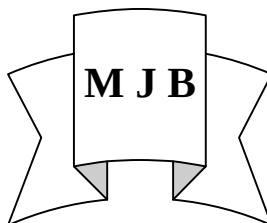


A histochemical study of the peroxidase enzyme activity associated with paradontal abscess

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

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

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



Abstract

Background: Histochemical and cytochemical techniques of peroxidase enzyme activity was determined semiquantitatively the severity of inflammation in periodontal lesions.

Aim: To determine the severity of inflammation in gingival tissues associated with paradontal abscess by histochemical method to determine peroxidase enzyme activity.

Patients and Method: six biopsies and six blood smears, four with paradontal abscess and two from healthy gingival. Histochemical method for peroxidase activity using the technique described by Deimann et al., (1991) was used.

Results: In tissue with acute inflammation the sections clearly showed intense and widely distributed enzyme activity both in the epithelium and subepithelial connective tissues, while a discrete low activity was obtained in sections taken from healthy gingiva.

Conclusion: It is concluded that the intensity of peroxidase activity is much increased in acute inflammation of the gingiva, (paradontal abscess), irrespective of its activity in PMNs of blood as demonstrated in blood smears.

الخلاصة:

التقنيات الكيميائية الخلوية والكيميائية النسيجية على فعالية إنزيم البروكسيداز ساعدت في بيان شدة الالتهاب في أمراض اللثة.

وقد لاحظت هذه الدراسة كثافة فعالية إنزيم البروكسيداز تزداد كثيراً في أمراض اللثة الحادة مثل الخراج اللثوي بغض النظر عن

مدى فعالية في خلايا الدم البيضاء المبينة في مسحات الدم.

Introduction

Acute periodontal abscesses are a crisis in the life of a tooth. Patient and dentist may opt for a treatment plan of early extraction rather embarking on prolonged conservative measures of uncertain outcome.

The periodontal abscess can be an acute exacerbation of chronic periodontal disease ^{1, 2, 3}. It may occur when the infection passes into tissues through the pocket epithelium. Such abscesses frequently result from the occlusion of the narrow mouths of tortuous or deep intrabony pockets. Since the virulence of the organism is an important factor, even shallow pockets may become involved.

Bacteria are not normally present in the tissue, and when they gain entrance a migration of leukocytes occurs to contain the infections. The area is walled off by thrombosis of the vessels and by a fibrinous blockade. The number of leukocytes and microorganisms continues to increase. This is followed by necrosis and liquefaction of the central area, with formation of pus ^{4, 5, 6}.

Peroxidases are any of a group of iron – prophyrin enzymes which catalyze the oxidation of certain organic substrates (e.g. phenols, reduced glutathione, ferrocytochrome C and NADH) in the presence of hydrogen peroxide (H₂O₂), which act as hydrogen acceptor, being converted to water (H₂O) in the process. But it was not until the end of the last century that the name “peroxidase” was first given by Linossier in 1898 that isolated free presentation of peroxidase from pus ^{7, 8, 19}.

The measurement of gingival fluid volume ⁹ has been widely used although there is poor correlation between the amount of fluid and the extent of inflammatory infiltrate in the tissue ¹⁰. The correlation between the peroxidase activity of gingival

exudates and the severity of inflammation is positive.

Aims of this study

The purpose of this study is to determine semiquantitatively the peroxidase activity in the gingival tissue associated with paradontal abscess and to compare it with clinically healthy gingiva as a control.

Patients and Method

Six gingival biopsy and peripheral blood smears were studied in this work. None of volunteers and patient had any systemic disease or received antibiotics or any medication that affect the results of the histochemical investigation. These were obtained from subjects attending the Periodontal and Oral Surgery Departments, College of Dentistry, University of Baghdad.

Patient from either sex were 30 –40 years olds. Both diseased and clinically healthy sites, when available, were selected from each patient.

The selection was based on clinical examination and radiography. Clinically healthy gingiva was chosen when no signs of inflammation were present and didn't bleed on probing. Periodontal abscess was diagnosed on the presence of gingival swelling and / or pain associated with deep pocket and vital pulp confirmed the diagnosis. Furthermore a periapical film was taken to exclude periapical pathology. Two abscesses involved mandibular molars and two abscesses involved maxillary molars. Three abscesses occur interstitially involved maxillary and one abscess localized mid – buccally. Gingival sections of both diseased and normal (two clinically healthy) were stained for H&E for general histopathological morphology.

At the same time sections of kidney, salivary gland of mouse were processed along with the gingival sections as positive controls.

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 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 consequently adherence.

Histochemical demonstration of peroxidase

The method followed in this study was that of Deimann et al., (1991)¹¹ 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) 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

The histopathological picture revealed by H & E stain (figure 1) shows intense inflammation with abundance of PMNs and macrophages with tissue destruction is evident.

The PMNs are widely distributed throughout the section but concentrated more towards the epithelium and in

figure (2) demonstrates a section of a healthy gingiva with minimal PMNs are seen in both epithelium and connective tissue.

Gingival histochemistry of peroxidase activity

Figure 3 (A, B, C) demonstrate intense inflammation, the enzymatic stain is widely distributed both in the epithelium and connective tissue. All the sections show loss of tissue architecture due to profound tissue destruction.

Figure 3 (C) with high magnification shows the stain has leaked from the connective to the epithelium towards the area of acute infection. Also in certain areas the stains is very dense illustrating the severity of the inflammation.

Figure 3 (A) show loss of tissue continuity, in fact sectioning is very difficult, due to the friability of the tissue because of the oedematous acute stage of infection. The junctional epithelium in figure 3 (A, B) has elongated rete pegs that penetrated the connective tissue indicating a sign of inflammation.

Figure 4 (A, B) are taken for healthy gingiva, the faint blue stain indicates minimal peroxidase activity, either in the epithelium or connective tissue.

Blood cytochemistry of peroxidase activity

The blood from all four patients with periodontal abscess show similar picture to blood from healthy individuals as shown in figure 5 A and B. Granulocytes show strong coarse granules that are evenly distributed throughout the cytoplasm, while the nucleus is devoid of peroxidase activity.

The final reaction product is gradually and relatively quickly transferred from blue to brown. This is a function of time in laboratory preparation procedure.

Lymphocytes showed non peroxidase activity neither in cytoplasm nor in the nucleus. The RBCs show dots of dark stain, representing pseudoperoxidase. Platelets exhibit normal features.

Discussion

Inflammatory lesions of periodontium are seen in the vast majority of the adult population and can result in progressive destruction of the soft tissues and bone that support teeth in their sockets^{3,7}. The accumulation of adherent, heterogenous bacterial plaque on the crowns and root surfaces of teeth has been linked as the major etiological event in the initiation and progression of gingival and periodontal disease¹².

Whole saliva and gingival fluid from subjects with periodontal inflammation contain greater amounts of peroxidase^{5,6}. However, it was pointed out by some studies, that peroxidase from PMNs could account for at least a portion of the increased enzyme activity¹¹.

PMNs release some of their contents upon ingestion of bacteria or during contact with plaque². Monocytes and eosinophils cells also contain peroxidase.

Neutrophil, monocytes and eosinophil peroxidase are granule – associated enzymes whose roles in defence mechanisms against infection are well established¹³. These enzymes have the potential to kill holminth larvae^{14,15,16}, protozoa, actinomycetes, bacteria and viruses^{13,17}.

When the cells are stimulated peroxidase enzyme are released either in the phagocytic vacuole containing the ingested organism or at the surface of large non – phagocytosable parasites thus killing them with the aid of hydrogen peroxidase are also released into the extracellular environment^{5,6,18}, where they can interact with

inflammatory mediators, interstitial macromolecules, basal membrane^{6,7,17,19} and neighbouring cell with possible influence on evolution of inflammatory process^{17,18}.

Periodontal pockets contain PNM – leukocytes, the amount of which increases according to the severity of inflammation, PNM – leukocytes contain a considerable amount of peroxides, one of the antimicrobial agents present in neutrophil^{3,9,10,11,12}.

During the inflammatory process, the neutrophils break down and liberate, e.g. peroxidase. The means that the peroxidase activity of inflammatory exudates correlates to the degeneration rate of leukocytes as observed with rats^{7,8,12}. Peroxidase activity in gingival exudates resembles that leukocytes and is increased in line with certain inflammatory changes in periodontium^{3,7,11}.

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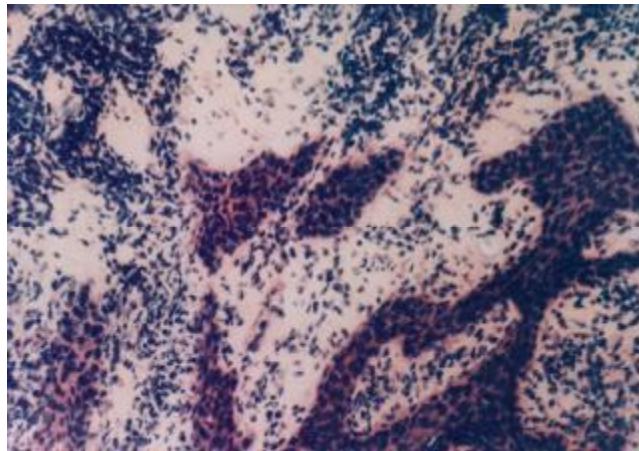


Figure 1. Photomicrograph of paradontal abscess showing severe inflammation with high number of PMN leukocytes. H & E (X 100).

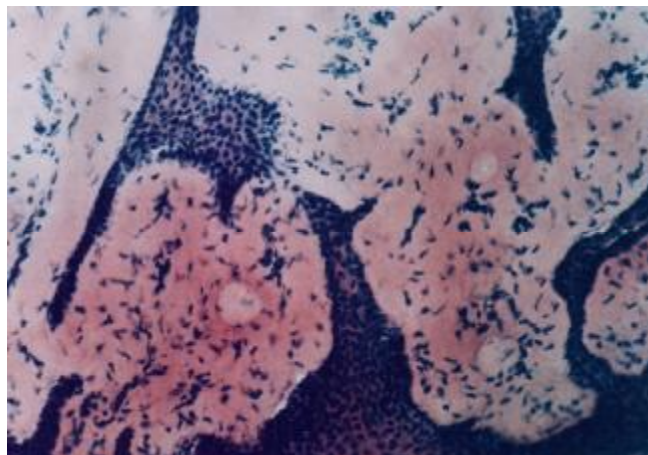
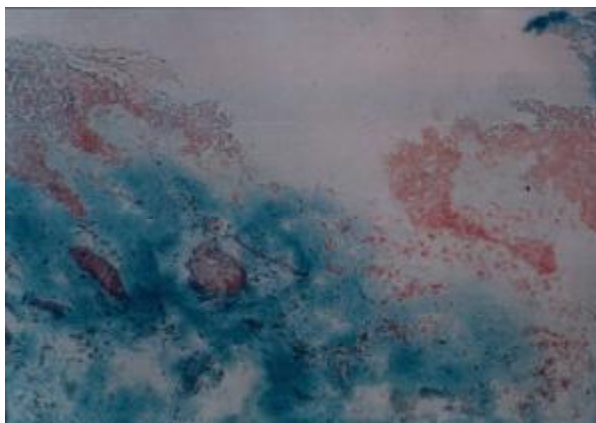
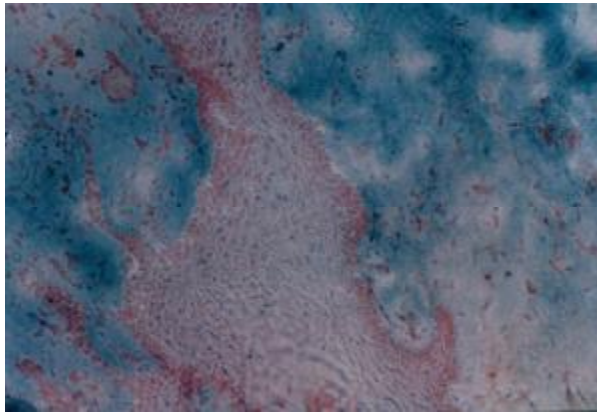


Figure 2. Photomicrograph of normal mucosa. H & E (X 100).

A.



B.



C.

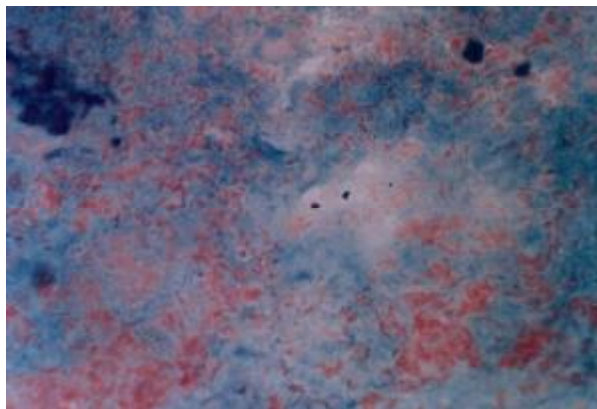
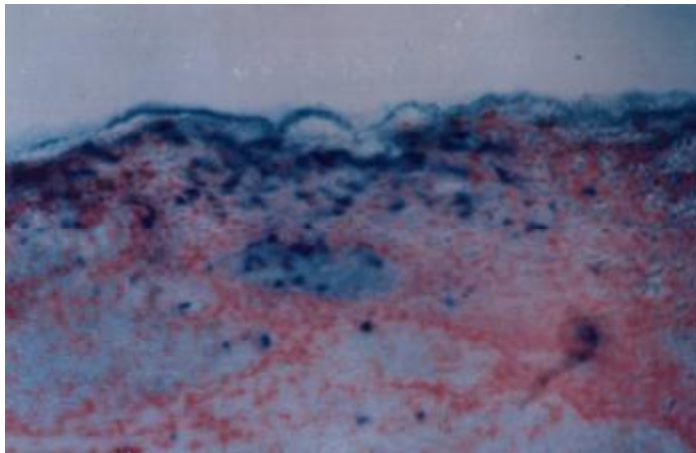


Figure 3 (A, B, C) : Histochemical reaction of peroxidase enzyme in tissue section of paradontal abscess, brown to blue colour fin granules stain final reaction product.

A.



B.

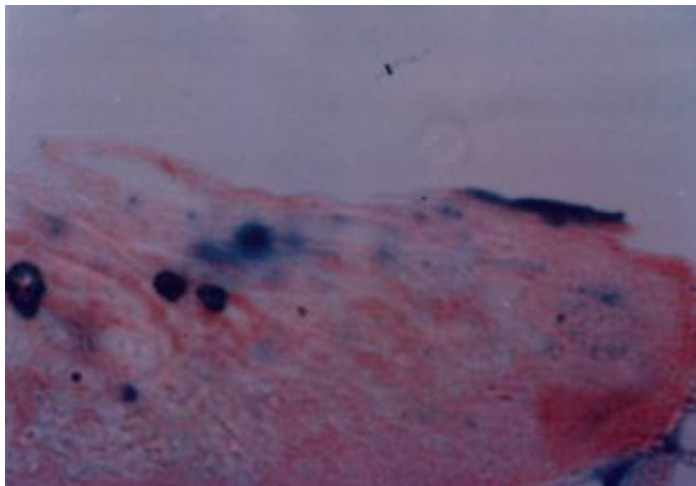
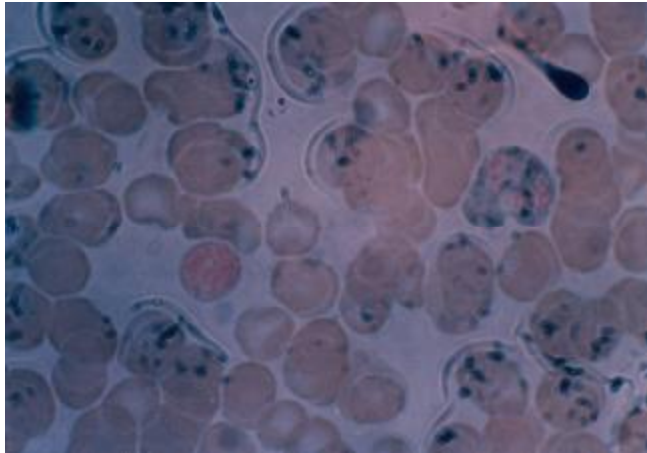


Figure 4 (A, B): Histochemical reaction of peroxidase enzyme in tissue section of normal mucosa, brown to blue colour fine granules stain of final reaction product. (X 40).

A.



B.

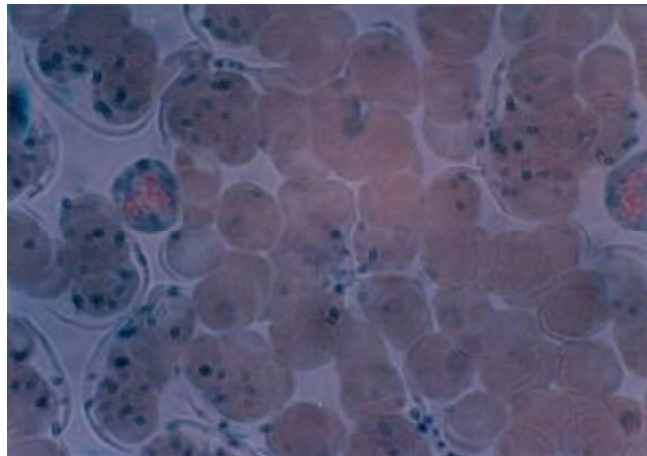


Figure 5. Blood cytochemistry of peroxidase activity showing normal picture of PMN (1000).

A. Blood from healthy person

B. Blood from patient suffering from paradontal abscess.