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# FREQUENCY OF ENTAMOEBA GINGIVALIS AMONG PERIODONTAL AND PATIENTS UNDER CHEMOTHERAPY

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## ABSTRACT

This study was carried out to investigate the frequency of *E. gingivalis* in the gingival crevices of patients with periodontitis compared to normal subjects. Flagyl therapy was tried in cases with periodontitis. Also the frequency of this amoeba was investigated in patients receiving chemotherapy to be compared to normal subjects. The results revealed that higher frequency was observed in patients with periodontitis and flagyl therapy reduced this frequency. Cases under chemotherapy showed higher frequency than the normal control. Histopathological sections from the gingiva of periodontitis patients did not show any amoeba invading the tissue.

## INTRODUCTION

*Entamoeba gingivalis*, an oral protozoan first discovered by Gros (1849) followed by Smith and Barret (1915). Since its discovery, several clinical studies have been conducted. The high incidence of *E. gingivalis* in individuals with periodontitis, led some investigators to believe that it may be one of the aetiologic agents of this condition (Lyons and Palmer, 1983). However the general presence of *E. gingivalis* in the healthy oral cavity, led others to consider it a scavenger that thrives in the nutritional environment induced by the presence of periodontal disease (Ockert and Schneider, 1987). Unfortunately, the

significance of these discoveries was ignored as insignificant to oral health. Studies on the ultrastructure of *E. gingivalis* added impetus to the need to review the pathogenicity of this protozoan (Keller et al, 1967). Metronidazole is one of the effective drugs against oral protozoa with a unique spectrum of activity against anaerobic bacteria (Sutter and Finegold, 1984). Several studies on animals (Ehrlish et al, 1974) and human beings (Vincent, 1981) described the histological alterations caused by cancer chemotherapeutic agents on different tissues but non of them have reported the effect of these drugs on the frequency of oral protozoa.

In this study, the frequency of *E. gingivalis* was investigated in the gingival crevices of healthy mouth and dental patients with advanced periodontitis, and, in patients who are under chemotherapy. The effect of metronidazole on this protozoan was also investigated.

## MATERIAL AND METHODS

Patients were divided into three groups as follows:-

**Group I:** Ten patients with advanced periodontitis were selected from out-patient dental clinic in Department of Oral Medicine, Dental Faculty of Alexandria University. Patients aged from 25-40 years with no systemic disease and no history of current use of antibiotics or other medications. These patients were given 3 metronidazole tables 250 mg daily for seven days. **Group II:** Ten patients of matching age, who are under chemotherapy were selected from the Oncology Department of Alexandria University Hospital. They were given a single agent 5 Fluoruoracil MTX Methotrexate. Subjects with risk factors (head and neck radiation, diabetes, corticosteroid therapy and recent use of antibiotics) were excluded from the study. **Group III:** Ten persons of matching age with no systemic disease and with good oral condition were selected.

Two samples were obtained from each patient in group I, one before and one 2 weeks after flagyl therapy. Whereas one sample was taken from each patient in group II and III. Samples were taken by a sterile curette, they were obtained at random from 5 disto-facial sites of the periodontium. Each sample was examined while fresh by placing it on wet mount slide, fixed in polyvenyl alcohol (Brooke and Goldman, 1949) stained by trichrome stain (Garcia and Ash,

1975) and examined by ordinary light microscope. Results were obtained by determining the number of *E.gingivalis* trophozoites per field. The mean number of 10 fields was calculated. The mean number of trophozoites in the obtained 5 smears of each patient was recorded.

T test and paired t test were applied in the statistical analysis of the results. Six patients who were diagnosed as advanced periodontitis cases and who needed tooth extraction were selected for a histopathological study. Prior to removal of the tooth a gingival specimen was excised from the diseased area, fixed in Carnoy's solution, dehydrated and embedded in paraffin. 5µm sections were cut and stained with trichrome to search for the presence of *E.gingivalis*.

## RESULTS

*E. gingivalis* was found in patients with periodontitis in a frequency of 64% i.e. 32 were positive from a total of 50 samplings. After flagyl treatment, the frequency decreased to 13 positive samples out of 50 (26%). In cases under chemotherapy it was 58%. On the other hand, in control cases the frequency was 34% (17 positive samplings out of 50) (table I). In patients with periodontitis

**Table I:** Frequency of *E.gingivalis* in the samples of the different groups of the study.

| Group | Total no of samples | no of +ve samples | no of -ve samples | +ve % |
|---|---|---|---|---|
| Group I: Periodontitis (before treatment) | 50 | 32 | 18 | 64% |
| Periodontitis (after treatment) | 50 | 13 | 37 | 26% |
| Group II: Under chemo-therapy | 50 | 29 | 21 | 58% |
| Group III: Control | 50 | 17 | 33 | 34% |

the mean number of *E.gingivalis*/field was increased as compared to the control (Table II). Treatment with flagyl caused significant decrease in the mean number of *E.gingivalis*/field (Table III). However, a significant increase was observed in patients under chemotherapy (Table IV). Sections of gingiva excised from patients with periodontitis did not reveal amoebae invading the tissue (fig.3 and 4).

**Table II:** Mean number of *E.gingivalis*, trophozoites/field in cases with periodontitis compared to the control.

| | Control group | | Periodontitis |
|---|---|---|---|
| X | 8.5 | | 15.9 |
| SD | 1.84 | | 4.48 |
| t | | 4.836* | |

* significant

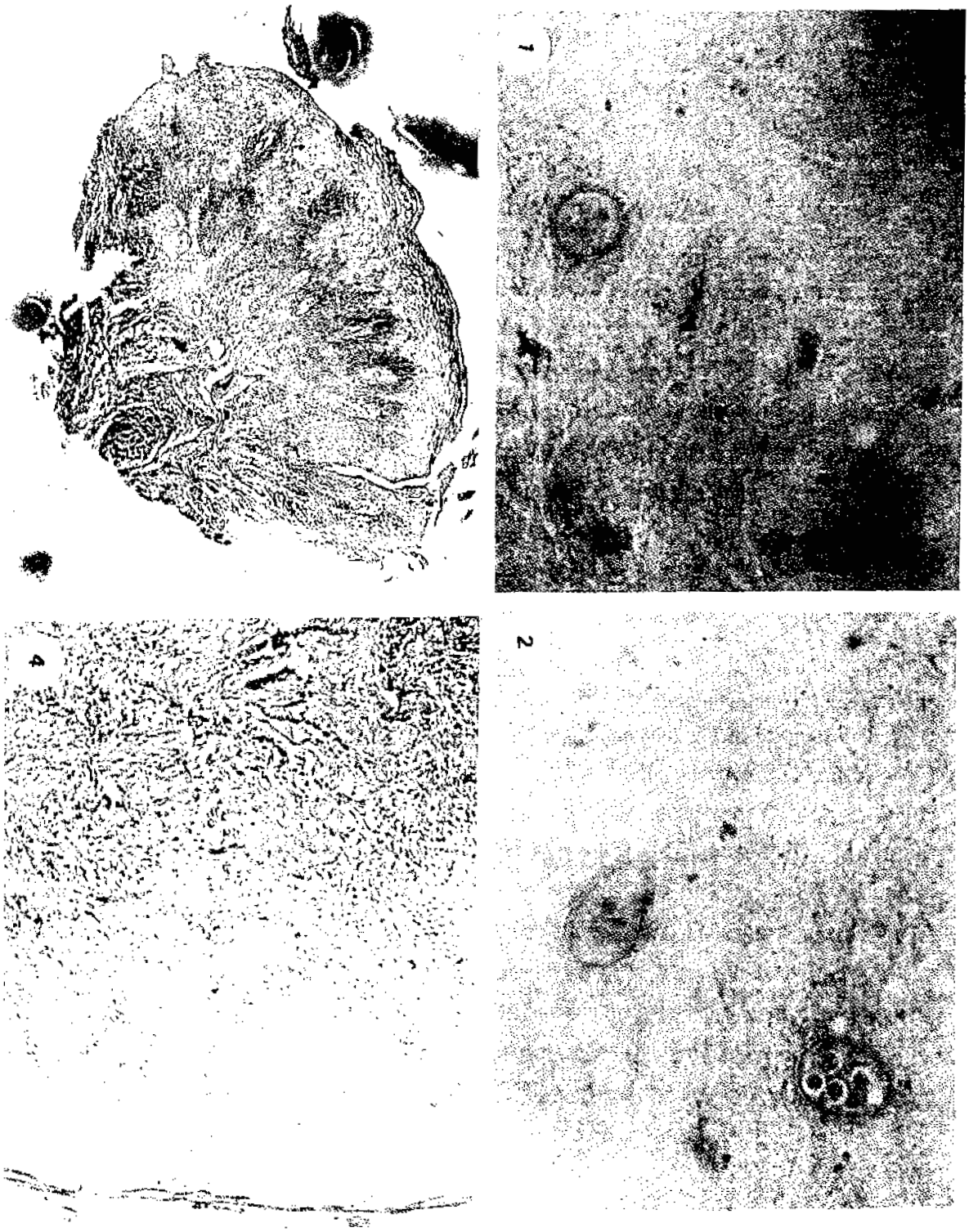
**Table III:** Mean number of *E.gingivalis*, trophozoites/field in patients with periodontitis before and after flagyl therapy.

| | Before Treatment | | After Treatment |
|---|---|---|---|
| X | 15.9 | | 6 |
| SD | 4.48 | | 1.5 |
| t | | 6.805* | |

* significant (paied t test)

**Table IV:** Mean number of *E.gingivalis*, trophozoites/field in cases under chemotherapy compared to the control.

| | Control group | Under chemotherapy |
|---|---|---|
| X | 8.5 | 14.5 |
| SD | 1.84 | 2.32 |
| t | | |




Figs. 1 & 2: *E.gingivalis* stained by trichrome in a smear X 1000.
Figs. 3 & 4: Section in the gingiva stained by trichrome showing no *E.gingivalis* X 100.

## DISCUSSION

*Entamoeba gingivalis* is known to be a non pathogenic amoeba which inhabits the gingival tissues and tartar of the teeth. It is found with a higher frequency with gingival infections or poor oral hygiene (Goldsmith and Hyenman, 1989). The results of this investigation indicate that the highest frequency of *E.gingivalis* was found in patients with periodontitis. This finding would support that *E.gingivalis* may play an active role in the periodontal disease. Lyous and Palmer (1983) found that after demonstration of *E.gingivalis* from apparently healthy gingival tissue, there was no periodontal decline unless the protozoan was eradicated in the mean time. Symptoms often observed in patients infected with the oral protozoan included itchy gingivae which bled easily, ulcers of the palate and halitosis. Wantland and Lawer (1970) stated that the rate of *E.gingivalis* infection was found to be directly proportional to the amount of calculus present on the teeth and the degree of coating on the surface of the tongue. These results could be supported by earlier studies which observed that *E.gingivalis* was not found in living gingival tissue but within plaque or cellular debris adjacent to sulcular epithelium (Gottieb and Miller, 1971). Therefore, it appears that if *E.gingivalis* contributes to the gingival lesion, it does so other than by invasion. It is conceivable that it actively transports bacteria, either attached to the cell surface or within vacuoles, which upon release could contribute to the progress of periodontal disease. This suggestion, might help to explain the cyclical nature of periodontal disease (Socransky et al, 1984). Also *E.gingivalis* might elaborate a proteolytic enzyme that oculd contribute to the pathogenesis of periodontitis (Gotteib and Miller, 1971).

Metronidazole was found to be effective in the treatment of some forms of periodontal disease (Duckworth et al., 1966). The results of this study revealed that metronidazole did not eliminate only anaerobic organism from subgingival microbiota, but also has a potent effect on *E.gingivalis*. This is shown by the reduction of frequency from 64% to 26%. Thus elimination of anaerobic organisms was also responsible for the improved of periodontal health.

Trophozoites of *E.gingivalis* were seen to ingest both erythrocytes and leucocytes in additon to the nuclei of leucocytes (Dao, 1985). Such finding indicates that the oral protozoan may affect the oral ecology in regard to dental plaque formation. The antineoplastic drugs have many side effects on the tissue

such as the oral mucosa. These stomatotoxic effects included denudation, ulceration (mucositis) and infection that orginates from an ulceration in the normal oral flora (Depaola et al., 1983). A high frequency (58%) of *E.gingivalis* was found in patients under chemotherapy. It seems logic to report that *E.gingivalis* might play an active role in the oral mucosa of myelosuppressed patient. The frequency of *E.gingivalis* in periodontal disease and patients under chemotherapy suggests the need for further investigations in the potential role of this parasite.

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