Research Article

Prevalence of Entamoeba Gingivalis and Its Potential As A Supportive Biomarker For Periodontal Disease Progression in Maysan, Iraq

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Abstract

Background and Objectives: This study investigated the prevalence of oral infection with the protozoan parasite Entamoeba gingivalis among 200 patients attending dental departments in Maysan Governorate hospitals (Al-Sadr Teaching Hospital, Al-Hussein Neighborhood Dental Center, and local medical clinics) between November 2022 and April 2023.

Methods: A total of 135 positive cases were diagnosed out of the examined individuals. The distribution of infections was analyzed in relation to various periodontal conditions and oral diseases.

Results: The findings revealed a strong association between E. gingivalis infection and several oral pathologies, most notably periodontal tissue destruction (periodontitis). Furthermore, the statistical analysis demonstrated that the parasitic infection is widespread and not restricted to any specific gender, age group, residential location, or educational level.

Conclusion: Entamoeba gingivalis exhibits a high prevalence among patients with oral complications in the region, emphasizing the need for enhanced diagnostic protocols and preventive dental awareness.

Keywords: Entamoeba gingivalis; Prevalence; Periodontal disease; Biomarker; Disease progression; Oral parasites.

1. Introduction

The protozoan parasite Entamoeba gingivalis inhabits the human oral cavity as an anaerobic organism, demonstrating elevated prevalence among individuals with inadequate oral hygiene and active periodontal pathologies (Mahmoud & Rahman, 2004). Parasite transmission occurs via direct oral contact (such as kissing), respiratory droplets, shared contaminated eating utensils or toothbrushes, and contaminated water. Notably, the parasite exhibits physiological resistance to ambient temperature fluctuations, maintaining viability in drinking water from hours to several days (Brook et al., 2007; Talaro & Talaro, 2002; Roberts et al., 2000). The life cycle of E. gingivalis lacks a cyst stage; consequently, propagation relies exclusively on binary fission of the trophozoite stage, wherein nuclear division precedes cytoplasmic separation (Hersh, 1985). The human oral cavity accommodates a complex microflora and presents distinct physiological features that establish a specialized microenvironment (Marsh, 2000; Feki & Molet, 1990). Taxonomically, E. gingivalis belongs to the family Entamoebidae within the phylum Sarcodina (Albert et al., 1988; Gharavi, 2004). The parasite colonizes carious dental cavities, suppurative gingival pockets, and tonsillar crypts, with extensive evidence linking its presence to active periodontitis (Sarowaska et al., 2004). It is classified as an opportunistic organism, existing commensally in healthy oral cavities while displaying marked proliferation under diseased periodontal conditions (Chen et al., 2001; Ghabanchi et al., 2010). Pestechyan (2002) observed E. gingivalis in up to 95% of patients with unhealthy oral status. Its clinical manifestations include severe periodontal support destruction, halitosis, gingival pruritus, palatal ulceration, headache, and physical fatigue (Vrablicova, 1992). Despite high clinical attendance for dental and periodontal ailments in regional healthcare facilities, comprehensive epidemiological investigations focusing on E. gingivalis remain limited. Thus, this study aimed to determine the prevalence of E. gingivalis and evaluate its associated clinical and demographic risk factors in Maysan Governorate .

Materials and Methods

1.1. Laboratory Instruments and Equipment

The current investigation utilized specific laboratory apparatus and glassware sourced from certified manufacturers and international origins, as detailed below:

Equipment:

Refrigerator: Marubeni, Japan.
Light Microscope: Olympus, Japan.
Laboratory Utensils:
Plain Plastic Tubes (10 mL): AFMA, Japan.
Glass Microscopic Slides: Citoclase, China.
Gauze Swabs: Huangzhou, China.
Medical Cotton Swabs: Kardelen, Turkey.

1.2. Chemical Reagents and Stains

Chemical solutions and diagnostic stains employed throughout the experimental procedures, along with their respective manufacturing entities and countries of origin, comprised:
Medical Alcohol (70% concentration): Samara, Iraq.
Giemsa Staining Solution: Himada, Syria.
Ethanol Alcohol: Tiba Baghdad Company, Iraq.

1.3. Experimental Workflow and Design

The procedural pathway of this research was structured sequentially, beginning with clinical sample acquisition, followed by comprehensive laboratory screenings encompassing macroscopic inspection, direct smear analysis, and wet-mount microscopic examinations.

1.4. Clinical Sample Collection

Cohort Demographics: The study population incorporated 200 patients of both genders, representing diverse age brackets and varying clinical health statuses.
Sampling Locations: Specimens were gathered from specialized healthcare facilities across Maysan Governorate, specifically Al-Sadr Teaching Hospital, Al-Hussein Specialized Dental Center, and regional medical outpatient clinics.
Timeline: Field and laboratory procedures were conducted between November 2022 and April 2023.
Data Documentation: Standardized clinical questionnaires were implemented to document patient parameters, including full name, chronological age, gender, residential locality, educational background, and underlying medical conditions.
Clinical Categorization: Participants (stratified into diabetic and non-diabetic cohorts) were further categorized based on their primary oral pathology into groups suffering from bleeding gingivitis, chronic gingivitis, acute gingivitis, and periodontal tissue destruction (periodontitis).

1.5. Laboratory Diagnostic Protocols

1.5.1. Macroscopic Inspection

The clinical assessment involved visual evaluation of supra-gingival plaque accumulation covering tooth surfaces, degree of dental mobility, gingival coloration and textural consistency, alongside the identification of greenish-yellow tartar deposits localized within periodontal supportive structures.

3.5.2. Microscopical Examination Procedures

Direct Smear Technique: Oral samples were collected directly from patients under the supervision of an attending specialist clinician, transferred immediately onto clean glass slides, and examined via light microscopy under both low and high magnifications. The implementation of phase-contrast microscopy was noted as advantageous for distinguishing active parasite motility patterns from cellular debris.
Wet Preparation Method: Saliva-moistened specimens acquired from the oral cavities of participants (encompassing both healthy and diabetic cohorts exhibiting oral manifestations) were placed onto sterile, pristine microscopic slides. Samples were gently mixed without the addition of external diluents such as distilled water or normal physiological saline; this precaution was strictly observed to preserve the fragile cellular morphology of the trophozoites and prevent diagnostic distortion. Slides were secured with cover slips and screened under high dry magnification (\times 400) to detect parasite activity and interactions with white blood cells and oral bacteria, supplemented by Giemsa staining protocols following established diagnostic guidelines (Garcia & Bruckner, 1995).

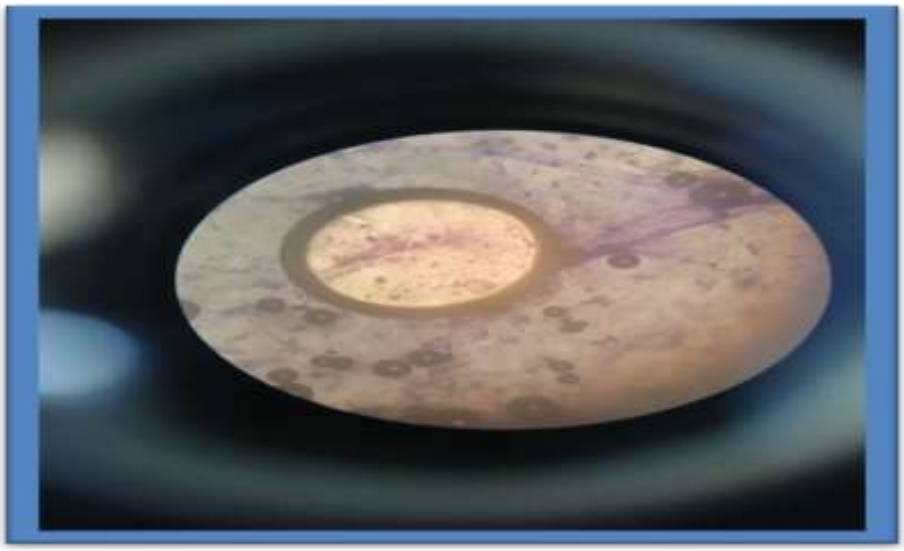
Results

2.1. Overall Prevalence of Entamoeba gingivalis

Out of 200 examined individuals in Maysan Governorate, 135 tested positive for Entamoeba gingivalis, corresponding to an overall prevalence rate of 67.5%. The rate of infection was significantly higher among individuals suffering from oral diseases (91.6%, 110/120) compared to healthy controls (31.2%, 25/80).

Table 4-1. Prevalence of Entamoeba gingivalis infection among individuals with and without oral diseases.

Health Status of Individuals	Number Examined (N)	Positive Cases (n)	Infection Rate (%)
Individuals with Oral Diseases	120	110	91.6%
Individuals without Oral Diseases (Healthy Controls)	80	25	31.2%
Total	200	135	67.5%



Imag 4-1 for microscopic identification of the Entamoeba gingivalis trophozoite stage under 40x and 100x magnifications).*

2.2. Prevalence of Entamoeba gingivalis Infection According to Gender

Gender-wise distribution showed no statistically significant difference in infection rates between males and females. Males recorded a prevalence of 68.5% (72/105), whereas females recorded 66.3% (63/95).

Table 4-2. Distribution of Entamoeba gingivalis infection according to gender in Maysan Governorate.

Gender	Number Examined (N)	Positive Cases (n)	Infection Rate (%)
Male	105	72	68.5%
Female	95	63	66.3%
Total	200	135	67.5%

2.3. Age-wise Distribution of Entamoeba gingivalis Infection

Statistically significant variations were observed among different age groups. The highest rate of infection was recorded in the age group 45–54 years at 90.0% (36/40), followed by 25–34 years at 71.9% (23/32), 35–44 years at 65.8% (25/38), 5–14 years at 63.6% (14/22), and 15–24 years at 62.5% (30/48). The lowest prevalence was detected in the age group 55–64 years, with 35.0% (7/20).

Table 4-3. Prevalence of Entamoeba gingivalis infection categorized by age groups in Maysan Governorate.

Age Group (Years)	Number Examined (N)	Positive Cases (n)	Infection Rate (%)
5–14	22	14	63.6%
15–24	48	30	62.5%
25–34	32	23	71.9%
35–44	38	25	65.8%
45–54	40	36	90.0%
55–64	20	7	35.0%
Total	200	135	67.5%



imag4-2 for clinical representations of affected oral structures across infected age groups).*

2.4. Distribution of Entamoeba gingivalis Infection According to Residence

The evaluation of infection rates based on residential setting demonstrated a higher prevalence in rural areas compared to urban settings. Rural residents exhibited an infection rate of 84.8% (95/112), whereas urban residents recorded a significantly lower rate of 45.5% (40/88).

Table 4-4. Relationship between Entamoeba gingivalis infection and residential setting.

Residence	Number Examined (N)	Positive Cases (n)	Infection Rate (%)
Rural	112	95	84.8%
Urban	88	40	45.5%
Total	200	135	67.5%

Discussion

This chapter addresses the interpretation and discussion of the results obtained in the current study, which focused on evaluating the prevalence of the oral parasite Entamoeba gingivalis and its relationship with various demographic, health, and behavioral variables among patients. Despite the clinical significance of oral parasitic infections and the potential local complications they may cause, a notable scarcity of comprehensive academic research dedicated to investigating this parasite precisely within dental tissues is observed, as most previous attempts have been limited in scope. This academic gap is attributed to the relative simplicity of diagnostic techniques used or the similarity of clinical symptoms of parasitic infection with various other periodontal disorders (Marsh & Bradshaw, 1994).

Summary of Discussion Results: The results showed that the overall prevalence rate of E. gingivalis infection was 67%, with statistically significant differences ($p < 0.05$) related to the patient's gender, place of residence, educational level, history of diabetes mellitus, and associated periodontal diseases.

3.2 General Prevalence and Demographic Factors

3.2.1 Overall Prevalence

The current study recorded an overall prevalence rate of E. gingivalis of 67%. This percentage is relatively high compared to some reference epidemiological surveys, where Dao et al. (1983) recorded a slightly lower infection rate in their study. This elevation is attributed to variations in environmental and health conditions among communities, as well as differences in sampling efficiency and laboratory diagnostic methods.

3.2.2 Gender Distribution

The results showed statistically significant differences in infection rates between genders, with the infection rate increasing among males to reach 68% compared to 66% in females. This finding is consistent with previous literature confirming an increased exposure of males to infection (Mahdi et al., 1996). This pattern can be explained by behavioral patterns, social and occupational habits that make males more socially active and exposed to external environmental conditions, in addition to individual differences in daily personal hygiene and oral care habits.

3.2.3 Geographical Location

The geographical distribution showed a notable variation, with the infection rate reaching 84% in rural areas compared to only 45% in urban areas (cities), representing a statistically precise and significant difference. These results confirm that the environmental residential nature and service levels directly affect prevalence rates. These conclusions agree with what Sonne et al. (1980) pointed out regarding the role of lacking health and social awareness in rural communities in increasing the spread of oral diseases. Furthermore, weak health infrastructure and limited access to preventive dental care services in rural areas enhance opportunities for infection transmission and persistence.

3.2.4 Educational Level

Statistical analysis revealed significant differences ($p < 0.05$) among different cultural and educational levels, with infections being more widely distributed among individuals with lower educational and cultural backgrounds. These results are entirely consistent with the findings of Winn (2001). This illustrates how educational level reflects sound health awareness, the practice of preventive behaviors, and the early recognition of the importance of treating gum and dental problems.

3.3 Economic, Environmental, and Behavioral Factors

Oral diseases are not limited to cultural awareness alone but interact with multiple factors:

Economic and Living Standard: This is considered the primary driver for high infection rates, as it determines the family's financial ability to acquire oral care supplies (such as medical toothpastes and mouthwashes) and bear the costs of regular dental treatments (Colle et al., 1998).

Behavioral Habits and Individual Environment: These include daily practices followed inside and outside the home, as well as combating surrounding environmental pollution.

Health Awareness Programs: A lack of preventive health guidance campaigns targeting dental health leads to weakened natural immunity and reduced resistance of oral tissues to pathogens.

3.4 Oral Health Status, Age, and Associated Conditions

3.4.1 Periodontal Status and Age

The results showed that the highest infection rate was concentrated among individuals within the age group (45–54 years) at 90%. This sharp increase is attributed to the cumulative effect of behavioral neglect and deteriorating periodontal tissue health with advancing age. This corresponds with the findings of Garcia (1995) and Albandar (2002), which proved that tissue damage in supporting structures increases directly with advanced age groups due to chronic tissue hypersensitivity.

As for the infections recorded among adolescents, they are attributed to an active lifestyle and poor personal oral hygiene habits, in addition to unhealthy habits such as the direct sharing of foods and sweets in school environments, which facilitates the transmission of the parasite via saliva (Lyons et al., 1983; Lindhe et al., 2002).

3.4.2 Diabetes Mellitus Correlation

The results of the current study confirmed a close correlation between diabetes mellitus and a high rate of E. gingivalis infection, where diabetic patients recorded infection rates three times higher than those recorded in non-diabetic individuals. These results agree with documented scientific literature (Lalla, 2006).

This proposition is further supported by Emmrich's (1991) studies indicating that the risk of periodontal disease doubles by about fivefold in diabetic adolescents compared to their healthy peers. Similarly, Grossi (1997) proved in a study of periodontal microorganisms involving 500 patients that the clinical risk of developing these disorders in diabetic patients increases by 3.2 times compared to healthy individuals, due to reduced immune response and altered biochemical environments within the oral cavity.

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