ORIGINAL ARTICLE

LEVEL OF VISFATIN IN OBESE AND DIABETIC BALB/c MICE

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Background: Visfatin is a novel adipocytokine, secreted by the visceral fat of humans and mice. Its level increases in plasma during progression of diabetes and development of obesity. The objective of study was to determine the levels of visfatin in obese and diabetic BALB/c strain of albino mice. **Methods:** It was a quasi experimental study. Ninety BALB/c strain albino mice were procured from NIH, Islamabad and divided into three groups. Animals in Group I (n=30) were grown obese by feeding high fat/high carbohydrate diet whereas Group II (n=30) were turned insulin dependent diabetic by injecting streptozotocin. Group III (n=30) served as control. Blood samples were collected to measure the blood glucose, lipid and visfatin levels. Visfatin levels were measured by enzyme-linked immunosorbent assay (ELIZA), and $p \leq 0.05$ was considered significant. **Results:** Visfatin levels were significantly raised in obese and diabetic mice. **Conclusion:** Diabetes Mellitus and obesity are strongly associated with increased serum visfatin levels in BALB/c strain of albino mice.

Keywords: Visfatin, obesity, adipocytokines, insulin dependent diabetes mellitus

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INTRODUCTION

The development of resistance to insulin and impaired metabolism of glucose in body is usually a gradually developing process, beginning with excessive gain of weight and uncontrolled obesity.[1] Several adipocytokines are released by the visceral adipose tissues like leptin, resistin, adiponectin, tumour necrosis factor alpha (TNF-α) and interlukin-6 (IL-6) that modulate on its own way the sensitivity to insulin and appear to have a predominant role in the progression of insulin resistance, extreme obesity, diabetes, dyslipidemia, atherosclerosis and inflammation.[2–4] Visfatin (52 KD) is a newly identified adipocytokine which is secreted particularly by visceral fat in both human and mice, and its adipose tissue expression and serum levels increase quite in parallel with increasing obesity.[5,6] Visfatin has been reported to increase the transport of glucose and enhances lipogenesis by visceral fat cells and muscle cells and to decrease the production of glucose by liver.[7,8] IV injection of recombinant visfatin decreases plasma glucose in mice in dose dependent manner and is as affective in lowering blood glucose, as insulin in insulin deficient diabetic animal's models.[9]

Plasma visfatin levels increase proportionately following high fat/high carbohydrate diet which suggests that it has been probably involved in the development of obesity or diet-induced insulin resistance[7–9], and that visfatin expression is under regulation of certain cytokines that are known to enhance insulin resistance. Such culprits are lipopolysaccharide (IL-1B), TNF-α and IL-6 etc. Visfatin binds with receptor for insulin (IR) at a separate location than the insulin. The affinity of visfatin and insulin IR is similar in mice but the levels of visfatin are ten times less than the levels of insulin.[10] The interesting feature of molecular mechanism of visfatin is that visfatin initiates the cascade of intracellular signalling of insulin. It stimulates the phosphorylation of tyrosine present in IR and IR-substrate-1/2 (IRS-1/2) and activation of enzyme protein kinase B. Strikingly, however, visfatin activates the receptor for insulin in a distinct manner other than that of the insulin.[10]

The objective of this study was to see the level of visfatin in obese and diabetic albino mice and to see any correlation of diabetes and obesity with visfatin level in blood.

MATERIAL AND METHODS

This was a quasi experimental study conducted in Physiology Department, Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad. Ninety albino mice of balb/c strain having age between 6 and 12 weeks weighing 21–40 grams, were procured from the animal house of NIH, Islamabad. Sampling was done through convenience sampling technique. The mice (n=90) were divided into three equal groups of 30 each. Animals were weighed and blood samples were collected by tail bleed for fasting levels of blood glucose and serum visfatin. Animals in Group-I (n=30) were made obese by giving high fat and high carbohydrate diet for four months. The feed composition was 60% fat, 26% carbohydrates, and 16% proteins.[11] Group-II animals were made type I diabetic, who were insulin dependent, by giving injection of streptozotocin intraperitoneally 40 mg/Kg body weight.[12] After a lapse of one week, their fasting glucose levels in blood were measured by tail bleed. Diabetic mice included in this study had blood glucose levels equal to or greater than 11.3 mmol/l. Animals were kept in good hygienic condition and at optimum temperature (22–24 °C) for 10 days and given food and

water *ad libitum*. Group-III were age, weight and sex matched normal mice (n=30) and served as control.

Three ml of blood was drawn under ether anaesthesia by intracardiac puncture, out of that one ml of blood was transferred to a vacutainer, containing a glycolytic inhibitor (potassium fluoride BD), and plasma glucose was determined. Two ml of blood was used for serum separation and determination of serum visfatin levels in a tube containing a polymer gel (BD Vacutainer® SST™) a spray-coated silica. Similarly, samples of blood for Group-II (insulin dependent diabetic mice) and Group-III (control) mice were analyzed. Measurement of blood glucose was done by using a commercially available kit of Linear Chemicals SL by glucose oxidase method. Serum visfatin levels were assayed by Mouse Visfatin/PBEF ELISA Kit, CircuLEX (MBL International, USA). Data was entered and analysed using SPSS-20. Student's *t*-test was applied and $p \leq 0.05$ was considered significant.

RESULTS

The means age of mice was 9.3±2.2 weeks with a range of 6–12 weeks. Comparison of mean values of serum visfatin, fasting blood glucose and weight of mice across the three groups is shown in Table-1. Table-2 shows pair-wise comparison of the three groups with all the possible combinations.

Table-1: Comparison of serum visfatin, plasma glucose and body weight of mice in three groups

Variable	Group-I (Obese mice) n=30 (Mean±SD)	Group-II (Diabetic mice) n=30 (Mean±SD)	Group-III (Controls) n=30 (Mean±SD)
Serum visfatin (ng/ml)	2.72±0.19	2.91±0.12	1.05±0.02
Fasting blood glucose (mmol/l)	15.18±1.39	14.5±1.20	4.3±0.48
Weight of animal (gm)	59.4±1.01	26.38±1.18	25.0±1.28

Table-2: Pair-wise comparison of serum visfatin, plasma glucose, and body weight among the groups

Parameter	Group pairs	Mean Difference	*p*
Serum visfatin (ηg/ml)	Group-I vs Group-III	1.67	<0.001
	Group-II vs Group-III	1.86	<0.001
Blood Glucose (mmol/l)	Group-I vs Group-III	10.88	<0.001
	Group-II vs Group-III	10.20	<0.001
Animal's Weight (gm)	Group-I vs Group-III	34.40	<0.001
	Group-II vs Group-III	1.38	0.53

DISCUSSION

Obesity and diabetes are closely associated and most prevailing epidemics of present days. Excessive storage of fat, a common feature of both diseases is now considered to be the missing link between the mechanism that obesity induces insulin resistance, diabetes mellitus and release of various adipocytokines secreted by adipose tissue.[9–11] Along with a variety of adipocytokines like leptin, resistin, adiponectin, TNF-α and IL-6[2–4], it has been well established that obesity causes the release of visfatin from adipocytes which is more pronounced in visceral fat as compared to the subcutaneous adipose tissue.[13]

It is postulated that increase in amount of the visceral fat of obese mice in our study have resulted in considerable increase in serum visfatin levels whereas hyperglycemia was because of diabetes mellitus due to insulin resistance as observed in another international study by Fukuhra *et al*.[10] However, it is yet not clear whether induction of visfatin production is in response to a compensation for tissue-specific resistance to insulin or is the function of adipose tissue-specific cytokines which are supposed to be the inflammatory markers. In our present study we tried to delineate the same relationship between serum visfatin and obesity induced diabetes mellitus in mice. We found in our study a statically significant difference between mean visfatin values of obese (Group-I) and diabetic (Group-II) mice, (2.72±0.19, 2.91±0.12) respectively ($p<0.001$) in both groups compared with the controls. Similar observation was demonstrated by Chen *et al*[14]. They found that visfatin expression is under regulation of inflammatory adipocytokines and the progressive development of insulin resistance is associated to tumour necrosis factor-α, which is markedly increased during obesity[14], a reason which can also be attributed to the obese and diabetic groups of mice in our present study. These results are also comparable to those observed in a study conducted by Jewish K *et al*[15] at the Faculty of Medicine, Damascus University, Syria, and Fukuhra *et al*[10] in Japan, who suggested that visfatin has insulin mimetic properties. They further established the similarity between mechanism of action of both insulin and visfatin that they use tyrosine kinase-B phosphorylation-dependent signalling mechanism of action like IR.[7] In our study blood glucose levels and weight of animals also raised proportionately with serum visfatin.

Fasting blood glucose in group I was 15.18±1.39 mmol/l and Group-II was 14.5±1.20 mmol/l as against controls (4.3±0.48), a statistically significant difference (p=0.000). Lo´pez-Bermejo *et al*. have demonstrated that secretion of visfatin increases substantially with progressive dysfunction of β-cell of Islets of Langerhans in pancreas[16], while as a consequence of destruction of β-cells hyperglycemia results and insulin resistant diabetes mellitus occurs. However, the dilemma of presence of hyperglycemia along with raised levels of visfatin is yet to be cleared. One possible explanation about raised levels of visfatin observed in diabetes and other hyperglycemic conditions may be due to ongoing excessive oxidative stress.[2,3]

In obesity, visfatin expression is enhanced in visceral adipose tissue and that plasma level of Visfatin is correlated directly with amount of visceral fat.[15] Similar results reached in our study as the weight of animal in Group-I was raised by using high fat high carbohydrate diet[11] to a value of 59.4±1.01 gms as compared to Group-III (25.0±1.28) a highly significant change in weight and serum visfatin correspondingly in two groups (*p*-value 0.000) while change in weights of Group-II (Insulin dependent diabetic mice) and Group-III (controls) was not statistically significant 26.38±1.18 in Group-II and 25.0±1.28 in Group-III, *p*-value 0.53 as expected, and we reach similar conclusion as in other international studies.[13,17] This finding corresponds to the fact that visfatin is secreted by the adipose tissue and this secretion increases proportionately with the amount and size of adipose tissue, this is closely similar to the findings of Berdent.[13] This enhanced production of visfatin might be a compensatory response to impaired insulin signalling in the early stages of development of resistance to insulin induced by obesity.[7]

There is need for further well-designed controlled-clinical studies to interpret the missing data on visfatin and to fully understand its relationship to insulin resistance, diabetes mellitus and its metabolic derangements, inflammation and the co-morbid complications.

CONCLUSION

Our study confirmed the increased levels of visfatin in obese and diabetic mice which might represent a causative mechanism and metabolic disturbance associated with insulin resistance, diabetes and obesity.

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