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## Prevalence and risk factors of oral cavity parasites in pregnant women in Western Iran

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### ABSTRACT

**Background:** We aimed to study the frequency and risk factors of oral cavity protozoa (*Trichomonas tenax* and *Entamoeba gingivalis*) in pregnant women in Lorestan province, western Iran.

**Methods:** The current cross-sectional descriptive work was performed on 200 pregnant women referring to health centers of Lorestan Province, Iran during August 2021 to February 2022. Two specimens were obtained from each women by means of sterile swabs from saliva and dental plaques. 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 41 (20.5%) and 46 (23%) of the pregnant women by microscopic and PCR test, respectively. Among positive samples, 29 (63.1%) of the pregnant women were infected with *E. gingivalis*; whereas 19 (36.9%) of the participants were positive for *T. tenax*. In the multivariate model, living in rural regions ( $p < 0.001$ ) and brushing teeth ( $p = 0.021$ ) were considerably linked with the prevalence of oral cavity parasites.

**Conclusion:** The current study evidently exhibited the high frequency of oral cavity parasites (*E. gingivalis* and *T. tenax*) in pregnant women in Lorestan province, Western Iran. Awareness of the main risk factors for oral cavity parasites especially teeth brushing is necessary in refining public and oral health approaches in pregnant women. Thus, dental practitioners and gynecologists must be alert of these risk factors to carefully identify and manage oral health concerns in pregnant women to prevent the oral diseases and infections.

## 1. Introduction

It has been proven that during pregnancy, a number of pathophysiological and mechanical modifications such as reduction in the respiratory volumes, hormonal changes, immune System alterations, stress, and microbiome markedly increase the susceptibility to infections, like hepatitis E virus, influenza virus, and *Plasmodium* parasites (Kourtis et al., 2014).

Studies reported that pregnancy increase the risk of dental health problems, such as premature birth, cavities, gingivitis, periodontitis. For example, elevating the progesterone and estrogen during pregnancy results in periodontium hypervascularisation and

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subsequently alteration in production of collagen which makes the gum tissue more susceptible to colonization of microorganisms and biofilm formation (Boutigny et al., 2016).

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 (Deo and Deshmukh, 2019). *Entamoeba gingivalis* and *Trichomonas tenax* are considered as anaerobic protozoan parasites observed in human oral cavity (Marty et al., 2017; Badri et al., 2021). 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 (Marty et al., 2017; Badri et al., 2021). 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 (Arpağ and Kaya, 2020). Studies showed that *T. tenax* can enter the respiratory tract through respiration and cause pulmonary trichomoniasis (Eslahi et al., 2021). In addition, investigations revealed that *E. gingivalis* may be observed in the progress of periodontal disease, osteomyelitis as well as infections caused by the intrauterine contraceptive device (Bao et al., 2020).

Considering the prevalence of these parasites, although several studies have been conducted in people with periodontitis and gingivitis (Mehr et al., 2015a), Down syndrome (Yaseen et al., 2021), patients undergoing chemotherapy (Al-Muathen et al., 2020); however, the frequency of these oral protozoa and their related risk factors in pregnant women has not been studied. Hence, this survey was designed to study the frequency and risk factors of *T. tenax* and *E. gingivalis* and in pregnant women in Lorestan province, West of Iran.

## 2. Materials and methods

### 2.1. Ethical statement

This epidemiological study was reviewed and permitted by Lorestan University of Medical Sciences ethical committee of, Iran (IR.LUMS.REC.1400.298). However, a printed informed consent form was acquired from all participant.

### 2.2. Participants

The current cross-sectional descriptive work was performed on 200 pregnant women referring to health centers of Lorestan Province, Iran during August 2021 to February 2022. Participants who have taken systemic antibiotics in the last 90 days and also immunocompromised patients were excluded from the study.

### 2.3. Questionnaire

Before sampling, a provided questionnaire with a number of demographical information and related risk factors such as age, education, residence, pregnancy month, brushing, flossing, and mouthwash was completed for each patient.

### 2.4. Sample collection

Two specimens were obtained from each women by means of sterile swabs from saliva and dental plaques for microscopic examinations. In addition, the third sample (obtained from saliva) was put into a tube with sterile physiological saline and kept at  $-20^{\circ}\text{C}$  for molecular tests.

### 2.5. Polymerase chain reaction (PCR) assay

Qiagen kits was used to extract DNA from 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' GCGCATTTGGAACAGGAATGTAGA-3') and reverse (5'-CAAAGCCTTTTCAATAGTATCTTCATTCA- 3') as well as 18S ribosomal RNA gene for *T. tenax* using the primers of forward (5'-ATGACCAG-TTCCATCGATGCCATTC -3') and reverse (5'- CTCAAAGATTCTGCCACTAACAAG -3') according the previous study (Peres et al., 2019). The thermal condition was 6 min at  $93^{\circ}\text{C}$  for early denaturation, 35 cycles of 30 s at  $93^{\circ}\text{C}$ , 30 s at  $57^{\circ}\text{C}$ , 60 s at  $72^{\circ}\text{C}$ , and a last extension phase of 10 min at  $72^{\circ}\text{C}$ . Then the obtained amplicons using agarose gel (1%) were electrophoresed. Positive and negative controls were DNA of standard strains and distilled water, respectively.

### 2.6. Microscopic examination

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

### 2.7. Statistical analysis

The analysis of the collected data was performed using SPSS software version 25.0. Chi-square-test, Fisher exact, univariate and multivariate regression analysis tests are applied to assess the relationship among the variables and the frequency of oral cavity protozoan parasites (*E. gingivalis* and *T. tenax*). Also, if the sample size is sufficient, the odds ratio and 95% confidence interval are

calculated using logistic regression. All statistical tests are performed using SPSS software version 25.0.  $P < 0.05$  will be considered as a significant level.

### 3. Results

#### 3.1. Participants

The mean age of the pregnant participants was  $28.3 \pm 9.2$  years. Among the participants, 73.5% (147 women) lived in urban areas and 26.5% (53 women) lived in rural areas. By education, 147 (73.5%) participants had diploma or higher, while 53 (26.5%) participants had lower education than diploma. Most participants (149, 74.5%) were over 20 years old. In term of month of pregnancy, most participants were in the 3-6th months (100, 50%) of pregnancy. Among the participating pregnant women, 124 (62%) women brushed their teeth daily. Floss and mouthwash were used by 81 (40.5%) and 20 (10%) of participants, respectively (Table 1).

#### 3.2. Prevalence of oral cavity protozoan parasites

The results showed that *E. gingivalis* and *T. tenax* parasites were found in 41 (20.5%) and 46 (23%) of the pregnant women by microscopic and PCR test, respectively (Fig. 1). Microscopic examination showed that 58.5% (24 women) and 41.5% (17 women) of the participants were positive for *E. gingivalis* and *T. tenax*, respectively. PCR test showed that among positive samples, 58.7% (27 women) and 41.3% (19 women) of the pregnant women were positive for *E. gingivalis* and *T. tenax*, respectively.

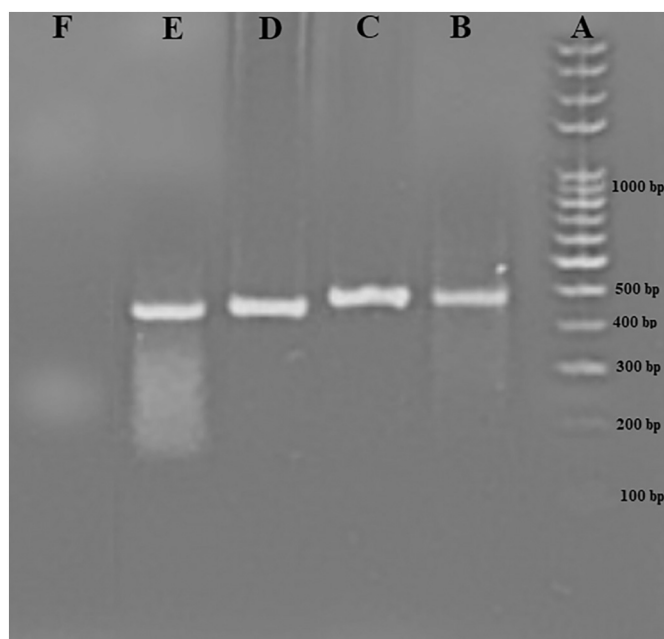
#### 3.3. Risk factors

There was no significant relationship between age ( $p = 0.340$ ), education ( $p = 0.707$ ), month of pregnancy ( $p = 0.197$ ), flossing ( $p = 0.235$ ), use of mouthwash ( $p = 0.784$ ) and prevalence of oral protozoa in pregnant women. However, a significant correlation was observed among between living in rural regions ( $p < 0.001$ ), brushing teeth ( $p = 0.037$ ) and prevalence of oral protozoa in pregnant women. Table 2 shows the prevalence of oral cavity protozoa in pregnant women in Lorestan province, western Iran according to the demographic features and related risk factors. In the multivariate model, living in rural regions ( $p < 0.001$ ) and brushing teeth ( $p = 0.021$ ) were significantly related with the frequency of oral cavity parasites.

**Table 1**

Frequency of oral cavity protozoa (*E. gingivalis* and *T. tenax*) in pregnant women in Lorestan province, western Iran according to the demographic features and related risk factors by univariate regression analysis.

Group	Total No. (%)	Oral cavity parasites		Crude OR	95%CI	P value
		Positive	Negative			
		No. (%)	No. (%)			
Age						
<20 yrs	51 (25.5)	9 (17.6)	42 (82.4)	1	1	–
≥20 yrs	149 (74.5)	37 (24.8)	112 (75.2)	1.54	0.68–3.46	0.295
Education						
<diploma	53 (21.5)	11 (20.8)	42 (79.2)	1	1	–
≥diploma	147 (78.5)	35 (23.8)	112 (76.2)	1.19	0.55–2.56	0.651
Residence						
Rural	53 (21.5)	23 (43.4)	30 (56.6)	1	1	–
Urban	147 (78.5)	23 (15.6)	124 (84.4)	0.242	0.12–0.48	<0.001*
Pregnancy month						
<3	35 (17.5)	10 (28.6)	25 (71.4)	0.878	0.37–2.7	0.767
03-Jun	100 (50.0)	26 (26.0)	74 (76.0)	1	1	–
>6	65 (32.5)	10 (15.4)	55 (84.6)	1	1	–
Brushing						
Yes	124 (62.0)	22 (17.7)	102 (82.3)	0.467	0.24–0.91	0.026*
No	76 (38.0)	24 (31.6)	52 (68.4)	1	1	–
Flossing						
Yes	81 (40.5)	15 (18.5)	66 (81.5)	1	1	–
No	119 (59.5)	31 (26.1)	88 (73.9)	0.645	0.32–1.29	0.216
Mouthwash						
Yes	20 (10)	5 (25.0)	15 (75.0)	1.3	0.38–3.2	0.823
No	180 (90)	41 (22.8)	139 (77.2)	1	1	–



**Fig. 1.** Agarose gel electrophoresis of SrRNA and 18 s 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*; F: negative control (distilled water).

**Table 2**

Comparison of oral cavity protozoa (*E. gingivalis* and *T. tenax*) in pregnant women in Lorestan province, western Iran based on the associated risk factors by multivariate regression analysis.

Group	Crude OR	95%CI	P value
Residence	0.224	0.1–0.468	<0.001*
Pregnancy month	0.621	0.36–1.06	0.08
Brushing	0.431	0.21–0.88	0.021*
Flossing	0.796	0.37–1.68	0.55
Mouthwash	0.688	0.21–2.1	0.528

\*  $p < 0.05$ , difference was statistically significant.

#### 4. Discussion

In the course of pregnancy various physiologic changes happen in the women's body, that may cause serious effects on oral health; so that oral and dental health care should be given special attention in pregnant women (Peres et al., 2019; Iida, 2017; Lachat et al., 2011; AlJehani, 2014). Here, we aimed to assess the frequency and risk factors of oral cavity protozoa (*E. gingivalis* and *T. tenax*) in pregnant women in Lorestan province, western Iran. Our results showed that *E. gingivalis* and *T. tenax* parasites were found in 41 (20.5%) and 46 (23%) of the pregnant women by microscopic and PCR test, respectively.

Considering the frequency of these parasites in Iran, Kashefi Mehr et al. (2015) showed the prevalence of *T. tenax* in Down syndrome patients with periodontal disease referred to Dental Clinics in Tabriz city was 18.8% by PCR assay (Mehr et al., 2015b). Gharavei et al. (2006) have reported that among 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 (Gharavi et al., 2006). Maraghi et al. (2012) also have reported that the frequency of *E. gingivalis* and *T. tenax* was 0.5% and 0% by microscopic examinations in patients with periodontitis in Khozestan Province, respectively (Maraghi et al., 2012). Another study conducted by Sharifi et al. (2020) on adolescents in Kerman province, the prevalence of oral cavity parasites by culture media and PCR was 9.2% and 11.4%, respectively (Sharifi et al., 2020). These differences in frequency of *E. gingivalis* and *T. tenax* can be attributed to some factors such as study population, sample size, type of sample collected, study method, and the region where the study subjects are located.

On the other hand, previous studies related to the prevalence of these parasites in the area under study showed that, the prevalence of *E. gingivalis* and *T. tenax* in periodontitis patients was 17.1% and 14.5%, respectively (Derikvand, 2018); where, this prevalence among patients referred to Khorramabad Dental clinics with at least one decayed tooth was 15.4% and 10.7%, respectively (Mahmoudvand et al., 2018). Subsequently, this difference in prevalence even in the study area, in addition to the previously mentioned cases (sample size, type of sample collected, and study method), is mostly due to the low level of oral and dental hygiene in pregnant

women, which causes a higher frequency of these parasites in them (Gharavi et al., 2006; Özçelik et al., 2010).

In the present study, there was no significant relationship between age, education, month of pregnancy, flossing, use of mouthwash and prevalence of oral protozoa in pregnant women. However, a significant correlation between was observed among those living in rural regions ( $p < 0.001$ ), brushing teeth ( $p = 0.037$ ) and prevalence of oral protozoa in pregnant women. In consistent with our findings, the studies conducted by Derikvand et al. (2018) and Mahmoudvand et al., (2018) have reported a significant correlation between teeth brushing and the prevalence of *E. gingivalis* and *T. tenax* (Derikvand, 2018; Mahmoudvand et al., 2018). Previous studies have reported people living in rural regions poor due to lack of attention to oral health, more frequency of tooth loss and gum diseases was observed (Zander et al., 2013; Chen, 2018). Similarly, our findings showed that there was a significant correlation between living in rural regions and the frequency of *E. gingivalis* and *T. tenax*; this considerable prevalence in rural communities may be due to some factors such as the low compliance with health standards, lower educational level, more contact between people, and lack of access to health services (Coughlin et al., 2019).

## 5. Conclusion

The current study evidently exhibited the high frequency of oral cavity parasites (*E. gingivalis* and *T. tenax*) in pregnant women in Lorestan province, Western Iran. Awareness of the main risk factors for oral cavity parasites especially teeth brushing is necessary in refining public and oral health approaches in pregnant women. Thus, dental practitioners and gynecologists must be alert of these risk factors to carefully identify and manage oral health concerns in pregnant women to prevent the oral diseases and infections.

## Funding

None.

## Declaration of Competing Interest

The authors declare no conflict of interest in this study.

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