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Original Article

The influence of smoking on the levels of matrix metalloproteinase-8 and periodontal parameters in smoker and nonsmoker patients with chronic periodontitis: A clinicobiochemical study

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ABSTRACT

Aims: Even though worldwide evidences tend to prove that smoking adversely influences periodontal health, there are few studies demonstrating the effect of levels of salivary matrix metalloproteinase-8 (MMP-8) in smokers. This study aimed to compare and quantify the levels of MMP-8 in smokers and also to evaluate the effect of smoking on periodontal indices in smokers and nonsmokers with chronic periodontitis.

Methods: A total number of 60 subjects were selected for the study and were divided into three groups: group I, healthy nonsmoking subjects; group II, nonsmoking patients with chronic periodontitis; group III, smoking patients with chronic periodontitis. Each group consisted of 20 subjects. Saliva sample was collected for the estimation of MMP-8 by enzyme-linked immunosorbent assay (ELISA) method using Quantikine human total MMP-8 immunoassay kit.

Results: The levels of the salivary MMP-8 of group III was highest followed by group II and group I, the least. The other periodontal indices, viz. plaque index (PI), probing pocket depth (PPD), clinical attachment level (CAL), were highest for group III, except for gingival index (GI).

Conclusion: This study suggests that MMP-8 is involved in periodontal destruction associated with smoking. Additionally, smoking exerts disastrous effects on immune response and can affect the pathogenesis of disease; hence, smoking results in increased severity of periodontal destruction.

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1. Introduction

Smoking is now considered as one of the most important risk factors in the development and progression of periodontal disease.[1,2] Smoking-induced changes in neutrophil reactive oxygen species (ROS) within periodontal tissues play a role in increasing oxidative stress and reducing innate immune responses to periodontal pathogens and their products, thereby contributing to periodontal tissue damage and disease progression.[3] It is also associated with increase in pocket depths, alveolar bone loss and increased rate of tooth loss.[4,5] Cigarette smoking affects neutrophil function which are major source of matrix metalloproteinase-8 (MMP-8).[6,7] Cigarette smoke contains a mixture of chemicals having carcinogenic potential, as well as various stable and free radicals species, and ROS that causes cellular oxidative stress, which is a factor for many smoking-related diseases. This smoke-induced oxidative stress has the potential to alter the MMP activity. It may increase the MMP expression via activation of inflammatory transcription factors.[8]

Human MMPs are a group of 23 structurally related but genetically distinct endopeptidase enzymes that cleaves the internal peptide bonds of proteins. They have the capacity to degrade practically all extracellular matrix, basement membrane matrices, and their components.[9] There are several groups of MMPs: Collagenase (MMPs 1, 8, 13), Gelatinase (MMPs 2, 9), Stromelysin (MMPs 3, 10, 11), and membrane-associated MMPs. Among these, MMP-8 (collagenase-2) has been considered as a key marker in chronic periodontitis, and is synthesized by differentiating granulocytes in the bone marrow and stored in specific granules of circulating neutrophils. It is detectable in saliva and gingival crevicular fluid (GCF).[10] It exhibits a unique ability to decompose Type I and III collagens,[11] and is highly correlated with bleeding on probing, attachment loss, and probing pocket depth (PPD). In healthy condition, the periodontal ligament apparatus is shielded from MMP-mediated proteolytic attack by tissue inhibitors of metalloproteinase (TIMPS). In pathologic conditions like chronic periodontitis, the amount of TIMP is low and thus inadequate to inactivate the elevated levels of MMPs, leading to aberrant connective tissue destruction. Hence, evaluating the level of MMP-8 will enable us to understand how tobacco smoke-induced alteration in MMP-8 levels may contribute to the increased susceptibility of periodontitis in smokers.

2. Methods

2.1. Patient selection

The present study was conducted in the Department of Periodontology of Dr. Z.A Dental College, Aligarh Muslim University, Aligarh, India. This study was approved by the Institutional Review Board. All patients were informed about the study and informed consent forms were signed in full accordance with the declaration of Helsinki.

2.2. Groups

A total of 60 study participants, in the age range of 35–55 years and having at least 20 teeth were enrolled into the study and were further divided into three groups: group I: healthy nonsmoking subjects, group II: nonsmoking patients with chronic periodontitis, and group III: smoking patients with chronic periodontitis.

2.3. Inclusion criteria

Group I: Periodontally and systemically healthy individuals with no apparent signs of clinical inflammation.

Group II: Subjects who never smoked and were systemically healthy but clinically diagnosed with moderate to severe chronic periodontitis (e.g. at least two or more interproximal sites with clinical attachment level (CAL) of ≥4 mm, or two or more interproximal sites with PPD of ≥5 mm, not on the same tooth).[12]

Group III: Subjects who had smoked ≥1 pack/day for at least past 10 years and were systemically healthy but clinically diagnosed with moderate to severe chronic periodontitis (e.g. at least two or more interproximal sites with CAL of ≥4 mm, or two or more interproximal sites with PPD of ≥5 mm, not on the same tooth).

3. Exclusion criteria

Pregnant and lactating women, patients with acute or chronic medical disorders, patients under any medication for the past 3 months, patients who had undergone any surgery in past 6 months, and subjects undergoing orthodontic treatment were not included in the study.

3.1. Periodontal assessment

Periodontal disease status of all patients was evaluated by the measurement of gingival index (GI),[13] plaque index (PI),[14] pocket probing depth, and CAL, using the same periodontal probe (University of North Carolina-15 probe (UNC-15), Hu-Freidy's, USA) and conducted by the same examiner to avoid bias. Clinical periodontal examination was performed by a trained and calibrated investigator who was masked to groups. The overall k for intra-examiner reliability was 0.75.

3.2. Method of collection of saliva sample

The clinical parameters described above were recorded and saliva samples necessary for the estimation of MMP-8 were taken from all groups. Subjects were told not to eat or drink 2 h prior to the examination. They were then asked to rinse and 3 ml of unstimulated whole expectorated saliva was collected from each subject and placed into sterile 5 ml saliva collecting tubes according to method described by Navazesh.[15] The collected saliva was centrifuged immediately to remove cell debris (1000 × g for 10 min at 4 °C). Then the supernatant was removed and sample was stored in small aliquots at −80 °C until analysis. Salivary MMP-8 levels were estimated by using Quantikine human total MMP-8 immunoassay kit using

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