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The Role Of Entamoeba Gingivalis In Periodontal Disease: A Literature Study

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ABSTRACT

Periodontal disease can affect the supporting structures of the teeth and can cause tooth loss. Periodontal disease is caused mainly by bacteria in dental plaque, but protozoa can also be found in deep pockets. The most common protozoa existing in periodontal disease is *Entamoeba gingivalis*. This study aims to determine the role of *E. gingivalis* in periodontal disease. This research was a rapid review which is a method of synthesizing and assessing existing research. Articles were searched using the PubMed database from February 2021 to April 2021 with inclusion criteria in English from 2010 to 2020 and discussing the presence of *E. gingivalis* in periodontal disease. Articles that comply with the inclusion criteria of this study are 11 articles. The study designs included case-control, cross-sectional, cohort, and case series. All articles concluded that *E. gingivalis* was found in patients with periodontal disease. The presence of *E. gingivalis* in large numbers in inflamed periodontal tissues indicates that *E. gingivalis* has a role in the course of periodontal disease.

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1. INTRODUCTION

Periodontal disease occurs due to the interaction between microorganisms in the subgingival plaque and inflammatory cells that develop in the periodontal tissues in response to microorganisms. Gingivitis may result in periodontitis. Gingivitis is an inflammation limited to the gingiva, while periodontitis is an inflammation of the supporting tissues of the teeth that extends to the periodontal ligament and alveolar bone. The periodontal ligament may be damaged by inflammation, which would lead to a loss of clinical attachment and alveolar bone resorption.[1]

Riskesdas data in 2018 shows that the number of periodontitis cases in Indonesia reached 74.1%.[2] The main etiological factor of periodontal disease is microorganisms found in subgingival plaque.[3] Infected periodontal tissue contains many neutrophils, bacteria, and protozoa.[4,5] The results of research conducted by Onyido et al. show that patients with dental and oral diseases are susceptible to protozoa colonization of the oral cavity, one of which is *E. gingivalis*. This shows that the presence of protozoa in the oral cavity is closely related to the oral health of a person.[6] *Entamoeba gingivalis* is found in many cases of periodontitis, a periodontal disease that has had a high prevalence among the public until now.[5]

The human oral cavity is habitat to the protozoan *E. gingivalis,* which belongs to the amoeba phylum. [4,7] It is present in 50% of individuals with healthy gingiva and 95% of individuals with periodontal disease.[8,9] *E. gingivalis* in the oral cavity of patients with good immunity usually does not cause pathological changes. These protozoa can be found on the tooth surface, gingiva, interdental space, gingival pocket, saliva, and dental plaque.[8] The inflammatory process produces an anaerobic environment that is favorable for the development of these protozoa. Factors that can increase the pathogenicity of *E. gingivalis* include age, oral hygiene, and periodontal disease. The presence of *E.*

gingivalis in the mouth can be prevented by maintaining oral hygiene and performing oral dental care every day.[8] This protozoon does not have a cystic stage, its infective form is trophozoite. Transmission of *E. gingivalis* can occur either directly or indirectly from one person to another through droplets or by using shared cutlery.[8,9] *E. gingivalis* can also be found in contaminated food and drink.[8]

Based on a search of articles on PubMed, many studies have been found proving that *E. gingivalis* has a role in the development of periodontal disease, however, there are still few reviews that discuss the role of this protozoa in periodontal disease. Therefore, the authors are interested in analyzing existing studies to review the impact of the presence of *E. gingivalis* on healthy and inflamed periodontal tissue. Research is carried out by collecting articles related to predetermined keywords from the Pubmed database which will then be synthesized to answer research questions.

2. METHOD

The research method used in this study was rapid review, which synthesizes findings and assesses the study's validity using a simplified systematic review method to produce information in a short time.[10] The study was conducted by comparing the results of previous studies on *E. gingivalis*. The tools and materials used are stationery, laptops, articles, and Microsoft Word software. The database used is PubMed with keywords ((((*Entamoeba gingivalis*) AND (gingivitis)) OR ((*Entamoeba gingivalis*) AND (periodontitis))) OR (((*Entamoeba gingivalis*) AND (gingivitis)) AND (periodontitis))) OR ((*Entamoeba gingivalis*) AND (periodontal disease)). Article searches were conducted online from February 2021 to April 2021.

The inclusion criteria in this study were articles published from 2010 to 2020, in English, discussing *E. gingivalis* with gingivitis and periodontitis in humans, and articles indexed by Scopus. The exclusion criteria in this study were articles discussing microorganisms other than *E. gingivalis* and animal studies. Article searches are carried out by entering predetermined keywords in Advance Search and using the "AND" and "OR" Boolean Operators in PubMed.

The process of selecting articles uses PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses). Article screening was carried out twice, namely (i) articles were selected by reviewing the title and abstract, articles that did not fit the inclusion criteria were filtered and (ii) articles were reviewed by reading the entire text, and screening was carried out on articles that met the exclusion criteria. The selected articles will be qualitatively synthesized.

The data from the articles to be extracted include the author's name, year of publication, title, research objectives, methodology, results, and conclusions. The data from the extracted articles will be presented in tabular form which suggests the role of *E. gingivalis* in periodontal disease.

3. RESULTS AND DISCUSSION

Sixty articles resulted from a PubMed search. A total of 49 articles were eliminated because they did not meet the inclusion criteria after reading the title and abstract, leaving 11 articles. The selected articles were read in their entirety and obtained the final results of 11 articles that could be used for this study according to the inclusion criteria. The results of selecting articles for research are depicted in the PRISMA diagram shown in Figure 1. Eleven articles were selected using various study designs, namely six articles using a case-control study design, one using a cohort study design, three using a cross-sectional study design, and one using a case series study design.[11–17] Two of the eleven articles included patients with systemic disease. [16,18]

The results of data extraction from each article are presented in Table 1. The measurement parameters of each article are not all the same. Based on the detected *E. gingivalis*, there were seven articles assessing the presence of *E. gingivalis* in general without differentiating subtype 1 (ST1) or subtype 2 (ST2)[16–18], and four articles assessed and compared the presence of *E. gingivalis* ST1 and ST2 in healthy individuals and individuals with periodontal disease.[13–15] Based on the location where *E. gingivalis* was found, seven articles discussed *E. gingivalis* in healthy gingiva,[5-12] four articles discussed *E. gingivalis* in gingivitis,[16-18] and ten articles discussing *E. gingivalis* in periodontitis.[11–17]

Research shows the presence of *E. gingivalis* in inflamed periodontal tissue is higher than in healthy tissue.[15,17] The presence of *E. gingivalis* in periodontitis patients without systemic disease reaches 74%, in patients with HIV/AIDS reached 63.4%, and in patients with hepatitis B reached

67.6%.[9,16,18] One of seven articles showed that *E. gingivalis* was not found in healthy areas.[17] One article stated the presence of *E. gingivalis* in gingivitis is more than in periodontitis. More gingivitis patients were infected with *E. gingivalis* ST2 than *E. gingivalis* ST1, while periodontitis patients were more infected with *E. gingivalis* ST1 than *E. gingivalis* ST2.[9,14,15] Patients with healthy gingiva had more *E. gingivalis* ST1 than those with *E. gingivalis* ST2.[14,15]

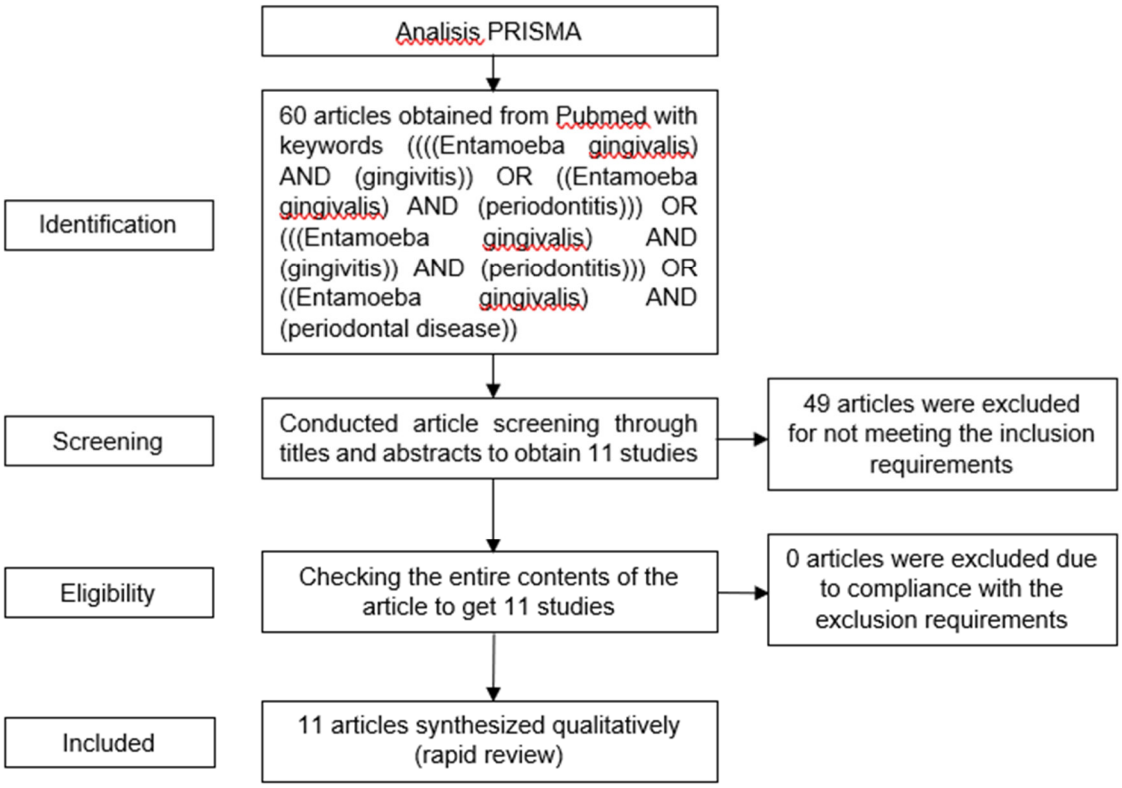

Figure 1. PRISMA Analysis

Table 1. Result data extraction

No	Author, year	Title	Research purposes	Design study	Research sample	Results	Conclusion
1	X. Bao et al, 2020	*Entamoeba gingivalis* Causes Oral Inflammation and Tissue Destruction	To find out the latest periodontitis infection process caused by *Entamoeba gingivalis*	Case-control	Periodontitis patient: 51 Healthy patient: 107 The samples obtained from the patients were examined using PCR and microscopy	Presence of *Entamoeba gingivalis* in the periodontitis group: 1. Pockets > 7mm: 77% (39 people) 2. 3-4 mm pocket: 22% (11 people) Presence of *Entamoeba gingivalis* in the healthy group: 15% (16 people)	*E. gingivalis*, which is abundant in inflamed periodontal tissues, has a strong virulence potential.
2	Zhi-Luo Deng et al, 2017	Dysbiosis in Chronic Periodontitis: Key Microbial Players and Interactions with the Human Host	to understand the taxa that result in functional alterations in dysbiosis	Cohort	14 pockets, consisting of: 4 pockets from individuals with chronic periodontitis 10 pockets from individuals with healthy gingiva	Presence of *E. gingivalis*: By rRNA deletion using SortMeRNA 1. Periodontitis: 4.72% 2. Healthy: 0.32% Without rRNA deletion using SortMeRNA 1. Periodontitis: 9% 2. Healthy: 2%	*E. gingivalis* can be found in large numbers in periodontitis but is rarely or even absent in healthy gingiva.
3	Jian Ming Huang *et al*, 2020	The First Study to Detect Co-Infection of *Entamoeba gingivalis* and Periodontitis-Associated Bacteria in Dental Patients in Taiwan	Identifying microbes containing protozoa (*E. gingivalis*) in periodontitis patients in Taiwan.	Case series	4 patients with generalized chronic periodontitis. Samples were taken from one moderate periodontitis area (≥5mm pocket) and one healthy area (<5mm pocket) from each patient. Subgingival plaque from the patient was examined by PCR	Presence of *Entamoeba gingivalis*: 100% 1. Presence of *E. gingivalis* in pocket ≥ 5mm: 75%(3) • ST1: 50% (2) • ST2: 50% (2) 2. Presence of *E. gingivalis* in pocket < 5mm: 100%(4) • ST1: 25%(1) • ST2: 75%(3)	The presence of *E. gingivalis* and bacteria that cause periodontitis can have an important role in periodontitis. *E. gingivalis* could be a potential pathogen in periodontitis.

4	Maryam Sharifi *et al*, 2020	Prevalence and Associated Factors for *Entamoeba gingivalis* in Adolescents in Southeastern Iran by Culture and PCR, 2017	Identifying the prevalence and potential factors associated with *Entamoeba gingivalis* in adolescents in Kerman City, Southeast Iran, 2017.	Cross-sectional	315 adolescents aged 13 to 16 years consisting of 189 boys and 126 girls. Potential factors: -Gender -History of antibiotics -Gingival index -Candida spp infection -DMFT Dental plaque taken from the gingival line was examined using PCR and light microscopy	Prevalence of *E. gingivalis*: 11.7%. Male: 15.9% Female: 5.6% 1. Results of univariate logistic regression model analysis • Gender - Male:15.8% - Female:5.5% • History of antibiotics - Yes: 12.03% - No: 11.5% • Gingival index - Without inflammation: 4.3% - Mild: 6.9% - Moderate: 10.1% - Severe: 76.9% • Candida spp infection - Yes: 11.02% - No: 15.38% • DMFT - Yes: - - No: - 2. The results of the multivariate logistic regression model analysis • Gingival index - Without inflammation: 5.8%	The prevalence rate of *E. gingivalis* is quite high. There is a relationship between *E. gingivalis*, demographics, and clinical risk factors.

						- Mild:5.7% - Moderate: 10.7% - Severe: 66.67% • Candida spp infection - Yes: 16.95% - No: 5.55% • DMFT - Yes: 11.74% - No: -	
5	Gabriela Garcia *et al*, 2018	Prevalence of Two *Entamoeba gingivalis* ST1 and ST2-Kamaktli Subtypes in the Human Oral Cavity Under Various Conditions	To determine the prevalence of infection with two subtypes of *E. gingivalis*, namely *E. gingivalis* ST1 (Eg-ST1) and *E. gingivalis* ST2, kamaktli variant (kamaktli-variant) during healthy and pathological conditions.	Case-control	The 287 patients consisted of: a. Periodontitis: 102 patients b.Orthodontic treatment (gingivitis): 80 patients c. Healthy: 105 patients Samples were taken from the gingival sulcus around the inferior molars and examined using PCR	Presence of *Entamoeba gingivalis* 1. Periodontitis patients • ST1: 23.4% • ST2: 15.7% • ST1 & ST2: 34.3% 2. Gingivitis patients • ST1: 7.5% • ST2: 33.8% • ST1 & ST2: 40% 3. The patient is healthy • ST1: 24.8% • ST2: 5.7% • ST1 & ST2: 23.8%	*Entamoeba gingivalis* ST1 and ST2 can cause periodontal disease. The two subtypes have different genetic and infectious behavior patterns. The presence of *E. gingivalis* ST2 in the three groups was different, most commonly found in the group with orthodontic treatment (gingivitis).
6	Mark Bonner *et al*, 2014	Detection of the Amoeba *Entamoeba gingivalis in* Periodontal Pockets	to study the potential association between colonization of the gingival sulcus by	Case-control	Patient cohort: 139 Samples that can be used to continue research are 105 Samples were taken from:	1. Clinical observation of periodontitis (+): 72 patients Presence of *E. gingivalis* -PCR: 80.6%	*E. gingivalis* infection and periodontitis have a relationship. *E. gingivalis* is

			Entamoeba gingivalis and periodontitis		- Periodontal pocket: 72 - Healthy gingiva: 33 Examined using a microscope and PCR	-Microscopy: 86.1% (-): 33 patients Presence of *E. gingivalis* -PCR: 33.3% -Microscopic: 9.1% 2. Microscopy of *E. gingivalis* (+): 65 patients (-): 40 patients 3. PCR *E. gingivalis* (+): 69 patients (-): 36 patients	rarely found in healthy patients. The presence of *E. gingivalis* before the occurrence of periodontitis can be considered the causative agent of the disease.
7	Marie Zaffino *et al*, 2019	Development of a New TaqMan PCR Assay for the Detection of Both *Entamoeba gingivalis* Genotypes	Detecting the presence of *E. gingivalis* ST1 and ST2 in clinical samples using the TaqMan PCR test and comparing the results of evaluating the presence of *E. gingivalis* in 50 pathological locations in patients with periodontitis using the TaqMan PCR and two different conventional PCRs.	Cross-sectional	50 periodontitis patients Samples in the form of subgingival plaque were taken from the periodontal pocket	Prevalence of *E. gingivalis* 74%, ST1: 50% ST2: 24% 1. Conventional PCR: Only detects ST1 • EGF/EGR: 50% (25) • EGO1/EGO2: 40% (20) 2. qPCR SYBR Green • ST1: 50% (25) • ST2: 24% (12) 3. TaqMan PCR • ST1: 50% (25) • ST2: 24% (12)	This new application of quantitative PCR can assist in assessing whether conventional non-surgical periodontal treatments targeting the microbiota and including amoebae are effective, by measuring the number of parasites before and after treatment.
8	Marie Dubar *et al*, 2019	Protozoans in Subgingival Biofilm: Clinical	Evaluated the two identified subtypes of *Entamoeba gingivalis*	Case-control	Periodontitis patients: 30 people Male: 13	Prevalence of *E. gingivalis* 88.3% 1. Healthy group	Scaling and root planing were not effective in

		and Bacterial Associated Factors and Impact of Scaling and Root Planing Treatment	and Trichomonas tenax and the impact of scaling and root planing on the prevalence of these protozoa in pathological and healthy areas in periodontitis patients.		Female: 17 samples were taken from 2 pathological areas (pocket ≥5mm) and 1 healthy area (pocket ≤3mm) Healthy patients: 30 people Male: 13 Female: 17 (sample taken from 1 healthy area) Samples in the form of subgingival plaque were examined using PCR	• ST1: 3.3%(1) • ST2: - 2. Periodontitis group • Before SRP - Pocket ≤ 3mm • ST1: 10% (3) • ST2: 3.3% (1) - Pockets ≥ 5mm • ST1: 70% (42) • ST2: 18.3% (11) • After SRP - Pocket ≤ 3mm • ST1: 13.3% (4) • ST2: 0% (0) - Pockets ≥ 5mm • ST1: 58.3% (35) • ST2: 13.3% (8)	eliminating the presence of *E. gingivalis* in the periodontal pocket. Actions of elimination and reduction of the presence of protozoa were not associated with healing and an increase in the parameter of periodontal pocket depth.
9	Sibeli B. S. Cembranelli *et al*, 2013	First Evidence of Genetic Intraspecific Variability and Occurrence of *Entamoeba gingivalis* in HIV(+)/AIDS	Evaluating the incidence, opportunistic conditions, and intraspecific genetic variability of *E. gingivalis* in HIV(+)/AIDS patients	Cross sectional	82 HIV(+)/AIDS patients with periodontal disease (gingivitis and periodontitis), age range 40.49 ± 10.52 years Male: 51 patients Female: 31 patients Dental plaque was taken from the gingival line and then examined using fresh examination, culture, and PCR	*E. gingivalis* was present in 63.4% (52) of the patients • Number of *E. gingivalis* based on research method: - Conventional PCR: 56.1% - FE: 36.6% • Number of *E. gingivalis* based on CD4+ count : - >200 cells/m3 : 63.8% - ≤200 cells/m3 : 62.5% • Number of *E. gingivalis* based on the amount of virus: - <50: 56.1%	High numbers of *E. gingivalis* in HIV(+)/AIDS patients may support the development of existing periodontitis.

						- 51-5000: 82.4% - 5001-30000: 60% - 30001-100000: 50% - >100000: 66.7%	
10	Robert D. Trim *et al*, 2011	Use of PCR to Detect *Entamoeba gingivalis* in Diseased Gingival Pockets and Demonstrate Its Absence in Healthy Gingival Sites	Developing a molecular biology approach to determine the presence of *E. gingivalis* in periodontal pockets and healthy gingiva	Case-control	Periodontitis patients: 26 people Healthy patient: 5 people Supragingival plaque samples were examined using conventional PCR and real-time PCR	Presence of *E. gingivalis*I: 1. Healthy group • Conventional PCR: 0 • Real-time PCR: 0 2. Periodontitis group • Conventional PCR: 27% • Real-time PCR: 69% Positive results infected using conventional PCR were detected positive using real-time PCR.	*E. gingivalis* is only found in diseased periodontal pockets. The sensitivity of real-time PCR is higher than that of conventional PCR.
11	Abdelhakam G. Tamomh *et al*, 2020	The Oral Parasitic Mivrobiome in Hepatitis B Virus Infected Sudanese Patients with Gum Disease	to detect and identify *E. gingivalis* as an oral parasite in salivary and gingival specimens and to determine the prevalence of parasitic infection and HBV in patients with gum disease in Sudan.	Case-control	448 patients with periodontal disease HBV(+) : 336 people HBV(-) : 112 people Samples taken from saliva and gingiva were examined using a light microscope	Presence of *E. gingivalis* 1. HBV(+) : 67.6% (227) • Saliva: 227 • Gingival scraping:198 2. HBV(-) : 32.1% (36) • Saliva: 36 • Gingival scraping: 28	The presence of *E. gingivalis* is not only associated with the severity of periodontal disease but also with HBV infection. *E. gingivalis* was detected more in saliva compared to samples taken from the gingiva.

Periodontal disease occurs due to an imbalance between microorganisms, the host, and the environment.[7,19] Periodontal disease begins with an imbalance of commensal microbes in the oral cavity which then interacts with the body's immune cells and causes inflammation of the gums which is called gingivitis.[20] Untreated gingivitis will develop over time and cause periodontitis. Periodontitis is a chronic inflammation that affects the supporting tissues of the teeth, including the periodontal ligament and alveolar bone. The main cause of periodontitis is bacteria in plaque. Microorganisms in dental plaque will trigger an inflammatory response which in turn causes alveolar bone resorption and tooth mobility.[21]

One of the microorganisms that can be found in the oral cavity is protozoa. Protozoa have an important role in the pathology of periodontal disease. The first and most common protozoa found in the oral cavity is *E. gingivalis*. The presence of *E. gingivalis* in the oral cavity was reported by G. Gros in 1849. Lyons further detected the presence of *E. gingivalis* in periodontal pockets.[22] The appearance of *E. gingivalis* in individuals may vary depending on age, oral hygiene, and the health of the periodontal tissues.[7,8] Epidemiological studies show that the infection rate of *E. gingivalis* in periodontitis patients is higher than in healthy patients.[23]

The results showed that there was an effect of the presence of *E. gingivalis* on periodontal disease. Eleven articles reviewed stated that *E. gingivalis* could be found in healthy gingiva, gingivitis, and periodontitis, although the numbers vary. This difference in number could be caused by differences in the number of samples used, the type of sample used, the sampling method, the time of the study, the inclusion and exclusion criteria, the research method, and the type of *E. gingivalis* studied.

Studies conducted by Bao et al., Deng et al., Sharifi et al., Garcia et al., Bonner et al., Dubar et al., and Trim et al. prove that the presence of *E. gingivalis* is more prevalent in patients with periodontal disease, which ranges from 40% -88.3% compared to healthy patients which range from 0%-33.3%.[15,17] The study conducted by Luszczak et al. also stated that a high prevalence of *E. gingivalis* was correlated with the condition of the periodontal tissues. The results of Luszczak et al.'s study proved that the prevalence of *E. gingivalis* in the group with periodontal disease reached 81%, but in the healthy group, it was also quite high, namely 65%.[24] The study of Onyido et al. also stated that the prevalence of *E. gingivalis* in patients with periodontal tissue inflammation was higher than in healthy patients, namely 31.67% while in healthy patients only 3.3%.[6] Based on the study of Deng et al., *E. gingivalis* contributes to periodontal disease by 4.72% while in healthy patients 0.32%.[12]

According to research by Garcia et al., *E. gingivalis* is found more often in gingivitis than in periodontitis.[14] These results are supported by Ramamurthy et al. who showed the presence of *E. gingivalis* was found more often in gingivitis patients by 88% compared to periodontitis patients by 76%[25] and Hassan et al.'s study of 40% in gingivitis patients and 15% in periodontitis patients.[26] The study by Garcia et al., which differentiated *E. gingivalis* ST1 and ST2 using real-time PCR in orthodontic patients with gingivitis, showed a total prevalence of 40%, greater than periodontitis sufferers only amounted to 34.3%.[14] *E. gingivalis* ST 1 and ST2 have widely differing genetic variations.[27,28] This may allow for differences in biological and pathological function between the two. 28 These two subtypes differ substantially across rRNA gene subunits and can be considered distinct species.[29]

Based on a study conducted by Trim et al., the conventional PCR test only detected 27% of *E. gingivalis* in inflamed periodontal tissue, whereas using SYBR Green real-time PCR could detect up to 69% of *E. gingivalis* although the distribution of ST1 and ST2 was not specified.[17] Zaffino et. al also said conventional PCR could only detect ST1 while SYBR Green real-time PCR could differentiate ST1 and ST2. *E. gingivalis* has a prevalence of 74% in periodontitis samples, with a prevalence in ST1 of 50% and ST2 of 24%.[9] A study conducted by Trim et al. showed that *E. gingivalis* was not found in the healthy group.[17] This may be due to the number of healthy patients who contribute little. *E. gingivalis* ST1 and *E. gingivalis* ST2 can be found in individuals with healthy periodontal tissue, indicating that the two amoebae act as commensal microorganisms.[14] Real-time PCR has advantages over conventional PCR, including being highly sensitive, carried out in a closed system, avoiding post-PCR handling, and saving time.[9] Real-time PCR can eliminate ambiguity from positive or negative interpretations that can lead to bias in conventional PCR. Real-time PCR detects the product at the

exponential stage whereas conventional PCR detects at the plateau stage. This allows real-time PCR to be less susceptible to product degradation at high reaction cycles as reagents run out.[30]

A study conducted by Cembranelli et al. using conventional PCR stated that the presence of *E. gingivalis* in HIV(+)/AIDS patients did not show opportunistic behavior because the presence of this parasite was not associated with low immunity.[16] This discrepancy may have occurred due to the use of highly active treatment antiretroviral therapy (HAART) can improve the immune response and reduce the severity, course of the disease, and prevalence of the periodontal disease.[16,33] Further studies need to be conducted to ascertain HAART therapy's effect on this protozoa's presence. The high presence of *E. gingivalis* in HIV (+)/AIDS patients can be influenced by multifactorial factors that are not directly related to CD4 which can hide the opportunistic ability of *E. gingivalis*.[16] The study by Lucht et al. said there was a significant correlation between the presence of *E. gingivalis* in saliva and plaque with periodontal disease in HIV patients. The presence of *E. gingivalis* is more in HIV patients compared to healthy patients and the presence of *E. gingivalis* increases with the severity of HIV/AIDS.[34]

Tamomh et al. in their study using light microscopy said that there was a very significant correlation between the presence of *E. gingivalis* in patients infected with the hepatitis B virus and those who were not infected. The prevalence of *E. gingivalis* is higher in patients infected with the hepatitis B virus. Tamomh et al. said *E. gingivalis* is easier to find in saliva than dental plaque. This is because saliva facilitates the movement of *E. gingivalis* compared to the gingiva. 18 This is in contrast to a study conducted by Ghabanchi who found less *E. gingivalis* in saliva than in dental plaque.[35]

Dubar et al. identified two subtypes of *E. gingivalis* in the subgingival microbiota, their association with bacteria, and periodontal parameters to demonstrate that this amoeba was relatively unaffected by non-surgical periodontal treatment. The number of *E. gingivalis* in pathological areas after scaling and root planing did not decrease significantly.[15] This statement is in contrast to Maybodi et al.'s study using a light microscope which found that there was a decrease in the number of *E. gingivalis* in pathological areas after three weeks of scaling and root planing treatments.[4]

Suggestions that can be given for future research further research is needed regarding the role of *E. gingivalis* in the gingivitis group because research on gingivitis is still small so, further experimental research that can prove *E. gingivalis* has a role in the development of periodontal disease, experimental research that differentiates the pathogenicity of the two subtypes currently known, and conducting clinical trial studies or using randomized controlled trial research methods.

4. CONCLUSION

E. gingivalis was found more frequently in inflamed periodontal tissue than in healthy gingiva. This suggests that *E. gingivalis* has a role in the course of periodontal disease although there are still few studies that prove a direct role of *E. gingivalis* in periodontal disease. The presence of *E. gingivalis* in healthy gingiva indicates that it can act as a commensal microorganism.

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