Retinol binding protein-4 and insulin resistance in obese patients with type 2 diabetes mellitus treated with oral antidiabetic agents at Baghdad city

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Abstract

Background: Type2 diabetes mellitus (T2DM) affects a large population (about 387 million people) worldwide and expected to increase by approximately 70% in the next 20 years. Adipose tissue is responsible for releasing various adipokines which have been related to insulin resistance. Retinol binding protein -4 (RBP-4) is one of these adipokine.

Objective: To detect the relation between insulin resistance and serum retinol binding protein -4 (RBP-4) in type 2 diabetic obese patients at Baghdad city.

Method: A case- control study was performed at Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq from March 2013 to August 2013. Forty five obese type 2 diabetic patients with mean body mass index (36.62± 0.8 kg/ m^2) compared to forty obese age matched apparently healthy control subjects with no family history of type 2 diabetes. Laboratory and anthropometric measurements including: fasting plasma glucose, glycosylated hemoglobin, lipid profile, urea, creatinine, insulin, retinol binding protein-4, body mass index and waist to hip ratio were measured.

Results: Serum retinol binding protein-4 (RBP 4) was significantly ($p < 0.001$) elevated in patients with type 2 diabetes mellitus (427.28 ± 23.28 µg/dl) compared with that in control group (244.19 ± 16.63 µg/dl). Fasting glucose (F.P.G), Glycosylated hemoglobin (HbA1c), were significantly greater in subjects with diabetes (p value for F.P.G and HbA1c). Whereas insulin and homeostatic model assessment of insulin resistance (HOMA-IR) showed no significant difference between patients and control group.

Conclusion: Although serum RBP4 concentration was significantly increased in T2DM, but there is no significant association between RBP4 levels and the insulin resistance in obese type 2 diabetic patients.

Key words: Retinol binding protein-4, insulin resistance, insulin sensitivity index, type 2 diabetes mellitus.

Running title: Retinol binding protein- 4 in type-2 diabetes mellitus

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Introduction

Type2 diabetes mellitus (T2DM) a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1-3]. DM associated with potentially devastating complication with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [4, 5]. This form of diabetes is most often associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity, and certain ethnicities[6]. Approximately 80% of patients with T2DM are obese [7]. An increase in adipose tissue mass is strongly associated with the pathogenesis of insulin resistance and T2DM [8]. Adipose tissue secretes many type of adipokines that modulate the action of insulin in other tissue [8]. Retinol binding protein-4(RBP-4), a new fat-derived adipokine that specifically bind to retinol, has recently been reported to provide a link between obesity and insulin resistance [9].

Retinol binding proteins contain two families: intracellular RBPs (CRBP I, CRBP II, CRBP III) and extracellular (cellular RBPs (RBP 4). Cellular retinol binding protein type I (CRBP I), a small cytosolic binding protein for retinol and retinaldehyde, is specifically expressed in preadipocytes but not in differentiated adipocyte. The absence of CRBP I in mice leads to increase adipocity.3T3-L1 cells deficient in CRBP I or mouse embryonic fibroblast derived from Cellular retinol binding proteintype I knock-out (CRBP-I-KO) mice had increase adipocyte differentiation and triglyceride (TG) accumulation [10].

Cellular retinol binding protein type II (CRBP II) is mainly expressed in small intestine and is involved in retinoid incorporation into chylomicrons [11, 12].

Cellular retinol binding protein type III (CRBP III) belongs to the family of intracellular lipid binding proteins. CRBP III binds to retinol its function to facilitate the esterification of retinol to retinyl ester in the mammary gland during lactation [13]. CRBP I and CRBP III have overlapping expression in heart, muscle, adipose, and mammary tissue [13].

Retinol binding protein-4 (RBP - 4) a novel protein secreted mainly by adipose tissue with a 21 KDa molecular mass [14- 16]. Retinol (vitamin A) is bound to RBP-4 in 1:1 molar ratio [17]. A major part of circulation RBP-4 form is complex with prealbumin ((transthyretin (TTR)) to increase the molecular weight of RBP-4 from approximately 21 KDa to approximately 76 KDa preventing its loss through filtration by renal glomeruli while only small fraction of free RBP-4 can be found in serum [7, 18]. RBP-4 has been studied since the 1960s, mainly as a transporter of retinol. Yang et al, suggest that RBP-4 may contribute to pathogenesis of T2DM [19]. The aim of this study was to investigate the role of retinol binding protein- 4 in obese type- 2 diabetic patients.

Methods

This case- control study was conducted at Department of Chemistry and Biochemistry, College of Medicine, Al-Nahrain University, Baghdad, Iraq from March 2013 to August 2013. The study was carried out with 45 patients with T2DM (7 men and 38 women, mean age 52.1± 1.01 years) and 40 controls (6 men and 34 women, mean age 47.38 ± 1.45 years). Most of the patients were on metformin or sulfonylurea or combination of them. Few were on other antidiabetic drugs like repaglinide or sitagliptins. Patients with chronic renal disease, pancreatic, liver diseases, diabetic patients treated with insulin were excluded from this study. The local ethics committee approved this study.

The body mass index (BMI, Kg/m^2) was calculated as weight (in kilograms) divided by square of height (in meters).The subjects' waist was measured with a soft tape midway between the lowest rib and the iliac crest. Fasting blood glucose (FBG) was measured on the same day as the blood was collected by an enzymatic colorimetric method (GOD-PAP method) [20, 21]. Glycosylated hemoglobin (HbA_1c) was measured using ion-Exchange High Performance liquid Chromatography (HPLC) [22, 23]. The serum was stored at -20 °c for insulin and RBP-4 levels measurement.

Serum insulin and RBP-4 levels were determined by enzyme immunoassay via enzyme-linked immunosorbent assay (ELISA) using demeditec kit (Germany) &Cusabio (China) respectively. Insulin sensitivity index was estimated for all patients and control subjects.

Insulin resistance was calculated as HOMA- IR using the following equation:
HOMA-IR= [fasting blood glucose (m mole/L)x fasting serum insulin (m U/L)] /22.5 .[24]

Statistical analysis

The data were analyzed using SPSS package version 16, all data are expressed as mean ± SD. Student's t- test was used for comparing the mean of two groups. Pearson's correlation coefficient was used for measuring the mutual correspondence between two groups of variables.

Results

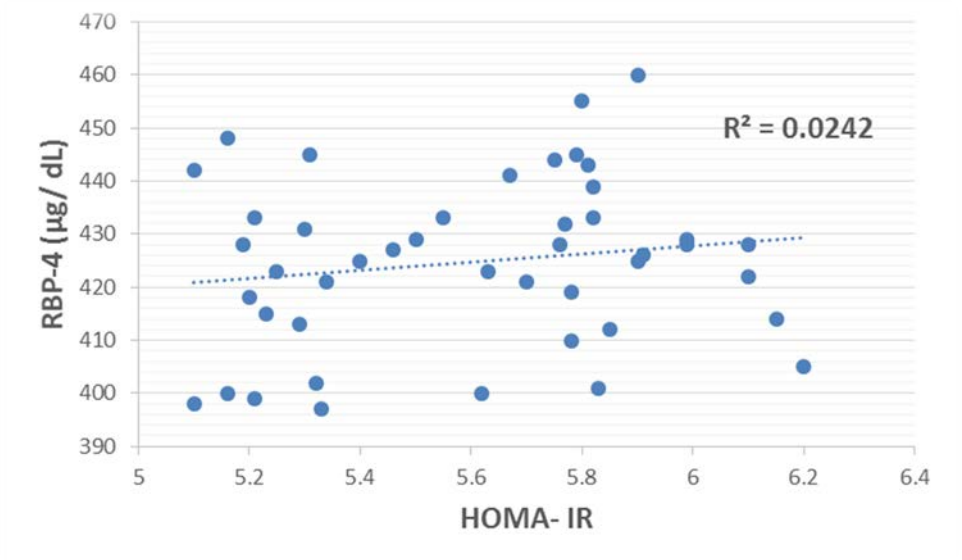
Forty five patients and thirty four controls participated in this study. The clinical characteristic of the 45 type 2 diabetes patients and 40 control subjects are shown in table 1.

Fasting plasma glucose, HbA1c, HOMA-IR and RBP 4 were significantly elevated in patients with diabetes, whereas insulin sensitivity index (ISI) was significantly ($p \leq 0.05$) decreased in patients with diabetes (table 1).

Table (1): Clinical characteristics of patients in the study

Parameter	T2DM (n= 45)	Control (n= 40)	P-value
Gender(Male/female)	7/38	6/34	
Age (year)	52.14 ± 1.01	47.38 ± 1.45	N.S
BMI (Kg/m^2)	36.62 ± 0.8	35.2 ± 1.00	N.S
W.C. (cm)	114.37± 1.76	108.9 ± 2.13	N.S
F.PG (mg/dl)	188.33±8.98	96.28±3.39	<0.001
Insulin (mU/L)	12.9 ± 1.6	10.06 ± 1.14	N.S
HOMA-IR	5.82 ± 0.66	2.44 ± 0.32	<0.001
ISI	1.2 ± 0.1	3.5 ± 0.3	<0.001
HbA1c (%)	9.12±0.33	5.69±0.15	<0.001
RBP 4 (µg/ dL)	427.28 ± 23.28	244.19 ± 16.63	<0.001
Cholesterol (mg/dL)	189.91 ± 30.2	143.25 ± 20.91	<0.001
Triglyceride (mg/dL)	192.88 ± 25. 8	86.25 ± 20.7	<0.001
LDL (mg/dL)	120.13 ± 8.8	101.73 ± 9.3	<0.001
HDL (mg/dL)	29.76 ± 3.1	51.81 ± 5.7	<0.001
Urea (mg/dL)	38.21 ± 6.1	31.71 ± 8.1	N.S
Creatinine (mg/dL)	0.98 ± 0.08	0.84 ± 0.06	N.S

There was no significant correlation between serum retinol binding protein 4 and insulin resistance in type 2 diabetic patients as shown in figure (1).



Figure(1): Correlation plot between retinol binding protein- 4 and HOMA- IR.

Discussion

Adipose tissue secretes many molecules, some of which confer insulin-sensitizing action, whereas others promote insulin resistance. Recent animals and human studies have suggested that the soluble form of retinol binding protein 4 is a major circulating adipokine implicated in systemic insulin resistance [28]. It was found that RBP 4 levels were significantly higher in patients with T2DM compared with control group (427.28 ± 23.28 µg/dl vs. 244.19 ± 16.63 µg/dl; p=0.001), but no causal relationship was suggested [29]. The results of the current study were in agreement with those reported previously in gestational diabetes mellitus [30] and T2DM[14,31]. In contrast to this study Erikstrup et al. found that RBP 4 was lower in individuals with T2DM compared with individuals with non-glucose tolerance test [15] but Lewis et al. reported that RBP levels were not significantly different between subjects with diabetes and subjects without diabetes

It is suggested that the GLUT4 is downregulated in the insulin resistance state in adipocytes that relates to an increased secretion of RBP 4. There are not enough data that prove this hypothesis [30]. On the other hand, this study did not find any association between total RBP 4 and HOMA-IR results which supports the previous remark that RBP 4 is not a useful marker of insulin resistance [30, 32].

Fasting blood sugar levels were highly significant in T2DM compared with control group (181.6 ± 57.3 vs. 94.1 ± 16.3 mg/dl, p<0.001). The glucose transporter GLUT4 facilitates the transport of glucose across plasma membrane into skeletal muscle cells and into adipocytes.

This process is the rate-limiting step in glucose uptake into those tissues and is subjected to stimulation by insulin. Insulin resistance occurs in the pathogenic condition of obesity, metabolic syndrome, and type 2 (non-insulin-dependent) diabetes. The expression of GLUT4 is then down regulated in adipose tissue, resulting in impaired glucose uptake [(33)]. The result of the present study were in consistent with those reported previously that fasting blood sugar was higher in patients group than controls group (188.40 ± 68.62 vs. 81.80 ± 11.35 mg/dl, $p<0.01$) [(34)].

Glycated hemoglobin A1c levels were significantly higher in T2DM comparable with control group ($p<0.001$, 9.12 ± 0.33 vs.5.69 ± 0.15) which reflects poor glycemic control of our patients. A previous study reported that HbA1C levels were significantly higher ($p<0.001$) in T2DM (13.1 ± 3.1vs. 3.1 ± 1.7) [(35)].

In this study no significant elevation in diabetic serum insulin and HOMA-IR in comparison to obese control group (12.9 ± 1.06 vs. 10.06 ± 1.14 µIU/ml) for insulin and (5.82 ± 0.66 vs. 2.44 ±0.32) for HOMA-IR respectively. These result in agreement with those reported previously in Italians type 2 diabetic, mean insulin (109.1± 8.13 vs. 127.4 ± 15.75 pmol/l), mean HOMA-IR (5.7 ± 0.49 vs. 4.6± 0.68) [(36)].

In T2DM group insulin sensitivity index was significantly reduced in comparison with each control groups and p value <0.001 this was in covenant with Wiegand et al. who found that ISI decreased significantly in T2DM (mean ISI = 1.2 ± 0.1) than normal glucose tolerance (mean ISI = 3.5 ± 0.3) $p<0.001$[(37)].

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