http://www.ljmr.com.ly/ eISSN:2413-6096

*Original Article*

# The Prevalence of Entamoeba gingivalis and Oral Trichomonas tenax among people in Zawia city, Libya.

Fawzia Shawesh[1], Lubna Mohamed Elbeshti[2], Abdurraouf Zaet[3]

1- Fawzia Shawesh, Department of Medical Laboratories, Faculty of Medical Technology,University of Zawia, Libya.

2- Lubna Mohamed Elbeshti, Department of parasitology, Faculty of Medicine, University of Zawia, Libya.

3- Abdurraouf Zaet, Department of Dental Technology, Faculty of Medical Technology,University of Zawia, Libya.

**Abstract**

*Entamoeba gingivalis* and *Trichomonas tenax* are protozoa existing in the oral cavity and are typically detected in the periodontal pockets of teeth in humans. This study aimed to determine the prevalence of *E. gingivalis* and *T. tenax* infections among people in Zawia city. Another objective was to find any relationship between parasitic infections and some factors of human behaviours. All the participants filled out questionnaires that covered the oral health status, as well as drinks and smoking.

The population for this study included 280 participants, the samples were taken from the tooth surface of the fouling materials using sterile swabs. Then they were examined by wet mount smear for the diagnosis of *E. gingivalis* and *T. tenax* under the light microscope. The prevalence of *E. gingivalis* infection was found to be 77.47 %. Specifically, 51.67% of males and 25.8% of females were infected. Very high occurrences of *T. tenax* among people were detected (74.29 %). Most of the *E. gingivalis* infections were accompanied by *T. tenax* (48.39 %). In males, the prevalence of the infection in those with good and bad oral hygiene was 14.3% and 62.5 %, respectively, and of a significant statistical difference. Similar findings were measured in females, where the infection prevalence was equal to 60.7 % and 16.1 % among those with good and bad oral hygiene, respectively. Moreover, a significant relationship between the occurrence of infection and smoking was established. The highest infection rate was found in smokers (78.4%) compared to those non-smokers (21.6 %) in males. In conclusion, oral protozoa *E. gingivalis* and *T. tenax* were found to be of high prevalence among people in Zawia city. Furthermore, the parasitic infection has a close relation to oral hygiene habits and smoking.

Keywords: Entamoeba gingivalis, Trichomonas tenax, infection, prevalence, Zawia city, Libya.

Citation Shawesh F, Elbeshti L, Zaet A.The Prevalence of Entamoeba gingivalis and Oral Trichomonas tenax among people in Zawia city, Libya.2022;16(1):https://doi.org/10.54361/ljmr.15203 **Received**: 08/04/22**accepted**: 08/05/22; **published**: 31/06/22

## Introduction

*E. gingivalis* and *T. tenax* are considered to be pathogenic parasites by several researchers and to be commensal by others. It lives and survives in the gums and in the area surrounding teeth particularly in spaces between teeth cavities and gingival tissue. Also, these parasites were the first amoeba described in the oral cavity of humans [1,2]. Moreover, the trophozoite stage only is recognized in clinical samples, therefore, it does not have a cysts stage[2].

Around the world, there are several studies on human mouth protozoa such as a study in Nigeria that assessed the prevalence of oral protozoa in 203 dental patients attending a dental clinic, of these, *E. gingivalis* was the most popular (11.3%). The findings of this study suggested that poor oral hygiene, calculus, childhood, and loss of attachment of periodontal fibres are factors that favour the proliferation of *E. gingivalis* [3].

In the previses study, oral trichomoniasis, specifically *T. tenax*, was prevalent among 20.6% of the population. The infection frequency between patients with periodontitis and gingivitis was 40% and 14.1%, respectively. Additionally, oral trichomoniasis was dominant at an age range of 31 – 40 years, and most of them were males (19%) – only 14% of females were infected, with no significant difference. As for *E. gingivalis*, it was prevalent among 43.1% of the subjects and 6.8% of controls by direct smear technique [4].

A recent study was performed in Iran to assess the prevalence of *E. gingivalis*, the result was showed that the occurrence was 11.7% in total. Whereas the frequency in males (15.9%) was higher than in females (5.6%).

As there is a correlation of *E. gingivalis* and *T. tenax* to periodontal diseases, the following study was carried out to evaluate the prevalence of parasites in the oral cavity of patients with periodontal disease compared to oral healthy individuals. The prevalence of *E. gingivalis* in the study was found to be 66.7%. The associations of some factors, including smoking habits, and history of antibiotic intake throughout the previous six months for each patient, were also investigated. Seven patients had wabbling of the teeth, one of them was a smoker and five had an earlier history of antibiotic consumption [6]. The prevalence of *E. gingivalis* was high as the infection rate was 41.7% as reported in participants' referrers to the Faculty of Dentistry in Tehran[7].

Due to the presence of various factors including tissue damage, poor oral health condition, tooth loss, dental caries, low educational level, cigarette smoking, drugs, and food, which are seen to have some roles in the prevalence of *E. gingivalis* entails periodontal [8, 9,10]. Accordingly, a study was carried out on students and staff at the Faculty of Biological Sciences, Baghdad University, that aimed to assess the prevalence of *E. gingivalis*. The research found that the frequency of *E. gingivalis* in males was 46%, whereas, in females, it was 30%. Besides, there was a significant relationship between the frequency of infection and smoking. The highest rate was found in smoker people (52.6%) compared

with non-smoker people (20.9%). The rate of parasite infections was higher among individuals with poor oral hygiene (32.4%), in contrast to those with good oral hygiene (12.6%) [11]. The presence of these organisms in the mouth cavity correlates with poor oral hygiene and is quite common among people with a poor standard of life, contrary to the notion that parasites avoid the oral cavity [12,13].

Study done by Petersen PE, the sample were nine patients all of which were infected, six patients of them were infected with *E. gingivalis while three* patients were infected with *T. tenax*. Seven patients out of the nine patients had mobility of the teeth, while one patient was a smoker. However, five patients of all had previous 18 times history of antibiotic consumption. In the control group, only one patient was infected with *E. gingivalis* without any history of smoking and antibiotic consumption.

**Material and Methods**

**Samples Collection and Procedure**

Amongst the 280 samples from Zawia city, 140 were taken from females and 140 from males, as well as 50 healthy subjects were evaluated and chosen as the control group (25 women and 25 men). A special questionnaire form was designed and filled out by each participant. The questionnaire included questions regarding age, sex, smoking habit, food and drinks, oral hygiene, oral care, and medical status.

Following, the wet smear technique via sterile swabs was performed to collect samples. The dental sign samples were obtained by scraping the area with a sterile swab cleaned round the surface of teeth

Parasitic infections are relatively common in patients with periodontal disease [14].

In Egypt, another study has investigated the frequency of *E. gingivalis* in the gingival crevices of patients with periodontitis compared to normal subjects. The results revealed that higher frequency was observed in patients with periodontitis (64%) and Flagyl therapy reduced this frequency to (26%) [2,10].

From the above reviews, it is obvious that numerous studies are investigating the prevalence of *E. gingivalis* and *T. tenax* conducted in the western region. However, very limited research has been carried out in Libya, and in particular, in Zawia City. Thus, the present study aims to assess the prevalence of *E. gingivalis and T. tenax* in Zawia City. Also, this current study objects to investigate the association of some factors with parasitic infection.

from the cavity and round the gingival and subgingival areas.

**Microscopical Analysis of the Samples**

At the time following sample collection, the swabs were curved in sterile vials containing normal saline. Then, the swabs were rolled on the sterile microscope glass slides with a drop of normal saline. All the slides were then examined under a light microscope at magnifications of x100, x200, and x400. The strains of *E. gingivalis* and *T. tenax* were identified through the observation of morphology and the characteristic movement of their pseudopodia. Profile depending on the expansion of the pseudopodia formation and presence of vacuoles containing inclusion bodies of the nuclear remains of

lymphocytes. Subsequently, the correlation between the prevalence of *E. gingivalis* and *T. tenax* infections and the participants' sex and age were assessed.

### Statistical Analysis

The data for numeral parameters were subject to statistical analysis via SPSS version 25, a software program using analysis of variance one-way ANOVA. The non-parametric data were analyzed via the chi-square test. The significance level was set at ($P < 0.05$). Excel was used to draw all figures of results.

### Results

Table 1 displays the distribution of *E. gingivalis* and *T. tenax* s among people, according to gender. The occurrence of *E. gingivalis* infection was found to be of higher frequency in males (51.67%), in comparison to female people (25.8%). There was a high rate of *T. tenax* in females (43.26%) in contrast to males (31.03%). Correspondingly, the results demonstrate co-infection of *E. gingivalis* and *T. tenax* in both male and female people, at rates of 15.01% and 33.30%, respectively, as shown in figure 1. Accordingly, the characteristics of the types of infections present in all people are described in more detail in Table 1. All samples used in this study were teeth swabs with and without calculus and plaque. Also, 230 people suffer teeth decay and 50 people are not.

*Table 1: The Prevalence of Entamoeba gingivalis and Trichomonas tenax in Population, according to Gender.*

| Gender | No. of samples | *E. gingivalis*% | *T. tenax* % | co-infection % |
|---|---|---|---|---|
| Males | 140 | 51.67% | 31.03% | 15.01% |
| Females | 140 | 25.8% | 43.26 % | 33.30 % |
| Total | 280 | 77.47 | 74.29 % | 48.39 % |

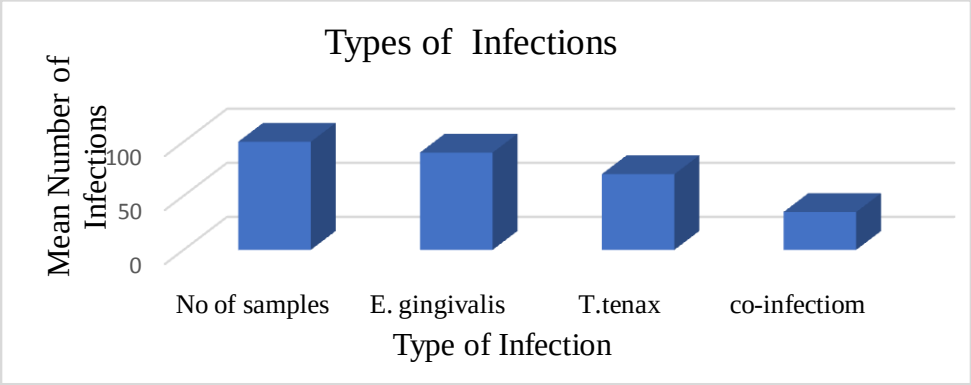


*Figure 1: Detailed Characteristics of the Types of Infections Present in all People.*

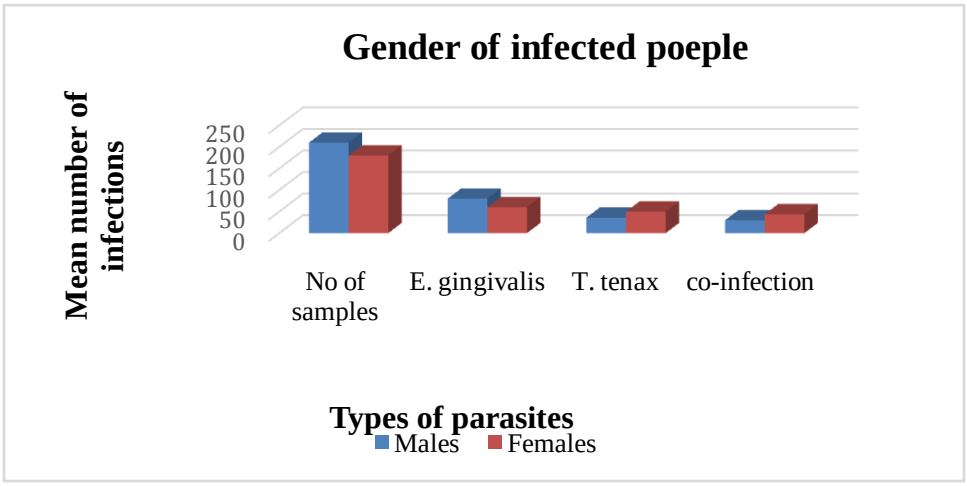


*Figure 2: Occurrence of Entamoeba gingivalis and Trichomonas tenax in Population, according to Gender ((P=0.03) P < 0.05 significant).*

The age of participating people ranged from 20 to 66 years. The correlation between the people's age and the presence of *E. gingivalis* and *T. tenax* (*P*=0.05), was noticeable among males (*P*=0.08). Nevertheless, the same pattern could not be observed in females (*P* = 0.16). Among the four age groups in men (20-25, 26-30, 31-40, and ≥41), the prevalence of *E. gingivalis* was observed in females, the *E. gingivalis* infection rate was considerably lower (figures 3 and 4). There was a significant relationship between age and the prevalence of *T. tenax* infection. In both genders, particularly, there was a positive relationship with age. In addition, figure 2 shows that the prevalence of *E. gingivalis* in males was higher compared with females (P=0.03). in contrast, the prevalence of *Trichomonas tenax* in females was higher than in males (P=0.02)

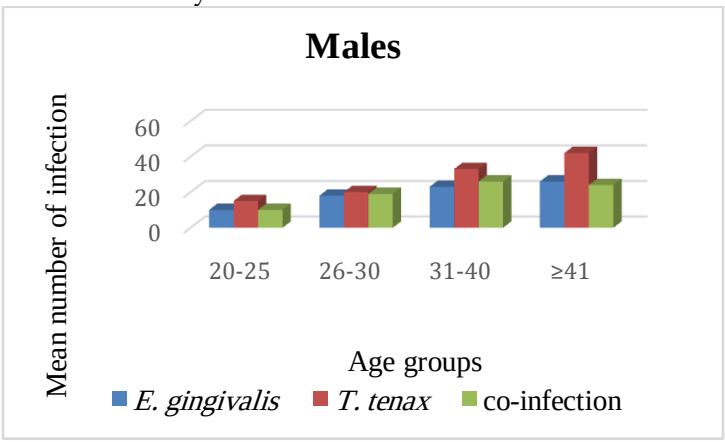


*Figure 3: The Prevalence of Entamoeba gingivalis and Trichomonas tenax in Males according to Age.*

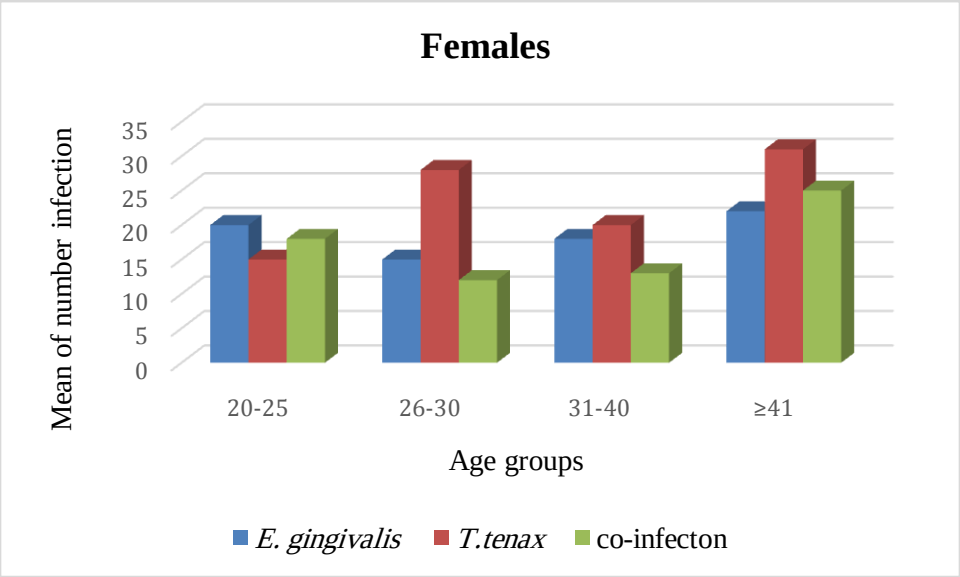


*Figure 4: The Prevalence of Entamoeba gingivalis and Trichomonas tenax in Females according to Age.*

In this study, 280 people were examined for available risk factors. The first factor was smoking. The highest rate of infection was found in male smokers (78.4%), compared to non-smokers (21.6%). A significant statistical difference was established ($P < 0.001$).

Secondly, oral hygiene was assessed. It was concluded that oral hygiene was mostly poor among the population, regardless of the routine. In males, *E. gingivalis* was found in higher rate with bad (62.5 %), then medium (23.2%), and good oral hygiene (14.3%). As well, the *T. tenax* infection was correlated with bad oral hygiene equal 44.6%. In female people, the prevalence of *E. gingivalis*, according to good, medium, and bad oral hygiene was 60.7 %, 23.2 %, and 16.1%, respectively. Meanwhile, the occurrence of *T. tenax* was seen at higher rates in male smokers (98.2 %) in contrast to non-smokers (5.6 %). The co-infections of *E. gingivalis* and *T.s tenax* were highly present among both genders with coffee consumption (50%) (Table 2). There was a statistically significant correlation between the risk factors in people and the presence of *E. gingivalis* infection ($\chi^2 = 6.175$, $P = 0.001$).

*Table 2: Risk Factors among studied Groups*

| Factors | *Entamoeba gingivalis* | | *Trichomonas tenax* | | Co-infection | | |
|---|---|---|---|---|---|---|---|
| | Males | Females | Males | Females | Males | Females | P<0.05 |
| Smoking | | | | | | | 0.001 |
| yes | 88(78.4%) | 0 | 55(98.2%) | 0 | 16(100%) | 0 | |
| No | 25(21.6%) | 56(100%) | 10(5.6%) | 90(100%) | 0 | 35 (100%) | |
| Oral hygiene | | | | | | | 0.001 |
| Good | 16(14.3%) | 34(60.7%) | 20(30.8%) | 45(50%) | 5(31.3%) | 25(71.4%) | |
| Medium | 26(23.2%) | 13(23.2%) | 16(24.6%) | 28(31.1%) | 2(12.5%) | 10(28.6%) | |
| Bad | 70(62.5%) | 9(16.1) | 29(44.6%) | 17(18.9%) | 9(56.3%) | 0 | |
| Drinks | | | | | | | 0.003 |
| Coffee | 51(45.5%) | 28 (50%) | 25 (38.5%) | 30(33.3%) | 8(50%) | 11(31.4%) | |
| Tea | 34(30.4%) | 24(42.9%) | 25(38.5%) | 40(44.4%) | 6(37.5%) | 10(28.6%) | |
| Both(co&T) | 27(24.1%) | 4(7.14%) | 15 (23.1%) | 20(22.2%) | 2(12.5%) | 14(87.5%) | |

## Discussion

*E. gingivalis* and *T. tenax* are free-living amoebae and transferable parasites, which are considered non-pathogenic to individuals but might be made a secondary effect. Nevertheless, the parasite has the potential to become devious pathogens or infrequently become hostile [1,2]. Hence, the present study was designed to determine the prevalence of *E. gingivalis T. tenax* among people in Zawia city.

One more significant finding that emerged from this study was that there was an excessive prevalence of *E. gingivalis* in both genders (77.47%). Also, this was found to be higher in males than females (51.67% and 25.8%, respectively). There was an increase in the rates of *T. tenax* in females (43.26%) compared to males (31.03%). Correspondingly, the results also show co-infections of *E. gingivalis* and *T. tenax* in males and females, at rates of 15.01% and 33.01%, respectively (table1 and figure1). These results are similar to those observed in earlier studies in several developing and developed countries [2, 3, 4, 7, 16]. It is important to bear in mind the possible bias in these results as women's oral care is more cared for than men. Consequently, it might also be since males seem to be more exposed to infection than females. Besides, men generally show inadequate oral hygiene

and wrong social habits regarding food and drinks. Accordingly, this result might reflect different social standards [7, 16,19].

The initial objective of this study was to assess the association of oral parasite infections and certain factors, such as smoking, oral hygiene, and drinks. First of all, smoking is identified as a key factor linked with global forms of basic periodontal disease [12, 13]. Our findings suggest that smoking was the largest characteristic significantly associated with factors of *E. gingivalis* and *T. tenax*, too. The highest infection rate was found in smokers (78.4%) compare to non-smokers (21.6 %) in men. This portrays a significant statistical difference with $P < 0.001$. Thus, this finding confirms the connection between smoking and the development of critical periodontal disease by some studies [3, 9, 15].

Moreover, the second factor evaluated by the study was oral hygiene. This research evaluated the level of people's oral hygiene (bad, medium, and good) and its relation with oral infections. There was a substantial relationship between the prevalence of *E. gingivalis* and the level of oral hygiene. In males, *E. gingivalis* was found in higher amounts in those with bad oral hygiene, compared to those with good oral hygiene, as shown in table 2. In females, the prevalence of the parasite according to the degree of oral hygiene was 60.7% in those with good and 16.1% in people with bad oral hygiene (Table 2). This result is in line with many previous studies that described an increased occurrence of *E. gingivalis* among individuals with bad oral hygiene [11, 24]. These results may be explained by the fact that bad oral hygiene encourages inflammation of the gingiva, mucous membrane, and cavity. Besides, it expenses the build-up of food residues and the development of dental panels, which establishes a good base for the growth of such microbes. This explains the significantly higher rate of *E. gingivalis* and oral *T. tenax* in cases presenting gingivitis in the present study [11, 18, 20].

A further interesting finding of the current study was the occurrence of *E. gingivalis* among people, of both genders, with regular coffee consumption (95%) (Table 2). Additionally, a large portion of other individuals assessing gum disease, gum expanding paying little mind to their immunological status was 50%, and just 0.2 % among the control group, yet with a higher force of contamination. The rate of infection in the control group is revealed by several investigations. It has been proposed that these protozoa could influence the commencement, improvement, and development of periodontal diseases [12,13,15,19,20].

## Conclusion

Even though *E. gingivalis* and *T. tenax* are not mainly pathogenic, their presence in the oral cavity is considered an indicator of oral trichomoniasis and gingivitis disease. The results of the current study suggest that *E. gingivalis* and *T. tenax* infection arise more frequently in people with periodontal and gingivitis disease. However, further studies are necessary for determining the real nature of the association between these species (*E. gingivalis* and *T. tenax*) and the other risk factors which may be the main

reason for several periodontal and oral diseases. Moreover, oral hygiene for people was poor. There were multiple records for the presence of tooth decay, gum diseases, and rarely or never using a toothbrush, especially in male people.

**Recommendations**

For further investigations, we recommend increasing the number of samples and generalizing them across different faculties, and also using different types of diagnosis via more advanced biological techniques, such as PCR and electron microscopy. Hence, this study recommends paying more attention to dental healthcare as well as raising education and awareness through lectures to increase oral hygiene knowledge.

*Disclaimer*

The article has not been previously presented or published, and is not part of a thesis project.

*Conflict of Interest*

There are no financial, personal, or professional conflicts of interest to declare

**References**


1. Al-Saeed W.M. 2003. The pathogenic effect of *Entamoeba gingivalis* on gingival tissue of rats. Al-Rafidain. Dent. J., 3, 70-73.
2. Foda A.A., El-Malky M.M. 2012. Prevalence of genital tract infection with *Entamoeba gingivalis* among copper T 380A intrauterine device users in Egypt. Contraception., 85, 108-12.
3. Ozumba, U.C., Ozumba, N and Ndiokwelu, E.M. (2004). Oral protozoa in a Nigerian population. African Journal of Clinical and Experimental Microbiology, 5:15-19.
4. PAri A, Ilango P, Subbareddy V, Katamreddy V, Parthasarthy H (2014). Gingival diseases in childhood–a review. J Clin Diagn Res, 8(10).
5. Maryam SHARIFI , Fatemeh JAHANIMOGHADAM , Zahra BABAEI, Mohammad Ali MOHAMMADI, Fatemeh SHARIFI, Nima HATAMI, Masumeh DANESH, Parnian POURESLAMI, *Hamidreza POURESLAMI. Prevalence and Associated-Factors for Entamoeba gingivalis in Adolescents in Southeastern Iran by Culture and PCR. Iran J Public Health, Vol. 49, No.2, Feb 2020, pp.351-359.
6. Chen J.F., 2001. Studies on periodontal disease caused by *Entamoeba gingivalis* and its pathogenetic mechanism. CMJ., 114, 12-15.
7. Gharavi, M.; Hekmat, S.; Ebrahimi, A. and Jahani, M. Buccal cavity protozoa in patients referred to the faculty of Dentistry in Tehran, Iran. Iranian J. Parasitol., 2006; 1(1): 43-46.
8. Gharavi MJ.,. 2006. Buccal cavity Protozoa in patients referred to the Faculty of Dentistry in Tehran, Iran. IJP., 1, 43-46.

9. Ponce de León P., 2001. Relation between buccal protozoa and pH salivary IgA in patients with dental prosthesis. Rev. Inst. Med. Trop. Sao Paulo., 43, 241-242.
10. El Azzouni, M and El badry, A (2003). Frequency of *E. gingivalis* among periodontal and patients under chemotherapy. Egyptian Society of Parasitology, 24: 649 – 655.
11. Sabaa T. Mohammed*, Nidaa M. Sulaiman and Sura B. Kamal (2015). Prevalence of *Entamoeba gingivalis* Among People of Collage of Science In Ai-Mustasyria University, Baghdad, Iraq, World Journal of Pharmaceutical Research, 4: 6, 2091-2096.
12. Sharma K, Kaur H (2015). Oral health about nutritional status, age and sex among 14-18 years children of Naraingarh, Haryana. Braz Dent Sci, 18(3):68-76.
13. Lin G.Y., Chen J.F., Ma W.M.: Investigation of infection of oral protozoan of parts of persons in Fuzhou, Strait. AJPM., 3, 129-130, 1997.
14. Petersen PE. The World Oral Health Report 2003: continuous improvement of oral health in the 21st century – the approach of the WHO Global Oral Health Programme. Community Dentistry and Oral Epidemiology 2003;32 Suppl 1:3-24
15. El-Dardiry MA (2016). Detection of *Entamoeba gingivalis* trophozoites in patients suffering from gingivitis versus healthy. Adv Environ Biol, 10(12): 222-226.
16. Ghabanchi, J, zibaei,M, Afkar Md and Sarbazie AH (2010). Prevalence of oral *E. gingivalis* and *T. tenax* in patients with periodontal disease and healthy population in Shiraz, southern Iran. Indian Journal Dental Research, 21: 89-91.
17. Bonner M., Detection of the amoeba *Entamoeba gingivalis* in periodontal pockets. Parasite., 21-30, 2014.
18. Athari, A., Soghandi, L., Haghighi, A. and Kazemi, B. (2007). Prevalence of oral Trichomoniasis. Iranian Journal of Public Health, 36: 33-37.
19. Wantland, W. W; Lauer D. Correlation of some oral hygiene variables with age, sex, and incidence of oral Protozoa. J Dent Res., 1970 49: 293-297.
20. Sheiham, Aubrey. "Oral health, general health and quality of life." (2005): 644-644.