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## Original Article

# Molecular Epidemiology and Associated Risk Factors of Parasites in Oral Cavity of Children with Malignancies in Western Iran

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Received 17 Dec 2022  
Accepted 14 Mar 2023

**Keywords:**  
*Entamoeba gingivalis*;  
*Trichomonas tenax*;  
Mouthwash; Malignancy

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### Abstract

**Background:** This survey was designed to study the molecular epidemiology and risk factors of *Entamoeba gingivalis* and *Trichomonas tenax* in children with underlying malignancies and those on chemotherapy in Lorestan province, West of Iran.

**Methods:** The present cross-sectional descriptive study was performed on children who suffering from different types of malignancies or receiving treatment by chemotherapy referring to oncology section of hospitals of Lorestan Province, Iran during May 2021 to April 2022. The frequency of oral cavity protozoa was investigated using microscopic and conventional polymerase chain reaction (PCR).

**Results:** *E. gingivalis* and *T. tenax* parasites were found in 23 (25.5%) by microscopic method and 28 (31.1%) using PCR in children with malignancy. Among positive samples, 20 (71.4%) were infected with *E. gingivalis*; whereas 8 (28.6%) of the participants were positive for *T. tenax*. In the multivariate model, living in rural regions (OR= 3.437; 95% CI= 1.22-9.63; p=0.019) and using mouthwash (OR= 0.082; 95% CI= 0.018-0.37; p<0.001) were significantly related with the frequency of oral cavity parasites.

**Conclusion:** Our results showed the high frequency of oral cavity parasites in children who suffering malignancies or receiving treatment by chemotherapy in Lorestan province, Iran. The awareness of the main risk factors for oral cavity parasites particularly using mouthwash is necessary in improving public and oral health strategies in children with cancer. Consequently, oncologist and dental practitioners must be aware to identify and manage oral health concerns in children who suffering from different types of malignancies to prevent the oral diseases and infections.



## Introduction

Adults are more likely to develop solid organ cancers, while leukemia and brain tumors are the most common malignancies in children (1). Leukemias and embryonic tumors such as neuroblastoma, Wilms tumor and retinoblastoma and liver tumors are more common in infancy and early childhood, and Hodgkin's disease and genital malformations and bone tumors are more common in adolescence (2). Today, it has been proven that malignancies, cancers and their drug therapies result in some immune system disorders and predisposes people to many infectious diseases (3). Bacterial, viral, or parasitic infection are observed about 20% of neoplasm patients around the world (4). Chemotherapy and therapeutic radiation for cancer treatment are also associated with some side effects such as immunological disorders (e.g. neutropenia, impaired humoral and cellular immunity) and disrupt the oral mucosa which leading to severe bacterial, viral, and parasitic infections (5, 6).

The human oral cavity contains numerous microorganisms so that a set of different bacterial and viral agents, like the oral microbiome, live inside the gum plaques (7). *Entamoeba gingivalis* and *Trichomonas tenax* are considered as anaerobic protozoan parasites observed in human oral cavity (8). Since, these parasites have no cyst forms; thus, they can transmit between individuals, generally through saliva, kissing or contaminated dishes, food, and drinking water, toothpicks, gum or other utensils (8, 9). Both *E. gingivalis* and *T. tenax* are oral protozoa that live near teeth, on dental plaque, gums and even tonsils, which can be an indicator of oral health status (10). *T. tenax* could enter the respiratory tract through respiration and cause pulmonary trichomoniasis (11). In addition, investigations revealed that *E. gingivalis* might be observed in the progress of periodontal disease, osteomyelitis as

well as infections caused by the intrauterine contraceptive device (12).

Considering the prevalence of these parasites, although several studies have been conducted in children with in people with periodontitis and gingivitis, pregnant women, patients undergoing chemotherapy (13-15); nevertheless, the frequency of these oral protozoa and their related risk factors in pediatric oncology children has not been studied. Hence, we aimed to study the molecular epidemiology and risk factors of *T. tenax* and *E. gingivalis* in children with underlying malignancies and those on chemotherapy in Lorestan Province, West of Iran.

## Materials and Methods

### Ethical statement

This work was reviewed and approved by Ethical Committee of Lorestan University of Medical Sciences, Iran (IR.LUMS.REC.1400.317). However, a written informed consent form was obtained from the parents of all participant.

### Participants

The present cross-sectional descriptive study was performed on children who suffering from different types of malignancies or receiving treatment by chemotherapy referring to Oncology Section of hospitals of Lorestan Province, Iran during May 2021 to April 2022. Participants who had taken systemic antibiotics in the last three month and immunocompromised patients were discarded.

### Questionnaire

Before sampling, a provided questionnaire with a number of demographical information and related risk factors, e.g. age, gender, residence, parent education, toothbrush, mouthwash, was completed for each patient.

### Sample collection

Two specimens were obtained from each patient by means of sterile swabs from saliva and dental plaques for microscopic examinations. In addition, the third sample was put into a tube with sterile physiological saline following growth of the trophozoite in the culture for molecular tests.

### Microscopic examination

The saliva and dental plaque specimens were smeared on a glass slide, and after staining by Giemsa and trichrome staining technique, they were examined by means of a light microscope (16).

### Polymerase Chain Reaction (PCR) assay

Qiagen kits was used to extract DNA from all specimens based on the protocol of producer. The extracted DNA was applied to amplify of the SrRNA gene for *E. gingivalis* using the primers of forward (5'-GCGCATTTCGAACAGGAATGTAGA-3') and reverse (5'-CAAAGCCTTTTCAA-TAGTATCTTCATTC-3') (17, 18) as well as 18S ribosomal RNA gene for *T. tenax* using the primers of forward (5'-ATGACCAG-TTCCATCGATGCCATTC-3') and reverse (5'-CTCCAAAGATTCTGCCACTAACAAG-3') according the previous study (13). The thermal condition was 6 min at 93 °C for early denaturation, 35 cycles of 30 sec at 93 °C, 30 sec at 57 °C, 60 sec at 72 °C, and a last extension phase of 10 min at 72 °C. Then the obtained amplicons using agarose gel (1%) were electrophoresed. The size of the PCR bands for the *E. gingivalis* and *T. tenax* was 454 and 496 bp, respectively. Positive and negative controls were DNA of standard strains and distilled water, respectively (17, 18).

### Statistical analysis

The analysis of the collected data was performed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Chi-square-test, Fisher exact, univariate and multivariate regression analysis tests were applied to assess

the relationship among the variables and the frequency of oral cavity protozoan parasites (*E. gingivalis* and *T. tenax*).

## Results

### Participants

Ninety pediatric oncology children with the mean age of the  $9.23 \pm 5.2$  years were recruited in this study. The most of participants were male participants (48, 53.3%); while the most of participants lived in urban regions (54, 60.0%) and the rest lived in rural region. By age, most of participants 57 (63.3%) were lower than 10 years old. By parent education, 75 (83.3%) of parents had higher education than diploma, while 15 (16.6%) participants had lower education than diploma. By brushing teeth, 52 (57.8%) children brushed their teeth daily. Furthermore, mouthwash was used by just 32 (35.5%) of participants, respectively (Table 1).

### Prevalence of oral cavity protozoan parasites

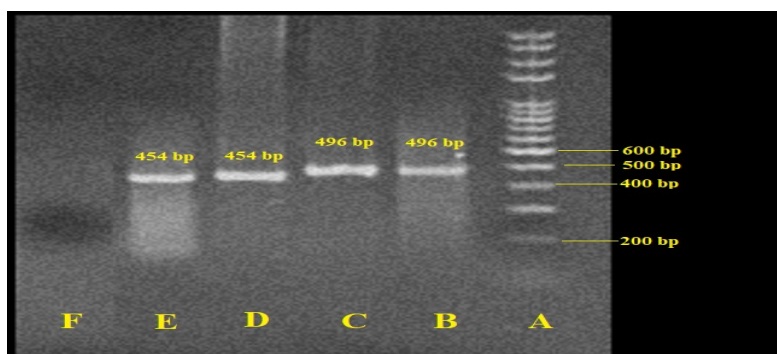
*E. gingivalis* and *T. tenax* parasites were found in 23 (25.5%) by microscopic method and 28 (31.1%) using PCR in children with malignancy. Among positive samples, 20 (71.4%) were infected with *E. gingivalis*; whereas 8 (28.6%) of the participants were positive for *T. tenax*. Figure 1 shows the gel electrophoresis of the typical *E. gingivalis* and *T. tenax* isolates obtained from children with malignancy.

### Risk factors

The findings of the chi-square analysis showed, there was no significant relationship between age ( $P=0.349$ ), gender ( $P=0.670$ ), parent education ( $P=0.769$ ), brushing teeth ( $P=0.170$ ) and prevalence of oral protozoa in pediatric oncology children. However, a significant correlation was observed between living in rural regions ( $P=0.02$ ), use of mouthwash ( $P=0.784$ ) and prevalence of oral protozoa in pediatric oncology children. Table 2 shows the prevalence of oral cavity protozoa

in pediatric oncology children in Lorestan Province, western Iran according to the demographic features and related risk factors. In the multivariate model, living in rural regions

( $P=0.022$ ) and brushing teeth ( $P=<0.001$ ) were significantly related with the frequency of oral cavity parasites.



**Fig. 1:** Agarose gel electrophoresis of SrRNA and 18s rRNA genes for *Entamoeba gingivalis* and *Trichomonas tenax*, respectively. A: Ladder (size marker), 100 bp; B: positive control (*T. tenax*, 496 bp); C: positive sample of *T. tenax*; D: positive control (*E. gingivalis*, 454 bp); E: positive sample of *E. gingivalis*; E: negative control (distilled water)

**Table 1:** Frequency of oral cavity protozoa (*E. gingivalis* and *T. tenax*) in pediatric oncology children from Lorestan province, western Iran according to the demographic features and related risk factors by univariate regression analysis

Group		Totally	Oral cavity parasites		P value Chi-Square	Crude OR	95%CI	P value
		No. (%)	Positive No. (%)	Negative No. (%)				
Age (yr)	<10	57 (63.3)	20 (35.1)	37 (64.9)	0.349	1	1	-
	≥10	33 (36.6)	8 (24.8)	25 (75.2)	-	0.592	0.22-1.55	0.287
Parent education	<diploma	15 (16.6)	4 (26.7)	11 (73.3)	-	1	1	-
	≥diploma	75 (83.3)	24 (32.0)	51 (68.0)	0.769	0.773	0.22-2.67	0.684
Residence	Rural	36 (40.0)	6 (16.7)	30 (83.3)	-	1	1	-
	Urban	54 (60.0)	22 (40.7)	32 (59.3)	0.02	3.437	1.22-9.63	0.019*
Gender	Male	48 (53.3)	14 (29.2)	34 (70.8)	-	-	-	-
	Female	42 (46.7)	14 (33.3)	28 (66.7)	0.820	1.214	0.49-2.98	0.670
Brushing	Yes	52 (57.7)	13 (25.0)	39 (75.0)	-	-	-	-
	No	38 (42.3)	15 (39.5)	23 (60.5)	0.170	0.511	0.20-1.26	0.146
Mouthwash	Yes	32 (35.6)	2 (6.3)	30 (93.8)	-	-	-	-
	No	58 (64.4)	26 (44.8)	32 (55.2)	<0.001	0.082	0.018-0.37	<0.001*

**Table 2:** Comparison of oral cavity protozoa (*E. gingivalis* and *T. tenax*) in pediatric oncology children from Lorestan province, western Iran based on the associated risk factors by multivariate regression analysis

Group	Crude OR	95%CI	P value
Age(yr)	1.503	0.467-4.837	0.494
Parent education	2.183	0.490-9.721	0.306
Residence	0.349	0.076-0.816	0.022*
Gender	1.261	0.414-3.842	0.684
Brushing	1.699	0.587-4.918	0.328
Mouthwash	13.01	2.67-63.34	<0.001*

\*  $P < 0.05$ , difference was statistically significant

## Discussion

Pediatric cancer as a stressful event can jeopardize various aspects of the physical, mental and social health of the child and family (19). Prevalence of childhood cancers accounts for 0.5-6.6% of all cancers, and in Iran, about 2.5% of children have cancer (20). This debilitating and common disease is the second leading cause of death of children aged 1-14 years in developed countries and is currently developing; whereas the overall incidence of cancer among Iranian children for girls and boys is reported to be 112-48 and 144-51, respectively, per one million people (21). The most common cancers in children are blood cancers, followed by cancers of the lymph nodes and central nervous system (20, 21). In recent years, due to advances in the treatment of various cancers, the survival rate in children with cancer has increased, and this has led to an increase in the need for parental care at home and at the time of your illness (22).

Since cancer and its therapeutic approaches such as chemotherapy and radiotherapy can lead to some side effects such as immunological disorders (e.g. neutropenia, impaired humoral and cellular immunity) and disrupt the oral mucosa which leading to severe bacterial, viral, and parasitic infections; therefore, oral care in cancer patients should be given special attention (5, 6).

Selecting the appropriate diagnostic method is definitely a very important step in epidemio-

logical studies. Detection of *T. tenax* and *E. gingivalis* microorganisms has previously been based on conventional methods such as direct microscopic observation and culture, which, although very rapid, do not produce very reliable results (23). In contrast, today, with the development of molecular methods, PCR is used to identify which has a very high sensitivity and specificity and can determine the presence or absence of microorganisms in a short time and in a small sample (24).

With respect to the prevalence of oral cavity parasites in Iran, in a study conducted on 315 adolescents in Kerman province by culturing and PCR, the findings exhibited that by culture media and PCR the oral cavity parasites was 9.2% and 11.4%, respectively. Whereas, 11.7% and 2.2% of the adolescents were positive *E. gingivalis* and *T. tenax* (17). The prevalence of *T. tenax* in 52 Down syndrome patients with periodontal disease referred to Dental Clinics in Tabriz, Iran was 18.8% by PCR assay (24). In addition, among 240 patients referred to the Dentistry clinics in Tehran, 41.7% and 9.2% of the patients were positive for *E. gingivalis* and *T. tenax*, respectively (25). The frequency of *E. gingivalis* and *T. tenax* was 0.5% and 0% by microscopic examinations in patients with periodontitis in Khuzestan Province, Iran, respectively (26). Considering the prevalence of these parasites in the present studied region, the prevalence rate of *E. gingivalis* and *T. tenax* in 76 patients with periodontitis was 17.1% and 14.5%, re-

spectively (16). In a study on 140 patients referred to Khorramabad Dental clinics with at least one decayed tooth, 15.4% and 10.7% were positive by microscopic examinations for *E. gingivalis* and *T. tenax*, respectively (27). However, it should be mentioned that the difference between the findings of the current study and previous surveys may be related to various factors, e.g. target group, sample size, used diagnostic methods, and lifestyles of studies regions (25, 28).

Our results displayed no significant relationship between age, gender, parent education, brushing teeth and prevalence of oral protozoa in pediatric oncology children. However, in the multivariate model, living in rural regions and brushing teeth were significantly related with the frequency of oral cavity parasites. Similarly, previous studies reported a significant association between mouthwash and the prevalence of *E. gingivalis* and *T. tenax* (16, 27). We also found that there was a significant correlation between living in rural regions and the frequency of *E. gingivalis* and *T. tenax*. In consistent with these results, it has been exhibited that people living in rural regions poor due to lack of attention to oral health, more frequency of tooth loss and gum diseases was observed (29, 30).

As the main limitation of the present study, the sample size was low, while the use of a larger sample size could lead to better conclusion.

## Conclusion

Our results showed the high frequency of oral cavity parasites (*E. gingivalis* and *T. tenax*) in children who suffering from different types of malignancies or receiving treatment by chemotherapy in Lorestan province, Western Iran. Our results also showed the awareness of the central risk factors for oral cavity parasites particularly using mouthwash is necessary in improving public and oral health strategies in children with cancer. Consequently, oncologist

and dental practitioners must be aware to identify and manage oral health concerns in children who suffering from different types of malignancies to prevent the oral diseases and infections.

## Funding

None.

## Acknowledgements

The authors thank all the patients and medical staff who contributed to this study.

## Competing interest

The authors declare that they have no competing interest.

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