ORIGINAL ARTICLE

CORRELATION OF SERUM VISFATIN WITH ANTHROPOMETRIC AND GLYCAEMIC PARAMETERS IN NON-DIABETIC SUBJECTS WITH AND WITHOUT PARENTAL HISTORY OF TYPE II DIABETES MELLITUS

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Background: Visfatin has insulin like metabolic effects and has a key role in insulin secretion in response to glucose stimulus. This existed link of visfatin with obesity and glucose metabolism is still to be explored and debatable. We aimed to find out the correlation between visfatin and selected anthropometric and biochemical parameters in non-diabetic subjects with type II diabetic parents and with non-diabetic parents. **Methods:** This cross-sectional analytic study was conducted at the Diabetes Clinic of Lahore General Hospital and Department of Physiology, Postgraduate Medical Institute, Lahore. It comprised of 40 on-diabetic subjects with non-diabetic parents (Group I) aged 30–50 years, and 40 age and sex matched non-diabetic subjects with type II diabetic parents (Group II). Blood pressure, BMI and waist hip ratio, fasting levels of serum visfatin, insulin and glucose were measured and indices of insulin resistance (HOMA-IR), sensitivity (HOMA-%S) and beta cell function (HOMA-%β) were calculated. **Results:** Serum visfatin did not correlate with any of the anthropometric and glycaemic parameters assessed in group I and II. However, in combined analysis of female non-diabetic subjects, a statistically significant negative correlation between serum visfatin and waist circumference/waist hip ratio, and a positive correlation of serum visfatin with insulin sensitivity index (HOMA-%S) was found. **Conclusion:** A decline in visfatin production is seen with increasing visceral obesity in non-diabetic female subjects. The fall in visfatin levels seems to play a part in lowering insulin sensitivity in them.

Keywords: Visfatin, diabetics, Type II diabetes mellitus, T2DM, Insulin resistance, Insulin sensitivity, Beta cell function

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INTRODUCTION

Obesity is a matter of great concern globally as its prevalence has nearly tripled between 1975 and 2016. Approximately 11% of adult men and 15% of women were found obese worldwide in year 2016.[1] Non-communicable and preventable diseases are showing increasing trend in Pakistan. In Pakistan 4.8% of the population is obese and 20.8% is overweight as per 2016 WHO findings.[2] Abdominal obesity is an integral component of the metabolic syndrome, associated with many chronic ailments such as type 2 diabetes mellitus and cardiovascular disease. It has been generally accepted that adipose tissue not only stores energy, act as thermal insulator but it has immune and endocrine function as well. It secretes a large number of hormones and cytokines involved in autocrine, paracrine, and endocrine signalling. In obesity, adipose tissue function is impaired and this dysfunction has a strong linkage with insulin resistance.[3]

Visfatin is a multifunctional protein found in all living beings with highly conserved amino acid sequences. Adipose tissue, hepatocytes, and circulating leucocytes such as granulocytes and monocytes are reported as its preferential sources.[4–6] Its adipokine function was first brought into notice by Fukuhara *et al*[5]. They showed that this protein is predominantly being produced by the visceral fat of humans and mice and functions like insulin in the body. Visfatin facilitates glucose entry in adipocytes and myocytes, encourages triglyceride accumulation in preadipocytes by inducing the expression of PPAR-γ and many others.[5] Visfatin's downregulation reduces the sensitivity of rat hepatocytes towards insulin.[7] Earlier, visfatin was named as pre-B cell colony-enhancing factor (PBEF) as it found to be involved in the growth of B lymphocyte precursors in conjunction with interleukin 7 and stem cell factors.[8] It also works as an enzyme (nicotinamide phosphoribosyl transferase/Nampt) regulating the synthesis of nicotinamide adenine dinucleotide (NAD) molecules within the beta cells of the pancreas and hence improves insulin secretion in response to glucose challenge.[9]

The relationship between visfatin, obesity and glucose metabolism has been studied a lot in the past years but it is still indecisive whether visfatin is a marker of obesity or a new key player in the pathogenesis of diabetes. The objective of this study was to find out whether circulating visfatin levels show any correlation with the selected anthropometric and glycaemic parameters in them.

METHODOLOGY

It was a cross-sectional study, conducted in 2018 at the Diabetes Clinic of Lahore General Hospital (LGH) and Department of Physiology, Postgraduate Medical Institute, Lahore. Forty non-diabetic subjects with non-diabetic parents (group I) and 40 non-diabetic subjects with type II diabetic parents (group II) were enrolled in the study. Individuals with history of any acute illness for the past 2 weeks or with chronic inflammatory disease, diabetes or any systemic disease, grade I hypertension, taking any kind of medication, smokers and morbid obese with BMI ≥30[10] were excluded. Pregnant women or with history of menstrual irregularities, acne and hirsutism were also excluded.

Approval of the study was given by Ethical Committee of Postgraduate Medical Institute Lahore. Written consent along with clinical history was sought from each subject. General physical examination was also carried out for each subject. Blood pressure was recorded twice in sitting posture at the left arm after having a rest for 15 minutes using a mercury sphygmomanometer. Weight was taken in minimal clothing and without shoes, height by a height chart, and BMI was calculated. Waist circumference was taken at a point midway between the lowest palpable rib and the uppermost lateral border of iliac crests. Hip circumference was measured at the widest part of the hips by a tape measure keeping the subject in upright position with feet together and without clothes. Waist hip ratio was obtained by dividing waist circumference (WC) by hip circumference (HC). WHR= WC/HC[11]

All the samples were drawn between 8 am to 9 am after overnight fasting under aseptic measures. Gel activated vacutainer tubes were used for the blood collection and sera was extracted after centrifugation. Serum glucose was determined and remaining amount of sera was shifted in properly labelled Eppendorf tubes and frozen at -20 °C for further analysis. Serum visfatin levels were determined by direct ELISA method, catalogue #11560, Glory science company, USA. Assay range of the kit was 1–20 µg/l, Intra-assay precision <9%, inter-assay precision <15% and sensitivity of the assay ≥1 µg/l. Serum Insulin levels were determined by direct Human ELISA kit, Diametra Italy, Ref # DK0076 using an analyzer STAT FAX 303 reader. Serum glucose was analyzed using GOD-PAP enzymatic colorimetric method of Human Diagnostics, Germany kit ref # 10260.

Homeostasis Model of Assessment was used for the calculation of insulin resistance, sensitivity and beta cell function indices.

HOMA-IR= fasting serum glucose (mg/dl)×fasting serum insulin (µIU/ml)/405

HOMA-%S= 1/HOMA-IR×100

HOMA-%β= 360×fasting serum insulin (µIU/ml)/ fasting serum glucose (mg/dl) –63.[12]

Data were analysed on IBM-SPSS-26. Normality of the distribution of the study variables was checked by Shapiro-Wilk's statistics. Mean±SD was given for normally distributed quantitative variables while median with interquartile range (IQR) for non-normally distributed quantitative variables. Independent-samples *t*-test and Mann Whitney U Test were applied for observing mean and median (IQR) difference of the two groups. Pearson's correlation was used to explore the correlation between serum visfatin and selected anthropometric and glycaemic parameters; $p<0.05$ was considered as statistically significant. Simple linear regression analysis was applied to assess the linear relationship between serum visfatin and other quantitative variables.

RESULTS

Demographic, anthropometric and biochemical parameters of the study population are shown in Table-1 and 2. Group I and II were of similar age and gender (20 males, 20 females in each group). There was no significant difference in BMI of males and females of group I and II respectively. However, the waist circumference and waist hip ratios of group II females were significantly higher than that of group I females. No significant difference was observed in the above-mentioned parameters of the males of group I and group II respectively. Group II had significantly lower serum visfatin than that of group I. Insulin resistance (HOMA-IR) was significantly higher while insulin sensitivity (HOMA-%S) was significantly lower in group II in comparison to group I respectively. Beta cell function (HOMA-%β) of group II was also significantly higher than group I.

No significant correlation was achieved between serum visfatin and BMI, waist, hip circumferences or waist-hip ratio in either of the group ($p>0.05$). Moreover, serum visfatin didn't show any significant correlation with serum glucose, insulin and indices of insulin resistance (HOMA-IR), insulin sensitivity (HOMA-%S), or percentage of beta-cell function (HOMA-%β) in group I ($p>0.05$) and II ($p>0.05$) as shown in Table-3. In the collective analysis of female subjects of group I and II, a significant but weak negative correlation was obtained between serum visfatin and waist circumference and waist-hip ratio and positive correlation with the insulin sensitivity index (HOMA-%S) was obtained. No significant correlation between serum visfatin and anthropometric or glycaemic parameters was found in the combined analysis of male subjects of groups I and II (Table-4). On applying simple linear regression analysis, it was found that among female non-diabetic subjects, a unit increase in the waist circumference decreases serum visfatin level 0.16 times. Waist circumference contributed 28% of variability in the fasting levels of visfatin (Table-5).

Table-1: Comparison of demographic and anthropometric parameters of group I and II

Parameters (unit)		Group I (n=40)	Group II (n=40)	*p*
Age (Years)° Median (IQR)		32.00(31.00–36.00)	34.00(32.00–38.00)	0.11
Systolic blood pressure (mmHg)° Median (IQR)		120.00(110.00–125.75)	120(110.00–120.00)	0.80
Diastolic blood pressure (mmHg)° Median (IQR)		80(70.50–85.00)	80.00(78.50–85.00)	0.93
BMI (Kg/m^2)° Median (IQR)	Males	23.96 (21.58–26.14)	25.66(22.07–27.74)	0.31
	Females	23.78(21.11–27.32)	26.36(22.69–27.64)	0.13
Waist circumference (Cm)▪ Mean±SD	Males	89.24±9.62	93.91±9.99	0.14
	Females	81.24±9.03	88.43±7.54	0.01*
Hip circumference (Cm)▪ Mean±SD	Males	97.87±7.92	100.77±8.79	0.28
	Females	97.57±6.13	101.53±7.86	0.08
Waist Hip Ratio▪ Mean±SD	Males	0.91±0.07	0.92±0.07	0.58
	Females	0.83±0.06	0.88±0.06	0.02*

*Statistically significant (p<0.05); Results are expressed as Median (IQR) and Mean±SD; compared by °Mann-Whitney U test and ▪Independent-samples t test respectively. Group I (non-diabetic subjects with non-diabetic parents) & group II (non-diabetic subjects with type II diabetic parents)

Table-2: Comparison of biochemical parameters of group I and II

Biochemical Parameters (unit)	Group I (n=40)	Group II (n=40)	*p*
Fasting serum glucose (mg/dl)▪ Mean±SD	81.67±10.24	85.72±11.17	0.10
Fasting serum insulin (µIU/ml)° Median (IQR)	4.25(3.43–5.47)	13.45 (11.40–15.88)	0.00*
HOMA-IR° Median (IQR)	0.90 (0.64–1.03)	2.73 (2.21–3.79)	0.00*
HOMA-%S° Median (IQR)	111.11 (97.09–156.25)	36.63 (26.37–45.29)	0.00*
HOMA-%B° Median (IQR)	70.90 (48.64–117.75)	234.01 (147.48–334.50)	0.00*
Fasting serum visfatin (ng/ml)° Median (IQR)	11.25 (9.50–13.00)	9.00 (8.00–10.88)	0.00*

*Statistically significant (p<0.05)

Results are expressed as Median (IQR) and Mean±SD; compared by Mann-Whitney U-test and Independent-samples *t*-test respectively

Table-3: Correlation of serum visfatin with anthropometric and glycaemic parameters in group I and II using Pearson correlation

Pearson's correlation of serum Visfatin with	Group I (n=40)		Group II (n=40)	
	p	*R*	*p*	*R*
BMI	0.09	-0.27	0.11	0.25
Waist circumference	0.07	-0.29	0.35	0.15
Hip circumference	0.09	-0.28	0.19	0.21
Waist hip ratio	0.25	-0.19	0.85	0.03
Serum glucose	0.54	-0.10	0.87	0.03
Serum insulin	0.89	-0.02	0.59	-0.09
HOMA-IR	0.96	-0.01	0.57	-0.10
HOMA-%S	0.98	-0.01	0.42	0.13
HOMA-%β	0.95	-0.01	0.76	-0.05

*Statistically significant (p<0.05)

Table-4: Correlation of serum visfatin with anthropometric and glycemic parameters in combined analysis of group-I & II males and females

Pearson's correlation of serum Visfatin with	Group I and II non-diabetic male subjects (n=40)		Group I and II non-diabetic female subjects (n=40)	
	p	*r*	*p*	*r*
BMI	0.93	0.02	0.05	-0.32
Waist circumference	0.80	0.04	0.00*	-0.53
Hip circumference	0.88	0.03	0.06	-0.30
Waist hip ratio	0.61	0.08	0.00*	-0.48
Serum glucose	0.67	0.07	0.14	-0.24
Serum insulin	0.16	-0.23	0.04*	-0.33
HOMA-IR	0.19	-0.21	0.05	-0.34
HOMA-%S	0.30	0.17	0.04*	0.36
HOMA-%β	0.24	-0.19	0.23	-0.21

*Statistically significant (p<0.05)

Table-5: Simple linear regression of visfatin with waist circumference in combined analysis of group I and II female non-diabetic subjects (n=40)

Strata	Variable	Constant	Co-efficient	R	R^2
Female	Waist Circumference	23.99	-0.16	0.53	0.28

Dependent variable; fasting serum visfatin

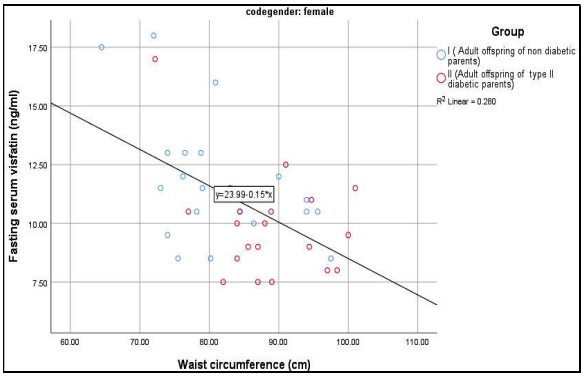

Figure-1: Scatter plot showing negative correlation between waist circumference and serum visfatin in combined analysis of group I and II female non-diabetic subjects (n=40) by Pearson's correlation

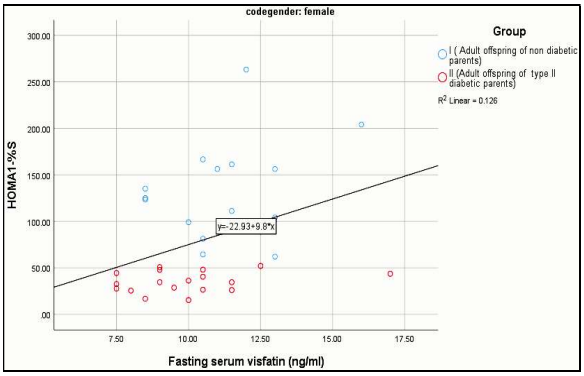

Figure-2: Scatter plot showing positive correlation between serum visfatin and HOMA-%S in combined analysis of group I and II female non-diabetic subjects (n=40) by Pearson's correlation

DISCUSSION

The relationship of visfatin with obesity and abnormal glucose metabolism is a complex one. Both positive and negative correlation of serum visfatin with anthropometric and glycaemic parameters has been reported in the past literature in non-diabetics.[13,14]

Results of our study support the inverse relation of visfatin with waist circumference and waist hip ratio and positive with insulin sensitivity in non-diabetic female subjects. Previously in a study, a significant negative correlation was reported between visfatin/insulin ratio and the body mass index and waist circumference of obese women with metabolic syndrome. The researcher proposed that the release of adipokines such as visfatin becomes greatly reduced as person gains fat especially in the abdominal area. This decline in visfatin/insulin ratio influence the development and progression of insulin resistance.[14] This fact is further supported by a study where glucose uptake was diminished in mouse adipocytes after/following visfatin down regulation.[15] In another study on obese females, visfatin levels were lowest in those who had highest BMI and waist circumference among others; however, no significant correlation between serum visfatin and above-mentioned obesity markers was documented.[16] Kaminska *et al*, found high levels of visfatin in obese subjects and a strong negative correlation of visfatin with the waist hip ratio and a nearly significant positive correlation with hip circumference. As majority of candidates were female, they proposed that raised visfatin levels in obese female subjects were probably associated with pear shaped rather than abdominal obesity.[17] Marked improvement in insulin sensitivity was observed after caloric restriction in a group of Spanish obese non-diabetic women and was associated with increase in their visfatin levels.[18]

In obese males, a positive correlation between visfatin level and their waist hip ratio was reported by Jian *et al*., emphasizing the direct relation of visfatin with visceral adiposity in them.[19] Studies' documenting the direct relationship between circulating visfatin levels with obesity markers suggest that with an increase in adiposity there is increase release of pro-inflammatory adipokines including visfatin. These cytokines are involved in promoting insulin resistance both locally and systemically.[20]

It may, therefore, be hypothesized that different associations between visfatin's circulating level and various anthropometric parameters could be due to the difference in visfatin's genetic expression in adipose tissue in both genders or it is related more with grades of obesity and abnormal carbohydrate metabolism.

Limitation of the study were, its small sample size, cross-sectional design and methods of assessment of insulin resistance and beta cell function.

CONCLUSION

Visfatin secretion decreases with increasing obesity in non-diabetic female subjects and has a role in insulin sensitivity reduction.

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