Evaluation of Entamoeba gingivalis and Trichomonas tenax in patients with periodontitis and gingivitis and its correlation with some risk factors

Sumaiah Ibrahim, B.Sc, M.Sc. (1)
Rasha Abbas, B.Sc., M.Sc. (2)

ABSTRACT

Background: It was shown that two protozoans, Entamoeba gingivalis and Trichomonas tenax may be responsible for oral parasitic infection. This study was designed to detect these parasites in oral cavity of patients with periodontitis and gingivitis with some risk factors and compare it with healthy oral persons.

Material and Method: A total of 60 patients with periodontitis and gingivitis and 25 healthy persons (control group) enrolled in the present study. Two samples were collected from each patient, dental plaque and saliva samples. These samples were stained with Giemsa stain and examined under light microscope. Some factors were taken such as pH saliva, age, sex, smoking habits, diabetes mellitus, blood pressure, heart disease and any history of antibiotic consumption during the last six months.

Results: This result shown Entamoeba gingivalis and Trichomonas tenax in dental plaque samples was higher than in saliva samples in patients with periodontitis (70%, 60%), (33%, 20%) respectively. While in gingivitis it was shown that these two parasites higher in saliva than the dental plaque (60%, 46.6%), (46.6%, 30.9%) respectively. These two parasites are present in healthy individuals less than in patients with periodontitis and gingivitis. The presence of these protozoans was related to the type of periodontal disease (p=0.0257), sex (p=0.043), age (p=0.0058), PH of saliva (p=0.043) and risk factors (p=0.0168).

Conclusion: This study showed Entamoeba gingivalis more common in patients with periodontal disease whereas Trichomonas tenax is considered a protozoan in oral cavity. There was relationship between the presence of these parasites and the type of periodontal disease and the risk factors.

Key words: Entamoeba gingivalis, Trichomonas tenax, periodontitis, gingivitis, risk factors. (J Bagh Coll Dentistry 2012;24(3):158-162).

INTRODUCTION

The oral cavity is suitable for invasion of many microorganism, the protozoan Entamoeba gingivalis and Trichomonas tenax are recognized eukaryotic representatives. Although these forms are not generally associated with pathogenesis, their presence in the oral cavity is taken as a sign of poor dental hygiene (1,2,3).

Since mouth infection symptoms derive from the interaction between pathogenic microbiota and the host’s defense mechanisms, it is extremely important to study the microorganisms that cause periodontitis and gingivitis in human (4). Entamoeba gingivalis and Trichomonas tenax were the first commensal foud in human oral cavity, they occur only as a trophozoit, and these are found in gingival tissues, particularly in suppurrative, inflammatory processes, due to there are preference for anaerobic environments (5).

Some authors believe that these commensal could be opportunistic, that these, capable of proliferating in a gingival environment modified by periodontal and gingivites disease (6). The trophozoites of Entamoeba gingivalis and Trichomonas tenax are most probably transmitted from person to person by close contact, since they exhibit only slight resistance to the environment. Kissing may be play a role in transmission, but direct passage by many ways such as mutual usages of cups, spoon, fork, and subjects contaminated by an infected person probably is a mode of transmission also (7). This study was designed to detected of these parasites in oral cavity of patients with periodontitis and gingivitis with some oral risk factors and compare it with healthy oral persons.

MATERIALS AND METHODS

Patients

This study was conducted on 60 patients(39 males and 21 females) with gingivitis and periodontitis and 25 samples presented a healthy periodontium attending the Teaching Hospital of the College of Dentistry/Baghdad University during the period first of February to the end of April 2012.

Samples collection and parasitological examination

The samples of saliva and dental plaque were collected from all patients at the morning, in the first visit for each patient. Saliva samples were placed in sterile cup and diluted with normal
The dental plaque samples were collected by scraping the area with sterile swab rubbed around the surface of teeth from caries and around the gingival crevices, the swabs were dipped in sterile vials containing normal saline, after that the swab rolled on the slide, also stained with Giemsa stain and examined under light microscope (40x,100x,200x,400x). The parasites of *Entamoeba gingivalis* were identified by their shape depending on the expansion of the pseudopodia formation and presence of vacuoles while *Trichomonas tenax* was identified by its flagella and characteristic locomotion. Questionnaire for these patients who entered in the present study, this includes: general health, age, sex, smoking habits, diabetes mellitus, Blood pressure, heart disease and any history of any type of antibiotic consumption during the last six months for each patients(most antibiotics used in this country). 

**Determination of saliva PH**

Universal indicator strips (PH 0-14) were used to determine the saliva PH and a possible relationship between PH and presence of the study microorganisms.

**Statistical analyses**

The data were analyzed by the non parametric chi square test($\chi^2$ test).The significance level was set at ($p< 0.05$).

**RESULTS**

Table (1) showed that the microscopical examination of the fresh samples revealed a total 35 dental plaque samples(58.3%) were positive for *Entamoeba gingivalis*, where 21 cases (70%) were taken from patients with periodontitis,14 cases (46.6%) were taken from patients with gingivitis and it found in 4 cases (16%) in control individuals,19 cases (31.6%)of dental plaque samples were positive for *Trichomonas tenax*, where 10 cases(33.3%)were taken from patients with periodontitis and 9 cases (30%)from patients with gingivitis and 5cases (20%) in control group. As for the saliva samples,36 (60%) were positive for *Entamoeba gingivalis*,where18 cases (60%) were from patients with periodontitis, 18 (60%) were from patients with gingivitis while it found in 6 cases (24%) in control individuals. *Trichomonas tenax* was identified in 20 saliva samples (33.3%), where 6 cases (20%) were from patients with periodontitis, 14 cases (46.6%) were from patients with gingivitis and only in 3 cases (12%) in control group. There was a significant correlation between presence of these two parasites and type of periodontal disease ($\chi^2 = 4.73,p=0.0257$).

Table (2)showed higher *Entamoeba gingivalis* and *Trichomonas tenax* in males than in females,(38.4%.28.5%)(5.1%.0) respectively, so there was a significant correlation between the sex of the patients and the presence of these protozoan parasites ($\chi^2 = 3.860,p=0.043$).

Regarding the relationship between patients age and presence of these protozoan, *Entamoeba gingivalis* was more common in patients age 61 to 70 years(60%), whereas *Trichomonas tenax* was more common in patients age 31 to 40years(28.5%), Both commensals were found in higher rate in age 21 to 30 years(80%) (table 3),There was statistically significant correlation between the age of patients and presence of these protozoan parasites ($\chi^2 = 10.307, P=0.0058$).

The saliva PH of the participants ranged from 5 to 7.5, *Entamoeba gingivalis* was found in higher rate in PH 6-6.5 (44.4%) while *Trichomonas tenax* was found in higher rate in PH 7-7.5(5%).Both protozoan were found in higher rate in PH 5-5.5(100%) (table 4), There was statistically significant correlation between the saliva PH and presence of these parasites($\chi^2 = 3.563,P=0.043$).

52 of 60 patients examined in this study presented risk factors,9 individuals were negative for these parasites and 43 were positive, *Entamoeba gingivalis* was found in higher rate in patients with hypertension in periodontitis and gingivitis (60%), while the parasite *Trichomonas tenax* was found in higher rate in patients who taken antibiotics at last six months (7.6%), Both parasites were present in higher rate in patients with diabetic (56.2%) (table5), There was statistically significant correlation between the risk factors of patients and presence of these parasites($\chi^2 = 8.175,P=0.0168$).
Table 1: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque and saliva samples according to the periodontal status of the patients.

<table>
<thead>
<tr>
<th>Clinical status</th>
<th>No. of samples</th>
<th><em>Entamoeba gingivalis</em></th>
<th></th>
<th></th>
<th></th>
<th><em>Trichomonas tenax</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dental plaque</td>
<td>saliva</td>
<td>Dental plaque</td>
<td>saliva</td>
<td></td>
</tr>
<tr>
<td>Periodontitis</td>
<td>30</td>
<td>21 70</td>
<td>18 60</td>
<td>10 33.3</td>
<td>14 46.6</td>
<td></td>
</tr>
<tr>
<td>Gingivitis</td>
<td>30</td>
<td>14 46.6</td>
<td>18 60</td>
<td>9 30</td>
<td>14 46.6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>35 58.3</td>
<td>36 60</td>
<td>19 31.6</td>
<td>20 33.3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>25</td>
<td>4 16</td>
<td>6 24</td>
<td>5 20</td>
<td>3 12</td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2 = 4.73 \ P=0.0257 \ P < 0.05$ significant

Table 2: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque and/or saliva samples according to the patients sex.

<table>
<thead>
<tr>
<th>Sex</th>
<th>No. of samples</th>
<th><em>Entamoeba gingivalis</em></th>
<th></th>
<th></th>
<th></th>
<th><em>Trichomonas tenax</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>39</td>
<td>15 38.4</td>
<td>2 5.1</td>
<td>16 41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>21</td>
<td>6 28.5</td>
<td>0 0</td>
<td>10 47.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>21 35</td>
<td>2 3.3</td>
<td>26 43.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2 = 3.860 \ P=0.043 \ P < 0.05$ significant

Table 3: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque and/or saliva samples according to the patients age.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Total</th>
<th><em>Entamoeba gingivalis</em></th>
<th></th>
<th></th>
<th></th>
<th><em>Trichomonas tenax</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>&gt;10</td>
<td>11</td>
<td>3 27.2</td>
<td>0 0</td>
<td>3 27.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11-20</td>
<td>9</td>
<td>5 55.5</td>
<td>0 0</td>
<td>3 33.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21-30</td>
<td>10</td>
<td>1 10</td>
<td>0 0</td>
<td>8 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>31-40</td>
<td>7</td>
<td>1 14.2</td>
<td>2 28.5</td>
<td>3 42.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>41-50</td>
<td>7</td>
<td>3 42.8</td>
<td>0 0</td>
<td>3 42.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>11</td>
<td>5 45.4</td>
<td>0 0</td>
<td>5 45.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>5</td>
<td>3 60</td>
<td>0 0</td>
<td>1 20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>21 35</td>
<td>2 3.3</td>
<td>26 43.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2 = 10.307 \ P=0.0058 \ P < 0.05$ significant

Table 4: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque and/or saliva samples according to the salivary PH.

<table>
<thead>
<tr>
<th>PH</th>
<th>No. of samples</th>
<th><em>Entamoeba gingivalis</em></th>
<th></th>
<th></th>
<th></th>
<th><em>Trichomonas tenax</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td>No. %</td>
<td></td>
</tr>
<tr>
<td>5-5.5</td>
<td>2</td>
<td>0 0</td>
<td>0 0</td>
<td>2 100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-6.5</td>
<td>18</td>
<td>8 44.4</td>
<td>0 0</td>
<td>6 33.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-7.5</td>
<td>40</td>
<td>13 32.5</td>
<td>2 5</td>
<td>18 45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>21 35</td>
<td>2 3.3</td>
<td>26 43.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$\chi^2 = 3.563 \ P=0.043 \ P < 0.05$ significant
DISCUSSION

There are only few reports on the role of oral commensals in the pathogenesis of periodontitis and gingivitis despite the high incidence of certain protozoa, such as *Entamoeba gingivalis* and *Trichomonas tenax*. In the present study, the result revealed that the incidence of *Entamoeba gingivalis* was higher than the parasite *Trichomonas tenax*, very similar results were found by other studies (3,4,7,10,11), and the rate of oral parasites higher than in control individuals, this also in agreement with many studies (4,7,11). This study indicating that the incidence rate of *Entamoeba gingivalis* was (35%), this rate approximately for many studies (4,7,11). It has been suggested that these protozoans could affect the formation of contribute to the development and progression of periodontal disease. The other researchers state that this protozoans may be opportunistic, since they are capable of proliferating in the microenvironment of the mucobuccal fold affected by periodontal disease (6).

Therefore, if *Entamoeba gingivalis* helps the development and progression of periodontitis and gingivitis, these diseases increasingly facilitate the proliferation of these protozoa, this vicious circle could explain the increased incidence of these microorganisms in the dental plaque and saliva samples of patients with periodontitis and gingivitis. A suspension of *Entamoeba gingivalis* was spread on the gingival margins of rats immunosuppressed with (prednisolone acetate), leading to the development of the clinical signs and inflammatory process of periodontal disease much faster than that observed in immunocompetent rats (13). These data indicate that immunosuppression may play role in the pathogenesis of periodontitis and gingivitis induced by this commensal.

The rate of incidence of *Trichomonas tenax* in this study was a little (3.3%), this approximately to other researchers (14). Prevalence of patients with *Trichomonas tenax* world wide ranges from 4.0 to 53% (15), but some reports found this parasite much less (2.17%) (3). the role of *Trichomonas tenax* in periodontitis and gingivitis is still controversial. Although a relationship between the increased occurrence of this protozoan and progression of this disease has been demonstrated recently (15), the precise mechanism of tissue damage is still unknown. However, further studies are necessary for the complete elucidation of this mechanism.

Saliva PH ranged from 5.5-7.5, other research suggested that the peak incidence of commensals in salivary samples occurred between 6-6.5 (14), while other study have not found a relationship between saliva PH and the presence of these protozoa (10).

Regarding gender both parasites were more common in male, these data are in agreement with those of other studies (7,16) and the difference was statistically.

Regarding age, several researchers reported that the presence of oral protozoan increases with age (6,17) as such in this study, where *Entamoeba gingivalis* was found in higher rate in age group (61-70), this finding support the idea that *Entamoeba gingivalis* may play an active role in the mouth disease, the both parasites were found in higher rate in age group (21-30), the parasite *Trichomonas tenax* was found in high rate in age group (31-40), this results are in general agreement with other studies (12,19). However, statistically significant correlation between age and presence of oral commensals was observed in this study.

The complexity of the oral environment and the multifactorial nature of the caries lesion, with the periodontal disease, requires the cooperation of other disciplines such as microbiology, chemistry and dietetics.

Other factors that increase the risk of periodontitis and gingivitis disease are diabetes, hypertension, smoking, heart disease and antibiotic consumption, in this study, there is statistically significance between these factors and the presence of these parasite, the reasons may be that the immunity which reduced in these patients,

**Table 5: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque and/or in saliva samples according to the risk factors of patients.**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>No. sample</th>
<th><em>Entamoeba gingivalis</em></th>
<th><em>Trichomonas tenax</em></th>
<th>Both</th>
<th>Negative sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>14</td>
<td>5 35.7</td>
<td>1 7.1</td>
<td>5 35.7</td>
<td>3</td>
</tr>
<tr>
<td>Diabetic</td>
<td>16</td>
<td>6 37.5</td>
<td>0 0</td>
<td>9 56.2</td>
<td>1</td>
</tr>
<tr>
<td>B.pressure</td>
<td>16</td>
<td>3 60</td>
<td>0 0</td>
<td>2 40</td>
<td>1</td>
</tr>
<tr>
<td>Herat.Dis</td>
<td>4</td>
<td>2 50</td>
<td>0 0</td>
<td>1 25</td>
<td>1</td>
</tr>
<tr>
<td>Antibiotic</td>
<td>13</td>
<td>7 53.8</td>
<td>1 7.6</td>
<td>2 15.3</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>21 40.3</td>
<td>2 3.8</td>
<td>19 36.5</td>
<td>9</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 8.175 \ P = 0.0168 \ P < 0.05 \text{ significant} \]
Evaluation of Entamoeba gingivalis and the presence of these parasite are making facilitates.

The present study recorded a relationship between the presence of Entamoeba gingivalis and Trichomonas tenax and these factors. In fact, risk factors for periodontitis and gingivitis were present in only a few participants, so any inference or deliberation on this subject should be cautious. Other studies did not find relationship between the presence oral parasites and these factors (4,10).

The results of this study suggest that Entamoeba gingivalis occur more frequently in the patients with periodontal and gingivitis disease, whereas Trichomonas tenax sees the oral cavity as the natural habitat given its commonness in individual with healthy gums. However, further studies are necessary for determining the real nature of the relationship between these species and the periodontal disease.

REFERENCES