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Isolation and real-time PCR detection of (Entamoeba gingivalis) in Gingivitis patients

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Abstract

Entamoeba gingivalis is an oral protozoan commonly found in dental plaque and particularly within the gingival sulcus, periodontal pockets, and tonsillar crypts. Periodontitis consider one of the most common illnesses in the world. The aims of current study isolation and diagnosis.

Entamoeba gingivalis from patients with gingivitis and healthy individuals.

The current study included 68 samples 34 samples was collected from healthy individuals and 34 samples was collected from dental plaque and gingivitis patients from the private dental clinics. The results shown 13 samples positive with Entamoeba gingivalis from gingivitis patients and 5 samples positive from healthy individuals. Diagnosis of this parasite was by using direct microscope examination and using real-time PCR technique

SSU rDNA gene 203 bp.

Highlights:

*Entamoeba gingivalis is not limited to patients with gum disease, but can even be found in healthy individuals.

Molecular method consider the best methods for diagnosis this parasite. *

Keywords: Entamoeba gingivalis, Real-time PCR technique ,Gingivitis patients

الملخص

إنّ الأميبا اللثوية **(Entamoeba gingivalis)** هي طفيليّ فمويّ شائع الوجود في طبقة البلاك السنية، وخاصةً في التلم اللثوي، والجيوب اللثوية، وتجاويف اللوزتين. يُعتبر التهاب دواعم السن من أكثر الأمراض شيوعًا في العالم. تهدف هذه الدراسة إلى عزل وتشخيص الأميبا اللثوية من مرضى الذين يعانون من التهاب اللثة ومن الاشخاص الأصحاء.

شملت الدراسة 68 عينة، جُمعت 34 عينة من اشخاص أصحاء، و34 عينة من مرضى التهاب اللثة في عيادات الأسنان الخاصة. أظهرت النتائج وجود الأميبا اللثوية في 13 عينة من مرضى التهاب اللثة، و5 عينات من الاشخاص الأصحاء. تم تشخيص هذا الطفيليّ باستخدام الفحص المجهري المباشر وتقنية تفاعل البوليميراز المتسلسل ريل تايم **(PCR)** لجين الحمض النووي الريبوزي الصغير **(SSU rDNA)** بطول موجي (203 زوج قاعدي)

أبرز النقاط:

* لا تقتصر الأميبا اللثوية على مرضى التهاب اللثة، بل يمكنان توجد أيضًا لدى الاشخاص الأصحاء.

* تعتبر الطرق الجزيئية أفضل الطرق المستخدمة لتشخيص هذ الطفيلي.

الكلمات المفتاحية: الأميبا اللثوية، تقنية تفاعل البوليميراز المتسلسل ريل تايم ، مرضى التهاب اللثة

Introduction:

Entamoeba gingivalis is a protozoan parasite establish in the oral cavity of human identified in gingival dental plaques ,tooth, in addition carious lesions (1). This parasite associated with periodontitis may act as a potential pathogen by triggering inflammation in host cells and promoting the destruction of gingival tissues (2).

The first diagnosis methods of this parasite by using microscopic detected in the oral cavity, By diagnosis identified trophozoite stage only (3).

Entamoeba gingivalis morphologically, is present only in the trophozoite stage and does not develop a cyst form, its transmission involves direct oral contact, such as kissing, sharing utensils, or the use of contaminated dental instruments. Its detection is often

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overlooked due to its fragile nature and the lim itations of conventional microscopic techniques. However, the best diagnostics by molecular, particularly PCR-based methods, have greatly improved both the sensitivity and specificity of identifying *E. gingivalis* in clinical samples (4,5).

Protozoan pathogens are often detected in periodontal pockets and are regarded as significant contributors to periodontal disease (6).

The first to successfully amplify the small subunit ribosomal RNA gene (SSU rDNA) by utilizing laboratory-cultured *E. gingivalis* and designing their own unique, specific primers were Kikuta and his colleagues (7).

Furthermore, recent studies suggest that *E. gingivalis* may alter the host immune response by regulating cytokine secretion and escaping neutrophil-mediated destruction. These immune-related changes can contribute to faster periodontal tissue breakdown and delayed healing, thereby worsening the progression and severity of periodontal disease (8,9). Therefore, elucidating these mechanisms is essential for designing targeted diagnostic and therapeutic approaches to minimize protozoan-associated periodontal damage (10).

Materials and methods

1- Collection of Samples

This study included 68 samples swab 34 collected from healthy individuals and 34 samples was collected from dental plaque and gingivitis patients from the private dental clinics. The study was from October 2025 to February 2026.

2- Microscopic examination

The samples collected by the researcher it was taken using a sterile swab from the gingival crevices and surrounding teeth in tub contain normal saline them diagnosis by direct microscope examination use clean slides and stained with Giemsa stain for detection *E. gingivalis* depended on morphological shape detection trophozoite stage by presence of vacuoles and the extension of pseudopodia, according to (11).

3- Molecular study

1- Genomic DNA extraction from samples

DNA isolation from saliva, gingival crevices and teeth samples was extracted by using human gSYNC™ DNA Extraction Kit, according to manufacturer's protocol. human gSYNC™ DNA Extraction Kit/ Geneaid/ Taiwan and Syber Green Master Mix Kit/ Promega/ USA.

2- Primers:

In this study use 18S-SSU rDNA gene. F: 5'AGGAATGAACGAACGTACA3'

R: 5'CCATTTCCTTCTTCTATGTTTCAC 3'.203bp.this primers according to (17).

3- Real- time PCR detection of *Entamoeba gingivalis* by18S-SSU rDNA gene.

Amplification the 18S-SSU Rdna. The gene was amplified using a primer ,performed in real-time PCR thermocycler system. The final volume of RT-PCR reaction tubes was 20µl, consist of 12.5µl Syber green Master Mix, 1µl of both forward and reverse of the primer specific for this gene, 3µl of DNA template and the volume was completed by adding nuclease free water.

The PCR cycling conditions consisted of an initial denaturation step at 95°C for one cycle, followed by 40 cycles of denaturation at 95°C for 20 seconds and annealing at 60°C for 30 seconds.

PCR reaction mixture was prepared as follows:

- 10 µL of SYBR Green Master Mix
- 1 µL of each primer (forward and reverse)
- 5 µL of double-distilled water (DDW)
- Total reaction volume: 20 µL

Results:

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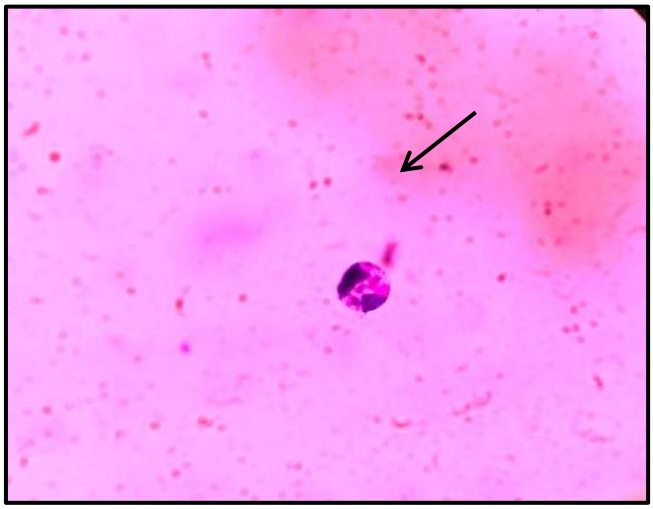
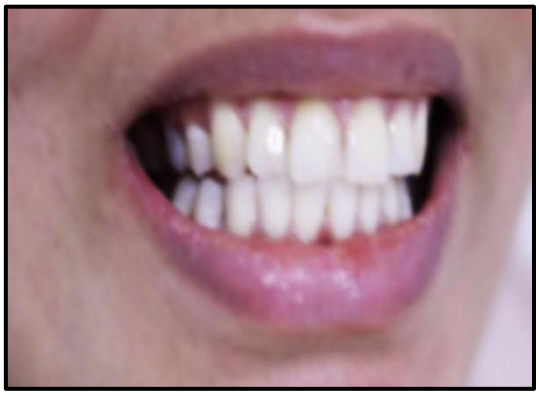
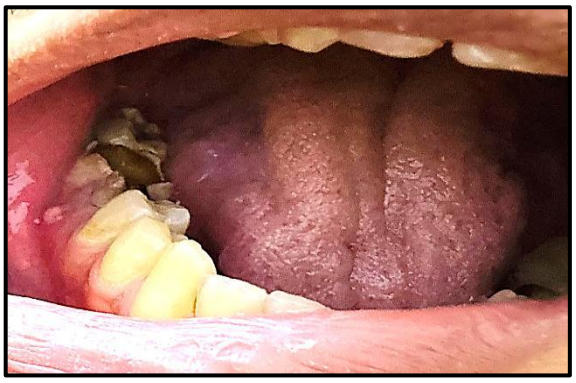
In current study the microscopic examination shown 13 samples were positive from dental plaque and gingivitis patients for *E. gingivalis*. and also shown 5 samples were positive from healthy individuals for *E. gingivalis*.



Fig(1): *Entamoeba gingivalis* (Trophozoite stage) in direct smear from oral cavity stained with Giemsa stain (40X).



Fig(2): Patient with gingivitis, dental plaque show *E. gingivalis*.



Fig(3): Healthy patient without gingivitis, and dental plaque and show *E. gingivalis*.

Diagnosis of *E. gingivalis* by real-time PCR technique

Completely samples (100%) of total DNA samples extracted and gave a positive results from gingivitis patients and healthy individual of 18S-SSU rDNA gene that used to detected this parasite by use by real-time PCR technique.

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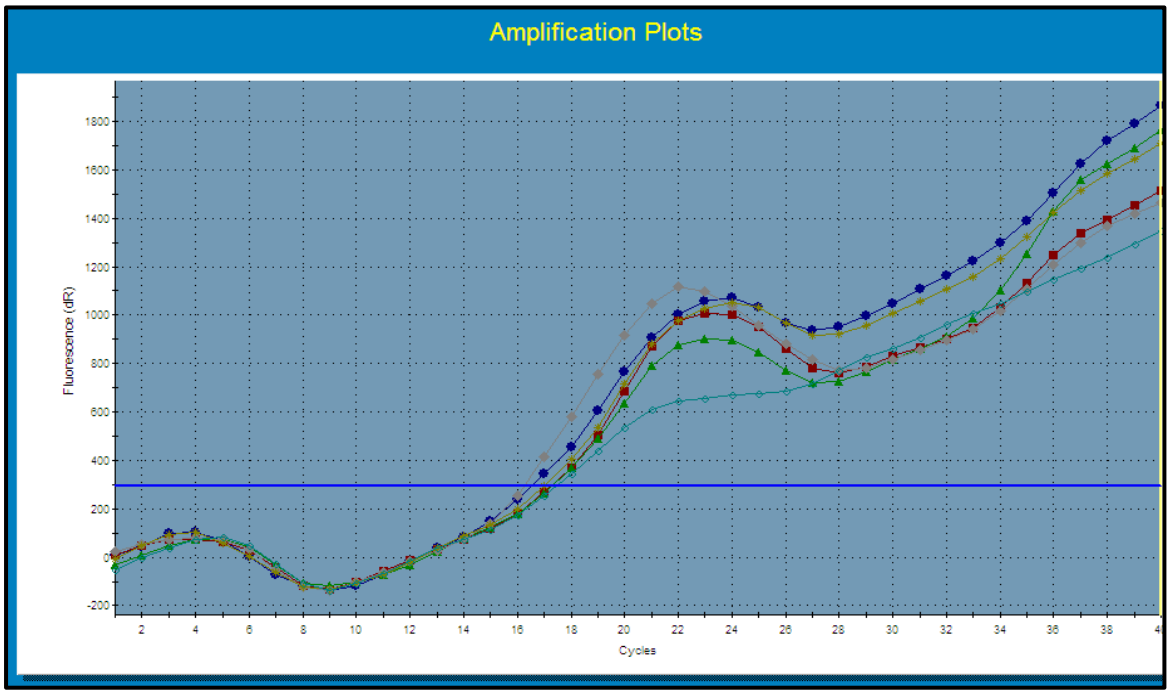

Fig (4) The amplification plot of 18S-SSU rDNA gene in *E. gingivalis* by real-time technique.

Discussion

Periodontal infection including gingivitis and periodontitis, are inflammatory conditions that can also be considered infectious diseases. (12). *E. gingivalis* found in approximately 15% of healthy adult oral cavities. Its prevalence rises markedly in cases of periodontal inflammation, where it may colonize As many as 80% of inflamed periodontal pockets are found in patients with periodontitis. (13).

This study appearance high infection with *E. gingivalis* in gingivitis and periodontitis patients while healthy patients also appearance infection with *E. gingivalis* but less from periodontitis patients this agree with may studies such as and agrees with Hussian (13) .Al-Dulaimi *et al.*,(14). Al-hamiary *et al.*,(15) Jabuk *et al.*,(16) who found prevalence
of the parasites was (46.6%), (42.9%), (42.9%), and (53%).

In real-time PCR, melting curve analysis provides a rapid and reliable means of characterizing amplified DNA products, removing the requirement for gel electrophoresis and producing reproducible sequence-specific signatures that can be utilized for PCR product typing (23). Another study also Showed that the prevalence of *E. gingivalis* was significantly greater in individuals with periodontal disease. (46%) related with healthy individuals (6%) This study by(17).

Our study agree with results occur in Thi-Qar Province of *E. gingivalis* with diseased periodontitis sites shown higher prevalence for *E. gingivalis* in microscopic examination this prevalence higher in periodontitis diseased than individuals without periodontitis by (18). A previous study present of *E. gingivalis* in the oral cavity of patients with plaque-induced gingivitis and compared with healthy individuals using the RT-qPCR techniquein dental center in Al-Hilla city by (19).

Molecular diagnostic techniques are increasingly being used either to complement or replace conventional microscopy-based methods for the detection of parasitic infections (20,21).

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Another study for *E. gingivalis* occur in Wassit University shown periodontal disease has a higher prevalence of *E. gingivalis* and also shown The presence of this parasite in the oral cavity is interpreted as an indication of inadequate dental hygiene (22). Consistent with this study, patients through gingival disease more susceptible to oral parasite infection and real-time PCR technique reflect a good diagnosis methods of this parasite.

As a final recommendation, sequence analysis using the NCBI database is advised for the accurate isolation and diagnosis of *E. gingivalis.*

Conclusions

In conclusion, this study demonstrated the higher in individuals with periodontitis than individuals with healthy periodontal sites and the 18S SSU rDNA gene may serve as a reliable target for the detection of *E. gingivalis*, complementing conventional diagnostic methods. And also shows present of *Entamoeba gingivalis* in healthy and increase in gingivitis patients and the molecular such as real-time PCR technique consider the best method to diagnosis of this parasite.

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