



VISFATIN BETWEEN FACT AND FICTION; A MARKER OF OBESITY OR A NEW PLAYER IN THE PATHOGENESIS OF TYPE 2 DIABETES MELLITUS

Yasser S. Sheta

From the departments of Internal Medicine Faculty of Medicine, Zagazig University

El-Sayed A. Elgohary

From the departments of Internal Medicine Faculty of Medicine, Zagazig University

Samar Sharaf

From the departments of Clinical Pathology Faculty of Medicine, Zagazig University

ABSTRACT

An adipose-tissue-derived protein termed visfatin was described recently with putative key role in pathogenesis of type 2 diabetes mellitus. It turns out, though, that the molecule was previously identified as a growth factor for early B-lymphocytes termed pre-B cell colony enhancing factor (PBEF). Visfatin was reported to be expressed almost exclusively in visceral adipose tissue and has insulin-like metabolic effects. These findings are very attractive and could provide a hot point of medical research to answer if visfatin provide a novel mechanism by which visceral fat accumulation can promote the development of type 2 diabetes mellitus (T2DM). However, its patho-physiological role in humans remains largely unknown. The Aim of this study is to investigate the role of plasma visfatin as a missing link between obesity and type 2 DM patients. Methods: Plasma visfatin concentrations were measured through ELISA in forty obese patients and compared to forty age and sex matched healthy non-obese subjects. Obese patients further subdivided into two subgroups according to presence of diabetes mellitus. Results: Plasma visfatin was found to be elevated in obese (38.7 ± 13.3 ng/ml) compared to lean subjects (20.8 ± 3.8 ng/ml), ($P < 0.01$). In obese patients no significant difference was found in diabetic obese patients (39.1 ± 7.3 ng/ml) in comparison to non diabetic patients (36.9 ± 7.4 ng/ml). In the other hand; no significant differences between diabetic patients according to diabetic control and line of treatments. Only waist to hip ratio was correlated with plasma visfatin level. Conclusion: Our results indicate that visfatin is elevated in obese patients apart from the presence of diabetes, diabetes control and line of treatments. Visfatin more reasonably to be a marker of obesity as a product of visceral fat cells rather than a new key in pathogenesis of type 2 diabetes mellitus. It seems that visfatin could not be one of the missing links between type 2 diabetes mellitus and its relation to visceral obesity. Further large scale studies are needed to determine its relation to immune status of obese patients and other adipo-cytokines and the effect of weight loss on its level.

Key Words: Visfatin, Visceral fat, Type 2 DM

INTRODUCTION

The world prevalence of diabetes among adults (aged 20–79 years) about 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7%, and 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw *et al.*, 2010). Accordingly, type 2 diabetes mellitus (T2DM), the most common form of diabetes that stems from peripheral insulin resistance or dysfunctional pancreatic beta-cell function, is also approaching pandemic proportions. These alarming health changes are primarily fueled by the prevalence of obesity arising in part from increasingly sedentary lifestyles and high-fat diets (Chang *et al.*, 2010). Although surfeit weight is notably deleterious, obesity appears to be heterogeneous because not every overweight subject presents with the same cardiometabolic risk profile. In fact, epidemiologic studies suggest that visceral (omental) adipose tissue is more pernicious than subcutaneous (gluteal) adipose tissue (2). Unlike all other fat depots, visceral adipose tissue are drained by the portal vein; thereby this adipose region has direct contact with the liver. Portal release of products from visceral fat could be of particular importance for inducing T2DM or protecting from this disorder due to effects on the liver (Chang *et al.*, 2010). Indeed, removal of abdominal subcutaneous (sc) adipose tissue from obese insulin-resistant subjects has no metabolic effect, whereas removal of visceral (i.e. omental) fat improves insulin sensitivity in such individuals (Klein *et al.*, 2004; Giorgino *et al.*, 2005). What factor(s) in adipose tissue causes insulin resistance? It was long thought that fatty acids produced by lipolysis in fat cells were the only culprits. Circulating fatty acids are elevated in T2DM and other insulin-resistant conditions, and they interfere with the action of insulin, glucose metabolism, and with the production of lipoproteins by mechanisms that have been discussed (Thorne *et al.*, 2002). However, during the last 15 yr or so it has become increasingly apparent that adipose tissue, besides releasing lipids, is a very active protein-secreting organ (Noriyuki *et al.*, 2011). The tissue secretes classical hormones such as leptin and adiponectin, cytokines such as tumor necrosis factor and interleukins, chemokines such as monocyte attractant protein 1, coagulation factors such as plasminogen activator 1 (PAI-1), and complement factors such as adipsin. Some of these proteins are termed adipokines, meaning that they are produced by the fat cells. Leptin and adiponectin are only produced by adipocytes, whereas most of the other proteins are produced by fat cells as well as by the stromal cells of adipose tissue (Arner, 2005; Noriyuki *et al.*, 2011). Visfatin, also known as pre-B cell colony-enhancing factor, is an adipokine that is highly expressed in visceral fat and was originally isolated as a secreted factor that synergizes with IL-7 and stem cell factors to promote the growth of B cell precursors (Samal *et al.*, 1994; Adegate, 2008; Chang *et al.*, 2010). It has been postulated to play a role in innate immunity (Samal *et al.*, 1994). However, the biological activity of visfatin is poorly understood. It is secreted by activated lymphocytes, monocytes, and neutrophils (Jia *et al.*, 2004); stimulates the expression of IL-6 and IL-8 in amniotic cells (Ognjanovic and Bryant, 2002); and prolongs neutrophil survival in clinical sepsis (Jia *et al.*, 2004) found that visfatin expression in visceral fat is increased in obese subjects and that

plasma concentrations of visfatin correlated much more strongly with the amount of visceral fat than that of subcutaneous adipose tissue. Visfatin exerts insulin-mimetic effects in stimulating muscle and adipocyte glucose transport and in inhibiting hepatocyte glucose production (Fukuhara *et al.*, 2005; Adeghate, 2008). Visfatin was also found to be bound to and activate insulin receptor, causing receptor phosphorylation and the activation of downstream signaling molecules. However, visfatin and insulin did not compete for binding to the insulin receptor, indicating that the two proteins were recognized by different regions of the receptor (Adeghate, 2008). Thus, visfatin might play a role in glucose homeostasis and pathogenesis of diabetes. In order to elucidate the role of visfatin in obesity and diabetes, we measured plasma visfatin level in obese patients in comparison of lean subjects with special focusing on diabetic obese patients and its status as regard control and line of treatments whether oral drugs or insulin.

SUBJECTS AND METHODS

This case control study included 80 consecutive subjects, recruited over a period of three months, from the medical outpatient Clinics of the Internal Medicine department, Zagazig university hospitals. The patient group included 40 obese patients (BMI) > 30 (20 men and 20 women). 22 of them had type2 diabetes, diagnosed according to the American Diabetes Association guidelines . Patients presenting with symptoms suggestive of type 1 diabetes, defined as diabetic ketoacidosis, acute presentation with heavy ketonuria, or continuous requirement of insulin within 1 yr of diagnosis, were excluded. Patients who had a diagnosis of urinary tract infection, urolithiasis, liver cirrhosis, congestive heart failure, macrovascular diseases, overt proteinuria, or other known major diseases were also excluded on the basis of interview, physical examination, and urinalysis. Forty age and sex-matched individuals served as the control group. They included healthy volunteers recruited from the blood bank. None of them had diabetes or overweight. All participants were subjected to the following after fully informed consents: complete physical examination and routine biochemical analysis of blood and urine as well as an assessment of the presence and extent of macrovascular or microvascular complications. Anthropometric parameters measured included body mass index (BMI) and waist to hip ratio (WHR). Waist and hip circumferences were measured to the nearest 0.1 cm at the narrowest point between the lowest rib and the uppermost lateral border of the right iliac crest. The hips were measured at their widest point. Plasma biochemical parameters were also measured after overnight fasting including fasting blood glucose, HbA1C , triglycerides, total cholesterol, low-density lipoprotein- cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), uric acid, and creatinine, , which were measured using routine methods of analysis.

Plasma visfatin measurement

All of the blood samples were drawn after overnight fasting .EDTA plasma samples were collected centrifuged for 15 minutes at 1000 x g within 30 minutes of collection. Plasma stored in aliquots at - 80°C for subsequent assay. Plasma visfatin levels were determined by enzyme-linked immunosorbent assay (ELISA) using Human Visfatin Kit from BioSource Europe S.A. Belgium.

The intra-assay coefficients of variation were 4.4–10.4%. Statistical Analysis was performed using a computer-based program (SPSS version 11). The data are presented as mean \pm standard deviation. Continuous data were compared with a two-tailed unpaired t test. The strength of the link between the continuous variables was tested by Pearson correlation coefficient r. P value less than 0.05 indicated statistical significance.

RESULTS

The clinical characteristics of our subjects are shown in Table 1 & 2: A total of 40 obese patients and 40 sex- and age-matched non-diabetic lean subjects were studied. Obese subjects had higher BMI, waist, WHR, serum triglyceride and lower HDL-C measurements than those of control subjects. Plasma visfatin levels were found to be elevated in obese patients (38.7 ± 13.3 ng/ml vs. 20.8 ± 3.8 ng/ml in controls, $P < 0.01$). However, when obese subjects further subdivided into two groups (diabetic [22] & non diabetic [18]); there is no significant difference as regard plasma visfatin level in diabetic obese group (39.1 ± 7.3 ng/ml) in comparison to non diabetic obese group (36.9 ± 7.4 ng/ml) But there is a highly significant difference between both subgroups (diabetic obese and non diabetic obese) and control subjects as regard plasma visfatin level ($P < 0.01$) figure 1.

Table-1: Clinical characteristics of study subjects

Factor	Obese (N 40)	Control (N 40)	P
Age(Years)	45.7 \pm 17.2	42.9 \pm 12.4	NS
Gender (Males)	20	23	NS
Diabetics	22	-----	-----
Total cholesterol(mg/dl)	189.4 \pm 26.5	187.6 \pm 21.9	NS
Triglyceride(mg/dl)	249.5 \pm 37.8	144.9 \pm 26.5	<0.001
HDL-C(mg/dl)	40.7 \pm 4.1	49.3 \pm 8.4	<0.001
LDL-C(mg/dl)	107.8 \pm 12.8	105.2 \pm 9.1	NS
BMI (kg/m ²)	33.2 \pm 6.4	24.8 \pm 0.9	<0.001
Waist (cm)	106.4 \pm 11.5	85.3 \pm 4.3	<0.001
WHR	1.06 \pm 0.08	0.87 \pm 0.07	<0.001
Serum creatinine(mg/dl)	1.1 \pm 0.2	1.1 \pm 0.3	NS
Plasma visfatin(ng/ml)	38.7 \pm 13.3	20.8 \pm 3.8	<0.001

13 diabetic patients were treated with oral hypoglycemic agents alone and 9 cases with combined insulin and oral hypoglycemic agents. The mean plasma level of visfatin in subjects receiving insulin treatment (38.9 ± 7.5 ng/ml) did not differ from that of patients receiving oral hypoglycemic agents (38.4 ± 13.4 ng/ml) (Fig. 2). 8 patients had HbA1c level ≤ 7 and the other 14 showed bad

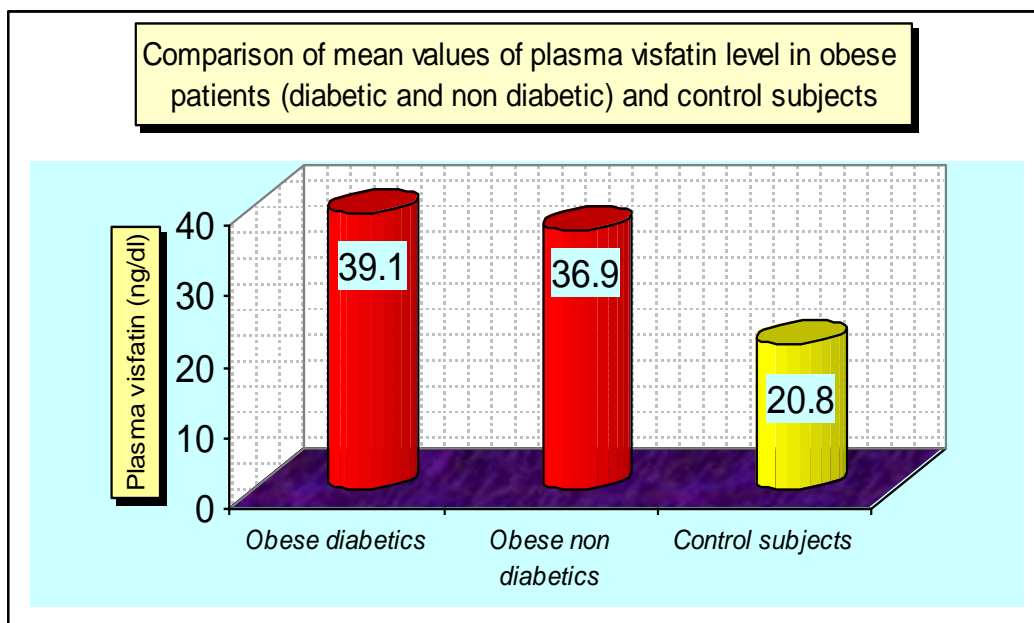
control and the mean HbA1c was 9.1 ± 2.3 however no significant difference between the two groups as regard plasma visfatin level (37.9 ± 13.6 ng/ml in the controlled group and $39.4.1 \pm 12.5$ ng/ml in the uncontrolled group) (Fig. 2).

Plasma visfatin concentration was significantly correlated with WHR ($r = 0.45$, $p < 0.01$) (Fig.3). While we did not find any significant correlations between plasma visfatin and metabolic biomarkers in individual groups.

Table-2: Clinical characteristic of obese patients

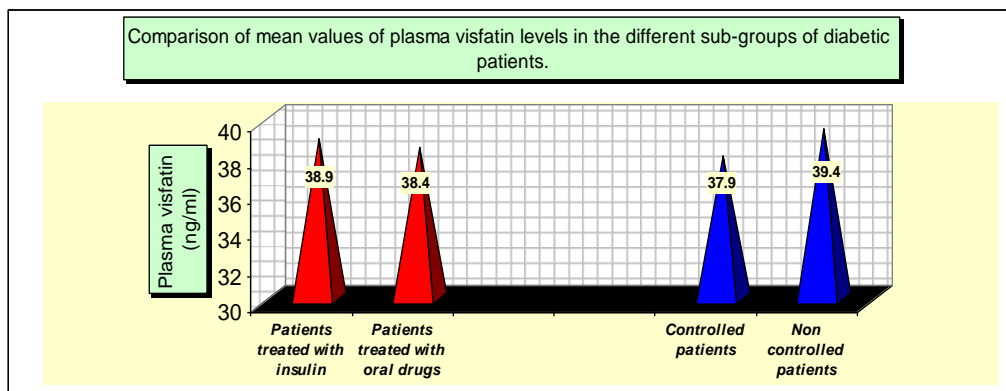
Factor	Diabetic obese (N 22)	Non diabetic obese (N 18)	P
Fasting blood glucose(mg/dl)	141.8 ± 37.6	86.4 ± 10.6	< 0.001
HbA1c(%)	8.4 ± 3.2	5.9 ± 0.6	< 0.001
Controlled diabetics	8	-----	
Patients treated with insulin	9	-----	
Plasma visfatin(ng/ml)	39.1 ± 7.3	36.9 ± 7.4	NS

Fig-1: Comparison of mean values of plasma visfatin level in obese patients (diabetic and non diabetic) and control subjects.



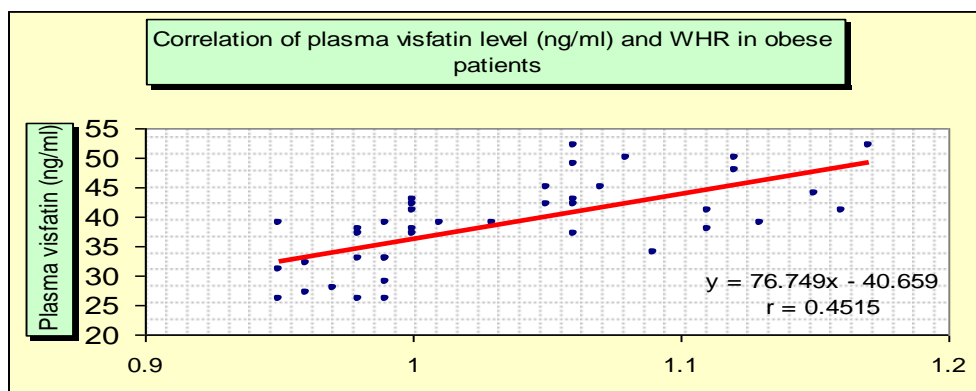
$P < 0.01$

Fig-2: Comparison of mean values of plasma visfatin levels in the different sub-groups of diabetic patients.



$P > 0.05$

Fig-3: Correlation of plasma visfatin level (ng/ml) and WHR in obese patients



$P < 0.01$

DISCUSSION

Visfatin as an adipokine has recently been identified and named as such because of its much greater expression in visceral fat than in subcutaneous adipose tissue (Adeghate, 2008). Conflicting results have been reported in the literature concerning the relationship between visfatin and body fat accumulation, as well as its potential involvement in glucose homeostasis. (Fukuhara *et al.*, 2005) reported that visfatin is predominantly secreted by visceral fat and that plasma visfatin concentration increases during the development of obesity in mice. Therefore, it is reasonable to expect an association between circulating serum visfatin concentration and trunk fat, which is a reflection of the amount of visceral fat. Visfatin stimulates glucose uptake in adipocytes and myocytes and inhibits glucose release from the liver (Fukuhara *et al.*, 2005). In keeping with its insulin-mimetic effects, visfatin was as effective as insulin in reducing hyperglycemia in insulin-deficient diabetic mice and also bound to activated insulin receptors, causing receptor phosphorylation and the activation of the downstream signaling molecules (Fukuhara *et al.*, 2005; Adeghate, 2008). However, (Chen *et al.*, 2006) reported that plasma visfatin concentrations elevated in patients with type 2 diabetes mellitus, compared with those in healthy subjects, and negatively correlated with adiponectin, which is an adipocytokine related to insulin sensitivity (Oh

et al., 2007; *Zhu et al.*, 2010). In contrast to the results of (*Fukuhara et al.*, 2005), this appears to suggest that visfatin is associated with insulin resistance, rather than insulin sensitivity. The main finding in our study is elevated visfatin level in obese patients compared to controls. Obesity is the core of insulin resistance. So this finding could augment the hypothesis that visfatin may be a marker to insulin resistant state. In the same direction (*Krzyzanowska et al.*, 2006) reported that visfatin significantly elevated in women with gestational diabetes which is a classic example of insulin resistance. The other exciting finding in our study that there is no significant difference between diabetic obese and non diabetic obese patients as if visfatin had nothing to do with diabetes. Also no significant changes in the level of visfatin in diabetic obese patients according to glycemic control or line of treatment whether insulin or oral drugs. These results are supported by that done in 2007 by (*Abbas*, 2007). In this study they founded statistically significant difference in the level of visfatin in diabetic patients in comparison to healthy subjects but when they divided the diabetic patients into obese and non obese ; visfatin was only high in obese patients while in non obese had no significant difference when compared to healthy volunteer. Also there is no significant correlation between visfatin and any diabetes parameters such as blood glucose or HbA1c or even any components of dyslipidemia (triglyceride, HDL etc...) characteristic to diabetes and insulin resistance. These results may point to different concept that visfatin is only marker of adiposity apart from diabetes status. In contrast to (*Guang Sun. et al.*, 2007) who found that serum visfatin concentration at base line were positively correlated with serum triglycerides independent to factors like age and percent of body fat. Another study done in Asian Indians diabetic patients by (*Sandeep et al.*, 2006) , they reported that serum visfatin levels are associated with obesity and visceral fat but not with subcutaneous fat. Although visfatin levels are also increased in type 2 diabetes mellitus, the association seems to be primarily through obesity and not with diabetes per se. Significant elevation of plasma visfatin in obese patients sounds acceptable because visceral fat is the source of visfatin. Accordingly, obese patients reasonably had huge amounts of all types of fat including visceral type and hence elevated plasma visfatin level. (*Haider et al.*, 2006) reported that elevated plasma visfatin level in morbidly obese subjects reduced after weight loss by gastric banding. . Another supporting point that in our work only WHR significantly correlated with plasma visfatin level while on the other hand, plasma visfatin did not correlate with BMI .This fact is consistent with findings that visfatin is mainly secreted in the visceral fat, not subcutaneous fat. The (*Fukuhara et al.*, 2005) was the first to positively correlate serum visfatin levels with visceral but not subcutaneous fat composition in both mice and humans. (*Sandeep et al.*, 2006) also reached to same fact using CT scan. Some limitations of this study need to be considered. Our analyses are based on single measurements of blood visfatin, which may not reflect the relationship over time. It would be interesting to measure serial changes of plasma visfatin levels in obese, insulin-resistant, or pre-diabetic subjects and in patients with metabolic syndrome to further clarify the role of visfatin in the pathogenesis of T2DM. The lack of association between the change in triacylglycerols with visfatin warrant further studies because elevated serum triacylglycerols is a marker of the metabolic syndromes, these results may have clinical implications. In conclusion: Our results indicate that visfatin is elevated in obese patients apart from the presence of diabetes, diabetes control and line of treatments. Visfatin more

reasonably to be a marker of obesity as a product of visceral fat cells rather than a new key in pathogenesis of type 2 diabetes mellitus . It seems that visfatin could not be one of the missing links between type 2 diabetes mellitus and its relation to visceral obesity. Further large scale studies are needed to determine its relation to immune status of obese patients and other adipo-cytokines and the effect of weight loss on its level.

REFERENCES

- Diagnosis and classification of diabetes mellitus. In: provisional report of a WHO consultation. *Diabet Med.* pp: 539–553.
- Abbas, O., 2007. Visfatin: The missing link between visceral fat and type 2 diabetes; true or false? *Zgzig university journal* 10: 654-661
- Adeghate, E., 2008. Visfatin: Structure, function and relation to diabetes mellitus and other dysfunctions. *Curr Med Chem*, 15(18): 1851-1862.
- Arner, P., 2005. Insulin resistance in type 2 diabetes: Role of the adipokines. *Curr Mol Med* 5: 333–339.
- Chang, Y., T. Chang, W. Lee and L. Chuang, 2010. The relationship of visfatin/pre-b-cell colony-enhancing factor/nicotinamide phosphoribosyltransferase in adipose tissue with inflammation, insulin resistance, and plasma lipids. *Metabolism*. 59 (1): 93-99.
- Chen, M., F. Chung and D. Chang, 2006. Elevated plasma level of visfatin/pre-b cell colony-enhancing factor in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 91(295- 9).
- Fukuhara, A., M. Matsuda, M. Nishizawa, K. Segawa, M. Tanaka, K. Kishimoto, Y. Matsuki, M. Murakami, T. Ichisaka, H. Murakami, E. Watanabe, T. Takagi, M. Akiyoshi, T. Ohtsubo, S. Kihara, S. Yamashita, M. Makishima, T. Funahashi, S. Yamanaka, R. Hiramatsu, Y. Matsuzawa and I. Shimomura, 2005. Visfatin: A protein secreted by visceral fat that mimics the effects of insulin. *Science*, 307: 426–430.
- Giorgino, F., L. Laviola and E. JW., 2005. Regional differences of insulin action in adipose tissue: Insights from in vivo and in vitro studies., 183: 13 - 30.
- Guang Sun., Jessica Bishop., Sammy Khalil., Sudesh Vasdev., Vicki Gill., David Pace., Donald Fitzpatrick., Edward Randell., Ya Gang Xie. and Hongwei Zhang., 2007. Serum visfatin concentrations are positively correlated with serum triacylglycerols and down-regulated by overfeeding in healthy young men. *Am J Clin Nutr* 85: 399-404.
- Haider, D., K. Schenidler and G. Prager, 2006. Increased plasma visfatin concentration in morbidly obese patients is reduced after gastric banding. *J clinic endo metab* 91: 1578-1581

- Jia, S., Y. Li, J. Parodo, A. Kapus, L. Fan, O. Rotstein and J. Marshall, 2004. Jc pre-b cell colony-enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. *J Clin Invest* 113(1318–1327).
- Klein, S., L. Fontana, V. Young, A. Coggan, C. Kilo, B. Patterson and B. Mohammed, 2004. Absence of an effect of liposuction on insulin action and risk factors for coronary heart disease. *N Engl J Med*, 350: 2549–2557.
- Krzyzanowska, K., W. Krugluger, F. Mittermayer, R. Rahman, D. Haider and N. Shnawa, 2006. Increased visfatin concentrations in women with gestational diabetes mellitus. *Clin Sci (Lond)* 110(605–9).
- Noriyuki, O., L.P. Jennifer, J.L. Jesse and W. Kenneth, 2011. Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology*, 11: 85-97
- Ognjanovic, S. and G. Bryant, GD. , 2002. Pre-b cell colony-enhancing factor, a novel cytokine of human fetal membranes. *Am J Obstet Gynecol* 187: 1051–1058.
- Oh, D., T. Ciaraldi and R. Henry, 2007. Adiponectin in health and disease. *Diabetes Obes Metab* 9: 282–289.
- Samal, B., Y. Sun, G. Stearns, C. Xie, S. Suggs and I. McNiece, 1994. Cloning and characterization of the cdna encoding a novel human pre-b-cell colony enhancing factor. *Mol Cell Biol*, 14: 1431–1437.
- Sandeep, S., K. Velmurugan and R. Deepa, 2006. Serum visfatin in relation to visceral fat, obesity and type 2 diabetes mellitus in asian indian. *Metabolism Clinical and Experimental* 55: 565– 570.
- Shaw , J.E., R.A. Sicree and P.Z. Zimmet, 2010. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes research and clinical practice* 87: 4-14.
- Thorne, A., F. Lonnqvist, J. Apelman , G. Hellers and P. Arner, 2002. A pilot study oflong-term effects of a novel obesity treatment: Omentectomy in connection with adjustable gastric banding. *Int J Obes Relat Metab Disord* 26: 193–199.
- Zhu, N., J. Pankow, C. Ballantyne, D. Couper, R. Hoogeveen, M. Pereira, B. Duncan and M. Schmidt, 2010. High-molecular-weight adiponectin and the risk of type 2 diabetes in the aric study. *J. Clin. Endocrinol. Metab*, 11: 5097–5104.