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Diagnosis of infection with amoebic parasite that cause stomatitis

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ABSTRACT

The current study dealt with the first detailed diagnosis of gingival amoebiasis (*Entamoeba gingivalis*) that affects humans from samples taken from both the gums and teeth, and the results showed that the parasite infects both sexes with a total percentage (12.14). Statistical analysis proved that there was no significant difference between male and female infection rates (52.94%, 47.06%), respectively, which is the lowest percentage of infection compared to what was recorded in previous studies. The results indicated that the parasite can infect all ages obtained in the current study, and that the highest infection rate was in the age period 30-39 (41.18%). While the lowest percentage was in the age period >50 (17.65), the highest percentage of periodontitis was 7 (41.18), followed by dental infection 6 (35.29), and the lowest percentage was recorded in both locations 4 (23.53) , as well as, there was not a significant difference between the percentage of inflammation in the gums and teeth, while there is a significant difference in the inflammation that it affects the gums and teeth together when compared to the percentage of gums and teeth separately The results recorded a relationship between amoebic and bacterial infections, and the high coefficient of Contingency value (0.988) indicated that there was a strong correlation between the presence of these two organisms.

Keywords: Non-pathogenic, Cavity, Gingivalis, Towels.

Introduction

Entamoeba gingivalis is described as a non-pathogenic amoeba that lives in the human gums and oral cavity and occasionally elsewhere, while it is often found in association with periodontal infection, no relationship to the nature of the relationship has been definitively established (Bonner et al., 2014).

The life cycle of this parasite is simple, that is, the parasite completes a life cycle in a single host (human, for example) (Burton & Thomas, 2012). There is no cystic stage, and the feeding stage is the infectious stage and it can be transmitted directly from an infected person to another healthy person through kissing or mouth spray from the infected person, or by sharing using some tools such as towels and toothbrushes, and the active stage of this parasite feeds on Lysate cells blood cells as well as bacteria in the infected mouth (Bao et al. , 2021; Christin et al., 2022). This parasite can be found in food and water (Mielnik-błaszczak et al., 2018).

E. gingivalis was the first symbiont described in humans (Gros,1849). Although it was noted in patients with periodontitis, presumed aetiological link, among the parasite later called "*Entamoeba gingivalis*" and Gum disease has been neglected for more than a century, the literature available on periodontitis focuses primarily on potential bacterial etiology, which is exacerbated by the genetic determinants of patients. However, this hypothesis failed to explain the difference between two types of inflammation (gingivitis and periodontitis) and some animals have been used to develop models to study periodontitis (Oz & Puleo, 2011). Therefore, the current research aimed to diagnose the parasite and its relationship to periodontal infections

Materials and Methods

Diagnosis and staining of samples: For the purpose of diagnosing and staining samples, the following steps were taken:

a- Diagnostic samples of the parasite were obtained in the form of swabs from 140 samples (the number of infected samples was 17 samples) between the ages of 20 and over 50 years.

The main indicator for epidemiological studies in oral health was calculated as "decayed, full and missing teeth" (Mielnik-błaszczak et al., 2018). The samples taken from the teeth, gum cavity, gums and swollen areas were examined using sterile swabs, after which the samples were transmitted to a special medium (TYGM-9 medium) and cultivated in anaerobic environments in glass Petri dishes at a temperature of 35°C. according to the method described by (Bao et al., 2021).

b- isolate and diagnose the parasite Entamoeba gingivalis. 30 minutes after sampling, the swabs of samples were washed in physiological buffer (0.9% NaCl). drops of each samples were placed on the glass slides , the information such as sex, age, etc. were fixed on slides , and then the samples were stained with Ziehl-Neelsen Stain, made by Company " Ismailia Company for laboratory medical supplies in Egypt " , contains the following: 1-concentrated carbol fuschin , 2-20% sulphuric acid , 3- methylene blue , The trophzoites of the parasite was observed in the microscopic preparations using a Novex-Holland compound microscope (40 × and 100 x), the study results of current research.

The infected people were treated using the treatment prescribed by the dentist (the treatment described in the results of the current research).

Ethics statement: It was approved to complete this research by submitting a request to the Department of Biology, College of Education for Pure Sciences, Al-Muthanna University.

Statistical analysis of the results: The Spss (20) program (To find statistically significant differences) and coefficient of Contigency test were used to determine the compatibility of the presence of the parasite and bacteria in the infected person.

Results and Discussion

The Percentage of infection with Entamoeba gingivalis according to sex: Table (1) shows that the total number of samples collected during the current research is 140 samples, and that the number of total infection (P.) is 17 samples (12.14%) from both sexes, with a ratio of (52.94 % , 47.06 %) respectively(Figure ,1). There are no statistically significant differences between the infection of males and females.

Table (1): The Percentage of infection with *Entamoeba gingivalis* according to sex.

Total Samples	No of total infection (P.)	% of total Infection	% of infection (M) From total P.	% of infection (F) From total P.
140	17	12.14	52.94	47.06
Statistical analysis				P≤ 0.05

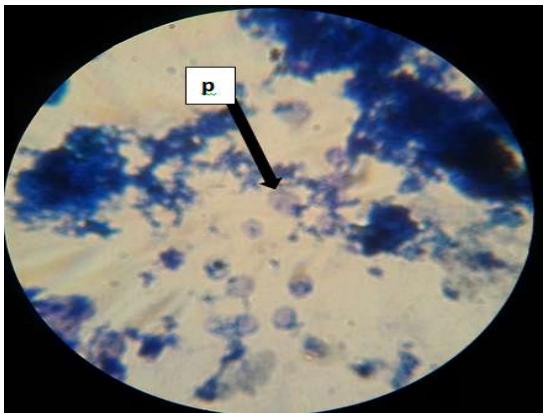

Figure (1): Shows Oral amebic parasite (*Entamoeba gingivalis*) obtained from a person with gingivitis.

Distribution of patients according to age intervals.

Table (2) indicates that the parasite can infect all ages, and the highest percentage of infection was in the age period 30-39 (47.14% , 41.18 %), while the lowest percentage was in the age period >50 (10%, 17.65).

Table 2: Distribution of patients according to age intervals.

Age group (year)	No.	% form the total samples	p.	% form the total p.
20-29	35	25	3	17.65
30-39	66	47.14	7	41.18
40- 49	25	17.86	4	23.53
>50	14	10	3	17.65
Total	140	100	17	100

The incidence of oral amebiasis according to the site of inflammation: It is clear from Table (3) that the highest percentage of stomatitis was in the gums 7(41.18), followed by inflammation in the teeth 6 (35.29), and the lowest percentage was recorded in both sites 4 (23.53), as well as the absence of a significant difference between the percentage of inflammation in the gums and teeth, while there is a significant difference in the inflammation that it affects the gums and teeth together when compared to the percentage of gums and teeth separately

Table (3): The incidence of oral amebiasis according to the site of inflammation.

site of inflammation in the mouth	Patients	
	No.	%
Gums	7	A 41.18
Teeth	6	A 35.29
both of sits	4	B 23.53
Total	17	100
Statistical analysis	P≤ 0.05	

*Vertically different letters indicate that there is a significant difference below the probability level ≤ 0.05.

The relationship of the parasitic infection of the current study with the bacterial infection: The highest correlation coefficient between the presence of amoeba and bacteria was recorded, as it was 0.988 (94.11) (Table (4).

Table (4): Showing the relationship of the parasitic infection of the current study with the bacterial infection.

No of total infection	% of Infection with bacteria	of Infection without bacteria
17	94.11	5.89
Coefficient of contingency		0.988

The percentage of infection with the parasite: *Entamoeba gingivalis*, compared with other studies from different regions of the world. Table (5) shows the percentage of infection of males, females, and both sexes with the *Entamoeba gingivalis* parasite, compared to other studies from different regions of the world. By looking at the table, it is clear that the percentage of infections recorded in previous studies is higher than that recorded in the current study. In a study conducted by Bonner et al. (2014) in France, it was recorded Percentage (78 for both gender), the study by Bao et al.(2021) in Germany recorded a percentage (15), a study by Yaseen, et al.(2021) in Jordan , recorded a percentage (M:54.7 , F: 45.3 and 88.9 for both gender), a study by Azadbakht et al. (2022) in Iran, recorded a percentage (F:63:1), while the percentage of infection in the current study was (M:52.94 , F: 47.06 and 12.14 for both gender). On the other hand, the percentage recorded in the current study is higher than what was recorded in the study by Mohammed & Binsaad (2023) in Yemen, where it was (M:23.7 , F: 12.5).

Table (5): shows the percentage of infection with the parasite *Entamoeba gingivalis*, compared with other sltudies from different regions of the world.

Author	Location	% (M)	% (F)	% Both Gender
Bonner *et al.*, 2014	France	--	--	78
Bao *et al.* 2021	Germany, Berlin	--	----	15
Yaseen, *et al.*, 2021	Jordan Amman	54.7	45.3	88.9
Azadbakht *et al.,* 2022	Iran	----	63.1	-----
Mohammed & Binsaad, 2023	Yemen	23.7	12.5	23.7
Alasadiy 2023	Present Study- Iraq- Al-Muthanna province, Samawah City	52.94	47.06	12.14

Treatment of parasitic infestation and other related infections: After diagnosing gum parasites by visiting the dentist, it was found that the treatment below is used to treat these conditions for a week:

1- Augmaxil (Amoxicillin and Clavlanate potassium, Tablets USP, 625mg).

2-Midagyl (Metronidazole ,Tablets , 500 mg).

3-Adolor strong (paracetamol,Tablets, 1000 mg).

In the event that the inflammation ends and the pain persists in the teeth, the damaged tooth is detected and extracted, and the following treatment is used:

1- Neomox (Amoxicillin,Tablets, 500 gm).

2- Tinidazole , Tablets , 500 mg.

3- Painoxia (Etoricoxib , Tablets, 90 gm).

The results of the current study were confirmed by)17(samples infected with the parasite out of (140) samples that were examined in the Environment and Pollution Laboratory.

Several studies indicate that *E. gingivalis* can be acquired by taking samples from the oral cavity of an infected person, sputum, and genital examination tools, especially in women , but the oral cavity and periodontal pockets remain one of the most common places for this parasite to live in both gender (Table ,1), the main reason for the existence of this parasite is due to the presence of nutrients that it feeds on, including gum bacteria (de Moraes-Ruehsen et al.,1980 ; Dao et al., 1983; Dao, 1985; Mirelman, 1987 & Alsaadawi et al., 2021).

The studyof Mohammed & Binsaad (2023) showed that the age group (40-49 years) recorded a percentage of 23.7, which is the highest percentage of infection compared to the rest of the age groups, and that the parasite infects both sexes, in addition to that the study recorded statistically significant differences between the people participating in the study, depending on some factors and habits, the difference between the current results and the previous study may be due to the difference in the sample size, its geographical distribution, the study area, and the different age periods of the samples of the two studies.

These results of current research (Table 1,2) are consistent with findings study by Researcher Aas et al. (2005) noted in a study conducted at Oslo University, that the infection occurs in both sexes, but the age group is more than 35 years and the predisposition to gum disease and the sex factor (females) is one of the most important indicators of gingival amoebiasis and study by Sarowaska et al.(2004) that this parasite does not ensue in young children and rarely occurs in mature adults.

Infection may occur directly as a result of following some customs and traditions by people, which is kissing between people as an indication of the strength of the relationship between them, as well as joint use of toothbrushes among members of the same family. The presence of the parasite in the places mentioned in the current study indicates the presence of preferential characteristics of the parasite, including the presence of bacteria, which many studies have shown to be present in the cytoplasm of the parasite, which confirms its ingestion by the parasite along with food debris.

Several studies have indicated that saliva contains little concentrations of nutrients and systems of antimicrobial resistance. A healthy oral cavity contains the numbers of more than 1,000 species of bacteria. These microorganisms and the layers of the mouth maintain the continuity of this symbiotic relationship, which has been proven through microscopic examination, and that the accumulation of food and food residues in the mouth leads to the survival of bacteria that colonize the teeth, and this is what helps the growth of other organisms, out of the 11 bacterial species that were discovered, six were highly present In 70% or more of the oral sites infected with various types of amoebic parasites, including gingival amoebas as well as flagellate parasites. The amoebic protozoa (Entamoeba gingivalis) is present in the oral mucosa in a healthy state, with an estimated incidence of 15%. It may be asymptomatic as non-pathogenic organism. In addition, it can be able to invade the ruptured oral mucosa, where it engulfs parts of living cells(Aas et al.,2005 ; Human & Project, 2012 ; Rosier, Marsh, & Mira, 2017; Bonner et al. , 2018, Marie, et al. , 2019 & Bao et el., 2021).

Other studies indicated that the prevalence of this parasite in 36 inflamed periodontal pockets increased by a percentage of more than 80 % (Bao et al.2020 ; Scannapieco & Dongari-Bagtzoglou, 2021). The results of the current research (Table 3,4) are consistent with the results of previous studies. Table (5) indicates that the the percentage of infection with gingival amoeba in previous studies differs or is close to the percentage of infection in the current study, and the reason for this may be due to several factors, including the difference in the geographical location, which leads to a difference in the virulence of the amoeba and according to environmental conditions, strain that were diagnosed for thespicies of amoeba, the cultural level of the population, the size of the study sample, the site of infection (gums, teeth, periodontal pockets), the kind of comorbidities of the patient (diabetes, etc.), the number of members of the same family and the usual living habits, the age group of the study, the diagnostic method used In each study (direct examination using light microscopy, immunological diagnosis, genetic diagnosis), as well as personal hygiene and the accompanying accumulation of plaque and their type(Bonner et al., 2014;Garcia et al. , 2018 ; Marie, et al. , 2019& von Huth et al., 2021)

Many studies indicated that there is a correlation between a number of factors and the increase in oral diseases, especially gum and dental diseases. These factors are divided into:

A- Modifiable factors, including interest in oral hygiene, which causes various biological infections, chronic diseases (diabetes and blood pressure), smoking and alcohol consumption.

B- Non-modifiable factors such as aging, infection with some diseases that are related to genetic predisposition, and osteoporosis (MARK, 2014; Wang; Mccauley, 2016; Koo; Hong, 2018; Masumoto et al. , 2018 & Alasadiy et al. , 2022)

Several microbiomes can be diagnosed in oral diseases (gums, teeth, and cavities), relying on some specific pathophysiological features, confirming that diversity in stomatitis is not specific, and that a common hallmark of inflammation is the presence of bone breakdown accompanying inflammation Thus, a good understanding of the mechanisms involved in infection (inflammatory and immune) observed in oral diseases is of paramount importance in any attempts to eradicate this widespread disease (Cekici et al. , 2015; Martínez-garcía & Hernández-lemus, 2021(.

The marital status, family history, demographics, methods of oral cleaning, the extent of kinship between parents, and the extent of addiction to smoking are among the methods that help doctors diagnose oral diseases better by developing a treatment program that identifies indicators of aggressive gingivitis, and this is what was indicated by a study conducted in Jordan by Ababneh et al. (2019).

Gingivalis (*E. gingivalis*) is highly abundant and more dispersed in the oral cavity especially in the gum and teeth pockets, which indicates that this environmental niche is suitable for this parasite to its survival or that the parasite causes changes leading to an appropriate adaptation of this parasite. additional, Reports will need to direct a view into the biological, physical and chemical properties of the mouth, and allow research relevant to parasite biology, in both in vitro and in vivo models.

The results of this study agree with previous studies indicating that the sites of infection in the mouth require the use of antibiotics to treat the types of bacteria present and at the same time the use of the treatment prescribed by the specialist doctor for the treatment of gingival amebiasis with monitoring of the disease condition to obtain the best results.

Conclusions

The gingival amoeba parasite is a common parasite in the mouths of both males and females and is an indicator of poor hygiene. It is a secondary infection as a result of people getting the primary bacterial infection as a result of the parasite feeding on the bacteria at the site of infection and on the remaining food scraps due to pain in the affected area due to insufficient attention to hygiene. The parasite is transmitted directly through kissing or through droplets of saliva from the mouth of an infected person to the mouth of a healthy person. All people should see a specialist for regular check-ups to monitor their oral health.

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