

Occurrence of *Entamoeba gingivalis* in patient with chronic periodontitis

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Abstract:

Recent studies have pointed the importance of *Entamoeba gingivalis* (*E. gingivalis*) as an active protozoa in periodontal pathology, its presence associated with the presence of pathological pockets, thus a clinical survey of *E. gingivalis* was conducted in patients with chronic periodontitis. A total of 240 specimens were taken from 48 patients (20 male and 28 female), their age ranged from 38 up to 60 years old. A direct smearing technique of deepest periodontal pocket (> 4mm) with a sterile universal curettes to pickup the plaque material and to be placed in phosphate - buffered saline pH7.4, then stained with Heidenhair hematoxyline modified technique, studied under light microscope, the results shown 33.3% of our patients were positive.

The occurrence of *E. gingivalis* appeared positively correlated with the pocket depth, 13.93% of 4-6mm pockets depth was positive, while in pockets of 7-8mm depth the percentage rises up to 38%.

Keywords:

Entamoeba gingivalis, periodontitis, periodontal pocket.

Introduction:

The concept of periodontal disease based on the fact that challenges of microorganism harboring the gingival sulcus leads sooner or later to the destruction of both soft and hard supporting tissues of the tooth^(1,2), these periodontal diseases are associated with constellation of bacteria, anaerobic or facultative gram negative species which envelope the root surfaces as a biofilm^(3,4), as long the plaque accumulated around a tooth as the attachment apparatus and the adjacent bone gone under a destructive process deeply and extensively^(5,6,7).

Adult periodontitis are frequently associated with pathogenics such as *Treponema denticola*, *porphyromonas gingivalis* and *Bacteroid Forsythus*⁽⁸⁻¹¹⁾. The Bacterial flora which harbors the gingival loges of human oral cavity include the *Entamoeba gingivalis* which can be easily gathered by gentle probing⁽¹²⁾ the *E. gingivalis* have been frequently demonstrated associated with diseased conditions rather than the healthy conditions, with high incidences of infection probably around 50%, but there is no evidences that *E. gingivalis* could cause the disease^(13,14) *Trichomonas Tenax* and *E. gingivalis* are

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both protozoas, positively found in the pathologic pockets^(1,10,13,15-19).

The highest prevalence of infection with these protozoas found in old age patients, 41-50 years old. Where 88% of this age group appeared infected^(20,21), the prevalence of *E. gingivalis* looks like higher in females than males⁽²²⁾, occasionally detected in gingivitis frequently in periodontitis^(14,23), uncorrelated with several hormonal disturbances, neither smoking habits⁽²⁴⁾.

The aim of this study was to investigate the occurrence of parasitic protozoa (*Entamoeba gingivalis*) in Iraqi patients having non-treated chronic periodontitis.

Materials and methods:

1. Patient selection:

Forty eight patients (28 female and 20 male) aged from 38 up to 60 years old were selected from the daily population of the department of perioontology, college of dentistry, Baghdad University as having a generalized chronic periodontitis when diagnosed clinically and radiologically according the probing depth and amount of bone loss. At least 5 sites of pockets depth measures 4-8mm were chosen for sampling.

Our subjects were medically healthy as reported by a physician according the general medical history and a clinical examination. Patients with less than 3 teeth in the posterior sextant, or having a history of

recurrent oral diseases other than periodontitis were excluded.

2. Sample collection:

From each selected 5 sites, subgingival samples were taken from the most apical portion of the periodontal pocket (4-8mm probing depth) Prior to sampling saliva, debris and plaque were gently removed from the gingival margin and subgingival area with sterile cotton. Sharp sterile universal curette was used to collect subgingival plaque, the plaque samples were pooled for each designated areas and placed into sterile Eppendorf tubes containing 1 ml phosphate - buffered saline (PH 7.4), the plaque smears were fixed with turdyer fixative solution and then stained with Heidenhain iron hematoxilyne modified technique (Lynch et al, 1969)⁽²⁴⁾

The samples were examined under light microscope using direct smear method for the presence of *E. gingivalis*.

Results:

The distribution of sample population according to the patients sites number is shown in table (1) on the basis of the clinical characteristics and light microscope examination, relation between periodontal condition and presence of the oral protozoa (*E. gingivakis*) were analyzed.

Table (1): Distribution of the sample patients / sites number .The age range of the patients 38-60 years.

Patient sex	No. of patients	No. of sites
Male	20	100
female	28	140
Total	48	240

In the present study *Entamoeba gingivalis* was detected in 33.3% of the patients, and in 21.7% of the sites as shown in table (2).

Table (2): The occurrence of E.G. among chronic periodontitis patients

Sample	Total no;	No. of sample with positive results	% of occurrence of E.G
patients	48	16	33.3
sites	240	52	21.7

In pockets with 4-6mm depth there were 13.97% positively containing *E. gingivalis*, as the pocket depth goes deeper the percents of positive occurrence increases successfully as shown in table (3)

E. gingivalis was detected in 21.7% pockets sites, with a positive correlation between the presence of *E. gingivalis* and the pocket depth.

Table (3): The relation ship between the E. G occurrence and the pocket depth.

Pocket depth (mm)	No. of sites	No. of sites with positive result	% percentage
4-6	136	19	13.97
6-7	94	17	18
7-8	42	16	38
Total	240	52	21.7

Discussion and conclusion:

Up date knowledge of periodontal diseases showed , clinically and experimentally , that the plaque micro organisms are the causative factor of all gingivitis and periodontitis

conditions ⁽²⁵⁾ their initiation , progression and final destination depends upon many essential factors , starting with bacterial antigenic invasion and penetration into the epithelial barrier and then the ability of plaque antigens to activate both , the cellular and the

humeral immune reactions with of course the reciproque activation of complement cascades , which ends with destruction of supporting connective tissues , deepening the gingival sulcus into a pathologic pocket ⁽²⁾ , this progression accompanied with the conversion of the intra pocket bacterial flora into a gram negative anaerobic bacterial associations ⁽¹⁾ with a variety of species that associate in a pathogenic colony ⁽²⁰⁾ , this flora includes , streptococci , spirochetes , *Bacteroides gingivalis*, *Bacteroid melaninogenicus* , *actinobacillus actinomycetes* , *Neisseria* ^(8, 9) . Also *trypomonas denticula*, *prophyromonas gingivalis*, *Bacteroides Forsythus* ^(10, 11) . Protozoa, such as *Entamoeba gingivalis* and *Trichomonas Tenax* with yeast were distinguished within the intra pocket flora ^(13, 14) .

As our results suggest, the earlier studies showed that *E. gingivalis* will be easily gathered and isolated from the deep untreated periodontal pockets ^(9,20,27,28) . *E. gingivalis* exist in deep pockets ranged from 4-8mm, their prevalence are frequently more in old age patients than in young ^(28, 29) .

In accordance , with our results on Iraqi subjects previous studies in Guatemala ⁽¹⁸⁾ France ⁽¹⁷⁾ , Chile , under scanning electron microscope ⁽²⁹⁾ , The *E. gingivalis* had been detected and isolated with prevalence ranged from 15% up to 50% , 15% of young adult having *E. gingivalis* in Guatemala ⁽¹⁸⁾ , 50 % of mid-age adult having *E. gingivalis* in France ⁽¹⁷⁾ , 33.3% of our subjects having *E. gingivalis*, particularly those of mid-age around 46-58 years old . It seemed to be the deepest intra-pocket environment is more favorable lodge for the growth of *E. gingivalis*. Our findings showed that the presence of *E. gingivalis*

in shallow pockets is occasional, Bartold ⁽³⁰⁾ showed that there is an interaction between the bacterial association including *E. gingivalis* by the ability of *E. gingivalis* to liberate oxygen free radical which rises the possibilities of tissular destruction, as well as rises the bacterial cooperation.

The Isolation of *E. gingivalis* from periodontal pockets could suggest an essential pathologic role of these parasites in aggravation of tissular destruction. Further studies concerning the pathogenesis of *E. gingivalis* in periodontal diseases seemed to be logic.

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