Original Article

Parasitological and molecular study of *Entamoeba gingivalis* and *Trichomonas tenax* in children from Lorestan Province, Iran

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ABSTRACT

Oral and dental hygiene plays an important role in the health and well-being of our bodies. Failure to take basic oral and dental hygiene measures not only damages the health of teeth and gums, but can also significantly increase the risk of heart disease, cancer, and diabetes. *Entamoeba gingivalis* and *Trichomonas tenax* are reported to be anaerobic parasites found in the human mouth. These parasites are transmitted through saliva, contaminated food containers, drinking water, and/or other utensils. Since the observance of personal hygiene and discipline in taking care of oral and dental health is less in children and this affects their performance in school and their future success, we aimed to evaluate a parasitological and molecular survey of *E. gingivalis* and *T. tenax* among children (2 to 15 years) in Lorestan Province, in western Iran. This descriptive cross-sectional survey was conducted from October 2021 to October 2022 on 660 children (aged 2-15 years) referred to health centers of Lorestan Province, Iran. The total prevalence of the parasites was 108 (16.4%) and 117 (17.7%) by microscopy and PCR, respectively. No significant association was reported among age (P=0.716), gender (P=0.289), parental education (P=0.812), tooth brushing (P=0.170), and prevalence of these parasites in children. Conversely, a significant association was reported between settling in rural areas (P=0.002), mouthwash (P=0.001), and the prevalence of these parasites in children. By multivariate test, settling in rural districts (P=0.007) and mouthwash (P<0.001) were considerably associated with the rate of these parasites. We found a significant prevalence of these parasites in children in Lorestan Province, western Iran, indicating that dentists as well as children's parents should pay special attention to oral health strategies in children.

Keywords: Parasites, Children, Prevalence, Iran

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1. Introduction

Oral and dental hygiene plays an important role in the health and well-being of our bodies. Failure to take basic measures to maintain oral and dental hygiene not only damages the health of teeth and gums, but can also significantly increase the risk of heart disease, cancer, and diabetes (1). The oral cavity, along with the teeth, is the body's first digestive organ (2). Scientifically, the initial digestion of food begins from the mouth, and its softening depends on the function of oral enzymes and the movement of teeth (3). With these interpretations, it is not surprising that any dysfunction of the mouth and teeth can cause the body to face serious digestive and functional disorders (4). Therefore, maintaining oral and dental hygiene is very important and, in some cases, it can even cause serious problems for the entire body structure (5). The statistics obtained from the World Health Organization show that despite the emphasis placed on oral and dental hygiene, a large percentage of people in the world, specially children, struggle with problems related to teeth and gum diseases, and this problem is also very common in the poor and disadvantaged groups of society (6,7). *Entamoeba gingivalis* and *Trichomonas tenax* have been reported as anaerobic parasites found in the human mouth (7, 8). These parasites are transmitted through saliva, contaminated food containers, drinking water, and or other utensils (9). These two protozoa live in the upper parts of the digestive system, such as the mouth, around the teeth, between the teeth, and at the margin of the gums, and in cases such as neglecting oral and dental hygiene or gum problems as well as the respiratory tract (10, 11). Although these two parasites are considered by most dentists as microorganisms not responsible for causing oral and dental diseases, however, recent reports indicate the role of these two parasites in causing some oral diseases such as periodontal disease, gingivitis, and osteomyelitis (12-14). Since the observance of personal hygiene and discipline in taking care of oral and dental health is less frequent in children and this affects their performance in school and their future success, we aimed to evaluate a parasitological and molecular survey of *E. gingivalis* and *T. tenax* among children (2 to 15 years) in Lorestan Province, in western Iran.

2. Materials and Methods

2.1. Participants and study area

This descriptive cross-sectional study was conducted from October 2021 to October 2022 on 660 children (aged 2–15 years) who were referred to health centers of Lorestan Province, in western Iran, bordering the provinces of Isfahan, Kermanshah, Markazi, Khuzestan, Hamedan, and Ilam. This province covers an area of 28.000 km^2 with a population of nearly 2 million people. Children who had received systemic antibiotics in the previous 90 days and immunocompromised children were not selected for the study.

2.2. Questionnaire

For each child, a questionnaire with several information, e.g. age, gender, residence, parental education, toothbrush, and mouthwash was filled in before the collection.

2.3. Sample Collection

For microscopic assessment, two samples from each child, were collected using sterile swabs from saliva and dental plaque; while, the third sample was transferred to tubes containing sterile normal saline for molecular testing.

2.4. Parasitological Examination

After preparation of the smears on a glass slides, the slides were stained with trichrome and Giemsa stains and checked under a light microscope (15).

2.5. Molecular Examination Using Polymerase Chain Reaction (PCR) Assay

First, the DNA of all samples was extracted using a commercial kit (Qiagen, Germany) according to the producer's instruction. SrRNA gene via the primers of F: 5'-GCGCATTTCGAACAGGAATGTAGA-3' and R: (5'- CAAAGCCTTTTCAATAGTATCTTCATTCA- 3' (17, 18) and 18S ribosomal RNA gene via the primers of F: 5'-ATGACCAG-TTCCATCGATGCCATTC-3' and R: 5'-CTCCAAAGATTCTGCCACTAACAAG -3' were used for *E. gingivalis* and *T. tenax*, respectively. The thermal conditions for PCR were performed based on the previous study (13). The band size for the *E. gingivalis* and *T. tenax* was 454 and 496 bp, respectively (17, 18).

2.6. Statistical Analysis

SPSS software (ver. 26.0) was used to analyze of the obtained data through using the Chi-square-and regression analysis tests.

3. Results

3.1. Participants

The mean age of the children was 8.75 ± 6.2 years; while 417 (63.2%) children were less than 10 years old. The 345 children (52.3%) were male while the majority of them lived in urban areas (393, 59.4%). Among the children, 552 (83.6%) of the parents had higher education. We found that 387 (58.6%) and 36 (5.5%) of the children brushed their teeth daily and used mouthwash, respectively (Table 1).

3.2. Prevalence of Protozoan Parasites in the Oral Cavity

The total prevalence of the parasites was 108 (16.4%) and 117 (17.7%) by microscope and PCR, respectively (Figure 1); while 86 (73.5%) and 31 (26.5%) children were positive for *E. gingivalis* and *T. tenax*, respectively.

3.3. Risk Factors

No significant association was found among age (P=0.716), gender (P=0.289), parental education (P=0.812), tooth brushing (P=0.170)and prevalence of these parasites in children. Conversely, a significant association was found among settling in rural areas (P=0.002), mouthwash (P=0.001), and the prevalence of these parasites in children (Table 1). By multivariate test, settling in rural areas (P=0.007) and mouthwash (P<0.001) were significantly associated with the rate of these parasites (Table 2).

Table 1: Frequency of the parasites in children based on the demographic items and correlated risk factors.

Group		Totally	Parasites		P value Chi-Square	Crude OR	*95%CI*	*P value*
		No. (%)	Positive No. (%)	Negative No. (%)				
Age (yr)	<10	417 (63.2)	78 (18.7)	339 (81.3)	0.716	1	1	-
	≥10	243 (36.8)	39 (16.1)	204 (83.9)	-	0.619	0.04-1.7	0.831
Parental education	≥diploma	552 (83.6)	96 (17.4)	456 (82.6)	-	1	1	-
	<diploma	108 (16.4)	21 (19.4)	87 (80.6)	0.812	0.768	0.42-2.87	0.768
Residence	Rural	267 (40.6)	21 (7.9)	246 (92.1)	-	1	1	-
	Urban	393 (59.4)	96 (24.4)	297 (75.6)	0.02	3.786	1.58-9.023	0.003*
Gender	Male	345 (52.3)	51 (14.8)	294 (47.7)	-	1	-	-
	Female	315 (47.7)	66 (21.0)	249 (79.0)	0.289	1.512	0.761-3.068	0.233
Brushing	Yes	387 (56.8)	60 (15.5)	327 (84.5)	-	1	-	-
	No	273 (43.2)	57 (20.9)	216 (79.1)	0.371	0.695	0.347-1.392	0.305
Mouthwash	Yes	104 (15.7)	3 (2.9)	95 (97.1)	-	1	-	-
	No	556 (84.3)	114 (20.5)	442 (79.5)	0.001	4.252	1.642-9.874	0.001*

* p<0.001 significant difference

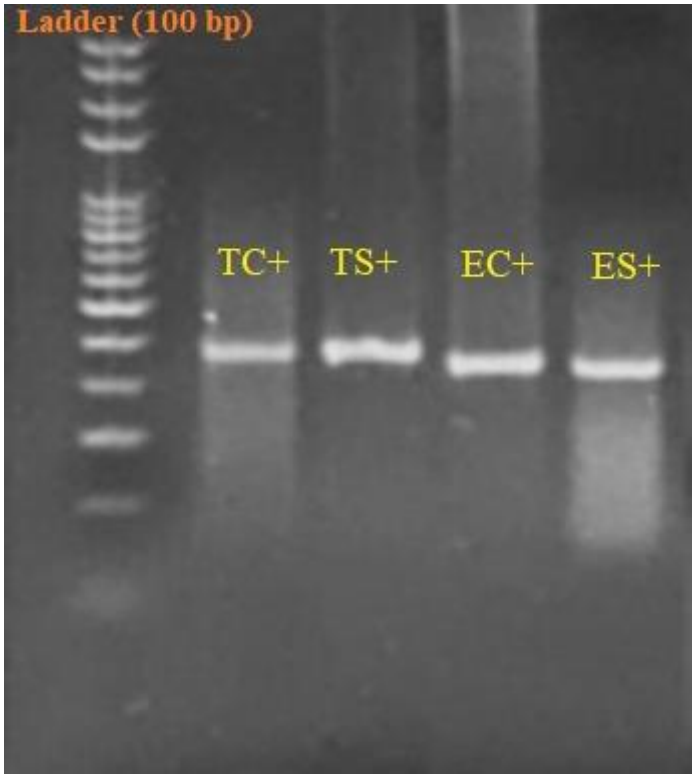

Figure 1. Agarose gel electrophoresis analysis of the PCR products.TC+: *T. tenax* positive control, 496 bp; TS+: *T. tenax* positive sample, EC+: *E. gingivalis* positive control, 454 bp, ES+:*E. gingivalis* positive sample.

Table 2: Evaluation of oral cavity parasites in children and associated risk factors through the multivariate regression analysis.

Group	*Crude OR*	*95%CI*	*P value*
Age (yr)	0.856	0.382-1.98	0.705
Parental education	2.183	0.363-2.617	0.960
Residence	0.285	0.115-0.709	0.007*
Gender	0.740	0.363-2.617	0.443
Brushing	1.319	0.623-2.79	0.469
Mouthwash	5.321	1.84-10.621	<0.001*

* $P<0.05$, difference was statistically significant

4. Discussion

Since, the observance of personal hygiene and discipline in taking care of oral and dental health is less in children and this affects their performance in school and their success in the future, we aimed to evaluate a parasitological and molecular survey of *E. gingivalis* and *T. tenax* among children in Lorestan Province, in western Iran. The total prevalence of the parasites was 108 (16.4%) and 117 (17.7%) by microscope and PCR, respectively; while86 (73.5%) and 31 (26.5%) children were positive for *E. gingivalis* and *T. tenax*, respectively. Considering the frequency of these parasites in Iran, Sharifi et al (2020) reported on 315 adolescents in Kerman Province, Iran, that 9.2% and 11.4% were positive by culture and PCR, respectively; while 11.7% and 2.2% were detected for *E. gingivalis* and *T. tenax*, respectively (17). Mehr et al (2015) showed that the frequency of *T. tenax* by PCR was 18.8% in Down syndrome patients with periodontitis in Tabriz, Iran (19). In Lorestan Province, Azadbakht et al (2023) reported that the frequency of *E. gingivalis* and *T. tenax* in periodontitis patients was 17.1% and 14.5%, respectively (16). Kooshki et al (2023) also reported that the frequency of *E. gingivalis* and *T. tenax* in children with cancer was 23 (25.5%) and 28 (31.1%) by microscope and PCR, respectively (20). This variation in the frequency of these parasites is probably associated with some factors, such as study group, the number of participants, and study method (20, 21). We found that no significant association was reported among age, gender, parental education, tooth brushing and frequency of these parasites in children. Conversely, settling in rural areas (P=0.007) and mouthwash (P<0.001) were considerably associated with the prevalence of these parasites. Similarly, a number of studies have shown a significant correlation among mouthwash and the incidence of *E. gingivalis* and *T. tenax* (16, 20). In line with our findings, Zander et al (2013) and Chen et al. (2018) reported that people living in rural areas have high rates of tooth loss, oral infections, and gum diseases due to lack of attention to oral health (22-25). One of the major weaknesses of the current study is the small sample size, whereas conducting such a study on a larger number of pollutants can lead to more realistic results. We found a significant prevalence of these parasites in children in Lorestan Province, western Iran, indicating that dentists as well as children's parents should pay special attention to oral health strategies in children.

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Authors' Contribution

FK, conceived and designed the study; AP, SM, and HM, conducted experiments and collected data; BS supervised, directed, and managed the study, BS and HM wrote the draft and AKK critically revised the paper; all authors approved the final version for publication.

Ethics

The Ethics Committee of Lorestan University of Medical Sciences approved the study protocol under the number. of IR.LUMS.REC.1401.064.

Conflict of Interest

The authors declare that they have no competing interests.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

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