± 2.23	98.3 ± 1.65	74.2 ± 1.02
Height (m)	1.76 ± 0.01	1.69 ± 0.01	1.68 ± 0.01	1.75 ± 0.01
BMI (Kg/m^2)	23.8 ± 0.26	35.0 ± 0.61	34.9 ± 0.53	24.2 ± 0.14
BF%	20.4 ± 0.54	34.3 ± 0.85	35.8 ± 0.78	22.2 ± 0.50

All results are expressed as mean ± SEM

Table (2): The clinical characteristics as well as the levels of circulating insulin, HOMAIR, RBP-4, leptin, TNF-alpha and CRP of the studied subjects.

Factor	Controls	Obese	Obese T2DM	Non-Obese T2DM	P1	P2	P3	P4	P5	P6
Creatinine(mg/dl)	0.95 ± 0.05	0.86 ± 0.02	0.88 ± 0.03	0.91 ± 0.04	NS	NS	NS	NS	NS	NS
ALT (U/L)	20.2 ± 1.60	20.33 ± 1.78	28.0 ± 1.71	27.7 ± 1.50	NS	0.002	0.002	0.004	0.005	NS
FBG (mg/dl)	89.5 ± 2.21	91.8 ± 2.04	212 ± 10.2	223 ± 9.40	NS	< 0.0001	< 0.0001	< 0.0001	< 0.0001	NS
HbA$_{1c}$%	5.01 ± 0.16	5.25 ± 0.43	8.78 ± 0.35	9.16 ± 0.36	NS	< 0.0001	< 0.0001	< 0.0001	< 0.0001	NS
Insulin (μU/ml)	5.57 ± 0.37	11.6 ± 0.76	17.4 ± 1.24	11.2 ± 0.81	< 0.0001	< 0.0001	< 0.0001	0.0003	NS	0.0004
HOMA$_{IR}$	1.26 ± 0.11	2.65 ± 0.19	9.58 ± 0.96	6.27 ± 0.60	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0114	NS
BF%	20.3 ± 0.52	34.3± 0.85	35.8 ± 0.78	22.2 ± 0.50	< 0.0001	< 0.0001	0.0138	NS	< 0.0001	< 0.0001
TAG (mg/dl)	110 ± 4.88	186 ± 6.44	213 ± 8.37	227 ± 13.1	< 0.0001	< 0.0001	< 0.0001	0.0130	0.0039	NS
TC (mg/dl)	167 ± 3.11	215 ± 6.79	244 ± 12.0	196 ± 6.48	< 0.0001	< 0.0001	0.0002	NS	NS	0.0037
HDL-C (mg/dl)	54.0 ± 2.16	36.3 ± 1.67	37.6 ± 1.86	42.5 ± 2.54	< 0.0001	< 0.0001	0.0010	NS	0.0368	NS
LDL-C (mg/dl)	91.0 ± 3.56	142 ± 7.13	163 ± 10.7	109 ± 7.78	< 0.0001	0.0001	0.0457	NS	0.0028	0.0004
Risk ratio type 1	3.20 ± 0.13	6.30 ± 0.35	6.71 ± 0.31	5.02 ± 0.33	< 0.0001	< 0.0001	< 0.0001	NS	0.0126	0.0005
RBP-4 (ng/ml)	4.84 ± 0.51	13.9 ± 0.99	25.1 ± 2.05	13.9 ± 1.10	< 0.0001	< 0.0001	< 0.0001	< 0.0001	NS	< 0.0001
Leptin (ng/ml)	5.88 ± 0.39	11.8 ± 0.79	15.2 ± 1.15	6.68 ± 0.57	< 0.0001	< 0.0001	NS	0.0182	<0.0001	< 0.0001
CRP (ng/ml)	139 ± 10.9	218 ± 19.2	305 ± 17.0	227 ± 26.4	0.0018	< 0.0001	0.0033	0.0010	NS	0.0115
TNF-α (pg/ml)	21.5 ± 0.91	26.0 ± 1.22	80.8± 3.27	83.0 ± 3.45	0.0070	< 0.0001	< 0.0001	< 0.0001	< 0.0001	NS

Results are expressed as mean ± SEM, *p*1 for control and obese healthy, *p*2 for control and obese T2DM, *p*3 for control and non-obese T2DM, *p*4 for obese healthy and obese T2DM, *p*5 for obese healthy and non-obese T2DM and *p*6 for obese T2DM and non-obese T2DM.

Table (3): Simple linear regression analysis using BMI, insulin, leptin, CRP and TNF-α as dependent variables.

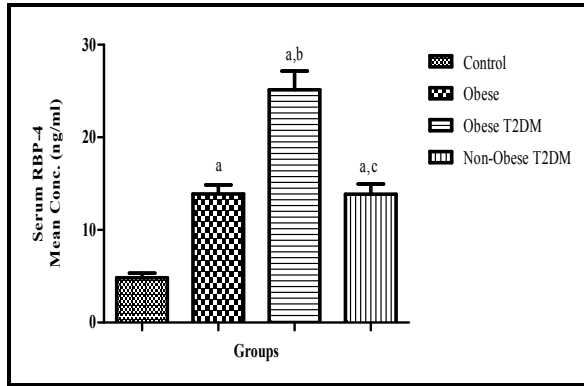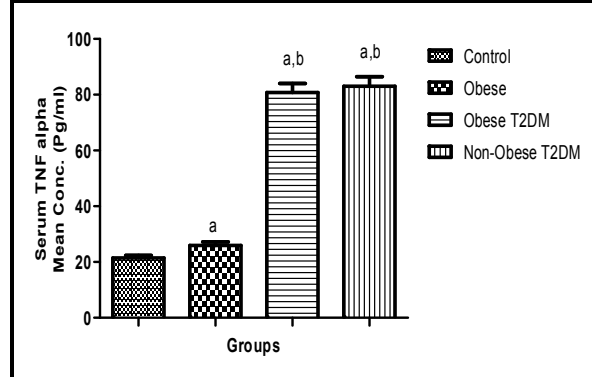| variable	BMI		Insulin		HOMA$_{IR}$		Leptin		CRP		TNF-α	
	r2	p	r2	p	r2	p	r2	p	r2	p	r2	p
RBP-4	0.3512	< 0.0001	0.5204	< 0.0001	0.5559	< 0.0001	0.5219	< 0.0001	0.5314	< 0.0001	0.2918	< 0.0001

Fig.(1): Serum RBP-4 in all studied groups
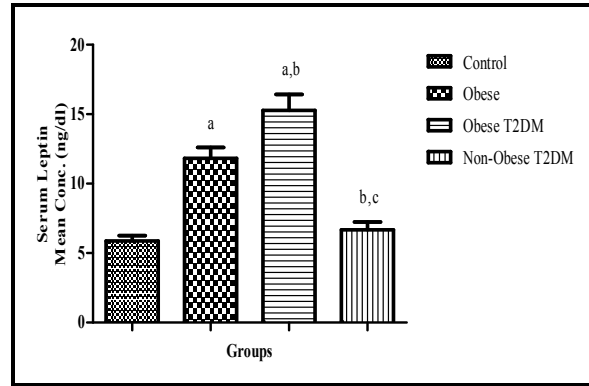
Fig.(2): Serum leptin in all studied groups
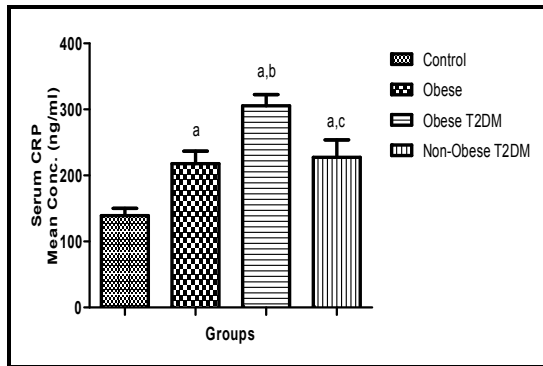
Fig(3): Serum CRP in all studied groups
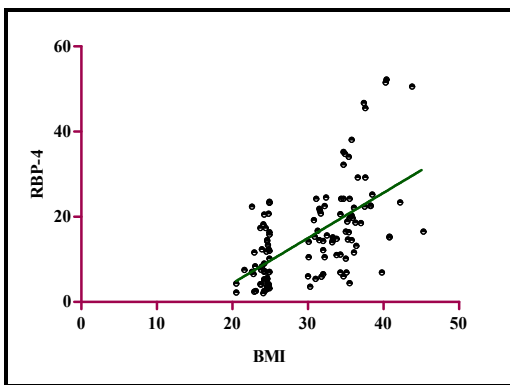

Fig(4): Serum TNF-α in all studied groups

[a]Significantly different from control group at *P* <0.05. [b]Significantly different from obese group at *P* <0.05.
[c]Significantly different from obese T2DM group at *P* <0.05.

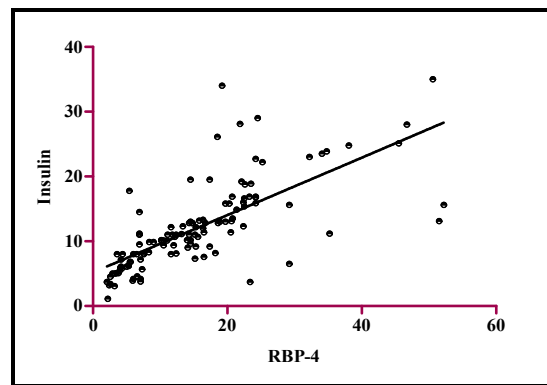Fig(5): Correlation coefficient between RBP-4 and BMI

Fig (6): Correlation coefficient between RBP-4 and insulin
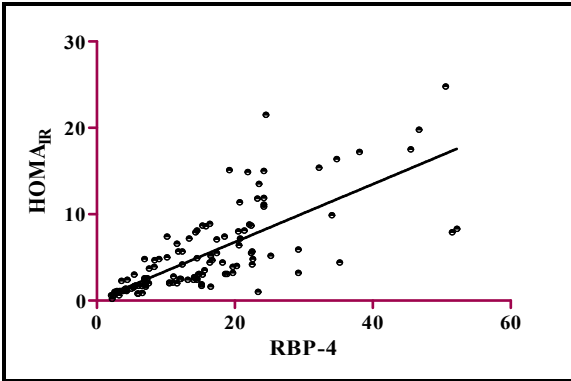
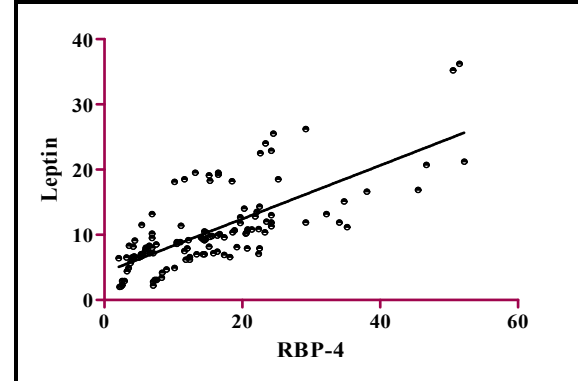Fig (7): Correlation coefficient between RBP-4 and
HOMA$_{IR}$

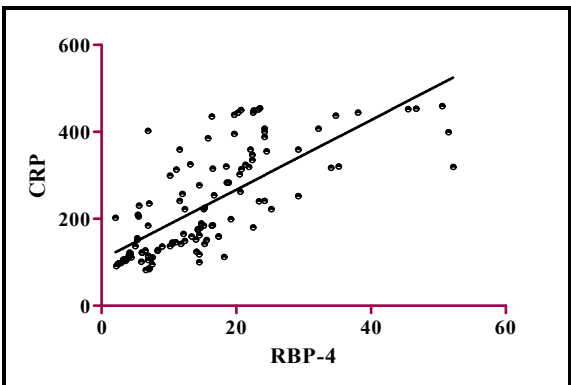Fig (8): Correlation coefficient between RBP-4 and
Leptin

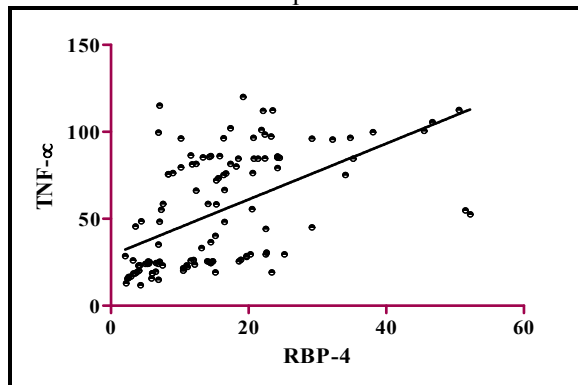Fig (9): Correlation coefficient between RBP-4and
CRP

Fig (10): Correlation coefficient between RBP-4 and
TNF-α

4. Discussion

The number and diversity of identified adipokines are growing rapidly [24]; and understanding of the diverse effects of distinct adipokines as well as the interplay between these bioactive mediators is still incomplete [25] and, if fully elucidated, would provide much better understanding for the molecular basis of T2DM.

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. Insulin deficiency in turn leads to chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism [26].

In the present study, serum levels of RBP-4 were significantly higher in obese, obese diabetic and non-obese diabetic groups in comparison to control ones. Also, serum levels of RBP-4 were significantly higher in obese diabetic subjects in comparison to healthy obese and non obese diabetic ones.

These results were supported by *Ji-Won et al., (2009)* who confirmed these findings regarding the relationship between circulating RBP-4 and insulin resistance [27].

Also, the resulted supported by Timothy *et al.* (2006) who found that the magnitude of increase in

serum RBP-4 correlates with insulin resistance among humans with obesity, impaired glucose tolerance, or T2D and among non-obese, nondiabetic subjects with strong family histories of T2D [28].

The serum RBP-4 level is correlated with a cluster of cardiovascular risk factors accompanying insulin resistance as part of the metabolic syndrome. Even though the serum RBP-4 level correlated with body-mass index, the relationship between the serum RBP-4 level and insulin resistance was independent of obesity, and non-obese, insulin-resistant subjects also exhibited increased serum RBP-4 levels. In these non-obese subjects, decreased expression of GLUT4 in adipocytes predicts increased serum RBP-4 levels and insulin resistance. The mechanism by which a decrease in adipocyte GLUT4 results in an increase in RBP-4 expression is unknown, but it might involve sensing of glucose by adipocytes [29].

The correlation of serum RBP-4 levels with plasma insulin levels suggests that the expression of RBP-4 in adipose tissue might be a direct consequence of hyperinsulinemia. Because the serum RBP-4 level correlates with insulin resistance and the clinical signs and biochemical components of the metabolic syndrome, measurement of serum RBP-4 could

become a noninvasive and accessible method for assessing the risks of impaired glucose tolerance, T2D, and cardiovascular disease. Altered levels of several adipocyte-secreted proteins (e.g., leptin and adiponectin), inflammatory cytokines (e.g., interleukin-6, monocyte chemoattractant protein 1, and tumor necrosis factor α), or inflammatory markers (e.g., C-reactive protein) have been observed in patients with obesity or insulin resistance. This study suggests that the serum RBP-4 level is correlated more specifically with insulin resistance and changes in insulin sensitivity than are the levels of several of these proteins (i.e., leptin, adiponectin, interleukin-6, and C-reactive protein) [29].

In mice, increased serum RBP-4 levels impair post receptor insulin signaling at the level of phosphoinositide-3 kinase in muscle and enhance the expression of phosphoenolpyruvate carboxykinase in liver [29].

Therefore, increased serum RBP-4 levels in humans might contribute to impaired insulin-stimulated glucose uptake in muscle and elevated hepatic glucose production, both of which are characteristic of T2D [30].

Regions near the RBP-4 locus on human chromosome 10q have been linked to hyperinsulinemia or early onset of T2D in two populations, a finding consistent with a pathogenic role for RBP-4 in insulin resistance and T2D [30].

Also supporting with this result, *Von Eynatten et al. (2007),* for patients with T2D, cardio artery disease (CAD) and control subjects, Type 2 diabetic patients had a higher mean BMI, elevated VLDL-cholesterol and triacylglycerol levels, increased fasting plasma glucose, insulin and HOMA-IR, as well as lower HDL-cholesterol and serum adiponectin compared with control subjects[31].

Xu et al., (2009) also reported: In both sexes combined, serum RBP-4 concentrations were significantly increased in participants with isolated impaired fasting glucose (IFG), isolated impaired glucose regulation (IGT), combined IFG and IGT, and newly diagnosed T2D compared with concentrations in participants with normal glucose regulation (NGR), after adjustment for sex, age, BMI, current smoking and current alcohol intake, the education received and the family history of diabetes. There was no statistical difference between these four groups with dysregulation of glucose metabolism [32].

Increased RBP-4 was associated with an increased risk of hyperglycaemia. The higher level of RBP-4 had a higher risk of impaired glucose regulation after adjustment for sex, age, BMI, current smoking and alcohol intake, family history of diabetes, insulin resistance, triacylglycerol, total cholesterol, HDL- and LDL-cholesterol [32].

Also Serum RBP-4 levels were positively correlated with several metabolic indices, such as BMI, waist/hip ratio, HOMA-IR, blood pressure, impaired glucose metabolism (IFG/IGT, T2D) and serum triacylglycerol [32]. These findings were in line with previous studies Graham *et al.* [33] and Takebayashi *et al.* [34].

Some recent studies *Broch et al.,* [35] and *Janke et al.,* [36] failed to detect difference in circulating RBP-4 levels according to obese status. *Janke et al.,* [36] argued that adipose tissue might be a less important source of circulating RBP-4 in humans than in animals and that it was possible that the increase in circulating RBP-4 in the insulin-resistant state was not explained by increased RBP-4 production in adipose tissue.

Increased RBP-4 levels increased the risk for hyperglycaemia, including impaired glucose regulation and newly diagnosed T2D, after excluding the effects of age, sex, central obesity, HOMA-IR, family history of T2D and the levels of serum lipid. *Yang et al. (2005)* reported that increasing RBP-4 could act directly to induce the expression of phosphoenolpyruvate carboxykinase (PEPCK) (also known as PCK2), increase glucose production and reduce insulin action to suppress glucose production in hepatocytes. In addition, there is a report explaining that RBP-4 attenuates insulin-induced phosphorylation of insulin receptor substrate 1 (IRS1) and extracellular regulated kinase (ERK1/2) in primary human adipocytes [37]. Several studies have shown that after treatment with thiazolidinediones (TZDs), an insulin sensitiser, or exercise, serum RBP-4 levels are decreased and insulin resistance improves [38,39,40].

In the present study, We found a striking association between serum RBP-4 and lipid levels, especially levels of risk ratio. Our results were in line with previous findings [32, 33, 34, 35].

Insulin resistance could be the potential mechanism for increased serum lipid levels. However, we found that when we controlled for serum insulin, insulin resistance and waist/ hip ratio, serum RBP-4 independently predicted the risk of hypertriacylglycerolaemia. It was suggested that RBP-4 might have a direct role in the progression of lipogenesis. In another study, RBP-4 increased the expression of the gene encoding fatty acid synthase (FASN) in adipose tissue in a manner predominantly correlating with visceral fat accumulation, impaired insulin sensitivity and levels of circulating adipokines [41].

In the present study, it was found significant relation between RBP-4 and inflammation, as indicated by the levels of C-reactive protein. In contrast to *Xu et al. (2009),* Inflammation is thought to suppress hepatic RBP-4 mRNA synthesis [42] and

decrease plasma concentrations of RBP-4 and retinol [43, 44].

The association of RBP-4 with inflammation remains controversial. *Sell and Esckle (2007)* studied the regulation of RBP-4 production by adiponectin and TNF-α in primary human adipocytes; they found that TNF- α strongly down regulates RBP-4 production in adipocytes, a completely unexpected effect as TNF-α-treated adipocytes are insulin resistant. The association of RBP-4 with inflammation, either acute or low-grade chronic inflammation, is of particular interest [45].

In obesity and T2D, expression of the GLUT4 glucose transporter is decreased selectively in adipocytes. Adipose-specific Glut4 (also known as Slc2a4) knockout (adipose-Glut4(-/-)) mice show insulin resistance secondarily in muscle and liver. Expression of retinol binding protein-4 (RBP-4) is elevated in adipose tissue of adipose-Glut4 (-/-) mice. Serum RBP-4 levels are elevated in insulin-resistant mice and humans with obesity and T2D. RBP-4 levels are normalized by rosiglitazone, an insulin-sensitizing drug. Transgenic over expression of human RBP-4 or injection of recombinant RBP-4 in normal mice causes insulin resistance. Conversely, genetic deletion of RBP-4 enhances insulin sensitivity. Fenretinide, a synthetic retinoid that increases urinary excretion of RBP-4, normalizes serum RBP-4 levels and improves insulin resistance and glucose intolerance in mice with obesity induced by a high-fat diet. Increasing serum RBP-4 induces hepatic expression of the gluconeogenic enzyme phosphoenolpyruvate carboxykinase (PEPCK) and impairs insulin signalling in muscle. Thus, RBP-4 is an adipocyte-derived 'signal' that may contribute to the pathogenesis of T2D. Lowering RBP-4 could be a new strategy for treating T2D [15].

In normal individuals, binding of insulin to its receptor on the cell membrane stimulates glucose uptake into muscle and fat cells through the GLUT4 transporter. It also inhibits glucose production in liver, thereby maintaining normal glucose levels in the blood. In adipose tissue, glucose provides fuel for the synthesis of fat stores, which serve as the body's main energy reservoir. *Yang et al., 2005* found that the decrease in GLUT4 expression that occurs in the fatty tissue of obese animals is accompanied by increased expression and secretion of the fat-derived factor RBP4. This factor, possibly working in concert with retinol (vitamin A), impairs insulin signalling in muscle, inhibiting glucose uptake, and interferes with insulin-mediated suppression of glucose production in the liver, causing blood glucose levels to rise [46].

In conclusion, we found that RBP-4 was significantly elevated in obese and T2DM patients either obese or non-obese compared with healthy control subjects. RBP-4 was found to be significantly correlated with various metabolic parameters. Our results indicate that RBP-4 might play a role in the pathogenesis of T2DM.

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