The Relationship of Plasma Visfatin and Retinol Binding Protein-4 to Fat Composition -assessed by Ultrasound-, Obesity, and type 2 Diabetes Mellitus

By

Dr/ Naglaa Mostafaa El-Sayed

Lecturer of Radiodiagnosis, Faculty of Medicine- Cairo University

Abstract

Background and objectives: Adipose tissue is responsible for releasing various adipokines which have been related to insulin resistance. Understanding the relationship of these adipokines to insulin resistance may help in early diagnosis and faster the development of new treatments for diabetes. The objective of this study was to determine the possible association between these adipokines (visfatin and retinol-binding protein-4 (RBP-4)) in type 2 diabetes and obesity. The role of ultrasound measurements was to determine which of the adipose tissue most closely relates to visfatin and RBP-4 levels. The subcutaneous fat or the visceral fat.

Participants and Research Design: According to the body mass index (BMI), 40 type 2 diabetes patients were divided into two equal groups, the 1st was non-obese diabetic patients with BMI < 25 Kg/ m^2 and the other was obese diabetic patients with BMI >25 Kg/m^2 . There were 40 healthy persons in the control group; also, divided into two equal groups according to the BMI. Anthropometric and biochemical measurements were done by using standardized techniques. Fasting plasma visfatin, RBP-4 and insulin levels were measured by enzyme-linked immunosorbent assay. Insulin resistance index was calculated by the homeostasis model assessment ($HOMA_{IR}$). Visceral fat in multiple sites, subcutaneous fat were measured by Ultrasonography in addition to assessment of presence or absence of fat deposition in the liver.

Results: The levels of plasma **visfatin** were increased significantly in diabetics compared to control group; moreover, visfatin concentration in diabetics obese was significantly higher compared to diabetics non obese. It was also highly related to the amount of visceral fat but not with subcutaneous fat.

The levels of plasma **RBP-4** were significantly higher in obese patients compared to control group. Furthermore, the levels of RBP4 were significantly higher in diabetics in comparison to non-diabetics with similar BMI values. Plasma visfatin was positively correlated with RBP-4, BMI, waist / hip ratio (WHR), insulin, insulin resistance index and visceral fat area in diabetic patients, while it was negatively correlated with systolic blood pressure. On the other hand, plasma RBP-4 correlated positively with visfatin, BMI, WHR, blood glucose, insulin and insulin resistance index, and fatty liver detected by ultrasound in diabetic patients. RBP-4 was not related to either visceral or subcutaneous fat.

Stepwise regression analysis revealed that plasma visfatin levels remained positively associated with visceral fat and WHR; while plasma RBP4 levels remained positively correlated with BMI, fatty liver in diabetic patients.

Conclusion: Plasma visfatin levels are significantly higher in diabetics than control subjects and positively correlated with visceral fat but not with subcutaneous fat.

So, increased visceral fat measured with US is highly related to the amount of visfatin in blood which increases the risk of developing diabetes. Moreover, plasma RBP-4 levels are increased significantly in type 2 diabetes mellitus and obesity. However, Circulating RBP4 is not correlated with the amount of visceral or subcutaneous fat, but, it correlates positively with liver fat which is easily detected with US as well as with the insulin resistance. The correlation between visfatin & visceral fat measurement confirm that visceral fat measurement by US is effective and reproducible.

Introduction

An increased adipose tissue mass is strongly associated with the pathogenesis of insulin resistance and type 2 diabetes [(1)] .Besides its role in energy storage, adipose tissue, an endocrine organ, produces several hormones and cytokines (such as leptin, tumor necrosis factor, interleukin-6 and adiponectin) that have wide-ranging effects on carbohydrates and lipid metabolism, and therefore appear to play an important role in the pathogenesis of diabetes, insulin resistance and atherosclerosis [(2)]. However, it is apparent that accumulation of visceral adipose tissue poses a greater cardio-metabolic risk than subcutaneous adipose tissue, even though the latter is the larger adipose tissue depot of the two [(3)] ; removal of visceral than subcutaneous tissue improves insulin sensitivity [(4)]. Additionally, differences in gene expression between adipocytes of visceral or subcutaneous origin do exist. The potential role of recently discovered adipokines (visfatin and retinol binding protein-4) in the development of obesity- related insulin resistance are increasingly understood.

Visfatin was recently identified as a protein highly expressed in visceral adipose tissue compared to subcutaneous adipose tissue [(5)]. Visfatin, previously known as a pre–B-cell colony–enhancing factor (PBEF), has a function also in the immune system, where it was described as a growth factor for early B-cells [(6)]. Visfatin/PBEF binds and activates the insulin receptor in different insulin-sensitive cells in vitro and treating mice with recombinant visfatin/PBEF elicited insulin-like effects also in vivo. Plasma glucose is lowered by treatment with visfatin/PBEF, while heterozygous mice knockout for the visfatin/PBEF gene have plasma glucose levels higher than wild-type littermates [(5)]. Visfatin/PBEF expression in adipocytes is upregulated by dexamethazone and is down regulated by growth hormone, isoproterenol, and cholera toxin. Insulin has no effect on visfatin/PBEF mRNA [(7)]. Moreover, visfatin/PBEF is upregulated by a peroxisome proliferators- activated receptor (PPARα and PPARγ) agonists in obese rats in association with improved glycaemic control and lipid profile, thus suggesting that PPARα and PPARγ agonists may act, at least in part, through the upregulation of visfatin/PBEF expression [(8)]. A recent study in humans reported plasma visfatin/PBEF to be directly correlated with body mass index and body fat content in males only and failed to find a different expression of visfatin mRNA between visceral and subcutaneous fat depots [(9)].Although, plasma levels of visfatin increased with obesity and correlated positively with visceral adiposity [(10)], others did not notice this association [(9)].

Retinol-binding protein (RBP)-4, secreted by liver and adipocytes, has important effects on systemic insulin sensitivity and glucose homeostasis [(11)]. Serum RBP-4 levels are elevated in insulin resistant states both in mice and humans. In mice, intraperitoneal injection of recombinant human RBP-4 induces systemic insulin resistance. Treatment of insulin-resistant mice with rosiglitazone, peroxisome proliferators activated receptor (PPAR)-γ agonist that improves insulin sensitivity, completely normalizes elevated RBP-

4 levels and reverses insulin resistance [12]. Elevation of RBP-4 might therefore play a causative role in insulin resistance and type 2 diabetes mellitus. In humans, serum RBP-4 levels correlate with the magnitude of insulin resistance and components of the metabolic syndrome. Improvement of insulin sensitivity by exercise training is associated with a reduction in serum RBP-4 levels [11]. Recently, serum RBP-4 levels were shown to be strongly correlated with the trunkal obesity and not with peripheral obesity [13] .However, others did not show any correlation between RBP-4 and either visceral or subcutaneous adiposity, but with the extent of fatty liver [14].

Effective methods for assessing visceral fat are important to investigate its role in the increased health risks in obesity. Simple anthropometric methods, such as waist-to-hip circumference ratio, waist circumference or sagittal diameter are widely used. However, these methods cannot differentiate between visceral and subcutaneous fat and are less accurate [15]. Dual energy x-ray absorptiometry (DEXA) measures body mass index, percentage of fat and total body fat mass [16].There have been a considerable number of researches involved in determining each component of body fat mass and body fat distribution. These methods are computed tomography (CT) [17] and magnetic resonance imaging (MRI) [18] the latter made it possible to assess subcutaneous fat (SF), and visceral fat (VF) volume. But they are expensive, time-consuming or require a relatively high radiation dose [16].

Moreover, in 1992, the method using abdominal ultrasonography (US) for assessment of subcutaneous and properitoneal fat thickness was reported, which made it possible to assess abdominal fat distribution in each individual frequently and repeatedly [19].US is a new, simple and accurate method of measuring visceral fat volume. Moreover, it can differentiate subcutaneous from visceral fat volume [20].

The main objectives of the current study were to investigate role of visfatin and retinol-binding protein 4 in type 2 diabetes mellitus and obesity and to measure using ultrasound method the visceral and subcutaneous abdominal fat as well as presence or absence of fatty liver, and whether these measures will be correlated with plasma visfatin and RBP-4.

Participants and Research Design

A total of forty type 2 diabetic patients (19 males and 21 females) were recruited from internal medicine outpatient clinics at Kasr El-Aini Hospital, as well as forty normal healthy adults (20 males and 20 females) from medical and paramedical stuff personals participated as control group. The subjects chosen for the study were categorized based on their body mass index (BMI) as non-obese (BMI < 25 kg/m2) and obese (BMI ≥ 25 kg/m^2).

Obesity was defined according to the World Health Organization criteria on the basis of the body-mass index (BMI) (the weight in kilograms divided by the square of the height in meters) [21]. Diagnosis of type 2 diabetes mellitus was made according to the American Diabetes Association. They considered an individual to be diabetic if fasting blood glucose (FBG) was ≥ 126 mg/dl and/or taking treatment of diabetes [22]. The definition of a non-diabetic is a subject who has a fasting blood glucose level lower than 100 mg/dl [22]. Additionally in the current study first and second degree relatives of type 2 diabetic patients were excluded from the control group. The diabetic patients were treated with diet and the oral hypoglycaemic drug metformin or sulfonylurea. No patients were receiving thiazolidinediones or insulin. Patients who had a diagnosis of urinary tract

infection, urolithiasis, liver cirrhosis, ischemic heart disease, macrovascular disease, overt proteinuria, or other known major diseases were excluded from the study. An informed consent was taken from all participants.

The following investigations were performed for all subjects:

Detailed history taking and physical examination to exclude the presence of cardiac, hepatic, renal, gastrointestinal or malignant disease which might affect the parameters to be investigated. Blood pressure was measured to exclude the presence of hypertension. **Anthropometric measurements** including: Body mass index (BMI) and Waist to hip ratio (WHR) were calculated for each participant. 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 right iliac crest. The hips were measured at their widest point.

Biochemical analysis

Blood samples were collected after an overnight fast from all subjects and divided into 3 parts. Part of each blood sample was withdrawn on EDTA for plasma separation and the separated plasma was kept frozen at – 80°C for further determination of Visfatin, Retinol-binding protein 4 and insulin. The other part was taken as whole blood for determination of glycosylated hemoglobin (HbA_{1c}) by the kit provided by Stanbio, Texas, USA [(23)].The third part was allowed to clot. The separated serum was used for determination of fasting blood glucose (FBG) [(24)], triglyceride [(25)]; total cholesterol [(26)], high density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c) [(27)] were estimated using commercially available kits.

Serum fasting insulin was assessed by the ELISA kit provided by BioSource, Nivelles, Belgium. Plasma visfatin was estimated by the ELISA kit provided by Phoenix Pharmaceuticals, Belmont, CA, USA. Assay sensitivity was 2 ng/ml and inter-assay and intra-assay Coefficient Variation (CV) were <10% and <5% respectively. Plasma Retinol-binding protein 4 (RBP-4) was measured by the ELISA kit provided by BioSource, Nivelles, Belgium. Assay sensitivity was between 0.001 and 5 µg/ml with inter-assay and intra-assay CV 5 % - 4.6 % respectively.

Insulin sensitivity was estimated using the homeostatic model assessment (HOMA-IR) index [serum fasting glucose level (mmol/l) × serum fasting insulin level (µU/ml) / 22.5] [(28)].

Fat composition

Thirty seven subjects were examined by US machine (Siemens elegra) and forty three subjects were examined using (General Electric voluson pro) US machine, Screening full abdominal examination was done first including assessment of the liver for presence of fatty changes, then the visceral fat thickness in different sites was measured by two experienced sonographers while the patient was in the supine position during normal quite respiration.

In the present study, the following parameters were measured;

(1) The distance between the internal surface of the abdominal muscle and the splenic vein. (Figure 1)

(2) The distance between the internal surface of the abdominal muscle and the posterior wall of the aorta just above the umbilicus. (Figure 2)

(3) The thickness of the fat layer overlying the posterior right renal wall. (Figure 3)

(4) The thickness of both the subcutaneous and properitoneal fat layers in the xiphoid process. (Figure 4)

A 3.5 –5 MHz convex-array probe was used to measure the first three parameters. The distance between the abdominal muscles and the splenic vein was scanned transversely in the mid line. In some cases of obese participants, the splenic vein was unclear in the mid line due to increased visceral fat in the mid line, so we tried to take this measurement in the anterior axillary line searching for the splenic vein at the splenic hilum. When the splenic vein could not be visualized clearly, this vein was detected by using color Doppler flow (Figure 1). The distance between the abdominal muscles and posterior wall of the aorta was measured also transversely in the mid line on the umbilicus (Figure 2).

The thickness of the fat layer of the posterior right renal wall was scanned longitudinally in the right anterior axillary line (Figure 3). For the fourth measurement (the thickness of the subcutaneous fat and the preperitoneal fat layers), they were measured using 7.5 MHz linear-array probe. The transducer was placed vertically against the skin as light as possible to prevent compression of the fat layers. It's to be mentioned that is some cases these measurements were taken using 3.5 MHz convex- array probe by decreasing the depth of the image. The volume of visceral fat was measured according to the following equation quoted from Hirooka et al 2005 : Visceral fat volume = -9.008+1.191 x (The distance between the internal surface of the abdominal muscle and the splenic vein) + 0.987 x (The distance between the internal surface of the abdominal muscle and the posterior wall of the aorta on the umbilicus in mm) + 3.644 x (thickness of the fat layer of the posterior right renal wall in mm) [(20)] .The visceral fat area of over 100cm^2 is widely accepted in diagnosing visceral fat obesity [(15)].

Statistical analysis

Statistical Package for social science (SPSS) program version 9.0 was used for analysis of data. Data was summarized as mean ± SD. T- test was used for analysis of 2 quantitative data. One way ANOVA was done for analysis of more than two variables followed by post Hoc test for detection of significance. Simple linear correlation (Pearson's correlation for quantitative data): It was done to detect the relation between the Visfatin and RBP4 with all other demographic and laboratory data. "r " value was considered weak if < 0.25, mild if $\geq$ 0.25-< 0.5, moderate if $\geq$ 0.5 -< 0.75 and strong if $\geq$ 0.75. Stepwise multiple regression analysis was done for detection of independent determining factors for visfatin and RBP-4 levels. P-value is considered significant if $\leq$ 0.05*.

Results

The clinical characteristics of the study groups are shown in Table 1. A total of 40 patients with type 2 diabetes mellitus and 40 sex and age matched nondiabetic subjects were studied . Diabetic patients had significantly higher fasting blood glucose, insulin, $HOMA_{IR}$, total cholesterol, triglyceride, LDL-c, uric acid, HbA_{1c} and systolic blood pressure than those of control subjects. While, HDL-c was significantly lower in diabetics than control subjects. Plasma visfatin levels were found to be significantly higher in

diabetic patients than control subjects (44.1 ± 13.4 *vs.* 32.8 ± 14.7 ng / ml, P = 0.001 respectively) as well as plasma RBP-4 (46.3 ± 10.3 *vs.* 26.8 ± 7.5 µg/ml, P = 0.001 respectively). As regards visceral fat area, was significantly higher in diabetic patients than in control subjects(95.2 ± 15.6 vs 83.3 ± 21.4 cm). However, no significant difference was found between diabetic and control subjects in age, BMI, WHR, diastolic blood pressure, subcutaneous fat thickness(13.7 ± 6.1 vs11.8 ± 4.5)and properitoneal fat thickness (10.7 ± 5.1 11.2 ± 4.6). No gender differences were observed in any of the studied parameters with plasma visfatin levels in diabetics (men, 44.7 ± 12 *vs.* women, 43.6 ± 14.9 ng / ml, P = 0.8) or in control subjects (men, 30 ± 11.1 *vs.* women, 35.2 ± 17.2 ng /ml, P = 0.3). Plasma RBP-4 levels in diabetics (men, 46.4 ± 10 *vs.* women, 46.1 ± 10.7 µg/ml, P = 0.9) and control (men, 26.7 ± 6.4 *vs.* women, 26.9 ± 8.5 µg/ml, P = 0.9).

Table 2 shows that there was a significantly higher visfatin levels in diabetic groups as compared to that in control groups ($p< 0.001$). Furthermore, visfatin levels were significantly higher in diabetic patients compared with controls with similar BMI values ($p< 0.001$), while plasma RBP-4 levels of non-obese and obese diabetics were significantly higher in comparison to that in control group with similar BMI values ($p< 0.001$). Moreover, RBP-4 level of obese diabetic participants was also significantly higher than that of non-obese diabetic group ($p< 0.001$). There were significant differences in blood lipid profile, systolic blood pressure, HbA_{1c} and uric acid levels between diabetics and controls groups, as given in Table 2. Cholesterol and triglyceride levels in non-obese and obese diabetic patients were significantly higher than that in control group with similar BMI ($p< 0.001$), but there was no significant difference between non-obese and obese diabetics. The levels of fasting insulin as well as $HOMA_{IR}$ were significantly different among the four studied groups ($P< 0.001$). Fasting plasma glucose in diabetics was significantly higher than that in control groups with similar BMI ($P< 0.001$), and there was a significant difference between non-obese and obese diabetics. As regards BMI, WHR, visceral fat and subcutaneous fat, there were significant differences between obese and non-obese groups in both diabetics and control subjects but there were no significant differences between obese diabetics and obese control as well as non-obese diabetics and lean groups; except for there was a significant difference in visceral fat between non-obese diabetics and non-obese control group. No significant differences were observed between groups in age, diastolic blood pressure and properitoneal fat.

Pearson correlation analysis was used to identify the factors that most closely related to visfatin and RBP-4 in diabetic patients, as shown in Table 3. Plasma visfatin correlated positively with RBP-4, BMI, WHR, insulin, insulin resistance index (respectively, $r = 0.4$, $P < 0.02$; $r = 0.8$, $P < 0.001$; $r = 0.9$, $P < 0.001$; $r = 0.5$, $P < 0.003$; $r = 0.5$, $P < 0.002$) and visceral fat ($r = 0.9$, $P < 0.001$) Figure 5; whereas it was negatively correlated with SBP ($r = -0.3$, $P < 0.04$). There was no significant correlation between plasma visfatin and the rest of the studied parameters. Plasma RBP-4 was correlated positively with visfatin ($r = 0.4$, $P< 0.02$), BMI ($r = 0.5$, $P < 0.002$), WHR

(r = 0.4, P< 0.006), fasting plasma glucose (r = 0.4, P < 0.01), insulin (r = 0.4, P < 0.004), insulin resistance (r = 0.6, P < 0.001) and fatty liver (r = -0.7, P < 0.001). There was no significant correlation between plasma RBP4 and age, sex, duration of diabetes, blood pressure, lipid profile, HbA_{1c}, uric acid, visceral, subcutaneous or properitoneal fat.

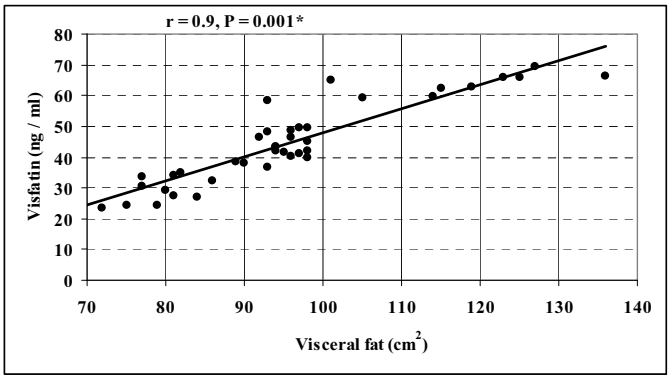

Figure 5: Correlation between plasma visfatin and visceral fat area in diabetic patients.

.

Table 3: Correlation between Visfatin and RBP-4 in diabetic patients

When each group was analyzed separately, in diabetic obese group visfatin correlated positively with RBP-4 (r = 0.5, P< 0.03), BMI (r = 0.8, P< 0.001), WHR (r = 0.9, P< 0.001), visceral fat (r = 0.8, P< 0.001) and properitoneal fat (r = 0.5, P< 0.001). RBP-4 correlated positively with visfatin (r = 0.5, P< 0.03), $HOMA_{IR}$ (r = 0.5, P< 0.05) fatty liver (r = 0.7, P< 0.001), and negatively with properitoneal fat only (r = -0.6, P< 0.01). In non-obese diabetic group, visfatin correlated positively with insulin (r = 0.4, P< 0.05) and visceral fat only (r = 0.9, P< 0.001), while RBP-4 correlated positively with insulin (r =0.5, P< 0.04), $HOMA_{IR}$ (r = 0.6, P< 0.002) and fatty liver (r = 0.9, P < 0.001).

While in control group, plasma visfatin levels correlated positively with RBP-4, BMI, WHR, FBG, insulin, $HOMA_{IR}$, uric acid, visceral fat and properitoneal fat (respectively, r = 0.6, P < 0.001; r = 0.9, P < 0.001; r = 0.9, P < 0.001; r = 0.6, P < 0.001; r = 0.8, P < 0.001; r = 0.8, P< 0.001; r = 0.4, P< 0.02 ; r = 0.9 ,P< 0.001 and r = 0.3 , P< 0.3). Plasma RBP-4 was correlated positively with visfatin (r = 0.6, P< 0.001), BMI (r = 0.6, P < 0.001), WHR (r = 0.6, P< 0.001), FBG (r = 0.5, P < 0.01), insulin (r = 0.7, P < 0.004), insulin resistance (r = 0.7, P < 0.001) and fatty liver (r = 0.7, P < 0.001). In obese control group, visfatin levels showed a significant positive correlation with BMI (r = 0.6, P< 0.006), WHR (r = 0.7, P< 0.001) and visceral fat (r = 0.8, P< 0.001). RBP-4 positively correlated with fatty liver only (r = 0.7, P< 0.001). However, in non-obese control group, visfatin correlated positively with visceral fat only (r = 0.7, P< 0.001). RBP-4 correlated positively with fasting insulin levels (r = 0.6, P< 0.001) and $HOMA_{IR}$ (r = 0.6, P< 0.001) in non-obese control subjects.

Table 4 shows stepwise multiple regression analysis using plasma visfatin as independent variable and various clinical and biochemical parameters as dependent variables in diabetic patients, only plasma visfatin level remained positively associated with visceral fat and WHR. When each group was analyzed separately, plasma visfatin level remained also positively correlated with visceral fat and WHR in obese diabetic group (P = 0.001, in both) and only with visceral fat in non-obese diabetic group (P = 0.001). While, in control group, plasma visfatin was positively correlated with visceral fat (P = 0.001), even after separation into obese and non-obese groups. Stepwise

multiple regression analysis using plasma RBP-4 as an independent variable and various clinical and biochemical parameters as dependent variables in diabetic patients, showed that fatty liver (P = 0.001), BMI (P = 0.001) and insulin (P = 0.05) were positively correlated with plasma RBP-4, and only fatty liver was positively correlated with RBP-4 in both obese and non-obese diabetic groups. However, in control group, plasma RBP-4 was positively associated with fatty liver (P =0.001) and $HOMA_{IR}$ (P = 0.04), and with fatty liver only (P = 0.001) in obese control group and with insulin only (P = 0.009) in non-obese control group.

Discussion

Type 2 diabetes mellitus is characterized by target-tissue resistance to insulin. It is strongly linked to obesity as over 80% of diabetics are obese [29]. Insulin resistance is the core pathogenic factor for diabetes. In addition, it is also strongly associated with obesity, hypertension and cardiovascular disease [29]. The new adipocytes derived hormones visfatin and RBP-4 may be an important link between increased fat mass and insulin resistance and disorder of metabolism of glucose in diabetes. To investigate whether the levels of visfatin, a recently characterized peptide, and the fat derived factor RBP-4 are related to adiposity and serve as a determinants of insulin sensitivity in diabetic and obese subjects. The present study was conducted to examine the relationship between plasma visfatin and RBP-4 and body composition, abdominal fat distribution, and insulin sensitivity in T2DM and obesity.

Visfatin corresponds to pre-B cell colony–enhancing factor, a 52-kD cytokine secreted by activated lymphocytes [6] and is up-regulated in neutrophils and monocytes after exposure to inflammatory stimuli [30-32]. Previous reports have raised questions regarding the origin and clinical relevance of visfatin, as it is ubiquitously expressed in different cell types [30, 33-39]. However, recent studies support the view that visfatin is a true adipokine that is clearly expressed in human adipocytes [32, 40, 41]. It was also shown that hyperglycemia induced visfatin overexpression in cultured human adipocytes [40]. There have been contradictory findings on the association between visfatin and obesity. Haider et al [42] showed that visfatin levels were substantially increased in morbidly obese individuals and gastric banding surgery lowered the circulating visfatin levels in them. However, a recent study showed that the plasma levels of visfatin were significantly lower in obese subjects [41]

In the current study, it was found that plasma visfatin levels were significantly higher in the diabetic compared with obese and lean subjects. Furthermore, visfatin levels in obese diabetics were significantly higher compared to non-obese diabetics. Similar findings were reported in a previous study [38], which had suggested that the increased circulating levels and messenger RNA expression of visfatin in the diabetic subjects may be related to their increased adipose tissue mass. Also, we found that plasma visfatin levels showed a significant positive correlation with BMI and waist / hip ratio. This finding supports the studies by Berndt et al [9] and Haider et al [42] that visfatin is associated with obesity.

Visfatin is preferentially secreted by visceral fat cells and increased in obesity and type 2 diabetes mellitus [5]. This is considered to reflect an impairment of visfatin signaling or a dysregulation in its biosynthesis [5, 7]. Visfatin levels correlate with visceral adipose tissue [5, 9] and consequently any increase in visfatin could indicate an increased visceral fat mass, which is usually associated with insulin resistance. Thus, considering

the insulin mimetic properties of visfatin, the increase in visfatin might be regarded as compensation for decreased insulin mediated glucose uptake, eventually by increased GLUT-4 transcription. Along this line, a significant positive correlation between visfatin and $HOMA_{IR}$, as well as insulin levels in the diabetic group in simple regression analysis but not in multiple regression analysis was observed. This finding supports other study by Chen et al [10] who showed a significant association between visfatin and $HOMA_{IR}$ and in contrast to other studies that showed a lack of association between visfatin and insulin resistance [9, 43]. Moreover, there was no association of plasma visfatin with any of the lipid parameters. This fact is consistent with findings that visfatin is mainly secreted in the visceral fat and not subcutaneous fat and suggest that the pathogenetic mechanism of visfatin in T2DM is different from that of insulin resistance.

RBP-4 is another factor derived from fat cells, has recently been reported to provide a link between obesity and insulin resistance modulating glucose homeostasis and therefore possibly involved in the development of insulin resistance [12]. RBP-4 expression is increased in the adipose tissue of adipose-glucose transporter 4 (GLUT4) knockout mice and the serum levels of RBP-4 are elevated in insulin-resistant mice, and in obese and type 2 diabetic subjects [12].

The current study extends the research on RBP-4 to humans and shows a correlation between RBP-4 levels and the magnitude of insulin resistance in subjects with obesity, and type 2 diabetes mellitus. In the present study, plasma RBP-4 levels were significantly higher in diabetic group when compared to control subjects. Moreover, RBP-4 levels were positively correlated with body-mass index, waist-to-hip ratio. This is in agreement with the recent study by Graham et al [11] who found a significant increase in RBP-4 in type 2 diabetes mellitus and obesity, and also found that the elevated RBP-4 levels correlate positively with components of the metabolic syndrome such as body mass index, waist circumference, triglycerides and systolic blood pressure. Also, this is in accordance with the previous study by Yang et al [12] who showed an unequivocal difference between normal and obese subjects, with or without diabetes, in terms of circulating RBP-4 concentrations. On the contrary to these findings, Cho et al [44] found that glucose tolerance status had only a small effect on plasma RBP-4 concentrations. This is probably attributable to the narrow BMI range shown by their study subjects. This was also in disagreement with Janke et al [45], who found that RBP-4 gene expression in adipose tissue was significantly reduced in obese subjects. They also detected no difference in RBP-4 serum levels between lean, overweight, and obese subjects [45]. The present study showed that RBP-4 was correlated with insulin resistance even in non-diabetic subjects. These results indicate that RBP-4 may be used as an index of insulin sensitivity. The present findings regarding the relationships of circulating RBP4 with insulin resistance are consistent with previous reports [11, 14]. However, other studies only detected a non-significant trend [46] or did not find significant relationships [45].

In the present study, there was consistent increase of visfatin and retinol binding protein-4 among the studied groups. The good correlation between the increase of visfatin and RBP-4 could indicate a common cause for the increased levels of both parameters, such as increased visceral obesity. Another explanation is that RBP-4 impairs insulin signaling in skeletal muscle [12]. Thus increased RBP-4 could lead to insulin resistance, which is followed by a compensatory hypersecretion of visfatin which is known to exert insulin mimetic properties [47].

Visceral and subcutaneous adipose tissue display important metabolic differences. Thus, visceral obesity is mainly associated with obesity-related cardiovascular and metabolic alterations [48]. Indeed, adipocytokines, such as visfatin, are mainly expressed in visceral fat [5]. Therefore, the present study looked at the association of visceral and subcutaneous fat as well as fat deposition in the liver with plasma visfatin and RBP-4 in the studied groups. Visceral fat area showed a significant association with plasma visfatin levels, whereas, subcutaneous fat, properitoneal fat and fatty liver did not show such an association with plasma visfatin levels. Furthermore, according to the results of stepwise multiple regression analysis plasma visfatin concentrations were correlated with visceral adipose tissue in all the studied groups. This study thus supports the report by Fukuhara et al [5] that visfatin is associated with visceral fat but not subcutaneous fat, but Berndt et al [9] did not find an association between plasma visfatin and visceral fat area. A recent study demonstrated visfatin messenger RNA may be differentially regulated in subcutaneous abdominal and visceral fat [41]. The presence of strong correlation between visfatin and visceral fat confirm the accuracy of measuring visceral fat by US rather than by other modalities .Being simple and non invasive other studies look for using US in assessment of visceral fat.

Long ago Armillini and his collaegues used to measure visceral thickness by ultrasonography from abdominal muscle to aorta at L4 level & concluded that Ultrasonographic intra-abdominal thickness correlated well with Ct intrabdominal fat area , supporting the hypothesis that ultrasonography could be useful in the direct evaluation of intra-abdominal fat deposits (56), the same authors also conclude in other study conducted on obese females before and after hypocaloric diet that Echography was also the most useful method to assess slight changes in visceral adipose tissue (57) , but in this study we use recent equation for evaluation of visceral fat area used by Hirooka et al [20], who concluded that measurement of visceral fat by US is effective as CT. Also Goodpaster and his colleagues found that as a component of central adiposity, subcutaneous abdominal fat has as strong an association with insulin resistance as visceral fat(58).

In the current study the properitoneal fat was positively correlated in the obese non diabetic group . This coincides with sabir et al who conclude All the abdominal fat layers, particularly the intra-abdominal Properitoneal fat, will decrease in response to loss of body fat by dieting. Sonography seems to be useful in monitoring small variations in the thicknesses of abdominal S and intra-abdominal P and V fat(59).

On the other hand, a prior study, revealed a correlation of RBP-4 to waist / hip ratio, suggesting an association between RBP-4 levels and abdominal obesity, however no correlation between RBP-4 levels and percent body fat was found [11, 44] . The current study showed that plasma RBP-4 levels did not correlate with visceral or subcutaneous fat but positively associated with fat deposition in the liver. This is in accordance with the recent study by Stefan et al [14] who found that RBP-4 correlated positively with fatty liver but not with viscera or subcutaneous fat. Also, a recent study by Gavi et al [13] found that serum RBP-4 levels were strongly correlated with the trunkal obesity and not with peripheral obesity [13].However, in disagreement with the current study, a recent study by Jia et al, [48] showed that serum RBP-4 level was positively correlated with visceral adiposity in Chinese subjects with and without type 2 diabetes. Since the liver is the major source of RBP-4 production in rodents and probably also in humans [50]. Ectopic

fat deposition in the liver represents an insulin resistant states [51- 55]. Therefore, a causative relation between both RBP-4 level and fat deposition in the liver may explain the results of the present study. Thus, the close correlations of circulating RBP-4 with these parameters may reflect stronger effects of RBP-4 on hepatic insulin sensitivity, than on insulin sensitivity of glucose disposal, possibly due to the relationship with liver fat and /or due to the stimulatory effects of RBP-4 on gluconeogenesis [12].

In conclusion, the present study reported that both plasma visfatin and RBP-4 are significantly higher in type 2 diabetics and obese individuals. Regarding fat composition, plasma visfatin was found to be strongly correlated with visceral fat only but not with subcutaneous fat, thus suggesting a divergent regulation of this adipokine in different fat depots. These data support that visfatin may be a feedback mechanism preventing the deleterious effects of the expansion of the intra-abdominal depots on insulin sensitivity or simply an epiphenomenon that might be useful as a surrogate markers of increased visceral fat mass and cardiovascular risk. On the other hand, plasma RBP-4 level correlates with insulin resistance and fat deposition in the liver, so, measurement of plasma RBP-4 could become a noninvasive and accessible method for assessing the risks of impaired glucose tolerance, type 2 diabetes mellitus, and cardiovascular disease. . The correlation between visfatin & visceral fat measurement confirm that visceral fat measurement by US is effective and reproducible .

Studies of the roles of visfatin and RBP-4 and the amount of visceral fat will shed new light on prevention and treatment of type 2 diabetes, and open a new field for the development of new drugs to improve insulin resistance.