



# ADIPOCYTOKINE VISFATIN - A LINK BETWEEN DIABETES MELLITUS AND PERIODONTITIS - A REVIEW

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## ABSTRACT

Visfatin is a newly discovered member of adipokine family. It is a visceral adipokine, it has been found in skeletal muscle, liver, bone marrow and lymph. It was originally identified as a cytokine-like secreted protein that synergizes the effect of IL-7 and stem cell factor in promoting the growth and differentiation of B-cell lineage precursors. It is expressed in lymphocytes of peripheral blood and plays a role in lymphocytes maturation and inhibition of neutrophil apoptosis.

It is an endocrine, autocrine as well as paracrine protein with many functions including enhancement of cell proliferation, biosynthesis of nicotinamide mono- and dinucleotide and induction of hypoglycemia. Visfatin has been shown to exert insulin mimetic and pro-inflammatory effects,

One of the fundamental functions of visfatin is the modulation of immune and inflammatory processes. It has been reported that visfatin activated nuclear factor-kappa B which has a crucial role in regulating immune responses. Up-regulation of visfatin has been also identified in a variety of pathophysiological conditions of the immune system including rheumatoid arthritis, psoriasis, clinical sepsis and acute lung injury.

Recently, there has been mounting body of studies investigating the association of visfatin with periodontal disease. This article gives an insight about visfatin and its correlation with periodontal disease.

**Key Words:** Visfatin, Periodontal Disease, Diabetes mellitus, Immunity

## INTRODUCTION

Periodontitis is an inflammatory condition of the supporting structures of teeth resulting from dental plaque biofilm attached to tooth surfaces. It is potentially an important nidus of systemic inflammation and its sequelae represents a unique opportunity for oral pathogens and their products to gain access to the systemic circulation.<sup>1</sup>

Diabetes mellitus is a group of metabolic disorders, characterised by hyperglycaemia, and excretion of sugar in the urine.<sup>1</sup> Type 2 diabetes mellitus is the most common form of diabetes accounting for 90-95% of all cases and usually has an adult onset. Diabetes mellitus plays an important role in the pathogenesis of periodontal infections sharing a common pathway that involves an enhanced inflammatory response which can be observed at the local and systemic level.<sup>2</sup> Periodontal infection itself represents a complication that may be involved in altering the systemic physiology in diabetic patients.

A dysregulated immune response stemming from an inappropriate cytokine production may act as a possible

mechanism underpinning the cross-susceptibility between periodontal disease and diabetes.<sup>3,4</sup> Another confounding factor related to periodontitis and type 2 diabetes mellitus is obesity or fatness which refers to excessive accumulation of body fat, caused by the consumption of more calories than the body can utilize.<sup>6,7</sup>

Obesity may be considered as a low – grade systemic inflammatory disease. Obese children and adults have elevated serum markers of inflammation and are closely associated with chronic inflammatory diseases. These findings explain the basis for association between obesity and periodontal disease.<sup>8</sup>

Currently obesity, particularly central obesity, heightens the pathogenesis of type 2 DM. Additionally, factors produced by adipose tissue (AT), referred to as adipocytokines, can influence the degree of insulin resistance as well as inflammation, due to their duality of function.<sup>7</sup> Visfatin is a recently discovered adipokine, synthesized and secreted primarily by visceral fat.<sup>9</sup> It is 52-kDa protein encoding a polypeptide of 491 amino acids.<sup>10</sup> Visfa-

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tin may be considered as a multifunctional protein acting as a hormone, cytokine and/or enzyme capable of exerting several pro-inflammatory functions<sup>11</sup>.

Visfatin levels are elevated in a wide variety of inflammatory conditions like rheumatoid arthritis and any acute and chronic inflammation. In periodontal inflammation, there is upregulation of IL-1, IL-6, TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ) etc., that has a profound effect on the expression of visfatin<sup>11</sup>. Elevated levels of proinflammatory cytokines and visfatin can worsen insulin resistance and thereby impair glycemic control. Thus, periodontal disease may have a significant impact on the metabolic status in type 2 diabetes mellitus.<sup>12</sup>

### PATHOGENESIS OF PERIODONTAL DISEASE

Periodontitis is a complex bacteria-induced infection, characterised by inflammatory host response to plaque microbiota and their by-products.<sup>18</sup>

Periodontal microbes colonizing the subgingival environment express various structural and metabolic substances, for instance, lipopolysaccharide (LPS). This is a surface component and a well-known virulence factor of these Gram negative subgingival bacteria, which has the ability to trigger the host cells to release cytokines and other mediators of inflammation, in an attempt to eliminate the infectious agents and initiate a defence mechanism. The chronic inflammation in periodontal tissues stems from inappropriate host-microbial interaction resulting in excessive production of proinflammatory cytokines (e.g. IL(interleukin)-1 $\beta$ , TNF(tumor necrosis factor)- $\alpha$ , IFN(interferon)- $\gamma$  and IL-6), leading to tissue destruction. Thus, LPS plays a key role in activation and perpetuation of tissue destruction in periodontal disease.<sup>14</sup>

Although the bacterial plaque is fundamental for periodontal disease initiation and propagation, the host defensive responses represented by inflammatory and immune reactions are the essential determinants of disease occurrence. Host related risk factors such as smoking, systemic disease, genetics, bacterial composition of microbial plaque and socioeconomic factors may exacerbate the host protective mechanisms against bacterial challenge and thereby increase the destructive nature of the processes.<sup>15</sup>

### PATHOGENESIS OF DIABETES MELLITUS

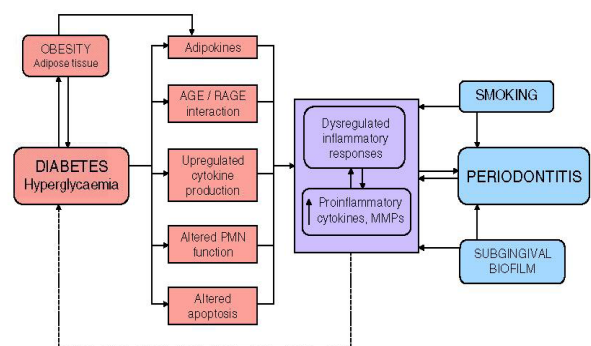
Body glucose homeostasis is mainly reliant on insulin (a hormone secreted by pancreatic  $\beta$ -cells). Diabetes mellitus, ensues from defects in insulin secretion, insulin action, or both resulting in the inability of glucose to be transported from the bloodstream into the tissues, which in turn, results in high blood glucose levels and excretion

of sugar in the urine.<sup>1</sup> The most likely cause of insulin secretion deficiency is the dysfunction in the pancreatic  $\beta$ -cells whilst the hypo responsiveness of the tissues to insulin action (termed Insulin Resistance IR) may represent one of the main complications of obesity.<sup>16</sup> Indeed, the regulation of insulin sensitivity and resistance depends on a number of factors including adipokines, inflammatory mediators, genetic factors and environmental stresses.<sup>6</sup>

During the initial stages in the natural history of type 2 diabetes mellitus, the body increases insulin output which results in hyperinsulinaemia to compensate for the reduction in insulin action and maintain normal glucose tolerance. As the insulin resistance worsens, the body is unable to control any further rise in metabolic load. There is a decline in insulin production affiliated with peripheral insulin resistance and diminished  $\beta$ -cell function. Ultimately, insulin secretions become diminished and inadequate to recompensate for the insulin resistance, thereby driving to impaired glucose tolerance and overt type 2 diabetes mellitus.<sup>17</sup>

### PERIODONTITIS ↔ TYPE 2 DIABETES MELLITUS RELATIONSHIP

There has recently been much emphasis on the 'two-way' relationship between diabetes and periodontitis. The exacerbation and dysregulation in the inflammatory responses play a central role in interrelationship between periodontal disease and diabetes. Hyperglycaemia drives numerous proinflammatory effects that influence various body tissues including the periodontium, leading to localized dysregulated immuno-inflammatory reactions.<sup>17</sup>



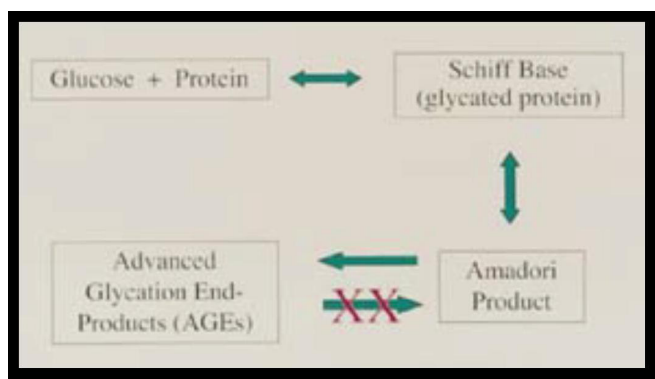
**Figure 1:** Schematic representation of the proposed bi-directional relationship between periodontal disease and diabetes<sup>17</sup>

The influence of diabetes on the periodontium has been thoroughly investigated. Although it is difficult to make definitive conclusions about the specific effects of diabetes on periodontium, a variety of changes have been described, including a tendency toward reduced salivary flow, burning mouth, enlarged gingiva, sessile or pedunculated gingival polyps, polypoid gingival proliferations,

abscess formation, periodontitis, and loosened teeth. Perhaps the most striking changes in uncontrolled diabetes are the reduction in defence mechanisms and the increased susceptibility to infections leading to destructive periodontal disease. Diabetes does not cause gingivitis or periodontal pockets, but there are indications that it alters the response of the periodontal tissues to local factors, hastening bone loss and retarding postsurgical healing of the periodontal tissues.<sup>1</sup>

## HYPERGLYCEMIA, ADVANCED GLYCATION END PRODUCTS (AGES) &

### PERIODONTAL HEALTH



**Figure 2:** Formation of advanced glycation end products (AGE)<sup>17</sup>

Binding of AGEs to its receptor (RAGE) resulted in the upregulated production of proinflammatory mediators such as IL-1 $\beta$ , TNF- $\alpha$  and IL-6. AGE formation results in the production of ROS and enhances oxidant stress, and the subsequent endothelial cell changes that occur contribute to the vascular injury implicated in many diabetes complications. Apoptosis may also play a role in the increased susceptibility to periodontitis associated with diabetes, and apoptosis of matrix-producing cells may limit the opportunities for repair in inflamed tissues.<sup>18</sup>

### IMPACT OF PERIODONTITIS ON DIABETES RELATED INFLAMMATORY STATE AND INSULIN RESISTANCE

In untreated severe periodontal disease, the cumulative surface area of ulcerated pocket epithelium has been estimated to range from 8 to 20 cm<sup>2</sup>, which approximates the size of the palm of an adult hand.<sup>19</sup> Bacteremia and endotoxemia can be induced by dental procedures, as well as by usual daily activities (such as chewing), leading to an elevated inflammatory state and stimulating increase in the levels of serum inflammatory markers.

Chronic inflammation, through the action of inflammatory mediators is found to be associated with the de-

velopment of insulin resistance which is influenced by genetically modified environmental factors, including decreased physical activity, poor nutrition, obesity and infection.<sup>20</sup>

Patients with inflammatory periodontal diseases often have elevated serum levels of proinflammatory cytokines mainly IL-6, IL-1, TNF- $\alpha$  and PGE-2. In diabetic patients with periodontitis, the hyperinflammatory immune cells can exacerbate the elevated production of proinflammatory cytokines which increases insulin resistance leading to greater risk of poor glycemic control when compared to diabetic patients without periodontitis.<sup>17</sup>

The inflammatory mediators originating from periodontal sources can interact systemically with lipids, free fatty acid and advanced glycation end products (AGEs), all of which are characteristics of diabetes. This interaction induces or perpetuates activation of the intercellular pathways, such as I kappa- $\beta$ , Nuclear factor kappa  $\beta$  and the Protein jun N-terminal kinase axes, all of which are associated with insulin resistance. The activation of these inflammatory pathways in immune cells (monocytes or macrophages), endothelium cells, adipocytes and muscle cells promotes and contributes to an increase in the overall insulin resistance, making it difficult to achieve metabolic control in patients with both type 2 diabetes and periodontitis.<sup>21</sup>

## VISCERAL ADIPOSITY, OBESITY, INSULIN RESISTANCE, TYPE 2

### DIABETES MELLITUS

In obesity, the initial deposition of triglycerides occurs in subcutaneous adipose tissue and as this increases, insulin resistance will arise and limit further subcutaneous lipid accumulation. Triglycerides will then be diverted to the visceral fat depot as well as to ectopic sites. This leads to a substantial rise in insulin resistance and the prevalence of its associated disorders. Supporting studies indicate that in lean subjects the prime determinant of insulin resistance is BMI (Body mass index), that is, subcutaneous fat, whilst in overweight and obese subjects, it is waist circumference and visceral adiposity. Accumulation of fat in abdomen regions has major implications for metabolism, particularly for insulin sensitivity.<sup>9</sup>

Visceral adiposity is considered a risk factor for type 2 diabetes mellitus in adults, as well as in first degree relatives of patients with type 2 diabetes mellitus with normal glucose levels. Adipocytokines, hormones secreted by the visceral adipocytes, generate the insulin resistant state and the chronic inflammatory profile that frequently goes along with visceral obesity.

## VISFATIN

### THE DISCOVERY, STRUCTURE & TISSUE DISTRIBUTION OF VISFATIN

Visfatin is a newly discovered member of adipokine family with the unique particularity within the group of having two variant forms, identical in sequence and coded by the same gene, but with different localizations - the first is intracellular nicotinamide phosphoribosyl transferase (iNampt), and the second extracellular (eNampt). The intracellular form of visfatin is ubiquitously expressed and it was proved so far to synthesize NMN, an intermediate of NAD, through its phosphoribosyltransferase activity. The extracellular form was found to be actively secreted by both white adipocytes<sup>9</sup> and brown adipocytes, justifying its inclusion in the adipokine group. The extracellular form of Nampt/visfatin is synthesized and released to the extracellular milieu, where it could exert a variety of actions in a paracrine or endocrine manner. Structurally, extracellular visfatin shows a slightly higher molecular weight than the intracellular isoform and seems to undergo post-transcriptional modifications.<sup>10</sup>

Despite the fact that visfatin is a visceral adipokine, it has been found in skeletal muscle, liver, bone marrow and lymph. Visfatin was originally identified as a cytokine-like secreted protein that synergizes the effect of IL-7 and stem cell factor in promoting the growth and differentiation of B-cell lineage precursors. It was originally called Pre-B cell colony enhancing factor (PBEF) which is protein with a enzymatic activity<sup>10</sup> and acts as a nicotinamide phosphoribosyltransferase (Nampt).<sup>10</sup> It is expressed in lymphocytes of peripheral blood and plays a role in lymphocytes maturation and inhibition of neutrophil apoptosis.<sup>22</sup>

Visfatin is a 52 kDa large protein, and its gene PBEF/Visfatin is located on chromosome 7q22.2. It consists of 11 exons and 10 introns and is 34.7 kb large.

**Fukuhara et al.**<sup>9</sup> presented Visfatin as a newly identified adipocytokine with many possible effects in physiological regulation in humans and a possible role in the development of some pathological conditions. Plasma Visfatin levels positively correlated with obesity development and had insulin-mimetic activity.

### VISFATIN IN REGULATION OF INSULIN SIGNALING

A very important finding is Visfatin's insulin-mimetic activity. The insulin-mimetic activity was explored in various experiments. Visfatin lowered plasma glucose level in mice. In heterozygous mice with mutated Visfatin gene, slightly higher plasma glucose levels were observed than in wild type individuals. The effect of Visfatin on cultured cells is found to be similar to that of insulin. Visfatin not only influenced glucose uptake into

3T3-L1 adipocytes and L6 myocytes, but also suppressed glucose release from H4IIEC3 hepatocytes.<sup>9</sup>

Further it was described that Visfatin binds to insulin receptor in a different binding site than insulin. Visfatin induced the phosphorylation of insulin receptor, IRS1 and also IRS2 (insulin receptor substrate).<sup>9</sup> Visfatin could act as an attractive target molecule with insulin-mimetic activity, which is non-competitive with insulin, in pharmacotherapy of insulin-resistant conditions. It is not surprising that **Fukuhara et al.**, considered the role of Visfatin in some metabolic disorders related to glucose homeostasis.<sup>9</sup>

### VISFATIN AND TYPE 2 DIABETES MELLITUS

Visfatin is an endocrine, autocrine as well as paracrine protein with many functions including enhancement of cell proliferation, biosynthesis of nicotinamide mono- and dinucleotide and induction of hypoglycemia. Visfatin has been shown to exert insulin mimetic and pro-inflammatory effects, also functioning as an intracellular enzyme to produce NAD.<sup>10</sup> The observation that visfatin has insulin-mimetic functions has raised the hypothesis that a dysregulation of the activity of this molecule may contribute to the metabolic syndrome and diabetes.

**Chen M. P et al.**, (2006) in their study investigated whether plasma visfatin level is altered in patients with type 2 diabetes mellitus. Plasma visfatin was found to be elevated in patients with type 2 diabetes mellitus when compared to age & sex matched controls. Increasing concentrations of visfatin were independently and significantly associated with type 2 diabetes mellitus.<sup>2</sup>

### VISFATIN AND OBESITY

Human body contains two types of adipose tissue – subcutaneous and visceral fat. Visceral fat is significantly related to metabolic disorders, especially metabolic syndrome, and represents a strong risk factor. Metabolic syndrome is associated with the central obesity, type 2 diabetes mellitus, insulin resistance, hypertension and higher cardiovascular risk. Obesity has also been associated with increased accumulation of macrophages in visceral fat and the amount of macrophages is positively correlated with the total fat mass of the body.

**Fukuhara et al (2005)**<sup>9</sup> reported that plasma Visfatin levels correlated strongly with the amount of visceral fat, but only mildly with the amount of subcutaneous fat in their experiment on 101 male and female human subjects. They also analyzed the mRNA Visfatin expression in the visceral and subcutaneous fat in KKAY mice which are models for obesity & type 2 Diabetes. In the time period, when the mice become obese (between 6 and 12 week of age), Fukuhara et al. found increased levels of plasma Visfatin which correlated with the increase of

mRNA Visfatin expression in visceral fat. On the contrary, no significant changes were found in mRNA expression in subcutaneous fat<sup>9</sup>

### VISFATIN AND IMMUNE AND INFLAMMATORY PROCESS

One of the fundamental functions of visfatin/PBEF/Nampt is the modulation of immune and inflammatory processes. **Moschen et al (2007)**<sup>10</sup> has revealed that human leukocytes can be activated by visfatin to produce several pro and anti-inflammatory cytokines. While stimulation of CD14+ monocytes by visfatin leads to the production of IL-1 $\beta$ , TNF- $\alpha$  and IL-6, on the other hand, anti-inflammatory cytokines such as IL-1Ra and IL-10 might also be produced by stimulation of monocytes by visfatin. Moreover, this adipokine enhanced the surface expression of the co-stimulatory molecules CD54, CD40 and CD80 in CD14+ monocytes. Furthermore, it has been noted that visfatin/PBEF/Nampt was able to activate antigen presenting cells (APCs) and enhance phagocytosis in monocytes.<sup>10</sup> In addition, trafficking CD14+ monocytes and CD19+ B-cells into sites of inflammation is another important function of visfatin which is deemed to be a strong chemotactic factor for these cells.<sup>10</sup>

Moreover, it has been reported that visfatin activated nuclear factor-kappa B which has a crucial role in regulating immune responses.<sup>9</sup> Indeed, it has been illustrated that serum visfatin levels positively correlated with IL-6 and CRP levels in human serum, which in turn corroborated the significance of visfatin as inflammatory cytokine. Up-regulation of visfatin has been also identified in a variety of pathophysiological conditions of the immune system including rheumatoid arthritis, psoriasis, clinical sepsis and acute lung injury.<sup>10</sup>

### VISFATIN AND PERIODONTAL DISEASE

Recently, there has been mounting body of studies many number of trials were have been done investigating the association of visfatin with periodontal disease. A pilot study exploring the gene expression signature in pathological gingival tissues has revealed Visfatin as one of the top 20 genes that have been distinguished in periodontitis lesions. Moreover, stimulating monocytic cell line with *Pgingivalis* and

*E.coli* LPS has yielded a differential up-regulation in Visfatin gene expression by

*E.coli* LPS when compared with *Pgingivalis* LPS.<sup>20</sup>

**Pradeep A R et al., (2011)**<sup>24</sup> assessed gingival crevicular fluid and serum visfatin concentration in subjects with periodontal health and disease. A total of 40 individuals (20 males and 20 females; age range: 23 to 53 years) were selected and divided into three groups based on the gingival index, probing depth, clinical attachment

level, and radiologic parameters (bone loss). GCF and serum samples were collected to estimate levels of visfatin using an enzyme-linked immunosorbent assay kit. Mean visfatin concentrations increased in GCF and serum with the severity of disease from healthy to gingivitis to periodontitis groups and differed significantly ( $P < 0.05$ ). However, it was found that GCF levels of visfatin were higher than serum values. The highest to lowest visfatin concentrations were found in chronic periodontitis patients, gingivitis patients and healthy subjects respectively. The results suggest that GCF and serum visfatin concentrations increase with the severity of periodontal disease.

### CONCLUSION

There is a plethora of evidence available demonstrating an association between higher circulating levels of visfatin and the development of type 2 diabetes. Visfatin is also found to exert insulin mimetic and proinflammatory effects and this observation has raised the hypothesis that a dysregulation of the activity of this molecule may contribute to the metabolic syndrome and diabetes.

The presence of Periodontal disease may influence the systemic inflammatory condition in individuals with diabetes. Periodontal inflammation causes elevation of circulating levels of proinflammatory cytokines including visfatin which can influence insulin resistance and modulate inflammation and immune responses.

### REFERENCES

1. Newmann M G, Takei H H, Klokkevold R P *et al.* Carranza's clinical periodontology. W B Saunders company 2006; 10<sup>th</sup> edition.
2. Brian Mealey. Periodontal disease and diabetes: a two way street; Journal of American Dental Association: 2006; 137(10 suppl): 26S-31S.
3. Amano. Host-parasite interactions in periodontitis: microbial pathogenicity and innate immunity; Periodontology 2000 October 2010; Volume 54(1): 9-14.
4. Philip M. Preshaw. Host response modulation in periodontics: Periodontology 2000 October 2008; Volume 48: (1): 92-110.
5. Giulietti A, Van Etten E, Overbergh L, Stoffels K, Bouillon K, Mathieu C. Monocytes from type 2 diabetic patients have a pro-inflammatory profile. 1, 25-Dihydroxyvitamin D (3) works as anti-inflammatory; Diabetes Res Clin Pract. 2007 Jul; 77: 47-57.
6. Wellen K E, Hotamisligil G S. Inflammation, stress, and diabetes: J Clin Invest. 2005 May; 115: 1111-9.
7. Kopelman P. Obesity as a medical problem. Nature. 2000; 404: 635-643.
8. Caroline FD. Overweight and Obesity as Risk Indicators for Periodontitis in Adults. Journal of Periodontology 2005; 76: 1721-1728.

9. Atsunori Fukuhara, Morihiro Matsuda, Masako Nishizawa, Katsumori Segawa, Masaki Tanaka, Kae Kishimoto *et al.* Visfatin: a protein secreted by visceral fat that mimics the effect of insulin. *Science*.2005 Jan 21;307: 426-30.
10. Moschen A R, Kaser A, Barbara Enrich, Birgit Mosheimer, Milan Theurl, Harald Niederegger and Herbert Tilg. Visfatin, an Adipocytokine with Proinflammatory and Immunomodulating properties: *J Immunol* 2007; 178:1748-1758.
11. S Ognjanovic, S Bao, S Y Yamamoto, J Garibay-Tupas, B Samal and G D Bryant Greenwood. Gnomonic organisation of the gene coding for human. *J mol.endocrinol* 2001;26:107-117.
12. Brian L Mealey and Thomas Oates. AAP-Commissioned Review Diabetes Mellitus and Periodontal Diseases: *J Periodontol* August 2006 ;77:1289-1300.
13. Socransky SS. Haffajee AD. Cugini MA, Smith C, Kent Jr. Microbial complexes in subgingival plaque: *J Clin Periodontol* 1998; 25: 134-144.
14. Richard P Darveau and Sumita Jain. Contribution of Porphyromonas gingivalis lipopolysaccharide to periodontitis: *Periodontology* 2000 2010; Vol. 54:53-70.