

THE RELATIONSHIP OF VISFATIN WITH MEASURES OF OBESITY IN PATIENTS OF DIABETIC NEPHROPATHY

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ABSTRACT

Objective: Visfatin is proposed as an adipocytokine secreted from visceral fat and its blood level correlate with obesity, diabetes mellitus and inflammation. Aim of this study was to examine association of serum visfatin with measures of obesity in a group of patients with diabetic nephropathy and normal controls.

Methodology: This was a cross sectional study analyzing 60 subjects including 30 patients of diabetic nephropathy and 30 controls. Anthropometric measurements were done using standard methods and visfatin was measured through EIA Kit.

Results: Serum visfatin in obese subjects among both groups was not different from non obese subjects (7.9 ± 6.1 vs. 6.4 ± 3.2 $p=0.238$). We found a positive correlation of visfatin with BMI ($r=0.313$, $p<0.05$) but no correlation with waist circumference ($r=0.148$ $p=0.695$) and waist to hip ratio (0.198 , $p=0.136$). Serum visfatin in subjects of diabetic nephropathy and non diabetics was (9.2 ± 5.4 vs. 5.2 ± 3.4 $p<0.05$).

Conclusion: Serum visfatin does not correlate with markers of visceral obesity including waist circumference and waist to hip ratio. However, a positive correlation is observed with BMI. Future studies involving larger sample size and quantifying visceral tissue expression of visfatin may explain its potential role in visceral obesity.

KEY WORDS: Visfatin, Obesity, Body Mass Index, Visceral Obesity.

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INTRODUCTION

Obesity and diabetes mellitus are emerging as important health problems worldwide. Rise in obesity is linked to a consequent increase in risk of diabetes mellitus. Prevalence of type 2 diabetes is expected to increase from 171 million in 2000 to 366 million in 2030.¹ Both obesity and diabetes further increase the risk of diabetic nephropathy which is the leading cause of end stage renal disease. Risk of diabetic nephropathy is sharply increasing especially in Asian countries and it accounts as the leading cause of end stage renal stage in 9 out of 10 Asian countries.²

This obesity pandemic has called for extensive research on adipose tissue biology. Research done over past decade has shed light on endocrine capability of adipose tissue and old concept of adipose tissue as just a site of nutrient storage has been shaken by uncovering the capability of adipose tissue to secrete several cytokines including leptin, adiponectin, resistin TNF- α , and IL-6.³

Another focus is the risk associated with regional difference in adipose tissue. It has been observed that it is mainly central or visceral fat seen around big bellies rather than subcutaneous fat seen around big buttocks and thighs which is responsible for detrimental ramifications of obesity. This distinction has drawn special attention by raising question about possible differences between visceral and subcutaneous fat. Fukuhara et al in 2005 came up with a possible explanation to this question by discovering a unique adipokine, Visfatin which was preferentially expressed in visceral fat.⁴ Later on it was observed that it shared resemblance with a factor that promotes the growth of B cell precursors (B-cell colony-enhancing factor, PBEF) which was discovered by Samal et al in 1994, as a growth factor for B lymphocyte precursor and it was concluded that visfatin was a rediscovery of PBEF (B-cell colony-enhancing factor, PBEF) in visceral fat.⁵

This exciting discovery has been the subject of intense research because of potentially diverse features. It has been proposed as insulin mimetic, research work done on mice has shown dose dependent decline in plasma glucose following intravenous administration of visfatin.⁴ Moreover, it acts as a Nicotinamide phosphoribosyl transferase⁶ and even more interestingly it acts as an inflammatory cytokine.⁷ Visfatin is produced not only by visceral fat but by other organs including brain, lung, kidney, spleen, and testis.⁸

Knowledge about the association of visfatin with visceral obesity was first described by Fukuhara et al and later corroborated by numerous observational and molecular studies. Visfatin might serve as a new connection between obesity and diabetes mellitus but in a

seemingly paradoxical manner, observational studies examining association of visfatin with adiposity and differential regional fat distribution have found great discrepancies in their findings and further studies to establish the role of visfatin in visceral obesity are awaited.

Aim of this study was to analyze association of visfatin with measures of obesity including BMI, waist circumference and waist to hip ratio in patients of diabetic nephropathy and normal controls. We hypothesized that serum visfatin level would be elevated in obese subjects having higher waist circumference.

METHODOLOGY

This study was conducted between January, 2009 and September 2009. We selected 60 patients including 30 patients of diabetic nephropathy and 30 normal controls. Patients of diabetic nephropathy were previously diagnosed and were recruited from department of nephrology JPMC whereas age matched controls were selected by convenient sampling from general population of same socioeconomic group. Subjects had to meet specific inclusion and exclusion criteria i.e. they had to be 40-60 years old, having BMI > 18kg/m² but less than 37kg/m²; should be free of ischemic heart disease, rheumatoid arthritis, liver disease and recent febrile illness. Purpose of the study was explained to participants and their informed consent was taken. History of diabetes and its duration, duration of nephropathy, family history of diabetes and smoking was asked through a structured questionnaire. Medications history was asked including use of anti hypertensive, oral hypoglycemics and insulin.

Each patient was examined including measurement of blood pressure, weight and height by standard techniques. Weight and height were measured while subjects were standing and barefooted. Waist circumference was measured by soft inch tape from the point midway between lowest rib and uppermost lateral border of right iliac crest just above the umbilicus. Hip circumference was measured at widest part of hip. BMI was calculated as weight in kg divided by height in meter square.

Obesity was defined according to WHO recommendations for Asian Indians; Asia Pacific criteria (APC- BMI $\geq 25 \text{ kg/m}^2$)⁹ as opposed to a cutoff value of $>30 \text{ kg/m}^2$ used for defining obesity in western world similarly visceral obesity was defined as a waist circumference $> 90 \text{ cm}$ in males and $>80 \text{ cm}$ in female (APC-WC) WHO recommendations for Asian Indians rather than $>102 \text{ cm}$ in men and $>88 \text{ cm}$ in women used for defining visceral obesity in western world.¹⁰⁻¹¹ The definition of non diabetic is a subject with fasting blood glucose lower than 110 mg/dl based on WHO Criteria.¹²

Blood samples were taken after overnight fast. After a fasting of 8-10 hrs venous blood samples were taken. After collection samples were centrifuged and serum was separated. Serum was immediately frozen at -70°C . Serum Visfatin was measured using EIA kit from Phoenix Pharmaceuticals, Burlingame, CA Catalog No: EK-003-80 LOT No.: 601344. The minimum detectable concentration with this method was 2.13 ng/ml and detection range was $0-1000 \text{ ng/}$

Table-I: Anthropometric and biological parameters of study subjects.

	DN	Non DM
n	30	30
Sex(M/F)	13/17	18/12
Age (yrs)	52 ± 6.9	48 ± 5.9
Obese(n)	15	15
Visceral obesity(n)	21	22
BMI(kg/m^2)	26.1 ± 5.8	25.9 ± 4.5
Waist Circumference (cm)	105 ± 23.2	113 ± 25.4
Waist to hip ratio	0.93 ± 0.06	0.88 ± 0.13
SBP(mmHg)	142 ± 9.94	$114 \pm 7.7^{**}$
DBP(mmHg)	96.4 ± 7.8	$73 \pm 9.8^{**}$
FBS (mg/dl)	142 ± 12	$86 \pm 11^{**}$
Visfatin (ng/ml)	9.2 ± 5.4	$5.2 \pm 3.24^*$

N, number of cases; SBP, systolic blood pressure; DBP, diastolic blood pressure; DN, diabetic nephropathy; Non DM, non diabetic controls; and FBS Fasting blood sugar. Data are presented as means \pm S.D. * $P < 0.05$; ** $P < 0.01$

ml. The research protocol was approved by ethical committee of Ziauddin University.

Statistical Analysis: The data feeding and analysis was done on computer package Statistical Package for Social Science version 11.0. The results are given in the text as frequency and percentage for categorical variables (gender, diabetes) and mean and standard deviation for continuous variables (age, and lab investigation, etc.). Baseline characteristics for obese and non obese individuals were compared by chi square test for qualitative variables and one way ANOVA for quantitative variables. Pearson's correlation was calculated to estimate linear correlation between variables. In all Statistical analysis only P-value < 0.05 was considered to be significant.

RESULTS

The clinical characteristics of subjects are shown in Table-I. Visfatin values did not differ between men and women ($n=31$, 6.8 ± 7.5 vs. $n= 29$, 7.5 ± 5.9 $p=0.58$). Mean visfatin concentrations were not different between obese in comparison to age matched non obese patients ($n=30$ 7.9 ± 6.1 vs $n=30$, 6.4 ± 3.2 $p= 0.239$) Fig-1. Similarly mean visfatin concentration in patients with central or visceral obesity and those without central or visceral obesity was ($n=43$, 7.3 ± 5.3 vs. $n=17$, 6.8 ± 3.6 $p= 0.75$). Correlation of visfatin with measures of obesity is given in Table-II. Correlation with waist circumference is shown in Fig-2.

On subgroup analysis mean plasma visfatin in obese patients with diabetic nephropathy and non obese patients with diabetic nephropathy was ($n=15$, 10.3 ± 7.0 v s. $n=15$, 8.0 ± 2.9 $p=0.546$)

Table-II: Correlation between serum visfatin and anthropometric parameters.

	Correlation coefficient (r^2)
Age	0.18
BMI(kg/m^2)	0.313*
Waist circumference (cm)	0.148
Waist hip ratio	0.198

Data are r^2 . * $p < 0.05$

and mean plasma visfatin in obese normal controls and non obese normal controls was ($n=15$ 5.8 ± 3.9 v s. $n =15$, 4.6 ± 4.9 $p=0.895$). Significant differences were observed between obese patients with diabetic nephropathy and both obese and non obese normal controls ($p<0.05$).

Among patients of diabetic nephropathy 12 were on oral hypoglycemic alone 18 were receiving combined insulin and oral hypoglycemic and their mean plasma visfatin was not significantly different (9.0 ± 3.4 vs. 9.4 ± 6.8 $p=0.819$).

DISCUSSION

In this study visfatin level was not significantly different between obese and non obese subjects. Moreover we could not appreciate any correlation of visfatin with measures of visceral obesity including waist circumference and waist to hip ratio but a positive correlation was observed with BMI. Our results were in accordance with results of Berndt et al as they also observed a weak correlation with BMI but no correlation with markers of visceral obesity.¹³

Also in concordance with our findings Chan et al in their study on patients with polycystic ovary disease found no difference in visfatin level between obese and non obese subjects but a positive correlation with BMI was observed.¹⁴ They also reported a positive correlation with

waist circumference which was not observed by us and Berndt et al. However there are great controversies regarding association of visfatin with measures of obesity. Some studies have shown a positive association whereas others were not able to replicate such results. Choi and colleagues observed a higher plasma visfatin in obese Korean women compared to healthy non diabetic subjects¹⁵, similarly Zahorska and colleagues observed a higher visfatin in their obese subjects.¹⁶ Contrary to that Pagano et al observed a lower visfatin level in their obese subjects compared to non obese¹⁷ whereas Jian and coworkers did not find any difference between overweight and normal weight subjects.¹⁸ Similarly Korner et al¹⁹ found no association with measures of obesity and no difference between visceral and subcutaneous fat mRNA of visfatin. We have no local data on association of visfatin with obesity from Pakistan; but a study was done by Sredharan et al in India to see the association of visfatin with measures of obesity and Type 2 Diabetes mellitus.²⁰ They observed a higher level of visfatin in type 2 diabetics compared to non diabetics. However, this association was lost after adjustment for BMI and waist circumference; suggesting that the association was primarily with obesity and since subjects with diabetes mellitus are usually obese a positive association was observed with diabetes mellitus. Moreover, they observed a positive association with visceral fat but not subcutane-

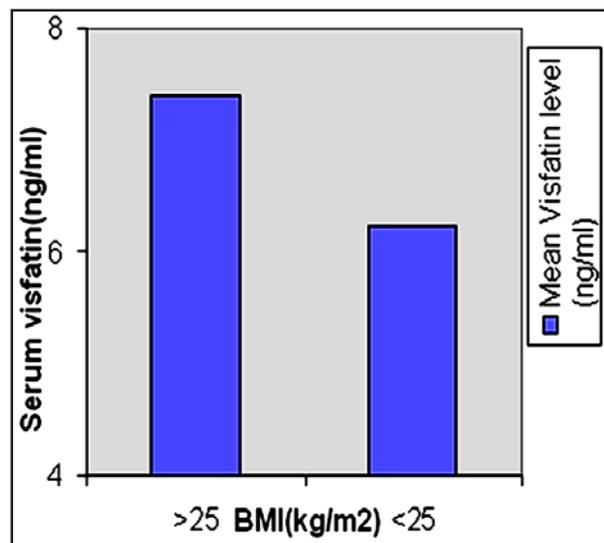


Figure-1: Association of visfatin with BMI

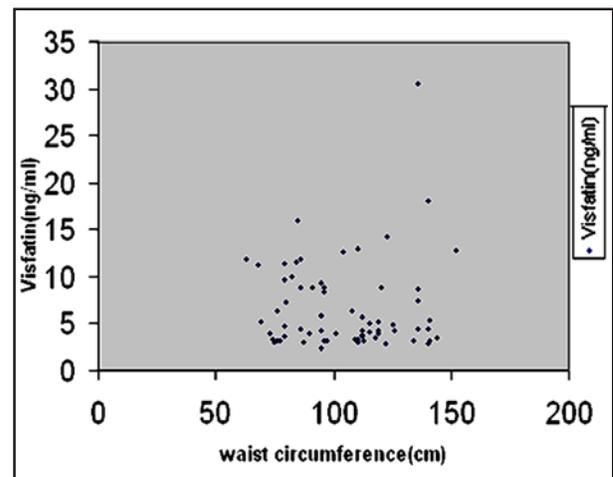


Figure-2: Correlation between visfatin and waist circumference.

ous fat. In contrast we did not find any association with both visceral and subcutaneous fat in both subjects of diabetic nephropathy and controls.

A careful look into biology of visfatin shows that it is ubiquitously expressed in many tissues and the different organ's contribution to circulating visfatin level still needs to be defined. Therefore merely restricting this ubiquitous molecule to adipose tissue is not justified and that could be the major reason why many studies could not detect its association with measures of obesity. Revello et al observed highest visfatin level in mouse brown adipose tissue, liver and kidney, whereas intermediate levels in visceral adipose tissue, lung, spleen, muscle and testis.⁶ In another research done on chickens to check visfatin mRNA expression in different tissues it was observed that its mRNA expression as well as protein expression was much higher in muscles compared to adipose tissue.²¹

Another possible explanation of lack of association between serum visfatin and visceral obesity observed by us and other studies could be because serum level may not be a true reflection of its expression within visceral adipose tissue. However Berndt et al have shown a positive correlation between serum visfatin and visceral fat visfatin mRNA expression and more interestingly a negative correlation between serum visfatin and subcutaneous fat visfatin mRNA expression.¹³ Further studies involving a comparison of its serum level with visceral and subcutaneous fat tissue expression may clarify this query.

Visfatin can be detected using three types of immunoassays including EIA (Enzyme immunoassay), ELISA (Enzyme linked immunoassay) and RIA (Radio immunoassay). Korner et al studied visfatin levels in 10 visceral obese, 10 subcutaneous obese and 10 normal healthy adults using three different immunoassays and they found no correlation between visfatin levels detected by the three different types of immunoassays.¹⁹ ELISA was shown as the most specific assay; however, we and majority of other studies have used EIA method for visfatin

level detection. This might be an important factor in the observed discrepancies in results as EIA detects the C-terminal visfatin as opposed to ELISA which detects full length visfatin.

We observed a higher visfatin level in subjects with diabetic nephropathy as compared to normal controls but whether, it is due to association of visfatin with diabetes or due to underlying inflammation in setting of chronic kidney disease is debatable as literature has shown a higher visfatin level in both conditions. If we review the pro inflammatory properties of visfatin a rise in subjects of diabetic nephropathy is well justified as chronic low grade inflammation is known to exist in setting of nephropathy.²²

Most of our diabetic patients were receiving anti diabetic treatment including oral hypoglycemic alone and combined insulin and oral hypoglycemics. We did not appreciate any difference in visfatin among two types of treatment. Korner et al¹⁹ studied newly diagnosed diabetics who were not yet started on any anti diabetic treatment. Lopez Bermejo²³ couldn't observe any difference in visfatin level between two different types of treatment. Pfutzner et al assessed the effect of treatment with pioglitazone, simvastatin or combined pioglitazone and simvastatin on visfatin level and they observed that after three months of treatment none of the above treatment regimen led to any change in visfatin level.²⁴ Similarly Kralisch et al found no effect in synthesis of visfatin in 3T3-L1 cells when they were treated with insulin making any link between insulin and visfatin unlikely.²⁵

Limitations of the study: First, our sample size was relatively small making it difficult to conclude inferences. Moreover severely obese individuals were not included making a possibility of missing a possible correlation between visfatin and severe obesity. Finally, diabetic patients in our study had nephropathy which it self is a state of inflammation and can lead to elevated visfatin level limiting the interpretation of results and decreasing their generalizability.

CONCLUSION

Serum visfatin does not correlate with markers of visceral obesity but a positive correlation is observed with BMI suggesting that visceral or central fat is not the primary source of visfatin overproduction. Future studies involving larger sample, subjects without any other comorbidities and severely obese individuals may further clarify its possible role in visceral obesity.

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