

Oral Colonization by *Entamoeba gingivalis* in the Biofilm and Saliva from Patients with Periodontal Disease

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Abstract

Background: In addition to human cells, healthy gums contain a variety of bacteria, fungi, and protozoa that may contribute to unhealthy sites. The effect of pathogens lysis or other immune cell lysis needs to be clarified in order to expand the current pathophysiological pattern of periodontitis. The purpose of this study was to survey the prevalence of *E. gingivalis* among patients with periodontitis and healthy individuals in order to support public health action. In addition, we investigated the relationship between the incidence rate and some parameters, such as patient age and gender.

Methods and Results: This study enrolled 51 subjects divided into two groups: The case group involved 31 patients (15 females and 16 males) with periodontal disease; the control group included 20 people (10 females and 10 males) with healthy oral cavities. Biofilm and saliva samples were collected and examined for the presence of *E. gingivalis*. A total of 22(71.0%) patients of various ages with periodontal disease were infected with *E. gingivalis*. Male patients were more likely to have the amoeba than female patients. *E. gingivalis* was also found in the control group, albeit at a lower frequency ($P=0.005$).

Conclusion: Infections with *E. gingivalis* should be considered as a potential pathological factor associated with the development of periodontal diseases. More research should be conducted to determine whether *E. gingivalis* is the primary cause of tissue destruction and disease progression, or if it is acting synergistically with the current pathogenic bacteria that should be investigated further. (International Journal of Biomedicine. 2022;12(3):409-411.).

Keywords: *Entamoeba gingivalis* • periodontal disease • oral cavity

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Introduction

In addition to human cells, healthy gums contain a variety of bacteria, fungi, and protozoa that may contribute to unhealthy sites.⁽¹⁾ The oral cavity is home to the amoeba *E. gingivalis*, an uncommon parasite that colonizes the mouth. *E. gingivalis* is a protozoan that is eukaryotic, motile, and unicellular. It can only be found in vegetative or trophozoite form.⁽²⁾ There is no mention of resistance or cysts.⁽³⁾

E. gingivalis morphologically resembles *E. histolytica*, and diagnosing such a protozoan necessitates expertise.⁽⁴⁾ This amoeba appears microscopically as a cell (10–35 μm) with granular endoplasm clearly distinguishing itself from the ectoplasm and active pseudopodia.⁽³⁾

Multiple foreign substances are seen in digestive vacuoles, including bacteria, cellular debris, and even the structure of changed nuclei of polynuclear cells and some red

blood cells.⁽⁵⁾ The anaerobic environment of deep periodontal pockets, which favors parasite development, shows that interactions between microbiota constituents may be critical for their function in the pathophysiology of the disease.⁽⁴⁾

According to a review of the literature, scientists have differing opinions about the pathogenicity of *E. gingivalis*; some believe it is a pathogenic protozoan that could play a role in the progression of periodontitis,^(3,4,6) while others believe it is commensal due to its presence in the oral cavity of healthy individuals.^(1,5) However, it is known that in immunocompromised individuals, *E. gingivalis* collaborates with some symbiotic bacteria to promote the progression of periodontitis.^(2,3,7) It is well recognized that people with low socioeconomic status and poor oral hygiene are the most vulnerable.

This commensal was found in HIV/1 patients with periodontal disease.⁽⁸⁾ This correlation may be due to the consequences of chronic diseases and their impact on the

variation of microbial communities in the oral cavity. *E. gingivalis* was the only microbe found in the oral cavity of people with severe periodontitis, implying that it is involved in disease progression and causes inflammation and destruction of surrounding tissues, as previously proposed by other researchers.^(3,4,6) The pathogenic activity of *E. gingivalis* has been established once the amoeba attaches to the surfaces of live epithelial cell models and induces cell killing and degeneration via trophocytosis.⁽⁴⁾

This study was conducted to survey the prevalence of *E. gingivalis* among patients with periodontitis and healthy individuals in order to support public health action. In addition, we investigated the relationship between the incidence rate and some parameters, such as patient age and gender.

Materials and Methods

Patients

Some demographic information was recorded, such as the age, gender, occupation, and residence of patients who attended private clinics in Najaf (Iraq). These parameters were collected prior to the collection of samples. Furthermore, the patients were questioned regarding the presence of systemic illness, and ingesting antibiotics or any medicine.

The case group involved 31 patients (15 females and 16 males) with periodontal disease. The following symptoms were found in these patients: swelling of the frontal mandibular deepening pocket, plaque and calculus, inflammation, and green or yellow gingiva. The control group included 20 people with healthy oral cavities. The age of participants in both groups ranged from 10 to 70 years.

Sample Collection and Microscopic Examination

Biofilm and saliva samples were collected and examined for the presence of *E. gingivalis*. From every patient, 3 spots were selected from which to collect the samples (interdental spaces, molar pockets, and palatogingival groove grooves). The cumulative materials were removed with sterilized periodontal curettes. Samples of saliva were collected using sterilized containers. Wet-mount smears of both samples were performed by placing one drop of physiological saline solution on the slide, mixed with samples and stained with Giemsa stain. The examinations were done in triplicate using 40X magnification to identify the amoeba with its unorganized shape, distinctive vacuoles, and pseudopodia.

Statistical analysis was performed using the statistical software STATISTICA (v10.0, StatSoft, USA). Group comparisons with respect to categorical variables are performed using Fisher's exact test. A value of $P < 0.05$ was considered significant.

The study was conducted in accordance with ethical principles of the WMA Declaration of Helsinki (1964, ed. 2013) and approved by the Kufa University Ethics Committee. Written informed consent was obtained from each research participant (or the participant's parent/guardian).

Results

In the case group, 22(71.0%) patients with periodontal disease were infected with *E. gingivalis* (Table 1). In the

control group of subjects who attended the clinics for monitoring general oral cavity health, only 4(20%) infected cases were observed.

Table 1.

Cases of *E. gingivalis* in the study groups.

<i>E. gingivalis</i>	Case group (n=31)	%	Control group (n=20)	%	Fisher's Exact Test (2-Tail)
Positive	22	71.0	4	20.0	P=0.0005
Negative	9	20.03	16	80.0	

Examination of the freshly prepared slides revealed that from the total confirmed infected cases, 15 were detected in the biofilm and one in saliva for patients with periodontitis. In patients with gingivitis, 4 cases were detected in biofilms and 2 in saliva. In the control group, only 3 cases of *E. gingivalis* were found in biofilm samples and one in saliva (Table 2).

Table 2.

Incidence of *E. gingivalis* in dental biofilm and saliva samples depending on the periodontal status in the study groups.

<i>E. gingivalis</i>	Biofilm n (%)	Saliva n (%)	Fisher's Exact Test (2-Tail)
Case group			P=0.000
Periodontitis	15 (68.18)	1 (25.0)	
Gingivitis	4 (18.18)	2 (50.0)	
Overall	19 (86.36)	3 (75.0)	
Control group	3 (13.64)	1 (25.0)	P=0.605
Total	22 (100)	4 (100)	

In terms of the relationship between patient age and the presence of *E. gingivalis*, individuals aged 31 to 40 years had the highest incidence rate, while those aged 41-60 years had the lowest incidence rate (Table 3).

Table 3.

Infection with *E. gingivalis* in biofilm and saliva samples based on patient age.

Age groups	Cases	%
10-20	3	13.64
21-30	5	22.73
31-40	10	45.45
41-50	2	9.09
51-60	1	4.54
60 and above	1	4.54
Total	22	100

In the case group, 59.09% of male patients were infected with *E. gingivalis* compared to 40.91% cases among women. In the control group, 75% of men were infected compared to 25% of females (Table 4).

Table 4.

The sex-related prevalence of *E. gingivalis* infection in biofilm and saliva samples in the study groups.

Gender	Case group	<i>E. gingivalis</i> presence	%	Control group	<i>E. gingivalis</i> presence	%
Male	16	13	59.09	10	3	75.00
Female	15	9	40.91	10	1	25.00
Total	31	22	100	20	4	100

Discussion

For over a century, numerous authors have commented on the presence of *E. gingivalis* in periodontal lesions of gingival infections in humans.⁽⁴⁾ Without being unanimous on its pathogenic or commensal abilities, the majority of authors have noted its consistent presence in periodontitis.^(2,3,5) Cell adhesion, neutrophil inactivation and phagocytosis, mass reproduction, and motility are all activities that give the amoeba essentially invasive pathogenic characteristics.^(4,5) Keeping this in mind, researchers discovered that patients with periodontal disease were more susceptible to *E. gingivalis* infection. According to a study conducted by Gharavi et al.,⁽⁹⁾ the incidence rate of this parasite was 41.7%. However, according to a study conducted by Sharifi et al.,⁽²⁾ the incidence rate of *E. gingivalis* was 11.7%, and their findings were lower than the current outcomes. Furthermore, we found *E. gingivalis* in 20% of individuals with healthy oral cavities, indicating commensal habitation. These differences can be explained by some parameters that may influence the variability of results, such as the diversity of samples examined (number of subjects, age, sex, etc.), as well as by the difficulty in detecting and identifying the amoeba in an accurate manner using direct examination on a fresh sample, examination after culture, or examination by PCR probe.⁽²⁾

The presence of *E. gingivalis* in dental biofilms of patients with periodontal disease and in the control group subjects validates previous hypotheses of studies that this protozoan may contribute to the progression of periodontal disease.^(2,4) This parasite may contribute to periodontitis development by assisting the bacteria that cause damage^(3,10) or by invading ruptured oral mucosa and ingesting fragments of live cells, as would be required to justify its periodontal pathogenicity.⁽⁴⁾

In terms of gender, *E. gingivalis* was found in more males (59.09%) than females (40.91%). These findings contradict another study, which found a high percentage of infection-related deaths among females.⁽¹¹⁾ However, according to our study, gender appears to influence the frequency of this amoeba. Regarding the relationship between patient age and the presence of *E. gingivalis*, the parasite was most common in ages from 31 to 40 years. Similarly, some authors propose a statistically significant link between *E. gingivalis* incidence and patient age (maximum infestation between 31-40 years).⁽¹¹⁾

Furthermore, periodontal tissue damage, poor oral health, tooth loss, *Candida* spp. infection, decay-missing-filled teeth, dental caries, cigarette smoking, drugs, frequency of brushing, and food allergies are all suggested to play a role in the prevalence of *E. gingivalis* infections.⁽¹²⁻¹⁴⁾

Conclusion

A total of 22(71.0%) patients of various ages with periodontal disease were infected with *E. gingivalis*. Male patients were more likely to have the amoeba than female patients. *E. gingivalis* was also found in the control group, albeit at a lower frequency (P=0.0005). Our study concludes that infections with *E. gingivalis* should be considered as a potential pathological factor associated with the development of periodontal diseases. More research should be conducted to determine whether *E.*

gingivalis is the primary cause of tissue destruction and disease progression, or if it is acting synergistically with the current pathogenic bacteria that should be investigated further.

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