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Original Article

Molecular Detection of *Entamoeba gingivalis* among Periodontitis and Gingivitis Patients by Singleplex Polymerase Chain Reaction

Mays Mahmood Yassin, Abeer Abbas Ali[1], Asal Aziz Tawfeeq[1]

Department of Dentistry, AL-Esraa University College, Baghdad, [1]Department of Medical Laboratory Techniques, Northern Technical University, College of Health and Medical Techniques, Kirkuk, Iraq

Abstract

Background: Periodontal disease is classically characterized by progressive destruction of the soft and hard tissues of the periodontal complex, mediated by an interplay between dysbiotic microbial communities and aberrant immune responses within gingival and periodontal tissues. It is being recorded as public health problems. **Objective:** The aim of study is to detect *Entamoeba gingivalis* parasite in the oral cavity of patients with periodontitis and gingivitis by using molecular technique with singleplex polymerase chain reaction (PCR). **Materials and Methods:** A total of 100 patients with periodontal diseases (periodontitis and gingivitis) were enrolled in the current study. Samples of dental plaque were collected from each patient and stained with Giemsa stain and studied under a microscope. Six samples were selected to be examined by using singleplex PCR technique for the detection of 18S-the small subunit of ribosomal RNA gene (SSU rDNA) gene with 203 bp and comparing the results. **Results:** This result showed a high significant prevalence of *E. gingivalis* in dental plaque samples in patients with periodontitis and gingivitis. About 46 (46%) samples were positive by microscope examination. In comparison, six positive samples (3 men and 3 women) were selected for parasitic investigation by singleplex PCR and the results of PCR with specific primers designed for the detection of 18S SSU rDNA gene of (203 bp) showed positive results for the six samples. **Conclusion:** It was concluded that *E. gingivalis* had a prevalence among male patients than female patients. In addition, singleplex PCR is the technique of choice for the detection of the target sequence of DNA.

Keywords: *Entamoeba gingivalis*, molecular detection, periodontitis, singleplex PCR

Introduction

The periodontal disease, which comprises gingivitis and periodontitis, is a common oral infection that affects the tissues that surround and support teeth.[1,2] This condition is often present as gingivitis which is characterized by bleeding, swollen gums, and pain, and if left untreated, it progresses to periodontitis which involves the loss of periodontal attachment and supporting bone.[3] The parasite *Entamoeba gingivalis* was among the first microscopic symbiont found in the oral cavity where they are found only in the trophozoite stage.[4] The trophozoite measures 10–30 μm, actively motile with multiple pseudopodia, and the cytoplasm (ectoplasm and the endoplasm) contains food vacuoles with ingested bacteria, leukocytes, and epithelial cells.[5] The *E. gingivalis* with periodontitis-associated bacteria may play an important role in periodontitis by the ability to induce host cell inflammation and gingival tissue destruction.[6] *Entamoeba gingivalis* disrupted the epithelial cell and fed on host cells by invading gingival tissue.[7] Hence, the parasite-bacteria interaction may cause severe periodontitis and change the microbiota in the gingival, not only in case periodontitis-associated bacteria but *E. gingivalis* will be as a potential pathogen on periodontitis.[8] Therefore, the recent study

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Address for correspondence: Mrs. Mays Mahmood Yassin, Department of Dentistry, AL-Esraa University College, Baghdad, Iraq.
E-mail: maysmahmood1989@ntu.edu.iq

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was designed to detect *E. gingivalis* in the oral cavity of patients with periodontal diseases (periodontitis and gingivitis) by using singleplex polymerase chain reaction (PCR) with specific primers of the the small subunit of ribosomal RNA gene (SSU rDNA) gene.

Materials and Methods

Collection of samples

Dental plaque samples were collected from 100 patients suffering from Periodontal disease, including 50 gingivitis and 50 periodontitis who attended the Dentistry Department, AL-Esraa University College, Baghdad, Iraq. In addition 50 samples from apparently healthy individuals as control, with age ranged between 20 and 64 years, from the beginning of October 2021 to the end of May 2022.

Isolation of *Entamoeba gingivalis*

The samples of dental plaque were collected by a sterile swab wiped around the teeth and around the gingival crevices, the swabs were immersed in tubes containing normal saline, and the swab smeared on the slide, and stained with Giemsa stain and scanned under a microscope and *E. gingivalis* were recognized by their shape depending on the presence of vacuoles and the expansion of the pseudopodia formation according to Ibrahim and Abbas.[9]

Genomic DNA isolation

The genomic DNA was extracted from the isolates using a commercial wizard genomic DNA purification kit (Promega, Madison, USA) and according to the manufacturer's instructions DNA concentration was determined by Nanodrop. After genomic DNA extraction, agarose gel electrophoresis has been adopted to confirm the presence and integrity of the extracted DNA.[10,11]

Singleplex polymerase chain reaction

PCR was used for the detection of the 18S-SSU rDNA gene. The primers used were 18S-SSU-F: 5' AGGAATGAACGAACGTACA3' rDNA-R: 5' CCATTTCCTTCTTCTATGTTTCAC 3', and then the general properties of these primers were checked by using the Oligocalc Oligonucleotide Properties Calculator program (OligoCalc, Microsynth AG; Switzerland). The thermal cycler conditions were 94°C 3.5 min denaturing followed by 40 cycles of 94°C 1 min, 60°C 1 min and 72°C 1 min, and 72°C 5 min for final extension.[12] Each PCR reaction was carried out in a final volume of 25 µL. The components o the reaction mixture includrd 12.5 µL of master mix, 5 µL of DNA sample (100 ng), 2.0 µL of each forward and reverse primer, and 3.5 µL free-nuclease distilled water. PCR products and 100 bp DNA molecular ladder (Promega) the positive result of the 18S-SSU rDNA gene is visualized in by 1.5% (w/v) agarose gel electrophoresis in 1× of Tris-acetate-EDTA buffer. Gels were stained with ethidium bromide solution (5 µg/mL)

Statistical analysis

The Statistical Analysis System—SAS (2018) program was used to detect the effect of different factors in the study parameters. Chi-square test was used to compare between percentages (0.05 and 0.01 probability) in this study.[13]

Ethical approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients verbal and analytical approval before sample was taken. The study protocol and the subject information and consent form were reviewed and approved by a local ethics committee according to the document number 137 (including the number and the date in October 12, 2021) to get this approval.

Results

In this study, the microscopical examination of the samples showed a total of 46 samples of dental plaque (46%) were positive for *E. gingivalis*, with significant difference at ($P < 0.01$) between control group and patient group [Table 1].

Table 2 revealed that *E. gingivalis* is higher in men than in women (50 % and 40%), respectively, with no significant difference observed between male and female groups. However, the severity of parasitic infection was more in men than women as shown in Figure 1.

Table 1: Distribution of *E. gingivalis* according to the periodontal status of the patients

Group	Total no sample	*E. gingivalis* infection percent
Periodontal	100	46 (46%)
Control	50	0 (0%)
Chi-square (χ^2)	–	11.07**
P-value	–	0.0001

** $P \leq 0.01$

Table 2: Distribution of *E. gingivalis* according to the patients' gender

Gender	Total no sample	*E. gingivalis* infection percent
Male	60	30(50 %)
Female	40	16 (40%)
Total	100	–
Chi-square (χ^2)	–	2.809 NS
P-value	–	0.184

NS: non-significant

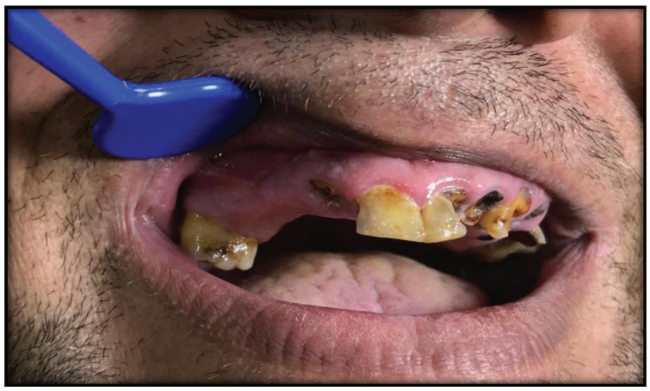

Figure 1: Male with *E. gingivalis* infection

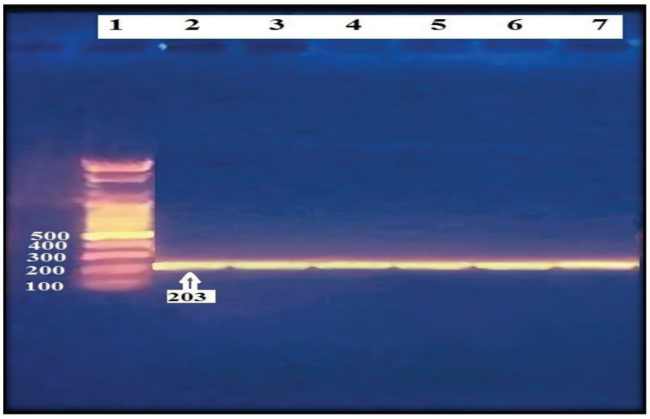

Figure 2: Gel electrophoresis of singlplex PCR product of *E. gingivalis* using 1.5% agarose gel at 7 volt/cm for 1 h. Lane 1: 100 bp DNA lader, lane 2–7: singlplex PCR product of 18S-SSU rDNA gene

Therefore, singleplex PCR was carried out to examine six positive samples out of the 46 microscopically detected *E. gingivalis* samples in order to confirm the results as shown in Figure 2.

In the molecular diagnostic methods, an amplification of 18S-SSU rDNA gene from 6 samples was performed to confirm *E. gingivalis* identification. The result showed that 6 (100%) of *E. gingivalis* had 18S-SSU rDNA gene with band (203 bp).

Discussion

Periodontal diseases including gingivitis and periodontitis are inflammatory conditions that can also be viewed as infectious diseases.[14] Periodontitis is a common oral disease affecting the global population, yet its etiology is not fully determined. Researchers are still investigating the role of many factors such as microorganisms and environmental or genetic factors in the pathogenesis of this multifactorial disease.[15] Approximately 15% of the healthy oral cavities of adults are infected with *E. gingivalis*. The prevalence of this protozoan strongly increases in periodontal inflammation, and it colonizes up to 80% of inflamed pockets of patients with periodontitis.[6]

The prevalence rate of *E. gingivalis* was high (46%) among individuals with periodontal disease, compared to those in the healthy group (6%) and agrees with the result of studies conducted by Hussian,[16] Al-Dulaimi *et al.*,[17] Al-hamiary *et al.*,[18] Jabuk *et al.*,[19] who found prevalence of the parasites was (46.6%), (42.9%), (42.9%), and (53%). The high prevalence rate of mouth protozoa *E. gingivalis* was related to mouth hygiene habits, periodontal tissue condition, gum bleeding, and degree of decayed and loose teeth.[20] According to Garcia *et al.*,[21] *E. gingivalis* positivity rates among individuals with periodontal disease range between 30% and 81%, and even positivity rates as low as 12% have been identified. These vast differences in positivity rates might reflect differences in diagnostic sensitivity among the methods used.[22] In contrast, Al-Saeed[23] suggested that if *E. gingivalis* supports or contributes to the development and progression of periodontal diseases (gingivitis and periodontitis), these diseases progressively facilitate the proliferation of this protozoa.

Regarding the distribution of infection in relation to gender, in the current study, the percentage of occurrence in men was higher than in women (50 % and 40%, respectively). The result of the current study matches with the results of different studies that stated men were more infected than women.[24-26] This result was probably due to lower oral hygiene followed by men in addition to smoking, energy drinks, and alcohol drinking.[27] Other studies by Jaffer *et al.*,[28] Hamady and Obaid[29] found the prevalence of parasites in the women group was significantly higher than in the men group in patients with oral diseases. The study by Stensvold *et al.*[30] found no relationship between basic demographic features such as reported for age and gender. Some natural factors, in addition to the complexity of the oral environment and rapid growth of germs, contribute to gum diseases and parasitic incidence.

Since molecular testing is increasingly used to supplement or replace conventional microscopic-based methods of parasite infection detection.[31-33]

This result agrees with the study conducted by Hussian,[34] which included 50 samples. Those were diagnosed by using PCR to insure found *E. gingivalis* with specific primers of the SSU rDNA gene. Tthe result of PCR found 20 samples with positive. So the PCR technique is the best method to detect this. Other studies detected *E. gingivalis* by PCR methods in respectively, 33.3%, and 16% of periodontitis patients.[35,36]

The study about children treated with orthodontic appliances in AL Muthanna Province, Iraq found *E. gingivalis* was in 15 (15.78%) samples by PCR technique.[37] They compared the morphological and genetic variabilities within *E. gingivalis* trophozoites isolated from diseased and healthy periodontal sites by

microscopic analysis and gene scanning of the 18S-SSU rRNA by real-time PCR.[38-40] In recent years, several PCR-based studies showed that *E. gingivalis* was significantly increased in periodontal pockets of periodontitis. The prevalence in patients with periodontitis and in individuals with periodontal health was 74%–88.9%.[41-43]

Amebae have the ability to colonize the human host extensively within the genus, depending on the sequence of this gene. They reflected their differences in the nucleus number of their cyst form. The mouth colonizing *E. gingivalis* has no identified cyst form.

In spite of its vicinity to species producing mono- or tetra-nucleated cysts, the detection of Entamoeba gingivalis in periodontal pockets depend on their small subunit ribosomal DNA (SSU rDNA) sequences.[12]

Conclusion

In conclusion, the present study revealed that the 18S-SSU rDNA gene could be considered as a target for the detection of *E. gingivalis* in addition to the conventional methods. Furthermore, the percentage of occurrence of *E. gingivalis* in males was higher in men than in women.

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Conflicts of interest

There are no conflicts of interest.

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