Original Article

Incidence of the oral protozoa - *Entamoeba gingivalis* in a hospital-based population in South India - A preliminary study

Shanmugapriya Ramamurthy[1], Sabitha Sudarsana[1], Shyam Sivasamy[2], Arunmozhi Ulaganathan[1], Kadhiresan Rathinasamy[1], Sujatha Govindarajan[3]

[1]Department of Periodontology, Sri Venkateshwara Dental College and Hospital, Thalambur, Chennai, Tamil Nadu, India, [2]Department of Community Dentistry, Sri Venkateshwara Dental College and Hospital, Thalambur, Chennai, Tamil Nadu, India, [3]Department of Oral Pathology and Microbiology, Sri Venkateshwara Dental College and Hospital, Thalambur, Chennai, Tamil Nadu, India

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Correspondence
Shanmugapriya Ramamurthy,
Department of Periodontology,
Sri Venkateshwara Dental College and Hospital, Thalambur, Chennai, Tamil Nadu, India. Phone: 044 27435060.
Fax: 044-27435059.
E-mail: drshanpriya@gmail.com

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Abstract

Background: Protozoa are single-cell animal organisms that can divide within a host organism. The oral protozoan *Entamoeba gingivalis* has held a rather chequered position in the history of research. Keyes in 1983 noted that this organism together with the cocci which colonize the surface of the actinomyces filaments was invariably found in close proximity to plaque, especially in patients with poor oral hygiene.

Aim: The aim of this study is to evaluate the incidence of the oral protozoan *E. gingivalis* in a hospital-based population (attending the outpatient clinic) in a dental institution in South India.

Materials and Methods: The objective of this clinical study was to investigate the prevalence of *E. gingivalis* in periodontal diseases and to compare its occurrence among gingivitis and periodontitis group and with healthy controls. It is a cross-sectional comparative clinico-microbiological study. Criteria-based sampling was undertaken among patients visiting a dental college in South India. The plaque sample was collected with a sterile curette (Gracey Curette) from the gingival crevice/periodontal pocket and immediately subjected to light microscopic examination by an original wet-smear technique for the presence of *E. gingivalis*.

Results: The results revealed that while 88% of gingivitis patients showed the presence of *E. gingivalis*, 76% of periodontitis patients and only 4% of healthy subjects showed its presence.

Conclusions: The results of this preliminary study showed an increased presence of *E. gingivalis* in plaque samples of both gingivitis and periodontitis patients when compared to healthy subjects. The incidence of the parasite, as well as the intensity of the gingival infection, is correlated.

Introduction

The oral microbial flora is constituted by a myriad collection of bacterial and protozoal species, which are seen to coexist in a relatively stable environment.[1] Only in recent years, it has been established that the species that have been associated with periodontal disease are not exogenous but are only a part of the normal oral microbiome. Moreover, when a metabolic disturbance occurs due to any systemic illness, pathological changes in the periodontal tissues ensue, which in turn favors the species composition of mixed protozoa, bacterial, and/or fungal infections.[2]

Entamoeba gingivalis and *Trichomonas tenax* are the protozoa found in the human oral cavity. *E. gingivalis* has held a rather chequered position in the history of research. It was the first parasitic amoeba recovered from human beings by Gros in the year 1849 from dental tatar.[3] It is classified under the phylum: Protozoa, subphylum: Sarcodina, class: Archamoebae, Genus: Entamoeba, and Species: *E. gingivalis*.[4]

This protozoa measures to about 10–35 μm in length. It has pseudopods that allow them to move quickly and a 2–4 μm spheroid nucleus and also a small endosome. *E. gingivalis* has a clear ectoplasm, and the endoplasm has few organelles, numerous

glycogen granules, and food vacuoles containing erythrocytes and leukocytes.[5] It is most often found in gingival tissues specifically undergoing suppurative changes due to the presence of pathogenic microorganisms around teeth, and sometimes, tonsils. The protozoa have a strong preference to habitat in an anaerobic environment.[6] Its occurrence has always been associated with age, poor oral hygiene, calculus, and periodontal disease.[7] They feed on oral epithelial cells, bacteria, food debris, and notably red blood cells and white blood cells. Transmission is by oral-oral contact, droplet spray, or sharing of eating utensils.[8]

The main activity of the amoeba *E. gingivalis* in the infected crevices is feeding on the white blood cell nucleus by negative suction. The amoeba targets neutrophils which gradually get digested inside the endoplasm. At times, the protozoa do phagocytose more than 20 polynuclear nuclei, thus resulting in a denucleated cell, which is unable to achieve its neutrophil extracellular trap activity or its programmed apoptosis. It then releases polymorphonuclear neutrophil-uncontrolled proteolytic enzymes into the surrounding tissues and could then be considered a pathogen from this phagocytic activity.[7]

Subsequently, many investigators studied its association with periodontal disease. To test its causal relation, treatment of some of these conditions with emetine hydrochloride[9] was also tried. Although many studies acknowledged its causal relation,[9] few authors disputed it.[10] However, it is still a widely accepted fact that this amoeba prevailed more commonly in patients with poor oral hygiene. Studies have found protozoa in 74.5% of the subjects, and *E. gingivalis* was almost twice as commonly isolated than *T. tenax*.[11,12] Hence, our exploratory study also focused only on the protozoan *E. gingivalis*.

The aim of this study was to evaluate the incidence of the oral protozoan *E. gingivalis* in a population attending a premium dental institution in South India.

Materials and Methods

After obtaining the Ethical Clearance from the Institutional Review Board of Sri Venkateshwara Dental College and Hospitals, the research project was initiated. It was a cross-sectional comparative clinico-microbiological study with criteria-based sampling. The plaque samples were obtained from 75 patients, who attended the outpatient pool of a private dental college and hospital in South India. Before the commencement of the study, the selected patients were explained about the nature of the study and then signed a consent form, following which they were categorized into the following groups based on the criteria suggested by the American Academy of Periodontology.[13]

- Group A (n = 25): Control group comprised of patients presenting with clinically normal gingival health with no evidence of clinical gingival inflammation.
- Group B (n = 25): Gingivitis group comprised of patients exhibiting generalized gingival inflammation evident clinically as mild bleeding on probing but with no probing pocket depths.
- Group C (n = 25): Periodontitis group comprised of patients described as having generalized chronic periodontal disease characterized clinically by moderate-to-severe bleeding and with probing depths ≥5 mm in at least 70% of sites and with radiographic evidence of bone loss.

Patients with the visible gingival recession, those with a history of systemic disease, smokers, and lactating women and in patients in whom oral prophylaxis had been performed within the previous 6 months were excluded from the study.

Plaque samples were collected from selected patients with a sterile Gracey Curette.

For patients in Group A (controls) and Group B (gingivitis group), samples were obtained from sites exhibiting maximum accumulation of plaque which was identified with the help of disclosing solution.

For patients with periodontitis in Group C, plaque samples were collected from sites exhibiting the greatest probing depths.

Light microscopic examination by original wet-smear technique was performed immediately for the presence of motile amoeba. The collected plaque samples were immediately mounted onto slides using the wet mount technique. The sample was added to a drop of saline which had been deposited on a clean microscopic slide. A coverslip was then dropped into position, and the material spreads by pressure on the coverslip. This prepared a thin film which was then viewed under the binocular light microscope with a magnification of ×100 and ×400. The results were statistically analyzed by the Epi Info 2000 software.

Results

Analysis of the plaque samples in this study revealed that 42 samples (56%) were positive for *E. gingivalis* in which 88% (22 samples) were from patients with gingivitis and 76% (19 samples) were from patients with periodontitis. *E. gingivalis* was found only in one (4%) of 25 control samples. There was a significant correlation between the presence of protozoa and periodontal status (Chi-square = 41.883, P = 0) (Table 1).

Discussion

The oral cavity harbors a plethora of microbiomes in the human body, including viruses, fungi, protozoa, archaea, and bacteria. Even though oral diseases are considered of bacterial origin, they are an outcome of a complex interaction between commensal microbiota, host susceptibility, and environmental factors such as diet and smoking.

Majority of the literature have studied only about bacteria as an oral microorganism in the pathogenesis of the periodontal disease. However, there are only a few studies that investigated the role and association of oral commensals with periodontal disease. This investigation, though started a century before, could not see the light of the day due to a dearth of concrete evidence in proving its causal nature and hence forgotten in the

Table 1: Prevalence of *E. gingivalis* according to the periodontal status of the patient

Periodontal status	*E. gingivalis*
	n (%)
Healthy (Group A - 25)	1 (4)
Gingivitis (Group B - 25)	22 (88)
Periodontitis (Group C - 25)	19 (76)
Total (75)	42 (56)

E. gingivalis: Entamoeba gingivalis

subsequent years. As parasitic infections have become relatively common among our patients currently, there is a growing interest to identify the role of protozoa in periodontal disease.

In the present study, it was found that 56% of patients with periodontal disease harbored *E. gingivalis* when compared to 4% of healthy controls. This is similar to many epidemiological studies that have shown that the presence of *E. gingivalis* in patients with periodontitis is much higher than that in healthy patients.[14] These results were comparable to the results of another study which analyzed the incidence of *E. gingivalis* and *T. tenax* in samples of dental biofilms and saliva from patients with gingivitis, periodontitis, and healthy subjects.[15] Among the biofilm samples collected and studied, almost 50% of gingivitis patients and 50% from periodontitis patients were positive for *E. gingivalis*. This led to the conclusion that *E. gingivalis* was far more common in the early stages of periodontitis.[15]

In another study, the occurrence of oral protozoa among all age groups was evaluated. They found that *E. gingivalis* and *T. tenax* do not occur in small children and were rarely found in older ones.[8]

In an animal model study, when the rats were injected with *E. gingivalis* at the base of the gingival pocket, it induced periodontal abscess in 78.9% of the samples. The authors were able to culture the protozoa from the purulent secretions. They proposed that salivary enzymes and free radicals in patients with *E. gingivalis*-induced periodontitis may be involved in the lysing of the plasmalemma and membranous organelles, damaging the epithelial cells of the gingiva.[16] In another comparative study, *E. gingivalis* was applied topically on the marginal gingiva of immunocompetent and immunosuppressed rats. The clinical signs of Periodontitis developed much faster in rats immunosuppressed by prednisolone than immunocompetent rats. These results suggest the role played by immunosuppression in the initiation of Periodontitis by oral commensal organism like *E. gingivalis*.[17] These results focus on the importance of immunocompetence and the chances of acquiring protozoal-induced diseases.

A similar study was carried out to investigate the effect of the antimicrobial imidazole group (Flagyl) on *E. gingivalis* in patients with periodontitis. The results revealed that higher frequency of the protozoa was observed in patients with periodontitis (64%), while treatment with antimicrobials reduced this frequency to half (26%).[18]

The fact that this species of protozoa has been frequently isolated in the dental biofilm and saliva of patients with periodontal disease[15] emphasizes that it may play a pivotal role in the development of periodontal disease, as supported from the results of others studies. On the contrary, very few authors have suggested that this protozoan is essentially opportunistic and proliferates in an environment made favorable by a host already affected by periodontal disease.[7,18]

Conclusion

The results of this study suggest that *E. gingivalis* is the species found to be prevalent in patients with periodontal disease. It appears that metabolic disturbances in the oral cavity due to poor oral hygiene resulting in gingivitis and periodontitis may influence colonization of this oral microbiome. This creates a roadmap for a mixed composition of protozoa, bacteria and fungi, thus paving the way for the initial subclinical destruction of periodontal tissues. Better diagnostic criteria using the phase contrast microscopy, polymerase chain reaction, current digital-based technology, and a larger patient base would serve to potentiate the fact that this much under-rated species does indeed create an ecological niche which may favor the aggregation and colonization of putative periodontal microorganisms, thus creating yet another dimension in understanding the complex nature of periodontal disease.

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