EPH - International Journal of Applied Science
ISSN (Online): 2208-2182
Volume 07 Issue 01-March, 2021

DOI: https://doi.org/10.53555/eijas.v7i1.74

PREVALENCE OF ENTAMOEBA GINGIVALIS AND TRICHOMONAS TENAX AMONG HEALTHY AND PATIENTS WITH PERIODONTAL DISEASE IN BENGHAZI – LIBYA.

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Abstract:-

The oral cavity of human is colonized by many microorganisms among these Trichomonas tenax and Entamoeba gingivalis, is known as common parasite of oral cavity and sub maxillary glands especially with poor oral hygiene and presence of periodontal disease. This paper describes the prevalence and patterns of Entamoeba gingivalis and Trichomona stenaxin person with and without periodontitis in Benghazi, Libya, emphasizing on the gender, age, pH of the saliva, smoking habit. Methods Bio film and saliva samples were taken from 70 patients with periodontitis and 30 healthy individuals. They were spread on sterile swab sample, diluted with saline and examined with a light microscope. Descriptive statistic and chi-square test were used. The overall prevalence of oral parasitic infections among individuals of the sample participant was 24\100 (24%), the prevalence of Trichomonastenax (31.6%) and Entamoeba gingivalis (68.4%) among individuals with periodontal disease, while Trichomonas tenax recorded (20%) and Entamoeba gingivalis (80%) among healthy gingival individuals there was a high significant difference between periodontal disease compared to healthy gingival group (P=0.000) in the rate of harboring these parasitic infections. Prevalence of oral parasitic infections among individuals of the sample participant was 24\100 (24%). However, further studies are needed to determine the relationship between these species and periodontitis.

Keywords:-*Entamoeba gingivalis, Trichomonas tenax, Gingivitis, Parasitology, Periodontitis.*

INTRODUCTION

The human oral cavity is an ecosystem suitable for the growth of numerous microorganisms, due to inner environmental conditions. As many as 20-25 diferent species have been isolated from the human oral cavity [1, 2]. The protozoa, *Trichomonas tenax* and *Entamoeba gingivalis* usually proliferates on the surface of teeth and gums associated with gingival pockets near the base of the teeth and in the tonsils. *Entamoeba gingivalis* itsbelong to Phylum: *Amoebozoa* Class: *Archamoebae*, Genus: *Entamoeba* [3]. It is important to note that *E. gingivalis* is the only ameba that ingests white blood cells. While *Trichomonastenax* belong to Phylum: *Metamonada*, Class: *Parabasalia*, Order: *Trichomonadida*, Genus: *Trichomonas* [4]. Despite both parasites are considered by some authors as nonpathogenic and is common in (*Trichomonas tenax* 10 %, *Entamoeba gingivalis* 95 %) individuals with unhygienic mouth [5], Others show that these parasites are linked to various pathological conditions in the tonsils, lungs, neck and pre-existing oral diseases, such as periodontitis, favored by the use of prostheses and orthodontic appliances [6, 7]. However, the evidence indicating their direct involvement in the etiology of these conditions is still not fully documented. Both two species the *Amoeba* and *Trichomonas*, due to absence of cystic forms requires direct personal contact, usually by kissing, droplet spray or shared eating utensils. However, indirect contamination may occur through sharing food, cups, cutlery and other fomites [8, 9]. Oral *Trichomonas* is more frequently found in elderly people [10, 11]. Compare to children and edentulous [10, 12].

Aims of the study:

The aim of the present study

1- To estimate Prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* among healthy and patients with periodontal disease in Benghazi - Libya.
2- To estimate the overall prevalence of oral parasitic infection in Benghazi - Libya.
3- Identify the factors that contribute to the spread of the protozoa parasites among patients with periodontal disease and healthy population and the relationship between Prevalence and sex, age, poor dental hygiene.

MATERIAL AND METHODS

Study areas:

This study was carried out from February to April 2017 in Benghazi City, (23°10 N /20°06 E), East Libya. The average temperature ranges from -19.5o to 34.4 ºC.

Study Population:

The study focused on periodontal patients who visit Department of Periodontics Faculty of Dentistry, University of Benghazi.The people who visited the dental clinic unit for routine checkup and healthy individuals in the study area served as control group.

Study design:

This was a case control study which used parasitological investigation conducted to determine the prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* among patients with periodontal disease and healthy population served as a control. One hundred Individuals, their ages ranged between 18 to 60 years were taken. Specimens were collected from seventy patients with periodontal disease who did not used antibiotics for the last one week and 30 from peoples with healthy gingiva. After obtaining consents from the participants a structured questionnaire was administered by student personnel to collect clinical information and socio-demographic characteristics including age, sex, oral hygiene by means of tooth brushing, using of antibiotics, oral health and dental problems of each patient was noted.

METHODS

Sample collection:

Samples of saliva and dental biofilm/calculi were collected from all patients in the morning, before any oral hygiene. After determining the frontal mandibular area most affected by periodontal disease, dental biofilm/calculi samples were collected by scraping the area with sterile periodontal curettes. Unstimulated saliva samples were collected as recommended by (Navazesh. 1993) [13]. Each participant was examined using a dental mirror and a periodontal probe. Then they learned how to use plaque disclosing tablets. All samples were placed in sterile cotton swaps and diluted with saline at room temperature (25 to 28°C).

Immediately after dilution, the samples were examined under a light microscope. Then, a control sample was taken from the cleansed tooth surface of each tooth investigated.

Microscopic examination:

Direct wet smear method:

The samples were examined by direct wet smear method.

1. Sterile cotton swaps were used to collect samples from gingival and teeth with complete aseptic precautions under the assistance of dentist.
2. All patients were requested to rinse the mouth with 5.0 mL of sterile saline solution for approximately one minute and, soon afterwards, spit this fluid into sterile vials duly dated and identified.
3. A drop of sediment from each tube was prepared on slides and analyzed with optical microscopy (400x).Sterile pipettes were used to put droplets of the samples on microscope slides, which were then examined under x10, x40 and x100 magnification. Amoebae were detected by their producing one lobose pseudopodium at a time, with dark

intracellular vacuoles and one nucleus containing a central karyosome and peripheral chromatin. Amoeboid movement is easy to detect, as well as phagocytic activity. In case of doubt – for instance, if the nucleus could not be clearly detected – the cell was not recorded as an amoeba and *Trichomonas tenax* was identified by its flagella and characteristic locomotion. Giemsa stain was used to facilitate *Trichomonas tenax* identification.

RESULTS

Prevalence of oral parasitic infection of all participants (both healthy and with periodontal disease) was high 24/100 (24%). The results revealed that the overall prevalence of infection with oral protozoa in periodontal disease group was 19\70 (27.1%) higher than healthy gingival "control group" 5\30 (16. 7%). Figure 1 although the results revealed that the prevalence rate was high in both periodontal disease and healthy gingival groups. The difference was significantly higher in periodontal disease group compared with healthy gingival group ($\chi^2 = 4.262^a$; df = 2; P-value = 0.000). The study showed that the prevalence of *Trichomonas tenax* which reported (31.6%) and *Entamoeba gingivalis* 68.4% among individuals with period on taldisease, while *Trichomonas tenax* recorded 20 % . and *Entamoeba gingivalis* 80 % among healthy gingival individuals. Table 1 The males have significantly higher prevalence (13/33, 39.4%), of oral parasitic infection in periodontal disease group compared to females (6/37, 16.2%) ($\chi^2 = 4.738a$; df = 1; P-value = 0.029). while the results shown that prevalence in males 10 % and females was 30 %. Among individuals with healthy gingiva with no significant differences (P = 0.166).

Figure 2

The results shows significant difference between age groups and oral parasitic infection in Periodontal disease group being higher in older persons (>40 years) ($\chi^2 = 9.702a$; df = 2; P-value = .008) While no statistical difference in Healthy gingival group ($\chi^2 = .029a$; df = 1 P-value =.865) . Table 2 illustrate the prevalence according to age groups. Oral hygiene is important part of public health. The study showed that people with neglected oral hygiene harboring the highest percentage of parasites. Figure (3)

The prevalence of parasitic infection among Smokers and stimulant consumers (tea and coffee) was highest among tea consumers (24/24, 100%) followed by coffee consumers (9/21, 42.85%), and the least was prevalence was among smoker (8/24, 33.33%) .Table 3

Tables and figure

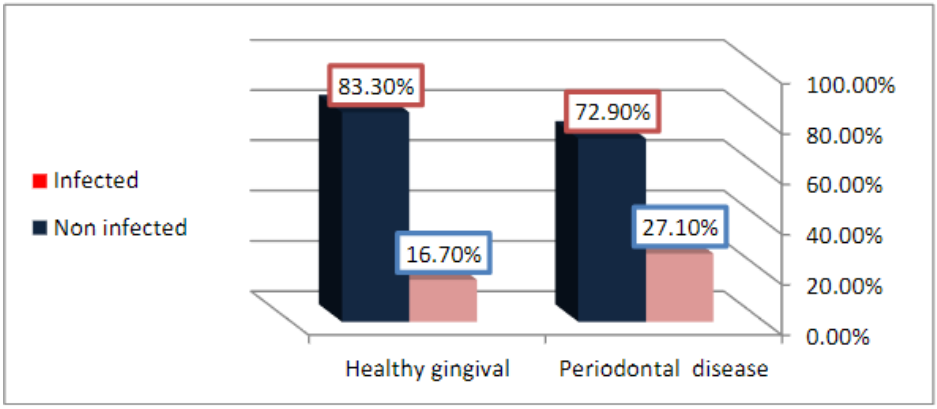

Figure (1): Prevalence of oral parasitic infection among examinedpatients (healthy and periodontal disease) (N=100)

Table (1): Percentage distribution of *Trichomonastenax* and *Entamoebagingivalis*among examined periodontal disease and healthy gingival Individual

Type of parasites	Oral serostatus (%)	
	periodontal disease (n=19)	Healthy gingival (n=5)
Trichomonastenax	31.6%(6/19)	20 % (1/5)
Entamoebagingivalis	68.4% (13/19)	80% (4/5)

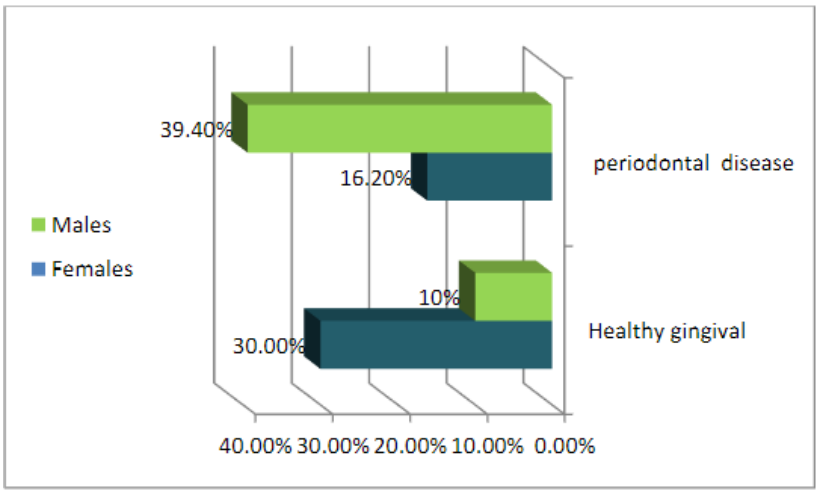

Figure (2): Relationship between prevalence of parasitic infection and sex among periodontal disease and healthy gingival groups

Table (2): prevalence of oral parasitic infection in each age groups among periodontal disease and Healthy gingival participants

Age groups (years)	Periodontaldisease		Healthy gingival	
	Examined (n=70)	infected (%) (n=19)	Examined (n=30)	Infected (%) (n=5)
18-29	24	4(16.7%)	0	0(0.0%)
30-39	**22**	3(13.6 %)	19	3(15.8%)
>40	**24**	12(50.0%)	11	2(18.2%)
Total	70	19(27.1%)	30	5(16.7%)

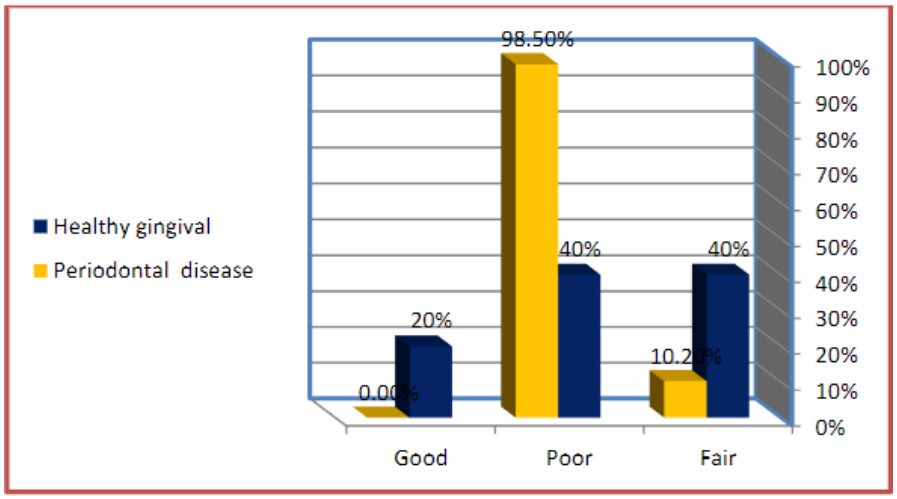

Figure (3): Distribution of oral isolates of parasites with respect to oral hygiene status of study participants

Table (3): The prevalence of parasitic infection and Smoking and drinking stimulant

	Periodontal disease and Healthy gingival both groups	
Exposure Status	Not infected	Infected
Smoker	16/24(66.66%)	8/24(33.33%)
Coffee	12/21(57.14%)	9/21(42.85%)
Tea	0/24(0%)	24/24(100%)

DISCUSSION

To our knowledge, this is the first study was carried out to detect the Prevalence parasitic infection emphasizing more of *Entamoeba gingivalis* and *Trichomonas tenax* among patients with periodontal disease in Benghazi – Libya . The results obtained in this study can provide important information for future understanding of oral parasitic infection. In the present study oral parasites was detected in both patients with periodontal disease and healthy gingival control groups. This finding is in agreement with previous reports from different countries of the world. The overall prevalence was 24 % this prevalence in concordance with that reported in the word [14, 15, 16, 17,18]. In the present study *Entamoeba*

gingivalis higher than *Trichomonas tenax* in both period on taldisease and healthy gingival group, 68.4% (13), 80% (4) respectively this result agreement with some previous studies [17]. The rate of infection in females was lower than in males in periodontal disease group. This is agreement with the Al-Najar and Ismaeel 1986 study [17]. The rate of infection varies in different age groups, it seems increased with the age of patients being highest > 40 years, and this might be related to oral unhygienic condition. Want land and Lauer found that the rate of infection increased with the age of patients up to the age of 40 years and then decreased.[12].

Conclusion:

In conclusion, oral parasitic infection appears to be an important infection in Benghazi, Libyan population. From the results of this study it is concluded that the prevalence of both T. *tenax* and *E. gingivalis* is high and proper care of teeth and gums, should be taken.

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