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Original Article

Assessment of Entamoeba Gingivalis and Trichomonas Tenax in Patients with Chronic Diseases and its Correlation with Some Risk Factors

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Abstract

Trichomonas tenax (*T. tenax*) and *Entamoeba gingivalis* (*E. gingivalis*) are two oral protozoan parasites that are universal and found in patients with poor oral hygiene, as well as chronic and periodontal diseases. This study was conducted to assess the efficacy of some parameters, such as age, gender, education, residency, smoking, and dental cleaning routines, on the incidence of these parasites in the oral cavity of the patients participating in this study. A total of 230 individuals with chronic diseases, 97 of whom were patients (44 females and 53 males) participated in the present study. Dental plaque and saliva samples were collected from each patient and examined under light microscopy with Giemsa staining. Out of 230 dental plaque samples, 60 (26.08%) samples were positive for *E. gingivalis*, while 37 (16.08%) cases were positive for *T. tenax*. Diabetic patients showed high *E. gingivalis* percentage (n=12; 20%) (*P*=0.000*). However, thyroid disorders showed a low percentage. Hypertensive patients showed a high percentage for *T. tenax* (n=6; 16.21%) (*P*=0.000*), while tuberculosis patients showed the lowest percentage. Patients with hypertension, smoking, heart disease, and diabetes showed statistical significance for the presence of these parasites. Equally, patients older than 30 years have shown a higher rate of infection. According to the findings, *E. gingivalis* was detected in 58.33% and 41.66% of the urban and rural populations, respectively. Furthermore, *T. tenax* was detected in 81.08% and 18.91% of the urban and rural populations, respectively. Parasitic contagions were comparatively popular in patients with chronic and periodontal illness.

Keywords: Chronic disease, *Entamoeba gingivalis*, Oral cavity, Periodontal disease, *Trichomonas tenax*

1. Introduction

Numerous microorganisms live in the human oral cavity. Interaction between pathogenic parasites and the defense mechanism of the host, as well as other irritating factors, of which the dental bacterial biofilm is of particular interest, have a crucial role in vulnerability to, as well as start and development of periodontal disease (1). When gingiva that supports the ligaments and bone is chronically inflamed, the condition is referred to as periodontal disease. The healthy gingiva is characterized as the absence or minimal amounts of periodontal inflammation, with appropriate support and no loss of attachment or bone. Symptoms, such as bleeding while probing a periodontium and/or apparent inflammation should be adequately identified to detect plaque-induced gingivitis. With careful management, such a situation can be reversed to a healthy state (2). Periodontitis causes the periodontal ligament, cementum, and alveolar bone to deteriorate. Periodontitis is treatable in terms of inflammation and bacteria; however, the tissues do not repair back to their original volume,

shape, and organization, necessitating ongoing good dental hygiene (3). *E. gingivalis* is an anaerobic amoebic protozoan greatly found in the mouth cavity of humans. The size of trophozoite (no cyst) ranges from 10 to 35 μm. Therefore, it is transmitted either by direct connection through kissing mainly, or indirectly through polluted food, toothpicks, chewing gum, or other cooking utensils. This opportunistic parasite resides in the gums, teeth surroundings, cavities between the teeth, gingival pockets, dental tartar, gum gingival fringes, and necrotic mucosa around the teeth.

T. tenax is a type of anaerobic commensal that originates in the human mouth. A number of researches were conducted concerning the frequency of *T. tenax* in patients with chronic gum disease. *T. tenax* transmission can occur through contaminated food or water, saliva or its spray, and kissing. It is widely spread in the mouth ranges from 4% to 53% (4). The importance of oral infections has lately increased because of the belief that *T. tenax* may enter the respiratory tract by aspiration from the oropharynx and causes bronchopulmonary trichomoniasis.

2. Materials and Methods

2.1. Samples

A total of 230 samples from patients were examined from October 2017 to April 2018. These include 100 females and 130 males who attended the College of Dentistry Clinic, University of Babylon, Hillah, Iraq. Oral swabs were collected from tooth surfaces. After that, they were examined using the wet mount technique utilizing physiological saline and eosin stain under the microscope to identify the parasite. The patients also completed a questionnaire form (age, gender, smoking habits, diabetes, atherosclerosis, asthma, and other diseases). During their first visit to the clinic, all patients had saliva and dental plaque samples taken. To obtain dental plaque samples, a sterile swab was used to scrape the area around the surface of the teeth from cavities and around the gingival fissures.

2.2. Microscopic Examination

Saliva samples were diluted with normal saline in a sterile cup at room temperature (25-28°C). The swabs were immersed in normal saline in sterile flasks after rolling the swab on a slide. Giemsa stain was used on samples directly after diluting them. The samples were examined using a lighting microscope (40×- 400×). Identification of *E. gingivalis* was dependent on their shape, the growth of the formation of the pseudopodia, and the vacuole's presence. Furthermore, the identification of the *T. tenax* was dependent on its flagella and its typical movement.

2.3. Statistical Analyses

Statistical analysis was performed using SPSS software (version 20). Moreover, nonparametric Fisher's exact test and Chi-square test were used to determine the correlation between the parameters (age, gender, smoking habits, diabetes, atherosclerosis, asthma, and other diseases) and the presence of parasites. A *P*-value less than 0.05 was considered statistically significant.

3. Results and Discussion

Chronic diseases, such as diabetes, asthma, and blood pressure lead to what is known as Global Burden Disease. In 2005, the World Health Organization reported that these chronic diseases could lead to 35 million deaths. *T. tenax* and *E. gingivalis* lead to many health problems. Whenever these parasites are found, it is a sign of unhealthy teeth with bad oral care. Infectious parasites can be categorized into parasites inducing local infections, and parasites causing systemic infections leading to incidental effects. However, Saprophytes, such as *E. gingivalis*, have the capability of turning into opportunistic pathogens, or free-living amoebas that become invasive in certain cases (1). *E. gingivalis* is a protozoan that lives in anaerobic environments largely found in the humans oral cavity. In addition to its participation in poor mouth odor, it is an index of oral health. In general, it is considered as an oral commensal, a pathogenic

potential associated with gingivitis in children. Eventually, it leads to periodontal diseases with age, particularly in minority assemblages, as immunocompromised patients (5, 6).

Table 1 tabulates the microscopic examination of fresh samples in the total of 230 dental plaque samples, 60 (26.08%) of which were positive for *E. gingivalis*, while 37 (16.08%) of which were positive for *T. tenax*. The results showed that the prevalence of *E. gingivalis* was greater than that of the *T. tenax*. A higher percentage of infection with *E. gingivalis* appears in patients with diabetes (n=12; 20%), while the lowest percentage of infection with *E. gingivalis* was in patients with thyroid disorders. A higher percentage of infection also appears in patients with hypertension (n=6; 16.21%), while the lowest percentage of infection with *T. tenax* was found in patients with tuberculosis.

Diabetes, hypertension, smoking, and heart disease are among other reasons that increase the possibility of periodontitis and gingivitis diseases. This study confirmed the presence of a statistically significant relationship between these issues and the prevalence of these parasites. The compromised immune system of patients is one possible reason for such a result. The pathogenicity of *E. gingivalis* has not been precisely determined. In people without immunological disorders, this amoeba does not usually produce pathological symptoms; however, the pathogenicity of this parasite is demonstrated amongst immunocompromised HIV-positive patients, as well as people with bad oral hygiene (7). Diabetic patients are more vulnerable to infectious diseases, decreased arterial perfusion, and neuropathy; moreover, suppressed immune response intensifies the frequency and severity of infectious diseases. Sucrose is the most cryogenic sugar as it causes a predisposition to buckle colonization by oral microorganisms, by increasing the viscosity of the plaque and allowing its greater attachment to the teeth (8). This study showed that males had a high incidence rate (53.33%, 56.75%) than females (46.66%, 43.24%) as shown in table 2.

Table1. Prevalence of *E. gingivalis* and *T. tenax* according to the category of chronic diseases

Disease	Tested (%)	*E. gingivalis* (%)	*T. tenax* (%)	*P*-value
Atherosclerosis	9 (3.91)	1 (1.66%)	0	-
Asthma	15 (6.52)	4 (6.66)	2 (5.40)	0.067
Diabetes	31 (13.47)	12 (20)	7 (18.91)	0.000*
Heart diseases	17 (7.39)	6 (10)	3 (8.10)	0.012*
Cancer	10 (4.34)	4 (6.66)	2 (5.40)	0.067
Chronic kidney disease	8 (3.47)	2 (3.33)	2 (5.40)	-
AIDS	4 (1.73)	1 (1.66)	1 (2.70)	-
Hypertension	30 (13.04)	10 (16.66)	6 (16.21)	0.000*
Tuberculosis	6 (2.60)	2 (3.33)	0	-
Psoriasis	11 (4.78)	1 (1.66)	2 (5.40)	0.333
Rheumatic arthritis	23 (10)	4 (6.66)	3 (8.10)	0.029*
Hepatitis	11(4.78)	1 (1.66)	2 (5.40)	0.333
Thalassemia	10 (4.34)	4 (6.66)	2 (5.40)	0.067
Thyroid disorders	12 (5.21)	0	1 (2.70)	-
Ulcerative colitis	16 (6.95)	4 (6.66)	2 (5.40)	0.067
Relapsing costochondritis	17 (7.39)	3 (5)	2 (5.40)	0.100
Total	230	60 (26.08)	37 (16.08)	
P-value			0.000*	

*Significant difference

Table 2. Prevalence of *T. tenax* and *E. gingivalis* according to gender and some oral symptoms disease

Disease state	NO%	*E. gingivalis*		*P*-value	*T. tenax*		
		Males%	Females%		Males%	Females%*-	*P*. value
Oral abscess	48 (20.86)	5 (15.62)	6 ((21.42)	0.002*	4 (19.04)	3 (18.75)	0.029*
Gingivitis	66 (28.69)	10 (31.25)	12 (42.85)	0.000*	8 (38.09)	6 (37.5)	0.000*
Periodontitis	44 (19.13)	6 (18.75)	4 (14.28)	0.005*	4 (19.04)	2 (12.5)	0.067
Dental caries	60 (26.08)	10 (31.25)	6 (21.42)	0.000*	4 (19.04)	3 (18.75)	0.029*
No symptoms	10 (4.34)	1 (3.21)	0	-	1 (4.76)	2 (12.5)	0.333
Total	230	32 (53.33)	28 (46.66)	0.000*	21 (56.75)	16 (43.24)	0.000*

*Significant difference (using Fisher's exact test at 0.05 level)

Regarding age (Figure 1), it causes an increase in oral protozoan as reported by several researchers (8, 9).

In this study, the age group (61-70 years) showed a greater prevalence of *E. gingivalis*. This finding agrees with the fact that *E. gingivalis* plays an influential role in mouth diseases. *T. tenax* was detected at a higher rate in the age group of 31-45 years. Furthermore, both parasites were detected at higher rates in the age groups of 30 years and more. This result agrees with the findings of other studies (10, 11). There is a statistically significant correlation between age and the existence of oral commensals. Some natural factors, in addition to the complexity of the oral environment and rapid growth of germs, contribute to gum diseases and parasitic incidence. Therefore, the inclusion of other disciplines (e.g., microbiology, chemistry, and dietetics) is required to achieve more accurate results. Gingivitis was detected in 58.33% of the urban population, according to the findings of this study, which investigated the prevalence and variables related to periodontitis in adults, compared to 41.66% in the rural population. On the other hand, *T. tenax* was estimated at 81.08% in the urban population, compared to 18.91% in the rural population as shown in figure 2.

Moreover, the results of the present study show the percentage of infection with gingivitis (75%) in patients with lower educational levels, white it was 67.56% in patients with *T. tenax* who had lower educational levels as shown in figure 3.

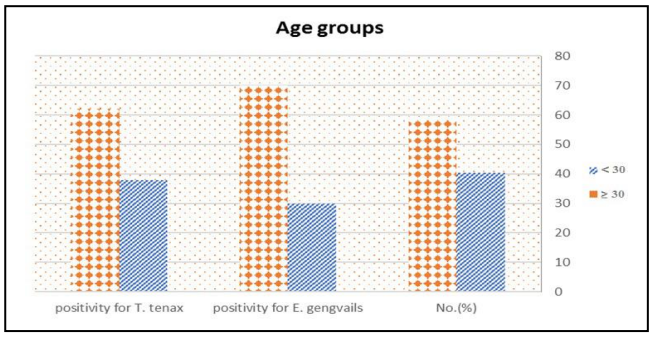

Figure 1. Prevalence of parasitic infection of *E. gingivalis* and *T. tenax* based on age groups

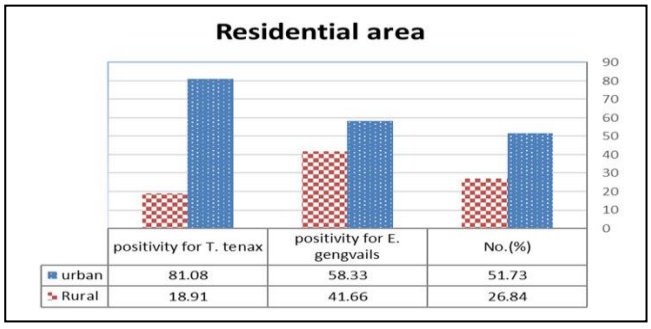

Figure 2. Prevalence of parasitic infection of *E. gingivalis* and *T. tenax* according to the residential place

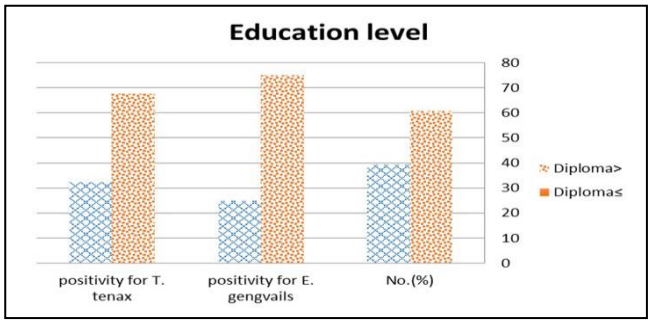

Figure 3. Prevalence of parasitic infection of *E. gingivalis* and *T. tenax* based on educational level

Oral health affects other body parts, and recently, it was suggested that some heart conditions are related to oral hygiene. In addition to the toothbrush, dental floss and oral mouthwash are important. Studies suggest that infection reaches 61% in those who clean their teeth once a day and 58.76% in those who spend less than one minute doing so. While infection percentages reached 67% and 71% with *E. gingivalis* versus *T. tenax*, respectively, in those who do not use any mouthwash solution or dental care tools. Oral mouthwash helps get rid of mouth infections. The cleaner the mouth, the less infected with parasites it is. Oral and dental cleaning is considered an important factor in limiting oral parasitic infections. Dental health awareness can be taught in many different and simple ways, such as using flyers as shown in table 3.

Table 3. Prevalence of parasitic infection of *E. gingivalis* and *T. tenax* based on oral hygiene habit

Variables	No. (%)	Positivity for parasites
Time of brushing		
Once a day	138 (60)	60 (61.85)
Twice or more	92 (40)	37 (38.14)
Duration of brushing		
One minute	142 (61.73)	57 (58.76)
More than one minute	88 (38.26)	40 (41.23)
Use flossing cleaning tools		
Yes	136 (59.13)	32 (32.98)
No	94 (40.86)	65 (67.01)
Use mouthwash (Rinsing)		
Yes	70 (30.43)	28 (28.86)
No	160 (69.56)	69 (71.13)

On the other hand, this study shows that the prevalence of parasites among smokers is greater than that in non-smokers, which is in line with the results of (12, 13). Smoking tobacco is the main factor associated with several acute periodontitis (Figure 4).

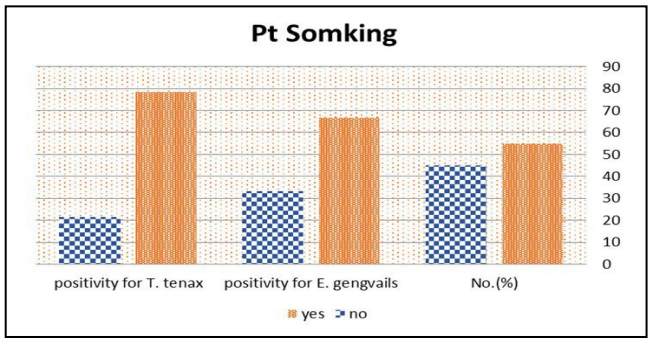

Figure 4. Prevalence of parasitic infection of *E. gingivalis* and *T. tenax* based on smoking habit

Epidemiological studies (14) commented on the relationship between periodontal disease and tobacco use. They consistently reported that non-smokers were five times less probably to develop severe periodontitis than smokers. One of the serious environmental factors in the inception and progression of devastating periodontal illnesses is tobacco smoking. It can influence periodontal pathogenesis and the result of its therapy (15, 16). The mechanism through which tobacco exercises its effect on the health of oral in humans has not been yet completely recognized; however, proofs gained from various risk evaluation studies propose that smoking is a causal factor in periodontitis initiation and progression (17-19).

This study indicates a statistically significant relationship between chronic diseases and periodontal diseases. The prevalence of *T. tenax* and *E. gingivalis* was also correlated with some variables. Oral parasites generally appear when the patient neglects or does not notice oral diseases. The presence of oral parasites is associated with periodontal diseases, gingivitis, diabetes, high blood pressure, and heart diseases. To

get a better healthy oral cavity, some bad habits, such as smoking must be avoided as it helps in spreading oral parasites. Oral parasites cause mild side effects, except in rare cases when the infections reach other vital organs, such as the lungs, causing cases, including bronchopulmonary trichomoniasis.

It is noticed that gum bleeding while brushing teeth or recurrent gum bleeding without touching were over indications of oral parasite presence. Additionally, it is preferred to use mouthwashes and floss, and implement health education in health centers and hospitals through posters, flyers, and training courses for medical personnel. This study focuses on the importance of developing healthy oral public strategies that encourage individuals to acquire healthy oral lifestyles.

Authors' Contribution

Study concept and design: S. F. A. M.
Acquisition of data: S. F. A. M.
Analysis and interpretation of data: B. A. A. A. A. J.
Drafting of the manuscript: B. A. A. A. A. J.
Critical revision of the manuscript for important intellectual content: S. F. A. M., B. A. A. A. A. J. and H. K. A.
Statistical analysis: S. F. A. M.
Administrative, technical, and material support: S. F. A. M., B. A. A. A. A. J. and H. K. A.

Ethics

All the procedures were approved by the ethics committee of the Babylon University, College of Medicine, Hillah, Iraq under the project number 1547-78-5424.

Conflict of Interest

The authors declare that they have no conflict of interest.

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