



Original Article

Association of Visfatin with Blood Glucose, Insulin Resistance and Body Mass Index in Patients with Type 2 Diabetes/Pre-Diabetes

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Received: 30 May 2021 Accepted: 20 Aug 2021

Abstract

Background & Objective: In recent studies visfatin plays a role in pathogenesis of many disorders such as obesity and type 2 diabetes. The aim of this study was to evaluate the association of visfatin with fasting blood glucose, insulin resistance and body mass index in patients with type 2 diabetes/ impaired fasting glucose (IFG) compared to non-diabetic subjects.

Material & Methods: This case control study was performed on 160 volunteers. 80 participants were categorized in type 2 diabetic group holding FBG ≥ 100 (mg/dl) and 80 participants were categorized in non-diabetic group holding FBG 70-100 (mg/dl). Serum visfatin and insulin levels were measured using enzyme-linked immunosorbent assay.

Results: The result was presented as mean \pm standard deviation and $p < 0.05$ was considered as statistically significant. Diabetic group showed a higher level of age in comparison with the non-diabetics. Meanwhile, the means of TG, FBG, BMI, WC, HC and HOMA were also significantly different between the two groups. Visfatin level was not different between diabetics and normal healthy controls. There was a positive correlation between visfatin and BMI in non-diabetics. Also, in diabetics there was a positive correlation between visfatin and BMI, HDL, Hip, insulin and HOMA-IR and a negative correlation with FBG.

Conclusion: Our study showed an association between visfatin and BMI but not with type 2 diabetes. Also, there was a significant association between this adipokine and insulin resistance in diabetic and pre-diabetic patients which indicates the pathological role of visfatin in insulin resistance.

Keywords: Type 2 diabetes, visfatin, insulin resistance, body mass index, fasting blood glucose

Introduction

Adipose tissue was traditionally regarded as a passive reservoir for energy depot. However, nowadays it has been widely accepted in various studies that, adipose tissue plays its metabolic and endocrine

role by mediating a group of hormones hormones that are now globally defined as adipokines (1-3). In general, the adipokines are categorized into two groups: proinflammatory adipokines” such as visfatin and leptin that promotes insulin resistance and inflammation and “anti-inflammatory adipokines that have the beneficial effect such as adiponectin. The imbalance between

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these two types of adipokines leads to pathogenic response and disorders (4, 5).

Visfatin is one of these hormones (adipokines) that has pro-inflammatory properties and are present in visceral fat tissue (6). Visfatin is a 52KD protein weight that was first detected and recognized by Fukuhara et al in 2005 (7). The adipokine visfatin was formerly known as nicotinamide phosphor ribosyl transferase (NAMPT), or pre-B cell colony-enhancing factor (PBEF) (4, 7, 8). This hormone is abundantly produced in musculoskeletal system (muscle, bone, synovium and cartilage (but the major visfatin source in the human body is visceral and subcutaneous fat (8).

In 2017, approximately 462 million people were affected by type 2 diabetes that is corresponded to 6.28% of the whole world population. Also, in Iran 11.4% of adult population showed 35% increase from the first estimation conducted in 2005. Among this population a significant percentage of 30% of diabetic patients are unaware of illness in Iran. These statistics well describe the necessity of urgent action in the field of diabetes and the delay in the diagnosis of DM that exacerbate the burden which is imposed on health care system (9-11). The alteration of plasma adipokines (with almost an unknown mechanism) is the result. The alteration of the plasma adipokine level has impact on glucose and lipid metabolism impairment. Therefore, the identification of modifiable risk factors is an urgent demand as the mission of research to at least slowing down the harsh trend of rising type 2 diabetes and obesity (12, 13).

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 (14-18) and in IFG (Impaired fasting glucose) patients of India (19). Studies 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, 20) and displayed a lower concentration of visfatin in T2DM and metabolic syndrome patients compared to controls in Vienna of Austria (21). In studies detecting visfatin level in human salivary was introduced as a biomarker in type 2 diabetic and pre-diabetic population (22). Therefore, we prepared the current study that necessitates another measurement of biomarkers to find a clinically acceptable proof for early identification of at-risk individuals and a successful prevention of type 2 diabetes.

Material & Methods

Study design

This case-control study consists of 160 participants as a whole (between 25-70 years old) with the mean age of 44.5, consisting of 80 volunteers holding fasting blood sugar upper than 100 mg/dl (FBG>100 mg/dl) categorized in type 2 diabetic/IFG group and 80 volunteers holding fasting blood sugar between 70-100 mg/dl categorized in healthy (non-diabetic) group. FBG 100- lower than 126 mg/dl regarded as pre-diabetic or IFG or impaired fasting glucose. The classification was defined according to WHO guidelines (23). The whole population had Iranian ethnicity. The samples were taken from people who referred to the hospitals of Toos (Tehran), Zanjan hospital and Pars (Tehran). The study started from June 2018 until October 2018. The whole procedure of the study involved human participants and was in accordance with the ethical standards of

the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards under the ethics code of IR.IAU.TMU.REC.1396.290. Initially, the final aim of the study was explained to the volunteers and if they were consent to participate in the study, a written consent was signed by every participant. Two groups underwent to fill the written questionnaire requesting information on their age, sex, diet, daily habits, drug or smoking consumption (The whole questionnaires are available up on request). The inclusion criteria were both genders with Iranian ethnicity, FPG<100 (mg/dl) and a normal health condition for non-diabetic group, and FBG>100 (mg/dl) for diabetic/pre diabetic group. The exclusion criteria for both groups were drug consumption of any type, cancer, pregnancy, addiction, alcohol and smoke consumption, any infection, well known diabetic patient, liver, heart, autoimmune, and any other well-known disease.

Estimation of anthropometric and laboratory markers

Anthropometric parameters such as body weight, BMI, waist circumference (WC) and hip circumference (HC) were determined. Weight was measured without shoes with minimum clothes dressed. Height was measured with tape in standing position. BMI (body mass index) was calculated by weight in kilogram divided by the square of height in centimeter (24). Venous blood was collected after 12-14 hour fasting condition and the FBG test was performed for each of the participants twice. 5 ml blood was taken in tubes and centrifuged. Then, it was kept in refrigerator in -70° centigrade for future examinations of Cholesterol, HDL, and LDL. The serum concentration of visfatin was measured by ELISA kits (enzyme-linked

immunosorbent assay) (Zell Bio company, Germany with intra assay CV% of 3.2 and sensitivity of 0.19 ng/ml). Insulin level was also measured by ELISA kits, Mercodia, Sweden, with sensitivity of 1 mU/L and expected value of 2-25 Mu/L.

Statistical analysis

Normally, distributed continuous variables were expressed as mean±SD, and skewed continuous variables were expressed as median and interquartile range (IQR 25-75%). Categorical variables were also reported as frequency (percentage).

Normally distributed variables were analyzed using two-tailed independent samples t-test, while variables with a skewed distribution were analyzed using Mann–Whitney test. Associations between HOMA-IR with skewed normal distribution and anthropometric or metabolic characteristics were assessed by Spearman correlation coefficient. All the analyses were performed using SPSS, version 20 (SPSS, Chicago, IL, USA), and the differences with P-values greater than 0.05 were considered significant (two-tailed test).

Result

Anthropometric and metabolic characteristics according to diabetics and non-diabetics

The present study consisted of 160 individuals including 80 diabetic patients, and 80 healthy controls all with Iranian nationality that were referred to the hospital. The demographics and clinical characteristics of the volunteers are given in Table 1. The significant levels were considered as $p < 0.05$.

Patients of type 2 diabetes showed a higher age (52.1 vs. 36.9, $P = 0.001$), TG (169.6 vs. 121 $P = 0.004$), FBG (165.5 vs. 87, $P < 0.001$), BMI (29.8 vs. 25.2 $P = 0.02$), HC (105.2 vs. 96.9) and HOMA-IR (2.84 vs. 2.17) values, but

reduced WC (96.3 vs.103 P=0.005) (Table 1). There was no significant difference between diabetic patients and non-diabetic healthy controls in other variables ($P \geq 0.05$).

The mean of fasting serum visfatin levels were found to be decreasing in diabetic group compared with non-diabetic group (21.3 vs 23.3, $P \geq 0.05$). However, this association showed no statistical significance (Table 1).

To find out the correlation of visfatin with biochemical and clinical variables, the

Spearman correlation analysis was performed. According to the Table 2, visfatin was positively correlated with BMI ($P=0.002$) in non-diabetic group. Also, in diabetic group a positive correlation was seen in visfatin with HDL, BMA, Hip, insulin and HOMA-IR. (Respectively P s = 0.004, 0.044, 0.13, 0.001, 0.017). Meanwhile, an inverse correlation was found between visfatin and FBG level in diabetic patients. (Table 2).

Table1. Anthropometric and metabolic characteristics according to diabetics and non-diabetics

	Diabetics (n=80)	Non-diabetics (n=80)	P-value
Sex female, n (%)	44(44.4)	50(55.6)	0.115
Age, year	52.1(14.5)	36.9(11.7)	<0.001
Anthropometric indices, mean \pm SD			
BMI, kg/m ²	29.8(16.8)	25.2(4.6)	0.021
Height, cm	163.8(12.1)	167.1(12.1)	0.125
Weight, kg	75.5(14.5)	71.9(13.7)	0.165
WC, cm	96.3(12.6)	103.1(13.1)	0.005
Hip circumference, cm	105.2(14.6)	96.9(15.7)	0.004
Metabolic indices, mean \pm SD			
HDL, mg/dL	52.9(35.9)	52.7(22.7)	0.975
LDL, mg/dL	115.3(42.0)	104.3(23.2)	0.143
Total cholesterol, mg/dL	185.8(49.0)	166.8(40.1)	0.055
Triglycerides*, mg/dl	169.6(103.5)	121.0(54.5)	0.004
FPG (mg/dL)	165.5(59.8)	87.8(8.7)	<0.001
Insulin, Mu/l	12.9(9.9)	11.3(10.6)	0.349
HOMA*, mole \times μ U/l ²	2.84(1.63-4.81)	2.17(1.30-3.50)	0.006

Categorical variables are represented as frequency (percent). Continuous variables are represented as mean \pm SD.

*Triglycerides and HOMA are reported as median (IQR 25-75).

FPG, fasting plasma glucose; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index; WC, waist circumference

**Table 2.** Spearman rank correlations coefficients of the visfatin with anthropometric and metabolic characteristics parameters in participants

	diabetic		Non-diabetic	
	Correlation coefficient	P-value	Correlation coefficient	P-value
BMI	0.261	0.044	0.347	0.002
FBG	-0.318	0.007	-0.158	0.170
Age	0.070	0.588	0.138	0.285
HDL	0.392	0.004	0.144	0.344
LDL	0.176	0.249	0.149	0.334
cholesterol	0.001	0.997	0.056	0.695
Triglyceride	-0.154	0.251	0.142	0.343
Weight	0.020	0.874	0.092	0.508
Waist Circumference	0.194	0.131	0.157	0.286
Hip Circumference	0.315	0.013	0.251	0.085
Insulin	0.423	<0.001	0.189	0.099
HOMA	0.267	0.017	-0.017	0.892

Categorical variables are represented as frequency (percent). Continuous variables are represented as mean \pm SD.

Discussion

The current biological studies regarding visfatin, expressed that visfatin with its insulin-mimic role, was as effective as insulin in decreasing hyperglycemia in insulin-deficient diabetic mice. Moreover, the visfatin caused receptor phosphorylation through binding and activating insulin receptors. We hypothesized that visfatin is associated with type 2 diabetes. To this end, a case-control study was conducted on type 2 diabetic, pre-diabetic population in Iran. We 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 our population. In a

meta-analysis conducted by Ekpe EL et al in Nigeria, it was revealed that there is still a contradicting result regarding the association of visfatin and diabetes in studies conducted so far that is in line with our result. In this study, also the level of visfatin was higher in diabetic group than non-diabetics but there was not any statistically significance (25). Also, Telejko et al suggested that plasma visfatin level did not differ in woman with gestational diabetes and normal glucose tolerance (26). Meanwhile Lopez and et al concluded that serum visfatin level increased in patients with long-standing type 2 diabetes (27) and Mujgan guler that resulted the difference of visfatin between diabetic and non-diabetic group is not statistically



significant (28). On the other hand, contradicting results also revealed that visfatin level elevated in diabetic and pre-diabetic samples in India, USA, Egypt and Korea (29-32).

This case-control study revealed a difference between means of age in diabetic and non-diabetic groups (diabetics: 52.1 vs non-diabetics: 36.9, $p < 0.001$). This result introduces age as a risk factor for type 2 diabetes. Central obesity and insulin resistance as the initial preconditions that are related to metabolic diseases are mainly found in elderly population. Aging creates the condition of decrease in insulin sensitivity that is mainly due to insufficient composition of beta cell function mass (33). These results are in agreement with those in United States that revealed that the prevalence of diabetes between age group 20-24 years was 3.7% while in 45-64 years this level reached to 13.7% and also 26.9% in ≥ 65 years old. Similar results were also achieved in England where the prevalence of type 2 diabetes increase with age. The risk of getting diabetes lowered as age decreased and the risk of diabetes was higher among elderly also in Saudi population and diabetic population had greater TG and BMI compared to non-diabetics (34, 35). In a meta-analysis also the overall prevalence of type 2 diabetes depends on age (36) that was similar to our findings.

The presence of type 2 diabetes affected the factors of TG, FBG, BMI, Hip and HOMA-IR in our study and showed a higher level in diabetic group. In a study done on a similar population in Egypt showed that age, BMI and WC did not differ between diabetic and control healthy groups. However, there was a significant difference in the means of TG, HOMA-IR and FBG between T2DM patients and healthy controls that was similar

to our findings in this regard (1). Increasing the level of TG is the main criterion of atherogenic dyslipidemia in people with insulin resistance. But in this population HOMA also showed a higher level in diabetics that was similar to our result. Results in Turkey also support our finding on elevating BMI among type 2 diabetic population in which only 10% of diabetic population had normal BMI while others were affected by being overweight (37). Large or even moderate increase in BMI had impact on the incidence of type 2 diabetic in Japan that also protect our result (38).

Regarding visfatin correlation with other parameters, there was a strong positive relationship between visfatin and HOMA-IR, insulin, HDL and Hip in type 2 diabetic patients. 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 (39-41). However, there was no correlation between visfatin and insulin resistance in Indonesian study which contradicts our findings. Also, in a study by Eid M El-Shafey et al in Egypt the positive correlation of visfatin with HOMA-IR was achieved (31). In Hep G2 cells, visfatin significantly increased the level of expression in TNF- α , IL-1 β and IL-6 and reduced the expression levels of the insulin-signaling pathway proteins phospho-IRS-1 (Tyr612) and phospho-AKT. Visfatin also increased the activity of STAT3 and NF- κ B pathway but not JNK, p38, or ERK pathway activities. The STAT3 and NF- κ B inhibitor blocked the synthesis of visfatin-induced pro-inflammatory cytokine and rescued insulin signaling. On the basis of our results, it may be possible to explain the significance of visfatin in insulin resistance.

Meanwhile, Chang YH et al also support this thesis in their meta-analysis that visfatin is related to insulin resistance (16, 42, 43). These observations suggest that visfatin is a specific marker for insulin resistance in diabetic patients.

In summary, this study gives attention to the fact that visfatin level does not differ significantly between healthy controls and type 2 diabetic patients but introduced visfatin as a risk factor for insulin resistance in type 2 diabetic patients. Meanwhile, type 2 diabetes is more predominant among elderly people and is associated with high level of triglyceride, BMI, FBG and WC as the risk factors of type 2 diabetes. The major limitation of our study is relatively a small sample size that was mainly due to strict criteria (no history of drug consumption and no well-known disease) that took a longer period of time that was predicted. On the other hand, our study benefited from a series of strict inclusion criteria that we referred as our study power. These inclusion criteria made a homogenous sample population specifically in diabetic/pre diabetic population that collected the first line diabetic patients with no effect of metabolic drugs.

Conclusion

Our result showed that there was not any significant association between visfatin level in diabetic and non-diabetic patients but visfatin showed an association with insulin resistance and BMI in diabetic and pre-diabetic patients that represents the pathophysiological role of visfatin in insulin resistance and BMI.

Acknowledgement

The study was approved by the ethical committee (IR.IAU.TMU.REC.1396.290)

at Islamic Azad University, Tehran Medical Sciences, Tehran, Iran. The authors of the study express special appreciation from Dr “Laleh Ghanei”, Endocrinologist and Metabolism Specialist, Dr. “Mehran Zaman zadeh”, Member of Iranian Association for Diabetes and Endocrinology and Member of the American Diabetes Association (ADA), for their guidance and support in sample gathering and diabetes investigations and Endocrine research center of Shahid Beheshti University for technical and equipment support.

Conflicts of Interests

The authors have no conflict of interests.

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