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Incidence of *Entamoeba Gingivalis* and *Trichomonas Tenax* in Periodontitis and Gingivitis Patients Who Attended to Private Clincs in Babylon Province

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Abstract

Trichomonas tenax, a commensal flagellated protozoan, inhabits in human oral cavity. This parasite is cosmopolitan and frequently found in patients with poor oral hygiene and advanced periodontal disease. By using wet mount smear and giemsa staining to detect the prevalence of oral protozoa in patients with oral diseases and a healthy control group. From October 2014 to April 2015, the subgingival dental plaques of 310 patients with gingivitis or periodontitis and 310 controls who attended to clinics periodontics , in Babylon province. 64 (20.6 %) of patients were positive (40.2 % periodontitis ,14.2 % gingivitis) by using wet preparation and Giemsa staining. The prevalence of oral *Trichomonas tenax* in our study (20.6%) and *Entamoeba gingivalis* was (42.9 %) was compatible with many other published reports which mostly has ranged from 12%-32%.The study revealed dependence between the frequency of occurrence of protozoa and the state of periodontitis.the age group (41-50)yr. Have high incidence of *T.tenax* compared with an other groups,as well as the males have high incidence(24.7 %) than females (16.8 %).

Key words: *Entamoeba gingivalis, Trichomonas tenax, gingivitis patients.*

Introduction

T. tenax is an anaerobic commensal of the human oral cavity. There are studies that relate to Its prevalence in patients with Marginal Chronic Periodontitis [4,6]. Transmission is through saliva , droplet spray, and kissing or use of contaminated dishes and drinking water [7, 14]. world widely, its prevalence in the mouth ranges from 4 to 53% [17,19,22].Since the organism is believed to enter the respiratory tract by aspiration from the oropharynx and then cause bronchopulmonary trichomoniasis, the importance of oral infections has been increased recently [1,12, 13]. Surprisingly in Iraq there is study of [11] which showes a prevalence of periodontis 8.4 % with *Entameba gingivalis* and *T. tenax* by direct smear. The number of trichomonads found in oral washing is rather low, and detection by conventional methods such as wet-mount preparations or staining may be sensitive. In addition, staining is useful for species identification, and culture techniques are routine use [2,5,15]. This study was carried out to determine the prevalence of oral trichomoniasis by by direct smear methods and giemsa staining with microscopic observation to detect of *T.tenax* and *E. gingivalis* [8].

Materials and Method

The *Study population* included 620 individuals; 310 patients (160 females and 150 males) aged ranged 18-60 years old with periodontitis or gingivitis who attended to periodontitis -clinics of periodontics- and 310 healthy controls, who were matched with case group. The kind of oral disease previously was established by periodontist. Direct observation For each pattient a sample of subgingival dental plaque from deep pockets obtained and preservedin an individual container of 2 ml Ringer's solution.The containers of fixed plaques duly labeled and examined to the department of Microbiology for identification of oral parasites. Microscopic observations were made three times under dry magnification (400x) and then each sample stained with Giemsa. The identification of *T. tenax* was established as a pear-shaped flagelatted trophozoite, about 5-13µ long and with circular movement. Another oral protozoan, *Entamoeba gingivalis*, if present, was differentiated by its size (10-20µ), presence of prominent pseudopodia, and sluggish

movement..The statistical analysis was performed by the Chi-square test (signification level 0.05) so as to study the correlation between the kind of oral disease, age and sex with the presence of parasite.

Results

Among the samples 33 (14.2% gingivitis) and 31 (40.2 % periodontitis) of those specimens were detected by wet preparation and Giemsa-stained smears. All the cases of oral trichomoniasis in control group were both detected by direct smear. The infection rate among the patient with periodontitis and gingivitis was 40.2 % and 14.2 %, respectively (Table 1). There was a significant difference between two last groups [$P< 0.005$].Oral trichomoniasis was prevalent at age ranged 31-40 yr, and in total males (24.7 %) than females (16.8 %) with no significant difference (Table 2, 3). *Entamoeba gingivalis*, the other oral protozoan, was found in 133 (42.9 %) distributed as 98(42.1%) in gingivitis patients and 35 (45.45 %) in periodontitis patients(Table 2).

Table(1): Prevalence of *Trichomonas tenax* according to type of oral diseases

Oral diseases	Examined No.	Infected No.	%
Gingivitis	233	33	14.2
Periodontitis	77	31	40.2
Total	310	64	20.6
X2 calculated=45.2* X2 tabled=6.63			

***Significant differences**

Table(2): Prevalence of *Entamoeba gingivalis* according to type of oral diseases

Oral diseases	Examined No.	Infected No.	%
Gingivitis	233	98	42.1
Periodontitis	77	35	45.45
Total	310	133	42.9
X2 calculated=71.1* X2 tabled=6.63			

*Significant differences

Table (3): Prevalence of oral protozoa with patients periodontal disease(experimental group) according to age.

Age (years)	Examined No.	Positive case	%
20-30	89	13.6	15.3
31-40	132	25.2	19.1
41-50	56	19.4	34.6
50>	33	5.8	17.6
Total	310	64	20.6
X2 calculated=30.4* X2 tabled=11.28			

*Significant differences

Table (4): Prevalence of oral protozoa with patients periodontal disease (experimental group) according to sex.

Sex	Examined No.	Positive case	%
Male	150	37	24.7
Female	160	27	16.8
Total	310	64	20.6
X2 calculated=14.6* X2 tabled=6.63			

*Significant differences

Table (5):Detection of oral protozoa in 310 patients with periodontal diseases and control

Groups	Examined No.	Positive case	Negetive case	%
periodontal diseases	310	64	246	20.6
control	310	6	304	1.94
Total	620	70	550	11.3
X2 calculated=19.2* X2 tabled=6.63				

***Significant differences**

Discussion

The prevalence of oral trichomoniasis in our study (20.6%) was compatible with many other published reports which mostly have ranged from 12%-32% [4,6,17,19,20,22] . [21] examined 700 patients with periodontitis and found a prevalence of 26.5% [22]. [4] in France reported a prevalence of 28% among the 300 patients [4] .Mahdi in Iraq examined the saliva of 143 patients with poor oral hygiene and reported a prevalence of 8.4% [11] ,but further investigation showed that saliva was not a suitable media for detection of parasite [8]. In Iran 50 patients with periodontitis were examined by wet mount and 46% were found to be infected by *T. tenax* or *E. Gingivalis* [16] but the prevalence of each parasite was not determined. In the most above- mentioned researchs, the methods for detection and identification of *T.tenax* from human oral samples have been based on conventional techniques, such as microscopic observation [22] and cultivation [21], which are poorly reliable in spite of being skill-requiring and time-consuming. Recently small ribosomal RNA (SrRNA) sequences or the corresponding genes have been utilized as targets for PCR [3] . Similar to our study, Kikuta in Japan [18] developed a PCR protocol for specific detection of *T. tenax* by using a pair of primers (PT3 and PT7 with nucleotide positions of 407 to 425 and 1164 to 1182, respectively). In his study 55.6% of patients were shown to carry *T. tenax* in subgingival- plaque but no parasites were observed by microscopic examination. Likewise, in present study, we were not able to detect *T. tenax,* using wet mount, in 9 cases that were positive by PCR. To find *T. tenax* in bronchoalveolar fluid, Mallat in France amplified the 5.8S rRNA gene.He suggested that the sequences of this gene presented the advantages of being present in multiple copies in the genome, even between very closely related species [13] .The occurrence of *T. tenax* was not correlated with the age in our study and this finding was not agree with some authors [4, 19,22] who found that the frequency of infection increased with age, while some were believed that oral protozoa were rarely found in children [17]. According to our experience, Ringer solution was better than normal saline for transportation and maintenance of samples. But [10] recommended Safranin mixed with patient's saliva as fixative and emphasized that mishandling the plaque, use of different staining techniques, plaque other than

from the extreme base of the pocket, recent medication or hygiene and some types of food, did result in false negatives [9]. As in other reports [10, 16,17,22] our results demonstrated a link between the presence of *T tenax* and periodontitis in comparison with gingivitis and it seems that in each case, oral parasites were only found in diseased sites.

It is perhaps appropriate to note here that *T. tenax*, whilst seen less frequently than *E. Gingivalis* in patients with poor oral condition, but due to its role to produce pulmonary trichomoniasis, deserves much closer attention. Conclusively, with development of PCR for detection of *T. tenax,* we suggest an investigation to evaluate the pulmonary trichomoniasis in patients with cancer and chronic lung diseases.

Conclusion

The prevalence of oral *Trichomonas tenax* in our study (20.6%) and *Entamoeba gingivalis* was (42.9 %) was compatible with many other published reports which mostly has ranged from 12%-32%.The study revealed dependence between the frequency of occurrence of protozoa and the state of periodontitis.the age group (41-50)yr. Have high incidence of *T.tenax* compared with an other groups,as well as the males have high incidence(24.7 %) than females (16.8 %).

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the University of Babylon, Iraq and all experiments were carried out in accordance with approved guidelines.

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