Detection of *Entamoeba gingivalis* trophozoites in patients suffering from gingivitis versus healthy subjects.

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ABSTRACT

This study included a total 160 case which were diagnosed according to their clinical examination. Samples from gingival pockets were collected from all cases which were divided into 2 groups; group 1: (80 cases) patients suffering from gingivitis and group 2: (80 subjects) healthy volunteers. *Entamoeba gingivalis* was detected in 38 samples (28.75%) out of the total 160 collected samples using Iron & Haematoxylin. Wet unstained samples only diagnosed *Entamoeba gingivalis* infection in 14 cases (8.75%). Twenty three out of the 38 positive cases were from group of cases complained of gingivitis and the remaining 15 subjects were from the control group which did not complain from any symptoms. The infection was found in 23 (28.75%) out of 80 cases with gingivitis. While in control group, *Entamoeba gingivalis* was observed in 9 of them (11.25%). Concerning the intensity of infection, it was significantly higher in cases which suffered from gingivitis than in control group. Samples related to the diseased subjects were found to be severely to moderately infected, while mild to moderate infection was recorded in control group. In general, diagnosis of such infection is vital to avoid development of chronic irreversible oral diseases. Repeated examination with more than one diagnostic technique by expert personal is recommended as a key of perfect diagnosis in such protozoan infection that has only trophozoite stage. Further studies on large scale are necessary for determining the actual nature of the relationship between these species and oral diseases.

KEYWORDS: *Entamoeba gingivalis* – periodontitis- Iron &haematoxylin stain

INTRODUCTION

*Entamoeba gingivalis* was the first commensal protozoa found in the human oral cavity. It is observed in individuals with oral disease as well as healthy subjects. In addition, some believe that this commensal could be opportunistic, that is, capable of proliferating in a gingival environment modified by periodontal disease [1].

*Entamoeba gingivalis* possess only trophozoite forms with no cyst stage. Therefore, direct contact from person to person is needed for transmission of this protozoan infection which exhibits only slight resistance to the environment. Kissing may play a role in transmission, but indirect contamination may occur through sharing food, cups, cutlery and other fomites that may transmit the infection as well [2].

On the other hand, up to 95% of the population with poor oral hygiene may be infected with *Entamoeba gingivalis* [3]. Many years have passed since the first descriptions of these protozoa, and unluckily, there is a lack of information in variable aspects related to this protozoal infection [4]. This study was designed to detect *Entamoeba gingivalis* in oral cavity of patients with gingivitis versus healthy, considering the intensity of infection in both groups.
MATERIALS AND METHODS

The study included a total 160 case which were diagnosed according to their clinical examination at outpatient clinic, department of oral medicine and periodontology, Faculty of Dentistry, Fayoum University. Dental plaque samples were collected from all cases and subsequent preservation in PVA was done. The study included 2 groups; group 1: (80 cases) patients suffering from gingivitis and group 2: (80 subjects) healthy volunteers. The study included male or female patients which diagnosed with gingivitis, aged from 35 to 55 years old and had not received any oral therapy for minimum of 6 months prior to the study. Pregnant females were excluded from the study. Considering ethical issues, subjects were subjected to the following: data collection, clinical examination, sample collection and parasitological examination.

Sample collection and parasitological examination:

The samples of gingival pockets were collected from all patients, in the first visit and were placed in a sterile epindorphe. Part of the sample was smeared on a slide and subjected to direct wet mount examination which was done immediately by mixing a small amount of fresh sample with a drop of 0.85% NaCl. This mixture provided a uniform suspension under a 20 x 50 mm cover-slip to be examined microscopically. The other part of the sample was diluted with PVA at room temperature (25-28°C) to be stained with Iron & haematoxylin stain (I&H). At least three smears were stained for proper parasitological examination, using 40x and oil immersion 100x magnification. Entamoeba gingivalis parasites were identified by their shape depending on the expansion of the pseudopodia formation and presence of vacuoles, inclusions and its characteristic nucleus [5]. Measurement of parasitic stages was performed according to Bailey and his colleagues [6] Objects seen under the microscope are measured using an eyepiece (ocular) micrometer that has been calibrated against a stage micrometer in combination with a specific objective lens.

Results:

Entamoeba gingivalis was detected in 38 samples (28.75%) out of the total 160 collected samples using Iron & Haematoxylin. While using wet unstained samples only diagnosed Entamoeba gingivalis infection in 14 cases (8.75%). Twenty three out of the 38 positive cases were from group of cases complained of gingivitis and the remaining 15 subjects were from the control group which did not complain from any symptoms. The present work relied, not only on the characteristic morphological criteria to report positive findings concerning Entamoeba gingivalis parasitic stage, but also, on measurement of the detected stages using micrometry to confirm such findings. The detected trophozoites were observed with a single nucleus that has a small prominent central karyosome with a peripheral rim of chromatin and finely granular cytoplasm. The size of the detected trophozoites in this study ranged from 13 to 18 µm. There was no statistical significant difference between cases with unhealthy oral condition and the control concerning the size of the identified Entamoeba gingivalis trophozoites. In stained samples with iron & haematoxylin, the cytoplasm of the detected trophozoites appeared lighter in colour while inclusions which represent leuko-phagocytosis (engulfed WBCs) appeared dark stained bodies. The nucleus appeared as a dark spot surrounded by a relatively darker rim. It is important to mention that, careful screening for the whole smear during examination and for additional smears in the current work was vital, not to miss any of the positive samples. Fungal infection was reported in some samples either alone or mixed with Entamoeba gingivalis infection. In some cases, there was difficulty to observe the trophozoite stage as mentioned before. Repeated examination for more than one slide helped us in the current study, not to miss the positive Entamoeba gingivalis infection.

Concerning the occurrence of Entamoeba gingivalis infection among cases and control. The infection was found in 23 (28.75%) out of 80 cases with gingivitis. While in control group, Entamoeba gingivalis was observed in 9 of them (11.25%) (table 1). The higher rate of Entamoeba gingivalis infection among cases suffered from gingivitis was statistically significant (P=0.001). Concerning the intensity of infection, it was significantly higher in cases which suffered from gingivitis than in control group. Samples related to the diseased subjects were found to be severely to moderately infected, while mild to moderate infection was recorded in control group (table 2).
Fig. 1: *Entamoeba gingivalis* trophozoites stained with Iron & Haematoxylin (orange arrows) with characteristic nucleus (black arrow in the larger view). Dark inclusions appear in the cytoplasm (arrow head in the larger view). Notice the scattered fungal infection.

Table 1: Comparison between I&H and wet mount used in this study to detect *E. gingivalis*.

<table>
<thead>
<tr>
<th>Method of detection</th>
<th>Gingivitis (80)</th>
<th>Control (80)</th>
<th>Total number (160)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct wet examination</td>
<td>N 9</td>
<td>5</td>
<td>14 (8.75%)</td>
</tr>
<tr>
<td>% 11.25%</td>
<td>6.25%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron &amp;H stain</td>
<td>N 23</td>
<td>15</td>
<td>38 (28.75%)</td>
</tr>
<tr>
<td>% 28.75%</td>
<td>18.75%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Intensity of infection among diseased and control group.

<table>
<thead>
<tr>
<th>Intensity of infection</th>
<th>Diseased cases</th>
<th>Control cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sever</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Low</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>38</td>
<td>15</td>
</tr>
</tbody>
</table>

Discussion:
The present study aimed to study *Entamoeba gingivalis* among patients suffering from gingivitis versus healthy individuals. To achieve this goal, 80 patients having gingivitis and 80 apparently healthy individuals were randomly allocated in this study. In the current work, 38 out of the total 160 subjects were positive for *Entamoeba gingivalis* infection. Only 14 cases were diagnosed by wet mount while the 38 samples were diagnosed by I & H which subsequently confirmed by micrometry. On many occasions, measuring the size of suspected parasites is helpful in their identification. Therefore, I&E stain identified (28.75%) of the cases infected by *Entamoeba gingivalis*. This may denotes the importance of examining more than one sample and more than one method. In an old study done by Becky *et al* [7] to compare wet mount with variable stain in order to identify *Entamoeba* species, they only recorded 4.8% positive results by direct mount while 58.5% were positive by permanent stain. The former workers concluded that, the direct unstained wet mount may be helpful in detecting cyst stages which was not a choice in our study. Additionally, the previous workers warned the parasitologist about relying solely on the direct wet mount for detection or identification of protozoan trophozoites. Instead, they recommended the use of a permanent staining technique which reported to be much more effective for detecting and identifying protozoan trophozoites in different specimens. More recently, Billetter and Dunn [8] re-documented the limitation of wet mount preparation that previously mentioned in variable researches. They recorded that; wet mount may fail to detect various organisms due to variable factors. Delay in reading the smear, preparation that is too thin or too thick, in addition to untrained or inexperienced staff members, are among the factors recorded to restrict the use of wet mount. These data may explain the low percentage of positive findings recorded among our cases. Al- Najar and his coworkers [9] carried on their study in Baghdad and reported infection rate 28% for *E. gingivalis* among their cases which is more or less similar to that reported by our study. Although, Goldsmid and Gericke [10] reported that the infection rate for *E.
gingivalis was (62.5%), diagnosed by using contrast microscopy and (81.25%) in permanent smears stained with Iron-Haematoxylin. This is much higher than that reported by our study. The difference might be related to variation in study design, demographic changeability, period of study or the performed diagnostic techniques.

Concerning the morphological data related to the trophozoite stages of Entamoeba gingivalis, the observed trophozoites were of variable size ranged from 13 to 18 µm. They were similar to Entamoeba histolytica to a great extent regarding the shape of the nucleus; central karyosome and the peripheral chromatin. The main difference in addition to the relatively smaller size trophozoites, are the cytoplasmic inclusions, which were the phagocytosed leucocytes. This in agreement with many previous works which documented the ability of E. gingivalis to ingest WBCs [2,11].

In fact, the main activity of Entamoeba gingivalis in the infected oral cavity, besides moving, consists of feeding on the nucleus of white blood cells. The amoeba penetrates into the cytoplasm to reach the nucleus and literally suctions its contents via the negative pressure of the pseudopod. The food so gulped down is gradually digested inside the endoplasm. Phagocytosis can sometimes continue for more than 20 polynuclear neutrophil nuclei. This activity leaves a de-nucleated cell, unable to achieve either its activity or its preprogrammed apoptosis. It will release polymorphonuclear leukocytes-uncontrolled proteolytic enzymes on the surrounding tissues and could be considered a pathogen from this vampirising activity [12].

In the present study, wet mount examination followed by examination of at least 3 stained smears was performed. Important information is often missed if careful microscopic visualization of the sample is not carried out. Stains like I&E helps not only in the identification of the parasite including protozoal infection, but also in the visualization of variable cellular morphology, hence the stain is recommended to permanently stain suspicious samples for protozoal infection [13 and 14].

As regards the data related to the occurrence of Entamoeba gingivalis among the healthy persons which is extremely varied from asymptomatic infection to significant association with oral diseases. In the present work, the infection was reported in 18.75% of the healthy control group. Close to our report. Glebski et al. [15] conducted a research on students in 1970s and disclosed the presence of this amoeba among 20% of health subjects. Also, Dao and his partners [16] found E. gingivalis in larger number of cases, 32% of 96 controls with good oral hygiene. Other studies did not find relationship between the presence of Entamoeba gingivalis and oral infections [17 and 18]. Actually, Entamoeba gingivalis was reported to be the first commensal protozoa found in the human oral cavity. This protozoan is found in gingival tissues, particularly in supplicative, inflammatory processes, due to its preference for anaerobic environments. The occurrence of E. gingivalis in individuals with oral disease suggests that these protozoa might have an important role in the etiology of this condition. However, since it is also found in the oral cavity of healthy individuals, some authors believe that this commensal could be opportunistic, that is, capable of proliferating in a gingival environment modified by periodontal disease [1]. Reczyk and his colleagues [19] reported increased prevalence of E. gingivalis only with diseases of the oral cavity, and in particular with periodontal diseases and recorded E. gingivalis in 60% among their group of patients. As well, William [20] did not find the parasite in mouths free of any oral disease. Al saeed [21] stated that, if Entamoeba gingivalis helps the development and progression of 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 gingivitis. This is in accordance with our study and explains the presence of such infection in higher number of our cases with gingivitis.

In general, diagnosis of such infection is vital to avoid development of chronic irreversible oral diseases. Important information is often missed if careful microscopic visualization of the sample is not carried out. Therefore repeated examination with more than one diagnostic technique by expert personal is recommended as a key of perfect diagnosis especially in protozoan infection that has only trophozoite stage as Entamoeba gingivalis. Further studies on large scale are necessary for determining the real nature of the relationship between these species and the oral diseases. In addition, frequent dental check-ups are recommended to prevent or minimize periodontal diseases.

REFERENCES


