ORIGINAL ARTICLE

CORRELATION OF SERUM VISFATIN LEVELS WITH DYSLIPIDEMIA IN OBESE BALB/C MICE

Raisa Naz, Shazia Tauqeer, Nadia Haleem*, Muhammad Ayub**

Department of Physiology, *Biochemistry, Ayub Medical College Abbottabad, **AJK Medical College, Muzaffarabad, Pakistan

Background: Visceral adipose tissues secrete several adipocytokines that modulate on their own way the extreme obesity, diabetes, dyslipidemia, atherosclerosis and inflammation. Visfatin is newly identified adipocytokine that is released by visceral adipose tissue. Its levels markedly increase during development of obesity. The aim of this study was to determine the levels of visfatin and its association with dyslipidemia in obese albino mice. **Methods:** It was a quasi experimental study. Sixty (60) Balb/c strain albino mice were divided into two groups of 30 each. Group I was taken as control non obese mice while Group II animals were fed high fat/high carbohydrate diet. Blood samples were collected to measure Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL-C), Low Density Lipoprotein (LDL-C), Very Low Density Lipoprotein (VLDL-C) and visfatin levels. **Results:** There was significant correlation among different classes of lipids with increasing visfatin levels in diet induced obese mice ($p<0.05$). TC, TG, LDL and VLDL showed significant elevation in serum levels (5.94±1.18 mmol/L, 2.92±0.15 mmol/L, 3.91±0.56 mmol/L, 1.56±0.31 mmol/L respectively) against controls (3.1±0.40 mmol/L, 1.62±0.01 mmol/L, 1.82±0.432 mmol/L, 0.60±0.13 mmol/L correspondingly). Pearson's correlation coefficient between rising serum visfatin and various classes of lipids showed strong positive correlation between visfatin and TC, TG, LDL, and VLDL, while negative correlation with serum HDL levels ($p<0.05$). **Conclusion:** Increased serum visfatin levels are strongly associated with obesity and dyslipidemia.

Keywords: Visfatin, obesity, adipocytokines, dyslipidemia

Pak J Physiol 2018;14(1):27–9

INTRODUCTION

Obesity has emerged as a global epidemic and presents a major health problem involving a multiplex of etiopathological consequences.[1] It implicates marked perturbations in a variety of biochemical and metabolic processes.[2] Adipose tissue is a major endocrine organ secreting variety of adipocytokines[3] involved in above mentioned disease processes and metabolic syndrome. Visfatin, also known as pre-B cell colony enhancing factor (PBEF), a recently identified adipocytes hormone, is secreted by activated lymphocytes, monocytes and neutrophils with poorly understood biological activity.[4] Recently visfatin has been discovered to be highly expressed in visceral fat in obese subjects showing strong association between obesity, diabetes and metabolic syndrome which includes a triad of dyslipidemia, hypertension and ischaemic heart disease.[5]

There is evidence of strong association between underlying inflammation that involves variety of cytokines released from adipocytes (adipocytes derived growth-factors) and metabolic derangements and cardiovascular disease.[6] Plasma visfatin level has been observed to increase following high fatty diet, showing that it has strong relevance with diet or diet-induced resistance to insulin.[7] It functions like insulin by increasing glucose transport into the cells and formation of lipids by adipocyte and muscle cells and decreasing production of glucose by hepatocytes. Dyslipidemia frequently occurs in metabolic disorders 'atherogenic triad of lipid' of high TG levels, low serum HDL-C levels, and high small dense, low LDL-C particles. All deranged processes that are involved in the pathogenesis of atherosclerosis are exaggerated by insulin resistance (IR) and metabolic syndrome[8,9] and possible role of visfatin may be considered.

Hypertriglyceridemia strongly predicts coronary artery disease. There is well known relationship between low serum HDL-C and high triglycerides in obese type II diabetics. It represents an independent risk factor for coronary heart disease. Small dense (sd-LDL-C) particles are highly atherogenic because they form oxidized LDL which are more prone to develop atherosclerosis and IR, which plays central role in the development of type II diabetes mellitus. Insulin resistance leads to high levels of VLDL-C with high concentration of TG, resulting in high levels of serum triglyceride and less serum HDL-C.[9] We tried to explore level of visfatin in obese mice and its correlation with lipoprotein metabolism which might occur as a result of metabolic disturbance associated with insulin resistance prior to development of diabetes.[10]

MATERIAL AND METHODS

The model of study was quasi experimental. The study was conducted in the Physiology Department of Army Medical College Rawalpindi, and National Institute of Health (NIH) in Islamabad. Sixty (60) Balb/C albino mice in age groups 6–12 weeks (mean age 9.3±3.1

weeks), and weight 24–60 grams (mean weight 54 grams) were purchased from NIH animal house Islamabad. Convenience sampling technique was used to select animals in study. These mice were then divided into two groups each of 30 albino mice. Weight of animals was recorded and collection of blood done through bleeding from tail of mice for fasting serum lipid profile and visfatin levels.

Animals in Group I (n=30) comprised control group and animal of Group II as diet induced obese mice who were made so by giving a diet rich in fat and carbohydrates for period of four months at least. The composition of their feed was 60% fat, 26% carbohydrates and 16% proteins.[16] After a period of one week, their fasting lipid l profile was assessed. Animal house was maintained in good hygienic conditions and an optimum temperature of 22–24 °C for ten days proper food and water supply was assured.

Ether anaesthesia was induced by inhalation and three ml of mice blood was withdrawn with the cardiac puncture, one ml blood was then transferred into a BD Vacutainer® SST™, containing Potassium Fluoride BD as an inhibitor of glycolysis, and serum lipid profile was evaluated. Out of two ml of blood was serum was extracted and serum visfatin level was determined. Blood TG levels were measured by an enzymatic colorimetric process by the use of readily available commercial kit prepared by Linear Chemical Company, Spain. High Density Lipoprotein was determined by ready to use kit of Linear Chemicals SL using the direct enzymatic colorimetric method. TC was measured by quantitative analysis method by using the kit of Pioneer Diagnostic company, New York USA. Levels of serum visfatin were analysed by ELISA Kit for Mouse Visfatin/PBEF, manufactured by Circulex MBL International, USA.

Data was entered into SPSS-20. Pearson correlation coefficient (r) was determined for serum visfatin and other study parameters. Student's t-test was applied and $p \leq 0.05$ were considered as significant.

RESULTS

The mean age of mice in study was 9.3±3.1 weeks. Mean weight of the group I (n=30) initially was 23.0±1.28 gm, while at the start of study, serum visfatin level measured to be 1.93±0.04 ηg/ml. The weight of obese mice in second group which was given high fat, high carbohydrates diet for four months (n=30) increased to 60.4±2.11 gm (p=0.000). Level of visfatin in these obese mice raised to 2.74±0.21 ηg/ml (p=0.001). There were significant differences between two groups regarding lipids and lipoprotein levels. TC, TG, LDL and VLDL lipoproteins showed significant elevation in serum (5.94±1.18, 2.92±0.15, 3.91±0.56, 1.56±0.31 respectively) against controls (3.1±0.40, 1.62±0.01, 1.82±0.432, 0.60±0.13 correspondingly). HDL exhibited reduction (0.97±0.87) against controls (1.84±0.08, (p=0.051) (Table-1).

Table-2 shows associations between serum visfatin and lipids categories with Pearson correlation coefficient showing strong positive correlation between visfatin and TC, TG, LDL and VLDL while negative correlation with serum HDL levels with $p<0.05$ in each analysis. In Table-3 Frequency distribution of biochemical variables in obese mice (n=30) is given along with number of mice.

Table-1: Serum visfatin and lipid profiles of mice in both groups (Mean±SD)

Variable	Group I (Controls)	Group II (Obese Mice)	p
Weight (gm)	23.0±1.28	60.4±2.11	0.000
Cholesterol (mmol/L)	3.1±0.40	5.94±1.18	0.001
Triglyceride (mmol/L)	1.62±0.01	2.92±0.15	0.003
HDL (mmol/L)	1.84±0.08	0.97±0.87	0.051
LDL (mmol/L)	1.82±0.432	3.91±0.56	0.000
VLDL (mmol/L)	0.60±0.13	1.56±0.31	0.006
Visfatin (ηg/ml)	1.93±0.04	2.74±0.21	0.001

Table-2: Correlation between serum visfatin and different parameters under study

Variable	Pearson's correlation coefficient	p
Weight (gm)	0.506	0.000
Cholesterol (mmol/L)	0.624	0.001
Triglyceride (mmol/L)	0.357	0.003
HDL (mmol/L)	-0.342	0.021
LDL (mmol/L)	0.654	0.040
VLDL (mmol/L)	0.875	0.016

Table-3: Frequency distribution of biochemical variables in obese mice (n=30)

Variables	No. of cases	Percentages
Cholesterol (mmol/L)		
4.6–5.5	18	60
5.6–6.5	12	40
Triglyceride		
1.8–2.5	21	70
2.6–3.6	9	30
High density lipoprotein		
0.6–0.9	24	80
1.0–1.3	6	20
Low density lipoprotein		
3.3–3.7	17	56.6
3.71–4.0	13	43.3
Very low density lipoprotein		
1.1–1.63	10	33.3
1.64–1.79	20	66.6
Visfatin		
2.5–2.7	11	36.6
2.72–2.9	19	63.3

DISCUSSION

Excessive adiposity is well established risk factor for the development of IR, type 2 diabetes, deranged lipids, atherosclerosis and inflammation.[11] The molecular mechanism underlying these pathological processes is yet to be cleared. Recently, visfatin has been recognized as a hormone with greater expression on visceral fat in comparison with subcutaneous fat deposits demonstrated by worldwide research.[12–15] In our study animals were made obese by giving a diet rich in fat and carbohydrates for period of four months at least. The composition of

feed was high fat high carbohydrate16 and it consisted of 60% fats, 26% carbohydrates and 16% proteins. There was significant difference in the weight of two groups after four weeks of high fat and high carbohydrate diet (p=0.000) as a result of visceral fat accumulation. These results have been reported in human studies who found positive correlation with rising visfatin and BMI and waist hip circumference of obese subjects implicating that visfatin levels are strongly determined by amount of central adipose tissue which is one of the sources of endogenous visfatin production.[1,15]

In our study serum visfatin and lipid analysis also revealed significant rise of total cholesterol and triglycerides. These findings are in agreement with Derosa *et al*[17]. They also observed abnormalities of HDL-C levels consistent with this study. HDL-C level in our study group II was decreased compared to controls. Our study group II LDL-C also showed significant elevation consistent with earlier studies in Korean population18 but not consistent with findings of Derosa *et al*[17], reason being difference of study population, settings, diet and co-existing diabetes mellitus and other features of metabolic syndrome in their study.

We observed significantly raised serum VLDL in obese group as compared to controls. Dyslipidemia and raised visfatin related to obesity might have strong prediction that it can be lowered by weight reduction or effective exercise programme. Araki *et al*[10] reported higher plasma visfatin in obese subjects than controls, the visfatin significantly correlated with weight, triglyceride, total cholesterol and insulin resistance; this is consistent with our study. Similar explanation was given by Chang YH *et al*[5] that plasma visfatin is significantly elevated in overweight obese patients with T2DM, metabolic syndrome, and cardiovascular disease. Plasma HDL has negative correlation as HDL decreased with rising visfatin, a typical feature of metabolic syndrome and atherogenic lipid profile.[5]

The frequency distribution of individual biochemical variables, in obese mice is given suggesting that visfatin and its molecular mechanics might be relating with development and progression of obesity, insulin resistance, and dysregulation of lipid metabolic pathways, lipogenesis, development of diabetes mellitus and metabolic syndrome.

CONCLUSION

Increased serum levels of visfatin are strongly associated with obesity and dyslipidemia in Balb/c strain of albino mice. Further study is needed to uncover these mysteries of visfatin and other adipokines.

REFERENCES

1. Jewish K, Qabalan Y, AL-Quobaili F. Study of elevated Visfatin levels in obese diabetic and non-diabetic subjects. Int J Pharm Sci Rev Res 2014;26(1):271–4.
2. Lu LF, Yang SS, Wang CP, Hug WC, Yu TH, Chiu CA, *et al*. Elevated visfatin, Pre B colony enhancing factor plasma concentration in ischemic strokes. J Stroke Cerebrovasc Dis 2009;18:354–9.
3. Antuna-Puente B, Feve B, Fellahi S, Bastard J. Adipokines: The missing link between insulin resistance and obesity. Diabetes Metab 2008;34(1):2–11.
4. Chen CC, Li TC, Li CI, Liu CS, Lin WY, Wu MT, *et al*. The relationship between visfatin levels and anthropometric and metabolism parameters: association with cholesterol levels in women. Metabolism 2007;56:1216–20.
5. Chang YH, Chang DM, Lin KC, Shin SJ, Lee YJ. Visfatin in over weight/obesity, Type 2 Diabetes Mellitus, Insulin resistance, Metabolic syndrome in cardiovascular diseases; A meta analysis and systemic review. Diabetese Metab Res Rev 2011;27:515–27.
6. Atacani I, Parlak A, Aydogan U, Sari O, Gok DE, Cayci T, *et al*. The effect of valsartan treatment on visfatin levels and lipid profiles in newly diagnosed hypertensives. Turk J Med Sci 2013;43:57–62.
7. Adeghate E. Visfatin: Structure, function and relation to diabetes mellitus and other dysfunctions. Curr Med Chem 2008;15:1851–62.
8. Jin H, Jiang B, Tang J, Lu W, Wang W, Zhou L, *et al*. Serum visfatin concentrations in obese adolescents and its correlation with age and high- density lipoprotein cholesterol. Diabetese Res Clin Pract 2008;79:412–8.
9. Kolsgaard ML, Wangenseen T, Brunborg C, Joner H, Holven KB, Halvorsen B, *et al*. Elevated visfatin levels in overweight and obese children and adolescents with metabolic syndrome. Scand J Clin Lab Invest 2009;69:858–64.
10. Araki S, Dobashi K, Kubo K, Kawagoi R, Yammoto Y. Plasma visfatin concentration as a surrogate marker for visceral fat accumulation in obese children. Obesity (Silver Spring) 2008;16:384–8.
11. Karrasch T, Leszczak S, Bala M, Ober I, Martin J, Schmid A, *et al*. Short term regulation of visfatin release in vivo by oral lipid ingestion and in vitro by fatty acid stimulation. Exp Clin Endocrinol Diabetese 2014;122:126–34.
12. Divkovic D, Selthofer-Relatic K, Cosic A, Drenjancevic I, Kristek J, Radic R. Serum visfatin concentration in eutrophic and overweight/obese male children in early child hood. Period Biol 2014;116(2):191–6.
13. Taskesen D, Kirel B, Us T. Serum visfatin levels, adiposity and glucose metabolism in obese adolescents. J Clin Res Pediatr Endocrinol 2012;4(2):76–81.
14. Stastny J, Bienertova-Vasku J, Vasku A. Visfatin and its role in obesity development. Diabetes Metab Syndr 2012;6(2):120–4.
15. EL-Shafey EM, El-Naggar GF, Al-Bedewy MM, El Sorogi H, Is there a relationship between Visfatin levels and type 2 diabetes mellitus in obese and non obese patients? J Diabetes Metab 2012;S11:001.
16. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinal Metab 2004;89:2548–56.
17. Derosa G, Maffioli P, Salvadeo SA, Ferrari I, Gravina A, Mereu R, *et al*. Sibutramine and L-carnitine compared to Sibutramine alone on insulin resistance in diabetic patients. Intern Med 2010;49:1717–25.
18. Choi KM, Kim JH, Cho GJ, Baik SH, Park HS, Kim SM, *et al*. Effect of exercise training on plasma visfatin and eotaxin levels. Eur J Endocrinol 2007;157:437–42.

Address for Correspondence:
Dr Raisa Naz, Department of Physiology, Ayub Medical College, Abbottabad, Pakistan. **Cell:** +92-300-9112050
Email: raisakhan660@gmail.com

Received: 16 Oct 2017 Reviewed: 25 Jan 2018 Accepted: 28 Jan 2018