COMPARISON OF SERUM VISFATIN LEVELS IN NON-OBESE NON-DIABETIC, OBESE NON-DIABETIC AND OBESE TYPE 2 DIABETIC SUBJECTS

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ABSTRACT

Background: Obesity and type 2 diabetes mellitus are the most frequent endocrine disorders. Visfatin is a recently discovered adipocytokine with insulin mimetic properties.

Objective: To evaluate and compare the levels of serum visfatin in obese and non-obese non-diabetic and obese type 2 diabetic subjects.

Methodology: This was an analytical cross sectional study conducted in department of Physiology, PGMI Lahore from 1st September 2016 to 31st August 2017. There were total 78 subjects in this study, which were divided equally into three groups of both genders, with age 40-55 years. The group A contained normal healthy individuals with BMI 18.5 to 22.9 kg/m^2, in group B there were obese individuals which had BMI ≥25 kg/m^2 and group C contained the cases that had obesity same as in group B with history of type II diabetes mellitus. The cases suffering from medical conditions like hypertension, current pregnancy, other endocrinological problems, and any acute or chronic medical/surgical illness, were excluded from the study. Serum visfatin, fasting blood glucose, serum insulin were measured in blood samples. Insulin resistance index (HOMA-IR) was determined by fasting blood glucose and insulin levels. The collected data was analyzed by using SPSS version 17.0.

Results: There were total of 78 subjects in this study, 26 in each group. The mean serum visfatin level in group A was 1.08±0.56 ng/ml, in group B was 3.87±0.90 ng/ml and in group C was 6.8±0.95 ng/ml. The difference was statistically significant among and between study groups (p<0.001).

Conclusion: Increased serum visfatin level has shown a significant association with obesity and T2DM. This finding can be due to a compensatory mechanism against insulin resistance.

Key words: Obesity, Diabetes mellitus, Visfatin

INTRODUCTION

Visfatin, a 52 KDa protein was first discovered by Fukuhara and initially renowned as pre-B cell colony-enhancing factor (PBEF).[1] It is secreted mainly from visceral fat hence named visfatin, which acts as endocrine, paracrine and autocrine mediator involved in adipocyte differentiation, fat deposition and altering insulin sensitivity in peripheral organs.[2-4]

Visfatin had revealed as insulin like agent functioning through the same insulin receptor at different binding site and causes hypoglycaemia by inhibition of glucose release from liver cells and increases uptake of glucose in adipocytes and myocytes.[5] Experimentation over mice have shown that administration of recombinant visfatin causes plasma glucose lowering effect in insulin resistant and insulin deficient mice.[5] Systemic nicotinamide adenine dinucleotide (NAD) biosynthesis which is mediated by visfatin is required for β cell function of pancreas in insulin secretion and may help in the regulation of glucose homeostasis.[6]

Visfatin is an important marker in obesity and DM. The incidence of DM especially type II DM is on the rise mainly in the developing world might be due to change in the life style and there are a number of other factors that are still under extensive evaluation. Currently, Pakistan belongs to the high prevalence area with 6.9 million affected people. The incidence is expected to be doubled by the year 2025, affecting 11.5 million people.[7,8]

Excessive fat tissue and its particular distribution is considered as one of the major risk factor for development of insulin resistance and type II DM. Adipose tissue is also associated with production of a

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number of cytokines like adiponectin, leptin, TNFα, IL-6, resistin and visfatin which are found in the regulation of insulin sensitivity and seems to affect insulin resistance, dyslipidaemias, DM, inflammation, and atherosclerosis.[7,8,9] Visfatin is a novel multifunctional protein acting as a hormone, cytokine and/or enzyme recently under extensive discussion.[4,8]

Research work till date shows controversy regarding levels and relationship of visfatin in obesity and T2DM. Therefore this study was designed to find out and compare the serum visfatin levels in obese and non-obese non-diabetics and obese type 2 diabetic subjects.

METHODOLOGY

This was an analytical cross sectional study conducted in department of Physiology, PGMI Lahore in cooperation with Lahore General Hospital, Lahore, from 1st September 2016 to 31st August 2017 after approval by the Advanced Science and Research Board of University of Health Sciences (UHS), Lahore. This study included 78 subjects of either sex, between the ages 40-55 years divided into three equal groups. Group A had normal healthy individuals with BMI 18.5- 22.9 kg/m^2, in group B there were obese individual which had BMI ≥25 kg/m.^{2} Group C contained the cases that had obesity same as in group B and had history of type II DM. The cases suffering from medical conditions like hypertension, current pregnancy, other endocrinological problems, and any acute or chronic medical/surgical problem were excluded from the study. All cases were selected by non probability purposive sampling technique. After consent, fasting blood samples were drawn. Standard enzyme-linked immunosorbent assay (ELISA) technique was used to determine the levels of serum visfatin and serum insulin. Blood glucose was measured by the kit based on enzymatic method while BMI was calculated according to proposed WHO modified criteria for South Asians. Body weight and height of each subject were recorded and BMI was calculated by the formula BMI = Body weight (kg) / Height (m^2). Insulin resistance index (HOMA-IR) was determined by fasting blood glucose and insulin levels. Analysis of the collected data was carried out by using SPSS version17.0. The quantitative variables of the cases and controls were presented as mean ± SD. One way ANOVA and Kruskal Wallis ANOVA were applied to compare the levels of serum visfatin, fasting blood glucose, serum insulin, HOMA-IR and BMI among three groups. Post hoc Tukey and Mann-Whitney U tests were applied to observe which group mean differs. The *p*-value of < 0.05 was considered as statistically significant.

RESULTS

In the present study, 78 subjects were included among these 26 obese diabetics, 26 obese non-diabetics and 26 non-obese non-diabetics were selected. There were 13 (50%) males in group A, 12 (46.15%) in B and group C had 13 (50%) males with p=1.0. Mean age for group A, B and C was 48.12±4.83 years, 48.50±4.22 years and 47.08±4.33 years respectively with p=0.87. Table I shows the comparison of study parameters in all groups. Group A had mean BMI 21.83±0.67 with all cases less than 23.0. Mean BMI for group B was 30.32±4.07 and for group C was 29.89±4.55.

The difference was statistically significant among groups with p≤0.001. Pair wise comparison for BMI revealed that in group B and group C it was significantly higher than in group A, both with p≤0.001. Non-significant difference was observed between group B and group C. The mean fasting blood glucose level for group A was 81.13±13.55 mg/dl, for group B was 84.94±13.05 mg/dl and for group C was 162.09±39.57 mg/dl. Comparison for fasting blood glucose level among three groups showed that the difference was statistically significant (p≤0.001). Regarding pair wise comparison of fasting blood glucose level, the difference between group A and group B was non-significant and the difference of group C from two non-diabetic groups was statistically significant, p≤0.001.

Mean serum insulin level in group A was 5.84±1.78 µIU/ml, in group B was 15.42±6.70 µIU/ml and in group C was 32.13±25.01 µIU/ml. Comparison of serum insulin level among three groups revealed that the difference was statistically significant (p≤0.001). Concerning the pair wise comparison for serum insulin level, the difference was statistically significant between group A and group B (p≤0.001), the difference of group C with group B and group A were also statistically significant (p≤0.001). For HOMA-IR level of group A (1.17±0.42), group B (3.21±1.47) and group C (12.85±10.54), the comparison among three groups showed statistically significant difference (p≤0.001).

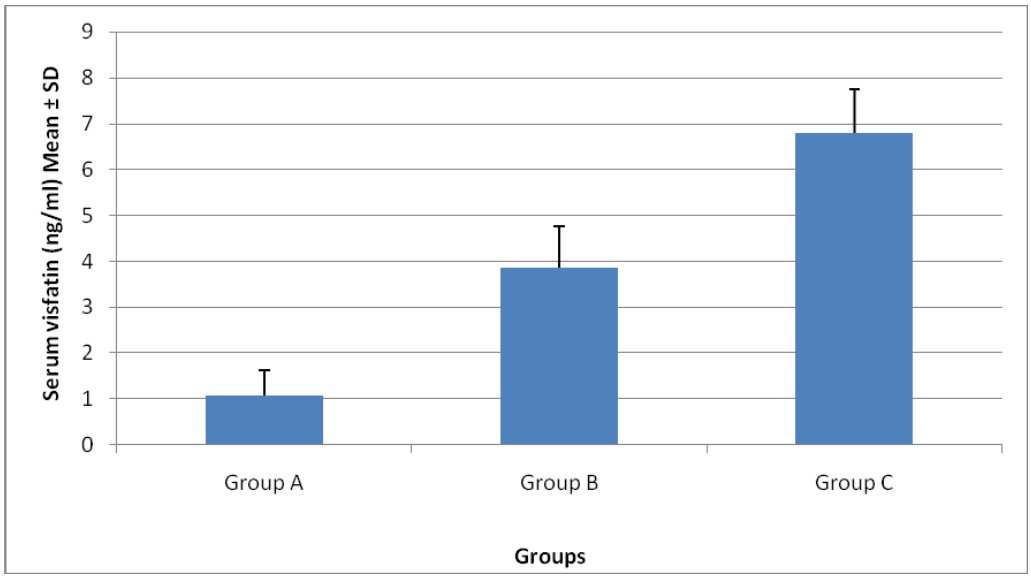
Pair wise comparison of HOMA-IR level showed that the difference between group A and group B was statistically significant ($p \leq 0.001$) and the difference of group C from two non-diabetic groups was also statistically significant ($p \leq 0.001$). Mean serum visfatin level presented in table I and figure I. In group A, it was 1.08±0.56 ng/ml, while 3.87±0.90 ng/ml was in group B, and 6.8±0.95 ng/ml was in group C. The difference was statistically significant among groups ($p \leq 0.001$). Pair wise comparison of serum visfatin showed significantly higher levels in group B and in group C as compared to group A ($p \leq 0.001$). Statistically significant difference ($p \leq 0.001$) was also observed between group B and group C.

Table No. I. Comparison of study parameters in three groups

Parameter	Group A n = 26 Mean ± SD	Group B n = 26 Mean ± SD	Group C n = 26 Mean ± SD	p-value	P-value Between two groups		
					A & B	A & C	B & C
Age (years)	48.12±4.83	48.50±4.22	47.08±4.33	1.0	1.0	0.87	0.87
Body mass index (BMI)	21.83±0.67	30.32±4.07	29.89±4.55	0.001	0.001	0.001	0.902
Fasting blood glucose (mg/dl)	81.13±13.55	84.94±13.05	162.09±39.57	0.001	0.184	0.001	0.001
Serum insulin (µIU/ml)	5.84±1.78	15.42±6.70	32.13±25.01	0.001	0.001	0.001	0.001
HOMA-IR	1.17±0.42	3.21±1.47	12.85±10.54	0.001	0.001	0.001	0.001
Serum visfatin (ng/ml)	1.08±0.56	3.87±0.90	6.80±0.95	0.001	0.001	0.001	0.001

Group A = non-obese non-diabetics **Group B** = obese non-diabetics **Group C** = obese type 2 diabetics

Figure I. Comparison of serum visfatin level among groups A, B and C



DISCUSSION

The prevalence of T2DM linked with obesity is rising rapidly worldwide. Obesity is the key element in pathophysiology of T2DM and insulin resistance principally because of excessive secretion of adipokines from expanded adipose tissue mass.[8]

Visfatin acts as insulin mimetic endogenous protein, abundantly produced by adipose tissue. Its plasma level increases with obesity and T2DM. A number of studies have shown controversial results regarding levels of serum visfatin and its relationship with glycemic and lipid parameters in obesity and T2DM.[9-14]

In this study, serum visfatin level was elevated significantly in the obese subjects with and without type 2 diabetes mellitus as compared to non-obese subjects which is in agreement with the study carried out by Jawish et al[11] and Taskesen et al.[12] It could be due to a greater amount of body fat as visfatin is generally produced by adipocytes. Serum visfatin level was highest in obese diabetics as compared to obese non-diabetic and controls (non-obese non-diabetics) in our study. This is in line with the studies of El-Shafey et al[8] and Rabo et al.[14]

They suggested that increased serum visfatin level has a link in obesity and T2DM. Hyperglycemia increases visfatin mRNA expression in human adipose tissue.[15] Elevation of serum visfatin level in T2DM may be due to impairment in visfatin signalling at target tissues or dysregulation of its biosynthesis.[16] Serum visfatin level might be elevated in hyperglycemic conditions owing to generation of reactive oxygen species and apoptotic cell lysis as hyperglycemia causes caspases activation or it could be due to counter regulation for decreased insulin sensitivity by stimulatory effect of visfatin on insulin receptor.[17,18] Chen et al,[16] El-Shaer et al,[18] Lopez-Bermejo et al[19] and Sandeep et al[20] found increased serum visfatin level in T2DM as in our study. Chen et al[16] and Lopez-Bermejo et al[19] found that plasma visfatin concentration was independently and significantly associated with T2DM. Lopez-Bermejo and colleagues[19] reported that serum visfatin concentration is increased with progressive beta cell deterioration, while Sandeep et al[20] suggested that the increased serum visfatin level in T2DM may be because of obesity.

Opposite to these findings, Yaturu et al[21] observed decreased serum visfatin level in type 2 diabetic subjects than in non-diabetics. They suggested that either decreased visfatin level might be partially due to pioglitazone therapy or due to difference in the population. On the other hand, Gligor et al[22] found no statistically significant change of serum visfatin level in type 2 diabetics or in obese non-diabetics than in apparently healthy normal weight subjects. These results are in disagreement with our study, may be due to visfatin is not a regulatory hormone of glucose metabolism.[9]

Elevated serum visfatin level was observed in obese subjects by Davutoglu et al,[10] Taskesen et al,[12] Kaminska et al[23] and Haider et al.[24] This is in consistence with our findings. Visfatin may be involved in weight reduction and in the improvement of insulin resistance as Haider et al[24] observed that weight loss following gastric surgery has decreased circulating visfatin concentration in morbidly obese subjects. Visfatin might have a role in lipid homeostasis as lipoprotein lipase may be upregulated by visfatin in preadipocytes and facilitates lipid uptake in differentiated adipocytes because it increases the gene expression of fatty acid synthase.[25] On the contrary, study reported by Pagano et al[26] showed significantly lower plasma visfatin level in obese subjects as compared to subjects with normal body weight. They have also observed visfatin mRNA expression in subcutaneous adipose tissue (SAT) and in visceral adipose tissue (VAT) and determined that SAT is the determinant of circulating visfatin which is only 30% suggesting the role of other sources of circulating visfatin. As the visfatin is also produced from skeletal muscles, liver and immune cells, it is possible that altered functions of these tissues in obesity may reduce circulating visfatin level or this could be due to its down regulation by TNF-α and IL-6 in obesity.[27,28] On the other hand Sun et al[9] found no significant difference in serum visfatin concentration between lean, overweight and obese subjects. This is in contrast to our findings. They suggested that visfatin may not be involved in regulation of glucose metabolism/insulin resistance in lean, overweight and obese subjects or the visfatin concentration is not affected by the amount of body fat in young men.

In this study fasting blood glucose level was significantly elevated in group C than in group B and group A in accordance with the results of Rabo et al[14] and Al-Dahr and Jiffri.[29] It could possibly be due to the excess release of FFA from ectopic fat which

prevents glucose uptake in peripheral tissues along with glucose production from liver.[29,30] The difference in fasting blood glucose level in group A and B was non-significant as both groups were non-diabetics.

According to our results, fasting insulin and HOMA-IR showed significant increase in diabetic subjects as compared to non-diabetics, similar to the studies carried out by Rabo et al[14]and Chen et al.[16]Davutoglu and colleagues[10] observed significantly elevated fasting insulin and HOMA-IR in obese group compared to non-obese group and significant increase in obese diabetics was observed in the study carried out by Rabo et al[14] as compared to non-obese non-diabetics. These findings are similar to our results. Impairment in the function of insulin receptor by lipotoxicity or ectopic fat build up may be attributable to insulin resistance as it inhibits the translocation of GLUT-4 towards the cell membrane. Decreased insulin sensitivity leads to increased production of insulin resulting in hyperinsulinemia in obesity and T2DM.[30]

CONCLUSION

Increased serum visfatin level has shown a significant association with obesity and T2DM. This finding can be due to a compensatory mechanism against insulin resistance. Further large scale studies are recommended to evaluate the role of visfatin in metabolic conditions with insulin resistance.

Authors Contribution: SI & AZ: Data Collection, Idea generation, Study Design. **SSH:** Writeup, data analysis. **MS:** Literature Search. **JL**: Discussion & Results. **SF:** Critically Reviewed. All authors critically revised and approved its final version.

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