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Evaluation of the Correlation between Tryptase Positive Mast Cells and Chronic Periodontitis using Immunohistochemistry

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Abstract

Background: Mast cells are mobile, bone-marrow-derived, granule-containing immune cells that are found in all connective tissues and mucosal environments. Mast cells are able to phagocytosis, process and present antigens as effectively as macrophages. Bacterial plaque has been implicated as the primary etiologic factor in inflammatory periodontal disease, but several studies have recently focused on the role of the immune system cells in periodontal disease.

Aim: The aim of the present study was to determine the relationship between tryptasepositive mast cells and chronic periodontal disease.

Methods: Seven cases of smoker chronic periodontitis, seven cases of nonsmoker chronic periodontitis, seven cases of smoker clinically healthy gingival tissues and seven cases if nonsmoker clinically healthy gingival tissues were obtained from patient undergoing periodontal surgery in Rasht private dental clinics. Samples fixed in 10% buffered formalin and stained with tryptase stain and observed under optical microscope.

Results: There is no significant difference between tryptase positive mast cells and chronicperiodontitis (P=0.829). However, a significant difference was seen between the number of tryptase positive mast cells in chronic periodontitis and non-smoker chronic periodontitis (P=0.012), similar to those in chronic periodontitis with systemic disease and chronic periodontitiswithout it (P=0.006).

Conclusion: considering the above findings which are in contrast withsimilar studies, more extensive studies with more precise methods are recommended in order to clarify the role of these mast cells in the pathogenesis of chronic periodontitis.

Keywords: Chronic periodontitis; Mast cells; Smoking

Introduction

Periodontal disease is a common disease that appears with periodontal ligament injury and bone loss, finally lead to missing of teeth. Scientists have implicated bacterial plaque as the initial cause of inflammatory periodontal disease, but recently several studies have emphasized on the role of the immune system in the development of periodontal disease[1]. Current documents indicate specific inflammatory and immune responses in different stages of periodontal disease, that includes invasion of immune cells and local cytokines and other mediators discharge[2].

Among the cells found in the periodontal tissues, mast cells have been detected in both healthy and inflamed gingiva, in different numbers at various sites[3]. Mast cells are tissue-resident immune cells that take part in a variety of allergic and inflammatory situations[4].

Mast cell's mediators are accumulated in secretory granules and released by degranulation or can be newly generated when mast cells are correctly activated[1].

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Mast cells are separated into connective tissue (CT) and mucosal phenotype based on their proteinase add-up. The CT phenotype owns both tryptase and chymase (MC (TC)), while the mucosal phenotype contains only tryptase (MC (T))[3].

Tryptase detection is a way to evaluating mast cells in the tissues, and it is the most stored mediator in the mast cell's granules that releases when the mast cell degranulates[5].

Given the importance of this issue, different outcomes and inadequate information about mast cell's role in creation or progression of periodontal disease this study aims to determinate the correlation between mast cells and periodontal disease. These results can be helpful in treatment and diagnosis of periodontal disease.

Method and Materials

In this experimental in vitro study that has been conducted in the form of a research project in Guilan University of medical sciences with scientific endorsement from research committee, 28 research samples were assessed.

These samples were contained, 14 people with periodontal disease and 14 healthy people, each group were divided into 7 smokers and 7 nonsmoker subset. The samples were taken from private health centers in Rasht and they should not be in specific hormonal status(pregnancy, maturation, postmenopausal), do not be on contraceptives or drugs that affect mast cells or periodontitis in last 2 months, have not allergies and does not existing acute inflammation, pain, pus exudation or tooth related infected lesion.

Existence of periodontitis, smoking, mast cell rate and existence of systemic disorders were evaluated in these samples. 14 biopsies from case group with more than 3-5 mm pocket depth were sampled by sub-margin cut, and the area between free gingival grooves to the end of cutting area was removed.

The control group was included 14 biopsies from patients under crown lengthening surgery or second stage of uncovered implant placement, underwent massacre treatment and health training from about a month ago.

Samples were fixed in neutral buffer of formalin (10%) and then preparation of paraffin blocks in a tissue processor started.

Two lam of each specimen for Haematoxylin-Eosin and IHC factor (tryptase) were provided. Provision of the specimens for lHC was performed on the basis of Master Diagnostic kit instruction.

Colored slides were assessed by a Japanese optical microscope with a magnification of 400. An average of three mast cells fields was considered for final rate of mast cells. Statistical analysis were performed by means of SPSS version22, Kobmogorovsmirnov, Independent T test, one-way ANOVA.

Results

In this experimental in vitro study after collecting and coding information's, SPSS utilized for analyzing. The specimens were classified to; chronic smoker periodontitis, chronic nonsmoker periodontitis, healthy smoker and healthy nonsmoker. Out of 28 samples, 17 were male and 11 were female.

The mean and the total number of mast cells in chronic smoker periodontitis was 10.57 ± 3.10 and 74 respectively (with maximum number of 15 and minimum number of 6), 20 ± 7.87 and 140 (max No. 26 and min No. 7) in chronic nonsmoker periodontitis, 14.28 ± 6.24 and 100(max No. 21 and min No. 5) in healthy smokers and 17.57 ± 0.80 and 123 (max No. 31 and min No. 3) in healthy nonsmokers.

Analyzing the data above with one-way ANOVA showed no significant difference.

Table 1: comparing the number of mast cells

disease	N	P
Chronic smoker periodontitis	7	0.184
Healthy smoker	7	
Chronic nonsmoker periodontitis	7	0.618
Healthy nonsmoker	7	
Chronic smoker periodontitis	7	0.012
Chronic nonsmoker periodontitis	7	
Healthy smoker	7	0.469
Healthy nonsmoker	7	

According to the table.1 T. test showed significant difference in comparison between chronic smoker periodontitis and chronic nonsmoker periodontitis mast cell's rate, and the other groups showed no differences.

Table 2: mast cells rate assessment with systemic status consideration

Systemic disease	N	P
Yes	12	0.059
No	16	
Chronic periodontitis (yes)	8	0.006
Chronic periodontitis (no)	6	
Healthy(yes)	4	0.767
Healthy(no)	10	

Based on the table.2 T. test indicated significant difference between mast cells rate of patient with chronic periodontitis and systemic disorder, and patient with chronic periodontitis and no systemic disorder, and no specific difference was observed with the ether groups.

Discussion

Periodontium is the supportive tissue of our dental structure, and periodontitis is the inflammation of periodontium that will destroy supportive structure of the teeth finally lead to teeth loss[6]. Inflammatory response is influenced in periodontitis by the bacteria, so it's useful to know the role immune cells and molecules[7]. In periapical discharge including macrophage, T cells, B cells and plasma cells, mast cells are so important because of their immune regulating properties[8].

Lead neutral proteases including Tryptase and Chymase are soluble executive responsible molecules of mast cells that release after stimulation and cell activation[1,9,10]. Tryptase have not been found in any other cells so it can be measured for mast cell activating diagnosis, because of its biologic effect in

tissue damages[4,5,11].

As for the importance of mast cell roles in development and progression of periodontitis and inadequate available knowledge, this study aims to evaluate the relation of mast cells and periodontitis.

In this study smoking or non-smoker and presence or absence of a systemic disease such as diabetes, heart troubles, blood pressure, blood problems, troubles of the joints, liver, thyroid and respiratory difficulties was considered which have not been surveyed in any other similar investigation.

Based on most studies prevalence and severity of periodontal disease is stronger in diabetics than nondiabetics with similar amount of plaque[6].

Also in women with periodontal disease and osteoporosis simultaneously, periodontal disease probably can be toughen up with osteoporosis[10].

Existence of various type of anemia in the patients cause generalized jaw osteoporosis, step ladder orientation in septum, changing the color of oral mucosa in the form of pruritus and jaundice, talent to neutropenia, glossitis and oral and oropharynx ulcer[6]. Additionally thrombocytopenia can cause self-hemorrhage.

Vitamin D deficiency also lead to reducing the density of supporting bone and loss of the trabecula[6].

Andrade et al.[8] in a 2016 investigation entitled Analysis of Tryptase-positive mast cells and 2 another enzymes in 3 periapical lesions, evaluated 3 periapical lesions with different degree of inflammation. However the comparison of periapical lesion was not possible with our study, but like our reviews their results do not show significant differences between different grades of inflammatory secretions.

As a contradictory result, in a 2014 survey Shiguang Hung et al. in China[9] offered that degranulated mast cell rate in healthy, moderate chronic periodontitis and severe chronic periodontitis reported respectively from the lowest to the highest. Perhaps this difference in outcome is due to the lack of systemic disease in their samples. Because systemic disease has a significant effect on the level of host immune response[6].

In an investigation carried out in 2011 by Dr Sushma S. Lagdive et al[1]. On correlation of periodontal disease and mast cells the average rate of mast cells in gingivitis associated with dental plaque in comparison with healthy periodontal and chronic periodontitis in comparison with healthy group significantly decreased. This outcome contradicts the present study that may be due to application of Tholuidine-blue and elimination of the patients with systemic disease[1].

In a 2013 study on correlation of mast cells and chronic periodontitis by Dr. Vahabiet et al.[12] an incremental connection between mast cells in area with chronic periodontitis than healthy areas was observed, which the reason of this difference in outcomes can be the elimination of general diseases and tobacco users, also despite our investigation the control group in this study included healthy persons and patients with gingivitis either.

Conclusion

Significant relationship in comparison of the rate of mast cells in chronic smoker periodontitis with mean and standard deviation 10.54+-3.10 and chronic nonsmoker periodontitis with 20+-7.87, also between tryptase-positive mast cell rate in patient with chronic periodontitis and systemic disorders with average and standard deviation 19.62+-6.84 and periodontitis without systemic disorders with 9.50+-3.50 can indicate the importance of more future studies specially in the field of mast cells, significantly tryptase-positive mast cells, in periodontitis destruction or the mechanism of the periodontitis defense.

Despite the various restrictions in this investigation, consideration of multiple influential factors can get more accurate results.

Research constraints

-lack of existing samples according to the study in Rasht
-Unavailability of complete patient information

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