

## Prevalence of Oral Protozoa in Periodontitis and Gingivitis Patients Whose Attended to Clinics Periodontics , Dentistry College\Babylon Univ.

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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 2009 to April 2010, the subgingival dental plaques of 310 patients with gingivitis or periodontitis and 310 controls who attended to clinics periodontics , dentistry college- Babylon university. 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 %).

### 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 (Feki & Molet,1990; Hayawan & Bayoumy,1992). Transmission is through saliva , droplet spray, and kissing or use of contaminated dishes and drinking water (Memlik,1968; Hersh,1985). world widely, its prevalence in the mouth ranges from 4 to 53% (Wantland&Lauer,1970;Varblic *et al.*,1991; Sarowska *et al.*,2004).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 (Mahmoud & Rahman,2004; Mallat *et al.*,2004 ; Chiche *et al.*,2005). Surprisingly in Iraq there is study of Mahdi & Al-Saeed (1993) which shows 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 (Osborn *et al.*,1984 ; Felleisen, 1997; Crucitti *et al.*,2004). 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* (Kikuta *et al.*,1997).

### Materials and Methods

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 Dentistry college-clinics of periodontics-Babylon University and 310 healthy controls, who were matched with case group. The kind of oral disease previously was established by periodontist. Direct observation For each patient a sample of subgingival dental plaque from deep pockets obtained and preserved in 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 flagellated trophozoite, about 5-13 $\mu$  long and with circular movement. Another oral protozoan, *Entamoeba gingivalis*, if present, was differentiated by its size (10-20 $\mu$ ), presence of prominent pseudopodia, and sluggish movement. The statistical analysis was performed by the Anova 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
F calculated=45.2*			
F tabled=6.63			

\*Significant differences

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

Oral diseases	Examined No.	Infected No.	%

<b>Gingivitis</b>	<b>233</b>	<b>98</b>	<b>42.1</b>
<b>Periodontitis</b>	<b>77</b>	<b>35</b>	<b>45.45</b>
<b>Total</b>	<b>310</b>	<b>133</b>	<b>42.9</b>
<b>F calculated=71.1*</b> <b>F tabled=6.63</b>			

\*Significant differences

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

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

\*Significant differences

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

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

\*Significant differences

**Table(٥):Detection of oral protozoa in 310 patients with periodontal diseases and control**

Groups	Examined No.	Positive case	Negative case	%
periodontal diseases	310	64	246	20.6
control	310	6	304	1.94
<b>Total</b>	<b>620</b>	<b>70</b>	<b>550</b>	<b>11.3</b>
<b>F calculated=19.2*</b> <b>F tabled=6.63</b>				

\*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% (Wantland & Lauer,1970; Feki & Molet, 1990 ; Vrablic et al.,1991; Hayawan & Bayoumy,1992;Vrablic *et al.*,1992; Sarowska *et al.*, 2004). Wantland *et al.*,1963 examined 700 patients with periodontitis and found a prevalence of 26.5% (Wantland & Lauer,1970). Feki & Molet (1990) in France reported a prevalence of 28% among the 300 patients (Feki & Molet,1990). Mahdi in Iraq examined the saliva of 143 patients with poor oral hygiene and reported a prevalence of 8.4% (Mahdi & Al-Saeed 1993)), but further investigation showed that saliva was not a suitable media for detection of parasite (Kikuta *et al.*,1997). In Iran 50 patients with periodontitis were examined by wet mount and 46% were found to be infected by *T. tenax* or *E. Gingivalis* (Pestechyan, 2002) 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 (Wantland&Lauer,1970) and cultivation (Wantland *et al.*,1963), 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 (Dix *et al.*, 1990). Similar to our study, Kikuta in Japan (Sambrook&Russel,2001) 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 (Mallat *et al.*,2004).The occurrence of *T. tenax* was not correlated with the age in our study and this finding was not agree with some authors (Wantland & Lauer,1970; Feki & Molet,1990; Vrablic *et al.*,1991) who found that the frequency of infection increased with age, while some were believed that oral protozoa were rarely found in children (Sarowska *et al.*,2004). According to our experience, Ringer solution was better than normal saline for transportation and maintenance of samples. But Lyone&Palmer(1983) 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 (Kox *et al.*,1995). As in other reports (Wantland & Lauer,1970; Lyone & Palmer1983; Pestechyan,2002; Sarowska *et al.*,2004) 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.

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انتشار الاوالي الحيوانية للمرضى المراجعين لعيادات طب الأسنان / جامعة بابل والمصابين بأمراض اللثة والفم

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الخلاصة

تعد الاوالي المسبوطة *Trichomonas tenax* و *Entamoeba gingivalis* من الطفيليات المتعايشة والموجودة في التجويف الفمي للإنسان وهي عالمية الانتشار ومتواجدة نسبيا في المجتمعات التي تعاني من سوء النظافة العامة والشخصية للفم ومرضى الفم واللثة ، باستخدام المسحة المباشرة وصبغة كمرزا لمعرفة انتشار هذه الاوالي ل ٣١٠ مريضا بأمراض الفم و ٣١٠ أصحاء كمجموعة سيطرة (مراجعين غير مصابين بأمراض

الفم) للمدة من شهر تشرين الأول ٢٠٠٩ ولغاية نيسان ٢٠١٠. كانت نسبة الإصابة ٦٤ (٢٠.٤%) إصابة موجبة توزعت بواقع (٤٠.٢%) إصابات محيطية بالأسنان periodontitis و١٤.٢% إصابات محيطية باللثة (gingivitis) باستخدام المسحة المباشرة وكان انتشار *T. tenax* هو ٢٠.٦% و *E.gingivalis* هو ٤٢.٩% عند مقارنتها مع بقية الدراسات المنشورة التي تراوحت نسب انتشارها من ١٢-٣٢% وأظهرت الدراسة علاقة هذه الطفيليات مع أمراض الفم، وكانت المجموعة العمرية (٤١-٥٠) سنة الأكثر انتشارا بهذه الطفيليات مقارنة مع الفئات الأخرى كذلك كانت نسبة الإصابة بالذكور أعلى من الإناث.