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Prevalence and Risk Factors of Oral Cavity Protozoa (*Entamoeba gingivalis and Trichomonas tenax*) among Patients with Dental Cavity Caries

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ABSTRACT

Background: The aim of this study was to evaluate and comparison the prevalence of oral cavity protozoa (Entamoeba gingivalis and Trichomonas tenax) in patients with dental cavity caries referring to Khorramabad Dental faculty, Lorestan Province, Iran.
Methods: This case-control study was done on 140 patients referring to Khorramabad Dental faculty with at least one decayed tooth (from all classes of caries, especially class 2) who needed to be restored were included in the study. The collected samples (saliva, dental plaque, and rotten dentin cavities.) were smeared on a glass slide, then were stained with Giemsa stain and examined under a light microscope. A questionnaire containing demographic data such as age, gender and residence, as well as some risk factors such as smoking, use of toothbrush, and dental floss were completed.
Results: Out of the 140 patient, totally 39 (27.85%) patient were found positive for oral cavity parasites including 22 (15.4%) patients for E. gingivalis, 15 (10.7%) patients for T. tenax, and also 2 (1.4%) patients for both parasite. There was no significant association between prevalence of E. gingivalis and T. tenax and age, education, residence, and smoking; however a significant association was found between the prevalence these oral cavity parasites and gender (male) dental flossing and teeth brushing ($p<0.05$).
Conclusion: The obtained results of the present investigation showed the high prevalence of E. gingivalis and T. tenax in patients with dental cavity caries; which suggests that these parasites can contribute to the development of cavity caries in the teeth. However, attention to oral and dental health standards, especially brushing and regular flossing, can prevent people from getting these parasites.

Key words: *Entamoeba gingivalis, Trichomonas tenax*, Tooth decay, Lorestan, Iran

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INTRODUCTION

Tooth decay (TD) is a condition in which the hard tissue of the tooth (enamel and dentin) is lost due to the loss of minerals, calcium and phosphorus as a result of acid secretion from cariogenic bacteria [1] or disappears because of a diet rich in fermentable carbohydrates [2]. Due to its multifactorial nature, it is difficult to eradicate the tooth decay [3]; therefore, the treatment is only possible by replacing the hard tissue with the dental restorative material [1]. If the tooth crown decay is not stopped and replaced at the root of the teeth, root canal treatment or neuropathy is needed and even in more severe cases it may be necessary to remove the tooth from the mouth. Most carious lesions occur in adults at proximal surfaces of posterior teeth [4].

TD is the most common oral health problem [5,6], and is currently abundant and distributed is different in several parts of the world [7]. World Health Organization (WHO) report shows the increasing prevalence of teeth caries in developing countries and its decline in developed countries [8]. However, tooth decay is one of the major issues in the field of oral health in industrialized countries, which affects 60% to 90% of school children and often adults [9]. There are several factors that cause teeth caries, but in a general category, there are four main causes of sugary matter, natural resistance to

disease, the time it takes to destroy hard surfaces and microbial pathogens [1,10,11].

Entamoeba gingivalis is an oral commensal organism that found in the interdental space, the depth of tonsils cysts, bacterial plaques, crevicular fluid, and saliva. Although there is still no sufficient evidence of its pathogenicity, However, some researchers have called it an organism responsible for dental caries, and some of them consider it an oral bad smell agent. There are up to 95% of the infection with *E. gingivalis* in people with low oral hygiene [12-16].Another protozoa that found in poor oral hygiene conditions, as well as periodontitis disease is *Trichomonas tenax*. The frequency of this parasite is estimated between 4% and 53% [11].The transmission of these protozoa occurs through the dispersal of oral secretions, the use of joint dishes, contaminated hands, dental instruments and kissing [17,18]. Previous studies have shown that these two protozoa have very high proteolytic and collagenolithic enzymes. Currently, one of the relatively strong theories about dental caries is that proteolytic enzymes solve enamel proteins and cause apatite crystals to lose their strength and eliminate tooth tissues. In addition, it seems to play the role of the shelter of pathogenic anaerobic pathogens [15]. Accordingly, the aim of this study was to evaluate and comparison the prevalence of oral cavity protozoa (*E. gingivalis* and *T. tenax*) in patients with dental cavity caries referring to Khorramabad Dental faculty, Lorestan Province, Iran.

METHOD

Participants

This cross-sectional descriptive study was done on 140 patients referring to Khorramabad Dental faculty with at least one decayed tooth (from all classes of caries, especially class 2) who needed to be restored were included in the study. All immunocompromised people and people who have taken anti-parasitic drugs recently were excluded from study.

Sample collection and microscopic examination

In this study, after obtaining written consent and before any treatment action, three specimens using sterilized swabs were collected including, saliva, dental plaque, and rotten dentin cavities.

The dental plaque specimens were obtained by means of scraping the area with sterile swab rubbed around the surface of teeth from caries. In addition, the samples of rotten dentin cavities were collected by dental drills from tooth decay cavities. The collected samples were smeared on a glass slide, then were stained with Giemsa stain and examined under a light microscope.

Questionnaire

For each person, a questionnaire containing demographic data such as age, gender and residence, as well as some risk factors such as smoking, use of toothbrush, and dental floss were completed.

Statistical analysis

Data were analyzed by using SPSS software 22.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were informed in terms of percent and mean. Chi-square test was applied to assess the univariate association between independent variables and outcome. Multifactorial logistic regression models were utilized to determine association between positivity and the potential risk factors. $P<0.05$ was described statistically significant.

RESULTS

Participants

In this cross-sectional investigation, 140 patients referring to Khorramabad Dental faculty with at least one decayed tooth were studied to evaluate the prevalence of *E. gingivalis* and *T. tenax* and the associated risk factors among them. The mean age of the children was 35.06 ± 11.23 years; so that the minimum and maximum age was 16 and 66 years, respectively. Most of the participants were female (82, 58.6%). Amongst the patients, 119 patients (85%) lived in urban areas, nonetheless the 21 (15%) living in rural areas. In term of education, 63 (45%) patients were under diploma; while the rest were diploma and above. Among the patients, smoking was observed in 36 (25.7%) of them. The results also exhibited that out of 140 patients, 23 (16.4%) and 90 (64.3%) patients were used the dental floss and toothbrush (Table 1). Prevalence and risk factors of *E. gingivalis* and *T. tenax*

Out of the 140 patient, totally 39 (27.85%) patient were found positive for oral cavity parasites including 22 (15.4%) patients for *E. gingivalis*, 15 (10.7%) patients for *T. tenax*, and also 2 (1.4%) patients for both parasite (Table 1).

Table 1: Demographic characteristics and the prevalence of *E. gingivalis* and *T. tenax* among the patients with dental cavity caries referring to Khorramabad Dental faculty, Lorestan Province, Iran

Variables	No. (%)	Positivity for parasite	*P* value
Gender			
Male	58 (41.4)	23 (39.7)	<0.001*
Female	82 (58.6)	16 (19.5)	-
Age groups			
<30yrs	61 (43.6)	18 (29.5)	-
≥ 30yrs	79 (56.4)	21 (26.6)	-
Residential place			
Urban	119 (85)	33 (27.7)	-
Rural	21 (15)	6 (28.6)	-
Education			
<Diploma	63 (45)	19 (30.1)	-
≥ Diploma	77 (55)	20 (25.6)	-
Smoking			

No	36 (25.7)	10 (27.7)	-
Yes	104 (74.3)	29 (27.9)	-
Flossing			
No	117 (83.6)	35 (30.8)	<0.001*
Yes	23 (16.4)	14 (13.4)	-
Brushing			
No	50 (35.7)	20 (40)	<0.001*
Yes	90 (64.3)	19 (21)	-

*P<0.05, difference is statistically significant

Results also showed that the prevalence of *E. gingivalis* and *T. tenax* was higher in male patients (23, 39.7%) compared with female patients (p<0.01); however no significant difference was observed in the prevalence these parasite in term of age. By residence, from 119 patients, who living in urban areas, 33 (27.7%) were found positive for *E. gingivalis* and *T. tenax*; while from 21 patients that lived in rural regions, 6 (28.6%) children tested positive for parasites. Therefore, there was no significant difference in the prevalence of *E. gingivalis* and *T. tenax* among the patients, who living in urban in comparison with those living in rural areas.

In term of education, among patients with high school diploma and less than diploma, 20 (25.6%) and 19 (30.2%) were found positive for *E. gingivalis* and *T. tenax*, respectively. Although the prevalence was higher than patients with less education than diploma; however no significant difference was observed.

The obtained findings also demonstrated that among the patients who smoked (29, 27.9%) and those who did not smoke (10, 27.7%), no significant differences were observed in the prevalence of *E. gingivalis* and *T. tenax.*

Out of the 117 patients, who did not use the dental floss, 36 (30.8%) patients were found positive for *E. gingivalis* and *T. tenax*; whereas among 23 patients, who use the dental floss, 3 (13.4%) were found positive for *E. gingivalis* and *T. tenax*. The statistical analysis demonstrated that there was a significant difference (p<0.001) was observed in the prevalence of *E. gingivalis* and *T. tenax* among these two groups of patients.

Among the 90 patients, who brushed their teeth, 19 (21.1%) patients were found positive for *E. gingivalis* and *T. tenax*; whereas among 50 patients, who did not brushed their teeth 20 (40%) patients were found positive. There was a significant (p<0.001) in the prevalence of *E. gingivalis* and *T. tenax* among these two groups of patients.

Based on the multifactorial logistic regression models, some risk factors including gender, the use of dental floss and teeth brushing were significantly related to positivity to *E. gingivalis* and *T. tenax* (Table 2).

Table 2: Logistic regression analysis of the potential factors associated with parasite positivity among the patients with dental cavity caries referring to Khorramabad Dental faculty, Lorestan Province, Iran

Variables	OR (95%CI)	*P* value
Gender		
Male	4.8 (1.7-9.9)	0.000*
Female	1	-
Flossing		
No	5.3 (1.9-10.6)	-
Yes	1	0.000*
Brushing		
No	4.6 (1.7-9.7)	-
Yes	1	0.000*

*P<0.05 was statistically significant

DISCUSSION

Nowadays, it has been proven that oral cavity infections might be related with considerable morbidity. Reviews have also shown that these infections may result in some serious problems such as cardiac and kidney disease, as well as diabetes [19]. Here we aimed to evaluate the prevalence of and associated risk factors of oral cavity protooa (*E. gingivalis* and *T. tenax*) in patients with dental cavity caries referring to Khorramabad Dental faculty, Lorestan Province, Iran.

Our findings demonstrated that totally 32 (22.85%) patients were found positive for oral cavity parasites including 17 (12.4%) patients for *E. gingivalis*, 13 patients for *T. tenax* (9.3%), and also 2 (1.4%) patients for both parasite.

So far, many studies have been conducted on the prevalence of *E. gingivalis* and *T. tenax* in patients with various oral and dental diseases. In a study conducted by Zakir et al. [20] in North West frontier Province of Pakistan demonstrated that the prevalence of *E. gingivalis* and *T. tenax* was 57 and 3.3%, respectively [20]. In the other study conducted by Ibrahim and Abbas [21], it has been shown that the prevalence of *E. gingivalis* (70%) and *T. tenax* (60%) in dental plaque of patients with periodontitis as well as in saliva of patients with gingivitis (*E. gingivalis* 60%, *T. tenax* 46.6%) was significantly higher than health subjects [21].

In Iran, Athari et al. [22] have reported the prevalence of *T. tenax i*n patients with periodontitis and gingivitis was 20.6% by PCR and 15.5% direct smear [22]. Maraghi et al. [23] have also studied the prevalence of *E. gingivalis* and *T. tenax* in patients with gingivitis and periodontitis referring to Dental Centers of Ahvaz University of Medical Sciences. They were reported *E. gingivalis* (0.5%) just in one patient; whereas no *T. tenax* infection was found [23]. Moreover, Gharavi, et al. [15] have

demonstrated that the prevalence of *E. gingivalis* and *T. tenax* in patients referred to the Faculty of Dentistry in Tehran was 41.7 and 9.2%, respectively [15].

Here we found that there was no significant association between age and the prevalence of *E. gingivalis* and *T. tenax*. In line with our results Gharavi et al. [15] and Abualqomsaan et al. [24] have reported no significant association between age and prevalence these oral cavity parasites [15,24].

The results of the present study demonstrated that the prevalence of *E. gingivalis* and *T. tenax* was significantly higher in male patients; similarly, in studies conducted by Ibrahim and Abbas [21] and Al-Khayat [25] in Iraq, the prevalence of these oral cavity parasites was significantly higher in male patients. This is probably due to the increased attention of women to the mouth and teeth [21,25]. Although in our study there was no significant association between prevalence of *E. gingivalis* and *T. tenax* and education, residence, and smoking; however a significant association was found between the prevalence these oral cavity parasites and dental flossing and teeth brushing; indicating that observing oral and dental health by regular brushing and flossing can prevent people from infection by these parasites.

CONCLUSION

The obtained results of the present investigation showed the high prevalence of *E. gingivalis* and *T. tenax* in patients with dental cavity caries; which suggests that these parasites can contribute to the development of cavity caries in the teeth. However, attention to oral and dental health standards, especially brushing and regular flossing, can prevent people from getting these parasites.

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