Investigation of Entamoeba gingivalis incidence in biofilm and saliva from periodontitis patients

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Abstract

Apart from and human cells, a few bacteria, fungi and protozoa are distinctly present in healthy gums, additionally, involved in diseased sites. The phenomenon of lysis by pathogens or other immune cells remains to be elucidated to improve the current pathophysiological paradigm of periodontitis. Protozoan, represented by Entamoeba gingivalis could play a determining role in the periodontitis progressing. Thirty-one individuals attending the private clinics in Najaf province/ Iraq were selected for this study. Twenty healthy oral cavity people were included in the present study as a control group. The samples from biofilm and saliva were collected and examined for the presence of the parasite. Twenty–two infected cases were confirmed, age group (31-40) and males patients were more common to have the amoeba in comparison to the female. E. gingivalis were also observed in the control group but with less incidence rate. These outcomes propose that E. gingivalis most abundant in periodontitis. Thereby, more investigations are required to establish the association between this microorganism and periodontitis.

Keywords: Entamoeba gingivalis, periodontal disease, oral cavity, correlation to the pathogenicity.

Introduction

The human oral cavity contains an abundant and polymorphous flora of microorganisms, the majority of which are bacteria (Oladokun et al, 2021). There are also mycoplasmas, yeasts and parasites. Parasites that colonize the mouth are rare, however particular amoeba Entamoeba gingivalis inhabits the oral cavity. Entamoeba Gingivalis is protozoan, eukaryotic, motile and unicellular organism, found only in vegetative or trophozoite form

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(Sharifi et al, 2020). No form of resistance or cyst is described (Bonner et al, 2018). From a morphological point of view, *E. gingivalis* mimics *Entamoeba histolytica*, and to diagnose such a protozoan requires proficiency (Bao et al, 2020). Microscopically, this amoeba appears as a large cell (30 to 50μm) in which the granular endoplasm is clearly distinct from the ectoplasm and active pseudopodia were also obvious (Bonner et al, 2018). Digestive vacuoles contain multiple foreign bodies: bacteria, cellular debris, or sometimes even the structure of altered nuclei of polynuclear cells and some red blood cells (Esenkaya et al, 2020). The anaerobic environment of the deep periodontal pockets, favorable to the development of parasites suggests that interactions between the constituents of the microbiota could be essential for their role in the pathophysiology of the disease (Bao et al, 2020).

Investigation through the literature demonstrate the opinions of scientists were conflicting about its pathogenicity, some of them suggested that it is a pathogenic protozoan and could play a determining role in the progression of periodontitis (Bonner et al, 2018, Bao, et al 2020, AL-Sarhan and Saeed, 2021), but others concluded that this protozoan commensal due to its presence in the oral cavity of healthy individuals (Esenkaya et al, 2020, Oladokun et al, 2021). It is recognized, however, that in immunocompromised individuals, *E. gingivalis* act in synergy with some symbiotic bacteria and promote the aggravation of periodontitis (How et al, 2016, Sharifi et al, 2020, Bonner et al, 2018). It is well documented that people with low economic standards of living and poor mouth hygiene are most likely to have this parasite than healthy people. Furthermore, systematic diseases such as diabetes and heart diseases increased the infection rate with this amoeba, Ghabanchi et al, 2010, Bao, et al 2020

Accordingly, to explore the role of this protozoan on periodontitis, this commensal was detected in the patients with HIV/1 diagnosed with periodontal disease (Cembranelli et al, 2013). This correlation may be due to the chronic diseases consequences and their impact on the variation of microbial communities in the oral cavity. *E. gingivalis* was the only microbe detectable in the oral cavity of people with severe periodontitis, suggesting its involvement of progression of the diseases and resulting in inflammation and destruction of the surrounding tissues as already proposed by other investigators (AL-Sarhan and Al-Saeed, 2003, Bonner et al, 2018, Bao, et al 2020, Bao et al, 2021).
Researchers have established the pathogenic activity of *E. gingivalis* once the amoeba attached to the live epithelial cell models surfaces induces cell killing and degeneration by trogocytosis (Bao et al., 2021).

For supporting action towards the field of public health and due to the abundance dispute about the pathogenicity of *E. gingivalis*, this study was carried out to survey the prevalence of this amoeba between patients with periodontitis and other healthy individuals. Besides, investigate the association between the incidence rate and some parameters such as patient's age and sex in the city of Najaf.

**Materials and methods**

**Patients**

Before the samples were collected, some information was recorded such as the age, sex, occupation and residence of the patients who attended private clinics in Najaf/Iraq. Additionally, the patients were questioned if he/she has any systematic illness, consumption of antibiotic or any treatments.

The study included 31 patients from both sexes. The patients were clinically examined and diagnosed by the physicians. The age group selected was between 10-70 years (15 females and 16 males) who were diagnosed with periodontal disease. The following symptoms (swelling of frontal mandibular deepening pocket, plaque and calculus, inflammation and green or yellow gingiva) were chosen as the case group. The other group with normal teeth, healthy gums and constant bone level) were nominated as a control group. This group was also confirmed with no systemic disease.

**Sample collection and Microscopic Examination**

From each patient, three sites were selected sites to collect the samples (interdental spaces, molar pockets, grooves and gingival. The accumulated materials were removed with sterile periodontal curettes. Whereas, samples of saliva were collected using sterilized containers. Wet-mount smears of both samples were performed by placing one drop of physiological saline solution on the slide, mixed with samples and stained with Giemsa stain. The examinations were done in triplicate using (40 x) to identify the amoeba with its unorganized shape, distinctive vacuoles and pseudopodia.

**Statistical analyses**
The data were analyzed by the non-parametric chi-square test. The significance level was set at 5% (p<0.05).

RESULTS

The study included 31 individuals: 22 cases were infected, while nine with no infection Table 1. In the studied group, 19 patients had periodontal disease infected with *E. gingivalis* (70.90), while the remaining 13 patients were attended the clinics for monitoring general oral cavity health only 3 infections were observed. Analysis of the fresh microscope slides revealed that from the total confirmed infected cases, 15 were detected in the biofilm and four in saliva. In the control group, only 3 people (13.63%) had the parasites in the biofilm samples and one sample in saliva Table 2.

Table 1: Percentage of patients infected *E. gingivalis* and healthy individuals

<table>
<thead>
<tr>
<th>E. gingivalis</th>
<th>Cases number</th>
<th>%</th>
<th>Control</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>22</td>
<td>70.9</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
<td>29.03</td>
<td>16</td>
<td>80</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>100</td>
<td>20</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2: Frequency of *E. gingivalis* in dental biofilm and salivary samples according to the periodontal status of the patients and healthy individuals.

<table>
<thead>
<tr>
<th>E. gingivalis</th>
<th>Biofilm</th>
<th>%</th>
<th>Saliva</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodontitis</td>
<td>15</td>
<td>65.21</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>4</td>
<td>17.39</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>Control (Healthy)</td>
<td>3</td>
<td>13.63</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>100</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>

Regarding the association between patient’s age and presence of *E. gingivalis*, protozoan was more common in individuals aged 31 to 40 years Table 3, whereas the lowest incidence rate was recorded between people aged 41-60 years.
Table 3: Frequency of infection with *E. gingivalis* in biofilm and salivary samples considering age in patient.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Cases</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>3</td>
<td>13.63</td>
</tr>
<tr>
<td>21-30</td>
<td>5</td>
<td>22.73</td>
</tr>
<tr>
<td>31-40</td>
<td>10</td>
<td>45.45</td>
</tr>
<tr>
<td>41-50</td>
<td>2</td>
<td>9.09</td>
</tr>
<tr>
<td>51-60</td>
<td>1</td>
<td>4.55</td>
</tr>
<tr>
<td>60 and above</td>
<td>1</td>
<td>4.55</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>100</td>
</tr>
</tbody>
</table>

Table below demonstrated the incidence of the protozoan *E. gingivalis* according to the sex of the patients. A Higher *E. gingivalis* rate was identified in males 59.59% (13 cases), while 40.90% (9 cases) were determined in male patients.

Table 4: Frequency of infection with *E. gingivalis* in biofilm and salivary samples considering age inpatient and healthy individuals.

<table>
<thead>
<tr>
<th>Patient's sex.</th>
<th>Cases number</th>
<th>Amoeba presence</th>
<th>%</th>
<th>Control</th>
<th>Amoeba presence</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>16</td>
<td>13</td>
<td>59.59</td>
<td>10</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>9</td>
<td>40.90</td>
<td>10</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>31</td>
<td>22</td>
<td>100</td>
<td>20</td>
<td>4</td>
<td>100</td>
</tr>
</tbody>
</table>

In the control group, males also showed a higher infection rate than females 75% (3 cases), while the female only one case (25%).

**Discussion**

The presence of *E. gingivalis* in periodontal lesions of gingival infections in humans has been commented on repeatedly by numerous authors for over a century (Bao et al, 2020). Without being unanimous on its pathogenic capacities or its commensal aspect, the majority of authors have noted its constant presence in periodontitis (Lbuquerque et al, 2011., Bonner et al, 2018., Bao, et al 2020., Sharifi et al, 2020).
All the activities encountered in the amoeba give it an essentially invasive pathogenic character: adhesion to cells, inactivation and phagocytosis of neutrophils, mass reproduction and motility (Esenkaya et al, 2020., Bao et al, 2021). With this in mind, this study revealed that those patients with periodontal disease were more disposed to infection with \(E.\) gingivalis. The incidence rate of this parasite was \(70.9\%\), this was accordant with the study conducted by (Lbuquerque et al, 2011., Luszczak et al, 2016., Bao et al, 2020), which mostly ranged from \(50\%\) to \(77\%). However, in the study implemented by (Gharavi et al, 2006., Sharifi et al, 2020) there was a certain disparity in the results, the prevalence ranging \(11.7-41.7\%\), their findings was less than the current ones. Moreover, the parasites were detected in the control group in 4 cases out of 20 cases \(20\%\), suggesting its commensal habitation. These differences can be explained by some parameters that may be influencing the variability of results for instance the diversity of the samples examined (number of subjects, age, sex, etc.), additionally, difficulty in detecting and identifying with certainty the amoeba (direct examination on a fresh sample, examination after culture, examination by PCR probe (Sharifi et al, 2020).

It should be pointed out that \(E.\) gingivalis, found at high frequency in the dental biofilm more than saliva of patients with periodontal disease and the control group, authenticates the assumption that this protozoan could be contributed to the progression of periodontal disease, as has been hypothesized by other studies Luszczak et al, 2016., Bao et al, 2020., Sharifi et al, 2020). It is possible that this parasite has a role in the development of periodontitis either by providing aid to the bacteria that cause damage (Bonner et al., 2018., Salman et al, 2021), or it can invade ruptured oral mucosa, where it ingests fragments of live cells, would be essential to justify its periodontal pathogenicity (Bao et al, 2021). It has therefore been observed that the more periodontal disease progresses, the more the number of protozoa increases.

Regarding gender, results indicated that the parasite was more common in males \(59.59\%\), while females \(40.90\%\). These data are incompatible with another study which revealed a high percentage of infection-related with females (Lbuquerque et al, 2011). This study, however, suggested that gender seems to influence the frequency of this amoeba. In respect of the link between patient's age and presence of microorganisms, \(E.\) gingivalis was increasingly frequent in patients aged 31 to 40 years. Likewise, some authors suggest a statistically significant connection between \(E.\) gingivalis incidence and the age of the patients: maximum infestation between 30-49 years (Lbuquerque et al, 2011., Luszczak et

**Conclusion**

It can therefore be concluded from this study that infections with *E. gingivalis* should be regarded as a potential factor associated with the pathological effects occurring in patients with periodontal diseases. Further research should be carried out to identify either if it is the main cause of tissue destruction and progressing such a disease, or it is acting synergistically with the present pathogenic bacteria, and this should be investigated in more depth.

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**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

All the authors approve the manuscript for publication.

**Competing interests**

The authors declare there is no competing interests
References


