Research Article

Comparison of salivary beta-defensin-2 levels in chronic periodontitis with or without diabetes

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ABSTRACT

Background: Chronic periodontitis (CP) is a common disease of an oral cavity consisting of chronic inflammation of the tissues. Patients with diabetes have increased incidence and severity of periodontal disease has been shown in earlier studies. **Objective:** The objective of this study was to study and compare the salivary levels of human beta-defensin (HBDF) protein concentration of healthy patients and people with gingivitis and CP. The different protein concentrations are not related to the frequency of periodontal pathogens, and it has a higher impact on salivary HBD-2 levels than bacteria. **Materials and Methods:** A total of 89 patients are enrolled in this study in which 31 periodontally healthy, 31 with CP with diabetes, and 27 with chronic diabetes. The HBD-2 ELISA kit will be obtained from bioarray technologies. The salivary BDF-2 will be quantified and evaluated using statistical analysis in one-way ANOVA. **Results:** The salivary HBD-2 protein concentration is higher in patients with CP compared with patients with diabetes. The salivary BDF-2 levels are higher in diabetic's patients with periodontitis than patients with non-diabetic periodontitis patients.

KEY WORDS: Beta-defensin-2, Chronic periodontitis, Diabetes, Periodontitis, Saliva

INTRODUCTION

Human beta-defensin-2 (HBDF-2) is a cysteine-rich cationic low-molecular-weight antimicrobial peptide stimulated by epithelial cells.[1] Chronic periodontitis (CP) is a common disease of an oral cavity consisting of chronic inflammation of the tissues. Patients with diabetes have increased incidence and severity of periodontal disease.[2] BDF levels in the CP with diabetes were found to be significantly decreased when compared with CP without diabetes, suggesting that BDF levels play an important role. Antimicrobial peptides, like HBD, play an important role in the epithelial innate defense response.

The aim of the present study was to investigate the quantitative expression of HBD-2 in inflammatory gingival diseases. Gingival biopsies were obtained from patients with healthy gingiva, patients with gingivitis, and patients with periodontitis. The clinical diagnosis was verified by histology. Gingival tissues were used for RNA extraction followed by reverse transcription. Gene expression was quantified by real-time polymerase chain reaction (normalization with GAPDH). Comparing the tissues with different clinical stages of health and disease, no significant differences in mRNA expression were found for any of the BDF studied. Similar levels of expression were found in healthy gingiva, whereas in gingivitis samples, there was a significantly higher expression of HBD-2 compared to HBD-1 ($P = 0.004$) and HBD-3 ($P = 0.016$). Likewise, in periodontitis samples, HBD-2 expression was significantly higher than HBD-1 ($P = 0.016$); however, HBD-2 expression was comparable to HBD-3. In the results of the present study showed a differential expression of human β-defensins (HBD-1, 2, and 3) in tissues with inflammatory gingival disease.

This study compares the salivary levels of HBD-2 protein concentration of healthy patients and patients with gingivitis and CP and correlates these levels with the presence of period onto pathogens. The antimicrobial activity is very likely based on their ability to form aggregates within the pathogen's

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membrane, thus serving as pores and leading to an osmotic shock.[3] Members of the defensin family are highly similar in their primary structure. All defensins share a β-sheet motif stabilized by intramolecular disulfides bridges. The α- and β-DFs differ in the position and connection of the conserved cysteine residues.

MATERIALS AND METHODS

The present study was conducted on subjects reporting to the Department of Periodontology at Saveetha Dental College and Hospitals, SIMATS, Chennai, Tamil Nadu, India. Sample size was determined based on the pilot study performed. A sample of 20 was determined as the minimum in each group to estimate and to calculate for the statistical analysis. Therefore, the study sample included a total of 40 subjects who were divided into the following groupings.

Group I: CP without diabetes ($n = 20$; 14 males and 6 females).

Group II: CP with diabetes ($n = 20$; 12 males and 8 females).

Sample Collection

Participants were instructed not to eat, drink, chew gum, or brush teeth for at least 30 min before sampling. Coronary heart disease patients were instructed to refrain from taking any medication 24 h before sampling based on the physician's consent. Saliva was collected from patients between 9 and 11 a.m. The patients were asked to sit comfortably and were instructed to allow the saliva to pool in the bottom of their mouth and drain it into plastic containers. The collected sample was centrifuged at 3000 rpm for 5 min, and the supernatant was collected and used for the assessment of salivary BDF levels by ELISA methods.

HBDF Elisa Kit

This kit is used to assay HBDF on the basis of the biotin double-antibody sandwich technology. This ELISA kit takes one-step method with which solutions do not need diluting because we simplify the dilute process by our laboratory techniques. This kit is for research only and is not for use in diagnostic procedures. This kit is used to assay the β-DFs in the sample of human's serum, blood plasma, and other related biological liquid.

Assessment of Salivary BDF-2 Levels by ELISA Methods

Procedure

Reagents and samples were prepared. Added prepared samples and standards together with secondary antibody labeled with biotin and ELISA solutions. Let them react for 60 min at 37°C. Then washed the plate 5 times. Add chromogen solution A and B. Incubate for 10 min at 37°C for color development. After that, stop solution was added. Read the OD value within 10 min. Calculated values by making concentration of standards the abscissa and OD value the ordinate. Draw the standard curve on the coordinate paper. According to the OD value of the sample, locate its corresponding concentration (which is the concentration of the sample) or calculate the linear regression equation of standard curve according to the concentration of the standard and the OD value. Then, substitute with the OD value of the sample to calculate its concentration.

Statistical Analysis

Using the Statistical Package for the Social Sciences version 12 (SPSS Inc., Chicago, USA 1999), data were analyzed. The patient's salivary SCN and clinical parameters were analyzed using one-way ANOVA and Kruskal–Wallis tests and in between comparison of all the four groups was carried out using Schiff's multiple comparison tests. $P < 0.05$ was considered as statistically significant.

RESULTS

Assessment of Salivary BDF-2 Levels in CP with or without Diabetes

In this study, BDF-2 levels in the CP with diabetes were found to be significantly decreased when compared with CP, suggesting that BDF levels play an important role [Table 1 and Figure 1].

DISCUSSION

The oral epithelium with its moist surface is extremely prone to microbial colonization. Thus, epithelial cells are in close contact with a wide variety of microorganisms and their metabolic products. Nevertheless, no infections occur under normal conditions. The epithelial compartment not only provides a physical barrier to microorganisms but also plays an important role in host defense.[3,4,5] Bacteria and yeasts surmount the epithelial barrier by disrupting the cell layer. Host targets being attacked by pathogens are mainly cell-cell junctions, for example, desmosomes, adherents, and tight junctions.[5] Desmoglein 1, a desmosomal protein mediating cell-cell adhesion, is the specific substrate for the proteolytic exfoliative toxin A (ETA) produced by *Staphylococcus aureus*, thus causing loss of cell adhesion. In addition to the physical barrier, the epithelium has established a chemical defense mechanism by expressing antimicrobial peptides. These peptides exhibit a broad range in specificity against Gram-positive and Gram-negative bacteria, as well as against yeasts and enveloped viruses. Defensins are positively charged antimicrobial peptides with molecular weights ranging from 3.5 to 6.5 kDa. A high number of basic amino acid residues are responsible

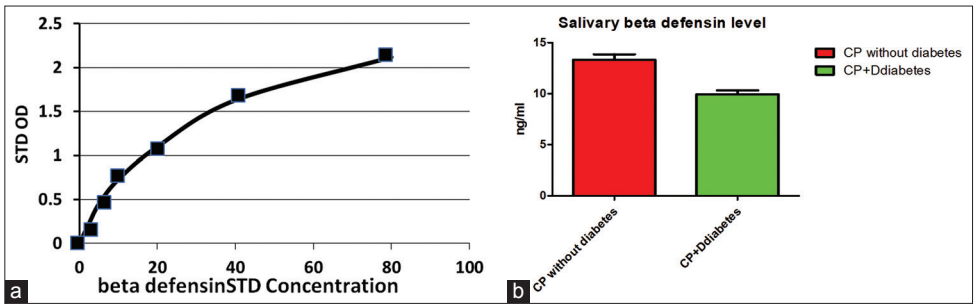

Figure 1: (a and b) Salivary beta-defensin-2 levels in chronic periodontics with or without diabetes. Each bar represents mean ± SEM of three observations from each sample. Significance at $P < 0.05$

Table 1: Chronic periodontitis without diabetes (ng/ml) chronic periodontitis with diabetes (ng/ml)

Chronic periodontitis (ng/ml) without diabetes	Chronic periodontitis with diabetes (ng/ml)
13.55828	12.29448
17.74233	11.46012
11.82822	7.092025
10.72393	7.656442
13.91411	9.865031
12.83436	9.006135
14.79755	8.957055
16.56442	10.11043
13.10429	12.31902
14.74847	10.38037
8.539877	9.276074
11.73006	11.8773
16.09816	10.45399
12.83436	12.83436
15.0184	9.865031
14.67485	11.46012
9.276074	7.092025
10.38037	7.656442
12.83436	10.11043
15.09202	8.736196

for their cationic feature. The antimicrobial activity is based on their ability to form aggregates within the pathogen's membrane, thus serving as pores and leading to an osmotic shock[3] Members of the defensin family are highly similar in their primary structure. All defensins share a β-sheet motif stabilized by intramolecular disulfide bridges. The α- and β-DFs differ in the position and connection of the conserved cysteine residues. HBD-2 was isolated from psoriatic skin.[10] It possesses a strong bactericidal effect on Gram-negative bacteria and a high antimycotic potency, but only a weak bacteriostatic activity against Gram-positive *S. aureus*.[6] In keratinocyte cell culture, hBD-2 was upregulated by exposition with TNF-α and bacteria.[7] This observation could also be confirmed for the expression of HBD-3. It has been shown in various epithelial tissues that expression of hBD-2 depends on the stage of inflammation.[5,8] In contrast, oral epithelial cells express hBD-2 even under non-inflamed conditions.[5,9] This has been suggested to be the result of exposure of the tissue to commensal non-pathogenic bacteria.[5,9] *In vitro* analysis of gene expression demonstrated a transcriptional upregulation of BDF-2 in cultured epithelial cells after treatment with bacteria or bacterial supernatant.[10] In addition, interleukin-1 and interleukin-1 receptor antagonist (IL-1 and IL-1RA) play important roles as interfering factors in transcriptional regulation of β-DFs[11] IL-1 alone activates gene expression of HBD-2, whereas IL-1RA has been shown to block this effect. In periodontitis, hBD-2 expression was significantly higher than that of hBD-1, while no statistically significant difference was observed for hBD-2 compared with hBD-3. The first stage of clinical oral inflammation (gingivitis) shows an early upregulation of hBD-2. Periodontitis leads to gene activation of hBD-3, while hBD-2 is still highly expressed. In summary, the expression of HBDFs was found to be similar in healthy gingiva, while the inducible defensin hBD-2 showed a higher transcriptional level in tissue with inflammatory disease.

CONCLUSION

The salivary BDF-2 level is higher in diabetic patients with periodontitis than patients with periodontitis without diabetes. This study clearly shows that BDF plays a significant role in periodontal health, and hence, assessing salivary BDF can be a biomarker for the periodontal diseases by non-invasive method.

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