ORIGINAL ARTICLE

ESTIMATION OF NEUTROPHIL COUNT FOR PERIODONTAL DISEASE ACTIVITY: A COMPARATIVE STUDY

[1]Yuvashri P, [2] Renuka Devi R, [3] Esther Nalini H,[4] Arun Kumar Prasad P, [5]Tamilselvi S, [6]Kokila G

[1]PG Student,
[2,3,4]Professor,
[5,6] Senior lecturer
Department of Periodontology, KSR Institute of Dental College and Research, Tamil Nadu

ABSTRACT

Background: The aim of the study is to evaluate the Neutrophil count in GCF for Periodontitis patient and compare it with Gingivitis and Healthy individuals. Methods: Totally 15 subjects from the age group of 20-65years were included in the study. Three groups with five subjects each designated as control(Healthy groups) and cases (Gingivitis and Periodontitis). Gingival crevicular fluid was collected using calibrated, volumetric 5µl, micropipettes and transferred immediately to the slide. Thin smear was prepared and Leishmann's stain was used. Neutrophils per hundred cells are counted under light microscope (100x magnification).Results: There was statistically difference between PMNs count in control and cases group. Mean percentage of PMNs in the Periodontits patients (68.8±5.40) was significantly higher than those with gingivitis patients (43.6±7.12) and healthy periodontium (31.8±1.92) at $p<0.05$
Keywords: PMN, GCF, MMP

INTRODUCTION:

Periodontitis is an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with increased probing depth formation, recession or both[1]. Neutrophils are the "professional phagocytes" that are critical to the clearance of bacteria that invade host tissues.

The presence of neutrophils and macrophages in the sulcus that phagocytose bacteria considered to be the innate and structural defense mechanisms. Neutrophils accumulate in the tissues and release their lysosomal contents extracellularly (in an attempt to kill bacteria that are not phagocytosed), thereby resulting in further tissue destruction. Neutrophils are also a major source of matrix metalloproteinase-8 (MMP-8; neutrophil collagenase) and MMP-9 (gelatinase B) and these enzymes are produced in large quantities in the inflamed gingival tissues as the neutrophils migrate through the densely packed collagen fiber bundles to enter the sulcus[2]. These inflammatory cells are usually present in small amounts in clinically normal gingiva. The present study is undertaken to estimate the number of PMNs in the GCF of periodontitis patients and compare it with healthy controls so as to know whether the levels of PMN count can serve as diagnostic marker of disease.

Access this article online

Quick Response Code:	Website: www.jiadsr.org

Address for correspondence:
Dr P Yuvashri
4/30 Karur road Ganapathipalayam (po)
Erode – 638 153
Email: sixthdec@gmail.com

MATERIAL AND METHODS:

This study was conducted in the department of periodontics, KSR institute of dental science and research, Tiruchengode, Tamil nadu, India. Fifteen subjects (8 males and 7 females) in the age range 20 to 65 years were recruited for the study. Informed written consent was obtained from all subjects and ethical clearance was obtained from the ethical board of this institution. Three groups with 5 subjects each were designated as controls (clinically healthy) and cases (Gingivitis and Periodontitis) respectively, according to the Gingival Index (Loe and Sillness, 1963)[3] and Russell's Periodontal Index(1956)[4].

Subject inclusion criteria:

- Subjects who were systemically healthy.
- Subjects with healthy individuals (Probing depth ≤3mm, no attachment loss,<10%bleeding sites)
- Subjects with gingivitis (probing depth ≤3mm, no attachment loss, ≥10%bleeding sites)
- Subjects with Periodontitis (Probing depth ≥4mm and clinical attachment loss ≥1mm
- No invasive periodontal therapy in the past six months

Subject exclusion criteria:

- Pregnant and lactating women
- Smokers and alcoholics
- History of systemic diseases
- Undergone periodontal treatment
- Anti-inflammatory drugs or any other drugs ≤6 months

A dental and medical history was compiled for all subjects with an oral examination. Clinical measurement of Gingival index and Russel's periodontal index was performed by the same examiner.

Collection of GCF:

The subjects were asked to gargle their mouth vigorously with water, so that it cleanses the teeth of loosely adherent debris. GCF samples were obtained from any healthy site by placing color coded, calibrated, volumetric, micro capillary pipettes with 0.5ml range, obtained from Sigma chemical company, Bangalore. The test site was dried and isolated with cotton rolls. Volumetric micropipettes were placed extra crevicularly at the entrance of the gingival crevice for each subject and the GCF sample collected. The pipettes which were contaminated with blood or saliva were discarded. Pooled volume of GCF was collected from healthy subjects and whereas for Gingivitis and periodontitis site, samples were collected from sites exhibiting severe inflammation and deepest probing depth (>5mm). Test sites which did not express any volume of GCF and Micropipettes contaminated with blood and saliva were not included in the study. Once the GCF was collected it was immediately transferred onto a slide. A thin smear was prepared and stained using Leishmanns stain[10]. It was then viewed under a light microscope with oil immersion lens (100xmagnification). The number of neutrophils per hundred cells were counted in a field. The above procedure was done for five healthy patients, five Gingivitis and five Periodontitis patients.

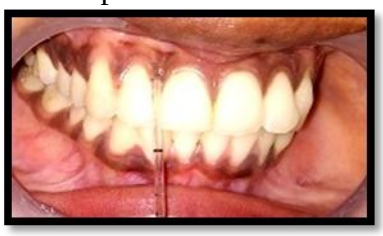


Fig 1: GCF collection

Statistical Analysis:

ANOVA test was done to compare the values of the cases and controls and a statistical significance was established at $p<0.5$.

RESULTS:

Mean percentage of PMNs in the Periodontits patients (68.8±5.40) was significantly higher than those with gingivitis patients (43.6±7.12) and healthy periodontium (31.8±1.92) at $p<0.05$.

Table 1:

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
1.00	5	31.8000	1.92354	.86023	29.4116	34.1884
2.00	5	43.6000	7.12741	3.18748	34.7501	52.4499
3.00	5	68.8000	5.40370	2.41661	62.0904	75.5096
Total	15	48.0667	16.70529	4.31329	38.8156	57.3177

Table 2: ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3572.133	2	1786.067	64.017	.000
Within Groups	334.800	12	27.900		
Total	3906.933	14			

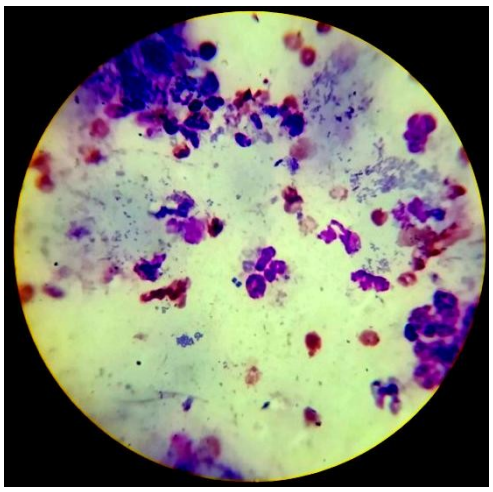


Fig 2: Microscopic field showing leucocytes in Periodontitis

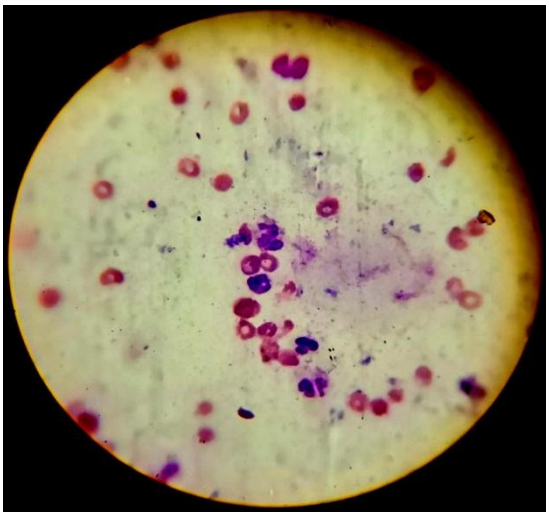


Fig 3: Microscopic field showing leucocytes in Gingivitis

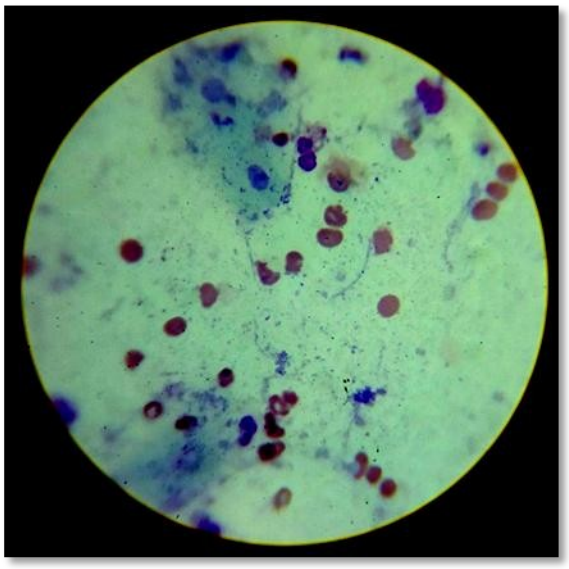
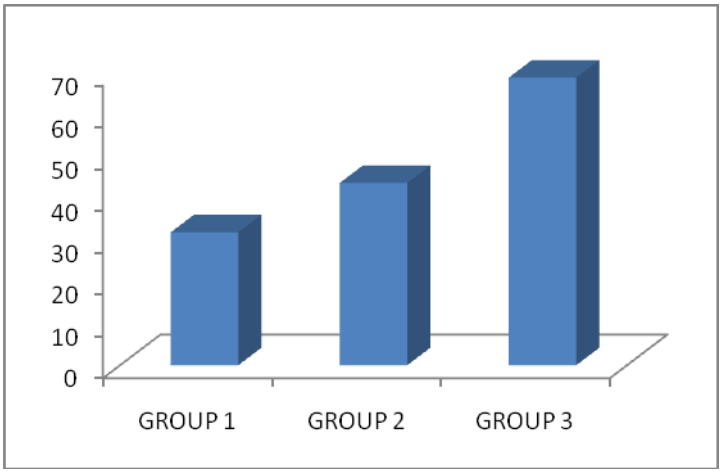


Fig 4: Microscopic field showing leucocytes in Healthy periodontium



Graph 1: Mean percentage of PMNs between groups

DISCUSSION:

The main sources of neutrophils in the oral cavity are from those migrating from the gingival sulcus[5]. PMNs are produced in the bone marrow and released at a rate of 80 to 160 x 107 cells/kg body weight/d ≈ 10^{11}. PMNs are released each day from the marrow of a 70-kg individual (Cartwright et al 1964, Dancey et al 1976) [19]. Leukocytes are the major systemic cells of phagocytosis and the first cells of the host defense mechanism against infective agents. During periodontitis neutrophils are initially predominant cells of the host defense mechanism and have a significant role in inflammation and pathogenesis of disease [17]. Patients with PMN defects, either quantitative or qualitative associated with increased susceptibility to severe periodontal destruction. The location of PMNs at the plaque interface, their phagocytic activity and signs of lysozomal enzyme release gives the morphological evidence that these cells protect the tissue from bacterial attack on the other hand, it may induce tissue damage and increased inflammation via release of lysozomal enzymes. Thus, high numbers of subgingival leucocytes could possibly indicate an active periodontal lesion[12]. The results of this study showed increase in the PMN leucocytes in GCF of Periodontitis patients compared to healthy controls. This might be attributed to increase in surface area of ulcerated epithelium and hence increase in the migration of PMN leucocytes[6].

By evaluating the neutrophil counts in the GCF, the periodontal disease activity has been used in earlier studies and has shown a positive correlation with the probing depth[7]. There are various methods, such as enzymatic activity and microbiologic testing that have evaluated periodontal disease activity. The most commonly used diagnostic tool is periodontal probing but it's a one dimensional measurement of a three dimensional space, the biggest advantage being speed of execution and immediate interpretation as compared to other microbiologic or immunologic methods. Periodontal probing provides clinical information regarding pocket depth and configuration, but periodontal pockets go through periods of exacerbation and quiescence. Periods of quiescence are characterized by reduced inflammatory response and little or no loss of bone and connective tissue attachment and the opposite in periods of activity. Thus, it is important to know current disease activity, which will have an implication on treatment options[13]. Alternate measures to assess periodontal disease activity can be used based on indicators of inflammatory process[20]. The use of Styroflex strips could not give accurate results, due to the clumping of the cells[14]. The washing method suggested by Skapski and Lehner in1976 and by Salonen and Paunio in 1991 has a shortcoming that the dilution factor cannot be determined accurately and it's not an ideal method. In this study we have used colour coded capillary micropippetes to collect GCF samples since it is considered to be one of the novel methods of collecting GCF sample. It is an extra crevicular method of GCF collection and there is lesser contamination with blood and saliva.

The physiologic evaluation of PMN from the peripheral blood of patients with acute bacterial infection and bacteremia revealed that the cells were hyperactive when compared with PMN of healthy controls (McCall et al., 1973; Simms et al. 1989; Barbous et al., 1980) [15]. This study showed a greater presence of PMNs in the cases(periodontitis) as compared to the controls(Healthy and Gingivitis) in accordance with the study by Anderson & Cimasoni in 1993[8]. In 2014 soumiya evaluated the number of PMNs in the GCF

of periodontitis patients and compare it with healthy controls so as to know whether the levels of PMN count can serve as diagnostic marker of disease activity. Neutrophils are found in higher numbers in GCF of chronic severe periodontitis disease, that reflects the inflammatory nature of the disease process[14]. This study also compared with Jaiswal in 2017 where the neutrophil count and its phagocytic activity is altered in chronic periodontitis as compared to healthy subjects, increase the susceptibility of individual to disease. Pejcic in 2011 investigated total count of leukocytes and polymorphonuclear cells (neutrophils) in the peripheral blood of patients suffering from chronic periodontitis showed that increase in total leukocyte and neutrophil counts in patients with chronic periodontitis especially its severe form can be an indicator of the possible exposure of the body to some systemic disease[16]. GCF neutrophils count could be used to assess the disease activity as a simple chairside diagnostic tool[8]. However the disadvantage is that it is not a method with very high specificity. Also the results of a pilot study not often right but it definitely throws light on the perspective of a future trial or research with a larger sample size. Further research could be directed to develop a chairside color changing agent that stains neutrophils in plaque which could help screen and monitor periodontitis subjects. Clinicians can use the plaque neutrophils to check the disease activity in subjects on supportive periodontal therapy [9]. However, this study was conducted with the intention of conducting a further research with larger sample size for a more coherent picture.

CONCLUSION:

Neutrophils can be seen in large numbers in inflamed periodontal tissues, and their presence correlates with the severity of the periodontal destruction. Therefore, this destruction seems to be collateral damage of hyperactive neutrophils. Neutrophils are specialized phagocytes that coordinate and execute inflammation. Alterations in the neutrophil homeostasis (defects in recruitment and proper function) can lead to periodontal diseases and even cause autoimmune and inflammatory diseases[11]. The studies of GCF chemistry have suggested the importance of an exuberant PMN response to subgingival plaque in the active phases of periodontal destructions. Also the functional status of PMN at sites of infection and inflammation suggest that the tissue destruction associated with an influx of PMN as a result of PMN hyperactivity [18]. Further studies should focus on identifying shifts in the equilibrium that occur with the related changes in GCF chemistry and different periodontal diseases.

REFERENCES:

1. Newman MG, Takei HH, Klokkevold PR. Carranza's Clinical Periodontology: A South Asia Edition.
2. Newman MG, Takei H, Klokkevold PR, Carranza FA. Carranza's clinical periodontology. Elsevier health sciences; 2011 Feb 14.
3. Rebelo MA, De Queiroz AC. Gingival indices: state of art. InGingival diseases-their aetiology, prevention and treatment 2011 Sep 22. IntechOpen.
4. Ramfjord SP. Indices for prevalence and incidence of periodontal disease. The Journal of Periodontology. 1959 Jan 1;30(1):51-9.
5. Dyke TE, Vaikuntum J. Neutrophil function and dysfunction in periodontal disease. CurrOpinPeriodontol1994; 28:19-27.
6. Skougaard MR, Klinkhammer JM. Correlation between gingivitis and Orogranulocyte migratory rate. J Dent Res1969; 48:716-18.
7. Aspey DJ, Kaciroti N, LoescheWJ. The diagnosis of periodontal disease in

private practice. J Periodontol 2006;77: 1572-81.
8. Andersen PE, Cimasoni G. A rapid and simple method for counting crevicular PMN leucocytes. JClinPeriodontol 1993; 20: 651-55
9. Jaiswal PG, Bhongade ML, Bhake A, Thakare K, Pande S, Bajaj P, Banode P. Comparison of The Phagocytic Activity Of Gingival Crevicular Fluid (GCF) Neutrophils At Healthy Sites With Periodontally Diseased Sites. Journal Of Applied Dental and Medical Sciences. 2017;3:1.
10. Nair SK, Ebenezer S, Jalaluddin MD, Faizuddin M, Jayanthi D. Leucocyte Count: A Window to Periodontal Disease. A Pilot Study. 2014;8:54-7.
11. Rosales C, Uribe Querol E. Neutrophil Role in Periodontal Disease. Role of Neutrophils in Disease Pathogenesis. 2017 Jun 7:67.
12. Miller DR, Lamster IB, Chasen AI. Role of polymorphonuclear leucocytes in periodontal health and disease. J Clin Periodontol1984; 11: 1-15.
13. Carranza FA, Camargo PM. The periodontal pocket. In: Newman MG, Takei HH, Klokkevold PR, Carranza FA (eds). Carranza's Clinical Periodontology, ed 10. St Louis: Elsevier, 2006:444–445
14. Attstrom R. Presence of leucocyte in crevices of healthyand chronically inflamed gingivae. J Periodont Res 1970;5:42-7.
15. Barbous, A. G., C. D. Alfred C. O. Solberg and H. R. Hill: Chemiluminescence by PMN's from Patients with Active Bacterial Infections. J. Infect. Dis. 141:14-26 (1980)
16. Pejcic A, Kesic L, Pesic Z, Mirkovic D, Stojanovic M. White blood cell count in different stages of chronic periodontitis. Acta Clinica Croatica. 2011 Jun 28;50(2):159-67.
17. Miyasaki KT. The neutrophil: mechanisms of controlling periodontal bacteria. J Periodontol 1991;62:761-4
18. Subbarao KC, Nattuthurai GS, Sundararajan SK, Sujith I, Joseph J, Syedshah YP. Gingival crevicular fluid: An overview. Journal of Pharmacy & Bioallied Sciences. 2019 May;11(Suppl 2):S135.
19. Cartwright, G. E., J. W. Athens and M. M. Wintrobe: The Kinetics of Granulopoiesis in Normal Man. Blood. 24:780-803 (1964).
20. Aspey DJ, Kaciroti N, LoescheWJ. The diagnosis of periodontal disease in private practice. J Periodontol 2006;77: 1572-81