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Evaluation Of Salivary Level Of Adipokines In Patients With Periodontal Health And Disease: A Biochemical Study

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Abstract

Objectives: Gingivitis and periodontitis are common bacterial infections caused by microorganisms. Adipokines such as resistin, visfatin and leptin mediate beneficial and detrimental effects in inflammation. Hence, the aim of the study was to evaluate the salivary adipokines level and compare them between the periodontally healthy subjects and patients with gingivitis and periodontitis.

Materials & Methods: A total of 90 patients were divided into 3 groups. Group 1 consisted of 30 individuals with healthy gingiva of probing depth ≤3mm, GI =<1, PI=<1 and CAL=0, Group 2 consisted of 30 individuals who had signs of gingival inflammation with probing depth ≤3mm, GI >1, PI>1 and CAL=0 and Group 3 consisted of 30 individuals who had signs of clinical inflammation, and a diagnosis of chronic periodontitis with PPD ≥ 5 mm, GI >1, PI> 1and CAL≥ 3mm. Whole saliva samples were collected and adipokines levels were evaluated by standard enzyme-linked immunosorbent assay.

Results: The level of salivary resistin and visfatin increased as the disease progressed from health to periodontitis and there was gradual decrease in leptin level in the saliva as condition progressed from health to periodontitis. A significant difference in resistin, visfatin and leptin levels in the saliva was found when group 1 was compared with group 3 and group 2 was compared with group 3.

Conclusion: These results demonstrated that the higher levels of resistin and visfatin and lower level of leptin in the saliva of patients with chronic periodontitis were correlated with the degree of tissue destruction.

Keywords: leptin, resistin, visfatin, periodontitis, saliva

Introduction

Periodontal disease is a chronic disease of the oral cavity comprising a group of inflammatory conditions affecting the supporting structures of the dentition.[1] It is characterized by overproduction of inflammatory mediators and tissue-destructive molecules against microbial pathogens.[2] It seems that patients with periodontitis has been linked to systemic conditions, including heart disease, diabetes, obesity, and metabolic syndrome. The association between periodontal diseases and these systemic conditions appears to be attributable to a low-grade inflammatory burden that links them through a common pathophysiologic mechanism.[3]

Saliva have a significant role in the establishment and progression of periodontal disease because of its importance in oral biofilm formation and host defense.[4] Assessment of the composition of saliva may provide valuable information about biochemical markers for assessment of periodontal diseases.[5]

Resistin is a cysteine-rich polypeptide hormone produced by adipocyte, monocyte and macrophages and plays a key role in inflammation and immune responses.[6,7] Resistin acts as a proinflammatory molecule and stimulates the synthesis and secretion of proinflammatory cytokines like tumor necrosis factor-α, interleukin6, interleukin-12 and monocyte chemoattractant protein-1.[8] The association of resistin with periodontitis investigated a positive correlation with bleeding on probing (BOP) when evaluated from the serum of patients with CP.[9]

Visfatin, also known as pre-B-cell colony-enhancing factor (PBEF) and nicotinamide phosphoribosyl transferase, is a novel adipokine that is preferentially produced by visceral adipose tissue and has insulin mimetic actions.[10] It has a several immunity functions that is secreted by neutrophils in response to inflammatory stimuli and upregulates the production of cytokines like interleukin 6 (IL-6), IL-1β and tumour necrosis factor alpha (TNFα) in human monocytes.[11] An increase in the levels of the proinflammatory cytokines in the periodontal tissues can induce visfatin production. Visfatin expression is up-regulated in a variety inflammatory diseases such as type 2 diabetes mellitus, inflammatory bowel disease and rheumatoid arthritis.[12]

Leptin is a 16 kDa adipocyte-derived circulating protein plays important roles in regulating lipid and glucose metabolism. Leptin inhibits appetite, stimulates energy expenditure, and it can modulate lipid and bone metabolism, coagulation, hematopoiesis, function of pancreatic beta cells, insulin sensitivity, etc.[13,14] Leptin is present within healthy gingiva and its concentration declines with the increase in severity of the gingival inflammation and periodontal pocket formation.[15]

In the light of the above facts, the current study was designed with an aim to evaluate the resistin, visfatin and leptin level and compare them between the periodontally healthy subjects and patients with gingivitis and periodontitis to determine whether these biomarkers in saliva can be used as an early diagnostic marker and help in the prevention of periodontal diseases.

Materials And Methods

This study was performed from September 2020 to March 2021 in the Department of Periodontology after obtaining ethical approval from the institutional review board of the college. A total of 90 male and female subjects aged between 25 and 50 years participated in this study. Written informed consent was taken from all the participants before the start of the study.

Participants were categorized into two groups based on the Gingival index(GI), Plaque index (PI), pocket probing depths (PPD) and clinical attachment level (CAL).

Group 1 (healthy) consisted of 30 individuals with healthy gingiva of probing depth ≤3mm, GI =<1, PI=<1 and CAL=0

Group 2 (chronic gingivitis) consisted of 30 individuals who had signs of gingival inflammation with probing depth ≤3mm, GI >1, PI>1 and CAL=0

Group 3 (chronic periodontitis) consisted of 30 individuals who had signs of clinical inflammation and a diagnosis of CP with PPD ≥ 5 mm, GI >1, PI> 1and CAL≥ 3mm.

Exclusion Criteria

cigarette smoking or tobacco use and alcoholism; (2) systemic diseases such as diabetes mellitus, hypertension, and rheumatoid arthritis; (3) pregnancy; (4) systemic bacterial, viral, or fungal infection; (5) history of antibiotic therapy or use of anti-inflammatory medications during the past 6 months; (6) periodontal therapy during the past 2 years; and (7) patients with aggressive periodontitis.

Sample Collection

Subjects were requested to refrain from eating and drinking for at least 2 h before saliva collection. Using the spitting method, unstimulated saliva was collected between 11:00 am and 13:00 pm for 5 min (one spit per minute). The saliva was collected in sterile tubes and immediately frozen at −70°C until the experiment. After thawing, ELISA kit (RD191016100; BioVendor Laboratory Medicine, Brno, Czech Republic) was used to determine the concentration of visfatin, resistin and Leptin. In favorable circumstances, the double-well assay is recommended. For the standard well, 100 µL of streptavidin-HRP was added; other protocols were the same as those with the test wells. 100 μl of anti-Visfatin antibodies, biotin labelled second polyclonal anti-human resistin antibody and biotinylated leptin-

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specific detection antibody were added to each well and incubated for 1.5 hours. The wells were washed 5 times with 300 µL of wash solution to eliminate the unreacted enzyme. 100 μl of prepared HRP-Streptavidin solution was added to each well and incubated for 45 minutes at room temperature. The solution was discarded and wells were washed 5 times with wash solution (200 μl each). 100 μl of TMB One-Step Substrate Reagent was added to each well and incubated for 30 minutes at 37°C in the dark environment. A total of 50 µL of stop solution was added into each well to stop the reaction (immediately the blue color changed into yellow). Absorbance of the substrate color reaction was read on ELISA plate reader (BioTeK Instruments Inc., Winooski, VT, USA) using 450 nm as primary wavelength carried out within 10 minutes after incorporation of stop solution. The visfatin, resistin and leptin concentration in each well was reported as ng/mL.

Data Analysis

Statistical analyses were performed using SPSS software version 21 (SPSS Inc., Chicago, IL, USA). The salivary level of resistin, visfatin and leptin was compared between the two groups using the Mann–Whitney test and t-test. $P < 0.05$ was considered statistically significant. Data were presented as mean ± standard deviation.

Results

Resistin, Visfatin and Leptin were detected in the Saliva of all patients. The mean values of the clinical and biochemical parameters were expressed as mean ± SD (Table 1). The highest resistin levels from the Saliva were detected in group 3 (13.37 ± 1.98 ng/mL), while the lowest were detected in group 1 (5.96 ± 1.04 ng/mL). Intergroup comparisons of the clinical and biochemical parameters are summarized in Table 2. A significant difference in resistin levels in the saliva was found when group 1 was compared with group 3 (P<0.0001) and group 2 was compared with group 3(P<0.0001).

The highest visfatin levels from the Saliva were detected in group 3 (33.85 ± 6.98 ng/mL), while the lowest were detected in group 1 (20.93±4.04 ng/mL). Intergroup comparisons of the clinical and biochemical parameters are summarized in Table 2. A significant difference in visfatin levels in the saliva was found when group 1 was compared with group 3 (P<0.0001) and group 2 was compared with group 3(P 0.0093).

The highest leptin levels from the Saliva were detected in group 1 (7.58 ± 1.58 ng/mL), while the lowest were detected in group 3 (4.21 ± 0.72 ng/mL). Intergroup comparisons of the clinical and biochemical parameters are summarized in Table 2. A significant difference in leptin levels in the saliva was found when group 1 was compared with group 2 and group 3 and group 2 was compared with group 3.

Statistically significant differences were observed when comparison of gingival index and Plaque index scores were made with groups 1 and 2 versus the periodontally diseased groups 3. Statistically significant difference in PPD was observed when group 1 and group 2 were compared with group 3.

Discussion

The potential role of saliva in the diagnosis of oral and systemic health is marked in researches. Salivary biomarkers could be used to screen periodontal health status and disease progression.[16,17] The analysis of saliva is a proven and accepted alternative to serum analysis. Saliva is an salient body fluid used for diagnostic purposes, which can be collected and persued easily and cost-effectively.[18]

Resistin is a recently discovered adipocyte-secreted polypeptide that has been implicated in the development of insulin resistance. Resistin was first described in 2001 during a search for genes that induced adipocyte differentiation. This led to the discovery of a protein that the investigators named as resistin (for resistance to insulin). Resistin is a member of a family of tissue-specific signaling molecules, called resistin-like molecules.[19]

The result of the present study revealed that the mean salivary resistin level of group 3 was higher than that of group1 and group 2 (p< 0.0001). This increase in resistin levels in patients with periodontitis could be attributed to, first, the fact that resistin is mainly expressed from polymorphonuclear leukocytes and macrophages in inflammatory conditions.[6,20] Second, with lipopolysaccharide stimulation, resistin is produced from neutrophils by putative periodontal pathogens such as Porphyromonas gingivalis and Escherichia coli.[21] The findings in this study are in accordance with the studies conducted by Eshafrood

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et al,[22] Karam et al,[23] and Akram et al[24] where resistin level was increased in chronic periodontitis patients.

Visfatin is responsible for inflammatory reactions in periodontal structures and the consequent bone loss. It has been shown that microbial infection (presence of *Fusobacterium nucleatum*) has a controlling influence on production of visfatin.[25]

The result of the present study revealed that the mean salivary vistafin level of group 3 was higher than that of group1 and group 2. This increase in vistafin levels in patients with periodontitis is due to polymorphonuclear leukocytes and macrophages in inflammatory conditions.[26] visfatin has more potent destructive and proinflammatory properties and has a key role in the diligence of inflammation through reticence of apoptosis and neutrophils.[27] The findings in this study are in accordance with the studies conducted by Tabari et al,[28] Roupas et al,[29] and Ozcan et al[30] where vistafin level was increased in chronic periodontitis patients.

Leptin is a hormone-like protein, which initially attracted attention due to its significant role in regulating weight and metabolism of the human body.[31] Leptin is a hormone that is secreted in the blood in varying quantities by adipocytes and controls the adipose tissue weight by stimulating lipid metabolism by the organism.[32]

The result of the present study revealed that the mean salivary leptin level of group 2 was higher than that of group1 and group 3. Purwar et al,[33] evaluated leptin levels in saliva and serum of patients with chronic periodontitis and healthy controls. Purwar et al,[34] found that appropriate non-surgical therapy can increase the leptin levels in the saliva of chronic periodontitis patients. Selvarajan et al,[35] examined and compared the GCF concentration of leptin in patients with perio-dontal disease and healthy subjects. They observed a substantial decrease in leptin levels in GCF as the periodontal disease progressed. They concluded that the level of salivary leptin in chronic periodontitis patients was significantly lower than that in healthy controls, consistent with the results of the current study. Johnson and Serio[15] evaluated leptin concentrations in healthy gingival tissues with a gingival sulcus index of ≤3mm and in inflamed gingiva with periodontal pockets >3mm, with the use of ELISA technique. Based on the results, leptin was present in the healthy and inflamed human gingival tissues and its concentration decreased with an increase in pocket depth and aggravation of periodontal disease, consistent with the results of the present study, in which the salivary leptin levels were higher in healthy subjects compared to patients with chronic periodontitis.

This reduction in salivary leptin levels in patients with chronic periodontitis could be attributed due to the increased expression of leptin receptors during periodontal inflammation due to the cytopathic changes leading to the increased binding of leptin to the receptors, thereby decreasing the concentration of leptin in the saliva of chronic periodontitis patients. Also, It appears that leptin is used as a substrate during the inflammatory process; thus, its concentration decreases.[33] Thus, leptin, at a high concentration locally, protects the host from inflammation and infection. Furthermore, leptin enhances the body's immune mechanism by inducing the proliferation of human peripheral blood mononuclear cells, chemotaxis and oxidative species production by stimulated polymorphonuclear cells[36] and the development/maintenance of natural killer cells.[37]

Statistically significant difference in PI, GI and PPD was observed when group 1 and group 2 were compared with group 3. Patients in group 1 and group 2 showed no statistically significant difference when the GI and PPD were compared. Statistically significant difference was observed when group 1 and group 2 were compared with group 3($p< 0.0001$). A mean probing depth of 6.80±1.21mm and a GI score of 2.32±0.49mm in group 3 established the presence of an active inflammatory component along with a destructive component to the prevalent periodontal disease.

Conclusion

At present there are several biomarkers, studied in relation to periodontitis. In the current study, level of resistin and visfatin was higher in the saliva of chronic periodontitis patients and the level of leptin was lower in the saliva of chronic periodontitis patients. These biomarkers may be used as an inflammatory marker for detection of periodontal disease. The limitation of this study was that we did not have information about these salivary biomarkers

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after treatment of periodontitis. Further long-term and interventional studies with larger sample sizes are required to assess the efficacy of these biomarker for early detection of periodontal disease and prevention of its progression. Finally, for considering these biomarkers of inflammation in periodontitis, further studies are needed with more sample size in different populations.

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Tables

Table 1: Values(mean ± SD) of resistin, visfatin, leptin, PPD, CAL, GI, PI in both Groups

Variable	Group 1	Group 2	Group 3
Resistin (ng/ml)	5.96±0.89	9.58±1.43	13.37 ± 1.98

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Visfatin (ng/ml)	20.93±2.74	27.58±3.23	33.85 ± 4.08
Leptin (ng/ml)	7.58±1.58	5.24±0.94	4.21 ± 0.72
PPD	1.47±0.57	1.75±0.84	6.80±1.21
CAL	0	0	4.13±1.31
GI	0.28±0.25	2.03±0.57	2.32±0.49
PI	0.22±0.19	2.38±0.84	2.13±0.41

ǂ PPD – Pocket probing depth; CAL– clinical attachment level; GI – Gingival index; PI – Plaque index; SD - Standard deviation

Table 2: Consolidated Pairwise Comparison (p value) Among the Three Groups (p<0.05)

Variable	Group	Mean difference (95% CI)	t	p
Resistin	Group1 vs Group2	-0.620 (−1.54-−0.31)	-1.358	0.1853
	Group1 vs Group3	-7.410 (−8.58-−6.21)	-12.832	< 0.0001*
	Group2 vs Group3	-6.790 (−8.08-−5.49)	-10.767	< 0.0001*
Visfatin	Group1 vs Group2	-6.650 (−10.14-−3.15)	-3.897	0.0006*
	Group1 vs Group3	-12.920 (−17.18-−8.65)	-6.205	< 0.0001*
	Group2 vs Group3	-6.270 (−10.87-−1.65)	-2.784	0.0093*
Leptin	Group1 vs Group2	2.000 (3694-0.30)	2.417	0.0224*
	Group1 vs Group3	2.370 (3.58-1.15)	3.991	0.0004*
	Group2 vs Group3	4.370 (5.83−2.90)	6.092	< 0.0001*
PPD	Group1 vs Group2	-0.280 (−0.65-−0.09)	-1.151	0.1363
	Group1 vs Group3	-5.330 (−6.33-−4.31)	-10.878	< 0.0001*
	Group2 vs Group3	-4.80 (−5.83-−3.75)	-9.525	< 0.0001*
GI	Group1 vs Group2	-1.750 (−2.03-−1.46)	-12.574	< 0.0001*
	Group1 vs Group3	-2.040 (−2.28-−1.78)	-16.585	< 0.0001*
	Group2 vs Group3	-0.290 (−0.63-−0.05)	-1.725	0.0926
PI	Group1 vs Group2	-2.160 (−2.54-−1.76)	-11.216	< 0.0001*
	Group1 vs Group3	-1.910 (−2.11-−1.70)	-18.903	< 0.0001*
	Group2 vs Group3	0.250 (0.67-0.17)	1.196	0.2391

*Statistically significant at P<0.05; p – Probability; Independent sample t-test; CI - Confidence interval, PPD – Pocket probing depth; GI – Gingival index; PI – Plaque index

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