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Comparison of salivary level of leptin in chronic periodontitis patients and healthy controls

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Objectives Periodontal disease is an inflammatory condition of the tooth-supporting structures. Leptin is a hormone produced by the human body under different circumstances such as infection. It affects the production of cytokines, phagocytosis and the inflammation process. This study aimed to compare the salivary level of leptin in chronic periodontitis (CP) patients and healthy controls.

Methods In this case-control study, saliva samples were collected from 43 subjects including 22 CP patients and 21 healthy controls. The salivary level of leptin was determined using the ELISA. Data were analyzed by the independent t-test.

Results Despite the presence of leptin in the saliva of CP patients and healthy controls, no significant difference was noted in its salivary concentration between the two groups ($p>0.05$).

Conclusion The salivary level of leptin in CP patients was not significantly different from that in healthy controls. Further studies with larger sample size are required to confirm the results of this study.

Keywords Leptin; Saliva; Chronic Periodontitis

Introduction

Chronic periodontitis (CP) is a chronic inflammatory condition with a bacterial origin. It occurs as the result of an imbalance between the bacteria present in dental biofilm and the host inflammatory immune response.[1] Destruction of periodontal tissue subsequently occurs as the result of release of pro-inflammatory mediators such as interleukin 1B, tumor necrosis factor alpha, and prostaglandin E2. These mediators cause destruction of periodontal fibers as well as bone resorption.[2] Assessment of the level of biomarkers in the saliva, serum, blood and gingival crevicular fluid (GCF) has the potential for detection and diagnosis of periodontal disease independent of the clinical and radiographic findings.

Saliva is a biological fluid that contains a number of biomolecules. It is easily accessible and enables the assessment of the level of biomarkers that are constantly present in the saliva and those entered into the saliva from the blood circulation of the gingival tissues due to the presence of a systemic condition.[3-5] Leptin is a non-glycosidic hormone, which is produced in large amounts by the adipocytes[6,7] and in smaller amounts by the placenta[8], gastrointestinal epithelium[9], T-cells[10], osteoblasts[11] and intralobular duct cells of the major salivary glands.[12] They have properties similar to those of cytokines in the process of inflammation.[13] The main role of leptin is to balance the energy in the human body. It also plays a role in thermal regulation of the body, bone metabolism, process of inflammation and host defense mechanisms.[14] Evidence shows that leptin helps in higher production of cytokines and phagocytosis by the macrophages.[15] Some studies have reported lower concentration of leptin in the GCF and serum of patients with CP compared to healthy controls.[16-18] Considering the confirmed role of adipokines such as leptin in resolution of inflammation, this study aimed to assess and compare the concentration of leptin in the saliva of CP patients and healthy controls using the ELISA.

Materials and Methods

This case-control study (Ethics Number: IR.SBMU.RIDS.REC.1395.304) evaluated 43 subjects (20 males and 23 females, aged between 22 to 60 years) including 22 patients with CP and 21 periodontally healthy controls. All subjects signed informed consent forms prior to participation in the study. Periodontal patients were selected according to the criteria set by the American Academy of Periodontology in 1999. The patients had to have a minimum of two teeth with pocket depth≥5 mm, clinical attachment loss≥4 mm and positive bleeding on probing. The healthy controls had gingival index<1 mm, probing pocket depth<3 mm and no clinical attachment loss.

Sample collection:

Unstimulated saliva samples were collected from all individuals between 10 a.m. to 12 p.m. The subjects were requested to refrain from eating and drinking for a minimum of 2 hours prior to sample collection. They were asked to rinse their mouth with water for one minute prior

to saliva collection. The oral cavity was then examined to ensure absence of debris. After 15 minutes, the subjects were requested to swallow their saliva and then spit into sterile test tubes every one minute for five minutes. The saliva samples were then centrifuged with 4000 g for 15 minutes at 4°C on the same day to separate the viscous portion of the saliva from the particles. The supernatant was removed by a sampler, transferred to microtubes (200 µL in each tube) and stored at -70°C until the experiment.

Detection of leptin:

An ELISA kit was used to determine the salivary concentration of leptin. All procedures were performed according to the manufacturer's instructions. The optical density was measured at 405 nm (the reference wavelength was 630 nm). The salivary concentration of leptin was determined by comparing the mean optical density of samples with the standard curve.

Statistical analysis

Data were analyzed using SPSS version 22 (SPSS Inc., IL, USA). The salivary concentration of leptin was compared between the two groups using the independent t-test. $p<0.05$ was considered statistically significant. The salivary concentration of leptin was reported as the mean and standard deviation values.

Results

Mean value of salivary concentration of leptin are shown in table1 and Fig 1. Despite the presence of leptin in the saliva of CP patients and healthy controls, the difference in the mean salivary level of leptin was not significant between CP patients and healthy controls (p=0.141).

Table 1- Salivary Leptin levels of study groups

Sample	Healthy (Group1)	periodontitis (Group 2)	
	Mean (SD)	Mean (SD)	p-value
Leptin (ng/ml)	0.17±0.2	0.32±0.06	0.141

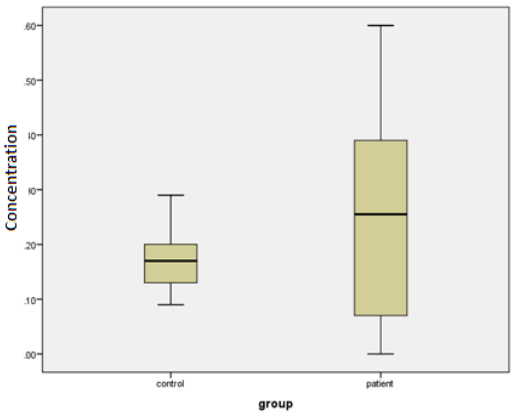

Figure 1- Comparison of salivary levels of leptin in study groups

Discussion

Oral cavity has several defense mechanisms that prevent entry of pathogens into the human body. Saliva and GCF are the main defense systems in the oral cavity since they contain several defense molecules that play a protective role. A high number of protective proteins and cytokines are found in the saliva, which play a role in innate and acquired immunity as well as inflammatory mechanisms.[19] Leptin is a peptide hormone that not only protects the adipocytes and regulates the metabolism, but also affects the endothelial, macrophage and T-cells and plays an important role in body defense via humoral and cellular immunity.[20] Leptin, present in the oral cavity as a cytokine, can decrease the secretion of mucin from the salivary glands. This reduction in mucin decreases the activity of bacteria. This is important considering the role of bacteria in periodontitis.[21] Moreover; leptin plays an important role in bone metabolism. It stimulates osteoblasts and induces their differentiation into mature bone cells with osteogenic potential. This is particularly important since bone loss is a common complication of CP.[22-24] Leptin acts as an inflammatory cytokine and triggers the immune system. Thus, its concentration often changes at the time of infection and inflammation. Periodontal disease is an inflammatory infectious condition, and bacteria such as Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis are involved in its occurrence. Thus, it is expected that periodontal disease changes the level of leptin.[25-28]

Considering the available information regarding leptin, we hypothesized that the salivary level of leptin would decrease in CP patients. However, our study failed to find any significant difference in the salivary level of leptin between CP patients and healthy controls ($p>0.05$). The salivary level of leptin had a wide variation in our CP group ranging from 0.02 ng to 0.51 ng. Although the mean value in patients (0.23) was higher than that in the control group, since the standard deviation was also high (0.18), the difference between the two groups did not reach statistical significance. The salivary level of leptin in the control group ranged from 0.10 ng to 0.26 ng. The mean value was 0.17 and the standard deviation was 0.05. Such variations in the salivary level of leptin can be due to unknown systemic conditions, although we excluded the patients with systemic diseases. It may also be due to the variations in the host immune response in different individuals. Khorsand et al. (2016) reported that the salivary level of leptin in 16 patients with aggressive periodontitis was lower than that in healthy individuals.[29] In their study, patients had aggressive periodontitis and the microorganisms involved in aggressive periodontitis are different from those involved in CP. Also, aggressive periodontitis has a much faster pace than CP. In the study by Sabir and Ahmed (2015) the salivary level of leptin in healthy controls was higher than that in CP patients, which was different from our finding.

However, the difference in salivary level of leptin between CP patients and healthy controls was very small (0.16 ng) in their study.[30] Purwar et al. (2015) indicated that the salivary level of leptin in CP patients was lower than that in healthy controls.[31] Difference between their results and ours may be due to the use of a different ELISA kit with lower accuracy in their study compared to ours. Also, racial differences in host response may explain the variability in the results.

The role of inflammatory biomarkers present in the saliva in diagnosis of periodontitis has attracted attention in the recent decades. However, search of the literature by the authors yielded only one previous study comparing the salivary level of leptin in CP patients and healthy controls. Saliva is easily accessible and its analysis for biomarkers is much easier than the assessment of biomarkers in the serum. This technique is cost-effective and yields valuable results.

Conclusion

According to the obtained results, leptin was present in the saliva of both CP patients and healthy controls and no significant difference was noted in its concentration between the two groups. Further studies with larger sample size are required to confirm the results of this study.

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