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Original article

Microscopic Detection of *Entamoeba gingivalis* and *Trichomonas tenax* in Oral Specimens from Healthy Individuals and Patients with Periodontal Disease in Misurata, Libya: A Clinic-Based Cross-Sectional Study

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Abstract
The oral cavity harbours a diverse microbial community that includes bacteria, fungi, viruses, and protozoa. Among the protozoa detected in the human oral cavity, *Entamoeba gingivalis* and *Trichomonas tenax* have been reported in saliva, dental plaque, gingival crevices, and periodontal pockets, although their precise clinical significance remains incompletely understood. This clinic-based cross-sectional laboratory study aimed to describe the occurrence of *E. gingivalis* and *T. tenax* according to periodontal status and to examine the distribution of periodontal categories by age and sex among individuals attending dental clinics in Misurata, Libya. One hundred participants aged 18–72 years were included, comprising 80 participants with periodontal disease and 20 participants with a clinically healthy periodontium. The periodontal-disease group included 53 participants with gingivitis and 27 with periodontitis. Four oral specimens were collected from each participant, yielding 400 specimens. The specimens comprised saliva; one paper-point specimen containing gingival crevicular fluid and periodontal-pocket material; dental plaque collected from tooth surfaces; and dental plaque collected from gingivitis-associated or periodontitis-associated sites. A sterile absorbent paper point (size 40) was placed for approximately 30 seconds. Samples were examined by direct light microscopy and Giemsa staining, and protozoa were identified according to their characteristic morphological features and motility. Data were analysed using SPSS version 25, and categorical variables were evaluated using the chi-square test, with statistical significance set at $P<0.05$. Among the 400 examined specimens, 89 (22.3%) showed detection of *E. gingivalis* alone, 16 (4.0%) showed detection of *T. tenax* alone, 82 (20.5%) showed co-detection of both protozoa, and 213 (53.3%) showed no protozoan detection. Of the 89 specimens showing *E. gingivalis* alone, 81 (91.0%) were associated with periodontal disease and 8 (9.0%) with a healthy periodontium. All 16 specimens showing *T. tenax* alone were associated with periodontal disease and specifically with periodontitis. Of the 82 specimens showing co-detection, 81 (98.8%) were associated with periodontal disease and 1 (1.2%) with a healthy periodontium. Within the periodontal-disease group, *E. gingivalis* alone was detected in 59 (72.8%) gingivitis-associated specimens and 22 (27.2%) periodontitis-associated specimens, whereas *T. tenax* alone was detected exclusively in periodontitis-associated specimens. Co-detection was observed in 31 (38.3%) gingivitis-associated and 50 (61.7%) periodontitis-associated specimens ($P<0.001$). Overall periodontal disease status did not differ significantly according to sex ($P=0.060$), although the distribution of gingivitis and periodontitis differed significantly between males and females ($P=0.025$). Age was significantly associated with periodontal disease status in both males and females ($P<0.001$). The findings support an epidemiological association between oral protozoan detection and periodontal status in this clinic-based sample from Misurata. However, because the analysis was performed at the specimen level and four specimens were obtained from each participant, the results should not be interpreted as participant-level prevalence estimates. Furthermore, the cross-sectional design does not establish a causal relationship between protozoan detection and periodontal disease. Larger participant-level studies using appropriate methods for clustered observations and molecular diagnostic techniques are warranted.
Keywords: Entamoeba Gingivalis, Trichomonas Tenax, Periodontal Disease, Gingivitis, Periodontitis.

Introduction

Periodontal diseases are among the most common chronic diseases affecting the oral cavity and represent a major cause of tooth-supporting tissue destruction and tooth loss worldwide. They include gingivitis and periodontitis, which differ in their clinical characteristics, tissue involvement, and long-term consequences. Periodontitis is a multifactorial inflammatory disease associated with a dysbiotic microbial community and an inappropriate host inflammatory response [1]. The oral cavity contains a complex microbial ecosystem comprising bacteria, fungi, viruses, and protozoa. Among the protozoan organisms reported in the human mouth, *Entamoeba gingivalis* and *Trichomonas tenax* have attracted increasing attention because of their detection in saliva, dental plaque, gingival crevices, and periodontal pockets, particularly among individuals with periodontal disease [3,4].

E. gingivalis is an amoeboid protozoan that has traditionally been identified by microscopic examination of oral specimens. It has been detected in dental plaque, gingival crevices, and periodontal pockets. *T. tenax* is a flagellated protozoan commonly associated with the oral cavity and has also been reported in periodontal sites. Although both organisms have historically been regarded as commensal or opportunistic oral microorganisms, accumulating evidence has raised questions regarding their possible association with periodontal inflammation and tissue destruction [3,4]. Several studies have reported higher detection rates of these protozoa among individuals with gingivitis or periodontitis than among

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individuals with healthy periodontium [5,6]. Molecular studies have also demonstrated differences in detection according to periodontal status, particularly for *E. gingivalis* and *T. tenax* [6,7].

Systematic reviews and meta-analyses have further suggested an association between oral protozoan detection and periodontal disease, although considerable heterogeneity exists among studies with respect to population characteristics, clinical classification, specimen collection, and diagnostic methods [5,7,8]. Microscopic detection remains relevant in settings where molecular diagnostic facilities are limited. However, microscopy may have lower sensitivity and specificity than molecular techniques and may be influenced by specimen quality, organism morphology, and examiner experience [4,6]. Therefore, findings obtained by microscopy should be interpreted within the methodological context of each study.

Previous investigations from Libya have reported the presence of *E. gingivalis* and *T. tenax* among individuals with periodontal disease in Benghazi and Zawia, indicating that oral protozoa are also detectable in Libyan populations [10,11]. However, epidemiological information from Misurata remains limited. The present clinic-based cross-sectional study aimed to describe the occurrence of *E. gingivalis* and *T. tenax* according to periodontal status and to examine the distribution of periodontal categories by age and sex among individuals attending dental clinics in Misurata, Libya.

Methods

Study Design and Setting

A clinic-based cross-sectional laboratory study was conducted between February and May 2022 in dental clinics in Misurata, Libya. The study included individuals attending the clinics for dental examination and treatment.

Study Population

A total of 100 participants aged 18–72 years were included. Eighty participants had periodontal disease, including 53 classified as having gingivitis and 27 as having periodontitis. Twenty participants had a clinically healthy periodontium. The study population included both males and females and represented different age groups. Participants underwent clinical periodontal examination before oral specimens were collected.

Clinical Examination and Periodontal Classification

A clinical periodontal examination was performed to classify participants according to their periodontal status. Participants with a clinically healthy periodontium were assigned to the healthy group. Participants with clinical signs of gingival inflammation without the periodontal-pocket characteristics used in the study protocol were classified as having gingivitis, whereas those meeting the study protocol criteria for periodontal-pocket involvement were classified as having periodontitis. For site classification, a probing depth of less than 5 mm was used for gingivitis-associated sites, whereas a probing depth of 5 mm or greater was used for periodontitis-associated sites. These criteria reflect the operational definitions used in the original study protocol and were not intended as a retrospective application of contemporary periodontitis staging and grading systems.

Specimen Collection

Specimens were collected in the morning before participants performed their usual oral hygiene procedures. Each participant was clinically examined using a dental mirror and a periodontal probe. Four oral specimens were obtained from each participant, resulting in a total of 400 specimens, comprising saliva, gingival crevicular fluid with periodontal-pocket material, and dental plaque.

1. Plaque Collection from Dental and Gingival Surfaces:

Dental and gingival surface plaque samples were collected using sterile cotton swabs under aseptic conditions. The material collected by the swabs was transferred into sterile collection containers containing normal saline and prepared for direct microscopic examination.

2. Collection of Wet Specimens:

- Saliva: Unstimulated saliva samples were collected into sterile clinical collection containers.
- Gingival crevicular fluid and periodontal-pocket material: Samples were collected using a sterile absorbent paper point (size 40), which was gently inserted into the periodontal pocket and left in place for approximately 30 seconds.
- Dental plaque from periodontal sites: Plaque samples were collected using a sterile periodontal curette or scaler from gingivitis-associated sites (<5 mm) and periodontitis-associated sites (≥5 mm).

Microscopic Identification of Protozoa

All specimens were placed in sterile collection containers and prepared in normal saline. A drop of the prepared specimen suspension was transferred onto a sterile glass slide using a sterile pipette/dropper and examined directly by light microscopy at ×10 and ×40 magnifications. This preparation allowed observation of the characteristic motility of the oral protozoa. A second portion of each specimen was spread onto a sterile glass slide, stained with Giemsa stain, and examined at ×100 magnification. *Entamoeba gingivalis* was identified based on characteristic amoeboid morphology, including an irregular cell outline, pseudopodia activity, and cytoplasmic vacuoles or inclusions. *Trichomonas tenax* was identified by the characteristic morphology of its trophozoite stage, including the presence of flagella and recognisable

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motility in fresh preparations when observed. The microscopic findings were recorded according to the presence of *E. gingivalis* alone, *T. tenax* alone, co-detection of both organisms, or absence of detectable protozoa.

Statistical Analysis

Data were entered and analysed using IBM SPSS Statistics version 25. Categorical variables were summarised using frequencies and percentages. Chi-square tests were used to examine associations between categorical variables, including protozoan findings, periodontal status, sex, and age group. Statistical significance was set at $P<0.05$. Because four specimens were obtained from each participant, the analyses were conducted at the specimen level. Consequently, observations from the same participant were not statistically independent. The chi-square analyses should therefore be considered exploratory, and the reported proportions should not be interpreted as participant-level prevalence estimates.

Results

Overall Distribution of Protozoan Findings

A total of 400 oral specimens were examined from 100 participants. *E. gingivalis* was detected alone in 89 specimens (22.3%), whereas *T. tenax* was detected alone in 16 specimens (4.0%). Co-detection of both protozoa occurred in 82 specimens (20.5%). No protozoan was detected in 213 specimens (53.3%). Of the 89 specimens with *E. gingivalis* detected alone, 81 (91.0%) were associated with periodontal disease and 8 (9.0%) with a clinically healthy periodontium. All 16 specimens with *T. tenax* detected alone were associated with periodontal disease, specifically periodontitis. Among the 82 specimens with co-detection of both protozoa, 81 (98.8%) were associated with periodontal disease and one (1.2%) with a clinically healthy periodontium.

Table 1. Overall distribution of microscopic protozoan findings according to periodontal status

Protozoan finding	Periodontal disease, n (%)	Healthy periodontium, n (%)	Total, n (%)
E. gingivalis alone	81 (91.0)	8 (9.0)	89 (22.3)
T. tenax alone	16 (100.0)	0 (0.0)	16 (4.0)
Both protozoa	81 (98.8)	1 (1.2)	82 (20.5)
No protozoan detected	142 (66.7)	71 (33.3)	213 (53.3)
Total	**320 (80.0)**	**80 (20.0)**	**400 (100.0)**

Distribution According to Periodontal Category

Among the 320 disease-associated specimens, 212 (66.3%) were associated with gingivitis and 108 (33.8%) with periodontitis. Among specimens with *E. gingivalis* detected alone, 59 (72.8%) were associated with gingivitis and 22 (27.2%) with periodontitis. In contrast, all 16 specimens showing *T. tenax* alone were associated with periodontitis. Among specimens showing co-detection of both protozoa, 31 (38.3%) were associated with gingivitis and 50 (61.7%) with periodontitis. The distribution of the four mutually exclusive protozoan findings differed significantly according to periodontal category ($P<0.001$).

Table 2. Distribution of microscopic protozoan findings according to periodontal category

Protozoan finding	Gingivitis, n (%)	Periodontitis, n (%)	Total, n
E. gingivalis alone	59 (72.8)	22 (27.2)	81
T. tenax alone	0 (0.0)	16 (100.0)	16
Both protozoa	31 (38.3)	50 (61.7)	81
No protozoan detected	122 (85.9)	20 (14.1)	142
Total	**212 (66.3)**	**108 (33.8)**	**320**

Chi-square test: P<0.001.

Distribution According to Sex

The study included 220 male-associated specimens and 180 female-associated specimens. Among male-associated specimens, 184 (83.6%) were associated with periodontal disease and 36 (16.4%) with a healthy periodontium. Among female-associated specimens, 136 (75.6%) were associated with periodontal disease and 44 (24.4%) with a healthy periodontium. The overall distribution of periodontal disease status according to sex did not reach statistical significance ($P=0.060$). Among disease-associated specimens, 112 male-associated specimens (60.9%) were classified as gingivitis and 72 (39.1%) as periodontitis. Among female-associated specimens, 100 (73.5%) were classified as gingivitis and 36 (26.5%) as periodontitis. This distribution differed significantly between males and females ($P=0.025$).

Table 3. Distribution of periodontal status according to sex

Sex	Gingivitis, n (%)	Periodontitis, n (%)	Total disease-associated specimens
Male	112 (60.9)	72 (39.1)	184
Female	100 (73.5)	36 (26.5)	136
Total	**212 (66.3)**	**108 (33.8)**	**320**

Among disease-associated specimens, chi-square test: P=0.025.

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Distribution According to Age

Age was categorised into three groups: 18–39 years, 40–61 years, and 62–72 years. Among males aged 18–39 years, 88 specimens (71.0%) were associated with periodontal disease and 36 (29.0%) with a healthy periodontium. All male-associated specimens in the 40–61-year and 62–72-year age groups were associated with periodontal disease. Among females aged 18–39 years, 52 specimens (61.9%) were associated with periodontal disease and 32 (38.1%) with a healthy periodontium. In females aged 40–61 years, 76 (86.4%) were associated with periodontal disease and 12 (13.6%) with a healthy periodontium. All female-associated specimens in the 62–72-year group were associated with periodontal disease. The association between age group and periodontal disease status was statistically significant in both males and females (P<0.001). However, among disease-associated specimens, age group was not significantly associated with the clinical periodontal category in either sex.

Table 4. Distribution of periodontal disease status according to age group and sex

Sex	Age group (years)	Disease, n (%)	Healthy, n (%)	Total
Male	18–39	88 (71.0)	36 (29.0)	124
Male	40–61	68 (100.0)	0 (0.0)	68
Male	62–72	28 (100.0)	0 (0.0)	28
Female	18–39	52 (61.9)	32 (38.1)	84
Female	40–61	76 (86.4)	12 (13.6)	88
Female	62–72	8 (100.0)	0 (0.0)	8

Association between age group and periodontal disease status: P<0.001 for both males and females.

Discussion

The present clinic-based study provides local data on the microscopic detection of *E. gingivalis* and *T. tenax* in oral specimens obtained from individuals with a clinically healthy periodontium and from patients with periodontal disease in Misurata, Libya. The principal finding was the distinct distribution pattern of the two protozoa according to periodontal category. *E. gingivalis* was detected alone in specimens associated with both gingivitis and periodontitis, whereas all specimens showing *T. tenax* alone were associated with periodontitis. Co-detection of both organisms was also predominantly observed in periodontal-disease-associated specimens. The predominance of *E. gingivalis* among the protozoan findings is consistent with previous reports describing this organism as a commonly detected oral protozoan. In the present study, 89 specimens showed *E. gingivalis* alone, of which 81 were associated with periodontal disease. Among disease-associated specimens, detection of *E. gingivalis* alone was more frequent in gingivitis than in periodontitis. This pattern is broadly compatible with molecular and microscopic studies demonstrating the presence of *E. gingivalis* across different periodontal conditions [6,9,12].

Differences in detection rates between studies are expected because of variations in diagnostic methodology, specimen collection, clinical definitions, study populations, and laboratory procedures. Molecular methods generally provide greater analytical sensitivity than conventional microscopy and may therefore produce higher detection rates [6,7]. The systematic review and meta-analysis by Martin-Garcia et al. also emphasised substantial methodological heterogeneity among studies investigating oral protozoa in periodontal health and disease [7]. The distribution of *T. tenax* in the present study was particularly notable. All 16 specimens in which *T. tenax* was detected alone were associated with periodontitis, while none were associated with gingivitis. This finding is consistent with evidence suggesting a stronger association between *T. tenax* and more advanced periodontal disease [4,5]. The 2025 systematic review and meta-analysis by Moosazadeh *et al.* reported a higher estimated detection of *T. tenax* in periodontitis than in gingivitis and found increased odds of detection in periodontal disease [8]. However, the present finding should not be interpreted as indicating a 100% prevalence of *T. tenax* in periodontitis because the analysis was performed at the specimen level and only specimens showing *T. tenax* alone were included in this particular comparison.

Co-detection of *E. gingivalis* and *T. tenax* was also observed frequently. Of the 82 specimens with both organisms detected, 81 were associated with periodontal disease, and only one was associated with a clinically healthy periodontium. The high frequency of co-detection may reflect the ecological characteristics of the periodontal environment, where changes in microbial communities and local environmental conditions may favour the presence of multiple organisms. Previous studies have similarly reported the simultaneous detection of oral protozoa in periodontal sites [6,7,8]. Nevertheless, the present cross-sectional design does not allow determination of whether one organism facilitates colonisation by the other or whether both simply reflect a periodontal environment favourable to their persistence. The findings are also broadly consistent with previous Libyan investigations. Younis and Elamami reported the presence of *E. gingivalis* and *T. tenax* among individuals with periodontal disease in Benghazi, while Shawesh *et al.* reported oral detection of these organisms among individuals from Zawia [10,11].

The present study extends the available Libyan evidence by providing data from Misrata and by examining the distribution of microscopic findings across healthy, gingivitis-associated, and periodontitis-associated specimens. The association between sex and periodontal status requires careful interpretation. At the specimen level, the overall distribution of periodontal disease status between males and females did not reach statistical significance (P=0.060). However, among disease-associated specimens, males had a greater proportion of periodontitis-associated specimens than females, and this difference was statistically significant (P=0.025). This result describes the distribution of periodontal categories within

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the study sample and should not be interpreted as evidence that sex independently determines protozoan detection or periodontal disease severity. Age was significantly associated with periodontal disease status in both males and females. The proportion of disease-associated specimens increased with age, particularly among the older age groups. This finding is compatible with the recognised cumulative nature of periodontal tissue damage and the increasing burden of periodontal disease with advancing age [1]. However, because the current study was cross-sectional and the age analysis was performed at the specimen level, the findings should not be interpreted as evidence of a direct causal effect of age on periodontal disease.

The present study has several limitations. First, its cross-sectional design prevents determination of temporal or causal relationships between protozoan detection and periodontal disease. Second, identification was based primarily on microscopic examination, without molecular confirmation. Molecular methods such as PCR may provide greater sensitivity and allow more definitive identification of the organisms [6,7]. Third, the study was conducted in a clinical population from a single geographic area, which may limit generalizability to the wider Libyan population. Fourth, four specimens were collected from each participant, resulting in clustered observations. The use of conventional chi-square analyses does not fully account for this within-participant clustering; therefore, the statistical findings should be regarded as exploratory rather than as definitive participant-level associations.

Despite these limitations, the study contributes useful local epidemiological information regarding oral protozoa in Misrata. The findings also emphasise the importance of distinguishing specimen-level detection from participant-level prevalence when multiple specimens are collected from the same individual. Future studies should include larger participant populations from multiple dental centres in Libya and should prioritise participant-level analyses or statistical approaches that appropriately account for repeated or clustered specimens. Molecular diagnostic methods, particularly PCR-based assays, would strengthen confirmation of microscopic findings and allow more accurate assessment of the epidemiological significance of these organisms. Longitudinal investigations would also be valuable for clarifying the temporal relationship between protozoan detection and periodontal disease. In addition, standardised periodontal diagnostic criteria and specimen-collection procedures should be adopted to improve comparability among studies. Further research should specifically investigate the biological and clinical significance of the co-detection of *E. gingivalis* and *T. tenax*.

Conclusion

This clinic-based cross-sectional study demonstrated the occurrence of *E. gingivalis* and *T. tenax* in oral specimens obtained from healthy individuals and patients with periodontal disease in Misurata, Libya. The distribution of mutually exclusive protozoan findings was significantly associated with periodontal status. *E. gingivalis* was detected alone in specimens associated with both gingivitis and periodontitis, whereas all specimens showing *T. tenax* alone were associated with periodontitis. Co-detection of both protozoa was predominantly observed in periodontal-disease-associated specimens. The findings support an epidemiological association between oral protozoan detection and periodontal status but do not establish a causal role for either organism in periodontal disease. Because the present study used specimen-level analysis with four specimens collected from each participant, the reported proportions should not be interpreted as participant-level prevalence estimates. Further studies involving larger participant populations, participant-level statistical analyses, methods accounting for clustered specimens, standardised periodontal diagnostic criteria, and molecular diagnostic approaches are warranted.

Conflict of interest. Nil

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