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Risk factors and approaches for detection of *Trichomonas tenax,* the silent culprit in periodontal disease: A narrative review

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ABSTRACT

Introduction: Periodontal disease is the inflammation of the periodontium tissues surrounding the teeth, potentially leading to loss of tooth attachment. In individuals with periodontal disease, the presence of *Trichomonas tenax*, a parasitic protozoan of the oral cavity has been observed and its frequency tends to rise as the disease progresses.

Methods: A literature search was conducted in the online databases of PubMed, Google Scholar, Web of Science, and Scopus using the combination of keywords: "*Trichomonas tenax*" AND "periodontal disease" OR "gum disease", OR "oral disease" OR "periodontitis". A total of 9 articles satisfied the inclusion criteria and were included in this study.

Results: This review highlights the incidence of *T. tenax* with periodontal diseases, the risk factors that contribute to the infection of *T. tenax* and available detection methods for the identification of the protozoan.

Conclusion: The inhabitation of the oral cavity by *T. tenax* prospers with the severity of periodontal diseases. Extensive research should be conducted to fully understand the potential pathogenic role and damaging effect of *T. tenax* in the oral cavity.

1. Introduction

Inflammation of the periodontium tissues that surround and support the teeth, as well as their underlying bone structure is defined as periodontal disease. Periodontitis represents the most advance and severe form of periodontal disease. It is an irreversible form of the disease, affecting about 20–50 % of the global adult population (Institute for Health Metrics and Evaluation, 2018). It is predicted that in the coming years, the worldwide periodontitis prevalence which is currently at 11.2 %, will increase concomitantly with the growth of the ageing population (Tonetti et al., 2017). Periodontitis is among the major non-communicable diseases (NCDs) that leads to tooth mobility and loss of teeth, which to a greater extent, would lead to edentulism and masticatory dysfunction that would deteriorate the wellbeing and self-confidence of affected individuals, as well as increasing the risk of systemic NCDs, such as diabetes, cancer, and cardiovascular diseases (Bracamonte-Wolf et al., 2019; Nazir et al., 2020; Dom et al., 2016).

The development of periodontal disease is initiated by bacterial in dental plaques due to poor practice of oral hygiene, which leads to dysbiosis of the host-oral microbiota and inflammation of periodontium tissues. The human oral cavity consists of a diverse microbiota that supports the growth of numerous microorganisms such as bacteria and protozoans (Deo & Deshmukh, 2019). *Trichomonas tenax* is a protozoan parasite residing in human's oral cavity and it is capable of producing diverse enzymes, such as cysteine proteases that could promote the degradation of periodontal tissue by periodontopathogens (El Sibaei et al., 2012). The occurrence of *T. tenax* is observed to be greater in individuals with periodontitis as compared to healthy individuals, with

Abbreviations: NCDs, Non-communicable diseases; S rRNA, Small regulatory ribonucleic acid; DNA, Deoxyribonucleic acid; PCR, Polymerase chain reaction; TYM, trypticase-yeast-maltose; TYI-S-33, trypticase-yeast-iron-serum.

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a prevalence range from 4 % to 53 %. (Albuquerque et al., 2011). The presence of *T. tenax* is also reported to increase exponentially with disease progression and is highly dependent on the presence of teeth, as *T. tenax* is found to be absent in babies and completely edentulous patients (Benabdelkader et al., 2019; Bracamonte-Wolf et al., 2019).

The intricate role of *T. tenax* in the progression of periodontal disease remains a puzzle, highlighting the urgent need to unravel its association with disease severity, risk progression, and potential adverse effects within the oral cavity. This knowledge gap underscores the significance of our endeavour to shed light on the intricate web of interactions between *T. tenax* and periodontitis, with a particular emphasis on deciphering prevalence patterns, elucidating contributory risk factors, and exploring diverse detection methods. By meticulously dissecting this association, our review aims to establish a foundational understanding, poised to catalyse further investigations into the potential pathogenic role of *T. tenax* in the realm of periodontal disease.

2. Materials and methods

A thorough search of literature was conducted through the online PubMed, Google Scholar, Web of Science, and Scopus databases using the combination of keywords: "*Trichomonas tenax*" AND "periodontal disease" OR "gum disease," OR "oral disease" OR "periodontitis". Articles that were deemed eligible for inclusion in this study were scrutinized according to the following inclusion criteria: (1) published in English; (2) studies that exclusively addressed the occurrence of *T. tenax* with periodontal disease, and (3) studies conducted on human subjects.

The initial search yielded 363 articles in total. A total of 281 articles were excluded after filtering out duplicates, non-human studies and articles that were not in English. The remaining 82 articles were evaluated for their eligibility. Of those, nine articles met the inclusion criteria and were incorporated into this study (Fig. 1).

3. Results and discussion

3.1. Trichomonas tenax and periodontal diseases

A human oral cavity is comprised of diverse oral microbiota that support the growth of numerous microorganisms, including bacteria, fungi, viruses, and protozoan, which coexist in a symbiotically manner inside the mouth (Deo & Deshmukh, 2019). Symbiotic destruction between the oral microbiota and the host would cause inflammation of the periodontium tissues, which could initiate the development of periodontal diseases (Radaic & Kapila, 2021). Periodontal diseases consist of gingivitis and periodontitis, which are distinguished by the degree of periodontal tissue destruction. Gingivitis is a reversible disorder that can be treated by removing bacterial plaque, whereas periodontitis causing permanent damage to the periodontium and the alveolar bone around the teeth that is irreversible (Kim and Amar, 2006). Nevertheless, it is controllable after removing causative factors through periodontal therapy.

According to the literatures included in this study, the *T. tenax* prevalence is observed to be higher in patients with periodontal diseases than healthy individuals, with a prevalence rate ranging from 8.09 % to 56.89 % (Abd et al., 2016; Abdulhaleem et al., 2018; Mohammed & Alwaaly, 2019). *T. tenax* is recorded to have a greater prevalence in periodontitis patients, with 40 % and 70 % prevalence rates as compared to gingivitis (14.1 % and 35.0 %), as reported by Athari et al. (2007) and Bracamonte-Wolf et al. (2019). These findings are evidence of the positive correlation between the progression of periodontal diseases and the increment of *T. tenax* colonization.

Transmission routes of *T. tenax* are diverse, encompassing saliva, droplet spray, contaminated utensil usage, and even interpersonal contact such as kissing (Abd et al., 2016; Hamad, 2021). Notably, Marty et al. (2015) presented a poignant case study involving an 8-year-old boy afflicted by acute periodontitis, with his parents grappling with chronic periodontitis. This scenario hints at the potential for parental

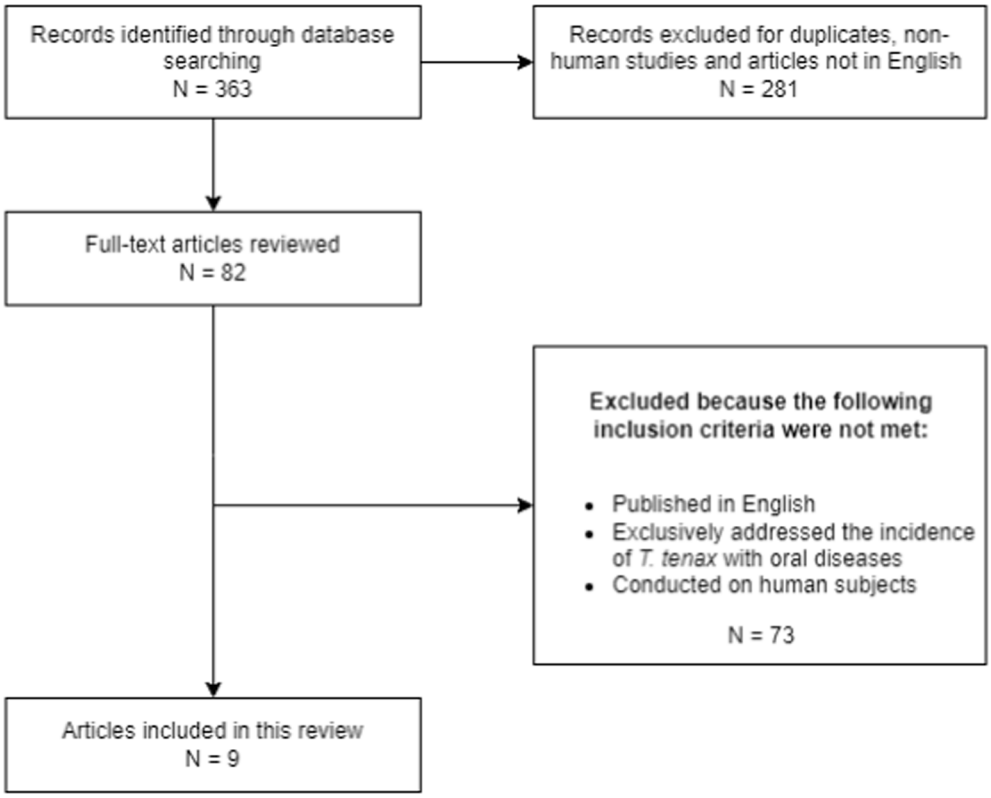

Fig. 1. Flow diagram illustrating the process for screening and selection of articles.

transmission of *T. tenax* to their offspring, a proposition corroborated by Filho et al. (2000).

3.2. Risk factors of T. tenax infection

An individual has a higher tendency of getting infected with *T. tenax* if they are present with any combination of the following risk factors, which includes smoking, poor oral hygiene, old age, and chronic diseases like diabetes and cardiovascular disease. Mohammed & Alwaaly (2019) reported that individuals who smoke exhibit a significantly elevated rate of *T. tenax* infection (15.7 %) as compared to non-smokers (4.58 %). This is due to the components of tobacco, such as nicotine, that are not only addictive but are also capable of altering the oral microbiota composition by boosting the colonization of pathogenic bacteria and impairing periodontal ligament cells (Zhang et al., 2019), which indirectly promote invasion of parasitic protozoans. On the contrary, Bracamonte-Wolf et al. (2019) found no association between smoking habits and the occurrence of *T. tenax,* as 68 % of patients presented with oral trichomoniasis in their study were non-smokers.

Regarding the practice of oral hygiene, individuals with poor oral care and who do not brush their teeth have higher chances of getting infected with *T. tenax*, with a prevalence rate of 29.1 % as compared to individuals who brush their teeth (11.9 %), as reported by Abd et al. (2016). The presence of *T. tenax* in the oral cavity of individuals within the age range of 40 to 60 years old is shown to be higher, and this is probably due to the build-up of dental plaque over time and minimal awareness of the causes and prevention of periodontal diseases. This finding is supported by Abd et al. (2016), Mohammed & Alwaaly (2019) and Athari et al. (2007), who found a notable rate of *T. tenax* infection, ranging from 12.19 % to 92.8 %. In contrast to other findings, a study conducted by Bracomonte-Wolf et al. (2019) reported a higher prevalence rate of *T. tenax* in the age group of 20 to 40 years old (50 %), although the difference is not significant.

Diabetes has a positive association with periodontitis, given that the frequency of diabetic patients with periodontitis is 86 % (Bracamonte-Wolf et al., 2019). Individuals with diabetes are also found to have a higher prevalence of *T. tenax* infection at 12.97 % as compared to healthy individuals (5.55 %), as reported by Mohammed & Alwaaly (2019). Nonetheless, Bracamonte-Wolf et al. (2019) found no positive correlation between diabetes and the presence of *T. tenax,* as the difference is not statistically significant, with a p-value of 0.393.

In terms of gender, most studies resulted in different *T. tenax* infection prevalence in males and females, varying from 6.79 % to 62.85 % and 11.01 % to 47.82 %, respectively. Despite that, gender has no affiliation with the incidence of *T. tenax* in the oral cavity (Athari et al., 2007; Abd et al., 2016; Abdulhaleem et al., 2018; Bracamonte-Wolf et al., 2019; Mohammed & Alwaaly 2019; Hamad, 2021).

According to the findings of Hamad, (2021) and Mehr et al. (2015), *T. tenax* is also likely to be present in the oral cavity of people with Down syndrome. They found the prevalence of *T. tenax* infection among people with Down syndrome to be ranging from 26.1 % to 37 %, which is higher as compared to healthy individuals. Hamad, H. K. (2021) also reported that the detection rate of *T. tenax* is much higher in Down syndrome children with heart disease (64 %).

3.3. Detection methods

Identification of *T. tenax* could be performed using samples of saliva, dental plaque, dental calculus, pulp tissue of root canal, carious dentine, and oral swab. The range of parasite detection for each type of sample varies, as the number of subjects and samples collected differ in all reported studies. The detection rate of dental plaque and dental calculus is the highest, ranging from 20.6 % to 63.63 %, whereas carious dentine has the lowest detection rate at 0 %. Saliva with an average pH of 7 to 7.7 is reported to have the highest presence of *T. tenax* at 56.1 % (Hamad, 2021). On the contrary, Abdulhaleem et al. (2018) and Mohammed & Alwaaly (2019) found that saliva samples had low detection rates, valued at 0 % and 8.09 %, respectively. On the other hand, the oral swab is reported to have a detection rate of 12.06 % to 31.89 %, while the detection of oral parasites by root canal sample is valued at 11.54 %. Sampling sites that are frequently favoured by studies included in this review are subgingival dental plaque from the periodontal pocket, followed by saliva and oral swab of the buccal mucosa. Based on this finding, we can infer that dental plaque provides a perfectly adequate environment for the growth of *T. tenax*.

Available methods employed for the detection of *T. tenax* comprise microscopic examination, culture, and molecular technique. The most common method of detection is microscopic examination, by the usage of a light microscope or phase-contrast microscope. The presence of *T. tenax* is identified by observing its movement and morphological criteria by wet mount method and staining using Giemsa stain or methylene blue stain, respectively. Trophozoite of *T. tenax* is flagellated and pear-shaped, measuring about 5 – 13 μm and moving in a circular motion (Bracamonte-Wolf et al., 2019).

Culture media that are extensively used for the *T. tenax* cultivation in related published articles are TYM (trypticase-yeast-maltose) and TYI-S-33 (trypticase-yeast-iron-serum) medium, which is the recommended growth medium by the American Type Culture Collection (ATCC). For instance, El Sibaei et al. (2012) incorporated TYI-S-33 medium for the growth of *T. tenax* for proteomic analysis, and they reported that 20 out of 110 plaque samples (18.18 %) were positive for *T. tenax*. Among studies included in this review, only Abdulhaleem et al. (2018) performed cultivation of living *T. tenax* trophozoites for parasite detection by using TYM medium that is supplemented with 10 % of heat-inactivated serum. *T. tenax* in the culture is smeared on a glass slide and observed under the light microscope for identification, where 40 out of 160 samples (25 %) were positive for *T. tenax*.

The molecular technique using Polymerase Chain Reaction (PCR), as stated by Athari et al. (2007) and Bracamonte-Wolf et al. (2019), is the most accurate detection approach with greater sensitivity and specificity. In both studies, the total genomic DNA is extracted according to the inorganic method of phenol–chloroform, while the primers used differ in their target regions. Athari et al. (2007) performed their PCR by using 18S rRNA gene primers and reported that 33 out of 160 samples (20.6 %) were invaded by *T. tenax*. Meanwhile, Bracamonte-Wolf et al. (2019) used beta-tubulin gene primers for their PCR assay, where 56 % of plaque samples from 50 patients with advanced periodontitis were positive for the *T. tenax* presence.

4. Conclusion

A positive link between periodontal disease and *T. tenax* can be inferred based on the findings of this literature review that focus on the prevalence, risk factors, and detection methods in studies that exclusively elucidate the incidence of *T. tenax* with periodontal diseases. *T. tenax* is observed not only among individuals with periodontal diseases but in healthy individuals as well, which suggests that *T. tenax* could be the oral cavity commensal. Nevertheless, the presence of *T. tenax* is also reported to increase along with the severity of the disease. Despite the existing evidence that corroborate the link between *T. tenax* and periodontal diseases, a comprehensive understanding of the exact mechanisms underlying its pathogenic role and the extent of damage it inflicts on the oral cavity remains notably underexplored. Previous studies have demonstrated an increase in *T. tenax* frequency with disease progression, yet a detailed exploration of the protozoan's specific contributions to the inflammatory processes and tissue degradation associated with periodontal disease remains lacking. As such, a significant gap exists in our knowledge, warranting focused research efforts to unravel this intricate relationship and its implications for oral health management.

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CRediT authorship contribution statement

Nurin Jazlina Nor Azmi: Data curation, Writing – original draft. **Suharni Mohamad:** Conceptualization, Funding acquisition, Writing – review & editing. **Wan Nazatul Shima Shahidan:** Writing – review & editing. **Haslina Taib:** Writing – review & editing. **Zeehaida Mohamed:** Writing – review & editing. **Emelia Osman:** Writing – review & editing.

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