Current global Status and the Epidemiology of *Entamoeba gingivalis* in humans: a systematic review and meta-analysis

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Systematic Review

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Abstract

Purpose *Entamoeba gingivalis (E. gingivalis*) is one of the members of the wide range of oral resident pathogens in humans, particularly found in dental plaques, surfaces of gingiva or teeth, interdental spaces and carious lesions. The purpose of the current review and meta-analysis was to determine the global prevalence of *E. gingivalis* infection and its association with oral diseases based on published literatures.

Methods Multiple English databases (PubMed, Scopus, Science Direct, Web of Science and Google Scholar) were explored for papers published until August 2020. A total of 52 studies (including 7596 participants) met the inclusion criteria.

Results The overall prevalence of *E. gingivalis* was estimated to be 37% (95% CI: 29% - 46%). With regard to different countries, the highest and lowest pooled prevalence of *E. gingivalis* infection were related to Jordan with 87% (95% CI: 81% - 92%) and Portugal with 3% (95% CI: 0% - 10%), respectively. Based on WHO regions, the highest prevalence was related to the region of the Americas with 56% (95% CI: 31%-79%). The infection was most prevalent in 46-55 mean age groups [61% (95% CI: 21% - 94%)]. Among different diagnostic methods, the highest rate of the pooled prevalence was related to the molecular [53% (95% CI: 24% - 81%)] and the direct methods [36% (95% CI: 25% - 47%)], respectively. Our analyses revealed that *E. gingivalis* infection was associated with 4.34-fold increased risk of oral diseases ($P < 0.05$).

Conclusion Our findings revealed a high prevalence rate of the infection among periodontal disease patients with 37% (95% CI: 20% - 57%). To conclude, it must be considered that *E. gingivalis* can be a risk factor associated with oral diseases and a wide range of research is needed to specify its role in the pathogenesis of these disorders.

Introduction

A wide spectrum of microorganisms dwell in the human oral cavity (1). The cosmopolitan *Entamoeba gingivalis* (*E. gingivalis*), initially described from dental plaque samples in 1849 (2), was the first amoeba found in human beings, although its pathogenic association with humans wasn't determined until 1914 (3). In the same year, a series of 46 pyorrhea (periodontitis) cases were shown to carry *E. gingivalis*, which were further treated with emetine (3). This amoeba is generally found in dental plaque, on gingival or tooth surfaces, in interdental spaces, and in carious lesions. It has also been isolated from bronchial mucus and tonsillar crypts (4). Approximately 50% of people with healthy gums and 95% of patients with gum disease host the parasite (5). Trophozoite is the only viable and parasite transmission takes place directly via droplet spray, kissing and/or shared eating dishes (6). The parasite is the only amoeba that ingests white blood cells and moves quickly using its numerous blunt pseudopodia (1).

The amoeba has been found scavenging in the healthy oral cavity, but there are still discrepancies regarding the true effect of the *E. gingivalis* on the hygienic status of the oral cavity (7, 8). Some

researchers suggest that this organism provokes periodontitis, while others remark that the protozoan is only an opportunistic survivor in the molecular milieu elicited by periodontal disease (9–11). However, it is recognized that *E. gingivalis* acts synergistically with symbiotic bacteria to cause periodontal disease in immunocompromised hosts (12). Nevertheless, the parasite prefers periodontal pockets, demonstrating a niche that is either conducive for parasite survival, or that the protozoan provokes micromolecular changes leading to these circumstances (1). Additionally, advanced molecular methods have shown that there exists important genetic variability in the *E. gingivalis*. For instance, there is considerable genetic distance between subtype 1 (ST1) and ST2 variants, accounting for possible differences regarding biological and pathological functions (13, 14).

Several studies have been conducted on the prevalence of *E. gingivalis* in healthy individuals and those with oral cavity disorders. However, the lack of compiled evidence regarding the global pooled prevalence of *E. gingivalis* infection and possible risk factors encouraged us to conduct the present systematic review and meta-analysis study.

Methods

The current study was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (15).

Systematic search strategy

Literatures published in English language on the prevalence of *E. gingivalis* among the worldwide human population were systematically searched via multiple English databases (PubMed, Scopus, Science Direct, Web of Science and Google Scholar) without time limitation until August 2020. The systematic search was performed using the following keywords: *Entamoeba gingivalis*, *E. gingivalis*, Periodontitis, Gingivitis, Periodontal disease, Prevalence, Frequency, Oral protozoa using AND and/or OR Boolean operators. Two independent researchers accomplished the systematic searching process. Moreover, the bibliographic lists of retrieved full-texts were manually explored to find related records not found during database searching.

Inclusion criteria, study selection and data collection

Screening of initially retrieved articles was done based on title, abstract and full-text, and duplicate and/or irrelevant records were excluded. Obtained full-texts were further evaluated by two independent reviewers, which separately made decision on the eligibility of papers. Any contradiction was obviated by consensus with a third reviewer. The following inclusion criteria was considered for article evaluation: **1**) peer-reviewed original articles without geographical limitation, **2**) cross-sectional studies on the prevalence of *E. gingivalis* among various subjects or case-control studies on the association between oral disease and amoeba infection, **3**) papers published only in English language without time limitation until August 2020, **4**) having accessible full-texts, and **5**) having total sample size and exact number of

positive cases. Those papers not meeting the above criteria, including review articles, letters, editorials and those having confusing/undetermined results were all excluded.

In order to extract information from qualified articles, a Microsoft Excel® datasheet extraction form was designed using following criteria: first author's name, country, year of publication, human development index (HDI), income level, WHO region, sample size, sample type, mean age, gender, number of infected individuals, candidiasis, smoking, gingivitis, periodontitis, immunodeficiency and systemic disease.

Meta-analysis

The current meta-analysis was conducted based on a protocol described previously (16–19). In the present review and meta-analysis, the pooled effect size and effect of each individual study with their confidence intervals (CI) was shown by using a forest plot, as a visual summary of the data. The weight of each study is shown by the size of squares, while the crossed lines demonstrate the specific CI. The heterogeneity index was determined among studies using Cochrane's Q test and I^2 index. Also, the probability of publication bias was checked by the Egger's regression test, drawn as a funnel plot. Packages of R software version 3.5.1 was used to perform statistical analysis. A *P*-value less than 0.05 was considered as statistically significant.

Quality assessment

To indicate the quality of each included study, Newcastle–Ottawa Scale was used as described before (20). Briefly, a maximum of 9 scores was determined for the criteria, including the subject selection (0–4 points), comparability of subjects (0–2 points), and exposure (0–3 points). The published paper can be awarded a maximum of one star for the numbered items from the selection and exposure categories and a maximum of two stars for comparability. A total score of 0–3, 4–6, and 7–9 points were considered as the poor, moderate, and high quality, respectively (21).

Results

In total, 3124 records were identified through English databases (PubMed, Scopus, Science Direct, Web of Science and Google Scholar). After removing duplicates and irrelevant papers, a total number of 151 relevant full texts were assessed, among which 99 entries were also excluded from the present systematic review for several reasons such as insufficient data, case reports or case-series, having not clear participants, non-original studies. Finally, 52 studies were eligible to be included in the present systematic review and meta-analysis. Figure 1 has shown the selection of studies for inclusion in the systematic review and meta-analysis. Among 7596 examined subjects, 2385 individuals were infected with *E. gingivalis*, indicated that the global pooled prevalence was 37% (95% CI: 29% − 46%) (Fig. 2). According to case-control studies, exposure to the parasitic infection increased the risk of oral disorder by 4.34-fold, which was statistically significant ($P < 0.05$) (Supplementary Fig. 1). Based on the country, a great number of studies on prevalence of *E. gingivalis* infection were done in Iran [15% (95% CI: 5% − 29%)], followed by Iraq (36%, 95% CI: 13% − 64%), Brazil (63%, 95% CI: 14% − 99%) and Poland (46%, 95% CI: 12% − 82%) (Supplementary Fig. 2). A QGIS3 software was used to provide a map representing the

global prevalence of *E. gingivalis* infection in humans. According to that, the highest and lowest pooled prevalence were related to Jordan (87%, 95% CI: 81% − 92%) and Portugal (3%, 95% CI: 0% − 10%), respectively (Fig. 3). With respect to WHO regions, the highest rate of prevalence was detected in the Americas with 56% (95% CI: 31%-79%) (Fig. 4). The age-based analyses showed that prevalence appears to steadily increase over life, with lowest prevalence in the youngest age group (1–25 years old) (11%, 95% CI: 3%-24%), followed by increased at each subsequent group (Supplementary Fig. 3). The association between *E. gingivalis* infection and gender of individuals was not statistically significant (OR, 1.11; 95% CI: 0.59–2.1) (Supplementary Fig. 4). There was no statistically significant difference between the groups in terms of sample type. However, the highest pooled prevalence was observed for the samples of saliva and dental plaque (41%, 95% CI: 28% − 56%), while the lowest prevalence was related to the samples of sub-gingival dental plaque (34%, 95% CI: 17% − 55%) (Supplementary Fig. 5). Several laboratory techniques were used to diagnose *E. gingivalis* infection in subjects examined in studies included. Our analyses based on the methods showed that the highest estimates for prevalence of the infection were seen in studies utilizing molecular methods (53%, 95% CI: 24% − 81%) and direct methods (36%, 95% CI: 25% − 47%), respectively. Furthermore, the lowest prevalence was estimated to be 25% (95% CI: 11% − 43%) for studies conducted using culture method. Also, among different staining techniques (Iron-Hematoxylin, Hematoxylin & eosin, Trichrome and Giemsa), the highest pooled prevalence was found with the Giemsa staining (29%, 95% CI: 13% − 48%) (Table 2).

Some of the studies included in the current systematic review and meta-analysis, considered possible risk factors for *E. gingivalis* infection. In this regard, candidiasis patients had a pooled prevalence of 35% (95% CI: 0% − 92%) for *E. gingivalis* infection, while this rate was 22% (95% CI: 12% − 34%) and 37% (95% CI: 20% − 57%) for gingivitis and periodontitis patients, respectively (Supplementary Figs. 6–8). In addition, smoking individuals had a prevalence rate of 31% (95% CI: 15% − 49%) for the infection (Supplementary Fig. 9). Also, the prevalence of *E. gingivalis* among patients suffering from immunodeficiency or systemic disease was 64% (95% CI: 19% − 97%) (Supplementary Fig. 10). Most of the studies were done in countries located in upper-middle income level, but the highest prevalence rate was detected in high income nations (47%, 95% CI: 32% − 64%) (Fig. 5). As well, the highest prevalence was observed among countries having a very high level of HDI (47%, 95% CI: 32% − 64%) (Table 2). According to Egger's regression test, which was applied to estimate publication bias, there was a statistically significant heterogeneity ($t = 2.25$, $P = 0.02$) (Fig. 6).

Discussion

Over the past three decades, the link between poor oral health and systemic diseases has been documented in a number of studies (22). As such, the association of oral infection, particularly periodontitis, with the pathogenesis of systemic and chronic diseases, such as diabetes mellitus, cardiovascular disease, rheumatoid-arthritis, low birth weight and bacterial pneumonia has been recognized (23–25). However, little information is known about the oral protozoan parasite *E. gingivalis* with these diseases. To the best of our knowledge, this is the first comprehensive systematic review and

meta-analysis of the global prevalence of *E. gingivalis* infection in humans and its association with oral diseases.

Our findings have shown that the pooled prevalence was 37% (95% CI: 29% – 46%) (Fig. 2). Interestingly, we could reveal that the infection is associated with a 4.34-fold increased risk of oral diseases, according to case-control studies (Supplementary Fig. 1). In most of the cases, *E. gingivalis* has been detected in periodontal pockets (1, 26, 27). However, the amoeba was also detected in pulmonary abscess (5), osteomyelitis of the mandible (28) and in neck nodules (29). Our results showed that the parasite was most prevalent in the samples of saliva and dental plaque (Supplementary Fig. 5). Dental plaque is a form of a microbial biofilm that covers the teeth and has a diverse microbial composition. It has benefits for the host by helping to prevent colonization by exogenous bacterial species (30, 31). The bacterial composition of plaque (microbial homeostasis) remains relatively stable despite exposure to some environmental perturbations, such as smoking, high frequency sugar diet or diabetes (30–31). Furthermore, smoking promotes the formation of a caries-susceptible environment by influencing saliva buffer capability, changing its chemical agent and bacterial composition (32). As a result, dental plaque could be a predisposing factor for gingival diseases and dental caries if their microenvironment is perturbed (33, 34). Our study reveals that smoking may be a risk factor for *E. gingivalis* infection (Supplementary Fig. 9). Also, this study has shown that oral candidiasis could be a risk factor of *E. gingivalis* infection, while the parasite was detected in 35% of candidiasis patients (Supplementary Figs. 6–8). On the other hand, previous studies have found that *Candida albicans* is significantly associated with dental caries (35, 36) and periodontal disease (37) through induction of oral microbial dysbiosis (38) and inflammatory responses (39). Immunodeficiency is also a predisposing factor for oral microbial dysbiosis (40, 41) and periodontal diseases (42). Similarly, we demonstrated that patients with immunodeficiency or systemic disease had a high prevalence rate of *E. gingivalis* infection (64%).

Although most studies have focused on the prevalence of *E. gingivalis*, recent studies have uncovered the role of this protozoa in oral inflammation and tissue destruction (43). In a case-control study among periodontitis patients, the parasite was detected in 77% of the inflamed periodontal sites and 22% of the inflamed healthy sites in the case group, while in the control group it was detected in 15% of healthy oral cavities (43). Moreover, histochemical staining revealed that in the presence of *E. gingivalis* infection, the inflammation of gingival epithelium is associated with abundant neutrophils (43). *In vitro* studies in primary gingival epithelial cells demonstrated that there was a strong upregulation of the inflammatory cytokine interleukin (IL)-8 and matrix metallopeptidase 13 (MMP13) during *E. gingivalis* infection, but not in the presence of *Porphyromonas gingivalis*, which is a common oral bacterial pathogen (43). Recent reports on dental diseases patients in Taiwan discovered the co-infection of *E. gingivalis* with three common oral anaerobic pathogenic bacteria, *P. gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (44). Therefore, it is probable that co-infection of the parasite with oral pathogenic bacteria and *Candida albicans* could promote tissue inflammation and predispose periodontal diseases.

Our analysis has suggested that molecular methods have higher sensitivity than conventional methods for detection of *E. gingivalis*. Thus, molecular methods could be helpful for detection of the parasite,

especially in immunocompromised patients who have diverse oral complications and microbial dysbiosis.

Conclusion

The results of this meta-analysis reveal a high prevalence rate of *E. gingivalis* among periodontal disease patients. Although the role of *E. gingivalis* in the pathogenesis of periodontal diseases is still not fully understood, some evidence such as its co-infection with oral pathogens and induction of tissue inflammation in the presence of the parasite can illustrate the possible role of *E. gingivalis* in the pathogenesis of oral diseases. Therefore, it seems that *E. gingivalis* may be a neglected risk factor for periodontal diseases and further research is needed to explore its role in the pathogenesis of these disorders.

Declarations

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Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Authors contributions

AVE and MB designed the study. EH, MZ, MGJ, AG and PAA searched for primary publications, screened and appraised primary studies. MB, AA, and AVE extracted the data and wrote the study manuscript. MO and MB contributed to data analysis and interpretation the manuscript. All authors read the manuscript and participated in the preparation of the final version of the manuscript. All authors read and approved the final manuscript.

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