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MOLECULAR DETECTION OF ORAL *TRICHOMONAS TENAX* AMONG INDIVIDUALS ATTENDING DENTAL CARE UNITS USING PCR IN DUHOK CITY – KURDISTAN REGION

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ABSTRACT

Background

***Trichomonas tenax* is a flagellated protozoan which inhabits theoral cavity of human and usually found in persons with poor oral hygiene and periodontitis. This is the first study in Duhok and Kurdistan region that aims at the detection of *T. tenax* in patients with oral diseases based on conventional PCR.**

Subjects and Methods

In this cross sectional study, a total of 184 individuals with different mouth conditions were included from October 2017 to February 2018 who attended the dental complex and outpatients dentistry clinic at the college of dentistry – Duhok University. Oral swab was taken from each subject for DNA extraction. The extracted DNA was subjected to PCR in order to detect *T. tenax* , using the 18s rRNA gene specific primers.

Results

Out of the 184 subjects involved in the study, 94(51.09%) were males and 90(48.91%) were females and the mean age was 48±5.2 years. Regarding the oral condition on examination, 40.24% have had healthy gingival and 8.67% of them have had gingivitis. Only 8(4.35%) of the total subjects were positive for *T. tenax* and all of them were among the female group while all of the males involved in the study were negative for *T. tenax.* Statistically the difference between males and females was significant(p=0.04) regarding the existence of oral *T. tenax*. The highest rate of *T. tenax* presence was found in the age group 20-30 years with healthy gingival (1.62%) followed by age group 51years and above with gingivitis and these differences were significant(p=0.03), and then the age group 31-40 years (0.54%) both with healthy gingival and gingivitis. Regarding the dentation condition of the participants, *T. tenax* was detected in complete dentate (1.08%), partial dentate without prosthesis (2.16%) with a significant differenc(p=0.03), partial edentulous with removable prosthesis (0.54%) and partial edentulous with wearing denture (0.54%).

Conclusion

***Trichomonas tenax* was detected in female group only and all males were negative. The dentation status had a significant effect on the presence of oral *T. tenax* in positive females.**

KEY WORDS: *Trichomonas tenax*, oral cavity status, gingivitis, PCR

INTRODUCTION

The oral cavity of human is colonized by specific bacteria, fungi and protozoa. Different species of bacteria are known to be associated with oral cavity pathogenesis (Subramani *et al.*, 2013). *Trichomonas tenax* is known as a common anaerobic parasite of the oral cavity (Duboucher *et al.*, 1995), it is commonly found in the oral cavity in patients with low socio-economic status, poor oral hygiene and periodontal disease. The

heterogeneity of tissue type in the oral cavity such as teeth, tongue and mucosa means that a variety of sites are available for colonization by specific bacteria, fungi and protozoa including *T. tenax* (Kolenbrander and London, 1993).

The host's age is highly determining the occurrence of *T. tenax*. The parasite can be transmitted by different factors, such as saliva, through kissing, or contaminated dishes and drinking water (Hersh *et al.*, 1985; Chiche *et al.*, 1994). The rate of oral *T. tenax* infection is reported between 0-25%, this variation depends on oral health status. Secondary *T. tenax* infections other than oral infections are frequently reported; more attention has been paid to oral *T. tenax* infection since the organism is believed to enter the respiratory tract through aspiration and leads to secondary broncho-pulmonary trichomoniasis (Chiche *et al.*, 2005; Mallat *et al.*, 2004). PCR techniques are the more accurate techniques for*T. tenax* identification, since staining is not useful for species identification, and culture techniques are not of routine use (Nicasio 2010). Amplification of the 18S rRNA gene by PCR followed by sequencing has become a reliable means for more rapid and specific detection and identification of trichomonads because these tools have been developed for both the detection and the identification of *Trichomonas* species (Felleisen, 1997; Crucitti *et al* 2004). Previous studies reported the existent of trichomonas in the oral cavity and probably it play a role in the development of oral diseases, this work is the first to be done in Duhok city / Kurdistan Region and aims at investigating the prevalence of *T. tenax* and it is relation to mouth's condition, age and sex of subjects.

Subjects and Methods

The current cross sectional study included 184 individuals, 90 males and 94 femaleswho attended the dental care units at the College of Dentistry and the dental complex in Duhok city/ Kurdistan region. An informed consent was obtained from each participant; the study was approved by the ethics Committee at the directorate general of health in Duhok city. The participants were clinically examined for having any history of consumption of systemic antibiotics within the three preceding months, periodontal therapy during the previous six months, medications that affect the periodontium (such as immunosuppressives), systemic diseases such as diabetes, heart disease or respiratory diseases, pregnancy, smoking or other drug abuse. A demographic form was filled out for each individual concerning age, sex, smoking habits, and medical status. They were grouped according to the age into four groups (20-30, 31-40, 41-50 and 51 and above) years. These participants were classified according to the clinical state in relation to missing teeth and wearing prosthesis into (completely dentate, partially dentate without prosthesis, partially dentate wearing prosthesis and completely edentulous wearing prosthesis), other factors like smoking and health of gingiva and residual ridge were considered. The patients were also asked about the use of medications and systemic conditions which might predispose them to the development of periodontal disease. Samples were collected from all patients before any oral hygiene using a sterile dental swab was rubbed around the surface of teeth, gingival crevices, soft tissue in areas of missing teeth and around prosthesis (fixed and removable) of each patient. DNA of each sample was extracted phenol-chlorophorm method according to Sambork and Russell 2001). In order to detect *T. tenax*, the 18s rRNA gene was amplified with the forward and reverse primers of TGBK gene (5′-AGCAGCTGCGGTAATTCCAG-3′ and 5′-CTTGTTACCACTTCTCCTTCC-3′), respectively (Thai *et al*.,2013).A volume of 10μL of each PCR product was electrophoresed in a 1.5%agarose gel. The results were visualized after staining with ethidium bromide in a U.V. light transilluminator. The statistical analysis was done via SPSS software (Version 11.5) to study the correlation between the oral diseases, age and sex with *T. tenax* infections. The X^2 and Fisher tests were used for data analysis to detect the significant differences at p value (<0.05).

RESULTS

The amplification of the 18srRNA gene of the *T. tenax* by PCR produceda 1000 bp band as shown in figure 1 which is compatible with data published by Thai *et al*.,2013 .

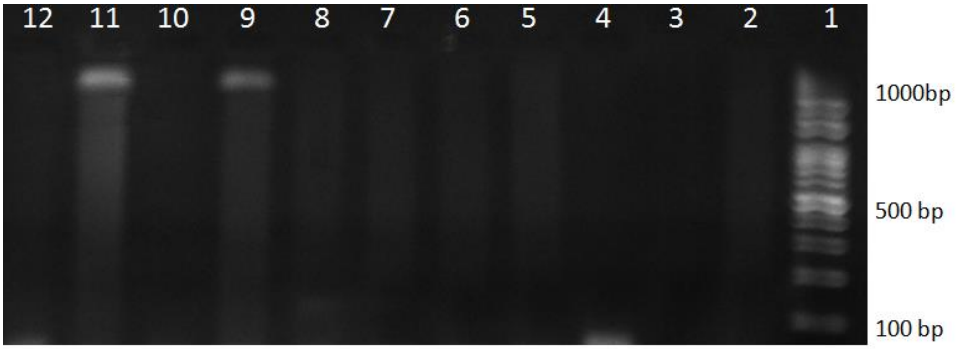

Fig (1): PCR products visualized on 1.5% agarose gel electrophoresis using primers targeting 18S rRNA gene of *Trichomonas tenax*. Lane 1: 100 bp DNA ladder; lanes 2,3,4,5,6,7,8,10,12: negative samples; lanes 9 and 11 represent 1054 bp PCR products of *Trichomonas tenax*positive samples.

Out of the 184 individuals involved in the current study, 8(4.32%) were positive by the PCR for the infection with oral *T. tenax*, all of them were females, while all males were negative, accordingly, the rate of infection among the female group was 8/90 (8.9%) as shown in table(1) and figure(1).

Table (1): The significance of sex in *Trichomonas tenax*infection

	***n*.(%)**	***Trichomonas tenax* positives (%)**
Males	94 (51.09)	0(0)
Females	90 (48.91)	8(4.32)
Total	184	8(4.32)

Regarding the age, groups in infected females, table 2 shows that the highest rate (1.62%) was found among the age group 20-30 years, which was significantly higher than the other age groups followed by age group 31-40 years and the age group of 50 years and above.

Table (2): *Trichomonas tenax* infections according to age groups among the infected females.

Female age group (years)	***Trichomonas tenax* positives (%)**
20-30	1.62
31-40	1.08
41-50	0.54
51 and above	1.08
Total	4.32

The effect of dentation condition of the participants on the infection rate is shown in tables (3,4,5,6). *Trichomonas tenax* was detected in complete dentate (1.08%), partial dentate without prosthesis participants (2.16%) with a significant difference (add p value), partial edentulous with removable prosthesis (0.54%) and partial edentulous with wearing denture participants (0.54%).

Table (3): The rate of *T. tenax* infection according to dentation status among group 20-30 years females

Dentation status	***Trichomonas tenax* positives (%)**
Complete dentate	1.08
Partial dentate without prosthesis	0.54
Partial dentate with fixed prosthesis	0

Total	1.62

Table (4): The rate of *T. tenax* infection according to dentation status among group 31-40 years females

Dentation status	***Trichomonas tenax* positives (%)**
Complete dentate	0
Partial dentate without prosthesis	1.08
Partial dentate with fixed prosthesis	0
Total	1.08

Table (5): The rate of *T. tenax* infection according to dentation status among group 41-50 years females

Dentation status	***Trichomonas tenax* positives (%)**
Complete dentate	0
Partial dentate without prosthesis	0.54
Partial dentate with fixed prosthesis	0
Total	0.54

Table (6): The rate of *T. tenax* infection according to dentation status among group 51 years and above females

Dentation status	***Trichomonas tenax* positives (%)**
Partial dentate without prosthesis	0
Partial edentulous with fixed prosthesis	0
Partial edentulous with removable prosthesis	0.54
Partial edentulous with wearing denture	0.54
Total	1.08

Regarding gingivitis (oral pathological status) the recruited subjects showed non-significant difference ($p>0.05$) between different age groups of males and females (table 7).

Table (7): Oral clinical condition of the study subjects

Age group (years)	**Healthy gingival (%)**	**Ginigivitis (%)**
20-30	23.35	1.08
31-40	7.12	1.08
41-50	7.6	1.63
51 and above	2.17	4.88
Total (%)	40.24	8.67

Comparison of positive infection with *T.tenax* between healthy gingival group and those with gingivitis using fisher test showed that the rate of infection was higher in the healthy gingival group (20-30 years) and thosewith gingivitis age group (51 years and above) , and these differences were statistically significant ($p<0.05$) (Table 8).

Table (8): The rate of infection with *T. tenax* among participants with healthy gingival and gingivitis

Age group (years)	**Healthy gingival (*T. tenax* positive) (%)**	**Gingivitis (*T. tenax* positive) (%)**
20-30	1.62	0
31-40	0.54	0.54
41-50	0.54	0
51 and above	0	1.08

DISCUSSION

Protists species, such as flagellates and amoebae, are not so often taken into consideration in clinical researches, butcan have a pathogenic impact on oral structures. The pathogenic effects of those protists such as fibronectin-like protein and collagenolytic effect of trichomonads in inflammatory gingivitis and in pathogenic dental pockets are underestimated (Ghabanchi *et al.*, 2010; Lunsford *et al*, 2015; Feki and .olet , 2016).The possible role of parasites in the development of periodontitis has been poorly studied.(Bonner *et al.*, 2014).Data from previous studies about *Entamoeba gingivalis* and Trichomonas is also limited (Haghighi *et al*., 2007)and have been conducted only in few countries. (Gharavi et al, 2006) . *Trichomonas tenax* is currently considered as a member of the oral biofilm. (Kurnatowska , 1985; Kurnatowska *et al*.., 2004). Its prevalence in the oral cavity ranges from 4 to 53% worldwide using different diagnostic tools. (Mallat *et al.*, 2004) however, in patients with periodontitis; it is 3 to 4 times more than healthy individuals (Albuquerque et al, 2011). Investigations have been performed on the correlation between the prevalence of *T. tenax* and the status of periodontitis (Lewis *et al*., 2003; Kucknoor *et al*., 2009) which is found particularly in patients with poor oral hygiene. (Lewis *et al.*, 2003; Athari *et al*., 2007). It can be simply transmitted through saliva, droplet spray, kissing, using contaminated dishes, and drinking water.The technological development, advances in amplification and sequencing tools, and large-scale genome analysis increase possibilities for diagnostics of species that cannot be maintend in *vitro*; this can impact the knowledge about microorganisms that can colonize the oral cavity (Lunsford *et al*., 2015). Oral protozoans are rarely found in children; they were more frequent in older persons. High prevalence and large number of microorganisms were found in persons showing pathological changes in their oral cavities: patients with systemic diseases and with decreased resistance connected with congenital disease, as well as in patients under chronic immunosuppression. For example, in 40–50-yearold patients with somatic and mental retardation connectedwith epilepsy or Down syndrome, the oral amoebae and trichomonads occurred with prevalence from 30% to 60%. These and other observations lead to a conclusion of an opportunistic nature of the protozoans (Chomicz *et al.*, 2002; Ghabanchi *et al.*, 2010; Perkowski *et al*., 2016). The result of the current study showed that percent of infection to *T.tenax* in the females group was significantly higher than the males group and the higher percent was found in the 20-30 years group with healthy gingival. In contrast, in a study conducted in Kirkuk-Iraq by Saadia *et al* have found that the highest rate of *Entamoeba gingivalis* and *Trichomonas tenax* was among age group (51-60) years old (Saadia *et al*., 2012) The high rate of infection among females and at the sexual active ages in the present study could be explained by the fact that humeral immune response in highly affected by sexual activity and sexual hormones fluctuation during menstrual cycle. However the classical detection methods are less sensitive, the PCR detection method also has been found to be with limited detection sensitivity (Naqwa and Eman 2008), the number of trichomonads found in oral washing is rather low, and detection by conventional methods such as wet-mount preparations or staining may not be sensitive enough (Pestechyan, 2002). While in another study in Iran among Down syndrome people, based on molecular detection of *T. tenax*, a rate of 18.8% of oral *T. tenax* was reportedin the patients as compared to 3% of the control group (Atabank., 2015). In anoter study conducted on Periodontitis or Gingivitis Patients in Kayseri-Turke by Yazar and collegues, they found a high percentage of *E. gingivalis* and *T. tenax* in periodontitis and gingivitis patients (Yazar *et al*, 2016).

The rate of infection among females at the sexual active ages in the present study could be explained by the fact that humeral immune response is highly affected by sexual activity and sexual hormones fluctuation during menstrual cycle increase the risk of infection. In a study conducted by Tierney et al. (2015), they examined cycle-related changes in two humoral immune parameters – mucosal (salivary) IgA and circulating (serum) IgG – and their interaction with sexual activity in healthy women. At menses, sexually active and abstinent women had similar levels of both IgA and IgG. However, at ovulation, sexually active women had higher IgG, but lower IgA, than did sexually abstinent women. Frequency of sexual activity

moderated cycle-related changes in IgA, with increased frequency of sexual activity associated with increased ovulation-related decline in Ig, (Tierney et al 2015), the instability of the salivary IgA levels may help the *Trichomonas tinax* protozoa to grow and cause the infection in the oral cavity . Furthermore, another study has supported the impact of the the immune system defect on the oral parasitic infections. Atabank *et al*. (2015) investigated the parasitic infection rate among Down Syndrome childern patients compared with a healthy group, they found a significant increasein rate of *Trichomonas tenax* infection among the DS patients (Tierney et al., 2015),since, it is noted that DS patients have intrinsic defect of the immune system (Kusters et al, 2009). Also in the present study we noticed an increased rate of infection among females of the age group 51 and above with gingivitis, this age group might have experienced more pathological changes in their oral cavities, or systemic diseases impacting immune system activity. In some studies conducted to examine the oral protozoan in patients with systemic diseases, 40–50-yearold patients with somatic and mental retardation connected with epilepsy or Down syndrome, the oral amoebae and trichomonads occurred with prevalence from 30% to 60%. These and other observations lead to a conclusion of an opportunistic nature of the protozoans (Chomicz *et al*., 2002; Ghabanchi *et al*., 2010; Perkowski *et al*., 2016). In the current study we did not find any significant impact of the oral helath condition on the rate of *Trichomonas tenax* detection, instead, the Trichomonas tenax was detected in higher rate among persons with healthy gingival (age group 20-30 years) rather than persons with gingivitis. This controversy in the presence of the *Trichomonas* protozoan regardless to the pathologic condition of the gingival establishes an unclear impact of the *Trichomonas* as a pathogenic protozoan rather than an opportunistic oral microbiota. Howerver, in a study by Sarah et al., (2019) they established a correlation between the severity of periodontitis and the presence of protists, they found *T. tenax* in severe periodontitis differing from other periodontitis, by the depth of the pocket as well as the loss of attachment (Sarah *et al*., 2019). Indeed, periodontitis is suspected to be due to an inflammatory response to microorganisms (Pihlstrom *et al.*, 2005; Kinane *et al*., 2017), in addition, no single microorganism is implicated but rather a combination of microorganisms act synergistically (Socransky *et al*.., 1998; Cappuyns *et al*., 2005; Bonner *et al*., 2014). Although in some studies these differences are statistically significant, it is difficult to determine whether they are a cause or a consequence of the disease. Regarding the dentation condition of the participants, however the *T. tenax* was detected in higher rate among partial dentate without prosthesis followed by complete dentate and in lower rate in partial edentulous with removable prosthesis and partial edentulous with wearing denture , and there was a significant ($p=0.02$) effect of dentation on the rate of *T. tenax*. These results are Atabak et al 2015) their result established that oral protozoa was found in children and teenagers groups with having cured or complete dentition. The very low rate of the *T. tenax* presence among partial edentulous with removable prosthesis and partial edentulous with wearing denture participants could be due to the hygienic conditions and regular cleaning of the oral cavity and the removable prosthesis. That is supported by Kurnatowska *et al*., 2004), since they showed that the frequency of occurrence of *T. tenax* is independence on state of periodontium and hygiene of oral cavity.

CONCLUSION

Trichomonas tenax was detected in female group only and all the males were negative, the result showed that dentation status had a significant effect on the presence of oral *T. tenax*.

Conflict of interests: Nothing to declare

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