Trichomonas Tenax in Human Oral Cavity

Trichomonas tenax u usnoj šupljini

Summary

Fifty-one patients were investigated in order to determine the presence of Trichomonas tenax in various oral structures (saliva, dental plaque from physiologically clean and unclean tooth areas, carious dentin, root canal contents). Samples were cultivated in liquid Diamond’s axenic broth medium. The results were considered positive if living oral Trichomonas was found in smears. The prevalence of Trichomonas tenax in the study patients was 35.5%. Trichomonas tenax was most frequently found in dental plaque (23.2%) and in the root canal content (30.0%) (p > 0.05). The variables observed in this study (patient’s age and sex, oral hygiene, gingivitis, number of carious teeth, smoking and coffee intake) did not influence the presence of Trichomonas tenax. According to the results obtained, a mature dental plaque appears to be the oral structure favoring the growth and survival of oral Trichomonas in the physiologic conditions. It is possible that Trichomonas tenax spreads from dental plaque into the diseased endodontium.

Key words: Trichomonas tenax, human oral cavity, dental plaque, root canal.

Introduction

The human oral cavity is an ecosystem very suitable for the growth and survival of numerous microorganisms, due to inner environmental conditions. As many as 20–325 different species have been isolated from the human oral cavity (1–3). The majority of these are probably transients, while resident or indigenous flora comprises approximately 20 species, both aerobic and anaerobic ones (1).

Among the anaerobic microflora in the oral cavity, Trichomonas tenax, a parasitic flagellate protozoon, was obtained. It is distributed world-wide, its prevalence ranging from 4% to 53% (4, 5).

Infection is spread through saliva, droplet spray and kissing, or by contaminated dental instruments, dishes, glasses and hands (4, 6, 7). Trichomonas tenax can survive for 48 hours in saliva (4, 7).

Oral Trichomonas is more frequently found in elderly people, in those suffering from periodontal diseases, and in those with poor oral hygiene (7–11).

Its prevalence is very low in children and in the edentulous (7, 12–14). However, it was also
obtained in the culture of subjects with healthy oral cavity (15).

Trichomonas is considered to be a commensal flora of the human mouth. When the conditions in the oral cavity are favorable for its growth and survival, oral Trichomonas can be obtained in and around the diseased and necrotic teeth and gums (4). Oral Trichomonas shows proteolytic and collagenolytic activity (16-18).

In humans, three distinct trichomonad species can be found: genitourinary Trichomonas vaginalis, intestinal Trichomonas hominis and oral Trichomonas tenax. The three trichomonad species could not be experimentally transmitted to each other's environment (4).

The aim of this study was to determine the presence of Trichomonas tenax on different structures of the human mouth (saliva, dental plaque from physiologically clean and unclean tooth areas, carious dentin, root canal content).

Furthermore, the influence of several variables (patient age and sex, oral hygiene, gingivitis, number of carious teeth, smoking, coffee intake) on the presence of oral Trichomonas was studied.

**Materials and Methods**

One-hundred-and-eighty-four samples were taken from 51 patients randomly selected at the Department of Dental Pathology, School of Dental Medicine, University of Zagreb.

Twenty-seven females (aged 18-68 years, mean age: 40 years), and 24 males (aged 19 to 74 years, mean age: 42 years) were included in the study.

The patients were classified according to:
- age;
- sex;
- oral hygiene degree (1 – up to 1/3 of tooth surfaces covered with dental plaque: 2 – up to 2/3 of tooth surfaces covered with dental plaque; 3 – more than 2/3 of tooth surfaces covered with dental plaque);
- gingivitis according to GSBI (gingival sulcus bleeding index) (19);
- number of carious teeth; and
- smoking and coffee intake.

None of the patients was under antibiotic or any other chemical therapy.

Fifty-one samples were taken from saliva, 50 samples from dental plaque in physiologically clean tooth areas, 49 samples from dental plaque in physiologically unclean tooth areas, 14 samples from carious dentin, and 20 samples from root canal contents of the teeth with necrotic pulp where endodontic spaces were opened by caries.

Saliva samples were taken by cotton pellets. Dental plaque samples from both physiologically clean and unclean tooth areas were taken by scraping the tooth surface with an excavator. Carious dentin samples (dg. caries profunda) were taken by a round stainless bur or by an excavator. Samples from the root canal contents were taken from the root canals of 20 teeth where endodontic spaces were opened by caries. After isolation of the teeth from the oral cavity by a rubber-dam, the tooth surface was cleansed with a polishing brush and cotton pellet soaked in 5% NaOHCl in order to prevent secondary contamination during sampling from root canals. Then, a control sample was taken from the cleansed tooth surface of each tooth investigated. Control samples taken in all the 20 teeth were negative for Trichomonas. Then, the carious mass was extracted by a sterile round steel bur and samples from the root canals were taken by sterile paper points (SANITARIA absorbent paper points No. 20, Vienna, Austria) prior to the root canal instrumentation and irrigation.

All samples were cultured in liquid Diamond's axenic broth medium (20–23), heated in a thermostat to 37°C (98.6°F) and the results read after 24, 48, 72, 96 and 120 hours by light microscopy of native smears, with magnification of 100 and 400 (21).

The results were considered positive if living oral Trichomonas was observed in the smear (21).

Statistical analysis was carried out by chi-square test with a continuity factor.

**Results**

In this study, 51 patients were examined. Oral Trichomonas was found in 18 (35.3%) out of 51 patients examined (Table 1).

The group of 51 patients had 27 females and 24 males. Trichomonas tenax was found in 11 (40.7%) females and 7 (29.2%) males (Table 1).

Trichomonas tenax was more frequently obtained in females than in males, but the difference was not statistically significant.
The frequency of positive Trichomonas tenax findings in the patients below the age of 30 and in those older than 50 was almost identical (23.1% and 25.0%, respectively) (Table 2). In the patients aged 30–50, oral Trichomonas was found in 11 (50.0%) out of 22 patients examined. Neither this difference was significant.

Trichomonas tenax was not found in the saliva of any of the 51 patients examined (Table 3). In 50 samples of dental plaque from physiologically clean tooth areas, oral Trichomonas was observed in 11 samples (22.0%). In 49 samples of dental plaque from physiologically unclean tooth areas, oral Trichomonas was found in 12 samples (24.5%). Trichomonas was not found in any of the 14 samples of carious dentin, but it was found in 6 (30.0%) out of 20 samples of root canal contents. The difference in the findings of Trichomonas tenax between the samples taken in physiologic human mouth conditions (samples of saliva and dental plaque) and the samples taken in pathologic human mouth conditions (samples of carious dentin and root canal contents) was not significant (p > 0.05).

With respect to the oral hygiene degree, oral Trichomonas was most frequently observed (six out of 13 patients or 46.2%) in the patients with poor oral hygiene, degree 3, but the difference did not reach statistical significance (Table 4).

Healthy gingiva (GSBI = 0) was present in one patient only and this one was Trichomonas tenax – negative (Table 5). GSBI 1 and 2 were present in 42 patients, 14 (33.3%) of them Trichomonas tenax – positive. GSBI 3, 4 and 5 were present in eight patients, four (50.0%) of them Trichomonas tenax – positive (p > 0.05).

In the dental plaque samples of the patients with low degree of gingivitis (BSBI 1–2), oral

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**Table 1. Oral distribution of Trichomonas tenax according to sex**

<table>
<thead>
<tr>
<th>Sex</th>
<th>N</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N0</td>
<td>%</td>
</tr>
<tr>
<td>F</td>
<td>27</td>
<td>11</td>
</tr>
<tr>
<td>M</td>
<td>24</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>18</td>
</tr>
</tbody>
</table>

\[ X^2 = 0.75 \quad p > 0.05 \]

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**Table 2. Oral distribution of Trichomonas tenax according to age**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>N</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N0</td>
<td>%</td>
</tr>
<tr>
<td>&lt; 30</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>30–50</td>
<td>22</td>
<td>11</td>
</tr>
<tr>
<td>&gt; 50</td>
<td>16</td>
<td>4</td>
</tr>
</tbody>
</table>

\[ X^2 = 3.68 \quad DF = 2 \quad p > 0.05 \]

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**Table 3. Oral distribution of Trichomonas tenax oral distribution**

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>N</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N0</td>
<td>%</td>
</tr>
<tr>
<td>Saliva</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>Dental plaque (clean area)</td>
<td>50</td>
<td>11</td>
</tr>
<tr>
<td>Dental plaque (unclean area)</td>
<td>49</td>
<td>12</td>
</tr>
<tr>
<td>Carious dentine</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Root canal</td>
<td>20</td>
<td>6</td>
</tr>
</tbody>
</table>

\[ *(1) samples taken in physiologic conditions in human mouth \]
\[ **(2) samples taken in pathologic conditions in human mouth \]

Difference between the Trichomonas tenax findings in region 1 and in region 2 is not significant (p > 0.05).

Dental plaque (clean area): root canal content \[ X^2 = 0.50 \quad p > 0.05 \]

Dental plaque (unclean area): root canal content \[ X^2 = 0.22 \quad p > 0.05 \]

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**Table 4. Oral distribution of Trichomonas tenax according to oral hygiene**

<table>
<thead>
<tr>
<th>Oral hygiene degree</th>
<th>N</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N0</td>
<td>%</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

\[ X^2 = 3.18 \quad DF = 2 \quad p > 0.05 \]
Table 5. Oral distribution of Trichomonas tenax according to the grades of gingivitis

<table>
<thead>
<tr>
<th>GSBI</th>
<th>N</th>
<th>Positive</th>
<th>( N_0 )</th>
<th>%</th>
<th>( X^2 )</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>42</td>
<td>14</td>
<td>33.3</td>
<td>50.0</td>
<td>0.90</td>
<td>0.34</td>
</tr>
<tr>
<td>3-5</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Legend:
GSBI (gingival sulcus bleeding index (19))
0 - no gingivitis; probing of gingival sulcus does not induce bleeding
1 - gingiva of normal appearance, but careful gingival sulcus probing induces bleeding
2 - probing of gingival sulcus induces bleeding and changes in the color of gingiva due to inflammation
3 - type 2 changes and slight changes of the gingival shape due to edema or inflammatory hyperplasia
4 - type 3 changes and obvious edema or hyperplasia
5 - type 4 changes and spontaneous bleeding, with or without gingiva ulceration

Trichomonas was found in 16 out of 81 samples (19.8%). In the dental plaque samples of the patients with progressive gingivitis (GSBI 3-5), Trichomonas tenax was found in seven out of 16 samples (43.8%). These differences were statistically significant (p < 0.05).

Concerning the number of carious teeth, Trichomonas tenax was found in five out of 11 patients free of carious lesions (45.5%); seven out of 25 patients with 1–5 carious teeth (28.00%); and six out of 15 patients with 5 and more carious teeth (40.0%) (p > 0.05).

Trichomonas tenax was recorded in 36.0% of the non-smokers (nine out of 25 patients), in 33.3% of those smoking one pack of cigarettes per day (six out of 18 patients), and in 37.5% of those smoking more than one pack of cigarettes per day (three out of 8 patients) (p > 0.05).

With respect to coffee intake, oral Trichomonas was most frequently observed in those drinking coffee occasionally (14 out of 37 patients or 37.8%), followed by those who never drank coffee (two out of six patients or 33.3%) and those drinking coffee quite often (two out of eight patients or 25.0%) (p > 0.05).

Discussion

Oral Trichomonas, an anaerobic flagellate protozoan, is more frequently found in elderly people, in those suffering from periodontal diseases, and in those with poor oral hygiene (7-11).

The prevalence of 35.3% of Trichomonas tenax found in 51 patients in this study is consistent with literature data (4%-53%) (4).

Trichomonas tenax was more frequently recorded in females (40.7%) than in males (29.2%), but the difference was not statistically significant. These results are in accordance with those reported by Cambone et al. (8, 10), who state that sex has no influence on the occurrence of Trichomonas tenax.

Lapierre et al. (5) report on the majority of oral Trichomonas findings in patients above the age of 30, while Wantland et al. (9, 12) and Cambon et al. (10) observed it in the 30–60 year age group.

In this study, Trichomonas tenax was most frequently found in the patients aged 30–50 years, which appears to be consistent with the above mentioned age range (9, 10, 12). Age-related differences in the Trichomonas tenax findings were not significant.

However, concerning the age and oral hygiene degree of the patients, the \( X^2 \) values were observed to approach the levels of significance, suggesting that the differences may have reached significance if more samples had been studied.

Two female subjects with oral hygiene degree no. 1 and a low degree of gingivitis had Trichomonas tenax in their dental plaque samples. In one of them, with no carious lesions and only 4 restorative fillings, the repeated samples were negative for Trichomonas tenax. This data appeared to support our hypothesis that a healthy clean mouth, as differentiated from unclean mouth, did not provide favorable anaerobic conditions for the growth and survival of Trichomonas tenax (9–11, 15, 25).
The increase in positive results in the patients with oral hygiene degrees 2 and 3 (40.0% and 46.2%, respectively), mostly in dental plaque, bearing in mind that dental plaque is a synonym for poor oral hygiene, indicated that plaque could be a predominant factor for the growth and persistence of Trichomonas tenax in the physiologic conditions in the human mouth.

Table 3 shows the distribution of Trichomonas tenax according to the site of sampling.

Oral Trichomonas was not found in any of the 51 saliva samples, indicating that saliva does not provide living conditions for Trichomonas tenax.

Likewise, carious dentin is not an oral structure where oral Trichomonas could live either, but because of a small number of samples (14 samples of carious dentin), no positive conclusions could be made.

Trichomonas tenax was recorded in dental plaque samples from clean tooth areas (22.0%), unclean tooth areas (24.5%) and in the root canal contents (30.0%) (Table 3). Dental plaque seems to be an oral structure where oral Trichomonas finds the best environment for its growth and survival in the physiologic conditions of the human mouth.

Dental plaque is a very dynamic ecosystem passing through several phases in its development, starting with the initial adherence phase where microorganisms pioneers adhere to the tooth surface (in this phase, oxygen may get into the plaque - aerobic conditions). Then, the intermediary phase follows, where an intensive struggle among microorganisms for predominance in the life environment takes place (oxygen may still get into the plaque), until a relative plaque metabolism stability in a microbe community is reached (the final phase when oxygen is not available, anaerobic conditions). The final phase lasts until the occurrence of enamel cavitation, whereby the microorganisms' life basis undergoes changes (26).

According to this, we may suppose that a mature dental plaque, the final phase of plaque development being reached, is the best life environment for anaerobic Trichomonas tenax.

This is in accordance with literature data, where Lange et al. (25) found Trichomonas tenax to be most frequently present in dental plaque as compared to other cavity structures.

The dental plaque samples, either from clean or unclean tooth areas, showed the same rate of presence of Trichomonas tenax (22.0% and 24.5%). We are inclined to explain it by clinical impossibility of delimiting a fine line between the clean and unclean tooth areas or by the method of sampling employed in the study. Microbiological tests may be influenced by many factors, such as: sampling methods, cultivating methods and sample amount (23, 28).

The method of dental plaque sampling may influence the results of dental plaque investigation, mostly because of a dynamic plaque metabolism (26, 29).

Lange et al. (30) found Trichomonas tenax in periodontal pockets. We did not examine the presence of Trichomonas in periodontal pockets, but recorded the presence of Trichomonas in dental plaque of the patients suffering from progressive gingivitis to be statistically significantly increased (Table 6), thus supporting the hypothesis that the presence of oral Trichomonas is associated with periodontal diseases.

Table 6. Distribution of Trichomonas tenax in dental plaque according to the grade of gingivitis

<table>
<thead>
<tr>
<th>GSBI</th>
<th>N</th>
<th>Positive</th>
<th>X²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N₀</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>81</td>
<td>16</td>
<td>19.8</td>
<td>4.25</td>
</tr>
<tr>
<td>3-5</td>
<td>16</td>
<td>7</td>
<td>43.8</td>
<td></td>
</tr>
</tbody>
</table>

p < 0.05

Twenty samples of root canal contents were examined. Trichomonas tenax was found in six of them (30.0%). We supposed the root canals of these patients to have been contaminated by Trichomonas tenax from dental plaque. This could be the subject of our future investigations.

In the category of carious teeth, Trichomonas was most frequently observed in the patients free of carious lesions (45.5%).

Similarly, Vrablicova et al. (31) failed to confirm the hypothesis that carious dentin, found in 41.3% of the children examined, stimulated the presence of Trichomonas tenax.

The mean number of carious teeth per patient in Wantland's group of patients contami-
nated with Trichomonas tenax is 1.99%, while in the non-contaminated group it is 1.63% (12).

In our study, the mean number of carious teeth per patient in the contaminated group was 17.74%, and in the non-contaminated group it was 17.41%.

This data and the absence of Trichomonas tenax findings in any of the 14 samples of carious dentin appear to suggest that carious dentin and the number of carious teeth do not influence the presence of oral Trichomonas.

According to Cambone et al. (8), smoking does not influence the presence of Trichomonas tenax. Our results also showed no effect of smoking and coffee intake on the presence of oral Trichomonas.

**Conclusion**

The prevalence of Trichomonas tenax in the study patients was 35.3%. The patient age and sex, oral hygiene, gingivitis, number of carious teeth, smoking and coffee drinking did not influence the presence of Trichomonas tenax.

According to the results of our study, mature dental plaque is an oral structure favoring the growth and survival of Trichomonas tenax in the physiologic conditions in the human mouth. It is possible that Trichomonas tenax spreads from dental plaque into the diseased endodontium.
References


