



Defective neutrophil chemotaxis in generalized aggressive periodontitis patients in Indian population

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ABSTRACT

Accumulation of neutrophils in the connective tissues and junctional epithelium of the periodontium is a characteristic feature of periodontal disease. The migration of neutrophils into the area may be in response to chemotactic substances elaborated directly by bacteria or by complement-derived chemotactic factors. The function of the neutrophil is thought to be clearance of infecting microorganisms and other noxious substances. It is, therefore, not surprising that a decrease in neutrophil number and function might result in more severe periodontal disease. The aim of the present study was to evaluate the chemotactic response of the neutrophils in a group of patients suffering from generalized aggressive periodontitis. Neutrophils from 10 normal subjects and 10 generalized aggressive patients were isolated from peripheral venous blood and chemotactic response was measured using casein as the chemoattractant. Of the 10 patients analyzed 5 patients showed depressed chemotaxis to casein. Elevated chemotactic responses were seen in remaining patients. Thus all patients recruited in the study showed abnormal neutrophil chemotaxis. The results obtained were not statistically significant. Based on these results it can be concluded that patients of generalized aggressive periodontitis show abnormal chemotactic response.

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Introduction

Periodontal diseases are inflammatory conditions of bacterial origin that involve large proportions of inflammatory cells and the sequential activation of different components of the host immune and inflammatory response, aimed at defending the tissues against bacterial aggression, reflecting the essentially protective role of the response. Bacteria and bacterial products have been implicated as important etiological agents in the development of gingival and periodontal disease. Neutrophils play a major role in the host response against invading periodontopathogenic microorganisms (Altman LC, Page RC, Vandestein GE, Dixon LI, Bradford C 1985). Accumulation of neutrophils in the connective tissues and junctional epithelium of the periodontium is a characteristic feature of chronic periodontal disease. The migration of neutrophils into the area may be in response to chemotactic substances elaborated directly by bacteria or by complement-derived chemotactic factors. The function of the neutrophil is thought to be clearance of infecting microorganisms and other noxious substances. Neutrophils protect the host tissues by killing various pathogenic bacteria either by non-oxidative or oxidative means in an intracellular or extracellular environment. Non-oxidative killing is mediated by various lysosomal enzymes, peptides and proteins, including lysozyme, bactericidal/permeability-increasing proteins, cationic proteins, defensins and lactoferrin. Generation of reactive oxygen species (superoxide, hydrogen peroxide, hydroxyl radicals and hypochlorous acid and chloramines) contributes in oxidative killing of the invading microorganisms (Nussbaum G, Shapira L 2011). It is therefore, not surprising that a decrease in neutrophil number and function might result in more severe periodontal disease. Several studies have documented suppressed polymorphonuclear leukocytes (PMN) chemotaxis in most patients with localized aggressive

periodontitis (Clark RA, Page RC, Wilde G 1977). Based on the recent findings localized aggressive periodontitis neutrophils are not "hypofunctional" or "deficient." They are "hyperfunctional," and their amplified activity is responsible for the tissue destruction in periodontal disease (Hart TC, Shapira L, Van Dyke TE 1994).

Data regarding PMN chemotaxis in patients with generalized aggressive periodontitis are very limited. The aim of the present study was to study the chemotactic response of the neutrophils in a group of patients suffering from generalized aggressive periodontitis.

Materials and Methods:

The study comprised of 10 patients with generalized aggressive periodontitis and 10 patients with healthy periodontium.

Patients with aggressive periodontitis (AP) were under 30 years of age, there was an involvement of at least three teeth other than 1st molars and incisors, pocket depth ≥ 6 mm, history of familial aggregation, radiologically bone loss ($\geq 50\%$ of the root length especially in first molars and incisors) (Tonetti, M. and Mombelli, A 1999). Patients with healthy periodontium were 25-45 years of age, with sulcus depth ≤ 3 mm and radiologically without bone loss. Patients were age and sex matched for the study (Leino L, Hurtia H 1999). Thorough medical and dental histories were taken from all patients and control subjects.

Criteria for exclusion included systemic illness likely to affect periodontal status, such as diabetes mellitus, chronic ingestion of drugs, including anti-inflammatory agents, antibiotics in the past month, smoking, previous periodontal therapy other than routine tooth cleaning, and a lack of a desire to participate.

Informed consent was obtained from all the patients who

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participated in the study. Clinical parameters like plaque index (Silness and Loe 1964), gingival index (Loe and Silness 1967), and probing pocket depth were recorded. These data were used only to establish a diagnosis and they are not presented in the present paper. Peripheral venous blood was collected and neutrophils were isolated to evaluate chemotactic response using casein as chemoattractant. Statistical analysis was carried out.

Sample collection- 5 ml of peripheral blood was collected in EDTA tubes from the antecubital vein. The EDTA blood was mixed with equal quantities of MEM (minimum essential medium) and 6% dextran solution of molecular wt of 150,000. The tube was kept upright without disturbing for about 45 min, such that the RBCs settled down at the bottom whereas the WBCs and the plasma would be in the upper layer. This upper layer was collected in a centrifuge tube and spun for about 10 min at 3000 rpm. The deposit contained the cells. This deposit was washed three times with phosphate buffered saline and the cell concentration was adjusted to 1000000 / ml with MEM.

Chemotaxis assay- For the chemotaxis assay a modified Boydens chamber was prepared (Thompson R.A. 1978), for this, the front portion of 1 ml plastic syringes was sawed off and 0.45u filter was pasted. The syringe was held in an inverted position with the filter facing down in a beaker through holes in a thermocol sheet. One beaker contained only MEM which served as the negative control and the other beaker contained casein, which served as a positive control. The syringes were filled with the WBC suspension and were immersed in the beaker for 75 min. After this the filters were removed, fixed in methanol, stained by hematoxylin and mounted on a glass slide. The slides were scanned under 40x power of the microscope. When the cells migrated deeper in to the filter, that area appeared blurred in relation to other fields and could be focused only by readjustment of the lens; known as the leading front method. The distance traversed by the cells through the filter was noted.

Results obtained were statistically analyzed using wilcoxon signed rank test.

Results:

Distance traversed by the neutrophils through the filter was noted, the distance traversed through the filter in healthy volunteers in minimum essential medium was 18-20 microns (19.20 ± 2.35) (fig.1). The distance traversed by the PMNs in generalized aggressive patients in minimum essential medium was 22- 60 microns (32.5 ± 11.31). Statistical analysis was carried out using wilcoxon signed test, the p value was statistically significant ($p \leq 0.005$) (bar graph: 1, table: 1). Thus the neutrophils from generalized aggressive patients showed elevated chemokinetic activity or random migration.

The distance traversed through the filter in healthy volunteers in casein medium was 110-136 microns (144.60 ± 8.28) (fig.2) while that in generalized aggressive patients in casein medium was 85-154 microns (119.30 ± 66.70) (fig.3). Statistical analysis was carried out using wilcoxon signed test, the p value was not found to be statistically significant ($p \geq 0.203$) (bar graph: 2, table: 2). With respect to chemotaxis, elevated chemotactic response was seen in 5 patients while depressed chemotactic activity to casein was seen in the remaining 5 patients (fig.4), thus all patients recruited in the study showed abnormal neutrophil chemotaxis.



Fig. 1. MEM (Healthy)

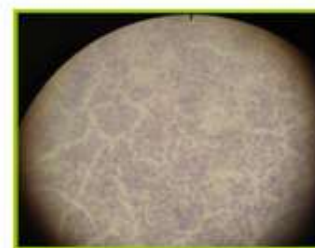


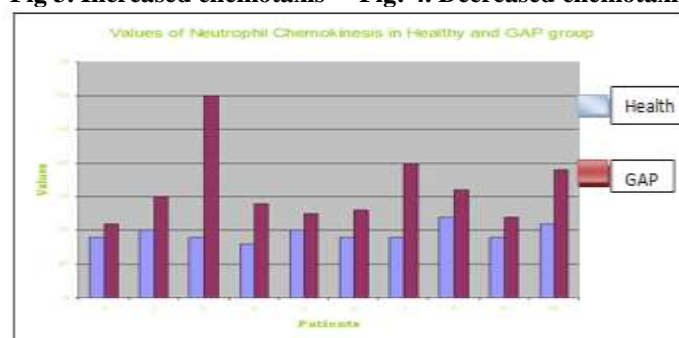
Fig. 2. Casein (Healthy)



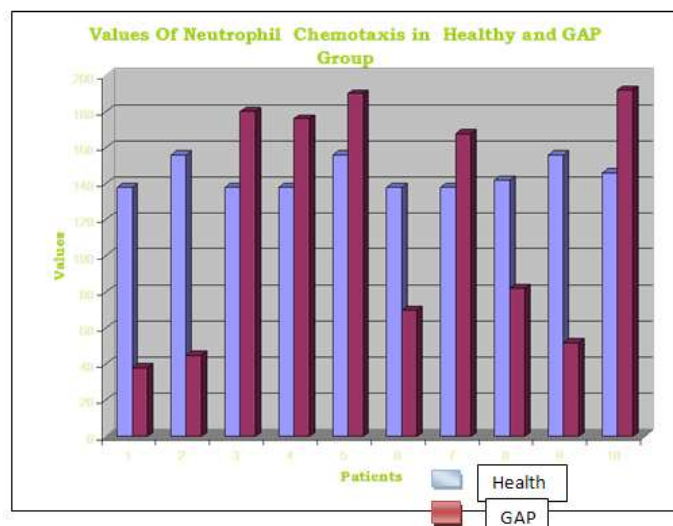
Fig 3. Increased chemotaxis



Fig. 4. Decreased chemotaxis



Bar Graph: 1



Bar Graph: 2

Discussion:

In this study an attempt was made to identify the neutrophil chemotactic defects in the generalized aggressive periodontitis patients, using MEM and casein to see the dysfunction in the form of chemokinesis and chemotaxis respectively. With reference to chemokinesis in healthy volunteers the range was 18-20mic. While in the diseased patients the chemokinesis was increased. When the two were compared using Wilcoxon signed rank test the difference in chemokinetic activity was statistically significant. This data corroborates with the findings of Page R.C, Sims TJ, Geissler F, Altman LC, Baab DA, who in the year 1985 demonstrated enhanced chemokinesis in 63% of the population and depressed chemotaxis in 85% of population. Similarly with reference to chemotactic activity the data showed impaired neutrophil chemotactic function in all patients.

Statistical Analysis Table: 1

S. No.	Group	Mean	Standard Deviation	Wilcoxon Signed Rank Test (P Value)
1.	Chemokinesis-healthy volunteers	19.20	2.35	P VALUE: 0.005
2.	Chemokinesis- GAP	32.5	11.31	

Statistical Analysis Table: 2

S.No	Group	Mean	Standard Deviation	Wilcoxon Signed Rank Test (P Value)
1.	Chemotaxis Healthy volunteers	144.60	8.28	P VALUE: 0.203
2.	Chemotaxis GAP	119.30	66.70	

This corroborates the findings of Van Dyke TE, Horoszewicz, Cianciola LJ, and Genco RJ, who in the year 1980 demonstrated depressed activity in 62.5% and increased activity in only 8% population. Mouynet P, Delamaire M, Le Goff MC, Kerbrol M, Yardin M, Michel JF in 1994 demonstrated random migration and increased chemotaxis with neutrophils in patients with aggressive periodontitis when compared with neutrophils from patients with chronic periodontitis and periodontally healthy controls. In 2001 Sigusch B, Erick S, Pfister W, Klinger G, Glockmann E demonstrated chemotactic defects in both crevicular and peripheral neutrophils in patients with localized aggressive periodontitis.

Conclusion:

In the above study increased chemokinesis was seen in all patients with generalized aggressive periodontitis. With reference to chemotaxis 50% cases showed increased chemotaxis and 50% cases showed depressed chemotaxis. From this we can conclude that there is a defect in neutrophil function. Since the study was carried out in 10 patients only, in future we require carrying out this study in a larger sample size to substantiate these findings. Once the nature of the defect is identified then future research may focus on the cause of neutrophil dysfunction and its significance in the pathogenesis of generalized aggressive periodontitis.

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