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

NADIA TAWFIQ JAFFER *, ALEEN SARDAR AL-NOORI **, AHMED MOHAMMED SALIH and SOLEEN SARDAR ZUHDI***

*Dept. of prosthetic and conservative dentistry, college of dentistry, University of Duhok, Kurdistan Region-Iraq
**Dept. of microbiology, college of medicine, University of Duhok, Kurdistan Region-Iraq
***Duhok Medical Research Centre (DMRC), college of medicine, University of Duhok, Kurdistan Region-Iraq

(Received: October 12, 2019; Accepted for Publication: December 3, 2019)

ABSTRACT

Background

Trichomonas tenax is a flagellated protozoan which inhabits the oral 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 51 years 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 difference(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 trichomoniases (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 females who 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’-AGCAGCTCCGTAATCCAG-3’ and 5’-CTTGTACCACCTTCTCC-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 X² 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 produced a 1000 bp band as shown in figure 1 which is compatible with data published by Thai et al., 2013.
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                                |
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 (%) | Gingivitis (%) |
|-------------------|----------------------|---------------|
| 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 those with 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

Protozoans species, such as flagellates and amoebae, are not so often taken into consideration in clinical researches, but can have a pathogenic impact on oral structures. The pathogenic effects of those protozoans 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 et al., 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 maintained 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-year-old 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). 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 is 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 (Pestechykan, 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 reported in the patients as compared to 3% of the control group (Atabank., 2015). In another study conducted on Periodontitis or Gingivitis Patients in Kayseri-Turke by Yazar and colleagues, 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 IgA. (Tierney et al 2015), the instability of the salivary IgA levels may help the Trichomonas tenax protozoa to grow and cause the infection in the oral cavity. Furthermore, another study has supported the impact of the immune system defect on the oral parasitic infections. Atabank et al. (2015) investigated the parasitic infection rate among Down Syndrome children patients compared with a healthy group, they found a significant increase in 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-year-old patients with somatic and mental retardation connected with epilepsy or Down syndrome, the oral amoeba 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 health 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. However, 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; Cappuyens 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

REFERENCES

Albuquerque, R.L.C., Moura, C., Alcântara, W., Lopes, A., Albuquerque, F. (2011). Incidence of Entamoeba gingivalis and Trichomonas tenax in samples of dental biofilm and saliva from patients with periodontal disease. RGO, 59, 35-40.

Atabank Kashefi Mehr, Ali Zarandi, keivan Anush. (2015). Prevalence of Oral Trichomonas tenax in Periodontal Lesions of Down Syndrome in Tabriz, Iran. Journal of Clinical and Diagnostic Research, 9(7), ZC88-ZC90.

Athari, A., Soghandi, L., Haghighi, A., Kazemi, B.(2007). Prevalence of oral trichomonirosis in
patients with periodontitis and gingivitis using PCR and direct smear. *Iranian J Public Health*, 36, 33-37.

Bonner, M., Amard, V., Bar-Pinatel, C., Charpentier, F., Chatard, J.-M., Desmuyck, Y., et al. (2014). Detection of the amoeba Entamoeba gingivalis in periodontal pockets. *Parasite*, 21, 30. https://doi.org/10.1051/parasite/2014029 PMID: 24983705.

Cappuyns, I., Gugerli, P., Mombelli, A. (2005). Viruses in periodontal disease—a review. *Oral Dis. Munksgaard International Publishers*, 11, 219–229. https://doi.org/10.1111/j.1601-0825.2005.01123.x PMID: 15984953.

Chomicz L., J. Piekarczyk, B. Starosciak et al. (2002). Comparative studies on the occurrence of protozoans, bacteria and fungi in the oral cavity of patients with systemic disorders. *Acta Parasitologica*, 47, 2, pp. 147–153. doi: 10.1128/CMR.00043-09

Duboucher, M, Mogenet, G Perie. Salivary trichomoniasis (1995). A case report of infestation of a submaxillary gland by Trichomonas tenax. *Arch Pathol Lab Med*.119(3): 277–79.

Feki, A. and .Molet.B. (1990). Importance des protozoaires *Trichomonas tenax* et Entamoebasingivalis dans la cavite buccale humaine. *Revue d’Odontologie et Stomatologie et Maxillo-faciale*, 19, pp. 37–45.

Ghabanchi J., M. Zibaee, M. D. Afkar, A. H. Sarbazie. (2010). Prevalence of oral Entamoebasingivalis and *Trichomonas tenax* in patients with periodontal disease and healthy population in Shiraz, southern Iran. *Indian Journal of Dental Research*, 21,1, pp. 89–91.

Gharavi, MJ., Hekmat, S., Ebrahimi, A., Jahani, MR.(2006). Buccal Cavity Protozoa in Patients Referred to the Faculty of Dentistry in Tehran, Iran. *Iranian Journal of Parasitology*, 1, 43-46.

Haghighi, A., Soghandi, L., Athari, A., Kazemi, B. (2007). Prevalence of Oral Trichomosis in Patients with Periodontitis and Gingivitis Using PCR and Direct Smear. *Iran J Public Health*, 36, 33–37.

Hersh, SM.(1985). Pulmonary trichomosisis and *Trichomonas tenax*. *J Med Microbiol*, 20, 1-10.

Kinane, DF., Stathopoulou, PG., Papapanou, PN. (2017). Periodontal diseases. *Nat Rev Dis Prim*. Macmillan Publishers Limited, 3, 17038. https://doi.org/10.1038/nrdp.2017.38 PMID: 28805207.

Kolenbrander, P.E. and London, J. (1993) Adhere Today, Here Tomorrow: Oral Bacterial Adherence. *Journal of Bacteriology*, 175, 3247-3252.

Kucknoor, AS., Mundodi, V., Alderete, J. (2009). Genetic identity and differential gene expression between *Trichomonas vaginalis* and *Trichomonas tenax*. *BMC Microbiol*, 9, 58.

Kurnatowska, AJ., Dudko, A., Kurnatowski, P. (2004). Invasion of *Trichomonas tenax* in patients with periodontal diseases. *Wiad Parazyt*, 50, 397-403.

Kusters, MA., et al. (2009). Intrinsic defect of the immune system in children with Downsyndrome: a review. *Clin Exp Immunol*, 156(2),189-93.

Lewis, KL., Doherty, DE., Ribes, J., Seabolt, JP., Bensadoun, ES. (2003). Empyema caused by *Trichomonas*. *Chest*, 2, 123: 291-292.

Lunsford R., D., A. A. Melillo, M. J. Somerman. (2015).“Guest editorial for special oral microbes edition. *Microbes and Infection*, 17, 7, pp. 471-472.

Mallat, H., Podglajen, I., Lavarde, V., Mainardi, JL., Frappier, J., Cornet, M. (2004). Molecular characterization of Trichomonas tenax causing pulmonary infection. *J Clin Microbiol*, 42, 3886-3887.

Nagwa MES, Eman MHM (2008). Detection of *Trichomonas tenax* in patients with periodontitis using microscopy and culture compared to PCR. *Egypt J M Sci*. 29(1-2); 537-550

Nicasio Mancini, Silvia Carletti, Nadia Ghidoli, Paola Cichero, Roberto Burioni, and Massimo Clementi (2010). The Era of Molecular and Other Non-Culture-Based Methods in Diagnosis of Sepsis. *Clin Microbiol Rev*. 23(1): 235–251.

Perkowski K., P. J. Zawadzki, B. Starosciak, B. Staro’ciak (2016). Składniki mikrobiomu jamy ustnej jako czynniki ryzyka zaka’ze’n lokalnych i uogólnionych u pacjent’ow bez oraz wadami wrodzonymi narządu ‘zucia. *Advances in Microbiology*, 55, 1, pp. 57–67, 2016.

Pestechyan N (2002). Frequency of Entamoeba gingivalis and *Trichomonas tenax* in patients with periodontal diseases and healthy controls in Isfahan Province, Iran. *Proceeding of 4th Iranian Congress of Parasitology, Mashad*, pp. 117.
Pihlstrom, BL., Michalowicz, BS., Johnson, NW. (2005). Periodontal diseases. *Lancet* (London, England), 366, 1809–20. https://doi.org/10.1016/S0140-6736(05)67728-8.

Saadia Shahab, Sanaa Huseein, Mohammad Kader (2012). prevalence's *Trichomonas tenax* and *Entamoeba gingivalis* among patients attending Dental Clinics in Kirkuk. *Journal of Babylon University/Pure and Applied Sciences*. 5:20.

Sambrook J, Russell DW (2001) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sarah Benabdellkader, Julien Andreani, Alexis Gillet, Elodie Terrer, Marion Pignoly2, Herve Chaudet, Gerard Aboudharam, Bernard La Scola. Specific clones of *Trichomonas tenax* are associated with periodontitis. PLOS ONE | https://doi.org/10.1371/journal.pone.0213338.

Socransky, SS., Haffajee, D., Cugini, M., Smith, C., Kent, RL.(1998). Microbial complexes in subgingival plaque. *J Clin Periodontol*, 25, 134–144. https://doi.org/10.1111/j.1600-051X.1998.tb02419.x PMID: 9495612.

Subramanian S, Hamed Emami, Esad Vucic, Parmanand Singh, Jayanthi Vijayakumar, Kenneth M. Fifer, Achilles Alon, Sudha S. Shankar, Michael Farkouh, James H.F. Rudd, Zahi A. Fayad, Thomas E. Van Dyke and Ahmed Tawako (2013). High-Dose Atorvastatin Reduces Periodontal Inflammation A Novel Pleiotropic Effect of Statins. *Journal of the American College of Cardiology*, 62; 25, DOI: 10.1016/j.jacc.2013.08.1627

Thai Kristina, Stander Duran, Rogers Joel, Shon Jae Ryong (2013). The Prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* in Tropical North Queensand Official. *Journal of The Australasian College of Tropical Medicine*. 14;(1):27.

Tierney, K., Lorenz, Gregory, E., Demas and Julia, R. (2015). Heiman. Interaction of menstrual cycle phase and sexual activity predicts mucosal and systemic humoral immunity in healthy women. *Physiol Behav*, 1, 152(0 0): 92–98. doi:10.1016/j.physbeh.2015.09.018.

Yazar S, Çetinkaya Ü, Hamamci B, Alkan A, Şisman Y, Esen Ç, Kolay M (2016). Investigation of *Entamoeba gingivalis* and *Trichomonas tenax* in Periodontitis or Gingivitis Patients in Kayseri. *Turkiye Parazitol Derg*, 40(1):17-21. doi: 10.5152/tpd.2016.4351.