

## Original papers

# Studies on prevalence of infection with *Trichomonas tenax* identified by molecular techniques – in respect to oral health of patients with various systemic disease requiring immunosuppressive therapy

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**ABSTRACT.** *Trichomonas tenax*, cosmopolitan flagellate inhabiting human oral cavity, is the etiological agent of oral trichomonosis associated with gingival and periodontium deterioration. Purpose of this studies was to investigate the prevalence of infection with *Trichomonas tenax* identified by molecular techniques amplifying the region of ITS1-5.8S rRNA-ITS2 specific for *T. tenax*. The study included 498 persons: diabetic, renal transplant, rheumatoid arthritis patients and the control group. Prevalence of *T. tenax* in oral cavity was 10.2% in control group, 14.1% in diabetics, 12.0% in renal transplant patients and 14.0% in rheumatoid arthritis patients. Comparative assessment of results showed symptoms of gingiva and periodontium deteriorations, at varying intensity in patients with various systemic diseases; higher prevalence of the trichomonad infection was revealed in adults in all groups. Simultaneously, renal transplantation, diabetes, rheumatoid arthritis and related therapy do not affect *T. tenax* incidences and no increased risk of the infection has been observed in the patients; the permanent medication used due to main disease should be taken into consideration as likely inhibitory factor.

**Key words:** *Trichomonas tenax*, PCR, diabetic, kidney transplant, rheumatoid arthritis patients, immunosuppression

## Introduction

*Trichomonas* species belong to the flagellates, evolutionary primitive protists, family Trichomonadidae (Wenyon, 1926). Trichomonad parasites generally inhabit the gastrointestinal tract (intestines, oral cavity), but also other systems such as urogenital or respiratory tract. They occur in invertebrate (leeches, molluscs, insects) and vertebrate animals (fish, amphibians, reptiles, birds, mammals). This group is very diverse in terms of location – the parasitism location as well as host species and pathogenicity.

*Trichomonas tenax* is a cosmopolitan flagellate, typically inhabiting human oral cavity, but also, not

specifically, other organs and tissues [1–13]; outside oral cavity, the protozoan was detected in lymph nodes, submaxillary glands, tonsils, bronchi, lungs, mammary gland and liver. Most of the patients with less frequent location of the trichomonad infection had decreased immunity due to chronic diseases and transplant surgery. The occurrence of *T. tenax* in the oral cavity of patients with systemic disorders, genetic diseases, HIV/AIDS, rheumatoid arthritis, and renal transplant has been described [14–21]. In these patients, the functions of the immune system were impaired due to the main disease and as a result of steroid and/or cytostatic therapy; moreover, the immunosuppression can lead to opportunistic parasitic diseases. Long-term use of drugs is often a

cause of changes in the periodontium and affects the condition of the oral mucosa.

Although in some literature data *T. tenax* is still discussed as a commensal, high proteolytic and collagenolytic activity of this flagellate accounts for its destructive effect on oral mucous membrane and tissues. Presence of many proteolytic enzymes in *T. tenax* cells, affecting pathogenicity, has been described [22–25]; the ability of the protist to deteriorate oral cavity status tied with its proteolytic activity is considered to be the proof of the pathogenic nature of the species.

The aim of the study was to assess the prevalence of infection with *T. tenax* identified by molecular techniques – in respect to oral health of patients with various systemic diseases: diabetes, with renal transplant and rheumatoid arthritis requiring immunosuppressive therapy.

## Materials and Methods

The study included 498 persons: 261 women and 157 men aged from 6 to 82 years categorized into four groups: diabetic, renal transplant, rheumatoid arthritis patients and the control group. Each person filled in a questionnaire including information on age, sex, oral and general health.

Diabetic patient group included 92 people: 54 women and 38 men aged 26 to 82, taking insulin *i.v.* or *p.o.* medication (Glucobay, Diabrezide, Diaprel).

The kidney allograft group included 50 patients: 21 women and 29 men aged 20 to 70 years. The kidney transplant was done between 4 weeks and 23 years before the material was taken. All patients received immunosuppression (cyclosporin A, Immuran, CellCept, Encorton).

The rheumatoid arthritis group involved 50 patients: 43 women and 7 men between 26 and 77 years of age receiving immunosuppressive and/or immunomodulatory drugs (Encorton, cyclosporin A, Endoxan). The duration of the disease ranged from one year to 26 years.

Control group included 226 generally healthy persons, not taking any drugs: 144 women and 82 men aged from 6 to 76 years.

**Collection of material from oral cavity of different groups of individuals.** The material from the oral cavities included in this analysis was collected by dentists and performed in accordance with the tenets of the Declaration of Helsinki. Medical history was gathered, including age, gender, course of disease and treatment of the

patient. The material was taken in the morning, before brushing and before eating, so as not to pollute the samples with food. The material taken were washings from the mouth. 5ml of PBS at pH 6.8 was administered orally. After 15 seconds of thorough rinsing of the mouth, the liquid was used to isolate the DNA.

**DNA extraction.** 100 µl of the washings were centrifuged at 5000×g for 10min. The pellet was washed twice in PBS, centrifuged at 5000×g for 10 min and pellet was dissolved in 100 µl PBS. The genomic DNA was extracted using a NucleoSpin kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions.

**PCR conditions.** The region of ITS1-5.8S rRNA-ITS2 of different trichomonad species sequences registered in GenBank served for the specific *T. tenax* primer design:

T1 (5'-GAGAAGTCGTAACAAGGTAACG-3'), T2 (5'-ATGCTTCAGTTCAGCGGGTCT-3') (ARK Scientific Pte Ltd, Maxwell House, Singapore). PCR reactions were performed in a volume of 50 µl. Reaction mixture consisted of 1µl DNA, 10pM of each primer, 0.2 mM of each dNTP, 2.5 mM MgCl<sub>2</sub> and 1 U Taq DNA polymerase (Qiagen, Hilden, Germany). PCR was performed in PTC-200 thermal cycler (MJ Research, Waltham, USA) in the following conditions: initial denaturation for 5 min at 94°C; 35 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 60°C, extension for 45 s at 72°C. The PCR products were analyzed under UV light in 2% agarose (MetaPhor, FMC BioProducts, Philadelphia, USA) gel stained with ethidium bromide.

## Results

DNA extracted from all patients was used as the template in separate PCRs to amplify region of ITS1-5.8S rRNA-ITS2. The product size specific for *T. tenax* was 368 bp. The positive results showing *T. tenax* product by amplification of ITS1-5.8S rRNA-ITS2 region are set together in Table 1.

Thirteen diabetic patients positive for *T. tenax* were at the age 32–76 years; among these 9 men and 4 women most were treated with insulin except one man getting Diaprel. Advanced dental caries, periodontitis, gingivitis and tooth loss were noticed most commonly in these patients. Kidney transplant patients infected with *T. tenax* were 4 women and 2 men at the age 41–62 (mostly 49–53) years; all were treated with Encorton and cyclosporin A. Three

Table 1. The prevalence of *T. tenax* identified by molecular techniques in oral cavities of different investigated groups

Group	No. examined F and M/total no. examined	No. infected F and M/total no. infected	Prevalence (%)
Control	144 F, 82 M/226	21 F, 12 M/33	14.6
Diabetics	54 F, 38 M/92	4 F, 9 M/13	14.1
Kidney allograft recipients	20 F, 30 M/50	4 F, 2 M/6	12.0
Rheumatoid arthritis patients	43 F, 7 M/50	5 F, 2 M/7	14.0

Explanations: F – women; M – men

patients received kidney transplant in a period of 4 weeks to several months, the other 3 patients – in a period of 3, 5 and 23 years. The conditions of their oral cavities showed dental plaque, advanced gingivitis and low value of bleeding index.

Seven patients with rheumatoid arthritis (5 women and 2 men) diagnosed positive for *T. tenax* were 53–73 years old and all received Encorton; the oral status was relatively good with low bleeding index, but also tooth loss was observed.

In the control group, 21 women and 12 men aged 48–72 years were found *T. tenax* positive.

## Discussion

The assessment of results of our study has confirmed some earlier observations about the higher prevalence of *T. tenax* in adults [19–20]. Individuals under 40 years of age usually have not been infected, despite the fact of the long term use of immunosuppressive drugs, e.g. for 7 years. The exception is 2 diabetes patients, aged 32 and 35. Similarly in the control group, the prevalence of *T. tenax* was in the same age range.

The condition of oral cavity of patients with rheumatoid arthritis and after kidney transplantation was assessed as relatively good. Probably this is related to the anti-inflammatory effects of the drugs applied. These patients were treated with Encorton and cyclosporin A. It is important to consider the fact, that medications used for treatment of each patient group, could have a direct impact on a relatively low rate of *T. tenax* infection. Parasitic infection is the effect of many interactions between the parasite and the host organism, where the common area of influence is the immune response. Cytotoxic effects of cytostatic drugs at their appropriate concentration in blood and saliva may lead to at least partial elimination of *T. tenax*. In

numerous experimental works it has been shown that, for example, cyclosporin A is toxic to many species of parasites; moreover, the drug is considered as modulator of the host-parasite relationship [26–28]. In both patient groups low value of bleeding index was observed, but some of them demonstrated high dental plaque, teeth loss and gingivitis.

The condition of oral cavities in course of diabetes differed significantly, as more symptoms, such as advanced gingival bleeding, dental caries, periodontitis, gingivitis and tooth loss were noticed. In diabetes mellitus the hyperglycemic environment induces immune dysfunction and higher frequency of infectious diseases and oral lesions in diabetic patients is observed, e.g. *Candida* colonization.

Higher *T. tenax* incidence in all studied patients was revealed in comparison with generally healthy patients of control group with a proper immune system (12.0–14.1%, and 10.2%, respectively). However, these prevalence differences are not very significant; similar results were obtained in other studies conducted on similar groups of patients, as well as in other diseases [14–16,18–21]. In our study, *T. tenax* infections in patients with kidney transplant occur with similar prevalence, both in the early post-transplant period, as well as in patients, who received transplant several years ago, in one case up to 23 years. According to epidemiological studies, trichomonosis is associated mainly with periodontitis, and the highest incidence of infections is found in humans with periodontitis [1,29–31].

The results obtained in our research shows that *T. tenax* infection is often asymptomatic, rather than a typical opportunistic disease. Specific opportunistic effect of parasitic disease along with reduced immunity would be observed in cases manifesting *T. tenax* invasion outside oral cavity e.g. to lungs in a case of a patient with cancer, treated with steroids,

chemotherapy and radiotherapy [4].

In conclusion, comparative assessment of results obtained in our study indicated that the frequency of infection with *T. tenax* differ depending on the human age, with the higher prevalence of it in adults; no trichomonads were found in persons under 32 years of age. All infected with *T. tenax* showed symptoms of gingival and periodontium deteriorations, at varying intensity in particular groups.

Serious systemic diseases in patients analyzed required permanent medication which influenced on the oral cavity state. Higher *T. tenax* prevalence was revealed in patients after kidney transplantation, with diabetes and rheumatoid arthritis – in comparison with generally healthy person of control group with a proper immune system; however, the differences were not significant. It should be taken into account that permanent medications used for treatment of each patient group, could have a direct inhibitory effect on a relatively low rate of *T. tenax* infections thus no increased risk of oral trichomonosis would be observed.

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