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Study of Serum Levels of Visfatin Amongst Pre-Diabetic Obese Patients

Salem Ali Alnoggah[1], Abdelmonem Fathy Zeid[1], Hatem M. Salem[1], Ahmad Baraka[2]
Department of [1]Internal Medicine and [2]Clinical and Chemical Pathology,
Faculty of Medicine, Zagazig University, Egypt
***Corresponding Author:** Salem Ali Alnoggah, **E-mail:** salemali20102000@yahoo.com

ABSTRACT

Background: Insulin resistance, pre-diabetes, and obesity have all been linked to excess adiposity. Proinflammatory adipokine visfatin is thought to play a key role in type 2 diabetes inflammation. **Objective:** This study aimed to estimate visfatin level among prediabetic obese patients and to observe and detect the interplay between visfatin, insulin resistance and obesity.

Patients and methods: 96 adult subjects were studied in case-control research at Internal Medicine Department and Clinical Pathology Department, Zagazig University Hospital. The study was carried out from January 2021 to November 2021. Subjects were divided into 3 groups: Group (1) included 24 healthy individuals as a control group, group (2) included 48 prediabetic individuals and group (3) that included 24 patients type 2 diabetes who never been treated in any of these. Serum visfatin was assessed in all participants.

Results: Diabetes mellitus (DM) patients had higher visfatin levels than those with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). Visfatin had a significant positive correlation with BMI, WC, FBS, PPS, HA1c, LDL, fasting insulin, and HOMA IR, while HDL had a significant negative correlation.

Conclusion: Visfatin levels were significantly linked to type 2 diabetes. HOMA-IR, fasting insulin, and BMI all showed a strong positive correlation with visfatin levels, suggesting that it may be a useful biomarker for detecting type 2 diabetes.

Keywords: Visfatin, Prediabetes.

INTRODUCTION

One of the most major risk factors related to prediabetes, insulin resistance as well as type 2 diabetes is obesity. Adipocyte-derived hormones, now known as adipokines, and various proteins and enzymes are all produced by adipose tissue, in addition to serving as a significant endocrine and metabolically active organ **(1).**

Adipocytokine visfatin, pre-B cell colony-enhancing factor (PBEF), is identified as a protein that is strongly produced then secreted by fat of viscera. It was isolated by **Fukuhara *et al.*** **(2)** in 2005 from patients who had blood levels of visfatin correlated with obesity.

Biosynthesis of Nicotinamide Adenine Dinucleotide is regulated by visfatin role as nicotinamide phosphoribosyl transferase (NAD). Stress-induced energy metabolism is controlled by visfatin, which possesses anti-apoptotic effects. Activation of the immune system is also an important purpose for this compound **(3, 4).**

It was previously hypothesized that visfatin, which is produced mostly by macrophages, would act as an insulin mimic because of its involvement in promoting triglyceride (TG) synthesis and glucose transfer. The novel adipokine, visfatin, plays an important part in the inflammatory process associated with type 2 diabetes **(5).** However, the results on visfatin levels are based on research using a non-specific test based on the C-terminus of visfatin, which led to significant inconsistencies. Visfatin concentrations in obese and healthy individuals are not known to be consistent **(6).** It has been suggested that increased visceral fat in these patients induces a state of inflammation, which may lead to insulin resistance. **Fukuhara and colleagues** **(2)** have previously showed that visfatin may have a glucose-lowering effect, but the study was later withdrawn by the Editor of "Science" because of the controversy surrounding the findings **(7).**

Insulin resistance and other metabolic illnesses such as obesity and type 2 diabetes have been related to persistent low-grade inflammation characterize by aberrant cytokines over the years, and that these inflammatory markers may be directly linked to an increased risk of developing diabetes. Type 2 diabetes and insulin resistance are both closely associated with obesity, and previous research suggests that adipocytokines and inflammatory markers may play a role in their development **(8, 9).**

The goal of this case-control analytic investigation was to estimate visfatin level among prediabetic obese patients and to observe and detect the interplay between visfatin, insulin resistance and obesity potentially enhancing our knowledge of this controversial marker.

PATIENTS AND METHODS

96 adult subjects were studied in case-control research at Internal Medicine and Clinical Pathology Departments, Zagazig University Hospital. The study was carried out from January to November 2021**.**

Ethical approval:

All participants signed informed consent forms that were submitted to Zagazig University's

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Research Ethics Committee where the study was allowed (ZU-IRB#6649). We followed the World Medical Association's ethical code for human experimentation (Helsinki Declaration).

Inclusion criteria: Age from 30 to 50 years old irrespective of sex and obese patients (BMI >30) following the criteria for determining pre-diabetic symptoms [FPG (fasting plasma glucose) between 100 and 125 mg/dl (5.6 and 6.9 mmol/l), (IFG=impaired fasting glucose), PPG for two hours after a 75-g OGTT (oral glucose tolerance test) range: 140 to 199 mg/dl (7.8-11.0 mmol/l) (IGT= impaired glucose tolerance) and HA1c of 5.7–6.4% (39–47 mmol/mol)] and newly diagnosed diabetic patients without treatment.

Exclusion criteria: Unwilling patients, patients with type 1 DM, pregnant patients, renal disease, macrovascular diseases, liver diseases, drugs influencing insulin sensitivity (e.g., metformin, thiazolidinediones, etc.), and BMI < 30.

Subjects were classified into three groups:

Group 1: A healthy control group of twenty-four people (based on fasting blood glucose, oral glucose tolerance test and HbA1c) There were 10 men and 14 women in total. The mean age of the group was 42.41 ± 4.9 years.

Group 2: Included 48 individuals had pre-diabetes (as determined by HbA1c and other markers of haemoglobin A1c and fasting glucose levels), 22 of them were males and 26 were females, who were subdivided to: **Group A:** 24 IGF subjects, 10 were males, while 14 were females. The average age of the group was 39.70 years old, and **group B:** 24 IGT subjects, males were 12 and ladies were 12, their average age was 41.29 ± 6.6 years.

Group 3: Involved 24 patients with type 2 diabetes mellitus never been treated in any of these. Males and ladies were 12 and 12 respectively. The average age of the group was 41.37 ± 5.6 years.

1- **Full medical history:** A detailed history was taken including personal history (age, sex and special history as smoking). History of medical diseases as hypertension and hyperlipidemia. Drug history, and past history of renal or hepatic diseases.
2- **Full clinical examination** was performed to rule out organic diseases for exclusion from the study. Blood pressure measurement. Anthropometric measurements (weighed in kilograms after voiding the bladder in light clothing without footwear). From the top of their heads to their soles, their height was measured in meters. Weighing in kilos divided by the square of the height was used to calculate the BMI.
3- **Laboratory tests:** CBC. 75-gram OGTT with fasting plasma glucose and 2-hour postprandial glucose. HbA1c. Triglycerides, HDL, and LDL cholesterol levels in the blood. CRP. HOMA-IR, and enzyme-linked immunosorbent test was used to assess serum visfatin levels.

Statistical analysis

In order to analyze the data acquired, it was loaded into a computer and run via the Statistical Package of Social Sciences, version 20. (SPSS). Tables and graphs were used to present the findings. The Shapiro–Wilk test was used to examine the distribution properties of variables as well as the homogeneity of variance. The quantitative data was reported in the form of the mean, median, standard deviation, and confidence interval. The frequency and proportions of qualitative data were used to present the information. For quantitative independent data, the student's t test (T) and the Mann-Whitney test (MW) were employed to examine the data as needed. To examine qualitatively independent data, researchers employed the Pearson Chi-Square Test and the Chi-Square for Linear Trend ($\chi 2$). P value equals or less than 0.05 was considered significant.

RESULTS

Age was distributed among groups as 42.41 ± 4.9, 39.70 ± 6.3, 41.29 ± 6.6 and 41.37 ± 5.6 with non-significant difference among groups. Also, there was non-significant difference among groups regarding height, weight, WC and BMI. Additionally, there was non-significant difference regarding sex distribution, smoking or HTN **(Table 1).**

In comparison to the IGT and IFG groups, the DM group had significantly higher FBS and PPS, as well as a significantly higher HA1c. While the control group had significantly lower cholesterol and LDL, as well as significantly higher HDL. Regarding TG, the IGT and IFG groups had significantly higher levels, while the control group and the DM group had significantly lower levels. HOMA IR was substantially greater in the DM group than in the IGT or IFG groups or in the control group compared to the fasting insulin levels **(Table 2).**

Visfatin levels were considerably greater in the DM group than in the IGT group and the IFG group than in the control group **(Table 3).**

Non-significant area under curve with cutoff >1.21 with sensitivity 66.7% and specificity 55.0% regarding prediabetes group. **(Table 4, figure 1).**

Significant area under curve with cutoff >2.45 with sensitivity 88.2% and specificity 83.3% regarding type 2 DM group. **(Table 5, figure 2).**

There was significant positive correlation as regards visfatin with BMI, WC, FBS, PPS, HA1c and LDL. There was a strong negative association between fasting insulin and HOMA IR and HDL cholesterol **(Table 6).**

Table (1): The study group's age and sex composition among studied patients

			Control group (N =24)	IFG group (N=24)	IGT group (N=24)	Diabetic group (N=24)	F	P
Age (Years)			42.41±4.9	39.70±6.3	41.29±6.6	41.37±5.6	0.844	0.473
Height (Meters)			1.58±0.11	1.60±0.08	1.61±0.12	1.64±0.07	1.592	0.197
Weight (Kilograms)			95.78±6.45	97.04±10.8	94.16±10.44	96.82±7.9	0.502	0.682
Waist circumference (CM)			98.66±7.69	99.62±9.5	102.0±7.9	103.20±7.9	1.525	0.213
BMI (kg / m^2)			38.50±4.23	37.82±4.79	36.40±4.55	35.91±3.91	1.814	0.150
Gender	**Female**	**N**	14	14	12	12		
		%	58.3%	58.3%	50.0%	50.0%		
	Male	**N**	10	10	12	12	0.67	0.88
		%	41.7%	41.7%	50.0%	50.0%		
Smoking	**NO**	**N**	20	19	20	18		
		%	83.3%	79.2%	83.3%	75.0%		
	YES	**N**	4	5	4	6	0.72	0.86
		%	16.7%	20.8%	16.7%	25.0%		
Hypertension	**NO**	**N**	21	18	20	22		
		%	87.5%	75.0%	83.3%	91.7%		
	Yes	**N**	3	6	4	2	2.76	0.42
		%	12.5%	25.0%	16.7%	8.3%		
Total		**N**	24	24	24	24		
		%	100.0%	100.0%	100.0%	100.0%		

Table (2): LAB data distribution among studied groups

	Control group	IFG group	IGT group	Diabetic group	F	P
FBS (mg/dl)	97.91±6.80	111.0±7.55	114.25±6.01	141.33±12.63*	118.8	0.00**
PPS (mg/dl)	116.87±7.82	134.33±3.26	171.5±16.02	206.41±18.26	230.7	0.00**
HBA1c (%)	5.46±0.43	6.01±0.22#	6.05±0.22#	7.70±0.68*	118.8	0.00**
Cholesterol(mg/dl)	177.87±20.2	202.75±13.6#	206.45±13.3#	199.20±18.9#	18.0	0.00**
LDL(mg/dl)	103.50±10.16	115.54±7.77#	118.16±9.33#	120.75±18.24#	9.594	0.00**
HDL(mg/dl)	56.58±2.94*	34.95±4.12	31.75±2.92	48.41±4.69	261.9	0.00**
TG(mg/dl)	95.54±5.42@	254.0±19.3#	263.91±18.2#	124.83±23.76	555.7	0.00**
CRP	0.68±0.18@	2.27±0.75#	2.21±0.69#	1.46±0.47	27.7	0.00**
Fasting insulin (mIU/ML)	21.25±3.27	28.79±3.71#	27.58±4.3#	37.62±6.30*	52.714	0.00**
HOMA_IR	1.24±0.25	1.82±0.37	2.52±0.41	3.15±0.53	100.82	0.00**

Table (3): Visfatin among studied groups

	Control group	IFG group	IGT group	Diabetic group	F	P
Visfatin (ng/*ml*)	0.77±0.16	2.10±0.55#	2.13±0.59#	7.60±1.51*	57.16	0.00**

Table (4): Visfatin cutoff regarding pre-diabetic

Area	Cutoff	P	95% Confidence Interval		Sensitivity	Specificity
			Lower Bound	Upper Bound		
0.631	>1.21	0.071	0.502	0.760	66.7%	55.0%

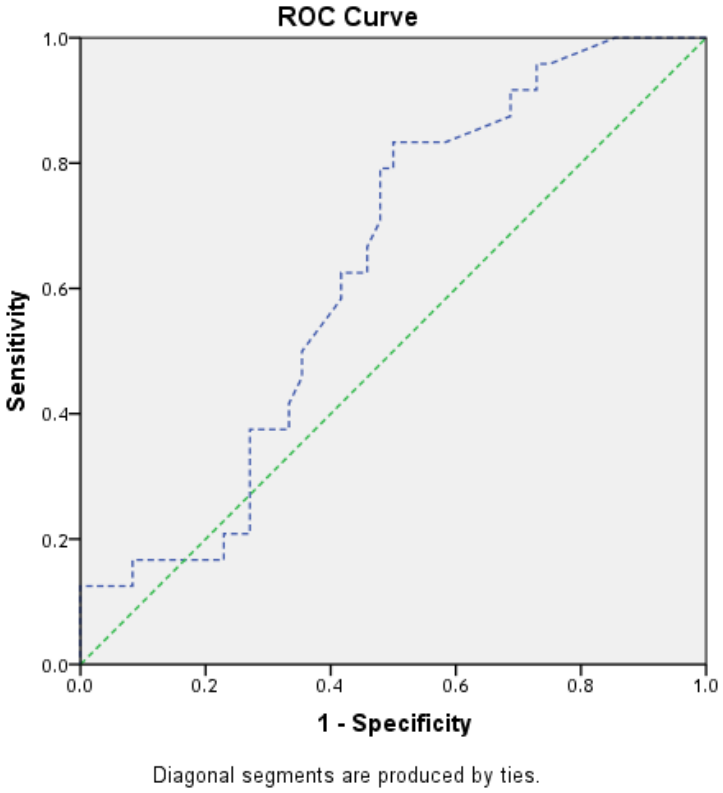

Figure (1): ROC Curve for visfatin cutoff regard pre-diabetic

Table (5): Visfatin cutoff regarding Type 2 DM

Area	Cutoff	P value	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.961	>2.45	0.001*	88.2%	83.3%

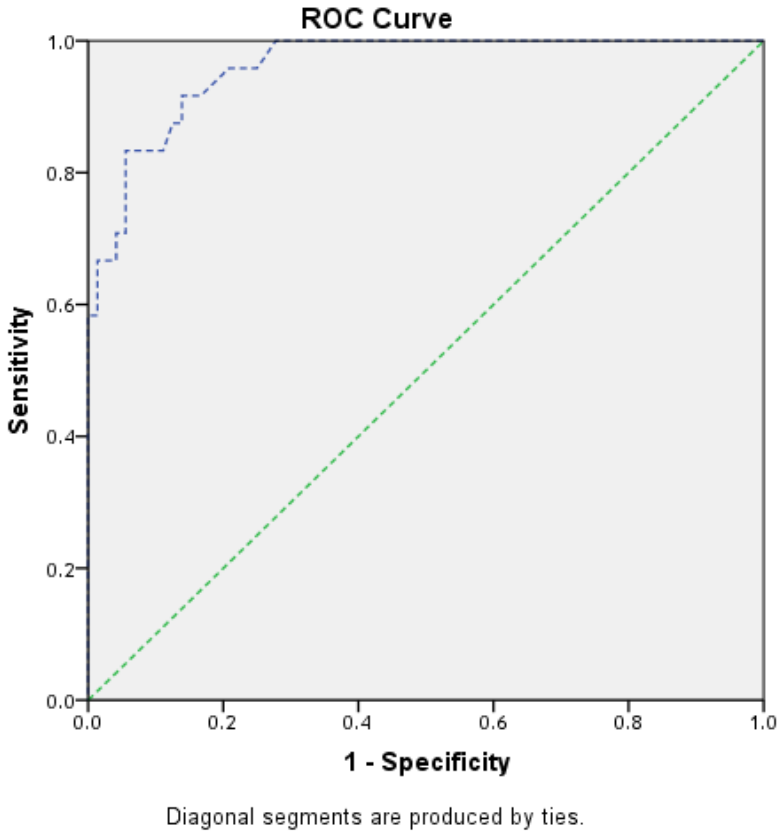

Figure (2): ROC Curve for visfatin cutoff regarding Type 2 DM.

Table (7): Correlation

		Visfatin
Age	r	-0.039
	P	0.709
Waist circumference	r	0.201*
	P	0.049
BMI	r	0.231*
	P	0.029
SBP	r	-0.111
	P	0.284
DBP	r	-0.016
	P	0.876
FBS	r	0.712**
	P	0.000
PPS	r	0.651**
	P	0.000
HBA1C	r	0.719**
	P	0.000
Cholesterol	r	0.040
	P	0.699
LDL	r	0.272**
	P	0.007
HDL	r	-0.203*
	P	0.047
TG	r	-0.155
	P	0.091
CRP	r	0.007
	P	0.948
Fasting insulin	r	0.641**
	P	0.000
HOMA_IR	r	0.693**
	P	0.000

DISCUSSION

It is reported in studies that visfatin plasma level is involved in pathogenesis of different metabolic disorders and multiple studies have been conducted around this issue. Increasing the plasma level of visfatin was reported in individuals with obesity, GDM (gestational diabetes mellitus) and insulin resistance and in IFG (Impaired fasting glucose) patients [(10)].

Age was distributed among groups as 42.41 ± 4.9 years, 39.70 ± 6.3 years, 41.29 ± 6.6 years and 41.37 ± 5.6 years with non-significant difference among groups. Also, there was non-significant difference among groups regarding height, weight, WC, BMI, sex distribution, smoking and HTN. Our findings are in conformity with the findings of a previous study by **Gunduz *et al.*** [(11)] as evidenced by the fact that their research involved 63 participants, 40 of whom had type 2 diabetes and 23 of whom did not. There were 44 women and 19 men in all (70% female to 30% male). In terms of age, gender, and BMI, there were no statistically significant differences between the T2DM patient and control groups (P>0.05).

Our study showed that in comparison to the IGT and IFG groups, the DM group had significantly higher FBS and PPS, and HA1c. While, the control group had significantly lower cholesterol and LDL, but significantly higher HDL. Regarding TG, the IGT and IFG groups had significantly higher levels, while the control group and the DM group had significantly lower levels. HOMA IR was substantially greater in the DM group than in the IGT or IFG groups or in the control group compared to the fasting insulin levels. Our results are supported by study of **Hetta *et al.*** [(6)] as they reported that there was a significant difference in the mean of cholesterol, HDL and TG (Triglycerides) between T2D patients and healthy controls. In the study of **Sheta *et al.*** [(12)], obese subjects had higher serum triglyceride and lower HDL-C measurements than those of control subjects.

For the DM group, fasting insulin levels were substantially higher than for the IGT group, the IFG group and the control group, according to the results of our research. It was found that the HOMA IR of the DM group was significantly greater than the IGT group, IFG group, and finally the control group. Patients with type 2 diabetes were found to have greater FBG (165.5 vs.87, P 0.001) and HOMA-IR (2.84) measurements, as reported by **Nezhadali and colleagues** [(13)].

In the study in our hands, visfatin was significantly higher among DM group (7.60±2.51 ng/ml P= 0.00) than IGT group (2.13±0.89 ng/ml, P= 0.00), IFG group (2.10±0.75 ng/ml P= 0.00) and control group (0.77 ± 0.26 ng/ml P =0.001). Our results are supported by study of **Hetta *et al.*** [(6)] as they showed a high significant difference in the mean serum level of visfatin. Study in Iraq showed higher visfatin and IR in type 2 diabetic patients but have failed to show any difference in visfatin level between diabetic and control group [(14)] while another study displayed a lower concentration of visfatin in T2DM and metabolic syndrome patients compared to controls in Vienna of Austria [(15)]. Moreover, study detecting visfatin level in human saliva was introduced as a biomarker in type 2 diabetic and pre-diabetic population [(16)]. In contrary to our results, study of **Nezhadali *et al.*** [(13)] concluded that visfatin level did not differ between diabetic and non-diabetic group and this rejects the hypothesis that visfatin could be a potential biomarker for diagnosis of type 2 diabetes in their population. Also, in contrast to our results, **Telejko *et al.*** [(17)] suggested that plasma visfatin level did not differ in woman with gestational diabetes and normal glucose tolerance.

Our results showed that using ROC curve for visfatin cutoff regarding pre-diabetic group, there was non-significant area under curve with cutoff >1.21

with sensitivity 66.7% and specificity 55.0%. As regards ROC curve for visfatin cutoff regard DM, there was significant area under curve with cutoff >2.45 with sensitivity 88.2% and specificity 83.3%. Visfatin showed significant positive correlation with BMI, WC, FBS, PPS, HA1C, LDL, fasting insulin and HOMA IR while was significantly negatively correlated with HDL. Our results are supported by study of **Naithani *et al.*** [(3)] as they reported that significant correlation was seen between visfatin and fasting plasma glucose ($p<0.01$), cholesterol ($p<0.01$), triglyceride ($p<0.01$), insulin ($p<0.01$) and HOMAIR ($p<0.01$). Positive correlations between visfatin and HDL cholesterol can support the hypothesis that visfatin has a protective effect and association with lipoprotein metabolism that was also found in Indonesia, Asian Indians, Caucasian subject and Taiwan [(18, 19)].

CONCLUSION

There is a significant association between visfatin level and type 2 DM. Visfatin level was significantly positively correlated with BMI, fasting insulin and HOMA-IR suggesting that it can play a role in pathogenesis of type 2 DM, and also could be a potential biomarker for diagnosis of type 2 DM.

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