Detection of Oral Entamoeba Gingivalis in a Kut Population with Gingivitis and Periodontal disease

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ABSTRACT

With regard to the parasitological confirmation, E. gingivalis trophozoites were evident microscopically within all 22 positive specimens.The study involved 30 persons: 15 women and 15 men. In the studied group. The study consisted of the observation of samples derived by way of buccal swabs under a microscope in a search for Entamoeba gingivalis trophozoites. Such protozoa were found in 22 patients, but no in 7 patients . Among the samples derived from women, the protozoa were in 13 (59.09%), and among men, in 9 subjects (40.91%).It was found during the examination that the percentage of non-smoking individuals was the highest, 95.54%, and the lowest percentage of smoking individuals was 4.55%. The number. The total number of people examined was N = 30.Out of 30 investigated studies, 30 specified a mean age for their sample size. Results showed an increase in prevalence on (21-30) the age of the patients, the lowest prevalence was reported lass 10 age and (51-60) age

Keywords: Entamoeba Gingivalis , Periodontal disease, women

Introduction

1.1 Introduction

Entamoeba gingivalis was the first commensal parasite detected in the human oral cavity. It is a cosmopolitan Anaerobic amoebic protozoan (Bhutta *et al,*2013),which exists as Trophozoite form that has variable size ranging from 10 To 35 µm in diameter. *E. Gingivalis* has no cyst form; Therefore, it spreads either directly via direct contacts, by Kissing or indirectly through contaminated food, or by Sharing eating utensils (Bogitsh *et al*,2013) *E. Gingivalis* is found most Frequently in tartar between and around the teeth, Tonsillar crypts and gingival tissues, particularly in Suppurative, inflammatory processes,due to its Preference for anaerobic environments (mielnik_Blaszczak *et al* ,2018).

E.gingivalis is more commonly found in persons with Bad oral hygiene. Also, *E. Gingivalis* is generally considered as an oral commensal but reports show that it displays a pathogenic role in periodontal disease (Bonner, *et al*, 2018).

In Addition, *E. Gingivalis* has been detected in conditions of Immune suppression. At present, there is evidence to Propose an association of *E. Gingivali*s in pathogenesi.periodontal disease (Yucel_lindberg, *et al,* 2013), and several studies reported That *E. Gingivalis* contributes to the initiation and Progression of gingivitis and periodontitis.

Moreover, the Pathogenic perspective of *E. Gingivalis* has been proven experimentally by the development of lesions in Immunosuppressed animals (Yucel_lindberg, *et al*, 2013).

However, there are Controversies concerning its pathogenicity characteristic As this trophozoite is commonly found in oral cavity of Healthy individuals and with gingivitis as well, and Several authors consider E. Gingivalis to be opportunistic [M.Bonner,etal,2018), F.Bhaijee etal,2011]. Accordingly, this data opens a scientific gate for More research to discover the pathophysiology of Gingivitis and

periodontitis. Data on the prevalence of this parasite remains limited in many developing Countries including Yemen, and due to controversies on Infectivity of these parasite, its correlation to periodontal Diseases, and to its public health importance, this study was carried out to determine the prevalence of *E. Gingivalis* and its association with some factors among Patients with and without periodontal disease attending three dental Clinics in Aden governorate Yemen.

1.2 The aim of the research

This study was conducted to detect. About the prevalence of Entamoeba (Gingivalis), the most common of which is in Wasit Governorate

1.3 Life Cycle of E. Gingivalis

In most species of the genus Entamoeba, two cellular forms have been identified in nature: the cyst, which is the contaminant form found in the environment, and trophozoites, the vegetative cell able to divide, that initially derives from encystations of cysts ingested by the host.

The survival of these Entamoeba species is ensured by their encystment in response to environmental changes (Mi-Ichi *et al*., 2016), permitting the survival in environments exposed to oxygen, like human stools, where identification of Entamoeba species is made by a simple morphological phenotyping that relies on the number of nuclei carried by the cyst.

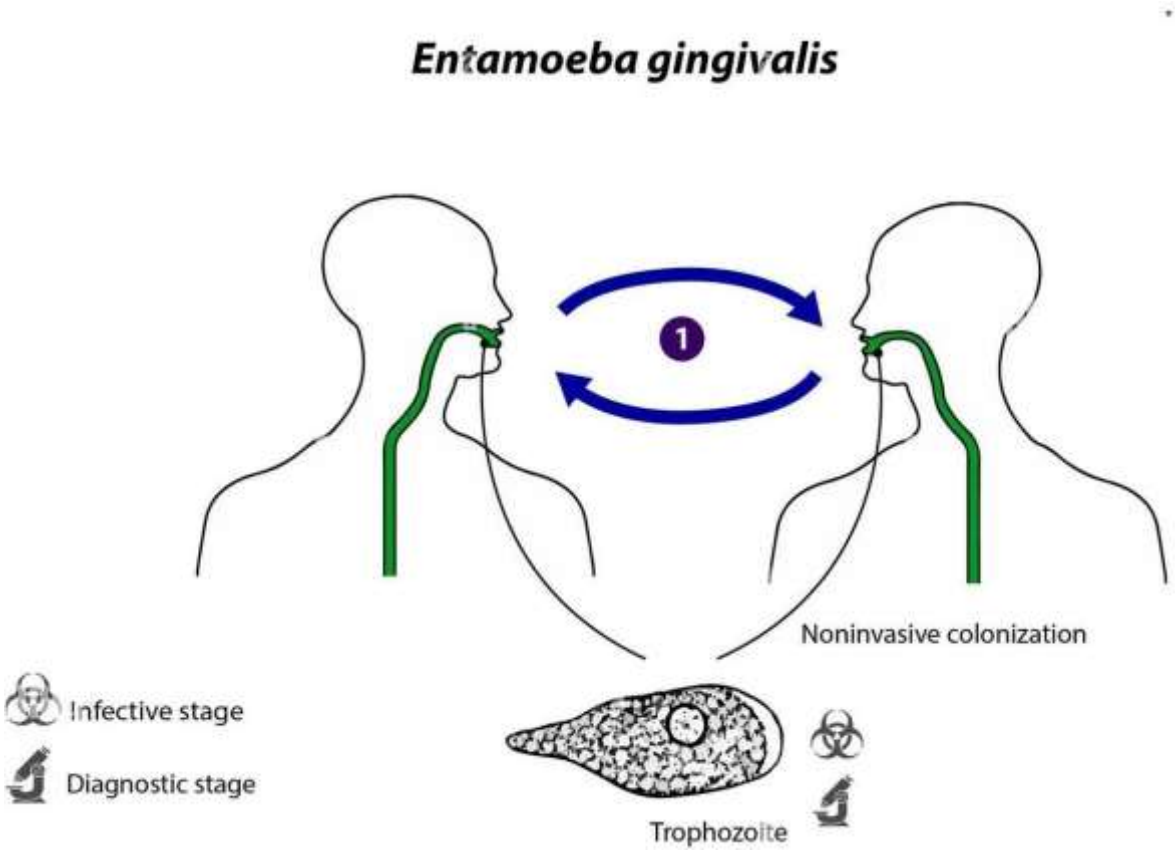
The sole *E. Gingivalis* would not encyst, though cysts of *E. Gingivalis* were reported in the literature at the beginning of the twentieth century (Chiavaro, 1914; Smith and Barrett, 1915b; Craig, 1916). However, it is now commonly accepted that

E. Gingivalis does not produce cysts, considering the absence of proof as a proof of absence.

Nevertheless, the parasite *E. Gingivalis* is essentially observed in periodontal pockets, suggesting that low oxygen levels are important for the survival of trophozoites, as in the case *of E. Histolytica and E. Dispar* (Olivos-Garcia *et al*. (2016).

Direct transmission of trophozoites to a new host would imply that they are resistant to oxygen, which raises questions about how *E. Gingivalis* is transmitted in nature, and what ecological niche serves as a reservoir for this microorganism. Unfortunately, the complete life cycle of *E. Gingivalis* is still missing and not addressed yet, hampering efficient prophylaxis.In the closely-related specie *E. Histolytica*, resistance to oxygen is modulated by interaction with bacteria (Varet et al., 2018), as well as virulence (Bracha and Mirelman, 1984; Galvan-Moroyoqui et al., 2008).

The microbiota could be of major importance in switching from commensal to pathogenic forms and explain why only a minor part of *E. Histolytica* intestinal infections are invasive and symptomatic. During periodontitis, bacterial virulence genes are strongly modulated (Deng *et al*., 2017) and the frequent and abundant detection of *E. Gingivalis* in periodontitis pockets (Bonner *et al.,* 2014) suggests and warns that interactions between constituents of the microbiota could be essential for their functions during the pathophysiology of the disease.



(Figure 1-1) show the life cycle of Entamoeba gingivali

1.4 Scientific classification

1.5 Pathogenicity

Gingivalis lives on the surface of the teeth and gum, gingival pockets near the base of the teeth and also seldomly in the crypts of the tonsils. The Organism is abundant in cases of gum and tonsil diseases but no evidence show that they are Involved in the etiology of these conditions. They are usually spread by direct contact from one person to another by kissing, droplet spray or sharing eating utensils. It is known that up To95% of individuals with unhygienic mouth may be infected with this amoeba. (Roberts et al., 2000)

1.6 Epidemiology of Entamoeba gingivalis

Entamoeba gingivalis is a single-celled protozoan found in the human oral cavity. While its pathogenicity remains debated, it's frequently associated with periodontitis, raising

questions about its potential role in this disease. Here's an overview of its epidemiology with specific sources.

1.7 Prevalence

Global: Varies widely, ranging from 20% to 70% depending on the studied population and detection methods. (Stensvold et al., 2021)Region-specific: Higher prevalence reported in developing countries compared to developed ones. (El- Askary *et al*., 2023) Gender: Some studies suggest higher prevalence in females, though not statistically significant. (Stensvold *et al*., 2021)

1.8 Risk.factors.:

Poor oral hygiene: Significant association with plaque accumulation and periodontal disease. (El-Askary et al., 2023)

Smoking: Increased risk compared to non-smokers. (Stensvold et al., 2021) Dental prostheses: Wearing dentures often with higher *E. Gingivalis* presence. (El- Askary et al., 2023) Age: Prevalence generally increases with age, possibly due to cumulative exposure to risk factors. (Stensvold et al., 2021)

Association with Periodontitis E. gingivalis frequently detected in periodontitis patients, but a causal relationship is yet to be conclusively established. (CDC, 2023) Some studies suggest it may contribute to periodontal inflammation or act synergistically with bacteria in disease progression. (Stensvold et al., 2021)

Research in this area is ongoing, with further investigations needed to elucidate the specific role of *E. Gingivalis* in periodontitis.

1.9 Diagnosing Entamoeba gingivalis

Entamoeba gingivalis is a single-celled organism found in the mouth, but its role in oral health remains controversial. While often associated with periodontal disease, it's unclear if it directly causes it or simply thrives in inflamed gums. Diagnosis primarily relies on microscopic identification of its trophozoites

1.10 Microscopic examination:

Gum and tooth scrapings: Collected from periodontal pockets and examined under a microscope for motile, pear-shaped trophozoites with a single nucleus. They may be seen ingesting white blood cells and epithelial cell nuclei. Direct wet mount: Scrapings are mixed with saline and viewed immediately for motile trophozoites. Stained smears: Scrapings are fixed and stained with dyes like hematoxylineosin to enhance visualization of trophozoite morphology.

Additional tests:

- Polymerase chain reaction (PCR): Detects Entamoeba gingivalis DNA in scrapings or saliva, offering higher sensitivity than microscopy but unable to distinguish viable parasites.
- Culture: Rarely used due to difficulty and lack of standardized methods.

Diagnosis of Entamoeba gingivalis alone is insufficient to confirm its role in periodontal disease.

Consulting a dentist or healthcare professional is crucial for proper diagnosis and management of oral health issues.

1.11 Prevention and treatment

Prevention

While there's no specific "prevention" for Entamoeba gingivalis since it's a common resident in healthy mouths, there are steps you can take to maintain good oral hygiene and reduce its presence, potentially lowering the risk of gum disease it's associated with:

- Brushing your teeth twice a day and flossing once a day: This removes food debris and plaque buildup where E. Gingivalis thrives.
- Using a mouthwash: Choose one with chlorhexidine gluconate, which has anti-amoebic properties.
- Regular dental checkups and cleanings: Your dentist can identify and address any underlying gum problems that might contribute to E. Gingivalis overgrowth.
- Quitting smoking: Smoking weakens your immune system and increases your risk of gum disease.
- Managing other health conditions: Certain medical conditions, like diabetes, can make you more susceptible to gum infections.

1.12 treatment

Metronidazole as a drug used to treat infections caused by protozoa in addition to fourteen agents is used as a mouthwash, with two pure compounds acting as a mouthwash. Ingredients, i.e. 20% benzocaine and 0.2% chlorhexidine, in addition to 12 commercially available formulations Azulane Colgate Plax Complete Sensitive Care Corsodyl 0.2% Coracept 205 ADS Dentosept, Dentosept A, Aludryl Classic, Listerine Total Care, Octinidol Oral-B Pro-Expert Sylvecos Clinic Line Tinctura salviae9.**)**

2.1 Materials and methods

Entamoeba gingivalis is a single-celled organism that can cause chronic periodontal disease, leading to gum inflammation, bleeding gums, and tooth loss. Diagnosing E. Gingivalis can be challenging because its symptoms overlap with other oral conditions. Here are some tools and materials used to diagnose it:

The Tools	Materials
1) Pipette	▪ Giemsa stain
2) Test tube	▪ Formalin
3) Wood sticks	▪ Samples
4) Slid	▪ Water
6) Gloves	
7) Compound light microscope	

2.2 The study area

Samples were taken from Wasit University Clinic and other external clinics

2.3 Microscopic Examination

The microscopic examination is carried out by an expert. Each specimen was divided in two parts one was used to prepare wet mount and the second to

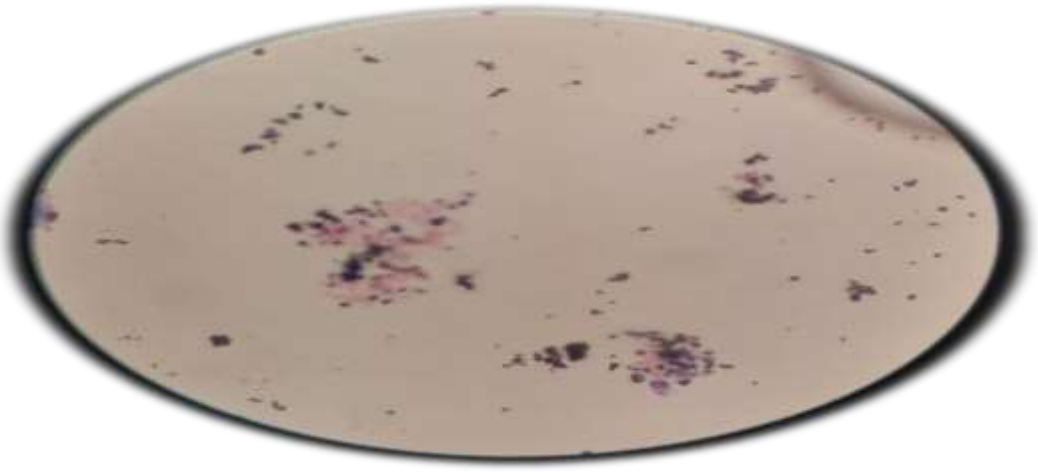
prepare smear for Giemsa staining. Wet mount examination Using sterile Pasteur pipette a drops of a diluted Specimen were placed on clean microscopic slides (25.4x76.2 mm) and a coverslip (24x 50 mm) was applied on the top, and the material spreads by pressure on the Coverslip. This prepared a thin film which was then examined immediately with a light microscope at 10X And 40X. The identification of E. Gingivalis was established by its shape depending on the expansion of Pseudopodia formation, and sluggish movement [11].

Three smears of wet mount method for each samples .Used to strength the chance of detection of parasites. Giemsa staining Thick smear were prepared on clean microscopic slide and allowed to dry. It was then stained with Commercially available Giemsa stain (Techno Pharmchem, India) diluted (1:50, vol/vol); (For a 1:50, Add 1ml Giemsa stock to 50 ml buffered water) for 50 Min. Then the slide was gently washed under clean water and let air dried in vertical position, and scanned for E. Gingivalis at 100X magnification. The characteristic Morphologic features for E. Gingivalis appear as irregular

Shaped with light red purple cytoplasm and an Intracellular vacuoles visualized as darker red – purple [12]. Three smears stained with Giemsa staining used for each sample to strength the chance of detection of Parasite. (lbrahim, R. et al., 2012),(Garcia,etal..,2016)

3-1 Results

With regard to the parasitological confirmation, *E. gingivalis* trophozoites were evident microscopically within all 22 positive specimens. The trophozoites were apparently smaller than that of *Entamoeba histolytica*. They were observed with the characteristic morphological features in the form of a single nucleus containing a tiny central karyosome with a rim of chromatin at the periphery and a delicately granular cytoplasm



(Figure 3-1) show the trophpzoite of E. gingivalis

The present study revealed significant morphological differences between *E. gingivalis* trophozoites within diseased and healthy sites including the size of parasitic stages, specific morphological variations in addition to the mean number of trophozoites between the two categories.

Detailed morphological features reported in this study were sufficient to differentiate the parasitic stages within the diseased and healthy periodontal sites. This was in contrast to the findings reported by (García *et al*., 2018) in which morphological characteristics were ignored and excluded by the authors.

They attributed this to the ineffectuality of microscopic examination to differentiate between amoebas. Similarly, other authors reported similar findings concerning morphology, in which no characteristic findings were reported (. Clark *et al*., 2006: Ponce-Gordo *et al*., 2010: Stensvold *et al*.,. 2011. And ; Jacob *et al.,* 2016).

Morphologically in this study, in addition to the observed characteristic nuclei, leukophagocytosis vacuoles were found within all the trophozoites isolated from affected periodontal sites. This may be related to certain virulence factors, facilitating the invasive power of *E. gingivalis* trophozoites, which is more or less similar to what is reported by. Bansal et al., 2009)

The significant difference regarding the larger size of *E. gingivalis* trophozoites identified within the diseased sites, possibly resulting from engulfing a number of white blood cells which may have cause cytoplasmic expansion. In fact, *E. gingivalis* is able to engulf one or more human cells at a time, mainly polymorphonuclear cells and neutrophils. These are the chief cells within the periodontal pockets, so engulfing these cells may exposes the first line of the innate immune mechanism to intense danger by consuming its powerful weapon (Bonner et al., 2014).

Thus, *E. gingivalis* finds a perfect environment involving damaged tissue, bacteria, and fungi, permitting its safe establishment and colonization. This may

explain the significantly higher number within samples isolated from the diseased cases, taking into consideration that all the enrolled subjects were immunocompetent. This intense colonization within immunocompetent subjects was previously (Hassan et al.,. 2019)

Table (1): The number of positive and negative samples of *E. gingivalis* by Microscopical examination depends on age and gender.

Sex	No. of Examined	*E .gingivalis*	
		No.	***%***
Male	15	9	40.91%
Female	15	13	59.09%

Total		30		22			

The study involved 30 persons: 15 women and 15 men. In the studied group. The study consisted of the observation of samples derived by way of buccal swabs under a microscope in a search for Entamoeba gingivalis trophozoites (Photo. 1, 2).

Such protozoa were found in 22 patients, but not in 7 patients (table 3-1). Among the samples derived from women, the protozoa were in 13 (59.09%), and among men, in 9 subjects (40.91%). No correlation was seen between gender and the presence or absence of protozoa (P = 0, 06).

Infectious parasites are categorized into those causing local infections, and those inducing systemic infections with indirect effects. However, saprophytes such as Entamoeba gingivalis (which belongs to the first group), have the potential to turn into opportunistic pathogens, or free-living amoebas that occasionally become invasive (Albuqerque R.L., et al 2011: Bergquist .2000).

The activity of *E. gingivalis* in oral cavity disease lesion progression is associated with a capacity for erythrocytophagy and the presence of enzymes important in the development of periodontitis. Lyon and workers described the possible pathogenic mechanism of Entamoeba gingivalis. Herein, the leukocyte metabolism altered by amoebas and the excessive release of elastase brought about the destruction of the periodontium, and it induced gingival bleeding.

Furthermore, the access of amoeba to the erythrocytes enables the neutralization of oxidants generated in the inflammatory process and the maintenance of the anaerobic conditions that are optimal for the amoeba (Chomicz L., *et al*., 2007).

Hormonal fluctuations Changes in hormone levels during menstruation and pregnancy may affect susceptibility to oral infections, but evidence for this with *E. gingivalis* is inconclusive. Other factors Additional factors such as general health status, immune function, and diet may play a role, but their impact on infection rates by sex needs to be further investigated.

Table (2): Prevalence Entamoeba gingivalis according to smoker

Samples	No. of Examined		*E. gingivalis*			
Smoker		2		1		4.55%	
Non-Smoker		28		21		95.45%	

Total		30		22			

Table 2 Table (2):shows the number of individuals examined, including smokers and non-smokers. Most of the participants were non-smokers.

It was found during the examination that the percentage of non-smoking individuals was the highest, 95.54%, and the lowest percentage of smoking individuals was 4.55%.

The number. The total number of people examined was N = 30, which corresponds to a research article by correspondent Adel Jumaan Awad bin Saad. In dental clinics in the city of Aden, the percentage of individuals who smoked was also the highest, at 81.3%, and of individuals who smoked 18.7%, as the total number of those examined was N = 300 (Adel Jumaan Awadh Binsaad 2023).

Tobacco reduce the synthesis of IgG and IgM by plasma cells, as well as the phagocytic activity and chemotactic response of gingival neutrophils, so the host's defense against bacteria in the gingival pocket is substantially impaired [Al-Qtaibi .,2004]. The use of tobacco increase the risk of periodontitis and gingivitis [Al-Saeed .,2003]

Table(3): 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			

Out of 30 investigated studies, 30 specified a mean age for their sample size. Results showed an increase in prevalence on (21-30) the age of the patients, the lowest prevalence was reported lass 10 age and (51-60) age

The occurrence of *E. gingivalis* is correlated with the age of the host, so oral protozoa are rarely found in children, while the frequency of infection increases with age [18Sarowska J., et al.: The Occurrence of *Entamoeba gingivalis* and *Trichomonas tenax* in patients with periodontal diseases, immunosuppression and genetic diseases.

The highest percentage for the category (21-30) was 54.55%, (Gharavi *et al.,* 2006)Buccal cavity protozoa in patients referred to the faculty of Dentistry in Tehran, Iran.by(J. Prasitol ..,etal)who noticed the high infection was among 21- 30 years old (Cielecka *..,et al*. 2000**(** and Linke *et al*..J.Parasitol., 1989; 19: 803-808.] reported an increased frequency of *E. gingivalis* infections among people with bad oral hygiene this report was in the line of our study. Improper oral care is conducive to inflammations of the mucous membrane, gingival diseases and caries.

It favours accumulation of food residue and the development of dental plaque, which constitutes an excellent base for the growth of fungi and bacteria, as well as protozoa. The most significant hygienic factors impacting the frequency of occurrence of *E. gingivalis*. For this purpose, the following have been taken into consideration: the frequency and duration of tooth brushing, the type of toothbrush used, the application of additional care products, and other procedures improving the state of oral cavity hygiene.[(Monika..,etal,2011).

which corresponds to a research article by correspondent Adel Jumaan Awad bin Saad. In dental clinics in the city of Aden, the number of those examined was n=300 also, the age group of 29 was the highest, with a rate of 19.7%. It also shows that the age group with the least infection was 10, with a rate of 4.55%. Also compatible with a 2019 study published in the Oman Medical Journal found that the prevalence of *E. gingivalis* was highest in the 16-25 age group, followed by the 26-35 age group. This study involved over 500 participants from Oman(Alaa Yaseen..,etal,2021).

A 2018 study published in the Parasitology Research journal reported similar findings, with the highest prevalence of *E. gingivalis* in the 19-25 age group in a population from India (Maria Mielnik-Blaszczak..,etal**,** 2018).

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.(Maryam Sharifi.., et al. 2020)

These results are not consistent with Studies have shown varying prevalence rates across different age groups. For example, one study in Iran found that adolescents aged 6-14 had a prevalence of 15.7%, while another study detected *E. gingivalis* in 41.7% of patients in Tehran (Maryam sharifl.., et al**,** 2017). Additionally, 2 studies (Rahdar..,etal.) and Periodontitis Patients by (Methods..,,etal.2019)

Reported a high prevalence of over 80% in the 45-60 years old study group. The results were similar to what was reported by J. Luszcak et al. in their study, recording the highest prevalence in the age groups of 40-49 and 50-59 years old.

These results suggests that the prevalence of *Entamoeba gingivalis* is correlated with age of the patients While some studies show higher infection rates in older age groups, the prevalence of *E. gingivalis* can vary across populations and age groups, suggesting that the highest infection rate may not consistently occur in the 21-30 age group.

However, the reason that the age group 12-30 is the most affected is due to several factors, the most important of which is oral hygiene habits: teenagers and young adults are more likely to engage in behaviors that can increase the risk of infection, such as not brushing and flossing teeth less, skipping dental examinations, and consuming sugary drinks and snacks. , Immune system: The immune system is still developing in adolescents and young adults, making them more susceptible to infections in general. Social factors: Sharing personal items such as tools or water bottles with others, as well as engaging in close contact activities such as kissing, can increase the risk of transmission of *E. gingivalis.* Hormonal changes: Fluctuations in hormones during puberty can also contribute to changes in the environment of the mouth, making it more susceptible to infection (Noor Mohammed Abdulla.., et al**,** 2023).

Conclusions:

There are only few reports on the role of oral commensals in the pathogenesis of periodontitis and gingivitis despite the high incidence of certain protozoa, such as Entamoeba gingivalis. In this study the prevalence of oral parasite it was found that the maximum number of patients with *E.gingvials* was reported within the age group 21-30 (54.55%), and that this disease was higher in females (59.09%) than in males. The disease rate in males was (4.55). The results also showed that non-smoking individuals had a higher incidence of the disease. 95.54% compared to individuals who smoked, had a 4.55% lower incidence of infection .Although *E.gingivalis* is not generally associated with pathogenesis, their presence in the oral cavity is taken as a sign of poor dental hygiene. We should stress that the state of oral cavity hygiene among patient is not Satisfactory. The oral cavity hygiene products which they use are largely ineffective.

Recommendations:

1. Investigate the ability of E. gingivalis to form cysts, as a recent study demonstrated their formation in response to antibiotics. Understanding cyst formation could provide insights into the organism's survival strategies and transmission dynamics.

2. Explore the relationship between E. gingivalis and co-infections with other oral parasites like Trichomonas tenax, as well as bacterial and viral agents involved in periodontal diseases

3. Analyze genetic variability within E. gingivalis populations, as suggested by a pilot study detecting a new genetic variant. Identifying distinct strains could help understand how they contribute to periodontal disease progression and inform targeted treatments.

4. Assess the impact of environmental factors, such as stress, alcohol consumption, and oral hygiene practices, on the prevalence and distribution of E. gingivalis in various patient populations

5. Collaborate across disciplines, such as dentistry, biomedical sciences, and epidemiology, to develop comprehensive models of E. gingivalis pathobiology and improve diagnostic capabilities.

6. Develop novel therapies targeting E. gingivalis, either alone or in combination with existing antimicrobials, to address the limitations of current treatments and prevent relapses.

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