Wasit Journal for Pure Science

Journal Homepage: https://wjps.uowasit.edu.iq/index.php/wjps/index
e-ISSN: 2790-5241 p-ISSN: 2790-5233

Prevalence of *Entamoeba gingivalis* Among Patients Attending Dental Clinics in Wasit Province

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DOI: https://doi.org/10.31185/wjps.461
Received 15 June 2024; Accepted 04 August 2024; Available online 30 September 2024

ABSTRACT: Gingivitis and periodontitis are two inflammatory illnesses that are also considered infectious diseases in the context of periodontal disease diagnosis, periodontitis is a widespread oral illness that affects people all over the world .The current study used the wet mount technique with physiological saline and Giemsa stain to ascertain the incidence of *Entamoeba gingivalis* among 30 subjects (15 men and 15 females). (73%), from the beginning of November 2023 to the end of January 2024 from some dental clinics in Kut.
E. gingivalis was prevalent overall (21 - 30) year olds had the highest frequency of *E. gingivalis* (54.55%). Infection was highly significant in female (59%) than male (41%).
The conclusion that periodontal disease has a higher prevalence of *E. gingivalis*. The presence of *E. gingivalis* in the oral cavity is interpreted as an indication of inadequate dental hygiene, even though it is not often linked to pathogenesis. It is important to emphasise that patients' oral hygiene practices are not up to scratch. They employ mostly ineffective oral cavity hygiene products.

Keywords: *E. gingivalis*, periodontitis, dental, gingivitis

1. INTRODUCTION

The mouth cavity has the second biggest quantity and variety of microorganisms in the body, containing millions of germs that compose the oral microbiota [1]. These microbes produce dental plaque and live in symbiotic balance with the host, but when there is a dysbiosis in the microbial equilibrium, several oral illnesses, including periodontal disease and dental caries, can arise [2] [3] . The most prevalent microbes are bacteria [4] . Protozoa like *Entamoeba gingivalis* and *Trichomonas tenax*, which have not gotten as much attention in periodontal investigations, can also be found in the oral cavity in addition to bacteria [5]. Some studies have explored the colonization of these parasites in the oral cavity of both healthy people and those with periodontal disease, with variable outcomes [6. 7]. The trophozoite form ranges in size from 10 to 35 µm in diameter *E. gingivalis* has no cyst form, so it spreads either directly through direct interactions, by kissing or indirectly through contaminated food, or by sharing eating utensils [8] Because *E. gingivalis* prefers anaerobic conditions, it is most commonly found in the tartar between and around teeth, gingival tissues, and tonsillar crypts, especially in suppurative, inflammatory diseases [9]. The protozoan microorganism *E. gingivalis* is often found in the human oral cavity, however reports of it in the genital tract have also been made [10]. Kissing, infected food or oral equipment, and mouth droplets all contribute to transmission [11]. Globally, *E. gingivalis* is distributed, with a 37% prevalence worldwide [12]. A connection between *E. gingivalis* and periodontal disorders has been suggested by some study [12]. The existence of *E. gingivalis* in healthy people and patients with various dental conditions has been the subject of numerous research published over the past few decades, with varying degrees [10. 13]. The use of various

approaches for the detection of *E. gingivalis*, the selection of various patient groups, and genetic diversity—which is still mostly unknown—can all be blamed for disparate or inconsistent results [14]. As a result, this information provides a scientific gateway for further investigation into the pathogenesis of periodontitis and gingivitis. Due to disagreements over the parasite's transmissibility, link to periodontal diseases and significance for public health, this study was conducted to ascertain the prevalence of *E. gingivalis* in dental clinics in the Wasit governorate of Iraq. Data on the parasite's prevalence are still scarce in many developing nations, including Iraq.

2. A STUDY AREA

Samples were taken from Wasit University Clinic and other external clinics

3. LABORARY METHODS

3.1 specimen collection

Collected samples from the beginning of November 2023 to the end of January 2024 from some dental clinics in Kut and under aseptic conditions and with the assistance of a dentist, a specimen of dental plaque and/or calculus was collected according to [15]. Specimens were transferred to the microbiological Laboratories/pure science within one hour of collection and quickly treated to preserve protozoan viability and avoid trophozoite lysis.

3.2 microscopical examination

Every specimen was split into two sections: one for wet mount preparation and the other for smear preparation for Giemsa staining.

3.2.1 wet mount examination

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, Using a sterile Pasteur pipette, droplets of a diluted material were put on clean microscope slides, then a coverslip was laid on top, and the material spread by pressure on the coverslip, This created a thin film, which was then quickly inspected at 10X and 40X magnification using a light microscope, *E. gingivalis* was identified by its slow movement and shape, which was determined by the expansion of pseudopodia development [16]. For each sample, three wet mount smears are employed to increase the likelihood of finding parasites.

3.2.2 Giemsa staining

On a sterile microscope slide, thick smears were formed and left to dry. Subsequently, Giemsa stain was applied to it, which was diluted (1:50, vol/vol) (to get a 1:50, 1ml Giemsa stock to 50 ml buffered water) and left for 50 min. after giving the slide a gentle wash with clean water and allowing it to air dry in a vertical posture, it was examined under a 100X magnification to check for *E. gingivalis*, the distinctive morphologic characteristics of *E. gingivalis* include an irregular form, light red-purple cytoplasm, and darker reddish-purple intracellular vacuoles [17]. For every sample, three smears that were stained with Giemsa were utilized to increase the likelihood of finding parasites.

4. RESULTS

In this study, the microscopical examination of the samples showed a total of 30 samples of dental plaque (73%) were positive for *E. gingivalis* as shown in Figure (1).

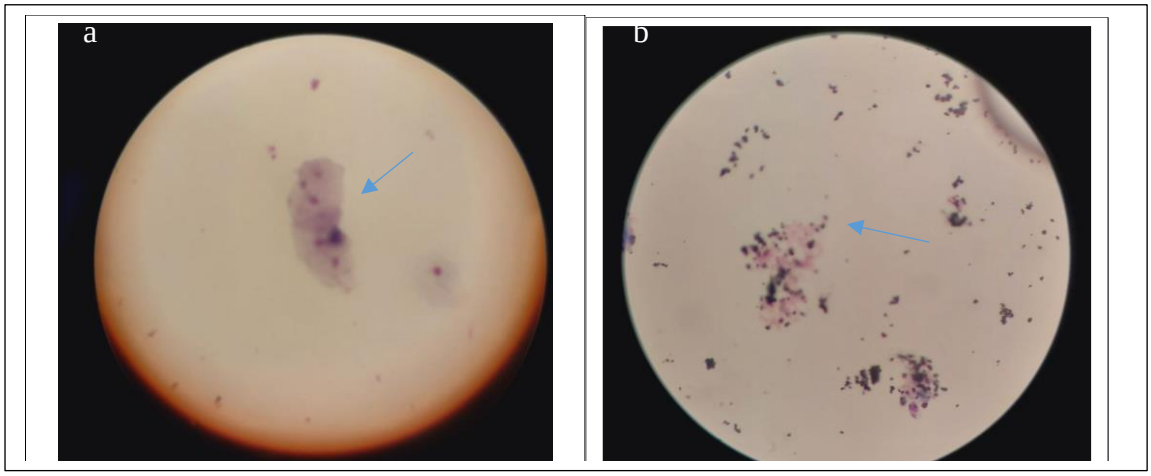

FIGURE 1. - (a), (b) the trophozoite of *E. gingivalis* stained with Geimsa stain (40X)

Gingivitis and periodontitis are two inflammatory illnesses that are also considered infectious diseases in the context of periodontal disease diagnosis [18]. Gingivitis and periodontitis are examples of periodontal disorders, although it's exact cause is unknown, periodontitis is a widespread oral illness that affects people all over the world [19]. Researchers are still looking into how many different elements, including genetic, environmental, and microbiological variables, contribute to the pathophysiology of this complex illness, can be classified as infectious diseases because they are inflammatory conditions[20] *E. gingivalis* is present in 15% of adult patients' healthy dental cavities, This protozoan is highly prevalent in periodontal inflammation and can colonize up to 80% of inflamed pockets in patients suffering from periodontitis [21].

The high incidence of the mouth protozoa *E. gingivalis* was linked to poor oral hygiene, periodontal tissue damage, gum bleeding, and the presence of decaying and loose teeth [22.23] *E. gingivalis* positive rates in people with periodontal disease range from 30% to 81%, with rates as low as 12% reported. These significant disparities in positivity rates may indicate differences in diagnostic sensitivity across the procedures used [24] In contrast, Al-Saeed [25] suggested that if *E. gingivalis* supports or contributes to development and progression of periodontal diseases (periodontitis and gingivitis), these diseases progressively facilitate the proliferation of this protozoa.

Table 1 revealed that *E. gingivalis* is higher in women than in men (59% and 41%), respectively, with no significant difference observed between male and female groups. However, the severity of parasitic infection was more in women than men.

Table 1. - number samples of *E. gingivalis* by microscopically examination depended on gender.

Sex	**No. of Examined**	***E .gingivalis***	
		No.	***%***
Male	15	9	41%
Female	15	13	59%
Total	30	22	

Regarding the distribution of infection in relation to gender, in this study, the percentage of occurrence in women was higher than in men (59 % and 41%, respectively). The findings of other research that suggested men were more infected than women are consistent with the findings of the current study [26.27] With patients with oral disorders, it was discovered that prevalence of parasites was much higher in a female group than in the male group [28]. discovered no connection between the gender and age stated, two essential demographic variables. Apart from the intricate oral environment and the swift proliferation of microorganisms, there exist certain inherent elements that lead to the prevalence of parasites and gum disease. In addition to smoking, consuming energy drinks, and drinking alcohol, men also likely practiced poorer dental hygiene, which contributed to this outcome [29]

Out of 30 investigated studies. The findings revealed a rise in prevalence among patients aged (21 - 30). The age groups with the lowest prevalence were those aged 10 and (51 - 60), according to Table (2).

Table 2. **Prevalence of *Entamoeba gingivalis* according to age.**

Age group	**No. Examined**	***E. gingivalis***	
		Positive case	%
>10	2	1	4.55%
11-20	4	4	18.18%
21-30	14	12	54.55%
31-40	5	3	13.64%
41-50	3	2	9.09%
51-60	2	0	0.00%
Total	30	22	

Because *Entamoeba gingivalis* is associated with host age, oral protozoa are uncommon in children, but infection prevalence rises with age. *E .gingivalis* and *Trichomonas tenax* occurrence in patients with immunosuppressive disorders, genetic diseases, and periodontal problems [30]. A 2016 systematic review published analyzed data from multiple studies and concluded that adolescents and young adults (aged 12-30) have the highest prevalence of *E. gingivalis* infection globally [23].

There are a number of reasons why the 12 to 30 age range is the most affected, the most significant of which is oral hygiene practices. Teens and young adults are more likely to participate in activities that raise the risk of infection, such as not brushing and flossing teeth as often, skipping dental exams, and consuming sugary drinks and snacks, Immune system: Adolescents and young adults are more vulnerable to infections overall because their immune systems are still maturing, Social factors: intimate contact activities as Kissing can raise the risk of *E. gingivalis* transmission. Other personal objects like tools or water bottles can be shared, Hormonal fluctuations: During puberty, variations in hormone levels can also lead to modifications in the oral environment, increasing the risk of infection. [31].

5. CONCLUSIONS

There are extremely few data on the function of oral commensals in the etiology of periodontitis and gingivitis, despite the high occurrence of specific protozoa, such as *Entamoeba gingivalis*. In this study the prevalence of oral parasite it was found that the maximum number of patients with *E. gingivalis* was reported within the young than adult and children, and that this disease was higher in females than in males. The presence of *E. gingivalis* in the oral cavity is interpreted as an indication of inadequate dental hygiene, even though it is not often linked to pathogenesis. It is important to emphasize that patients do not currently maintain adequate oral hygiene. Most of the products they use for oral hygiene are useless.

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