Section A-Research paper

Carotid artery intima-media thickness and Visfatin among Type 1 Diabetic Children

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Abstract

Type 1 diabetes is an autoimmune disease in which β-cells of pancrease that secrets insulin have impaired function due to destruction by cell-mediated immune response. This disease results from immune system breakdown, which is predominantly mediated via T helper 1 (Th1) cells, accompanied by the activation and islet infiltration of immune cells, which collaborate to destroy pancreatic β cells and cause overt hyperglycaemia. Carotid artery Intima-Media Thickness (CIMT) is a new noninvasive ultrasound test that is being recommended by the American Heart Association and the American College of Cardiology to screen for heart disease in apparently healthy individuals. the CIMT of the patients with microvascular complications was significantly increased compared with the patients without complications indicating a strong relation between CIMT and early stages of microangiopathy and macroangiopathy. Thus, when microvascular complications have developed, one should be alert to take precautions for atherosclerosis. Visfatin is a novel adipokine originally described to be produced predominantly by visceral fat tissue. It also synthesized by bone marrow cells, activated lymphocytes, liver cells, skeletal muscle cells, cardiomyocytes and brain cells. It is a hormone which exerts adipogenic effects and insulin-mimetic. Visfatin is ubiquitous cellular enzyme also called nicotinamide phosphoribosyl-transferase (NAMPT) and pre-B cell colony enhancing factor (PBEF). It was found that estimated visfatin level among prediabetic obese patients, diabetes mellitus (DM) patients had higher visfatin levels than those with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). Visfatin had a significant positive correlation with BMI, WC, FBS, PPS, HbA1c, LDL, fasting insulin, and homeostasis model assessment for insulin resistance (HOMA IR), while HDL had a significant negative correlation, suggesting that it can play a role in pathogenesis of type 2 DM, and also could be a potential biomarker for diagnosis of type 2 DM

Keywords: Carotid artery intima-media thickness, Visfatin

Introduction

Patient and family education is a key, as is an acknowlegment of the normal developmental stages and the challenges this brings in the context of daily living with a chronic disease. With proper care and support, children and adolescents with T1DM can expect to lead long and fulfilling lives **(1).**

Epidemiology:

Its incidence varies around the world, with the highest rates in northern Europe and in individuals of European extraction **(1).** In the Middle East and North Africa, about 129,000 children with type 1 diabetes mellitus (T1DM), among them Egypt represents half of the region's total (8.0 per 100,000 population).

Studing the T1DM modifiable risk factors in Egyptian children can help the specialists in planning and applying preventive polices to reduce its burden **(2)**.

Age:

Although T1DM may present at any age, but most cases detected in early life with a peak around puberty **(1).** It starts in children aged 4 years or older with the peak incidence of onset at age 11-13 years (ie, in early adolescence and puberty). There is also a relatively high incidence in people in their late 30s and early 40s, in whom the disease tends to present less aggressively **(3)**.

Sex:

Both sexes are equally affected in childhood, but men are more commonly affected in early adult life. An international survey of sex ratios in children presenting under the age of 15 years noted a minor male excess in Europe and populations of European origin, while a female excess was noted in populations of African or Asian origin. In contrast, clear male preponderance has emerged from most studies of patients with T1DM diagnosed at 15–40 years **(4).**

Race:

Type 1 diabetes is more common among non-Hispanic whites, followed by African Americans and Hispanic Americans, it is comparatively uncommon among Asians **(5)**.

Carotid artery intima-media thickness

Carotid artery Intima-Media Thickness (CIMT) is a new noninvasive ultrasound test that is being recommended by the American Heart Association and the American College of Cardiology to screen for heart disease in apparently healthy individuals.This is a noninvasive test which is performed with a high-resolution B-mode ultrasound transducer. The test is safe, painless and takes about twenty minutes. After applying conducting jelly to the skin over neck, a small hand-held transducer is applied to image the carotid arteries. The sonographer measures the combined thicknesses of the intimal and medial layers of the carotid artery walls **(5)**.

Torkar et al. (6) determined CIMT normative values in a healthy cohort of Caucasian children aged 6 to 18 years. CIMT increased significantly with age. An increase in CIMT is thought to reflect the precedent of vascular aging and the physiological adaptive remodeling of the vessel walls in response to the developmental changes. In addition to age, gender does appear to play an essential role in the interpretation of CIMT values in the pediatric population. A steady mean CIMT increase was observed in girls, while in boys, the most significant increase was determined in pre-pubertal years when the annual increase was 10μm per year, followed by more gradual yearly thickening.

It Was showed that the CIMT of the patients with microvascular complications was significantly increased compared with the patients without complications indicating a strong relation between CIMT and early stages of microangiopathy and macroangiopathy. Thus, when microvascular complications have developed, one should be alert to take precautions for atherosclerosis.

Bayır et al. (7) evaluated carotid intima-media thickness in young children with T1DM. They measured arterial wall IMT in the common carotid arteries in young children with T1DM and in healthy control subjects matched for age, sex, and body size and assessed the effects of vascular risk factors. They suggested that carotid IMT and stiffness are increased in T1DM children. As diabetes is a chronic disease and cardiovascular morbidity is very high among individuals with diabetes, noninvasive methods for monitoring vascular changes might be useful in clinical practice.

CIMT was directly correlated with age in studied diabetic patients. A strong direct correlation was found between mean CIMT and patient's systolic and diastolic blood pressure. A high proportion of early vascular damage, especially an increased CIMT, is present in children and adolescents with T1DM in whom SBP seems to be a common determinant. In children and adolescents with T1DM, a special focus should be on hemodynamic risk factors beyond metabolic ones **(8)**.

The relationship between increased CIMT and blood pressure suggests that smooth muscle proliferation plays a role in the early diffuse thickening of the arterial wall. No significant correlation was found between mean CIMT and metabolic control parameter HbA1c, this agreed with many studies **(9)**.

El Samahy et al. (10) assessed carotid intima media thickness in children and adolescents with T1DM in relation to plasma nitric oxide and plasma total antioxidant capacity levels and with diabetes duration, glycemic control and microvascular complications. They concluded that the significant elevation in nitric oxide and reduction in total antioxidant capacity in children and adolescents with T1DM together with their correlation with carotid intima media thickness may reflect the role of oxidative stress in the development of atherosclerosis in young T1DM subject. It Was found that oxidative stress is increased in youth with T1DM and only partially predicted by gender, age, glucose control, and anthropometry.

The study of **Łukawska-Tatarczuk et al. (11)** demonstrated that, the groups of patients with T1D were similar in diabetes duration, HbA1c, daily insulin requirement, lipid parameters, and thyroid hormones. However, patients with T1D and Hashimoto's disease (HD) exhibited significantly thicker cIMT and lower left ventricular global longitudinal strain (GLS) compared to women with T1DM only. These observed differences may be due to the relationship between autoimmunity and CV risk.

Visfatin

Visfatin is a novel adipokine originally described to be produced predominantly by visceral fat tissue. It also synthesized by bone marrow cells, activated lymphocytes, liver cells, skeletal muscle cells, cardiomyocytes and brain cells. It is a hormone which exerts adipogenic effects and insulin-mimetic. Visfatin is ubiquitous cellular enzyme also called nicotinamide phosphoribosyl-transferase (NAMPT) and pre-B cell colony enhancing factor (PBEF) **(12).**

Visfatin exists in an intracellular (iNAMPT) and extracellular (eNAMPT) form. Intracellularly, visfatin/iNAMPT plays a regulatory role in NAD+ biosynthesis and thereby affects many NAD-dependent proteins such as sirtuins, PARPs, MARTs and CD38/157. Extracellularly, visfatin is associated with many hormone-like signaling pathways and activates some intracellular signaling cascades. Importantly, eNAMPT has been associated with several metabolic disorders including obesity and type 1 and 2 diabetes **(13)**.

Structure:

It has a molecular weight of 52 KDa. It is identical to pre-B cell colony-enhancing factor (PBEF), described in 1994 as a cytokine produced by lymphocytes, acting on lymphocyte maturation and inflammatory regulation. Visfatin was also soon recognized as the formerly described Nicotinamide phosphoribosyltransferase (Nampt) the limiting enzyme in nicotinamide adenine dinucleotide (NAD) biosynthesis. Nicotinamide phosphoribosyl transferase (Nampt) synthesizes nicotinamide mononucleotide (NMN) from nicotinamide in a mammalian NAD+ biosynthetic pathway. Nampt has also been presumed to be a cytokine (PBEF) or a hormone (visfatin). The crystal structure of Nampt in the presence and absence of NMN shows that Nampt is a dimeric type II phosphoribosyltransferase and provides insights into the enzymatic mechanism **(13).**

Gene:

The gene encoding visfatin/PBEF/Nampt (PBEF1) was first isolated from a human peripheral blood lymphocyte cDNA library. The gene is located on the long arm of chromosome 7 between 7q22.1 and 7q31.33 and encodes a polypeptide of 491 amino acids with a molecular mass of 52 kDa**.** The visfatin/PBEF gene consists of 11 exons and 10 introns spanning 34.7-kb **(12).**

Action of visfatin:

Visfatin in inflammation:

Visfatin originally, when discovered as PBEF, was believed to be an immune modulating cytokine. It has been reported to regulate about 50 different inflammatory genes in peripheral blood mononuclear cells

(PBMCs). In line with this, visfatin has been proven to stimulate the release of many inflammatory mediators. Additionally, it has been shown to induce monocyte chemoattractant protein 1 (MCP-1) production and matrix metalloproteinases (MMPs) expression **(14)**.

Visfatin is implicated in the activation of many inflammatory pathways such as NF-kB, mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3 kinase (PI3). Visfatin also may act as a cytokine mediating vascular remodeling by upregulating vascular endothelial growth factor (VEGF) and fibroblast growth factor 2 (FGF-2). Visfatin-induced cytokine production in leukocytes has been also linked to p38 mitogen-activated protein kinase (p38MAPK) and NF-kB p65 signaling pathways. Therefore, it is clear that visfatin has abundant wide-ranging functions beyond immune modulation **(14)**.

It Was argued that the inflammatory effects of visfatin are due to its ability to incresae expression of lipoxygenase in human endothelial cells. This is consistent with **Romacho et al. (15)** showing that visfatin induces endothelial dysfunction via NLRP3-inflammasome and paracrine IL-1β signaling.

Visfatin stimulates the production of proinflammatory cytokines (IL-6, TNF-α, and IL-1β) and potentially acts as a chemotactic factor for monocytes. Furthermore, its expression is upregulated by IL-6, TNF-α, and IL-1β . Enhanced mRNA expression of visfatin was observed in inflamed mucosa of patients with inflammatory bowel disease. Visfatin has also potency for activation of T cells by upregulation of costimulatory molecules (CD40, CD54, and CD80) on monocytes. And was observed that ther is a positive association between visfatin and antithyroperoxidase antibodies (TPOAb), and the latter is considered the best serological marker of chronic autoimmune thyroiditis **(14).**

Insulin-mimetic function of visfatin:

PBEF/NAMPT is secreted by visceral fat and hence, has been denoted visfatin. It has been shown that visfatin/eNAMPT elicited insulomimetic effects via binding to and activating insulin receptor in hepatocytes, myocytes and adipocytes. Similar to insulin, visfatin/eNAMPT exerted a glucose-lowering effect and enhanced glucose transport and lipogenesis. Moreover, it increased insulin sensitivity in diabetic mice. Additionally, it has been shown that elevated blood glucose levels resulted in increased plasma PBEF/visfatin, which was abrogated by co-infusion of insulin or somatostatin. However, the involvement of insulin receptors in mediating visfatin/eNAMPT's actions became controversial **(13)**.

The binding affinity of visfatin/PBEF/Nampt to the insulin receptor (IR) was found to be similar compared with that of insulin, and, in a competitive binding assay, visfatin did not bind to the same site as insulin, suggesting that visfatin stimulated the insulin receptor in a different way compared with insulin. Visfatin stimulates glucose uptake in adipocytes and myocytes and inhibits glucose release from the liver. 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 **(16)**.

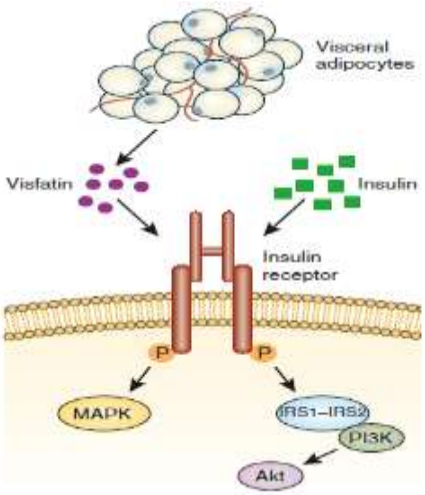

Figure (1): Visfatin is secreted by visceral fat and binds to the insulin receptor at a site different from that of insulin, phosphorylating a number of components of the insulin signaling cascade **(16)**.

Other Insulin-mimetic effects, includs glucose uptake in hepatocytes, preadipocytes where it stimulates accumulation of triglycerides similarly to cells treated with insulin. Also on human osteoblasts, Visfatin increases glucose uptake, stimulates expression of osteogenic markers at the mRNA and protein levels, and also causes an increase in mineralization of osteoblasts in a manner similar to insulin **(17)**.

Visfatin and cardiovascular system:

Visfatin/nicotinamide phosphoribosyltransferase (NAMPT) is an adipokine expressed predominately in visceral fat tissues. High circulating levels of visfatin/NAMPT have been implicated in vascular remodeling, vascular inflammation, and atherosclerosis, all of which pose increased risks of cardiovascular events. In this context, increased levels of visfatin have been correlated with several upregulated pro-inflammatory mediators, such as IL-1, IL-1Ra, IL-6, IL-8, and TNF-α. Furthermore, visfatin is associated with leukocyte recruitment by endothelial cells and the production of adhesion molecules such as vascular cell adhesion molecule 1, intercellular cell adhesion molecule 1, and E-selectin, which are well known to mediate the progression of atherosclerosis. Moreover, diverse angiogenic factors have been found to mediate visfatin-induced angiogenesis. These include matrix metalloproteinases, vascualr endothelial growth factor, monocyte chemoattractant protein 1, and fibroblast growth factor 2. In addition, visfatin has been found to upregulate CD40 inhuman monocytes CD40, a protein known to promote adhesion **(13)**.

Visfatin/PBEF/Nampt further promotes the endothelial release of several cytokines and chemokines by endothelial cells, including interleukin (IL)-6, IL-8 or MCP-1 and its putative receptor CCR2, and thus promotes the adhesion of human THP-1 monocytes to endothelial cells. The MAPK ERK 1/2 and p38, as well as PI3K and the intracellular generation of reactive oxygen species, have been involved in endothelial cell inflammation induced by visfatin/PBEF/Nampt **(17)**.

In human vascular smooth muscle cell cultures, visfatin/PBEF/Nampt consecutively activates ERK 1/2 and NF-κB, that in turn stimulates the expression of inducible nitric oxide synthase (iNOS), a NO- and peroxynitrite-forming pro-inflammatory enzyme playing a key role in vascular damage and endothelial dysfunction **(15)**.

Importantly, a positive correlation has been found between the expression of visfatin/PBEF/Nampt in the periaortic and pericoronary fat and coronary atherosclerosis, highlighting that perivascular visfatin/PBEF/Nampt might play an important paracrine role in the development of atherosclerotic lesions **(13)**.

The pro-inflammatory action of visfatin/PBEF/Nampt in human vascular smooth muscle cells is not mediated by the insulin receptor, but rather relies on Nampt intrinsic activity. Accordingly, the pro-inflammatory action of visfatin/PBEF/Nampt is prevented by the pharmacological Nampt inhibitor APO866 and mimicked by nicotinamide mononucleotide (NMN), the final product of the reaction catalyzed by Nampt **(15)**.

Immunoreactive visfatin/PBEF/Nampt has also been detected in vascular smooth muscle cells within the atherosclerotic plaque. So, visfatin/PBEF/Nampt can be a relevant molecule in plaque weakening **(13**

Through its multiple actions promoting cytokine and chemokine secretion, macrophage survival, leukocyte recruitment by endothelial cells, vascular smooth muscle and endothelial cell inflammation and matrix degradation, visfatin/PBEF/Nampt, either circulating or locally synthesized, may contribute to the development of atherosclerotic lesions and plaque vulnerability and rupture. Visfatin/PBEF/Nampt is therefore gaining relevance as a potential therapeutic target to interfere with the inflammatory response associated to obesity-related complications, especially to atherosclerosis **(15)**.

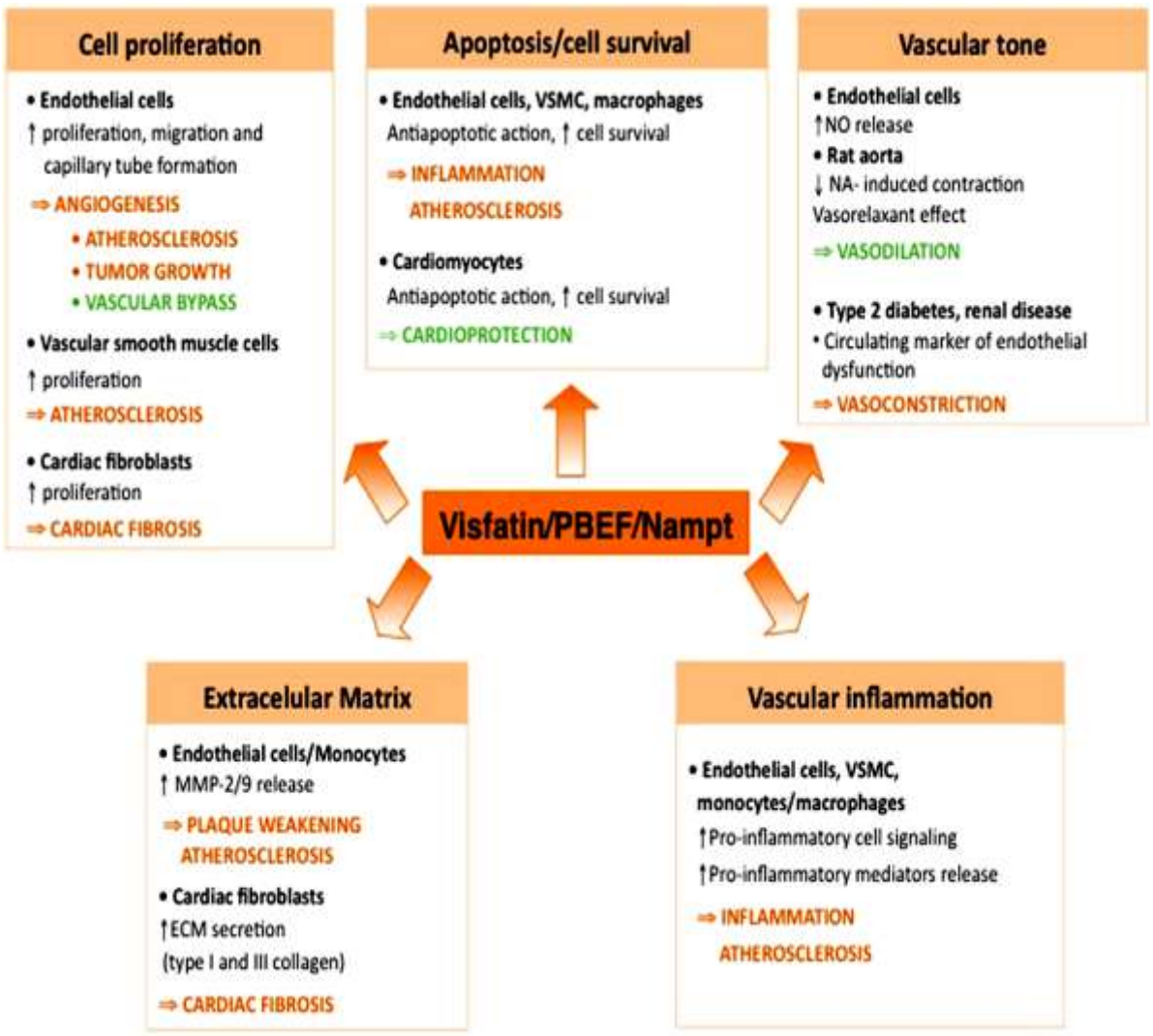

Figure (2): Diagram summarizing the main reported actions of visfatin/Nampt/PBEF in the cardiovascular system. The *arrows* indicate the potential clinical and therapeutical consequences of visfatin/PBEF/Nampt action (*orange arrows*, detrimental actions; *green arrows*, beneficial actions) **(15).**

Clinical importance:

Visfatin/PBEF/Nampt levels are positively associated to a series of inflammatory conditions, including osteoarthritis, acute lung injury, inflammatory bowel disease and Crohn's disease, gynecological disorders, such as infection-induced pre-term birth, sepsis, or psoriasis **(12)**.

It Was Reported That various effects and correlations between visfatin plasma levels with various medical conditions. It possesses antiapoptotic effects on neutrophils in both animal and clinical models of sepsis.

Visfatin is also diminished in patients with steatohepatitis when compared to pure steatosis. However, increased visfatin levels correlated positively with portal inflammation **(15)**.

Allam et al. (18) studied the visfatin level and its relation to the disease activity and severity in rheumatoid arthritis (RA) and osteoarthritis (OA) diseases. They concluded that serum visfatin may be involved in the pathogenesis of RA and OA diseases, and may act as a good predictor for activity and severity of RA, as well as the severity of OA.

Visfatin has been shown to play a detrimental role in the progression of OA. It increases the production of matrix metalloproteinases and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), induces the production of interleukin (IL)-1β, IL-6, and tumor necrosis factor-α, affects the differentiation of mesenchymal stem cells to adipocytes, and induces osteophyte formation by inhibiting osteoclastogenesis. Although some side effects of chemical visfatin inhibitors have been reported, they

were shown to be successful in the treatment of diabetes, cancer, and other diseases that can utilize Chinese herbs, further suggesting that similar therapeutic strategies could be used in OA prevention and treatmen9t **(1)**. However, It Was demonstrated that visfatin has proinfllammatory and prodegradative properties suggesting that visfatin could be a target for treating osteoarthritis in the future.

An inhibitor of visfatin (FK866) is being tested as a potential antitumor agent due to NAD depletion leading to apoptosis on highly metabolically active cells **(15)**.

Visfatin serum concentration in hypothyroidism has been analyzed in a few studies It Was reported That elevated level of this adipocytokine in hypothyroidism with further increase after restoration of thyroid function. It Was observed that visfatin level decreased after recovery .

Farghaly et al. (20) provided evidence of significantly higher levels of visfatin in children and adolescents with autoimmune thyroiditis (AIT). Visfatin might have a potential role in the pathogenesis AIT, which needs to be validated by measuring immunological responses in children and adolescents with AIT.

It Was hypothesized that these findings might result from heterogeneity of study groups. Visfatin in hypothyroidism depends on thyroid hormones level and coexisting autoimmunity.

Visfatin in diabetes mellitus:

It was first reported that patients with uncomplicated type 2 diabetes mellitus had elevated plasma visfatin levels. Conversely, plasma visfatin levels were reported to be decreased in women with gestational diabetes mellitus (DM). Visfatin expression and plasma levels of visfatin were associated with obesity, insulin resistance and the level of albuminuria in type 2 diabetic patients **(21)**.

Hetta et al. (22) reported that there was a significant difference in the mean of cholesterol, HDL and TG (Triglycerides) between T2D patients and healthy controls. They showed a high significant difference in the mean serum level of visfatin. **Dakroub et al. (13)** have reported association between visfatin levels and various types of diabetes ranging from gestational, type 1, and type 2 diabetes. Low circulating visfatin levels were found in gestational and other forms of diabetes.

Alnoggah et al. (23) estimated visfatin level among prediabetic obese patients. They found that dibetes mellitus (DM) patients had higher visfatin levels than those with impaired glucose tolerance (IGT) or impaired fasting glucose (IFG). Visfatin had a significant positive correlation with BMI, WC, FBS, PPS, HbA1c, LDL, fasting insulin, and homeostasis model assessment for insulin resistance (HOMA IR), while HDL had a significant negative correlation, suggesting that it can play a role in pathogenesis of type 2 DM, and also could be a potential biomarker for diagnosis of type 2 DM.

Kang et al. (24) study on type 2 diabetic patients with and without microalbuminuria stated that plasma visfatin levels were significantly increased in type 2 diabetic patients irrespective of the degree of microalbuminuria. Glomerular mesangial cells have been shown to secrete visfatin under high glucose conditions, with visfatin being shown to increase angiotensinogen protein expression, which may increase renin-angiotensin system activation, which would alter GFR **(25)**.

Mageswari et al. (26) concluded that visfatin was significantly elevated in stage IV nephropathy compared with stage III nephropathy. Irisin and visfatin are elevated in diabetic nephropathy and can be an index of its severity.

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