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RESEARCH ARTICLE

Oral parasitic protozoan *Entamoeba gingivalis* in periodontal disease patients, northeastern Thailand

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ABSTRACT

Entamoeba gingivalis is present in the oral cavity of humans and is associated with periodontal disease. Consequently, this study aimed to comprehensively investigate the *E. gingivalis* infection and the associated risk factors among individuals suffering from periodontal conditions. A cross-sectional descriptive study was carried out within a cohort of periodontal patients. Dental plaque specimens were meticulously collected and subsequently subjected to thorough examination using the polymerase chain reaction (PCR)-based technique targeting the small subunit ribosomal RNA (SrRNA) gene of the organism. The occurrence of risk factors for *E. gingivalis* infection was analyzed by the chi-square test and binary logistic regression. Out of the 230 participants, 60 were clinically diagnosed with periodontitis, while 170 were afflicted with gingivitis. Out of the 230 patients, 25 (10.9%) tested positive for *E. gingivalis* infections. An in-depth analysis unveiled that a significant majority of infections were recorded within subgroups characterized by a marital status (15.45%), manifestation of periodontitis (25.00%), and concomitant presence of underlying disease (20.83%). Furthermore, the high risk factor associated with *E. gingivalis* infection was the female (OR_{adj} = 13.65, 95% CI = 1.08-173.21), followed by periodontitis (OR_{adj} = 3.30, 95% CI = 1.21-9.00), respectively. The study employs a molecular diagnostic approach to screen for *E. gingivalis* enrichment within a subset of periodontal patients with advancing disease. The findings emphasize the necessity for further research to elucidate the pathogenesis of *E. gingivalis* and advocate for vigilant surveillance within a substantial population of periodontal patients.

Keywords: *Entamoeba gingivalis*; polymerase chain reaction (PCR); small subunit ribosomal RNA (SrRNA) gene; Thailand; periodontal disease.

INTRODUCTION

Entamoeba gingivalis, an amoebic protozoan, inhabits the oral cavity of individuals exhibiting inadequate oral hygiene practices. It is detected within dental plaques on gingival and tooth surfaces, interdental spaces, and carious lesions (Alhammza Abbass *et al*., 2020). While the amoeba's trophozoite form could potentially be transmitted, the infective stage exclusively spreads through direct droplet exposure or intimate contact such as kissing (Bonner *et al*., 2018; Mielnik-Blaszczak *et al*., 2018). *E. gingivalis* scavenges dental plaques within the oral cavity, yet the accuracy of its impact on oral hygiene remains inconsistent (Smith & Barrett, 1915; Craig, 1916). Prior investigations have reported *E. gingivalis* as an opportunistic pathogen that aggravates periodontitis within the complex molecular milieu shaped by periodontal disease (Ponce de León *et al*., 2001). Acting synergistically with symbiotic bacteria (*Porphyromona gingivalis, Treponema denticola*, and *Tannerella forsythia*), *E. gingivalis* contributes to the onset of periodontal disease in immunocompromised hosts (Chen *et al*., 2001; Socransky & Haffajee, 2005; Dubar *et al*., 2020). Moreover, studies employing progressive molecular methodologies have explored the prevalence of *E. gingivalis* in both healthy individuals and those affected by oral cavity diseases, effectively capturing the genetic variability of the organism (Badri *et al*., 2021). The occurrence of *E. gingivalis* prevalence has been examined across different countries, with the highest rates documented in Jordan (87%) and comparatively lower rates observed in Portugal (3%). In Thailand, a previous small-scale study in Suphanburi Province revealed that 9 out of 95 participants (9.5%) had tested positive for *E. gingivalis* infection in dental plaque samples. The infection rates were 9.7% for males and 9.4% for females (Siriba *et al*., 2016). Among diverse diagnostic techniques, molecular approaches (53%) and other methodologies (36%) have exhibited the highest combined prevalence (Badri *et al*., 2021). Furthermore, research efforts have documented *E. gingivalis*

prevalence within periodontal pockets, ranging from approximately 6% to 69% (Kikuta *et al*., 1996; Trim *et al*., 2011). This periodontitis-associated *E. gingivalis* assumes the role of a potential pathogen, eliciting host cell inflammation and inciting the degradation of gingival tissue (Bao *et al*., 2020). The pathogenesis of periodontitis is commonly characterized by pain, halitosis, and gingival bleeding, yet patients typically endure such discomfort (Steele *et al*., 2004; Huang *et al*., 2021). Therefore, this study employed a molecular investigative approach to examine the prevalence of *E. gingivalis* and its associated risk factors in individuals with periodontal conditions at a teaching hospital in northeastern, Thailand.

MATERIALS AND METHODS

Study population and sample collection

The purposive sampling of the population was calculated according to the sample size formula for a cross-sectional descriptive study (Daniel, 1999) using the equation: $n=[(Z^2)P(1–P)]/d^2$, n is the number of sample size, Z is a statistic for a level of confidence (99%), P is the expected prevalence or proportion; the last update on the prevalence of *E. gingivalis* infection in Thailand was 9.5% (9/95) (Siriba *et al*., 2016), and d is the precision (0.05) (Daniel, 1999). The principle sample size was calculated to be 230 participants based on the inclusion and exclusion criteria. The periodontal patient criteria from the clinical diagnosis were included. The periodontal screening and record (PSR) index was used to confirm the periodontal status referred to the WHO guideline avoidance of the bias that has been supervised by a periodontology dentist. Whereas, subjects still have permanent molars. Male and female aged 18 to 85 years to attend the oral health center at Suranaree University of Technology Hospital (SUTH), Nakhon Ratchasima Province, northeastern Thailand, from October 2021 to September 2022 were enrolled. Moreover, we excluded the patients that did not diagnose the periodontal disease and were treated with the scaling and root planning within six months before enrolment. The participants provided informed consent and basic information before dental plaque collection. This study was approved by the human research ethics committee of NRPH 055 (KHE 2021-055). The specimens were obtained from all participants using a sterile curette and placed into a microcentrifuge tube containing 500 µL of ultrapure distilled water (Invitrogen, Waltham, USA), kept in an ice box, and transported to the Parasitic Disease Research Center (PDRC), Institute of Medicine, SUT, within 1 hour of collection and immediately processed for examination.

Sample preparation

Genomic DNA (gDNA) was extracted from the dental plaque specimens by a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The DNA extracts served as a template for PCR amplification. The DNA was resuspended in elution buffer, quantified using NanoDrop spectrophotometry (Thermo Fisher Scientific, Wilmington, USA), and then stored at -20°C until PCR proceeded.

Molecular techniques

PCR was performed to detect the *E. gingivalis*-based small subunit ribosomal RNA (SrRNA) gene and specifically amplify a DNA fragment of *E. gingivalis*. The primers were used according to previous reports and were designed from the DNA sequence of the *E. gingivalis* SrRNA gene (D28490) (Yamamoto *et al*., 1995). The forward primer (EgF: 5'-GAATAGGCGCATTTCGAACAGG-3') and reverse primer (EgR: 5'-TCCCACTAGTAAG GTACTACTC-3') (Kikuta *et al*., 1996) were used to generate amplicons of a 1,412 bp fragment inside the SrRNA gene. PCR was performed in a reaction volume of 25 µL. The amplification was performed with 20 ng/µL per reaction of DNA, 10X Taq Buffer with (KCl) $MgCl_2$, 2.5 mM dNTP mix, 25 mM MgCl2, 10 µM of each primer, and 2.5 U of Taq DNA polymerase (Thermo Fisher Scientific, Waltham, USA). Thermocycler was used by following reaction conditions: an initial denaturing step at 94°C for 3.5 min was followed by 35 cycles at 94°C for 1 min, 58°C for 1 min, and 72°C for 1 min. The amplification products were separated by 1% agarose gel electrophoresis and visualized under UV light using gel documentation (Vilber Smart Imaging, Marne-la-Vallée, France). The amplicons were sequenced using Barcode-Tagged Sequencing (BTSeq™) (U2Bio, Seoul, South Korea), a next-generation sequencing (NGS)-based innovative sequencing platform for DNA sequencing, and analysed the product sequences via BLASTN (http://blast.ncbi. nlm.nih.gov/Blast.cgi). Sequences were compared with those reported in GenBank, and amplicons were verified as corresponding to the SrRNA gene sequence of *E. gingivalis*. Then, the obtained sequences and other sequences from 4 *Entamoeba* species [*E. gingivalis* (D28490), *Entamoeba coli* (AB444953), *Entamoeba dispar* (Z49256), and *Entamoeba histolytica* (AB426549)] that colonize humans were aligned to nucleotide sequences and analyzed by BioEdit version 7.2 software.

Statistical analysis

Statistical analyses were performed with STATA/SE version 17.0 for Windows (StataCorpLLC, USA). The independence and alternative hypotheses of an association between the variables were analyzed by the chi-square test. Including, the pattern of the probability of *E. gingivalis* presence based on the predictive variables was analyzed by binary logistic regression to investigate the association between risk factors and *E. gingivalis* infection, while accounting for different potential confounding variables, we carried out both univariate and multivariate analyses (log likelihood) for crude odds ratio (OR_{cru}) and adjusted odds ratio (OR_{adj}), respectively. A P-value of <0.05 was considered to statistically significant difference.

RESULTS

E. gingivalis-positive SrRNA gene detection

Dental plaque specimens were procured from periodontal patients, as illustrated in Figure 1, which delineates the collection sites for specimens from patients with dental plaque-induced gingivitis and periodontitis. Positive detection of the *E. gingivalis* SrRNA gene, discernible through the presence of a 1,412 bp PCR product, was identified in 25 individuals who were clinically diagnosed with periodontal disease [25 out of 230 patients (10.87%)], as depicted in Figure 2.

Multiple alignments of *Entamoeba* species

The SrRNA gene sequences underwent alignment against nucleotide sequences representative of a range of *Entamoeba* species. The DNA sequences within the primer binding regions of the *E. gingivalis* SrRNA gene were aligned with sample sequences. Remarkably, these sequences displayed substantial conservation within each *Entamoeba* species, particularly between *E. gingivalis* and the positive sample sequences, as well as between *E. dispar* and *E. histolytica* sequences (Figure 3).

General characteristics and prevalence

Predominantly, the participants were female (61.74%), aged between 25 and 59 years (54.35%), possessed a body mass index (BMI) of ≤25 (60.87%), were married (53.48%), and held employed (55.65%). Prevalent habits and chronic conditions encompassed non-smoking (86.52%), abstaining from alcohol consumption (61.74%), tooth brushing more than once daily (96.09%), and no-underlying disease (79.13%). The participants were dichotomized into 60 individuals diagnosed with periodontitis and 170 individuals with gingivitis by a dental practitioner (Table 1).

Table 1 portrayed the prevalence and characteristics linked with *E. gingivalis* infection. Contrasting attributes such as gender, age, BMI, educational attainment, occupation, smoking, alcohol

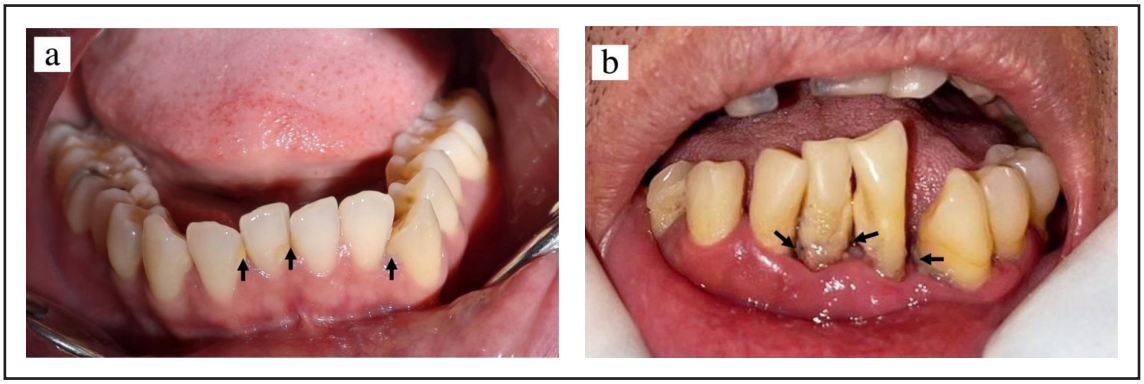

Figure 1. Representative image of periodontal disease. (a) Characteristic image from a dental plaque-induced gingivitis patient and (b) a periodontitis patient. The black arrows indicate the collection sites of dental plaque specimens.

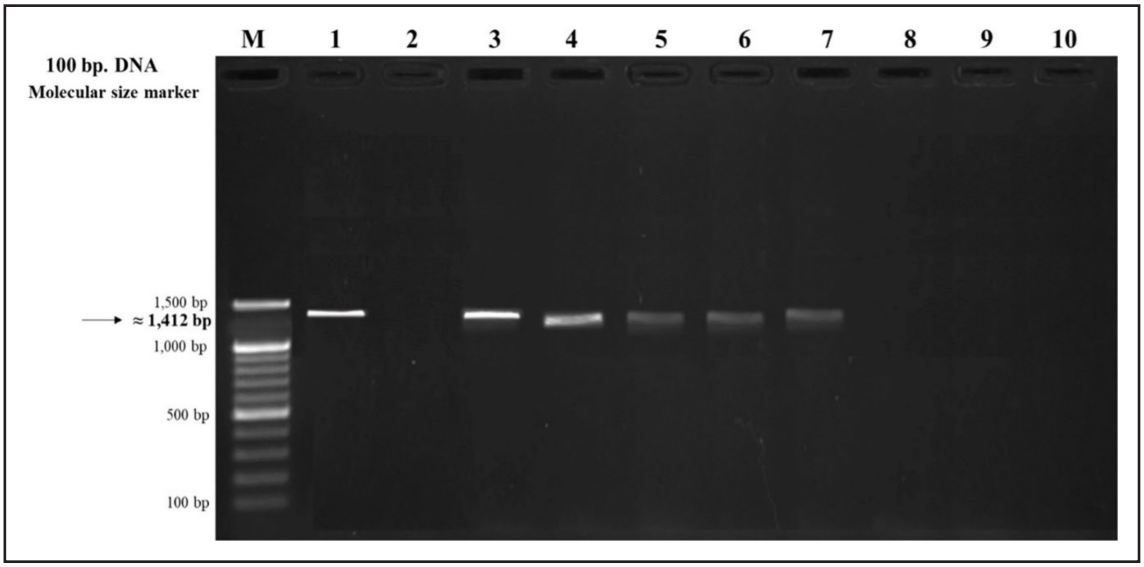

Figure 2. Gel electrophoresis of the amplified bands of targeted gene for *Entamoeba gingivalis* detection. The black arrows indicate the targeted amplicons of a 1,412 bp fragment. M: DNA ladder molecular size marker 100 bp, 1: positive control (*E. gingivalis* gDNA), 2: negative control (free nuclease water), 3-7: positive samples, 8-10: negative samples.

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(a)  ----------GAATAGGCGCATTTCGAACAGGAATGTAGAAAAGAAG--TTTATTAAGAAAAAGA-- 222
(b)  ----------.....--.....-- 53
(c)  ATAATCTACTGAG..GGG.AG.ATCC.TATGGTCCTT.CA.T.T.TT.CCAC..CT..GT...T...GGT 279
(d)  ACAA----------.G.G.C.AAT..AT.T.AGTA...TG.....T..CA--..CTAAGT..GTT..G-- 248
(e)  ACAA----------...G.C.AAT..ATTT.A.T....TG.....T..CA--..CTAAGT..GTT..G-- 248

(a)  -------ACAAATTTACAATTGTAGAAATGAAATACATTTTGACAAGGAATCAATGAAAATATCTGATCT 285
(b)  -------..... 116
(c)  GGATTAT.TGCCAAG.G.....CG-.A.G.....C.....TC....T.A.G.....C.. 348
(d)  -------.TGCCACG.....CAC--.C.GTG...A.....T..C.....G...T..... 309
(e)  -------.TGCCACG.....CAC--.C.GTG...A.....T..C.....G...T..... 309

(a)  AAAGGAAGCGTTAAGCAATAACAGGTCTGTGATGCCCTTAGACATCTTGGGCTGCACGCGCGCTACAATG 1548
(b)  ..... 1379
(c)  G.....C.....T..... 1735
(d)  .....A..C.....C..... 1583
(e)  .....A..C.....C..... 1582

(a)  AAAATACTAAAGAGTAGTACCTTACTAGTGGGATTTTTTATTCCATTATATTGTATAATGGAGTAAAAAGA 1618
(b)  .....---------- 1412
(c)  ...G......C....-T.T.GC.GT.T.C...TGG--..A.GAG..TCGCAACTA.T..CTA.TTGC..G 1802
(d)  G.GT.....G....C.T.TTA.C.T.TACACCT.A......AGGC...G.--CTA.TA..TAGGG.T..T 1651
(e)  G.GT.....G....C.T.TTA.C.T.TACACCT.A......AGGC.T.G.--CTA.TAATTAAGG.T..T 1650
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Figure 3. Alignment of the SrRNA gene sequence. (a) *Entamoeba gingivalis*, (b) *E. gingivalis*-positive human dental plaque specimens to detect with specific primers by PCR amplification, (c) *Entamoeba coli*, (d) *Entamoeba dispar*, and (e) *Entamoeba histolytica*. The forward primer and reverse complement sequence for this study are underlined with red lines. The dashes indicate the absence of residues. The dots specify identical residues with *E. gingivalis*, and identical residues were found in all species.

Table 1. The Chi-square test for analysis the prevalence of *Entamoeba gingivalis* (n=230)

Characteristics	Category	n (%)	*E. gingivalis* infection (%)	P-value
Gender	Male	88(38.26)	7(7.95)	0.286
	Female	142(61.74)	18(12.68)	
Age	15-24 years	42(18.26)	2(4.76)	0.184
	25-59 years	125(54.35)	18(14.40)	
	≥60 years	63(27.39)	5(7.94)	
BMI	≤25	140(60.87)	13(9.29)	0.388
	>25	90(39.13)	12(13.33)	
Status	Married	123(53.48)	19(15.45)	0.019*
	Single	107(46.52)	6(5.61)	
Education	Undergraduate	85(36.96)	9(10.59)	1.00
	Higher education	145(63.04)	16(11.03)	
Occupation	Unemployed	102(44.35)	12(11.76)	0.832
	Employed	128(55.65)	13(10.16)	
Smoking	Yes	31(13.48)	7(22.58)	0.055
	No	199(86.52)	18(9.05)	
Alcohol use	Yes	88(38.26)	8(9.09)	0.644
	No	142(61.74)	17(11.97)	
Tooth brushing	1 time/day	9(3.91)	3(33.33)	0.062
	>1 time/day	221(96.09)	22(9.95)	
Periodontal disease	Gingivitis	170(73.91)	10(5.88)	0.000*
	Periodontitis	60(26.09)	15(25)	
Chronic disease	No- underlying disease	182(79.13)	15(8.24)	0.019*
	Underlying disease	48(20.87)	10(20.83)	

*Significantly different P-value <0.05; n: number of sample size; BMI: body mass index.

Table 2. The binary logistic regression analysis of the relationship between characteristics and *Entamoeba gingivalis* infection

Characteristics	Category	n (%)	*E. gingivalis* infection (%)	Crude OR(95% CI)	Adjusted OR(95% CI)	P-value
Gender	Male	88(38.26)	7(7.95)	1	13.65(1.08-173.21)	0.044*
	Female	142(61.74)	18(12.68)	1.68(0.67-4.20)		
Age	15-24 years	42(18.26)	2(4.76)	1		
	25-59 years	125(54.35)	18(14.40)	3.36(0.75-15.16)	0.63(0.09-4.71)	0.655
	≥60 years	63(27.39)	5(7.94)	1.72(0.32-9.00)	0.17(0.02-1.72)	
BMI	≤25	140(60.87)	13(9.29)	1	1.53(0.54-4.37)	0.425
	>25	90(39.13)	12(13.33)	1.50(0.65-3.46)		
Status	Married	123(53.48)	19(15.45)	1	0.37(0.10-1.35)	0.134
	Single	107(46.52)	6(5.61)	0.33(0.13-0.85)		
Education	Undergraduate	85(36.96)	9(10.59)	1	0.89(0.31-2.60)	0.835
	Higher education	145(63.04)	16(11.03)	1.05(0.44-2.49)		
Occupation	Unemployed	102(44.35)	12(11.76)	1	0.82(0.30-2.19)	0.690
	Employed	128(55.65)	13(10.16)	0.85(0.37-1.95)		
Smoking	Yes	31(13.48)	7(22.58)	1	0.08(0.01-0.84)	0.035*
	No	199(86.52)	18(9.05)	0.34(0.13-0.90)		
Alcohol use	Yes	88(38.26)	8(9.09)	1	0.88(0.23-3.39)	0.849
	No	142(61.74)	17(11.97)	1.36(0.56-3.30)		
Tooth brushing	1 time/day	9(3.91)	3(33.33)	1	0.48(0.07-3.16)	0.443
	>1 time/day	221(96.09)	22(9.95)	0.22(0.05-0.95)		
Periodontal disease	Gingivitis	170(73.91)	10(5.88)	1	3.30(1.21-9.00)	0.020*
	Periodontitis	60(26.09)	15(25)	5.33(2.24-12.68)		
Chronic disease	No- underlying disease	182(79.13)	15(8.24)	1	2.26(0.72-7.08)	0.162
	Underlying disease	48(20.87)	10(20.83)	2.93(1.22-7.02)		

*Significantly different P-value <0.05; n: number of sample size; OR: odds ratio (odds ratio as 1 refer to the reference group); CI: confidence interval; BMI: body mass index.

consumption, and tooth brushing showed no statistically significant differences. The infection was identified in both males (n=7, 7.95%) and females (n=18, 12.68%). Notably, a higher prevalence of *E. gingivalis* infections was evident among adults aged 25 to 59 years (n=18, 14.40%), individuals with higher education (n=16, 11.03%), with a BMI >25 (n=12, 13.33%), and those with unemployed (n=12, 11.76%), thereby signifying no statistically significant difference (P>0.05). Furthermore, married individuals (n=19, 15.45%) exhibited an increased frequency of *E. gingivalis* infections (P=0.019).

Moreover, the relationship between behaviors and *E. gingivalis* infections was explored, revealing that the smoking subgroup (n=7, 22.58%) displayed a no significantly higher infection rate compared to non-smoking group (P>0.05). Additionally, no significant association of *E. gingivalis* infection was found between no alcohol consumption (n=17, 11.97%) and in individuals who brushed their teeth once daily (n=3, 33.33%) (P>0.05).

Noteworthy is the higher prevalence of *E. gingivalis* infections among those diagnosed with periodontitis (n=15, 25%) compared to those with gingivitis, indicating a statistically significant difference (P=0.000). In the realm of chronic diseases and their association with *E. gingivalis* infections, patients with underlying disease (n=10, 20.83%) exhibited markedly higher infection rates than those with no-underlying disease (P=0.019).

Associated risk factors

Exploring the interplay between *E. gingivalis* infection and general characteristics unveiled that infection was notably linked with a female [crude odds ratio (OR_{cru}) = 1.68, 95% confidence interval (CI) = 0.67-4.20; adjusted odds ratio (OR_{adj}) = 13.65, 95% CI = 1.08–173.21, P = 0.008], followed by periodontitis (OR_{cru} = 5.33, 95% CI = 2.24–12.68; OR_{adj} = 3.30, 95% CI = 1.21-9.00), respectively (Table 2).

DISCUSSION

E. gingivalis is notably prevalent within dental plaques, representing one of the diverse array of oral pathogens affecting humans. Its presence has also been identified in pulmonary abscesses and mandibular osteomyelitis (Jian *et al*., 2008). In the present study, *E. gingivalis* was detected by PCR in 25 out of 230 participants (10.87%) diagnosed with periodontal disease. Previous investigations have also examined this observed variability, identifying a 77% protozoan infection rate among periodontitis patients (Bao *et al*., 2020). Employing PCR, the presence of *E. gingivalis* was confirmed, accounting for 15.78% and 11.25% of prevalence rates (El-Dardiry & Shabaan, 2016; Alhammza Abbass *et al*., 2020). Furthermore, *E. gingivalis* has been reported to have a prevalence of 33.3% among healthy sites, with a range varying from 27% to 81% (Trim *et al*., 2011; Bonner *et al*., 2014; Garcia *et al*., 2018). The occurrence of this finding was comparable to that observed in previous study of around seven years ago in Thailand, *E. gingivalis* was detected in 9.5% of dental plaque specimens (Siriba *et al*., 2016). Therefore, our findings, which used the intensive method for this investigation, are an update on the infection rate of *E. gingivalis* in Thailand.

These findings also uncovered a higher frequency of *E. gingivalis* infection in females than males, with previous studies reporting infection rates in females as 63.8–73.3% higher compared to males (26.7–36.3%) (Garcia *et al*., 2018). Among participants diagnosed with periodontitis, the infection rate was observed in males (88.7%) compared to females (89.2%) (Yaseen *et al*., 2021). Regarding the age-range, *E. gingivalis* infections among adults aged 25-59 years were predominantly associated with periodontal disease. This pattern concurs with a previous study reporting that most infections (33.3%) were observed within the age range of 40-49 years (Siriba *et al*., 2016). Moreover, a substantial proportion of *E. gingivalis* infections were detected in patients over 38 years old (Yaseen *et al*., 2021). In agreement with these findings, periodontal disease is prevalent in adults over 30 years old, constituting over 50% of *E. gingivalis* diagnoses (Eke *et al*., 2012), and is often associated with ages above 20 years (Ghabanchi *et al*., 2010) or over 30 years (Eke *et al*., 2015). In the older age group, *E. gingivalis* infection is associated with gingivitis and periodontitis (Yaseen *et al*., 2021), and periodontal disorder is a major factor of this protozoan infection (Bonner *et al*., 2014). Therefore, this age group should be concerned about oral hygiene instruction.

Regarding BMI, this study identified a higher prevalence of *E. gingivalis* infection among participants with a BMI >25. Following this report, we suggested that people with a BMI >25 may consume sweet foods more than those with a BMI ≤ 25, which could be the reason for dental caries and may contribute to the development of periodontitis disease. Marital status emerged as a factor associated with *E. gingivalis* infection, with a higher presence among married subjects, likely due to a more established history of periodontal disease. Additionally, unemployment was linked with *E. gingivalis* infection, consistent with previous findings connecting the infection to lower income and poor oral health (Yaseen *et al*., 2021). The smoking group exhibited a higher prevalence of *E. gingivalis* infection compared to non- smoking group. Likewise, a previous study demonstrated a higher infection rate (51.6%) in individuals with current or past smoking habits compared to never-smokers (46%) (Yaseen *et al*., 2021). This study revealed that *E. gingivalis* infection was more common among those who brushed their teeth once a day, suggesting improved oral hygiene practices among individual people and Thai people enhanced their oral hygiene to decrease periodontitis and *E. gingivalis* infection, which were lower than those in a previous study in Thailand over the past several years (Siriba *et al*., 2016). In fact, *E. gingivalis* resides in oral cavities with inadequate hygiene practices, and it has been found in individuals who maintain poor oral hygiene as well (Yazar *et al*., 2016). However, despite the high percentage of *E. gingivalis* infection, no significant statistical association was identified with oral health in this regard (Hussian, 2017).

Notably, *E. gingivalis* was frequently detected in periodontal donors, with a higher infection rate observed in the periodontitis group compared to the gingivitis group, signifying a risk factor for *E. gingivalis* infection (Yaseen *et al*., 2021). Previous research has firmly linked *E. gingivalis* infection with periodontitis, with most cases occurring within periodontal pockets (Bonner *et al*., 2014; Bonner *et al*., 2018) and advanced periodontitis cases showing close to 100% infection rates (Wantland & Wantland, 1960). This concurs with the 88.9% prevalence in periodontitis patients and 84.9% in gingivitis patients (Yaseen *et al*., 2021). Presently, the study also presented *E. gingivalis* infection was found in chronic diseases or underlying conditions such as diabetes mellitus (DM), hypertension (HT), dyslipidemia (DLP), and cardiovascular disease (CVD), consistent with a limited number of published studies. *E. gingivalis* infection has been implicated in the pathogenesis of systemic and chronic ailments such as DM, CVD, rheumatoid arthritis, and bacterial pneumonia (Li *et al*., 2000; Jenkinson & Lamont, 2005; Jeftha & Holmes, 2013; Moodley *et al*., 2013; Shangase *et al*., 2013; Atanasova & Yilmaz, 2015). The connection between *E. gingivalis* and a history of DM was supported by the study's findings (Yaseen *et al*., 2021). Additionally, periodontitis has been associated with the pathogenesis of DM and CVD (Nascimento *et al*., 2018). In the context of periodontal conditions, *E. gingivalis* may serve as a reservoir for pathogens that migrate to other periodontal tissues, contributing to inflammation (Trim *et al*., 2011). Protozoa may interact with target cells by secreting substances or being ingested by the cell (Jiao *et al*., 2022). This study underscores the sensitivity advantage of molecular techniques in detecting the prevalence of *E. gingivalis*, highlighting its close association with periodontal disease.

However, the precise mechanisms underlying the interplay between periodontal pathogens and *E. gingivalis* remain subjects for future investigations.

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Conflict of interest

The authors declare no conflict of interest.

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