Assessment of retinol-binding protein-4, fibroblast growth factor-21, and dipeptidyl peptidase-4 in relation to obesity and insulin resistance of type 2 diabetes mellitus among Egyptian patients

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Received: 28 March 2021
Revised: 18 April 2021
Accepted: 28 April 2021
Published: xx xx 2020

Journal of The Arab Society for Medical Research 2021, 16:32–39

Background/aim
The relation between obesity, inflammation, and insulin resistance (IR) shows that adipose tissue plays a significant secretory role. Adipokines such as retinol-binding protein-4 (RBP-4), fibroblast growth factor-21 (FGF21), and dipeptidyl peptidase-4 (DPP4) exhibit pleiotropic biological activities and might be valuable biomarkers involved in the pathogenesis of type 2 diabetes mellitus (T2DM). The present study aims to assess serum levels of RBP-4, FGF21, and DPP4 and correlate their relation with obesity and IR in Egyptian patients with T2DM.
Patients and methods
This study included 130 patients with T2DM (70 obese and 60 nonobese) enrolled from the inpatient and outpatient clinics of the National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt, in addition to 70 age-matched and sex-matched healthy individuals (35 obese and 35 nonobese). Serum level assessments of RBP-4, FGF21, and DPP4 were carried out on all participants using Enzyme Linked Immuno-Sorbent Assay technique.
Results
Serum levels of RBP-4, FGF21, and DPP4 showed statistically significant differences in all studied groups ($P<0.01$). RBP-4, FGF21, and DPP4 were all correlated positively with BMI, fasting insulin, and HOMA-IR. RPB-4 was negatively correlated with high-density lipoprotein-cholesterol. Both RBP-4 and FGF21 were significantly associated with IR (odds ratio=1.264; $P<0.001$, and odds ratio=1.059; $P<0.01$, respectively), whereas receiver operating characteristic curves analysis revealed that serum levels of RBP-4 were most significant [area under curve (AUC)=0.826, $P=<0.001$], followed by FGF21 (AUC=0.774, $P<0.001$) and finally DPP4 (AUC=0.677, $P<0.001$).
Conclusions
Obesity and IR were found to be significantly associated with RBP-4, FGF21, and DPP4. They were higher in all obese groups, with the diabetic obese group having the highest concentrations. Of the three adipokines studied, RBP-4 has the strongest link. This finding will bolster the adipose-derived factors use as biomarkers and targets for treating and managing obesity and T2DM.

Keywords:
adipokines, insulin resistance, obesity, type 2 diabetes mellitus

J Arab Soc Med Res 16:32–39

1687-4293

Introduction

Obesity and type 2 diabetes mellitus (T2DM) are epidemic diseases that affect people all over the world [1]. They are two of the most challenging public health issues linked to life-threatening diseases like cardiovascular disease and cancer [2]. Increased lipolysis from large adipose tissues (ATs) and the inflammatory factors secretion are two main mechanisms that cause insulin resistance (IR). ATs as endocrine organs secrete peptides or proteins known as 'adipokines.' Adipokine secretion patterns are frequently altered in metabolic diseases like obesity and T2DM, and it is essential to gain a deeper understanding of the diseases' underlying pathogenesis. Retinol binding protein-4 (RBP-4), fibroblast growth factor-21 (FGF21), and dipeptidyl peptidase-4 (DPP4) are three adipokines that deserve more attention because they are linked to obesity, IR, and T2DM in humans [3,4].

RBP-4 is a 21-kDa hepatocyte-secreted factor located in chromosome 10 (10q23–q24). It is a carrier for vitamin A (retinol) and its derivatives. Mature

adipocytes and macrophages also secrete it [5]. RBP-4 is thought to stimulate gluconeogenesis enzymes in hepatocytes while impairing insulin signaling pathways in skeletal muscle. Significantly higher RBP-4 concentrations were found with IR, either owing to a glucose transporter-1 (GLUT-4) gene defect in ATs or as a result of CD4+ T-helper cell polarization induction and ATs inflammation. Injection of recombinant RBP-4 is also thought to have caused IR [6]. In obese patients with normoglycemia and T2DM, RBP-4 expression was significantly increased [7]. Higher levels of RBP-4 in obese and diabetic patients were also associated with IR [8]. RBP-4 was found to have similar associations in children and adolescents, suggesting that it may be implicated in metabolic syndrome in early stages [9]. Finally, lowering RBP-4 levels could be an effective treatment for IR and obesity-related diseases [10].

FGF21 is a circulating protein encoded by a gene in chromosome 19 (19q13.33). It is a member of the FGF superfamily that regulates lipid and glucose metabolism and energy homeostasis. The primary sites of expression are the liver and white AT [11]. It binds to receptors called FGFRs and a cofactor located in adipocytes called β-Klotho [12]. By inducing the GLUT-1 gene, glucose uptake in differentiated adipocytes is stimulated by FGF21, resulting in triglyceride storage and lipolysis stimulation. Fatty acids released into the bloodstream cause liver gluconeogenesis and ketogenesis, raising glucose in the blood and lead to IR. Numerous studies indicate that it could be an independent risk factor for IR, T2DM, and metabolic syndrome [13,14]. Obese children and adults have also been found to have higher serum FGF21 concentrations as obesity is a FGF21-resistant state [15,16].

DPP4 is a transmembrane glycoprotein enzyme that cleaves N-terminal dipeptides from a number of different substrates, including cytokines, growth factors, neuropeptides, and the incretin hormones. Hydrolysis and inactivation of GIT hormones are the most relevant DPP4 biological activities, such as glucagon-like peptide-1 and glucagon-dependent insulinotropic peptide, which are produced by the intestinal mucosa and contribute to postprandial insulin secretion by ~60% [17]. DPP4 inhibitors are used in T2DM as oral potential hypoglycemic and insulin-sensitizing drugs [18]. DPP4 inhibitors were shown to have anti-inflammatory properties in diabetic and cardiovascular patients in several studies [19,20].

This study aims to assess serum levels of RBP-4, FGF21, and DPP4 and correlate their relation to obesity and IR in Egyptian patients with T2DM.

Patients and methods

Patients

This case–control study was performed on 130 patients with T2DM and 70 age-matched and sex-matched healthy participants. All those with T2DM were enrolled from the inpatient and outpatient clinics of the National Institute of Diabetes and Endocrinology (NIDE), Cairo, Egypt, from 2018 to 2020.

Ethical consideration

The present study was conducted according to the Declaration of Helsinki principles. The General Organization of Teaching Hospitals and Institutes Ethics Committee has approved this study with approval number IDE/00216. Each participant provided written informed consent before recruitment.

Study design

A total of 200 participants (81 males and 119 females) were enrolled in this study, with age ranged from 35 to 70 years old. They were categorized into four groups as follows:

(1) Group 1: diabetic obese group, including 70 patients with T2DM with BMI $>30\,kg/m^2$.
(2) Group 2: diabetic nonobese group, including 60 patients with T2DM with BMI $<30\,kg/m^2$.
(3) Group 3: control obese group, including 35 healthy participants with BMI $>30\,kg/m^2$.
(4) Group 4: control nonobese group, including 35 healthy participants with BMI $<30\,kg/m^2$.

Patients with T2DM are diagnosed according to the criteria of the American Diabetes Association [21].

Exclusion criteria

T1DM, critically ill patients, a known history of inflammatory and autoimmune diseases, renal impairment, liver cirrhosis, and malignancies were excluded from the study.

Methods

A detailed history was obtained for all participants, including diabetes duration, smoking history, hypertension, current medications, and other comorbidities. BMI [the weight (in kilograms) divided by the square of the height (in meters)] was measured on the day of sample collection.

Blood sampling and biochemical analysis

A venous blood sample was withdrawn from each participant and divided into three sample tubes after overnight fasting, two of them for serum and the other tube is an EDTA tube. The first tube was serum separator tube for blood chemistry and was left to clot. By centrifugation, the serum was separated at 3000 rpm for ten minutes. It was tested for fasting blood glucose (FBG) by the glucose oxidase method and lipid profile [total cholesterol, triglycerides, high-density lipoprotein-cholesterol (HDL-c), and low-density lipoprotein-cholesterol (LDL-c)] using BT 3500 (Biotecnica Instruments Inc., Roma, Italy). HDL-C was assessed by the direct assay method [22], and Friedewald's formula estimated LDL-C [23]. The second tube was EDTA vacutainer for HbA1c analysis by high-performance liquid chromatography (HPLC) Biorad Variant II Turbo (Biorad Medical Diagnostics, Dreieich, Germany) [24]. The last serum separator tube was left to clot, and serum was separated after centrifugation for 15 min at 3000 rpm. Serum was stored at −80°C until analysis of RBP-4, FGF21, DPP4, and insulin levels by a commercially available Enzyme Linked Immuno-Sorbent Assay (ELISA) technique according to manufacturer's instructions. RBP-4, FGF21, and DPP4 serum concentrations were assessed using ELISA kits (NOVA Bioneovan Co., China), whereas serum insulin was assessed by an ELISA kit (NOVA Bioneovan Co.) (DRG Instruments GmbH, Marburg, Germany). The HOMA-IR was used to calculate the IR index: [FPG (mg/dl)×fasting insulin (μIU/ml)]/405 [25] applying normal cutoff value less than 2.6 [26]. Pancreatic B-cell function (B%) (HOMA-β) was obtained from the HOMA2-IR index by the program HOMA2 Calculator v 2.2.3 provided by Oxford University [27].

Statistical analysis

Statistical data were analyzed using Microsoft Excel 2010 and SPSS version 13 for Windows (SPSS Inc., Chicago, Illinois, USA). Quantitative data were expressed as mean ±SD and compared using *t*-test when normally distributed, and as median and range using Mann–Whitney *U*-test when not normally distributed. Categorical data were represented as frequencies (%). The differences in frequencies of categorical parameters were analyzed by χ^2-test. Correlations were done using Pearson's correlation coefficient test (*r*) or Spearman's coefficient. Binary logistic regression analysis and receiver operating characteristic (ROC) curve were constructed with area under curve (AUC) to detect the association of each adipokine with IR. A *P* value of less than 0.05 was considered significant.

Results

The present study comprised 200 participants (81 males and 119 females), with age ranged from 35 to 70 years. They were categorized into four groups, 70 T2DM obese patients (28 males and 42 females), 60 T2DM nonobese patients (22 males and 38 females), 35 healthy obese patients (13 males and 22 females), and 35 healthy nonobese patients (18 males and 17 females).

Demographic characteristics and laboratory parameters were summarized for all the studied participants in Table 1, where the data presented showed no significant differences regarding age and sex between the four groups. BMI showed a significant increase ($P<0.05$) in obese T2DM (G1) and control (G3) groups than that of nonobese diabetic (G2) and control (G4) groups. Regarding lipid profile, the two obese groups (G1 and G3) revealed significantly higher serum triglycerides and lower HDL-c levels than nonobese groups (G2 and G4) correspondingly, whereas LDL-c was significantly higher ($P<0.05$) in the diabetic groups (G1 and G2) than the control groups (G2 and G4), with no significant differences in the serum levels of total cholesterol. On studying glycemic control indices, the mean FBG level and HbA1c were significantly higher ($P<0.05$) in diabetic obese, and nonobese groups than control obese and nonobese, respectively. The serum insulin level was significantly higher ($P<0.05$) in diabetic obese group compared with all other studied groups. Moreover, HOMA-IR showed a gradual increase through the studied groups with significant high level in diabetic obese group compared with all other studied groups. On the contrary, HOMA-β was decreased in diabetic obese group with statistically significant differences when compared with all studied groups ($P<0.05$). Serum RBP-4 showed statistically significant differences in all studied groups ($P<0.05$) except when comparing control obese with control nonobese groups ($P>0.05$), whereas FGF21 and DPP4 concentrations showed statistically significant differences ($P>0.05$) in all studied groups ($P<0.05$) except when comparing diabetic non obese with control obese groups.

Considering the correlations of the three adipokines with demographic and laboratory parameters in the diabetic group, RBP-4, FGF21, and DPP4 serum levels were positively correlated with BMI, fasting insulin, and HOMA-IR. Serum levels of FGF21 and DPP4 were positively correlated with TG and FBG, whereas only RPB-4 showed a negative correlation with HDL-c, with no significant correlation with the other parameters (Table 2).

Table 1 Demographic characteristics and biochemical parameters for all studied groups

Parameters	Diabetic group (*N*=130)		Control group (*N*=70)	
	T2DM obese (G1) (*N*=70)	T2DM nonobese(G2) (*N*=60)	Control obese (G3) (*N*=35)	Control nonobese (G4) (*N*=35)
Age (years)	52 (44–58)[a]	50 (43–56)[a]	48 (40–55)[a]	48 (41–57)[a]
Sex (male/female)	28/42 [a]	22/38 [a]	13/22 [a]	18/17 [a]
Duration (years)	8 (3–13)[a]	8 (5–11)[a]	-	-
BMI (kg/m^2)	32.8±0.21[a]	27.2±0.21[b]	32.9±0.23[a]	27.8±0.23[b]
Triglycerides (mg/dl)	211±7.3[a]	153±9.8[b]	212±9.2[a]	175±8.8[c]
Total cholesterol (mg/dl)	202±5.8[a]	209±4.4[a]	191±8.6[a]	182±6.1[a]
HDL-c (mg/dl)	33±0.7[a]	39±1.3[b]	30±1.2[a]	40±1[b]
LDL-c (mg/dl)	137±5.5[a]	132±3.2[a]	118±4.4[b]	120±4.5[b]
FBG (mg/dl)	119±2.8[a]	115±2.1[a]	88±2[b]	81±2.1[b]
HbA1c (%)	9.1±0.26[a]	9.4±0.29[a]	5.6±0.15[b]	5.8±0.1[b]
Fasting insulin (μIU/ml)	17±0.3[a]	13±0.3[b]	14±0.5[b]	10±0.2[c]
HOMA-IR	5±0.1[a]	3.7±0.09[b]	2.6±0.11[c]	1.8±0.05[d]
HOMA %B	79±5.1[a]	97±4.9[b]	136±11.2[c]	153±11.4[d]
RBP-4 (μg/ml)	64±0.8[a]	51±0.7[b]	47±0.6[c]	44±0.6[c]
FGF21 (pg/ml)	169±2[a]	154±1.2[b]	156±2.2[b]	142±2.4[c]
DPP4 (ng/ml)	310±7.2[a]	278±5.9[b]	275±9.5[b]	238±9[c]

Age, sex, and duration values are expressed as median between (lowest–highest) values. Other parameters are expressed as mean±SE. All values with different letters (a, b, c, d) within the same row are significant at $P<0.05$, using Mann–Whitney test for age, sex, and duration, and ANOVA post-hoc test for rest of parameters. Dpp4, dipeptidyl peptidase-4; FGF21, fibroblast growth factor-21; FPG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL-c, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of IR; HOMA-β, homeostasis model assessment of β-cell function; LDL-c, low-density lipoprotein-cholesterol; RBP-4, retinol-binding protein-4.

Table 2 Pearson's correlation between RBP-4, FGF21, and DPP4 and other studied parameters in the diabetic group

Parameters	Diabetic patients (*N*=130)					
	RBP-4		FGF21		DPP4	
	r	*P* value	*r*	*P* value	*r*	*P* value
Age (years)	–0.079	0.374	–0.107	0.227	0.061	0.491
Duration (years)	0.128	0.146	–0.101	0.254	0.002	0.981
BMI (kg/m^2)	0.310	<0.001*	0.598	<0.001*	0.269	0.002*
Triglycerides (mg/dl)	–0.031	0.724	0.234	0.007*	0.264	0.002*
Total cholesterol (mg/dl)	–0.100	0.259	0.107	0.227	0.056	0.528
HDL-c (mg/dl)	–0.188	0.032*	–0.022	0.802	–0.064	0.473
LDL-c (mg/dl)	–0.065	0.465	0.119	0.176	0.058	0.510
FBG (mg/dl)	0.033	0.710	0.363	<0.001*	0.187	0.033*
HbA1c (%)	–0.167	0.057	0.031	0.730	0.056	0.525
Fasting insulin (μIU/ml)	0.248	0.004*	0.334	<0.001*	0.186	0.034*
HOMA-IR	0.236	0.007*	0.609	<0.001*	0.306	<0.001*
HOMA %B	0.105	0.234	0.107	0.226	–0.023	0.792
RBP-4 (μg/ml)	–	–	–0.024	0.790	0.110	0.215
FGF21 (pg/ml)	–0.024	0.790	–	–	0.152	0.084
DPP4 (ng/ml)	0.110	0.215	0.152	0.084	–	–

DPP4, dipeptidyl peptidase-4; FGF21, fibroblast growth factor-21; FPG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL-c, high-density lipoprotein-cholesterol; HOMA-IR, homeostasis model assessment of IR; HOMA-β, homeostasis model assessment of β cell function; LDL-c, low-density lipoprotein-cholesterol; RBP-4, retinol-binding protein-4. *Statistically significant.

Binary logistic regression analysis was performed to study the association between serum levels of RBP-4, FGF21, and DPP4 and IR (HOMA-IR cutoff=2.6) in all the studied groups. RBP-4 showed a highly significant association (odds ratio=1.264; $P<0.001$), and then FGF21 (odds ratio=1.059; $P=0.010$); on the contrary, DPP4 failed to reach a significance level (odds ratio=1.007; $P=0.056$), as shown in Table 3.

To study the diagnostic performance of RBP-4, FGF21, and DPP4 in evaluating the risk of IR, ROC analysis demonstrated that a cutoff value of 48.7 μg/ml for RBP-4 yielded a sensitivity and specificity of 77.50 and 77.60%, respectively, whereas a cutoff value of 148.6 pg/ml for FGF21 yielded a sensitivity and specificity of 85.90 and 70.70%, respectively, and a cutoff value of 241.5 ng/mL for DPP4 yielded a

Table 3 Association of RBP-4, FGF21, and DPP4 with insulin resistance by binary logistic regression analysis

Independent variable	OR (95% CI)	*P* value
RBP-4	1.264 (1.156–1.382)	<0.001*
FGF21	1.059 (1.025–1.095)	0.010*
DPP4	1.007 (1.000–1.015)	0.056

95% CI, 95% confidence interval; DPP4, dipeptidyl peptidase-4; FGF21, fibroblast growth factor-21; OR, odds ratio; RBP-4, retinol-binding protein-4. *Statistically significant at $P<0.01$.

Table 4 Diagnostic performance of RBP-4, FGF21, and DPP4 in evaluating the risk of insulin resistance (HOMA-IR index >2.6)

Variables	Cutoff	Sensitivity (%)	Specificity (%)
RBP-4 (μg/ml)	48.7	77.50	77.60
FGF21 (pg/ml)	148.6	85.90	70.70
DPP4 (ng/ml)	241.5	87.30	41.40

DPP4, dipeptidyl peptidase-4; FGF21, fibroblast growth factor-21; RBP-4, retinol-binding protein-4.

sensitivity and specificity of 87.30 and 41.40%, respectively (Table 4).

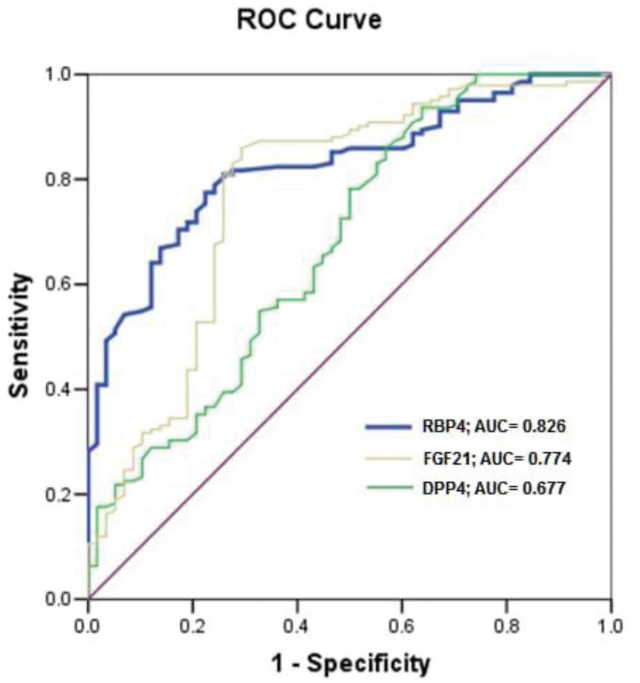
Furthermore, ROC curves and the area under the curve (AUC) were carried out to evaluate the discriminatory capacity of RBP-4, FGF21, and DPP4 levels with IR (HOMA-IR >2.6) in all the studied groups. ROC analysis revealed that serum level of RBP-4 was the most significant (AUC=0.826, $P<0.001$), followed by FGF21 (AUC=0.774, $P<0.001$), and finally, DPP4 (AUC=0.677, $P<0.001$), as shown in Table 5 and obtained in Fig. 1.

Table 5 Area under curve of RBP-4, FGF21, and DPP4 ROC in discrimination of insulin resistance (HOMA-IR index >2.6)

Variables	AUC	95% CI	*P* value
RBP-4	0.826±0.029	0.769–0.884	<0.001*
FGF21	0.774±0.040	0.695–0.852	<0.001*
DPP4	0.677±0.044	0.591–0.763	<0.001*

Values are expressed as value±SEM. 95% CI, 95% confidence interval; AUC, area under the curve; CI, confidence interval; DPP4, dipeptidyl peptidase-4; FGF21, fibroblast growth factor-21; RBP-4, retinol-binding protein-4. *Statistically significant at $P<0.01$.

Figure 1



Receiver operating characteristics curve of retinol-binding protein-4, fibroblast growth factor-21, and dipeptidyl peptidase-4 with insulin resistance (HOMA-IR index >2.6).

Discussion

Obesity and T2DM are the major noncommunicable public health problems of the twenty-first century [28]. Studies on the relationship between Ats and IR and T2DM have inspired researchers to look for new laboratory parameters that reflect adipocyte metabolism abnormalities and their proinflammatory potential. Adipokines are categorized into proinflammatory and anti-inflammatory ones, and the imbalance between them leads to pathogenic changes. As a typical example, patients with obesity and T2DM exhibit altered adipokine profiles, leading to increased metabolic risk and alterations in insulin sensitivity [3]. In this study, we assessed the potential role of RBP-4, FGF21, and DPP4 associated with obesity and IR in T2DM Egyptian patients.

In all obese groups, our study found that serum levels of RBP-4, FGF21, and DPP4 were significantly higher than the nonobese ones, with the diabetic obese group having the highest concentrations. Besides, these levels were correlated positively with BMI, fasting insulin, and HOMA-IR. These findings reveal that their levels are linked to IR and obesity, which agree with many studies.

The RBP-4 in our study showed the highest association and was the most significant among the three studied adipokines with a strong positive correlation with BMI and IR indices (FBG, fasting insulin, and HOMA-IR) and a negative correlation with serum HDL-c. Our results were consistent with those who reported that RBP-4 was higher in AT and serum of obese and/or T2DM people as shown by Kotnik *et al.* [6] Moreover, a significantly higher RBP-4 expression in obese normoglycemic patients and T2DM was observed by Kelly et *al.* [7] Similarly, in obese nondiabetic and diabetic patients, serum RBP-4 level was high and correlated with BMI, and under IR

conditions related to obesity and T2DM, it was highly expressed [4,9]. RBP-4 expression was found to be higher in visceral fat than in subcutaneous fat, according to Kloting *et al*. [29], with a significant increase in individuals with obesity and T2DM. Significant weight loss achieved through diet, exercise, or bariatric surgery results in lower circulating and/or AT RBP-4 levels and increased insulin sensitivity [30,31]. Furthermore, Wu *et al*. [32] reported that circulating RBP-4 level also correlated with other components of the metabolic syndrome, suggesting that RBP-4 is an atherosclerosis predictor. In a retrospective study, an increase in RBP-4 with time was associated with increased odds of worsening IR owing to a strong correlation between RBP-4 levels and the quantity of visceral adiposity and impaired glucose tolerance (IGT)/type 2 diabetes [33]. Data supporting a causative role for RBP-4 in IR/T2DM include the fact that a gain-of-function polymorphism in the RBP-4 promoter, which increases adipose RBP-4 expression, is responsible for 80% increased risk of T2DM [34]. These findings indicate that increased RBP-4 levels in the serum or AT of morbidly obese people can trigger hepatic and systemic IR by stimulating basal lipolysis and activating AT macrophages, causing the release of proinflammatory cytokines that disrupt lipolysis suppression [35]. An alternative explanation for the increased circulating RBP-4 levels in patients with T2DM could be renal insufficiency, decreasing its renal clearance [36]. Lowering inflammatory RBP-4 levels could be an efficient treatment target for IR and obesity-related diseases [10]. In disagreement with our results, circulating RBP-4 levels were not correlated with IR, IGT, T2DM, or altered insulin secretion which may reflect heterogeneity in the performance of different assays for measuring RBP-4 in insulin-resistant patients and potential racial/ethnic differences in the relationship between RBP-4 and IR. Moreover, no association was determined with metabolic syndrome and its complications [37,38]. In addition, Korek *et al.* [39] concluded that the mean RBP-4 concentration did not vary between obese and nonobese persons and that there were no significant correlations with HOMA-IR, anthropometric, or body composition parameters. The difference in the results of the many studies could be attributed to shortcomings in the methodology of measuring in RBP-4 levels. Serum RBP-4 levels should be measured with extreme care. According to Graham *et al.* [40], the most reliable method for assaying serum RBP-4 elevations associated with IR is quantitative Western blotting. Other measurement methods, such as ELISA and enzyme immunoassay, are, however, widely used in a variety of populations [41].

According to our study, all obese groups showed significantly higher serum levels of FGF21 than nonobese groups, with the diabetic obese group having the highest concentrations. It was positively correlated with obesity (BMI), glycemic indices (FBG, fasting insulin, and HOMA-IR.), and TG. Our results matched several studies that found higher levels of circulating FGF21 in people who already had IR, IGT, or hypertriglyceridemia with a significant correlation with BMI, TG, and insulin [13,42]. In agreement, Zhang *et al.* [16] reported that circulating FGF21 levels were elevated in various metabolic disease states, such as obesity, IR, and T2DM. Similarly, Sahar and colleagues in 2017 reported that serum FGF21 levels were significantly increased in newly diagnosed prediabetics and patients with T2DM compared with healthy individuals, with statistically significantly higher FGF21 levels in diabetic patients. FGF21 was correlated positively with obesity (BMI and waist-hip ratio), glycemic (FBG and HbA1c) and IR (fasting insulin and HOMA-IR) parameters, and atherogenic lipid profile [43]. To assess FGF21 predictive value on T2DM and metabolic syndrome incidence over 5 years, the Metabolic Syndrome Berlin Potsdam (MeSyBePo) recall study, which included white participants, found higher FGF21 levels in patients with incident metabolic syndrome. FGF21 was correlated significantly with TG, BMI, age, HbA1c, FBG, and HOMA-IR [14]. Another two studies in Chinese and Asian individuals revealed that FGF21 was related to metabolic syndrome and T2DM [44,45]. Furthermore, the level of FGF21 was an independent biomarker of progressive chronic kidney disease in patients with early-stage diabetic nephropathy [46]. In patients with T2DM and coronary artery disease, it can predict cardiac events and mortality rates [47,48].

Finally, this study found that serum DPP4 levels were increased in obese groups more than in nonobese groups, with the diabetic obese group having the highest concentrations. Similarly, significant positive correlations were found with obesity (BMI), glycemic indices (FBG, fasting insulin, and HOMA-IR), and TG. As obesity is a main T2DM risk factor, adipose-associated increase in plasma DPP4 is shown to have a crucial role in the relation between obesity and T2DM [49]. DPP4 cleaves N-terminal dipeptides from various substrates, including glucagon-dependent insulinotropic peptide and glucagon-like

peptide-1, thereby disrupts insulin secretion from pancreatic beta cells [50]. Our findings are supported by Reinehr *et al.* [51], who found that serum DPP4 showed a significant increase in obese more than nonobese individuals and was linked to IR factors and metabolic syndrome components (BMI, TG, and HDL-c). They also found that DPP4 was expressed in differentiated adipocytes, and it decreases insulin action in both adipocytes and muscle cells. Another study found that, regardless of BMI distribution, serum concentration and activity of DPP4 showed a significant increase in patients with T2DM more than controls. Although they found considerably higher plasma DPP4 levels in obese patients with T2DM, they did not find any difference in DPP4 activity [52]. In 2019, a study by Alameey and colleagues evaluated DPP4's potential as a target for improving metabolic syndrome components in Egyptians. They found that after losing weight, serum DPP4 enzyme activity, lipid panel except for HDL, and HOMA-IR were all significantly suppressed owing to exercise and nutritional regimen [53]. In support, during AT differentiation, DPP4 expression is increased with elevated secretion rate from AT in obesity, so serum DPP4 enzyme activity was positively correlated with BMI in young, healthy Japanese participants [54]. On the contrary, most studies support the link between increased plasma DPP4 activity and/or levels and T2DM. Inhibitors of DPP4 are thus used clinically as 'incretinergic' drugs in the treatment of T2DM [55]. DPP4 may be used as a diagnostic or prognostic marker for diabetes and coronary artery disease [56]. On the contrary, Eun-Hee Cho and colleagues demonstrated that DPP4 levels were significantly correlated with serum creatinine levels and eGFR in patients with T2DM, with no associations between DPP4 levels and BMI or waist circumference. They hypothesized that DPP4 might be a biomarker for deteriorating renal function in patients with T2DM [57].

Conclusion

The present study supports the role of RBP-4, FGF21, and DPP4 as adipose-derived biomarkers involved in the pathogenesis of IR in Egyptian patients with T2DM. RBP-4 showed a strong correlation and was the most significant among the three studied adipokines. However, the usage of new biomarkers in routine diagnosis is difficult as high analytical sensitivity is required to detect metabolic changes resulting from low-grade inflammation. The absence of standardized, automated assay methods is a limitation for the usage of described adipokines. Moreover, the relatively small number of participants and controls requires further large studies to confirm our findings and identify the role of pathologically disturbed adipokine profiles in various pathogenic conditions.

Acknowledgments

The authors are deeply thankful to the National Institute of Diabetes and Endocrinology (NIDE) for their help and support of this study.

Ayat I. Ghanem, Atef A. Bassyouni, and Ghada A. Omar contributed to the design and implementation of the study, analysis of the results, and manuscript preparation, editing, and review.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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