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Serum Visfatin in Type II Diabetes Mellitus and Its Implication in Development of Diabetic Complications

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Abstract **Background:** Diabetes mellitus is a multifactorial disease that is characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia in diabetes is associated with long-term complications, including micro vascular disease such as retinopathy, nephropathy, neuropathy and macro vascular disease including fatal and non-fatal myocardial infarction and peripheral vascular disease. Excess adiposity is the most important risk in the development of insulin resistance and diabetes mellitus II. Adipose tissue produce several factors called adipokines, visfatin is one of them, it acts as insulin mimetic, rate limiting enzyme in NAD synthesis and pro inflammatory cytokine. The study aims to evaluate serum visfatin level in type II diabeticpatients and elucidate its possible role in development of diabetic complications. **Methods:** This study was carried out on sixty patients with type II diabetes mellitus. They are classified into two groups according to the presence of complications or not. Group I: included fifteen patients with uncomplicated type II diabetes mellitus. Group II: included forty five Patients with complicated diabetes mellitus, divided according to the type of complication into three subgroups as following: with cardiovascular complications, with diabetic nephropathy and with diabetic foot respectively. Each subgroup included fifteen patients. In addition, fifteen clinically healthy subjects were recruited as control group. All studied subjects were subjected to complete medical history, clinical examination and routine laboratory investigations including fasting serum glucose level, post prandial serum level, serum creatinine, lipid profile and complete urine analysis, serum nitric oxide levels and serum visfatin by ELISA. **Results: the study revealed that:** ▪ Serum visfatin levels were significantly elevated in diabetic patients (complicated & uncomplicated) compared to healthy control subjects. ▪ Serum visfatin levels were significantly decreased in patients with cardiovascular complications and diabetic foot compared to uncomplicated group. ▪ Visfatin correlated negatively with HDL in cardiovascular complications and correlate positively with ESR in diabetic foot. It did not correlate with age, BMI, FBS, PP, NO, cholesterol, TG, LDL or creatinine. ▪ A significant decrease in serum nitrite in uncomplicated, cardiovascular complications and diabetic nephropathy groups but non-significant decrease in diabetic foot group compared to control group. ▪ A significant increase in serum nitrite in cardiovascular complications & diabetic foot compared to uncomplicated group. ▪ A significant decrease in serum nitrite in cardiovascular complications & diabetic nephropathy compared to diabetic foot group. **Conclusions:** Visfatin andnitrite may have a role on diabetes pathophysiology. Serum visfatin levels reduced with insulin treatment in complicated type II diabetes mellitus patients.

Keywords Visfatin, Type II diabetes mellitus, Cardiovascular complications, Diabetic nephropathy, Diabetic foot

1. Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Type II diabetes is characterized by three pathophysiologic abnormalities: Insulin Resistance, Impaired insulin secretion and deregulated hepatic glucose production [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [1]. A group of micro and macro vascular complications are affecting multiple organ systems. The rate and the severity of complications increase as the duration of diabetes increases [3]. A significant but poorly defined genetic predisposition exists to the development of diabetes mellitus, complicated by the heterogeneous nature of the disease and the influence of acquired factors [4]. However, excess adiposity is the most important risk in the development of insulin resistance and diabetes mellitus II [5].

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Adipose tissue produces several proteins (adipocytokines) such as leptin, adiponectin, resistin, TNFα, and IL-6, that modulate insulin sensitivity and appear to play an important role in the pathogenesis of insulin resistance, diabetes, dyslipidemia, inflammation, and atherosclerosis [6, 7]. These adipokines can partly explain the link between obesity, insulin resistance, beta cell dysfunction, endothelial dysfunction, and atherosclerosis [8]. Unbalanced production of pro- and anti-inflammatory adipocytokines in adipose tissue may contribute to many aspects of the metabolic disorders [9].

Visfatin is one of these adipocytokines which is largely secreted by visceral adipose tissue and its effects in the development of diabetes and inflammatory reactions are similar to insulin. It acts synergistically with insulin in increasing glucose cellular uptake, stimulating glucose transfer to the muscle and adipose tissue, as well as in preventing hepatic glucose production. Its insulin-like effects are mediated through direct connection and activation of insulin receptors without any change or competition with the insulin [10]. Visfatin is also known as B-cell colony-enhancing factor (PBEF) and Nicotinamide phosphor ribosyltransferase (Nampt), was first identified by Fukuhara and his colleagues [11]. The gene encoding visfatin is located on the long arm of chromosome 7 between 7q22.1 and 7q31.33 [12] and encodes a polypeptide of 491 amino acids with a molecular mass of 52 kDa [8]. Interestingly and strikingly, as its name visfatin implies, it mainly expressed in and secreted from visceral fat as opposed to subcutaneous fat [11]. Visfatin was found to be released predominantly from macrophages rather than from adipocytes in visceral adipose tissue [13]. In addition to be produced in human leukocytes and adipose tissue, visfatin is also expressed in human and animal hepatocytes and muscles [14].

In mammals, Nampt has two different forms: intracellular and extracellular Nampt (iNampt and eNampt, respectively) [15, 16].

It is now believed that visfatin actions can be endocrine, paracrine, and autocrine as well. These autocrine effects of visfatin may play an important role in regulating insulin sensitivity in the liver [17]. At least three specific functional properties have been suggested for visfatin: (i) as an enzyme in the catalysis of the rate-limiting step in the production of NAD from nicotinamide [18] (ii) as a novel insulin-mimetic fat-secreted factor [11] and (iii) as a regulatory factor in pro-inflammatory and immunomodulating processes [19]. In this context, visfatin might play a role in glucose homeostasis, not through a direct activation of the insulin receptor, as initially suggested by ***Fukuhara et al***, but through an indirect mechanism that facilitates insulin secretion [20].

Association between visfatin and development of vascular inflammation induced by obesity and type II DM, indeed, it has been shown that visfatin induces the adhesion of leukocytes to endothelial cells and aortic endothelium by activating intercellular adhesion molecule $(ICAM)^{-1}$ and vascular cell adhesion molecule $(VCAM)^{-1}$. This phenomenon appears to be mediated through the pro-inflammatory transcription factor nuclear factor-KB9 NF-kB) in a reactive oxygen species (ROS) dependent manner. This same study has also shown that visfatin significantly increases the transcriptional activity of NF-kB in human vascular endothelial cells, resulting in the activation of the matrix-metalloproteinases $(MMP)^{-2/9}$. These findings provide supportive evidence for a potential role of eNampt in the pathogenesis of vascular inflammation in obesity and type II DM [21].

Moreover visfatin was shown also to activate endothelial nitric oxide synthase (eNOS) via activated protein kinase and mitogen-activated protein (MAP) kinase and monocyte chemotactic protein-1(MCP-1) and improve endothelial cell function, angiogenesis, and atherosclerosis [22].

Several studies have demonstrated that peripheral insulin resistance plays an important role in the development of endothelial dysfunction and cardiovascular disease. In addition, impaired nitric oxide (NO) bioavailability may also be responsible for the vascular complications noted in T2DM. At this point, asymmetric dimethylarginine (ADMA), the major endogenous inhibitor of NO synthase (NOS), may be suggested as a causative factor for endothelial dysfunction. Increased plasma levels of ADMA are associated with several clinical conditions involving endothelial dysfunction, including hypertension, hypercholesterolemia, diabetes mellitus and cardiovascular disease. Hyperglycemia and poor glycemic control may be the underlying mechanism of endothelial dysfunction in T2DM. Hyperglycemia impairs dimethylarginine dimethyl amino hydrolase (DDAH) and causes the accumulation of ADMA, resulting in disturbances in NO pathways in vessels [23].

Nampt itself or any component in Nampt-mediated systemic NAD biosynthesis could be an effective therapeutic target/reagent for the prevention and the treatment of metabolic disorders including obesity and type II DM, inflammation, and cancer. Because downstream regulators, such as sirtuins and poly ADPribose polymerase (PARPs), have pleiotropic functions, more rigorous investigations will be necessary to clarify possible benefits from the manipulation of Nampt-mediated NAD biosynthesis [14].

2. Subjects and Methods

This study was carried out on sixty patients with type II diabetes mellitus attending the clinic of endocrinology departments in Al -Zahraa University Hospital. They are classified into two groups according to the presence of complications or not.

Patients of group II included 15 patients have no any apparent complications of diabetes mellitustype II and their ages ranged from 40 -65 years old.

Group III included 45 Patients with complicated diabetes mellitus, their ages ranged from 45 -72 years old and divided equally into three subgroups (A, B, C) as following: with cardiovascular complications, with diabetic nephropathy and with diabetic foot respectively.

In addition, Group I fifteen clinically healthy subjects were included in the study as a control group with their ages ranged from 42-66 years old. For subjects of all groups oral consent and history was taken (with a special emphasis on age, symptoms of diabetes mellitus). Also full clinical examination was done including neurological and fundus examination taking in consideration exclusion of cases complaining from other diseases. Special attention was paid to the body mass index.

Routine laboratory investigations including fasting and post prandial serum glucose level according to Spin react kit [24].

3. Determination of Lipid Profile

*Determination of cholesterol according to Spin react kit [25]

*Determination of serum triglyceride

Enzymatic calorimetric method according to Diamond kit [26].

*Estimation of serum high density lipoprotein (HDL)

Phosphotungstic precipitation method according to Spin react kit [25]

*Estimation of serum low density liopoprptein.

According to the Friedewald Formula:-

$$(\text{LDL}) = \text{Total cholesterol} - \frac{\text{triglycerides}}{5} - (\text{HDL})$$

Serum creatinine, and complete urine analysis

4. Specific Investigations

- Estimation of serum visfatin by ELISA method. (Glory Science Co., Ltd, USA).
- Estimation of serum nitrite as a surrogate to nitric oxide by spectrophotometry serum nitric oxide levels [27].

Sample preparation: from each case, 10 ml of blood were withdrawn from antecubital vein after 10 -12h overnight fast. Two ml of blood were added to a tube containing EDTA. The tube was shacked gently to mix the contents which were then subjected to microscopic examination for complete blood picture.

Eight ml of blood were left to clot at room temperature. The samples were then centrifuged at 3000 rpm for 10 minutes. Some of the separated sera were used fresh for measurement of serum fasting glucose, urea, creatinine, lipid profile and nitrite levels .The remaining sera was stored at -80°C until used for visfatin assay for a period not exceeding 6 months.

Another two ml blood was withdrawn 2 hours after light meal, left to clot at room temperature. The samples were then centrifuged at 3000 rpm for 10 minutes and used for measurement of 2 hours postprandial serum glucose level.

- Data was analyzed by Microsoft Office 2003 (excel) and Statistical Package for Social Science (SPSS) version 16.

5. Results

Tables (1 to 6) conclude the statistical data of laboratory results.

Table (1). Statistical data of laboratory results in the diabetic patients compared to control group

	Group (2)	Group (3)			Group (1)
		A	B	C	
FBS (mg/dl)	120.33±41.63 P<0.05	153.07±55.40 P<0.05	158.60±38.60 P<0.05	176.73±71.37 P<0.05	82.78±13.88
PP (mg/dl)	181.07±53.20 P<0.05	257.20±83.23 P<0.05	284.93±132.43 P<0.05	263.33±65.69 P<0.05	110.33±10.45
ESR	28.33±9.85 P<0.05	70.47±41.26 P<0.05	72.20±34.74 P<0.05	89.60±26.47 P<0.05	11.13±4.79
Creatinine	1.03±0.32 P<0.05	1.27±0.73 P<0.05	5.05±3.39 P<0.05	1.90±2.19 P<0.05	0.57±0.22
HDL (mg/dl)	35.78±6.82 P<0.05	32.13±4.29 P<0.05	32.75±8.75 P<0.05	34.13±7.75 P<0.05	56.49±8.09
LDL (mg/dl)	126.22±14.17 P>0.05	125.35±18.06 P>0.05	150.08±37.59 P>0.05	128.73±33.82 P>0.05	123.66±23.09
Cholesterol	224.47±78.88 P>0.05	204.00±38.45 P>0.05	182.27±49.02 P>0.05	216.60±56.99 P>0.05	199.37±31.16
Trig (mg/dl)	156.07±55.89 P<0.05	143.93±10.19 P<0.05	172.33±101.10 P>0.05	120.40±30.54 P>0.05	118.53±41.61

Group (1): Control
Group (2): Uncomplicated
Group (3A): Cardiovascular complications
Group (3B): Diabetic nephropthy
Group (3C): Diabetic foot
FBS: Fasting blood sugar
PP: Postprandial blood sugar
HDL: High density lipoprotein
LDL: Low density lipoprotein,
Trig: Triglycerides
ESR: erythrocyte sedimentation rate
P>0.05: Non significant P<0.05: significant

Table (2). Statistical data of serum visfatin levels in the studied groups

	Group (2)	Group (3)			Group (1)
		A	B	C	
Visfatin(ng/ml)	29.02±9.22	20.82±9.73	22.79±12.61	20.49±6.41	14.58±2.78

Group (1): Control
Group (2): Uncomplicated
Group (3A): Cardiovascular complications
Group (3B): Diabetic nephropthy
Group (3C): Diabetic foot

Table (3). Statistical data of serum nitrite levels in the studied groups

	Group (2)	Group (3)			Group (1)
		A	B	C	
Nitrite (µmol/1)	5.75±2.26	8.28±3.54	5.98±3.62	13.26±3.31	15.65±8.58

Table (4). Correlation of serum visfatin with nitrite in diabetic groups

	Uncomplicated	Cardiovascular complications	Diabetic nephropathy	Diabetic foot
0.115 r	0.115	-0.253	0.234	0.184
P	> 0.05	> 0.05	> 0.05	> 0.05

Table (5). Correlation of serum visfatin with laboratory data in diabetic groups

	Uncomplicated	Cardiovascular complications	Diabetic nephropathy	Diabetic foot
F.B.S	0.472 P>0.05	0.183 P>0.05	-0.292 P>0.05	-0.180 P>0.05
P.P	0.138 P>0.05	-0.001 P>0.05	-0.157 P>0.05	-0.061 P>0.05
ESR	-0.710 P<0.05:	0.191 P>0.05	-0.118 P>0.05	0.610 P<0.05:
Cholesterol	-0.088 P>0.05	-0.215 P>0.05	0.099 P>0.05	0.112 P>0.05
LDL	0.141 P>0.05	-0.312 P>0.05	0.318 P>0.05	-0.178 P>0.05
Trig	0.279 P>0.05	0.174 P>0.05	0.163 P>0.05	0.127 P>0.05
HDL	-0.022 P>0.05	-0.724 P<0.05:	0.363 P>0.05	-0.510 P>0.05
Creatinine	-0.080 P>0.05	0.258 P>0.05	-0.390 P>0.05	0.103 P>0.05

P>0.05: Non significant P<0.05: significant

Sensitivity and specificity: Receiving operating characteristic Curve (ROC curve) was done to detect the best cut off value of serum visfatin in diabetic patient which was 9.55ng/ml with 93% sensitivity and 100% and specificity (Table 6 & Figure 1).

Table (6). Sensitivity and specificity of serum visfatin in prediction of diabetes mellitus II

	Cut off value	Sensitivity	Specificity	PPV	NPV
Visfatin	9.55 ng/ml	93%	100%	100	86

PPV: Positive predictive value NPV: Negative predictive value

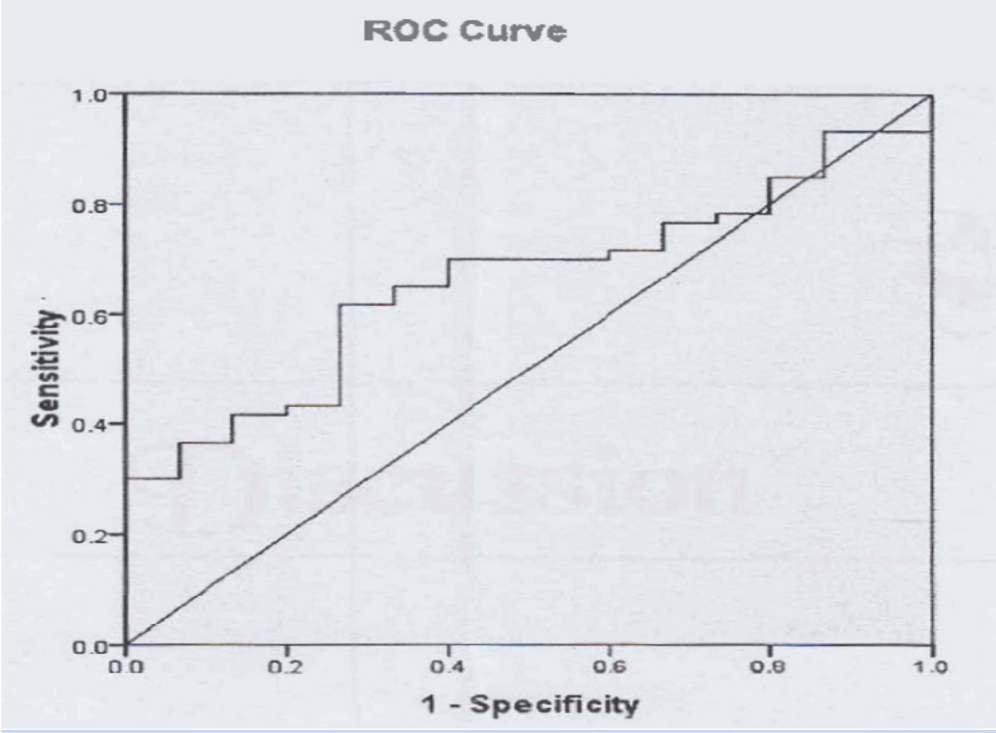


Figure (1). Receiving operation characteristic Curve (ROC curve)

6. Discussion

Type II diabetes mellitus accounts for 90-95% of those with diabetes, previously referred to as non-insulin dependent diabetes or adult-onset diabetes encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency [28].

It is clear that sub clinical inflammation is a key factor that triggers type II diabetes mellitus and is directly influenced by weight gain. Current studies highlight that obesity, particularly central obesity, heightens the pathogenesis of type II DM. additionally, factors produced by adipose tissue, referred to as adipocytokines, can influence the degree of insulin the degree of insulin resistance as well as inflammation, due to their duality of function [29]. Adipokines have the potential to predict individual treatment success and disease progression, to monitor clinical responses to therapeutic and lifestyle interventions, to early identify non-responders to specific interventions, or to monitor treatment adherence [30].

Visfatin is a hormone acts synergistically with insulin in increasing glucose cellular uptake, stimulating glucose transfer to the muscle and adipose tissue, as well as preventing from hepatic glucose production and its anti-diabetic insulin effect through insulin mimetic is documented. Visfatin does its insulin-like effects through direct connection and activation of insulin receptors without any change or competition with the insulin. Many clinical studies demonstrated the direct association of higher circulating levels of Visfatin with body fat percentage, type II diabetes and obesity [10]. Visfatin, also known as per-B cell colony-enhancing factor (PBEF), is highly conserved protein found in living species form bacteria to humans. It is also called NAMPT because of its significant sequence and functional homology with nicotinamide phosphor ribosy1 transferase, an enzyme involved in nicotinamide adenine dinucleotied biosynthesis form nicotinamide [31]. Visfatin is the rate limiting enzyme in NAD biosynthetic and thereby to play an important role in the regulation of cellular metabolism and insulin secretion [32].

Visfatin relevance in immune, metabolic and stress responses depends on both extra cellular (cytokine-like) and intracellular (enzymatic) functions. Visfatin was initially reported to mimic the effects of insulin by binding to the insulin receptor (IR) and by stimulating the phosphorylation of insulin-receptor substrate-1 (IRS-1) and IRS-2 and the downstream tyrosine protein kinsase B and mitogen activated protein kinase (MAPK). However, the original work describing visfatin binding to IR has been retracted. Therefore, IR is not considered as a visfatin receptor anymore, although there is evidence that regulation of insulin signaling pathways at the level of IRSs, tyrosine protein kinsase, and MAPK is implicated in producting the pathophysiological effects of visfatin [33]. Clinical studies have provided controversial findings concerning the role of visfatin in obesity and glucose metabolism. With positive, negative or no associations found [32].

The present study was designed to investigate serum visfatin in uncomplicated type II diabetes mellitus and those with diabetic complications including cardiovascular, diabetic nephropathy and diabetic foot complications.

The results of this study showed that serum visfatin level was significantly increased ($P< 0.05$) in uncomplicated DM patients compared to healthy control subjects (Table 2).

Clinical and laboratory results including BMI, FBS, PP, triglycerides, ESR and creatinine were significantly increased while LDL and cholesterol showed non-significant increase & HDL showed significant decrease (P<0.05) in uncomplicated DM patients as compared to healthy control subjects(Table 1).

There was non-significant correlation between visfatin and clinical and laboratory parameters including FBS, PP, ESR, creatinine, triglycerides, LDL, HDL and cholesterol. (Table 5) By measuring the cutoff value of visfatin was 9.55ng/ml. (Table 6). These results are in accordance with the results of ***Uslu et al***., who found increased visfatin levels in type II DM patients, in addition, visfatin levels were positively correlated with HOMA-IR, L/A ratio and insulin levels in type II DM patients [23]. Also ***Chen et al.,*** showed elevation of plasma levels of visfatin in diabetic patients compared to control group even after controlling for anthropometric variables and its elevation may suggest impairment of visfatin signaling in targets tissues or the deregulation in biosynthesis or in response to hyperglycemia, hyperinsulinemia, or adipocytokines [34]. Another study was performed and reported that plasma visfatin was higher in diabetic patients as compared to control group and did not correlate with BMI, glucose and lipid levels and that result indicate hyperglycemia cause increase in plasmvisfatin levels [35]. Also ***Hammarstedt et al.,*** reported increased visfatin level in obese type II diabetics in comparison to control group [36].

El-Masallamy et al., provided several explanations for the increased visfatin levels noted in patients with type II DM. firstly, increased visfatin levels in diabetic patients may be due to impaired visfatin signaling in target tissues. Secondly, due to the insulin mimetic effects, increased plasma visfatin levels could be a compensatory mechanism in response to hyperglycemia that ameliorates the functional consequences of insulin deficiency or resistance, thirdly, visfatin mediated NAD biosynthesis that regulates glucose-stimulated insulin secretion may explain increased levels of visfatin in type II DM patients as a compensatory mechanism of B-cell functioning. Finally, because of the pro-inflammatory properties, these elevated levels could be attributed to the chronic low-grade inflammation present in type II DM [37].

However, other study demonstrated that visfatin levels were significantly decreased in diabetic patients compared to control subjects [38]. While other authors**,** demonstrated that no association between plasma visfatin and diabetes [39]. Also ***Berndt et al.,*** found no relationship between plasma visfatin and insulin sensitivity or insulin itself [40].

As regard correlation with lipid profile previous studies showed conflicting results as some researchers reported no significant association to lipid profile [34] and others reported no significant association between visfatin and HDL [41].

Moreover, ***Kaminska et al.,*** revealed non-significant correlation between visfatin and cholesterol, triglycerides, DHL and LDL [42]. However other study found that visfatin levels were positively correlated with markers of lipid metabolism such as cholesterol, triglycerides and LDL [23]. The relationship of visfatin with lipid profile might be a compensatory mechanism for diabetic dyslipidemia, since visfatin up regulates peroxisome proliferators-activated receptor y activity [37]. ***Sandeep et al.,*** showed that the visfatin secretion from adipocytes depends on the duration and extent of hyperglycemia [43]. Accordingly, reported that high visfatin levels were positively related to glycemic control. Therefore, higher glucose and HbA1C levels may contribute to, at least partly, the increased visfatin levels found in type II DM patients [44]. The conflicting results of visfatin assay may be due, in part, to the types of populations studied, small sample size, and/or variability in the types of assays used to measure serum plasma visfatin [45]. Also, in this study there was a significant increase in serum visfatin in complicated subgroups compared to control group (Table 2).

As regard cardiovascular complications the results of the current study showed that serum visfatin level was significantly increased (P<0.05) as compared to healthy control subjects (Table 2). Also clinical and laboratory results including FBS, PP, triglyceride, ESR and creatinine were significantly increased, however HDL showed significant decrease (P<0.05), & LDL and cholesterol showed non-significant increase (P>0.05) in patients with cardiovascular complications compared to healthy control subjects (Table 1).

There was non-significant correlation between visfatin and clinical and laboratory parameters including FBS, PP, cholesterol, triglycerides, ESR, creatinine, LDL, however between visfatin and HDL there was a significant negative correlation (r=-0.724 & P<0.01) (Table 5). These results go in accordance with the results of ***Dahl et al.,*** who found increased plasma visfatin levels in patients with carotid atherosclearosis and the metabolic syndrome, and were independently associated with the maximum carotid intima media thickness (CIMT), impairment of the nitric oxide-dependent endothelial function, could confer as a potential mechanism to explain the dysfunction of vascular endothelium induced by this adipokine. A potential proinflammatory activity has also been described to visfatin [46]. Other study showed high level of visfatin significantly associated with advanced carotid atherosclearosis in patients with type II DM [47]. In addition, ***Liu et al.,*** showed that plasma visfatin levels were significantly associated with coronary artery disease [48].

Visfatin-provoked increase in markers of vascular oxidative stress, such as the production of reactive oxygen species, which are known to associate with visfatin may increase inflammatory cell adhesion to the aortic endothelium by an adipokine – provoked induction of several cell adhesion to the aortic endothelium by an adipokine-provoked induction of several cell adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1) [49]. On the whole, visfatin may be regarded as an inflammatory mediator, localized to foam cell macrophages within unstable atherosclerotic lesions and may potentially play a role in the destabilization of

atherosclerotic plaques and subsequently the occurrence of cardiovascular events [46]. On the other hand, ***lim et al.,*** suggested a cardiac protective effect of visfatin in an animal model of non-athereosclerotic ischemia [50].

Another study, found that visfatin activate endothelial nitric oxide synthase (eNOS) via activated protein kinase and mitogen-activated protein (MAP) kinase and monocyte chemotactic protein-1 (MCP-1) and improve endothelial cell function, angiogenesis, and atherosclerosis [22].

As regard diabetic nephropathy the results of the current study showed that serum level of visfatin was significantly increased (P<0.05)compared to healthy control subjects (Table 2) were also clinical and laboratory results including FBS, PP, ESR and creatinine were significantly increased (P<0.05), while HDL showed significant decrease & LDL, triglyceride and cholesterol showed non-significant increase (P<0.05) in patients with diabetic nephropathy as compared to healthy control subjects (Table 1).

There was non-significant correlation between visfatin and clinical and laboratory parameters including FBS, PP, triglycerides, ESR, creatinine, cholesterol, HDL, and LDL (Table 5). These results were in agreement with that of ***Yilmaz et al.,*** who suggested that the endothelial dysfunction in early diabetic nephropathy is associated with altered circulating levels of visfatin [51]. Later in 2009, ***Yilmaz and colleagues*** observed that decrease in visfatin level in patients after renal transplantation and associated with improvement in endothelial function [52]. ***Song et al.,*** found that visfatin was synthesized in renal glumerularmeasangial cells, up regulated by high glucose stimuli, in addition, exogenous visfatin stimulation in renal cells up regulated the synthesis of profibrotic molecules, including transforming growth factor (TGF)- B_1, plasminogen activator inhibitor (PAI)-1, and type I collagen, given these findings, the pathophysiologic relevance of visfatin seems to be as a proinflammatoryadipokine regarding type II diabetes and other metabolic complications [53].

Thus the fibrotic buildup observed by ***song et al.,*** and possibility of reactive oxygen species via its activity as a nicotinmaide phosphoribosy-1 transperase (Nampt) strongly supports the concept that visfatin could be one of the cytokines responsible of renal damage in diabetic nephropathy [53].

Other researchersconcluded that, visfatin might play an important role in the progression and/or the associated complications, especially inflammatory complications, of obesity and type II DM [21]. In addition, ***Kang et al.,*** observed that plasma visfatin levels were elevated in patients and animals with type II diabetic nephropathy [54].

Regarding diabetic foot the results of the current study showed that serum level of visfatin was significantly increased (P<0.05) as compared to healthy control subjects (Table 2) also clinical and laboratory results including FBS, PP, ESR and creatinine were significantly increased while HDL showed significant decrease (P<0.5) & LDL, triglyceride and cholesterol showed non-significant increase (P>0.05) in patients with diabetic foot as compared to healthy control subjects (Table 1). There was non-significant correlation between visfatin and all the parameters except a significant positive correlation between visfain and ESR (r=0.610&P<0.05) (Table 5). ***Jia et al. (2004)*** reported that it functioned as an inhibitor of apoptosis resulting from a variety of inflammatory stimuli [55]. Other study, showed that there was a link between visfatin and inflammatory cytokine secretion by leukocytes [56].

Visfatin might confer to cells of the immune system, the ability to survive during stressful situations such as inflammation. Moreover, visfatin enhanced the surface expression of the co-stimulatory molecules important for the activation of T cells Such as CD_{54} ($ICAM^{-1}$), CD_{40} and CD80 in monocytes, and was able to act as a potent chemotactic factor for CD^{14+} monocytes and CD^{19+} B cells [57].

Moreover, ***Cekmez et al.,*** had shown that resistin and visfatin levels in sepsis correlate positively with CRP, procalcitonin and IL-6 levels. Also suggested that visfatin and resistin could be used as acute phase reactants similarly to CRP and procalcitonin in the diagnosis of neonatal sepsis [58].

The results of this study showed that serum visfatin levels in complicated groups of type II DM was lower than of uncomplicated group where serum visfatin levels were significantly decreased (P<0.05) in patients with cardiovascular complications and diabetic foot compared to uncomplicated group also there was non-significant decrease (P>0.05) in serum visfatin in diabetic nephropathy compared to uncomplicated group. In addition there was non-significant difference (P>0.05) in serum visfatin levels between complicated studied sub groups (Table 2).

Zhu et al., observed that visfatin concentrations is reduced after 3 months of intensive glycemic control, that change of visfatin concentration may be a compensatory mechanism to ameliorate insulin deficiency due to pancreatic-B cell dysfunction [59]. In contrast, other observed that there was increased circulating visfatin concentration in states of hyperglycemia and type II DM, which reduced with insulin treatment [29]. Discrepancy between visfatin level and insulin resistance, lipid profile in different studies can be explained by; criteria for recruitment were different in various studies regarding age, sex, lifestyle, duration of diabetes mellitus, and degree of obesity and ethnic heterogeneity may affect visfatin level or visatin sensitivity [41] in addition to genetic background with different genetic single nucleotide polymorphisms in the visfatin gene and metabolic parameters [60] Plus confounding factor that circulating visfatin is increased with progressive B-cell dysfunction [61].

Studies have shown that the synthesis and secretion of visfatin is modulated by glucocorticoides, TNFa, IL-6, and growth factors. This means that visfatin is upregulated in the course of pro-inflammatory cytokines and under inflammatory conditions [31]. Also the findings of ***McGee et al.,*** have suggested that visfatin may represent a pro-inflammatory cytokine that is influenced by insulin/insulin sensitivity [29]. ***Chang et al.,*** suggested that

the use of visfatin may be promising for predicting obesity, diabetes status, insulin resistance, metabolic syndrome and cardiovascular disease [62].

In our study there was a significant decrease ($P<0.05$) in serum nitrite levels in type II DM uncomplicated, cardiovascular complications and diabetic nephropathy groups, but non-significant decrease ($P>0.05$) in diabetic foot group compared to control group (Table 3). Comparing of serum nitrite levels in uncomplicated and complicated type II DM groups there was a significant increase ($P<0.05$) in serum nitrite levels in cardiovascular complications and diabetic foot compared to uncomplicated group, no-significant increase ($P>0.05$) in serum nitrite levels in diabetic nephropathy group compared to uncomplicated group (Table 3). As regard to serum nitrite levels in complicated subgroups there was a significant decrease ($P<0.05$), in serum nitrite in cardiovascular complications and diabetic nephropathy compared to diabetic foot group non-significant increase ($P>0.05$) in serum nitrite in cardiovascular complications compared to diabetic nephropathy group (Table 3).

These results are in accordance with the results of ***Ghosh et al.,*** stated that serum NO was significantly low in diabetic participants as compared who to control [63]. Also other study results showed a significant decrease in the nitrite levels among diabetic patients when compared with healthy controls. They concluded that serum nitrite levels might as well be considered a predictor of onset of vascular complication in type II DM [64]. In addition, ***Dhananjayan et al.,*** observed that HDL-cholesterol and serum nitrite levels were significantly decreased in type II DM patients, as compared to those of healthy controls [65].

It was established that reduction in NO bioavailability is a predictor of dyslipidemia as it is an endogernous anti-atherosclerotic molecule [66].

Several explanations for the decreased serum nitrite levels noted in patients with type II DM. Firstly, nitric oxide synthase (NOS) from which NO is derived is a pH dependent enzyme. It is active at slightly alkaline (basic) conditions but is suppressed by acidotic conditions. In diabetes, glycolysis and ketoacidosis force pH toward acid conditions and this may account, in part, for the reduced production of NO. Secondly, adequate Oxygen is necessary for the activity of NOC and therefore NO circulation is notoriously impaired in diabetic patients, which limits available NOS and NO. Thirdly, people with diabetes often experience elevated glucose levels. Some of this glucose becomes incorporated into hemoglobin and is measured as glycosylated hemoglobin (Hgb) or HgbA1C. glycosylated hemoglobin binds NO in the form of nitrosothiols very tightly so that any NO that is formed cannot be easily released from RBC to help maintain blood flow through smooth muscle cell relaxation. Finally, in diabetes mellitus where there is increased oxidative stress there will be decreased nitric oxide synthesis leading to endothelial dysfunction. Hence the decreased amount of nitric oxide will give rise to less amount of nitrate and nitrate [64].

However, a Japanese study showed that plasma NO levels were significantly higher in diabetics than in controls, when measured by high-performance liquid chromatography with the Griess method [67]. In a Karachi study, a non-significant increase was observed in the levels of nitric oxide metabolites in diabetic patients as compared to non-diabetics, but diabetic patients with hypertension showed significantly higher levels as compared to controls, but the levels were not significantly different in patients with and without hypertension [68].

7. Conclusions and Recommendations

The results of the present study demonstrated that increased serum visfatin levels and decreased serum nitrite levels in uncomplicated type II diabetes mellitus and those with diabetic complications including cardiovascular, diabetic nephropathy and diabetic foot complications, clearly suggesting that they may be have a role on diabetes pathophysiology. Serum visfatin levels reduced with insulin treatment in complicated type II diabetes mellitus patients.

Visfatin is an adipokine involved in inflammatory phenomena, atherosclerosis, and possibly in insulin secretion. It may be an important link between increased fat mass, insulin resistance, disorders of lipid and glucose metabolism and endothelial dysfunction in diabetic patients. Its physiology has begun to be described but its role on diabetic complications pathophysiology is still controversial.

Further studies with larger sample size are needed to elucidate the physiological relevance of visfatin function in normal individual and patients with metabolic and other disease in humans since biological functions of visfatin had been conducted in cell culture and mouse models and the studies on possible correlation between human visfatin levels and metabolic parameters had been contradictory.

REFERENCES

[1] American Diabetes Association (2012): Diagnosis and Classification of Diabetes Mellitus. Diabetes Care; 35: S64-S71.

[2] Powers AC (2001): Harrison's Text book of internal Medicine. 15thed.New York, McGraw-Hill; 2109-2137.

[3] National Diabetes data Group (1995): Diabetes in America, 2nd. Bethesda (MD): National institute of health. NIH Publication; No 95-1468.

[4] Gómez R, Conde J, Scotece M, Gómez-Reino JJ, Lago F and Gualillo O (2011): What's new in our understanding of the role of adipokines in rheumatic diseases?Nat Rev Rheumatol; 2;7(9):528-36.

[5] Bloomgarden ZT (2002): Adiposity and diabetes. Diabetes Care; 25:2342–2349.

[6] Arner P (2005): Insulin resistance in type 2 diabetes—role of the adipokines. CurrMol Med; 5:333-339.

[7] Fantuzzi G (2005): Adipose tissue, adipokines, and inflammation. J Allergy ClinImmunol; 115: 911–919.

[8] Saddi-Rosa P, Oliveira C, Giuffrida F and Reis AF (2010): Visfatin, glucose metabolism and vascular disease: a review of evidence Diabetology&metabolic Syndrome; 2:21.

[9] Coelho M, OliveiraT and Fernandes R (2013): Biochemistry of adipose tissue: an endocrine organ. Arch Med Sci; 9(2): 191-200.

[10] Hajianfar H, Bahonar A, Entezari M H, Askari GH, and Yazdani M (2012): Lipid Profiles and Serum Visfatin Concentrations in Patients with Type II Diabetes in Comparison with Healthy Controls Int J Prev Med. 3(5): 326–331.

[11] Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T et al (2005): Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science; 307(5708): 426-430.

[12] Jia SH, Li Y, Parodo J, Kapus A, Fan LZ, Rotstein OD and Marshall JC (2004): Pre-B cell colony- enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest; 113: 1318-1327.

[13] Varma V, Yao-Borengasser A, Rasouli N, Bodles A, Phanavanh B, Lee M, Starks T, Kern L, Fried S and Kern P (2007): Human visfatin expression: relationship to insulin sensitivity, intramyocellular lipids, and inflammation. J ClinEndocrinolMetab; 92: 666–672.

[14] Garten A, Petzold S, Barnikol-Oettler A, Körner A, Thasler WE, Kratzsch J, Kiess W and Gebhardt R (2010): Nicotinamidephosphoribosyltransferase (NAMPT/ PBEF/ visfatin) is constitutively released from human hepatocytes. BiochemBiophys Res Commun; 391(1):376–81.

[15] Revollo JR, Grimm AA and Imai S (2004): The NAD biosynthesis pathway mediated by nicotinamidephosphoribo syltransferase regulates Sir 2 activity in mammalian cells. J BiolChem; 279: 50754-63.

[16] Revollo JR, Korner A, Mills KF, Satoh A, Wang T, Garten A, Dasgupta B, Sasaki Y and Wolberger C et al. (2007): Nampt/PBEF/Visfatin regulates insulin secretion in beta cells as a systemic NAD biosynthetic enzyme. Cell Metab; 6(5):363-375.

[17] Wang, T, Zhang, X, Bheda, P, Revollo, J R, Imai, S and Wolberger, C (2006): Structure of Nampt/PBEF/visfatin, amammalian NAD+ biosynthetic enzyme. Nat. Struct. Mol. Biol; 13, 661–662.

[18] Skop V, Kontrová K, Zídek V, Sajdok J, Pravenee M, Kazdova L, Mikulik K and Zidkova J (2010): Autocrine effects of visfatin on hepatocyte sensitivity to insulin action. Physiol Res; 59:615-618.

[19] Moschen AR, Kaser A, Enrich B, Mosheimer B, Theurl M, Niederegger H and Tilg H (2007): Visfatin, an adipocytokine with proinflammatory and immunomodulating properties. J Immunol; 178: 1748-1758.

[20] Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, Matsuki Y, Murakami M, Ichisaka T et al. (2007): Retraction: Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science; 318:565.

[21] Adya R, Tan BK, Chen J and Randeva HS (2008): Nuclear factor-B induction by visfatin in human vascular endothelial cells: its role in MMP-2/9 production and activation. Diabetes Care; 31(4):758-760.

[22] Lovren F, Pan Y, Shukla PC, Quan A, Teoh H, Szmitko PE, Peterson MD, Gupta M, Al-Omran M and Verma S (2009): Visfatin activates eNOS via Akt and MAP kinases and improves endothelial cell function and angiogenesis in vitro and in vivo: translational implications for atherosclerosis. Am J PhysiolEndocrinolMetab; 296:E1440–E1449.

[23] Uslu S1, Kebapçi N, Kara M, Bal C. Relationship between adipocytokines and cardiovascular risk factors in patients with type 2 diabetes mellitus. ExpTher Med. 2012 Jul; 4(1): 113-120.

[24] Young, (2001). Young DS (2001): Effects of diseases on clinical lab.tests;4thed AACC

[25] Burtis CA and Ashwood ER (1999): Tietz textbook of clinical chemistry. 3rd ed. Philadelphia: W.B. Saunders.

[26] Fassati P. and Prencipe L. (1982): Serum triglycerides determined- colormrtrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28(10): 2077-2080.

[27] Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JK, Tannenbaum SR (1982): Analysis of nitrate, nitrite and 15N in biological fluids. Anal Biochem; 126, 131–136

[28] Micheal J and Fowler (2010): Diabetes: Magnnitude and Mechanisms clinical diabetes; 28(1): 42-46.

[29] McGee KC (2009): The role of visfatin in adipose tissue metabolism and metabolic disease. PhD thesis, University of Warwick. Official URL: http://webcat.warwick.ac.uk/record =b2279747~S1.

[30] Matthias Blüher (2014): Adipokines – removing road blocks to obesity and diabetes therapy MolMetab. 2014 Jan; 3(3): 230–240.

[31] AL-Suhaimi and Shehzad (2013): Leptin, resistin and visfatin: the missing link between endocrine metabolic disorders and immunity. European Journal of Medical Research 18:12.

[32] Friebe D, Neef M, Kratzsch S, Erbs S, DittrichK, Garten A, Petzold-Quinque S, Blüher S, Reinehr T, StumvollM, Blüher Met al. (2011): Leukocytes are a major source of circulating nicotinamidephosphoribosyl-transeferase(NAMPT)/per b cell colony (PBEF)/visfatin linking obesity and inflammation in humans. Diabetologia; 54(5):1200-1211.

[33] Jacques C, Holzenberger M, Mladenovic Z, Salvat C, Pecchi E, BerenbaumF and Gosset M (2012): Proinflammatory Actions of Visfatin/ NicotinamidePhosphoribosyltransferase (Nampt) Involve Regulation of Insulin Signaling Pathway and Nampt Enzymatic Activity. J BiolChem; 287(18): 15100–15108.

[34] Chen MP, Tsai JC, Chung FM, Yang SS, Hsing LL, Shin SJ and Lee YJ (2006): Elevated Plasma Level of Visfatin/Pre-B Cell Colony-Enhancing Factor in Patients with Type 2 Diabetes Mellitus. J clinic endocrinology and metabolism; 91(1): 295-299.

[35] Dogru T, Sonmez A, Tasci I, Bozoglu E, Yilmaz MI and Genc H (2007): Plasma visfatin levels in patients with newly

diagnosed and untreated type 2 diabetes mellitus and impaired glucose tolerance. Diabetes Res ClinPract; 76:24–9.

[36] Hammarstedt A, Pihlajamaki J, Rotter Sopasakis V, Gogg S, Jansson PA and Laakso M (2006):Visfatin is an adipokine, but it is not regulated by thiazolidinediones. J ClinEndocrinolMetab; 91: 1181–1184.

[37] El-Mesallamy HO, Kassem DH, El-Demerdash E and Amin A (2011): Vaspin and visfatin/Nampt are interesting interrelated adipokines playing a role in the pathogenesis of type 2 diabetes mellitus. Metabolism; 60: 63-70.

[38] Li L, Yang G, Li Q, Tang Y, Yang M, Yang H and Li K (2006): Changes and relations of circulating visfatin, apelin, and resistin levels in normal, impaired glucose tolerance, and type 2 diabetic subjects, ExpClinEndocrinol Diabetes; 114 : 544–548.

[39] Ingelsson E, Larson M, Yin X, Lipinska I (2007): Clinical Correlates of Circulating Visfatin Levels in a Community Based Sample. Diabetes Care; 30:1278–1280.

[40] Berndt J, Klöting N, Kralisch S Kovacs P, Fasshauer M, Schön MR, Stumvoll M and Blüher M (2005): Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. Diabetes 2005; 54: 2911–2916.

[41] Jin H, Jiang B, Tang J, Lu W, Wang W, Zhou L, Shang W, Li F, Ma Q, Yang Y et al. (2008): Serum visfatin concentrations in obese adolescents and its correlation with age and high-density lipoprotein cholesterol. Diabetes Research and Clinical Practice; 79 412–418.

[42] Kamińska A, KopczyńskaE,Bronisz A, Żmudzińska M, Bieliński M, Borkowska A, Tyrakowski T and Junik A (2010): An evaluation of visfatin levels in obese subjects. Pol J Endocrinol; 61 (2): 169–173.

[43] Sandeep S, Velmurugan K, Deepa R and Mohan V (2007): Serum visfatin in relation to visceral fat, obesity, and type 2 diabetes mellitus in Asian Indians. Metabolism; 56: 565-570.

[44] Shaker O, El-Shehaby A, Zakaria A, Mostafa N, Talaat S, Katsiki N and Mikhailidis DP (2011): Plasma visfatin and retinol binding protein-4 levels in patients with type 2 diabetes mellitus and their relationship to adiposity and fatty liver. ClinBiochem; 44: 1457-1463.

[45] Korner A, Garten A, Bluher M, Tauscher R, Kratzsch J and Kiess W (2007): Molecular characteristics of serum visfatin and differential detection by immunoassays. Journal of Clinical Endocrinology & Metabolism; 92: 4783–4791.

[46] Dahl TB, Yndestad A, Skjelland M, Oie E, Dahl A, Dahl A, Michelsen A, Damås JK, Tunheim SH, Ueland T et al. (2007): Increased expression of visfatin in macrophages of human unstable carotid and coronary atherosclerosis: possible role in inflammation and carotid and coronary atherosclerosis: possible role in inflammation and plaque destabilization. Circulation; 115: 972-80.

[47] Kadoglou NP, Sailer N, Moumtzouoglou A, Kapelouzou A, Tsanikidis H, Vitta I, Karkos C, Karayannacos PE, Gerasimidis T and Liapis CD (2010): Visfatin (nampt) and ghrelin as novel markers of carotid atherosclerosis in patients with type 2 diabetes. ExpClinEndocrinol plaque destabilization. Circulation; 115:972–80.

[48] Liu SW, Qiao SB, Yuan JS and Liu DQ (2009): Association of plasma visfatin levels with inflammation, atherosclerosis and acute coronary syndromes (ACS) in humans. ClinEndocrinol; 71:202-207.

[49] Kim SR, Bae YH, Bae SK, Choi KS, Yoon KH, Koo TH, Yun I, Kim KW, Kwon YG, Yoo MA et al (2008): Visfatin enhances ICAM-1 and VCAM-1 expression through ROS-dependent NF- activation in endothelial cells. BiochimBiophysActa; 1783:886–895.

[50] Lim SY, Davidson SM, Paramanathan AJ, Smith CC, Yellon DM, Hausenloy DJ (2008): The novel adipocytokinevisfatin exerts direct cardio protective effects. J Cell Mol Med; 12(4):1395-1403.

[51] Yilmaz MI, Saglam M, Carrero JJ, Qureshi AR, Caglar K, Eyileten T, Sonmez A, Cakir E and Yenicesu M (2008): Serum visfatin concentration and endothelial dysfunction in chronic kidney disease. Nephrol Dial Transplant; 23: 959-65.

[52] Yilmaz MI, Saglam M, Carrero JJ, Qureshi AR, Caglar K, Eyileten T, et al. (2009): Normalization of endothelial dysfunction following renal transplantation is accompanied by a reduction of circulating visfatin/NAMPT. Anovel marker of endothelial damage?Clin Transplant; 23: 241-8.

[53] Song HK, Lee MH, Kim BK, Park YG, Ko GJ, Kang YS, Han JY, Han SY, Han KH, Kim HK and Cha DR (2008): Visfatin: a new player in mesangial cell physiology and diabetic nephropathy. Am J Physiol Renal Physiol; 295: 1485-94.

[54] Kang YS, Song HK, Lee MH, Ko GJ and Cha DR (2010): Plasma concentration of visfatin is a new surrogate marker of systemic inflammation in type 2 diabetic patients. Diabetes Res ClinPract; 89:141–149.

[55] Jia SH, Li Y, Parodo J, Kapus A, Fan LZ, Rotstein OD and Marshall JC (2004): Pre-B cell colony- enhancing factor inhibits neutrophil apoptosis in experimental inflammation and clinical sepsis. J Clin Invest; 113: 1318-1327.

[56] Busso N, Karababa M, Nobile M, Rolaz A, Van Gool F, et al (2008): Pharmacological inhibition of nicotinamidephosphor ibosyltransferase/visfatin enzymatic activity identifies a new inflammatory pathway linked to NAD. PLoSONE;3: e2267.

[57] Rongvaux A, Shea RJ, Mulks MH, Gigot D, Urbain J, LeoO and Andris F (2002): Pre-B-cell colony-enhancing factor, whose expression is up-regulated in activated lymphocytes, is a nicotinamidephosphoribosyltransferase, a cytosolic enzyme involved in NAD biosynthesis. Eur J Immunol; 32: 3225–3234.

[58] Cekmez F, Canpolat FE, Cetinkaya M, Aydinöz S, Aydemir G, Karademir F, Ipcioglu OM, Sarici SÜ (2011): Diagnostic value of resistin and visfatin, in comparison with C-reactive protein, procalcitonin and interleukin-6 in neonatal sepsis. Eur. Cytokine Netw; 22(2): 113-7.

[59] Zhu J, Schott M, Liu R, Liu C et al (2008): Intensive glycemic control lowers plasma visfatin levels in patients with type 2 diabetes.

[60] Johansson LM, Johansson LE and Ridderstråle M (2008): The visfatin (PBEF1) G-948T gene polymorphism is associated with increased high-density lipoprotein cholesterol in obese subjects. Metabolism; 57:1550-1562.

[61] Lopez-Bermejo A, Chico-Julia B, Fernandez-Balsells M, Recasens M, Esteve E, Casamitjana R, Ricart W and

Fernandez-Real JM (2006): Serum visfatin increases with progressive beta-cell deterioration. Diabetes; 55(10): 2871-2875.

[62] Chang YH, Chang DM, Lin KC, Shin SJ and Lee YJ (2011): 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; 27: 515-527.

[63] Ghosh A, Sherpa ML, Bhutia Y, Pal R and Dahal S (2011): Serum nitric oxide status in patients with type 2 diabetes mellitus in Sikkim. Int J App Basic Med Res; 1:31-35.

[64] Rani A J, Mythili S V & Nagarajan S (2012): Serum nitrite levels in relation to malondialdehyde in type 2 diabetes mellitus: a pilot study. Recent Research in Science and Technology; 4 (6): 2076-506.

[65] Dhananjayan R, Malati T, Brindha G & Kutala V K (2013): Association of family history of type 2 diabetes mellitus with markers of endothelial dysfunction in South Indian population. IJBB; 50(2): 93-98.

[66] Boger RH (2007): The Pharmacodynamics of L-Arginine. Nutr; 137: 1650S–1655S.

[67] Izumi N, Nagaoka T, Mori F, Sato E, Takahashi A and Yoshida A (2006): Relation Between Plasma Nitric Oxide Levels and Diabetic Retinopathy. Jpn J Ophthalmol; 50: 465-8.

[68] Khan MA (2011): Nitric Oxide and Lipid Peroxidation Status in Diabetes Mellitus and Hypertension. PhD thesis, University of Karachi, Karachi. ID Code: 1134.Coded from Ghosh et al., 2011.