A Cross-Sectional Study to Assess and Correlate Serum Levels of Visfatin with Insulin Resistance amongst Newly Diagnosed Diabetes Mellitus, Metabolic Syndrome, and Pre-Diabetes Patients

Manisha Naithani[1], Sarama Saha[1], Anisha Mathew[1], Jyoti Bharadwaj[2], Ravi Kant[3], *Anshuman Darbari[4]

[1] Department of Biochemistry, AIIMS, Rishikesh, India.
[2] Department of Nutrition, State Govt. Nursing College, Dehradun, India.
[3] Department of General Medicine, AIIMS, Rishikesh, India.
[4] Department of Cardiothoracic Vascular Surgery, AIIMS, Rishikesh, India.

***Correspondence:** darbarianshu@gmail.com

Received: Sep 19, 2020; Accepted: Oct 26, 2020

Citation: Naithani M, Saha S, Mathew A, Bharadwaj J, Kant R, Darbari A. A cross-sectional study to assess and correlate serum levels of visfatin with insulin resistance amongst newly diagnosed diabetes mellitus, metabolic syndrome, and pre-diabetes patients. Recent Adv Biol Med. 2020; 6(4): 1222516. https://doi.org/10.18639/RABM.2020.1222516

Abstract

Visfatin is an adipocytokine that has been thought to play a role in the pathogenesis of insulin resistance. Clarifying the role of Visfatin along with its correlation to insulin resistance in Diabetes and Metabolic Syndrome (MS) patients would enhance our knowledge of this novel biomarker. It was a clinic-based cross-sectional study. Serum levels of Visfatin assessed randomly in 118 patients having diabetes, Pre-diabetes, or metabolic syndrome, attending OPD in our institute. Visfatin levels measured along with BMI (Body Mass Index), Blood Pressure (BP), Lipids, Glucose, Insulin, Hs-CRP (High Sensitivity C Reactive Peptide) levels, Homeostatic model assessment is a method for insulin resistance (HOMA-IR) indexes. One-way ANOVA and linear regression analysis were carried out using SPSS 23. Average Serum Visfatin (SV) levels were significantly different in MS (n=34, 9.50±8.22), Diabetic (n=43, 12.04±7.6) and Pre-Diabetic (n=41, 9.07±8.6) groups. SV shows a significant association with fasting blood sugar (p=0.009) and BMI (p=0.038) only in MS patients. Significant correlation not found between Visfatin and insulin resistance (p=0.173) in either of the 3 groups. In the present study, SV levels were not correlated with insulin resistance in metabolic, diabetic, and pre-diabetic patients but SV showed a significant association with fasting blood sugar and BMI in MS patients.

Keywords: Visfatin; Metabolic Syndrome; Diabetes; Insulin Resistance; Pre-Diabetics; Blood Sugar.

1. INTRODUCTION

In India, 6.5 percent of people are suspected to be suffering from Diabetes Mellitus (DM) [1]. Excess adiposity has been heralded as the most important risk in the development of insulin resistance, pre-diabetes, and type-2 DM [2,3]. DM can be associated with MS if it is associated with central obesity defined as waist-hip ratio > 0.9 in men and >0.85 in women or Body Mass Index (BMI) > 30 kg/m^2), microalbuminuria, hypertension, and deranged plasma triglyceride [4]. These cut off are not applicable for South Asians or "Asian Indian phenotype". These are people whose ancestry can be traced to South Asia (countries of Afghanistan, Bangladesh, Bhutan, Maldives, Nepal, India, Pakistan, and Sri Lanka). They have been observed to exhibit higher indicators of cardiometabolic risk relative to Caucasians. These include lower cut-offs for BMI and Waist Circumference (WC). BMI 23–24.9 kg/m^2 for overweight and ≥25 kg/m^2 for obesity, WC ≥80 cm for women and ≥90 cm for men for abdominal obesity for South Asians [5].

Serum Visfatin (SV) is also known as the Pre-B Cell Enhancing Factor (PBEF). It has been named Visfatin as it is preferentially expressed in visceral fat. Visfatin acts as nicotinamide phosphoribosyl transferase, catalyzing the rate-limiting step in the biosynthesis of Nicotinamide Adenine Di-Nucleotide (NAD). SV has anti-apoptotic properties and regulates energy metabolism during stress responses. It also plays a role in immune activation. We evaluated SV levels in patients with DM, patients with MS, and also in patients with pre-diabetic features. In our study, SV levels were significantly raised in pre-diabetics, DM, and MS patients ($p<0.01$) which corroborate with SV findings reported previously [6]. It has been suggested that increased visceral fat in these patients induces a state of inflammation which may lead to insulin resistance. Fukuhara *et al.* had demonstrated that SV may have a glucose-lowering effect however it was controversial, and the article was later retracted by the Editor of "Science" [7,8]. This cross-sectional analytic study aimed to estimate SV level among patients having diabetes, pre-diabetes, or MS and to observe and deduct the interplay between SV, insulin resistance, obesity potentially enhancing our knowledge of this controversial marker.

2. METHOD(S)

2.1. Study Population

Institute research review board clearance and institute ethical clearance (ECR/736/Inst./UK/2015) were taken before starting this study. Ethical approval reference number: AIIMS/IEC/15/154. All patients' detailed consent was also taken for their participation and availability. 126 patients having diabetes, pre-diabetes, or MS attending General medicine outpatient clinics of our Institute in this study period of 6 months (1 January 2019 to 30 June 2019) were selected and clinical examination with blood sample analysis were done. Since many prescribed drugs can influence insulin sensitivity so, only treatment naïve patients were enrolled in this study. Proper written informed consent in local understandable language was taken from patients and protocol was explained to them.

2.2. Inclusion Criteria

- Age more than 21 years, irrespective of sex.
- Diagnosed as a case of DM according to the standard definition of Fasting blood sugar >126 mg/dl or 2 hours Postprandial blood sugar > 200 mg/dl or both (American Diabetic Association Guidelines) [9].
- Pre-diabetes criteria adapted from the International Expert Committee [9].
- MS patients based on diagnosis criteria: Glucose intolerance, IGT or diabetes and/or insulin resistance together with two or more of Blood pressure ≥ 140/90 mmHg, Raised plasma triglycerides: ≥ 150 mg/dl and/or HDL-cholesterol Men: <35 mg/dl, Women: < 39 mg/dl, Men: waist–hip ratio > 0.90, Women: waist–hip ratio > 0.80 and/or BMI > 30 kg/m2 and Urinary albumin: creatinine ratio ≥ 30 mg/g [10].

2.3. Exclusion Criteria

- Unwilling patients.
- Type-1 Diabetes.
- Patients on insulin therapy as a treatment for DM.
- Renal disease, macrovascular diseases, Overt proteinuria, or other unknown major diseases.
- Drugs influencing insulin sensitivity (e.g. metformin, thiazolidinediones, etc.).

2.4. Study Design

Cross-sectional analytical study.

2.5. Anthropometric Parameter

- Body Mass Index (BMI)
  - Height in meters (m) and Weight in Kilograms (Kg).
  - Formula: Weight (Kg)/ Height (m^2).
- Waist circumference.

2.6. Analytes and Estimates

- SV.
- Lipid profile.
- Homeostasis model assessment of insulin resistance (HOMA-IR)
  - Formula: (Glucose (mg/dL) x Insulin (I.U.))/405

2.7. Methods Used for Analysis

- SV: Enzyme-linked immunosorbent assay (ELISA) for the quantitative determination of Visfatin.
- Total Cholesterol: Cholesterol oxidase peroxidase (CHO-POD method) enzymatic method.
- Triglycerides: Glycerophosphate oxidase peroxidase (GPO-POD method) enzymatic method.
- High-density Lipoprotein: Enzymatic immune inhibition.
- Creatinine: Jaffes Kinetic.
- Glucose: Hexokinase.
- Insulin: Siemens Immunlitechemiluminescent insulin immunoassay.
- hs-CRP: Latex enhanced immunoturbidimetric.
- HOMA-IR: Homeostasis model assessment of insulin resistance (HOMA-IR) used.

2.8. Statistical Analysis

The normality of data was verified by Kolmogrov Smirov Method. Data following Gaussian Distribution were present as mean ± SD whereas, distributed data that did not follow Gaussian Distribution were presented as median (IQR). Group comparison was carried out by Mann Whitney U and Kruskalwallis Test. Correlation of various parameters with Visfatin was by Spearmen's Rank Correction. IBM SPSS version 23.0 was used for all statistical analysis. $P<0.05$ was considered statistically significant.

2.9. Cases Excluded

Six cases were excluded from the study on the basis of predetermined exclusion criteria, as these patients had deranged Renal Function Tests. 02 patients had been taking Oral Metformin as treatment, so they have been excluded as well. There was some unwilling patient to consent and so they were excluded.

3. RESULTS

The total number of patients selected was 126 but 118 patients were the final participants for this study (n=118). Out of these, 41 patients were in pre-diabetic groups, 43 were diagnosed as diabetic and 34 patients were diagnosed with MS based on established diagnostic criteria. Mean weight in pre-diabetic, diabetic, and MS groups were 68.1 Kg, 65 Kg, and 69.8 Kg respectively. There was no significance of weight between the groups. Significant correlation was seen with fasting plasma glucose ($p<0.01$), Cholesterol ($p<0.01$), Triglyceride ($p<0.01$), Insulin ($p<0.01$), HOMA-IR ($p<0.01$) and Visfatin ($p<0.01$) (Table 1).

Table 1: Significance of parameters in 3 groups.

Variable	Pre-Diabetes (n=41)	Diabetes (n=43)	Metabolic Syndrome (n=34)	p-value
Weight (kg)	68.1 (30-94)	65 (39-105)	69.8 (40-125)	0.304
Waist Circumference (cm)	96.5 (78-147)	96.28 (70-137)	97 (80-132)	0.965
Hip Circumference (cm)	100.6 (69-137.16)	98.49 (77-137.16)	99.56 (71-132)	0.701
BMI (kg/m^2)	26.29 (13-36)	24 (16-44)	27.06 (18-43)	0.09
Waist Hip Ratio	0.96 (.79-1.16)	0.97 (85-1.10)	0.97 (89-1.18)	0.610
Fasting Plasma Glucose (mg/dL)	88.3±9.24	166.3±78.295	188.4±98.22	<0.001
Cholesterol (mg/dL)	189.1±46.94	174.7±43.94	216.7±61.57	0.002
Triglyceride	156.8±87.3	142.8±53.52	326.6±251.3	<0.001
High Density Lipoprotein	40.5±6.81	41.9±7.97	38.9±8.17	0.235
Insulin (µIU/ml)	11.9±6.79	8.57±6.77	15.7±11.72	0.002
HOMAIR	2.64±1.73	3.57±3.51	6.61±4.35	<0.001
Hs-CRP	6.85±11.1	4.99±6.65	4.38±4.70	0.382
Visfatin (ng/ml)	7.07±8.79	12.69±7.25	9.50±8.22	0.007

Correlation of Visfatin was performed with BMI between all 3 groups and also seen individually in patients of MS where increased BMI is a major cause of the condition. Visfatin did not show any significant correlation between the three groups ($p>0.05$) but there was a significant correlation between Visfatin and patient BMI in the MS patient group ($p<0.05$). This may suggest that Visfatin increases with an increase in BMI.

As BMI and Visfatin levels have a significant correlation with each other, a correlation was done to see if there was any significance between Visfatin and waist: hip ratio to see a correlation between Visfatin level and visceral fat, however, there was no correlation between these two parameters. As raised blood glucose levels are an important part of the diagnosis for all three groups, a correlation study was done between SV levels and Fasting blood sugar (FBS) separately for all three groups. There was a significant correlation between FBS and Visfatin level in patients of the MS group ($p<0.05$) (Figure 1) but there was no significant correlation seen in Pre-diabetic and Diabetic groups ($p>0.05$) (Figure 2 and 3). As MS patients have high levels of glucose with other deranged parameters, increased glucose levels may be linked with increased secretion of Visfatin. Insulin levels are raised in diabetic and MS patients due to resistance against the hormone. Resistance in insulin causing deranged glucose levels and even play a role in increasing abdominal girth. So, a correlation was carried between insulin level and Visfatin levels in all 3 groups. No significant correlation was seen between Insulin and Visfatin in all 3 groups. The correlation was studied between Visfatin and HOMA-IR (a measure of insulin resistance), which had been calculated for every patient, to see if there is an increase in Visfatin levels depending on insulin resistance. However, there was no significant correlation seen between insulin resistance and Visfatin levels in either of the 3 groups ($p>0.05$).

4. DISCUSSION

Subjects with MS are at high risk of cardiovascular disease and metabolic complications. The prevalence of MS in India is as high as 33.5% overall in a recent study [11]. Because insulin resistance is thought to be the underlying defect of this syndrome, the term insulin-resistance syndrome has also been given. Interestingly distribution of adipose tissue correlates more with the risk of MS than the overall amount of fat [12]. People with more visceral fat (apple shape morphology with fat around abdominal viscera) are more at risk of obesity-related complications like Type 2 diabetes and coronary artery disease compared to people with fat in the gluteo-femoral region fat (pear shape). Adipose tissue is no longer considered just a passive storage unit for excess fat but an active organ, secreting substances now known as adipokines [13]. Some of these adipokines, like TNF-alpha, HB-EGF, and serpin E1, are nonspecific to adipose tissue while others like leptin, are adipose-specific [14]. An adipokine was identified, having preferential expression in visceral fat and was hence labelled as SV in 2005,

but it was previously known as PBEF found in 1994 [15]. It was also rechristened as Nicotinamide Phosphoribosyl Transferase (NAmPRTase or Nampt) in 2007 [16].

Figure 1: Correlation between Visfatin and FBS in pre-diabetic patients' group (p=0.565).

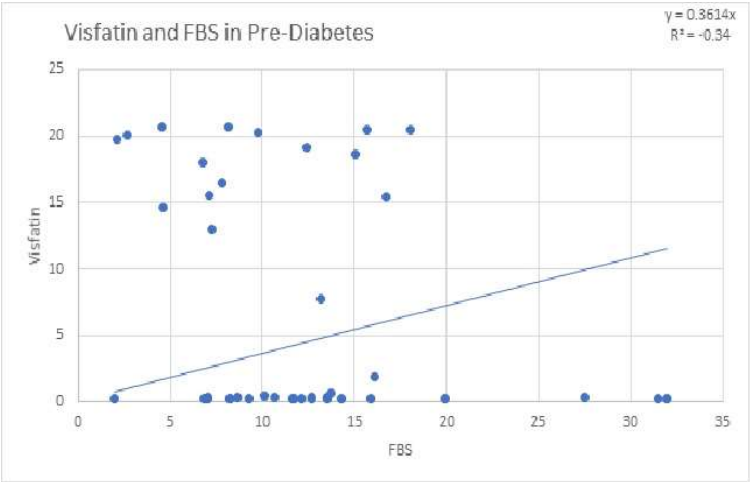


Figure 2: Correlation between Visfatin and FBS in diabetic patients' group (p=0.111).

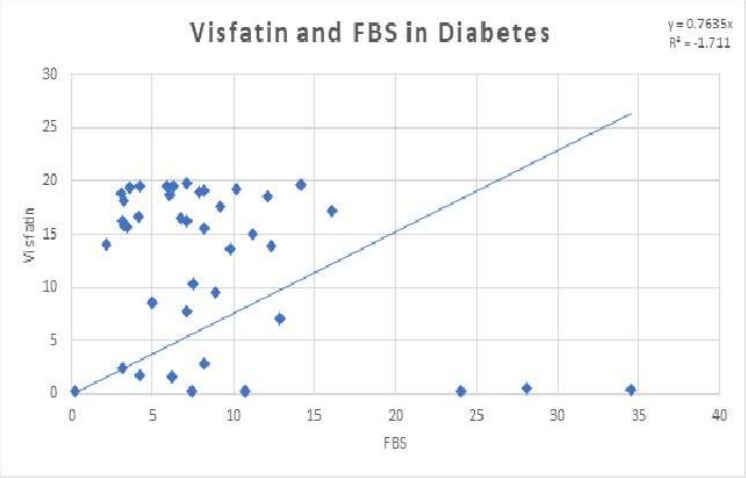
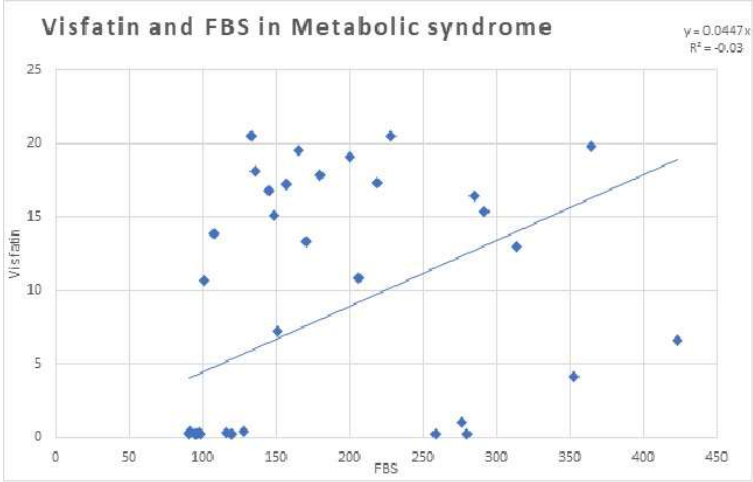


Figure 3: Correlation between Visfatin and FBS in metabolic syndrome patients' group (p=0.009).



Studies found a correlation between the level of SV and Type-2 DM [17]. The relationship between SV and glucose metabolism is still under evaluation. Obesity certainly and diabetes questionably relates to SV levels, thus has been labelled as a predictor of obesity, diabetes status, and MS. The aim of our study was to estimate SV level among patients having diabetes, pre-diabetes, or MS and to observe and deduct the interplay between all these. On correlating SV levels of all three patients' groups with BMI, there was no significant correlation seen ($p>0.05$), however, when correlating BMI with patients of only the MS group, there was a significant correlation between BMI and Visfatin levels ($p<0.05$). Our study found a significant correlation between SV and patient BMI in the MS patient group ($p<0.05$). This may suggest that SV increases with an increase in BMI. Although this finding has also been inconsistent in various studies, it has been hypothesized that with obesity adipose tissue is subjected to hypoxia, which can induce expression of SV via the transcription factor HIF-1[18]. Macrophages present in adipose tissue, secrete the inflammatory mediators which are characteristic of MS. which may lead to elevated SV [19]. In MS patients, there is an increase in BMI and visceral fat, however, there was no significant correlation between Waist:Hip ratio and SV level. The significance of this finding is unclear as and there may have been inter-observer variation in recording these anthropometric measurements.

If SV levels are increased with raised levels of glucose, it may have an association with raised insulin levels, due to the resistance seen in patients of pre-diabetes, diabetes, and MS patients. In diabetes and MS, the main pathophysiology is due to Insulin resistance leading to high levels of insulin which release many adipocytic inflammatory markers. The association of SV with raised glucose levels and inflammation in DM and MS patients has been studied in various studies [7,19,20]. In one of the studies, it was shown that SV levels rise in presence of a sustained blood glucose elevation lasting from 90 to 240 minutes [21]. Blood from visceral adipose tissue drains into the hepatic portal vein and adipokines released from visceral adipose can induce rapid responses in the liver influencing glucose metabolism [22]. Clinical evidence regarding the association of SV with DM has been inconsistent. Some studies have suggested that sustained elevated levels of SV may be associated with beta-cell damage [20,22]. In our study, there was no significant correlation seen between insulin levels and SV levels. Bermejo *et al*. study showed that insulin had no effect on either plasma Visfatin or SV expression even though insulin administration prevented the rise in SV concentrations under these conditions [23]. This observation is consistent with our finding as no significant correlation was found between HOMA-IR levels and SV levels in all three groups. The inconsistency of the correlation between SV and insulin among different studies suggest that there may be confounding factors between insulin and SV, which are not yet clear. In the current study, we could not find a correlation of SV levels which may be due to the selection of treatment naïve patients who may have preserved β-cell function. A recent study has shown diverse mechanisms linking SV to insulin resistance. One study concluded that SV induced proinflammatory cytokine production and inhibited insulin signalling via the STAT3 and NF-κB pathways in HepG2 cells may be causing insulin resistance [24]. Yet another labelled increase in the expression of ECM proteins and therefore WAT fibrosis and remodelling being central to the link between SV and insulin resistance [25]. Though SV promotes β-cell proliferation and improves insulin sensitivity, it is possible that oxidative factors induced by increased glucose levels may play an important role in the elevation of SV in the patients, and insulin may or may not have any role in it.

4.1. Limitation of Study

The majority of participants had a BMI of less than 30, which may be the reason for a poor correlation between increased body weight and Visfatin levels. We could have taken a larger number of patient size after proper calculation of sample size and also regrouping for randomization may have given more definitive results.

5. CONCLUSION

Our study shows that SV is elevated with MS but it is not yet clear and equivocal if it should be treated as a marker, causative agent, or even a compensatory response. We found that SV levels correlated significantly with fasting blood sugar levels in both DM and MS patients ($p<0.01$), implying that SV increases with rising blood glucose levels rather than play a role in decreasing glucose levels. Serum levels of Visfatin could not be correlated with Insulin resistance in Diabetic, pre-diabetic, and MS patients in this study. Further randomized control studies are required to delve into mechanisms linking SV with insulin resistance and if it can be targeted further as a potential therapy.

Acknowledgment

This study was supported by an Intramural Research Project Grant by AIIMS, Rishikesh, Uttarakhand, India (covering for patient blood investigation charges on subsidized rates only).

Author Contributions

MN, SS, and AD researched literature and conceived the study. MN and RK were involved in protocol development, gaining ethical approval, patient recruitment, and data analysis. AM extracted the data. SS and AM developed statistical modeling and performed the analysis. AD wrote the first draft of the manuscript. MN, JB, and AM wrote, edited, reviewed, and approved the article. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

Conflict of Interest

None.

References

1. Sinha KS. Maharashtra tops high blood pressure tally, foodie Punjab at bottom: Survey [National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular diseases and Stroke (NPCDCS) Survey Report-2012]. The Times of India, 17th Dec. 2012.
2. Bloomgarden ZT. Adiposity and diabetes. Diabetes Care 2002; 25:2342–2349.
3. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol. 2010; 316(2):129-139.
4. Deepa M, Farooq S, Datta M, Deepa R, Mohan V. Prevalence of metabolic syndrome using WHO, ATPIII and IDF definitions in Asian Indians: the Chennai Urban Rural Epidemiology Study (CURES-34). Diabetes/Metab Res Rev. 2007; 23(2):127-34.
5. Unnikrishnan R, Anjana RM, Mohan V. Diabetes in South Asians: Is the phenotype different? Diabetes. 2014; 63:53–5. doi: 10.2337/db13-1592
6. Teoman D, Sonmez A, Tasci I, Bozoglu E, Yilmaz MI, Genc H, *et al*. Serum Visfatin levels in patients with newly diagnosed and untreated type 2 diabetes mellitus and impaired glucose tolerance. Diabetes Res Clin Pract. 2007; 76(1):24-29.
7. Chang Y-H, Chang D-M, Lin K-C, Shin S-J, Lee Y-J. Visfatin in overweight/obesity, type 2 diabetes mellitus, insulin resistance, metabolic syndrome and cardiovascular diseases: a meta-analysis and systemic review. Diabetes/Metab Res Rev. 2011; 27(6):515–27.
8. Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, *et al*. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science. 2005; 307(5708):426-30.
9. The International Expert Committee. International Expert Committee Report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 2009; 32:1327–34.
10. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, *et al*. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement. Circulation. 2005; 112(17):2735-2752.
11. Prasad DS, Kabir Z, Dash AK, Das BC. Prevalence and risk factors for metabolic syndrome in Asian Indians: A community study from urban Eastern India. J Cardiovasc Dis Res. 2012; 3(3):204-211. doi: 10.4103/0975-3583.98895
12. Sethi JK, Vidal-Puig A. Visfatin: the missing link between intra-abdominal obesity and diabetes? Trends Mol Med. 2005; 11(8):344-7.
13. Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. Mol Cell Endocrinol. 2010; 316(2):129-139.
14. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat Rev Immunol. 2006; 6(10):772-83.
15. Samal B, Sun Y, Stearns G, Xie C, Suggs S, McNiece I. Cloning and characterization of the cDNA encoding a novel human pre-B-cell colony-enhancing factor. Mol Cell Biol. 1994; 14(2):1431-7.
16. Tanaka T, Nabeshima YI. Nampt/PBEF/Visfatin: a new player in β cell physiology and in metabolic diseases? Cell Metab. 2007; 6(5):341-3.
17. Chen MP, Chung FM, Chang DM, Tsai JCR, Huang HF, Shin SJ, *et al*. Elevated serum level of visfatin/pre-B cell colony-enhancing factor in patients with type 2 diabetes mellitus. J Clin Endocrinol Metab. 2006; 91(1):295-299.
18. Bae SK, Kim SR, Kim JG, Kim JY, Koo TH, Jang HO, *et al*. Hypoxic induction of human visfatin gene is directly mediated by hypoxia-inducible factor-1. FEBS Lett. 2006; 580(17):4105-13.
19. Peiró C, Romacho T, Carraro R, Sánchez-Ferrer CF. Visfatin/PBEF/Nampt: A new cardiovascular target? Front Pharmacol. 2010; 1:135. DOI: 10.3389/fphar.2010.00135
20. Saddi-Rosa P, Oliveira CS, Giuffrida FM, Reis AF. Visfatin, glucose metabolism and vascular disease: a review of evidence. Diabetol Metab Syndr. 2010; 2(1):21.
21. Haider DG, Schaller G, Kapiotis S, Maier C, Luger A, Wolzt M. The release of the adipocytokine visfatin is regulated by glucose and insulin. Diabetologia. 2006; 49(8):1909-14.
22. Arner P. Visfatin - a true or false trail to type 2 diabetes mellitus. J Clin Endocrinol Metab. 2006; 91(1):28-30. doi: 10.1210/jc.2005-2391
23. López-Bermejo A, Chico-Julià B, Fernàndez-Balsells M, Recasens M, Esteve E, Casamitjana R, *et al*. Serum Visfatin increases with progressive β-cell deterioration. Diabetes. 2006; 55(10):2871-5.
24. Heo YJ, Choi SE, Jeon JY, Han SJ, Kim DJ, Kang Y, *et al*. Visfatin induces inflammation and insulin resistance via the NF-κB and STAT3 signaling pathways in hepatocytes. J Diabetes Res. 2019; 4021623. doi: 10.1155/2019/4021623
25. Nourbakhsh M, Ezzati MS, Malekpour DZ, Nourbakhsh M, Razzaghy AM. Involvement of visfatin in adipose tissue fibrosis through modulation of extracellular matrix proteins. In 58th Annual ESPE 2019, Aug 22 (Vol. 92). European Society for Paediatric Endocrinology.