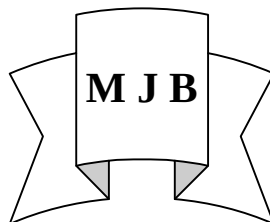


Peroxidase enzyme activity in gingival tissues associated with aggressive periodontitis: Histochemical investigation

*Lehadh M. Al – Azzawi, **Khalid B. Mirza

* Department of Oral Pathology, College of Dentistry – University of Baghdad, Baghdad – Iraq.

** Department of Periodontics, College of Dentistry – University of Baghdad, Baghdad – Iraq.



Abstract

Background: Cytochemical studies on peroxidase have contributed significantly to understanding of the movement of lysosomal enzymes and secretory protein through the various compartments of secretory pathway. The peroxidase in leukocytes (myeloperoxidase) could help in determining the severity of inflammation.

Aim: To determine the severity of inflammation in gingival tissue associated with aggressive periodontitis by histochemical method and to determine peroxidase enzyme activity semiquantitatively.

Material and Method: Five gingival biopsies and five blood smears, three with aggressive periodontitis and two from healthy gingiva. Histochemical method for peroxidase activity using the technique described by Deimann et al (1991) was used.

Results: sections clearly show a wide and intense distribution of the enzyme activity in gingival tissue with inflammation specifically in the subepithelial connective tissue, while a discrete low activity was obtained in the epithelial tissue. Control section showed small or few amounts of enzyme activity. In blood smears from aggressive periodontitis and control showed similar peroxidase activity in the section.

Conclusion: PMNs dysfunction observed in periodontal lesions may be a localized phenomenon caused by plaque bacterial products not found in healthy sites.

الخلاصة

أثبتت الدراسات الكيميائية الخلوية إن إنزيم البيروكسيداز يساهم بشكل ملحوظ في حركة إنزيمات اللايزوزوم والبروتينات الإفرازية خلال مختلف الممرات الإفرازية

إنزيم البيروكسيداز الموجود في خلايا الدم البيضاء قد تساعد في تحديد شدة الالتهاب.

عدم فاعلية كريات الدم البيضاء الموجودة في مناطق الالتهاب اللثوي يعود إلى منتجات البكتيريا الموجودة في الصفيحة الجرثومية الغير موجودة في المناطق الخالية من الالتهاب.

Introduction

Aggressive periodontitis is seen most commonly in young adults. During the active phase, the gingival tissues are extremely inflamed and there is haemorrhage, proliferation of the marginal gingiva and exudation. Destruction is very rapid with loss of much of the alveolar bone occurring within a few weeks to months. This phase may be accompanied by general malaise, weight loss and depression, although these symptoms are not seen in all patients. The disease may progress, without remission to tooth loss. Most patients with aggressive periodontitis have serum antibodies specific for various species of *Bacteroids*, *Actinobacillus*, or both and manifest defects in either neutrophil or monocytes chemotaxis, phagocytosis or both^{1, 2, 3}.

At the present time it is not possible to distinguish prior to treatment which individuals will respond to therapy and which will not. So far studies have found no clinical features which permits identification of these patients prior to treatment³.

Several indices have been reported which attempt to record the presence and extent of periodontal inflammation^{3, 4}. These indices depend on clinical and visual assessment of inflammation and have several disadvantages. They allow only semiquantitative measurement of the degree of inflammation and are prone to considerable variation between examiners^{5, 6}.

The measurement of gingival fluid volume has been widely used although there is poor correlation between the amounts of fluid and the extent of inflammatory infiltrate in the tissue. PMN leucocytes are present in gingival tissue and fluid and their numbers increase concurrently with the severity of inflammation^{2, 4, 6}. The PMN leucocytes contain peroxidase, which could provide a measure of the severity of the inflammatory process. It has been observed that the

peroxidase activity in gingival tissue and exudates resembles that of leucocytes and that there is an increase in activity with certain inflammatory changes in the periodontium^{6, 7}.

Aim of the study

The purpose of this study is to determine semiquantitatively the peroxidase activity in the gingival tissue associated with aggressive periodontitis.

Materials and Methods

Five gingival biopsies (3 aggressive periodontitis and 2 clinically healthy) and five smears of peripheral blood films from the thumb were obtained from volunteers and patients attending the Periodontal and Oral Surgery Department, College of Dentistry, University of Baghdad.

None of the subjects had contributing systemic disease or received antibiotics or medication in the past six months known to affect of the results of this histochemical investigation. Patient from either sex were 20 –32 years olds. Both diseased and clinically healthy sites, when available, were selected from each patient.

The selection of clinically healthy gingiva was chosen when no signs of inflammation were present and didn't bleed on probing. Aggressive periodontitis was diagnosed on clinical and radiographic basis. The clinical features are florid, highly acute inflammation with bleeding and proliferation of marginal gingiva. The amounts of associated microbial plaque vary greatly and pus may or may not ooze from the deep pockets (more than 7 mm). Radiographic evidence shows destruction of alveolar bone. Gingival sections of both diseased and normal were stained for H&E for general histopathological morphology.

Fresh frozen tissues block (0.5, 1, 1 cm) were immediately transferred to the cryostat (Slee Medical Equipment Ltd, Lanier works Hither Green Lane S. E., 13, London). After placed on a piece of cork with a drop or two of 10% gum acacia (BDH chemicals Ltd.,

Poole England). The cork with tissue on top were placed on the cryostat chuck with a drop or two of distilled water underneath, and then quenched in liquid nitrogen. Fresh frozen sections of 6 –8 μ thickness were received on clean cover slips, left for 15 minutes at room temperature for dryness and consequent adherence.

Histochemical demonstration of Peroxidase:

The method followed in this study was that of Deimann et al., (1991)⁸, which in principle depends on the sequential oxidation of benzidin to a blue and to a brown reaction product.

Accordingly fresh frozen sections and blood smears were incubated for 8 minutes at 15 – 20 °C in the dark medium consisting of 1ml saturated (42 g in 100 ml), ammonium chloride (BDH, Analar), 1 ml 5 % EDTA (Fluka AG, Buchs SG) and one drop of 3 % H₂O₂ (BDH, Analar), at pH 5.0 and containing 7.5 % sucrose (May and Baker Ltd, England). After incubation sections were post fixed for 6 minutes in formalin vapour, rinsed briefly with distilled water, counterstained for 1 –2 minutes in 1 % neutral red and mounted in PVP (BDH, Lab reagent).

Results

Histopathology:

H &E stain used in figure 1 (A & B) show inflammation with increasing number of leucocytes and macrophages these two cell types are widely distributed throughout the section, but mainly concentrated towards the epithelium. In comparing this picture with a section from healthy gingival, the latter shown minimal number of PMNs and macrophages in both epithelium and connective tissue (figure 2).

Gingival histochemistry of Peroxidase activity:

Figure 3 (A & B) show intense inflammation the enzymatic stain is widely distributed in both connective tissue and

epithelium. These sections show tissue destruction and loss of architecture the stain has concentrated in the area of severe inflammation, it has leaked from the connective tissue towards the epithelium. Increase in stain density is seen in area of inflammation. The gingival epithelium show elongated retepegs indicating the presence of inflammation.

Blood cytochemistry and Peroxidase:

The blood from all patients with aggressive periodontitis show similar picture to blood from healthy control (figure 4 A & 4 B). The reaction product is brown; this has been transferred from the blue due to a function of time in the laboratory preparation procedure.

Granulocytes show strong coarse granules that is evenly distributed through out the cytoplasm, the nucleus show no peroxidase activity. Lymphocytes show no peroxidase activity neither in the cytoplasm nor in the nucleus, the dots of dark stains in the RBC represent pseudoperoxidase.

Discussion

Many authors have reported that several pathogens isolated from periodontal lesions have the potential to cause PMNs dysfunction or even to kill PMNs. However, PMNs were found in higher numbers in periodontal lesion as compared to gingivitis sites¹². In aggressive periodontitis the viability of PMNs collected from diseased sites was to be lower⁹. The reasons for the decreased PMN viability are not known, but it has been suggested that local factors may alter cell viability as well as cell functions. In this context, it should be recalled that specific bacteria such as *Actinobacillus actinomycetemcomitans*, which has the potential to kill human PMNs, might alter PMN viability in the sulcus⁶. In contrast with these observations, a study showed no difference in crevicular cell viability between periodontitis and healthy patients^{2, 10}. Since in general the literature there is certain confusion in the nomenclature of

juvenile and aggressive periodontitis, it is possible that the clinical criteria of selection were not uniform between their various studies.

In vitro phagocytic function of sulcular PMNs has been shown to be impaired in aggressive periodontitis^{3, 9, 11}. In addition, in juvenile and aggressive periodontitis as well as in other patients with periodontal disease, it was found that the phagocytic capacity of PMNs from periodontal lesions was decreased when compared to the function of the cells isolated from healthy sites^{9, 11}. This could suggest differences in the number of receptors for immunoglobulins present on PMN membranes between the two cell populations¹².

It is also possible that PMN dysfunction observed in periodontal lesions might be a localized phenomenon caused by plaque bacterial products not found in healthy sites. This hypothesis is supported by the fact that several oral pathogens are able to produce functional abnormalities in PMNs¹¹, and that PMN phagocytic function can be modulated by factors from Gram – ve bacteria^{13, 14}. Their findings are in contrast with the data of the longitudinal study which did not show any significant variations in PMN functions during the experimental gingivitis period^{12, 15, 16}. These observations may reflect the differences in the clinical gingivitis and periodontitis situations. Gingivitis is a clinical entity which differs from periodontitis, not only in its bacterial etiology, but also in its histopathologic features. It is generally thought that gingivitis progress with time to periodontitis but the conditions for the progression is not fully understood.

It appears that in gingivitis, when there is a balance between the host and the bacteria, PMNs are functionally competent. Could it be possible that the progression from gingivitis to periodontitis may be due to factors impeding on PMN functions with

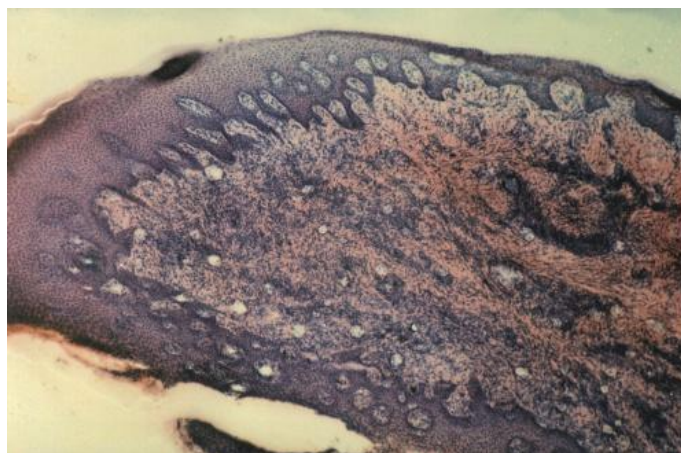
bacterial proliferation as a consequence? This hypothesis remains to be proven.

References

1. Page, R. C.; Attman, L. C.; Ebersole, J.L.; Vandesteen, G. E.; Dahlberg, W. H.; Williams, B. L. and Osterberg, S. K. (1983): Aggressive periodontitis. A distinct clinical condition. *J. periodontol*, 197: 209 – 215.
2. Eley, BM. and Cox, SW. (1996): The relationship between gingival crevicular fluid cathepsin B activity and periodontal attachment loss in chronic periodontitis patients: a 2 years longitudinal study. *J periodontal Research*, 31: 381 – 392.
3. Lindsokg, S.; Pierce, AM.; Blomlof, L. (1998): Chlorhexidine as a root canal medicament for treating inflammatory lesions in the periodontal space. *Endod Dent Traumatol*, 14: 186 – 90.
4. Nakamura, T.; Kido, J, Kido, R.; Yamauchi, N. (2000): The association of calprotection level in gingival crevicular fluid with gingival index and the activities of collagenase and separate aminotransferase in adult periodontitis patients. *J. Periodontol*, 71: 361 –7.
5. Magnusson, I.; Persson, R.G., Page, R. C.; Derouen, T. R.; Crawford, J.M. (1996): A multicenter clinical trial of a new chairside test in distinguishing between diseased and healthy periodontal sites. II: Association between site type and test outcome before and after therapy. *J. Periodontol*, 67: 584 – 596.
6. Garito, ML.; Prihoda, JJ.; McManus, LM. (1995): Salivary PAF level correlate with the severity of periodontal inflammation. *J Dent Res*, 74: 1048 – 56.
7. Paunio, K.; Malinen, K.K.; Scheinin, A. and Ylitalo, K. (1976): Turku sugar studies (IX) principal periodontal findings. *Acta Odontologica Scandinavia, supplementum*, 70: 217 – 222.
8. Deimann, W.; Angermuller, S.; Stoward, H. and Fahimi, H.D. (1991): Peroxidase. In *Histochemistry: Theoretical and Applied*.

- Eds. Stoward, P. J. and Pearse, AGE. 4th ed. Churchill Livingston. pp: 135 –160.
9. Murray, P.A. and Patters, M. R. (1980): Gingival crevice neutrophil function in periodontal lesion. J periodontal research, 15: 463 – 469.
 10. Newman, H. N. and Addison, I. E. (1982): J periodontology, 53: 578 – 586.
 11. Hauser, GJ.; Fekete, Z.; Livingstone, DH.; Adams, J. (2000): Major trauma enhances store operates calcium influx in human neutrophils. J trauma, 48: 592 – 8.
 12. Thurre, C.; Robert, M.; Cimasoni, G. and Baehni, P. (1984): Gingival sulcular leukocytes in periodontitis and in experimental gingivitis in humans. J. Periodontal Res, 19: 457 – 468.
 13. Taichman, N.S.; Bohringer, H. R.; Loi, C. H.; Shenker, B.J.; Tasi, C.C. ; Berthold, P. H.; Listgarten, M. A. and Shapiro, I. S.(1982): Pathobiology of oral spirochetes in periodontal disease. J. Periodontal research, 11: 449 – 451.
 14. Woodson, D.C. and Oneill, P.A. (1982): Effect of oral bacteria on pheripheral and oral PMN. J. Dental Research, 61: 310 – 319.
 15. Aarli, V. and Heyeraas, KJ. (1995): Effect of venous stasis and hypoproteinemia in gingival fluid formation in rats. J periodontal Res, 30: 231 –7.
 16. Kunimatsu, K.; Ozaki, Y.; Hara, Y. Aoki, Y. (1997): Immunohistochemical study of cathepsin G and medullasin in inflamed gingival tissue from periodontal patients. J periodontal Research, 32: 264 – 70.

A.



B

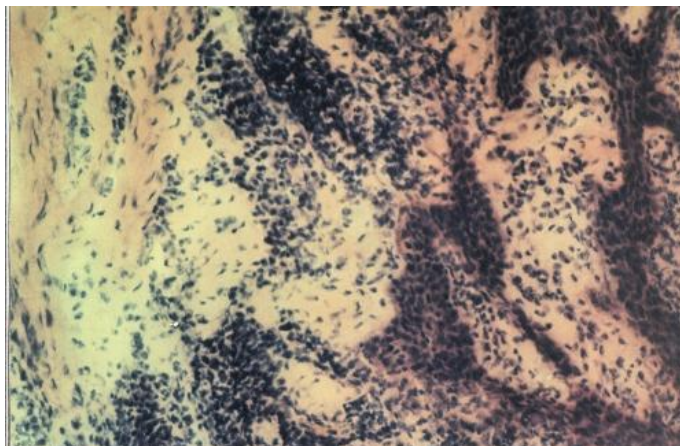


Figure 1. Photomicrograph of aggressive periodontitis showing severe inflammation with high number of PMN leukocytes.

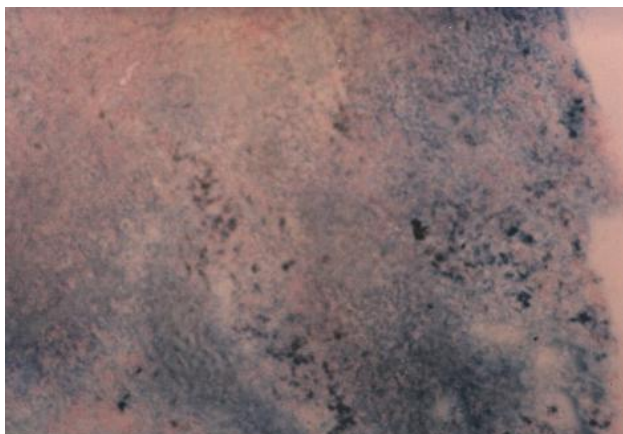
A. H & E (X 40).

B. H & E (X 100).



Figure 2. Photomicrograph of healthy gingiva.

A

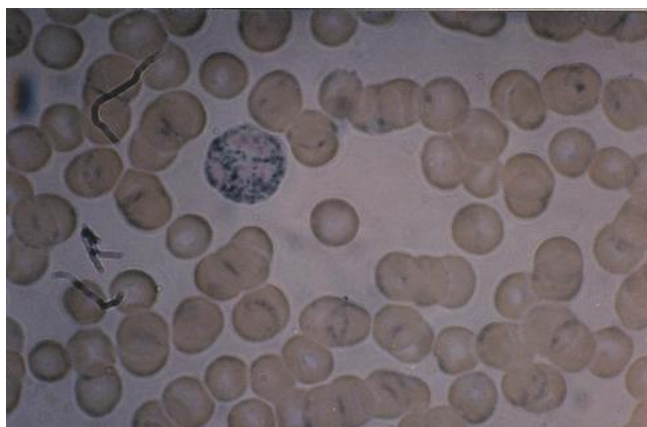


B



Figure 3. Histochemical reaction of peroxidase enzyme in tissue section of aggressive periodontitis, brown to blue colour fine granules stain final reaction product. (A & B X 40).

A



B

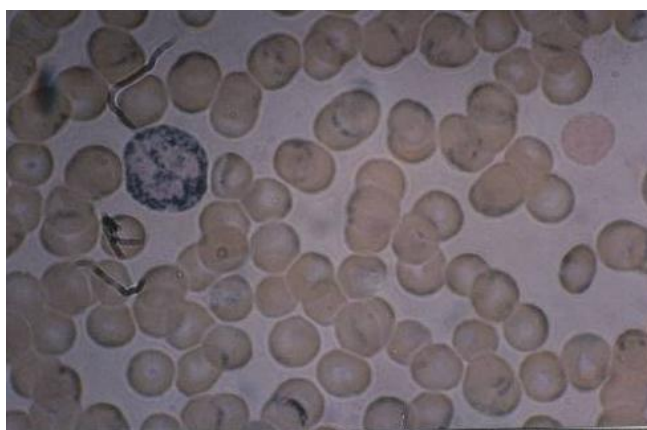


Figure 4. Blood cytochemistry of peroxidase activity showing the final reaction product granules in the cytoplasm of PMN (X1000).

A. Blood from healthy person

B. Blood from patient suffering from aggressive periodontitis