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Comparative Evaluation of CD4/CD8 Ratio and Absolute Eosinophil Count in Chronic Periodontitis Patients versus Healthy Subjects -A Clinico-Biochemical Study

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Abstract: Periodontal disease results from an interaction between the host's defense mechanisms and the microorganisms that constitute the dental plaque biofilm. Therefore, the progression and severity of the disease are strongly modulated by dysfunctions in cellular immune responses, particularly, T cell responses. These dysfunctions can be reflected through blood parameters such as CD4:CD8 ratio, absolute eosinophil count and complete blood count, measurement of which can provide insight into the disease process. The aim of the present study is to comparatively evaluate the differences in CD4:CD8 ratio in the peripheral blood, absolute eosinophil count and complete blood picture in healthy subjects and chronic periodontitis patients. 10 subjects with clinically healthy periodontium and 10 patients with moderate to severe chronic periodontitis were recruited from the outpatient department of Periodontics, Sri Sai College of Dental Surgery, Vikarabad. Following a comprehensive periodontal examination, 5 mL of non-fasting blood was obtained by venipuncture from each participating subject. Blood samples was processed and analyzed for values of CD 4: CD 8 ratio, absolute eosinophil count, and a complete blood picture that includes Hemoglobin, RBC count, MCV, MCH, MCHC, Total WBC count, Differential leukocyte count, and platelet count. Results were evaluated with appropriate statistical analysis. The mean values of CD8 counts were higher in test group. The mean values of CD3, CD4, CD4/CD8 ratio, and AEC were higher in control group. Although there were differences in the mean values, the difference was not statistically significant. The immune responses that contribute to the pathogenesis of periodontal diseases are complex and variable. Many of these responses may be restricted to the site of infection. Smaller sample size of the present study, warrants further studies with larger samples to confirm the results of the present study.
Keywords: chronic periodontitis, CD 4: CD 8 ratio, absolute eosinophil count

INTRODUCTION

Periodontitis is a multifactorial disease caused mainly due to imbalance between plaque microbial virulence factors and host immune system. Chronic periodontitis is a slowly progressing form of the disease. T cells play a crucial role in immunoregulation as they regulate both cell mediated and humoral immune responses. The progression and severity of periodontal disease are strongly modulated by dysregulation of cellular immune responses, particularly, T cell responses [1]. These dysfunctions can be reflected through the measurement of CD 4: CD 8 ratio. Previous studies [2-5] that attempted to evaluate the changes of T cell subsets in the peripheral blood of chronic periodontitis patients yielded disparate and inconclusive results.

Eosinophils are recognized as the primary effectors of allergic responses. Recent evidence suggests the role of eosinophils as antigen presenting cells, and in immunomodulation by enhancing the Th 1 response [6]. Some studies also disclosed that Major Basic Protein (MBP) which is one of the secretory products of eosinophil, the alternative and classical pathways, induce non-cytotoxic activation of neutrophils[7]. Taking this into consideration, it is reasonable to hypothesize that an altered eosinophil activity can be expected in chronic periodontitis patients. Such alterations can be deciphered by the measurement of absolute eosinophil count. Lack of evidence in this field provided an impetus to carry out this study. To our knowledge this is one of the first studies which attempted to decipher differences in

absolute eosinophil count in healthy subjects and chronic periodontitis patients.

The purpose of the present study is to evaluate the differences in CD 4: CD 8 ratio, absolute eosinophil count in peripheral blood in healthy subjects and chronic periodontitis patients.

MATERIALS AND METHODS

Study population

In the present study, 10 subjects with clinically healthy periodontium and 10 patients with moderate to severe chronic generalized periodontitis were recruited from the Outpatient Department of Periodontics, Sri Sai College of Dental Surgery, Vikarabad. All the subjects were explained about the study procedure and a written informed consent was obtained. Ethical clearance was obtained for this study from the Institutional Ethics Committee.

Inclusion criteria

Chronic periodontitis patients who had atleast ≥ 14 teeth, and >50% sites with PD≥5mm and CAL≥6mm[8]. Clinically healthy subjects who had Modified Gingival Index scores ≤ 1 and clinical attachment loss (CAL)=0.

Exclusion criteria

Subjects who had a systemic disease, who are current or ex-smokers, who received periodontal treatment within the previous year, who received antibiotic and anti-inflammatory drugs in the past 3 months, pregnant and lactating women were excluded from the present study.

Clinical examination

A comprehensive periodontal examination was performed which included the measurement of Plaque index (Loe and Sillness) [9], Modified Gingival Index Lobene *et al*. [10], probing pocket depth, and clinical attachment loss. Probing pocket depth and clinical attachment loss was measured at 6 sites of the teeth i.e mesial, distal and middle sites of facial and lingual surfaces) using a William's periodontal probe. All the examinations were performed by a single trained examiner.

Sample collection and processing

5 mL of peripheral blood was obtained by standard venipuncture using EDTA containing vacutainer tubes (B&D system) from each participating subject. The blood samples were processed and analyzed for values of absolute CD 4 count (normal value in peripheral blood is 25-29%), absolute CD 8 count (normal value in peripheral blood is 19-48%), CD 4: CD 8 ratio and absolute eosinophil count,

Absolute CD4, CD8 counts and CD4/CD8 ratio was determined using a flow cytometer (BD FACS caliber system, BD Biosciences). Flow cytometer is a device that illuminates cells as they flow in front of a light source and measures the antigenic properties of cells with the help of antibodies labelled with different fluorochromes. Absolute eosinophil count was established using the eosinophil counting chamber.

STATISTICAL ANALYSIS

Acquired data was analyzed statistically using SPSS software (Version 20.0. Armonk, NY: IBM Corp). The values are expressed in terms of mean ± SD. Inter-group differences were analyzed using unpaired t test. Any p value less than 0.05 was considered statistically significant.

RESULTS

The present study evaluated the CD4/CD8 and absolute eosinophil count in healthy subject versus chronic periodontitis. The mean values of CD8 counts were higher in test group. The mean values of CD3, CD4, CD4/CD8 ratio, and AEC were higher in control group (Table 1). Although there were differences in the mean values, the difference was not statistically significant.

Table-1: Intergroup comparison between parameters

Parameter	Test (mean±SD)	Control (mean±SD)	P value
CD3 count	1502.13±482	1528.38±601.78	0.915
Absolute CD4 count	755.79±194.2	795.42±360.38	0.763
Absolute CD8 count	640.27±299.78	588.40±260.21	0.684
CD4:CD8 ratio	1.36±0.5	1.47±0.82	0.714
Absolute eosinophil count	355±187.74	418±257.58	0.540

DISCUSSIONS

Evidence suggests that most of the destruction of hard and soft tissues in periodontal disease is mediated by the immune-inflammatory reactions triggered by the microbial virulence factors of dental plaque. Thus, the host response has a crucial role in the periodontal disease progression. It has often been discussed whether local periodontal inflammatory reactions could have systemic effects. The present study attempted to explore the differences in the host immunoregulatory mechanisms between the chronic periodontitis patients and healthy individuals, by means

of CD4/CD8 ratio and absolute eosinophil count in the peripheral blood.

Numerous studies have been undertaken concerning the cell count and function of lymphocytes in peripheral blood of periodontitis patients and reported conflicting results. In the present study no statistically significant difference was observed in CD4/CD8 ratio among healthy individuals and chronic periodontitis subjects.

These results are consistent with the findings of Okada *et al.* [2] study who reported no significant differences in the T lymphocyte subtypes in periodontal health and disease.

Zafiropoulos *et al.* [3] showed that CD4/CD8 ratio was increased in the test group for men as well as for women, although no significant differences were evident. They suggested that local inflammatory reactions and immunoregulatory dysfunction are limited to the periodontium in the patients with advanced periodontitis, with no significant quantitative effects on the peripheral lymphocyte subpopulations.

Buduneli *et al.* [4] and Emingil *et al.* [5] analyzed the differences in T lymphocyte subsets in periodontitis patients and, reported that no significant difference was observed between the study groups with regard to the relative counts of CD4, CD 8 and CD4/CD8 ratios. In addition, Petit et al (2001)[11] showed that the numbers of CD3 +, CD4 + and CD8 + T cells as well as the CD4/CD8 ratio, and the proliferative capacity of T cells, were not different between healthy and periodontitis subjects.

In a recent study, Sigusch *et al.*[12] evaluated the differences in T lymphocyte subsets in the gingival crevicular fluid and reported that the mean CD4+/CD8+ ratio was reduced in chronic periodontitis which was not significant. Another study by Cifcibasi *et al.* [13] determined the phenotypic profile of peripheral mononuclear cells in chronic as well as aggressive forms of periodontitis and found increased CD 8 cells when compared to healthy subjects.

Erciyas *et al.*[14] evaluated the changes in T lymphocyte subsets in chronic periodontitis patients after periodontal treatment using biopsy samples. They found that the CD 4/CD8 ratio decreased post curettage and post flap operation and suggested that local immune responses are poor in chronic periodontitis patients, and also emphasized on the significant role of CD 4, CD 8 cells in the pathobiology of periodontitis.

There are reports of altered CD4/CD8 ratio in aggressive periodontitis. Depressed CD4/CD8 ratios were reported by Kinane *et al.*[15] while others reported elevated levels of CD4/CD8 ratios[16-18]. Overall there is no consistent alteration of CD4/CD8 ratio in either chronic or aggressive forms of periodontitis which can attributed to variations in patient groups.

Localization of both CD4 and CD8 T cells in periodontal tissues has been confirmed by many studies[19]. Some researchers analyzed the cells extracted from human gingival tissues. Among them few studies reported decreased CD4/CD8 ratios in periodontitis lesions compared with gingivitis or healthy sites[20]. Whereas, some reported an increased CD4/CD8 ratio [21].

Some researchers found decreased CD4/CD8 ratios in periodontitis lesions compared with healthy sites or peripheral blood [22, 23]. This decrease appears to be due to both a reduction in the number of CD4+ cells and a slight increase in the number of CD8+ cells.

It is possible to measure the CD4 and CD8 cells in peripheral blood using a variety of techniques. Flow cytometry was used in this study because this method is cost effective, objective, faster, and the physical and biological features of the cells can be accurately and quantitatively measured using this technique.

Alterations in the absolute eosinophil count are commonly seen in allergic conditions, parasitic infections and in early stages of cushing's disease. As there is increased understanding regarding the potential role of eosinophils in the pathogenesis of periodontitis, the present study considered to assess the changes in absolute eosinophil count in chronic periodontitis compared to a healthy condition?

The results indicated that there is no statistically significant difference in the absolute eosinophil count in chronic periodontitis and healthy subjects. As there are no previous studies analyzing the absolute eosinophil count to evaluate the quantitative differences in eosinophils in healthy and periodontitis subjects a direct comparison could not be made.

The limitations of this study include

- Small sample size as a result of which significance in variation could have perhaps been undetected.
- A longitudinal analysis of these cells following the history of periodontal disease could have provided definitive results.

CONCLUSION

Within the limitations of this study, it can be concluded that there are no significant differences in CD3, CD4, CD8, CD 4/CD 8 ratios, and absolute

eosinophil count between chronic periodontitis and healthy subjects.

The immune responses that contribute to the pathogenesis of periodontal diseases are complex and variable in nature. Most of these responses may be restricted to the site of infection and may not be elicited systemically. Smaller sample size of the present study warrants further studies with larger sample to throw additional light and definitive directions in this possible association or otherwise.

REFERENCES

1. Okui T, Aoki-Nonaka Y, Nakajima T, Yamazaki K. The role of distinct T cell subsets in periodontitis-Studies from humans and rodent models. Curr Oral Health Rep. 2014; 1:114-123.
2. Okada H. Phenotypic and functional characterization of peripheral blood T cells in adult periodontitis. J Periodont Res. 1991; 26: 289-292.
3. Zafiropoulos GGK, Flores-de-Jacoby L, Schoop B, Havemann K and Heymanns J. Flow-cytometric analysis of lymphocyte subsets in patients with advanced periodontitis. J Clin Periodontol. 1990; 17: 636-641.
4. Buduneli N, Bicakci N, Keskinoglu A. Flow-cytometric analysis of lymphocyte subsets and mCD14 expression in patients with various periodontitis categories. J Clin Periodontol. 2001; 28: 419–424.
5. Emingil G, Karaarslan F, Keskinoglu A, Coker I, Atilla G. Phenotypic and functional analysis of peripheral blood mononuclear cells in generalized aggressive and chronic periodontitis patients. J Int Acad Periodontol. 2001; 3:87-94.
6. Lopez R, Loos BG, Baelum V. Hematological features in adolescents with periodontitis. Clin Oral Invest. 2012; 16:1209-16.
7. Suzuki T, Sugita N, Yoshie H, Hara K. Presence of activated eosinophils, high IgE and sCD23 titers in gingival crevicular fluid of patients with adult periodontitis. J Periodont Res. 1995; 30: 159-166.
8. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol. 1999;4:1-6
9. Loe H, Silness J. Periodontal disease in pregnancy. Prevalence and severity. Acta Odont Scand. 1963; 21:533–551.
10. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L. A modified gingival index for use in trials. Clin Prev Dent. 1986;8:3-6
11. Petit MDA, Hovenkamp E, Hamann D, Roos MTL, van der Velden U, Miedema F. Phenotypical and functional analysis of T cells in periodontitis. J Periodont Res. 2001; 36: 214-220.
12. Sigusch BW, Wutzler A, Nietzsch T, Glockmann E. Evidence for a specific crevicular lymphocyte profile in aggressive periodontitis. J Periodont Res. 2006; 41: 391–396.
13. Cifcibasi E, Ciblak M, Kiran B, Badur S, Firatli E, Issever H. The role of acyivated cytotoxic T cells in etiopathogenesis of periodontal disease. Does it harm or does it heal? Sci Rep. 2015;5:9262.
14. Erciyas K, Orban R, Karvut F, Demir T, Kaya H. The changes in T lymphocyte subsets following periodontal treatment in patients with chronic periodontitis subjects. J Periodontal Res. 2006;41(3):165-70.
15. Kinane DF, Johnston FA, Evans CW. Depressed helper-to suppressor T-cell ratios in early-onset forms of periodontal disease. J Periodont Res. 1989; 24:161-164.
16. Katz J, Goultschin J, Benoliel R, Schlesinger M. Peripheral T lymphocyte subsets in rapidly progressive periodontitis. J Clin Periodontol. 1988; 15:266-268.
17. Nagasawa T, Nitta H, Watanabe H, Ishikawa I. Reduced CD8 + peripheral blood T lymphocytes in rapidly progressive periodontitis. Arch Oral Biol. 1995; 40:605-608.
18. Takahashi K, Nagai A, Satoh N, Kurihara H, Murayama Y. Studies on phenotypic and functional characterization of peripheral blood lymphocytes from patients with early-onset periodontitis. J Periodontol. 1995; 66:391-396.
19. Mathur A, Michalowiccz BS. Cell-mediated immune system regulation in periodontal diseases. Crit Rev Orat Biol Med. 1997; 8:76-89.
20. Cole KL, Seymour GJ, Powell RN. Phenotypic and functional analysis of T cells extracted from chronically inflamed human periodontal tissues. J Periodontol. 1987; 58(8):569–73.
21. Stoufi ED, Taubman MA, Ebersole JL, Smith DJ, Stashenko PP. Phenotypic analyses of mononuclear cells recovered from healthy and diseased human periodontal tissues. J Clin Immunol. 1987;7(3):235–45.
22. Johannessen AC, Nilsen R, Kristoffersen T, Knudsen GE. Variation in the composition of gingival inflammatory cell infiltrates. J Clin Periodontol. 1990; 17(5):298–305.
23. Modeer T, Dahllof G, Axio E, Sundqvist KG. Subpopulations of lymphocytes in connective tissue from adolescents with periodontal disease. Acta Odontol Scand. 1990; 48(3):153–9.