Evaluation of Lipocalin-2 and Visfatin, and Vitamin (D,C, and E) in Serum of Diabetic Patients with Chronic Periodontitis

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Abstract

Objective: The aim of the present study is to verify the hypothesis that salivary and serum lipocalin-2 and visfatin, and vitamin (D,C, and E) used as indicators for chronic periodontitis among DMT2 patients. Subjects and methods: We enrolled 41 type 2 diabetic patients with chronic periodontitis -18 males and 23 females, aged 60± 14 years and 49 controls- 26 males and 23 females, aged 61.8 ± 12.5 years. Blood samples were collected after an overnight fast and routine biochemical parameters such as glucose, lipocalin-2 and visfatin, and vitamin (D,C, and E)were determined in all samples. Data were considered significant at a level of $p < 0.05$.Periodontitis status was established using classic clinical parameters, Plaque index (PI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL). Results: 1) The mean serum of blood glucose, Visfatin, and LCN2 were higher in patients group than in those with normal control group ($P<0.001$ for all comparisons) ; 2) patients with had a lower Vitamin D,C, and E than those with a normal group ($P < 0.001$ for all comparisons).Conclusions: The activity of lipocalin-2 and visfatin in saliva and serum can be useful in diagnosis, monitorization and treatment of this disease. Further studies are needed to elucidate the role of lipocalin-2 and visfatin in periodontitis and systemic disease interactions.

Key words: *Type2 diabetes; Visfatin; Lipocalin-2;Serum.*

Introduction

The oral cavity is constantly being exposed to bacterial, viral, and fungal activity, and is dependent on a symbiosis between a strong resident microbiome and the innate immune responses for the maintenance of health. Human saliva is a biological fluid with myriad of biological functions important for the maintenance of oral and general health, it is considered as the gold standard in biochemical assays and analysis[1,2].

Periodontitis is a chronic inflammatory disorder mediated by specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone, with periodontal pocket formation, gingival recession, or both[3,4]. The condition is started by pathogenic microbes in dental plaque attached to the tooth surface and harbors a complex microbiological community by anaerobic bacteria (*Porphyromonas gingivalis*, *Treponema denticola*, *Prevotella intermedia*, *Prevotella nigrescens*, *Eikenella corrodens*, *Aggregatibacter actinomycetemcomitans*, among others) [5] , which increase the level of fructose in the body, causing insulin resistance to worsen[6].

In the same context, periodontitis can provide risk factor for diabetes, as chronic inflammation in periodontitis causes a systemic response to bacteria causing insulin resistance predisposing to DM or aggravating glycemic control and increasing the hazard of diabetic complications[7].

The adipose tissue is a complex, essential, and highly active metabolic and endocrine organ. that releases a large number of bioactive mediators such as adipokines, hepatokines and myokines, involved in inflammatory processes, pointing toward comparable pathways involved in the pathophysiology of DM, periodontitis, and related inflammatory diseases among these mediators lipocalin and visfatin[8-10].

The present study was undertaken to evaluate the levels of lipocalin and visfatin, and vitamin (D,C, and E) in patients with both the chronic diseases, i.e., type 2

diabetes and chronic periodontitis.

Materials and Methods

Forty-one type 2 diabetic patients with chronic periodontitis -18 males and 23 females, aged 60± 14 years and 49 non-diabetic individuals 26 males and 23 females, aged 61.8 ± 12.5 years were selected from the population referred to the Periodontal Clinic of Tikrit University, from December 2019 to February 2020.The data were collected through a standard questionnaire. All subjects were interviewed regarding a full medical history that included age, sex, occupation, duration, and family history of diabetes mellitus. The biochemical parameters such as glucose, lipocalin-2 and visfatin, and vitamin (D,C, and E) were measured. The material for the study was the peripheral venous blood. Samples were drawn after an 8 to 12 hour of overnight fast. All the tubes were subjected to centrifugation at 3000 rpm for 10 minutes followed by storage at -40ºC until assayed.

Clinical periodontal parameters, including plaque index (PI), and bleeding on probing (PPD) were measured. PD and CAL were measured at six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual and disto-lingual), except for third molars[11]. All clinical examinations were carried out by a single examiner, who was trained, calibrated, and masked to the systemic condition of the patient.

Statistical analysis was executed by SPSS 20 (SPSS inc., Chicago, IL) program. The independent *t*-test was used for comparison of means of quantitative variables. *P*<.05 was considered statistically significant.

Results

The mean age of patients in the patient group was 60± 14 years, whereas the mean age of participants in the control group was 61.8 ± 12.5 years, there was no significant difference between the groups as regards age (p>0.05). Among the 41 patients in the diabetic with CP group, 18 (44%) were male and 23 (56%) were female. Among the 49 participants in the control group, 26 (53%) were male and 23 (47%) were female.

Table No. 1 Basic characteristics of study groups:

Parameters	Diabetic subjects with CP N = (41)	Control subjects N = (49)
No. of subjects	41	49
Sex (M/F)	18 (44%) /23 (56%)	26 (53%) /23 (47%)
Age (years)	60± 14	61.8 ± 12.5

As expected, the mean values of PI(1.5±±0.089 vs 0.37±0.178) GI (1.63±0.08 vs. 0.00), PPD (1.88±0.121 vs. 3.74±0.34), and CAL (2.9±0.38 vs. 0.00) between the study group and control group were statistically highly significant (*P*<0.001) [Table 2].

Table No. 2 Comparison of mean periodontal parameters PI, PPD and CAL between the both groups.

	Group 1 (n=50) Patients	Group 2 (n=50)
Plaque index	1.5±±0.089	0.37±0.178*
Gingival index	1.63±0.08	0.00
Pocket probing depth (mm)	1.88±0.121	3.74±0.34*
CAL (mm)	2.9±0.38	0.000

Table No. 3: Comparison of study biochemical parameters in PCOS and control subjects.

Parameters	Group A (Control)	Group B (Patients)	P value
Fasting blood glucose (mg/dl)	101.2 ± 7.18	184 ± 10.2	<0.0001
Visfatin (pg/ml)	16.17 ± 0.93	23.59 ± 1.13	P<0.001*
Vitamin D (ng/ml)	33.1 ± 14	20.71 ± 10.03	P < 0.05
vitamin C (mg/dL)	1.18±0.182	0.71±0.151	P < 0.001
Vitamin E (mg/L)	13.36±1.432	5.62±0.811	<0.001*

The mean Vitamin D level in case group was 18.726 ± 6.259 ng/ml, while in control group, it was 42.851 ± 14.516 ng/ml with $P < 0.05$ which is highly significant.

Discussion

Type 2 diabetes mellitus (T2D), a subclass of diabetes mellitus that is not insulin responsive or dependent, is a metabolic disorder that is categorized by chronic hyperglycemia caused by increased production of glucose in the liver and increased peripheral insulin resistance, which might eventually lead to a reduction in insulin secretion[12-14].

Visfatin,also known as nicotinamide phosphoribosyl transferase a 52 kDa molecule , is one of the most recently identified adipokine that is highly enriched in the visceral fat, It is a pleiotropic adipocytokine, which acts as a cytokine, a growth factor and an enzyme[15], with a potential glucose-lowering effect due to its nicotinamide phosphoribosyltransferase (NAMPT) activity[16].

Insulin bind to different sites of visfatin non-competitively, leading to the insulin signaling cascade, increasing glucose uptake, and inhibiting glucose release[17]. The reason for the higher level of serum visfatin in these patients may be due to the following[18-21] :

I. Visfatin may be related to the aging-dependent circadian cycle, which lead to the decline of pancreatic cell function, insulin mimetic effects, increased plasma visfatin levels could be a compensatory mechanism in response to hyperglycemia that ameliorates the functional consequences of insulin resistance.

II. impairment in visfatin signaling in target tissues or dysregulation of its biosynthesis in response to hyperglycemia, hyperinsulinemia, or adipocytokines in the condition of diabetes.

III. visfatin mediated nicotinamide adenine dinucleotide biosynthesis that regulates glucose-stimulated insulin secretion

IV. It has more potent destructive and pro-inflammatory properties and has a crucial role in the diligence of inflammation through reticence of apoptosis and neutrophils

Neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kD , recently identified adipokine. which mainly released from granules of activated neutrophils. It is expressed in several tissues including adipocytes, neutrophils, salivary gland, stomach, liver, lung, kidney endothelial cells, macrophages, and vascular smooth muscle cells which induced by many pro- and anti-inflammatory cytokines , factors like lipopolysaccharide (LPS), tumor necrosis factor-α (TNF-α), IL-1β, IL-6, IL-7, or IL-17, and anti-oxidant enzymes reducing free radicals in a variety of cell types[22-23]. It is an acute phase protein plays a pivotal role in glucose homeostasis and insulin sensitivity vascular remodeling, plaque instability specifically LCN2 expressed in macrophages[24].

Lipocalin-2 as a novel autocrine and paracrine adipokine, acts as an antagonist to the effect of inflammatory molecules on inflammation and secretion

of adipokines[25]. LCN2 was significantly elevated in patients with diabetes compared with controls.LCN-2 might be an indicator for β cell dysfunction, it is consider as iron delivery protein. Increased LCN-2 levels will increase cytoplasmic iron which may result in β cell oxidative stress and impaired insulin secretory capacity and activation of ferritin[26].

Vitamin D refers to a group of fat-soluble a fat-soluble biomolecule, which was first discovered in 1919–1924 discovered in 1922 by McCollum as an antirachitic agent[27] synthesized in the skin by the action of ultraviolet irradiation from the sun related to bone metabolism and skeletal integrity as well. Currently, vitamin D, and especially its most reactive metabolite, 1,25(OH)2D3 (calcitriol), is considered a hormone involved in complex endocrine systems and modulating growth and differentiation of cells from various lines, [28]. As well its best known for its role in regulating calcium and phosphate metabolism[29]. The synthesis and secretion of insulin is effected by existence of the vitamin D response element (VDRE) in the human insulin gene promoter and transcriptional activation of the human insulin gene triggered by 1,25(OH)2D[30].

Because chronic periodontitis is characterized by bone loss triggered by a host immune response reaction to bacterial plaque[31]. Vitamin D is believed to improve the body's sensitivity to insulin and thus reduce the risk of insulin resistance. It can also regulate the production of insulin in the pancreas through control of the insulin receptor gene[32]. Vitamin D deficiency could negatively affect the periodontal health. Its anti-inflammatory effect is mediated through inhibition of cytokine production, stimulation of monocytes and macrophages, and secretion of peptides with potent antibiotic activity. Recently, its effect on periodontitis through its anti-inflammatory effect[33].

Although evidence for the relationship between vitamin D status and DM is sparse, The possible mechanisms assumed that role of VDD in DM systemic inflammation by the formation and the effects of modulating cytokines, which lead to enhanced insulin sensitivity and promote beta-cell survival, and insulin resistance through induces the expression of the insulin receptor, thus increasing insulin sensitivity during transportation of glucose and pancreatic beta cell malfunction via a change of calcium flux, which can adversely affect beta cell function[34].

Vitamin C (ascorbic acid), a water-soluble vitamin, is an essential nutrient, that exerts a reducing and anti-oxidant effect, which scavenges free radicals and protect cells from oxidative stress, acts as an as an essential co-factor for the hydroxylation of proline and lysine which is vital to collagen biosynthesis in connective tissue, and probably reduce insulin resistance by improved endothelial function and lowering oxidative stress[35-37]. It is structurally similar to glucose and can replace it in many chemical reactions and thus is effective for prevention of nonenzymatic glycosylation of protein[38-45].

In DM subjects with CP, serum vitamin C was significantly lower compared with healthy individuals. Possible mechanisms for reduced vitamin C levels in diabetes assumed that the elevated free radicals caused by hyperglycemia reduce vitamin C, renal reabsorption of vitamin C is reduced and increase in clearance, increased turnover of vitamin C, due to the increased oxidation of ascorbate to dehydro-ascorbic acid in tissue mitochondria[38-40]. Deficiency of vitamin C increases susceptibility to infection, impair the function of neutrophils and macrophages, reduces antibody -mediated, cell- mediated, phagocytic and delayed type of hypersensitivity reactions and depletion of antioxidants[41].

Vitamin E is the most important lipophilic membrane radical-scavenging antioxidant antioxidant and that protect the cells from reactive oxygen species prevent damage of lipids particularly of polyunsaturated fatty acids. The decreased level of vitamin E results in damage to internal structures by enhanced free radical production[42,43.44].

Ethical Considerations: All Research participants haven't been subjected to any kind of harm in any way and the samples were collected according to the guidelines of blood sample collection guidelines provided by WHO.

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