

## Prevalence of Oral Trichomoniasis in Patients with Periodontitis and Gingivitis Using PCR and Direct Smear

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### Abstract

**Background:** *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. There is only one published study that rebound the prevalence of this parasite in Iran. This PCR based study compared the prevalence of oral trichomoniasis in patients with oral diseases and a healthy control group.

**Methods:** From May 2005 to April 2006, the subgingival dental plaques of 160 patients with gingivitis or periodontitis and 160 controls who attended to Dental School of Shaheed Beheshti Medical University, Iran were taken and examined by wet mount smear, and Giemsa staining. Likewise, a PCR protocol was developed for specific detection of *T. tenax* using a pair of primers designed for its 18S rRNA gene.

**Results:** Thirty three (20.6%) of patients were PCR positive while 28 (15.5%) were diagnosed using wet preparation and Giemsa staining. In the other hand, 2 (1.9%) of control group were identified positive by PCR procedure. The prevalence of oral trichomoniasis in our study (20.6%) was compatible with many other published reports which mostly has ranged from 12%-32%.

**Conclusion:** The study revealed dependence between the frequency of occurrence of *T. tenax* and the state of periodontitis. The present PCR procedure could provide a simple and rapid detection method of *T. tenax* in dental plaque.

**Key words:** *Trichomonas*, *Periodontitis*, *Gingivitis*, *Protozoa*

### 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 (1-3). Transmission is through saliva, droplet spray, and kissing or use of contaminated dishes and drinking water (4, 5). World widely, its prevalence in the mouth ranges from 4 to 53% (6-8).

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 (9-11). Surprisingly in Iran there is only one published article in this regard (12) which shows

a prevalence of 46% for mixed infections with *Endameba 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 not be sensitive enough. In addition, staining is not useful for species identification, and culture techniques are not of routine use (13). In comparison, molecular diagnostic tools have been developed for both the detection and the identification of *Trichomonas* species (14, 15). Amplification of the 18S rRNA gene by PCR followed by sequencing has become a reliable means for more rapid and specific detection and identification of trichomonads.

This study was carried out to determine the prevalence of oral trichomoniasis by a PCR protocol compared with microscopic observation (16).

## Materials and Methods

**Study population** The study included 320 individuals; 160 patients (85 females and 75 males) aged ranged 20-60 yr old with periodontitis or gingivitis who attended to Dental School of Shaheed Beheshti Medical University, Tehran, Iran and 160 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 sent to the Department of Parasitology and Mycology of Shaheed Beheshti Medical University, Tehran, Iran 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.

**DNA extraction** Clinical subgingival plaque samples were collected with sterile curette scrape, dispersed in 100  $\mu$ l of NTE buffer (100mmol NaCl, 20 mmol Tris-HCl with pH 7.4 and 1 mmol EDTA). Total genomic DNA extracted by phenol-choloroform method according to Sambrook (17).

**PCR conditions** In order to identify *T. tenax*, the 18S rRNA gene was amplified directly from dental plaque with the forward and reverse primers TGBK-F and TGBK-R (5'-AGC AGC TGC GGT AAT TCC AG-3' and 5'-CTT GTT ACC ACT TCT CCT TCC-3'), respectively. PCR was carried out in a 30  $\mu$ l reaction mixture containing 2  $\mu$ l of genomic DNA solution, 1.5

mmol MgCl<sub>2</sub>, 100  $\mu$ mol each of dNTPs, 0.4  $\mu$ mol each of two primers (TGBK-F and TGBK-R) and 1 U of *Taq* DNA polymerase (Cinagene Co., Ltd, Tehran, Iran). The specificity of the primers for *T. tenax* was approved by the mentioned manufacture. The reaction was performed in an Automatic Thermocycler (Techne, Thouchgene-Gradiante, Co., Ltd, England) with following cycling parameters: (i) *Taq* activation at 94 °C for 5 min; (ii) 30 cycles of denaturation at 93 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 2 min; and (iii) final extension at 72 °C for 5 min. A volume of 10  $\mu$ l of each PCR product was electrophoresed in a 1% agarose gel. The results were visualized after staining with ethidium bromide in a U.V. transilluminator (UVIdoc Deluxe GAS 9000, England). DNA extraction from clinical specimens and PCR were performed in a protected molecular biology area.

The statistical analysis was performed by the Chi-square test (signification level 0/005) 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 (20.6%) were positive for *T. tenax* by PCR (Fig. 1). Twenty eight (17.5%) of those specimens were detected by wet preparation and Giemsa-stained smears. All the three cases of oral trichomoniasis in control group were both detected by PCR and direct smear (Fig. 2). In present study PCR was used as reference standard. All direct smear positive were PCR positive showing a product of 1054 bp by agarose gel electrophoresis and giving 100% sensitivity (Fig. 3). No PCR negative specimens were positive by any other methods. Five specimens were direct smear negative, PCR positive and remained PCR positive when retested again.

The infection rate among the patient with periodontitis and gingivitis was 40% and 14.1%, 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 (19%) than females (14%) with no significant difference (Table 2, 3). *Entamoeba gingivalis*, the other oral protozoan, was found in 69 (43.1%) among the patients and 11(6.8%) controls by direct observation.

## 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% (1-3, 6-8). Wantland examined 700 patients with periodontitis and found a prevalence of 26.5% (7). Feki in France reported a prevalence of 28% among the 300 patients (3). Mahdi in Iraq examined the saliva of 143 patients with poor oral hygiene and reported a prevalence of 8.4% (18), but further investigation showed that saliva was not a suitable media for detection of parasite (16). In Iran 50 patients with periodontitis were examined by wet mount and 46% were found to be infected by *T. tenax* or *E. gingivalis* (12) but the prevalence of each parasite was not determined. In the most above- mentioned reports, the methods for detection and identification of *T. tenax* from human oral samples have been based on conventional techniques, such as microscopic observation (7) and cultivation (19), 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 (20, 21). Similar to our study, Kikuta in Japan (17) 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 sub-gingival-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 5 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 (10).

The occurrence of *T. tenax* was not correlated with the age in our study and this finding was not agree with some authors (3,7,8) who found that the frequency of infection increased with age, while some were believed that oral protozoa were rarely found in children(6).

According to our experience, Ringer solution was better than normal saline for transportation and maintenance of samples. But Lyone 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 (22).

As in other reports (6, 7, 12, 22), 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.

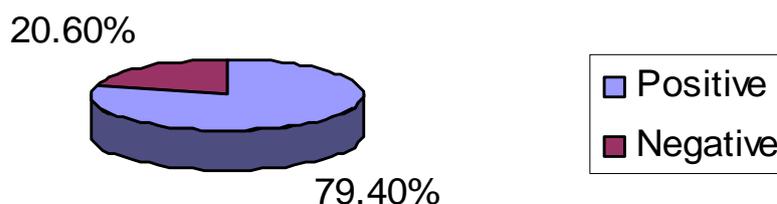
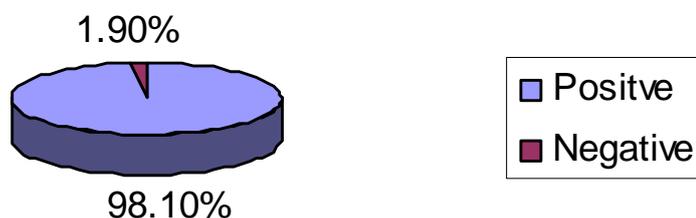
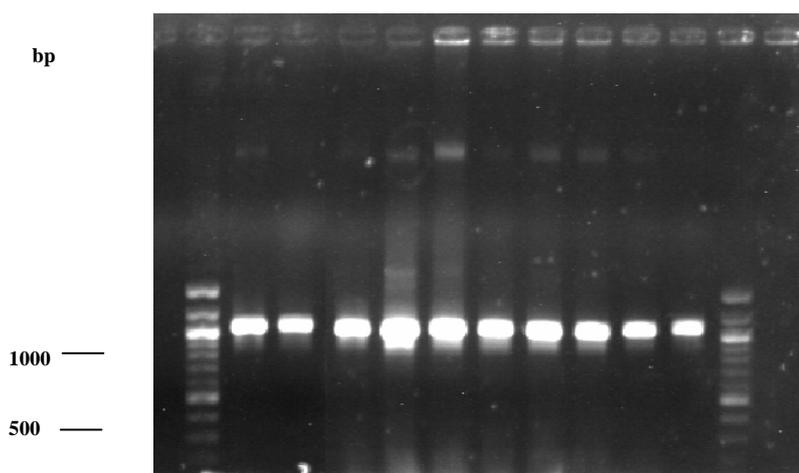


Fig. 1: Detection of *Trichomonas tenax* in 160 patients with periodontal diseases by PCR



**Fig. 2:** Detection of *Trichomonas tenax* in 160 controls by PCR



**Fig. 3:** PCR products on the basis of *Trichomonas tenax* 18S rRNA gene. M: molecular size marker; 1-10, 1054bp band obtained from positive cases; 12, negative control.

**Table 1:** Prevalence of *Trichomonas tenax* according to type of oral disease

Oral Disease	Examined No.	Infected No.	%
Gingivitis	120	17	14.1
Periodontitis	40	16	40
Total	160	33	20.6

**Table 2:** Prevalence of *T. tenax* in patients with periodontal diseases (experimental group) according to age

Age (years)	Examined No.	Positive Cases	%
20-30	46	7	15.2
31-40	68	13	19.1
41-50	29	10	34.5
50<	17	3	17.6
Total	160	33	20.6

**Table 3:** Prevalence of *T. tenax* in patients with periodontitis (experimental group) according to sex

Sex	Examined No.	Positive Cases	%
Male	75	19	25.3
Female	85	14	16.4
Total	160	33	20.6

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