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Isolation *Trichomonas tenax* and study of the relationship between parasite prevalence and periodontal disease and diabetes in Nineveh Governorate/ Iraq

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Abstract---The current study included the collection and examination of 286 samples taken in the form of swabs from the periodontal pocket from people with periodontal disease, distributed among 186 samples from the auditors of the periodontal diseases branch of the Teaching Hospital at the College of Dentistry/University of Mosul, some outpatient clinics for dentists, and 100 samples from health centers in Tal Afar and Sinjar districts. Affiliated to Nineveh Governorate to investigate the parasite *Trichomonas tenax*, where the total infection rate was 0.07% and the percentage was higher in males .09% than in females 5.22%. The highest infection rate appeared in the age group 49-59 years, reaching 14.06%. Infection with oral diseases such as inflammation and bleeding of the gums had a clear effect on the presence of the parasite *T. tenax*, where a clear significant effect was observed in the high rate of infection, which amounted to 8.33% among people with gum disease, compared to the rate of infection among people without gum disease, which amounted to 2.86%, and it showed The results showed that the presence of dental plaque and the layer of calcifications had a significant and clear effect on increasing the parasite infection rate, which amounted to 8.28% with the presence of dental plaque and 8.8% with the presence of calcifications. On the other hand, diabetes had a clear impact on increasing the rate of infection with *Trichomonas tenax*, as it reached 12.5% of people with diabetes compared to those without diabetes, which amounted to 6.1%, and the smoking habit of males was related to an increase in the incidence of *Trichomonas tenax*, and clear significant differences were noted confirming this, as the infection reached 11.68% among smokers compared to the rate among non-smokers, which amounted to 7.77%.

Keywords---*Trichomonas tenax*, periodontal disease, diabetes.

1-Introduction

Microorganisms are found on human epithelial tissues such as the skin, oral cavity, respiratory system, digestive system, urinary system and reproductive system. The number and type of these microbes varies with age, diet and levels of personal hygiene, and they are referred to as normal microorganisms in the human body (Berger et al., 2015). Oral organisms are diverse due to the constant contact of the oral cavity with the external environment. Temperature, ecosystem, pH and feeding habits are important factors that contribute to the formation of the oral microbiota (Neetu et al., 2018).

The oral cavity contains different genera of microorganisms, but bacterial infection occurs when these organisms penetrate into tissues with cases of immunodeficiency (Loesche et al., 2007). The oral cavity contains approximately 700 or more different types of bacteria that live in the mouth and from It is important to maintain oral health, as the mouth is a suitable environment for the presence of many microbes Such as lactobacillas Steptococcus and parasites namely *Entamoeba gingivalis*, *Trichomonas tenax* (Rosier et al., 2018). Studies show that in addition to tooth decay and gum disease, microorganisms can have negative effects on other aspects of human health (Jabuk et al. 2015). *Trichomonas\$ tenax* is one of the parasites that cause trichomoniasis Trichomoniasis pulmonary and this disease requires a high technique to diagnose the parasite, which includes the technique of Polymerais chain reaction (Bracamonte et al., 2019). The morphological characteristics of *T. tenax* were identified by using light microscopy Pardi et al(2002), using electron microscopy, showed that the parasite is characterized by a pear-shaped nucleus, an oval nucleus, and four flagella protruding from the anterior side, with a fifth flagella attached to the corrugated membrane. Undulating membrane axostyle consists of microtubules producing a hard edge at the posterior pole, the parasite lacks Golgi apparatus and mitochondria, which are replaced by chromatin granules, the cytoplasm is characterized by its containment of many food vacuoles that have the ability to digest other bacteria and bacteria Phagocytosis (Duboucher et al., 1995) The life cycle of the parasite includes one stage, the vegetative stage, and it lacks the accumulating stage. The parasite reproduces by longitudinal binary fission of the active stage (Hersh, 1985). The parasite is usually transmitted by kissing, flying spray or using contaminated food utensils and drinking contaminated water. Ghabanchi et al. 2010 (The parasite is resistant to changes in temperature and can live in drinking water from hours to many days, Roberts 2000 and others).

2- Materials and working methods

Sample collection

A total of 286 samples were taken, taken in the form of swabs from the Gingival Pocket, from people with Gingivitis disease, distributed among 618 samples from the patients of the Gum Diseases Branch of the Teaching Hospital at the College of Dentistry / University of Mosul, some outpatient clinics for dentists, and 100 samples from health centers in Tal Afar and Sinjar districts of the governorate. Nineveh during the period from 1/10/2013 to 31/5/2014. The samples included

both males and females from different age groups, ages ranged between 16-59. A special information form (questionnaire) was organized for the injured, which included the patient's age, gender, environmental regression, educational level, as well as the smoking habit and diabetes, as well as some health habits, including the use of toothpicks. Detale and the layer of calculus. Samples were taken from patients with Gingivitis disease with the help of the specialist by taking a swab from the periodontal pocket where parasites are present (Jian et al., 2008) using sterile paper pointer sutures size 40 and left for 30 seconds and then placed. The samples were then transferred to the laboratory for the purpose of examination and investigation for the presence of *Trichomonas tenax* and to find out whether the result was negative or positive, and this is done by preparing Direct wet smear by placing a drop of the samples taken and placed in the center on a clean glass slide, then the slide cover is placed and examined with a magnification of 40X to detect *Trichomonas tenax* by its characteristic movement (Hersh, 1985).

Statistical Analysis

Statistical analysis was carried out using Chi-square at the level of probability ($P < 0.05$) (Al-Rawi, 2000). To know the effect of a number of factors on the percentage of oral parasites in people with Gingivitis disease.

3- Results and Discussion

The total percentage of infection with the parasite *Trichomonas tenax* 286 samples were collected from people suffering from dental and gum problems of different age groups, and samples were collected from the Teaching Hospital of the College of Dentistry / University of Mosul and some outpatient clinics for dentists and health centers in Tal Afar and Sinjar districts of Nineveh governorate to know the total percentage of oral trichomonas infection. After diagnosing the parasite with the sample, as shown in Figure (1), the total percentage of the parasite is 7%, and as shown in Table (3-1).

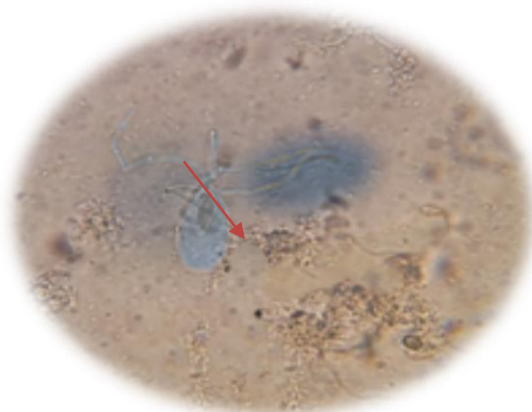


Figure 1. *Trichomonas tenax* in a swab taken from the periodontal pocket (40X)

Table (3-1) shows the percentage of total infection with *Trichomonas tenax* and its relationship to the sex of the affected person

sex	n.examin	n.infected	rat of infection(%)
Male	133	12	9
feamle	153	8	5.22
total	286	20	7
chi-square test	p=0.210	df=1	X ₂ =1.575

Among the studies that were conducted in the countries of the world and neighboring countries, is a study conducted in Turkey. The study included 175 samples, only(5)samples were infected with a rate of 2.9% (Yazar et al., 2016). There is also a study conducted in Babil Governorate, out of a total of 653 samples starved, the number of infected was 51 sample with a percentage of 7.81% (AL-Quraishi et al., 2015). Mohammed and Alwaaly (2019) also conducted a study on the parasite in Al-Qadisiyah Governorate, the number of infected people was 31 out of a total of 383, with a rate of 8.09%, and in another study conducted in Iran, the infection rate was 9.6% (Mehr et al., 2015) *T.tenax* is considered one of the parasites that burrows itself In solid waste that helps it stay in the mouth, it is difficult to diagnose. The process of diagnosing the parasite is a difficult process and requires high accuracy and expertise in diagnosis, due to the parasite's ability to lose flagella and transform into an amoebic form, in addition to its internal structures becoming invisible. One of the important points in the diagnosis is its distinctive spiral movement (Marty et al., 2017).It is clear from the above table (3-1) that the sex factor has an effect on the percentage of oral trichomonas, as the infection rate in males was higher than in females, reaching 9% compared to 5.22% in females, and no significant differences appeared also when applying the statistical analysis. The high incidence of oral trichomonas in males and lower in females may be due to practicing some healthy habits such as using toothpicks and flossing to clean the teeth. The effect of the patient's age factor on the incidence of oral trichomonas The results of the current study showed a clear difference between the incidence of *Trichomonas tenax* among the different age groups 16-59 years, as shown in Table (3-2). The highest incidence of gingival amoeba was found in the age group (49-59 years).

Table (3-2) shows the percentage of infection with *Trichomonas tenax* and its relationship to the age group of the infected

age group (year)	Male			Female			Total			chi-square test
	n. examine	n. infected	Rat of infection(%)	n. examine	n. infected	Rat of infection (%)	n. examine	n. infected	Rat of infection (%)	
26-16	49	2	4.08	22	0	0	71	2	2.82	X ₂ =7.340
37-27	32	3	9.38	65	2	3.08	97	5	5.15	df=3
48-38	20	2	10	34	2	5.88	54	4	7.41	P=.062
59-49	32	5	15.62	32	4	12.5	64	9	14.06	

This applies with the studies of many researchers who recorded the highest rate of infection in the large age groups of more than 50 years, including a study conducted in the province of Babylon, where the percentage of infection in males was more than in females, where the percentage reached within the age groups (55-60). 6.06% compared to the age groups (37-42), which amounted to 2.85% (AL-Quraishi et al., 2015).

The effect of some periodonal diseases on the presence of *Trichomonas tenax*

That were taken into consideration in the current study is inflammation and bleeding of the gums. Table (3-3) shows a high rate of *T.tenax* infection among people with gingivitis, as it was 8.33% in people with gingivitis, compared to the percentage of the parasite in people without gingivitis, where the percentage was 2.86%.

Table (3-3) shows the percentage of *Trichomonas tenax* infection and its relationship to gingivitis

Gingivitis	Male			Female			Total			chi-square test
	n. examine	n. infected	Rat of infection (%)	n. examine	n. infected	Rat of infection (%)	n. examine	n. infected	Rat of infection (%)	
yeas	92	11	10.87	124	7	5.65	216	18	8.33	X ₂ =2.438
no	41	1	4.88	29	1	3.45	70	2	2.86	p=118

It was documented between Bracamonte et al. (2019) in Chile, the relationship of a number of factors, including periodontal disease, and the presence of *T.tenax*, where the incidence of people with periodontal disease compared to those without it was 56% and 44%, respectively.

The rate of oral *Trichomonas* infection in people with bleeding gums was 9.29% compared to 4.79% in non-infected people, but it was higher in males than in females. Where the infection rate was 11.27 and 7.25%, respectively.

Table (3-4) shows the percentage of infection with *T.tenax* and its relationship to bleeding gums

Bleeding gums	Male			Female			Total			chi-square test
	n. examin	n. infected	Rat of infection (%)	n. examin	n. infected	Rat of infection (%)	n. examin	n. infected	ratof infection (%)	
yeas	71	8	11.27	69	5	7.25	140	13	9.29	X ₂ =2.216
no	62	4	6.45	84	3	3.57	146	7	4.79	p=0.00

The effect of the presence of dental plaque and the layer of calcifications on the presence of the parasite *T.tenax*

A high rate of infection with *T.tenax* parasite was observed, as shown in Tables (3-5) And (3-6) with the presence of dental plaque and calculus on the teeth, where

the parasite infection rate was recorded at 8.28% with the presence of the dental plaque layer, as shown in Table (3-5),

Table (3-5) shows the percentage of infection with *T.tenax* and its relationship to the presence of a layer of dental plaque

dental plaque	Male			Female			Total			chi-square test
	n. examin	n. infected	Rat of infection (%)	n. examin	n. infected	Rat of infection (%)	n. examin	n. infected	ratof infection (%)	
yeas	81	8	9.88	88	6	6.81	169	14	8.28	X ₂ =1.059
no	52	4	7.69	65	2	3.07	117	6	5.13	p=0.304

As for the effect of the presence of calcifications on the teeth, and as shown in Table (3-6), it was noted that the infection with *T.tenax* parasite increased, as the current study showed a high incidence of *Trichomonas oralis*, which reached 8.8%.

Table (3-6) shows the percentage of infection *T.tenax* and its relationship to the presence of a layer of calculus

calculus	Male			Female			Total			chi-square test
	n. examin	n. infected	Rat of infection (%)	n. examin	n. infected	Rat of infection (%)	n. examin	n. infected	Rat of infection (%)	
yeas	65	7	10.77	60	4	6.66	125	11	8.8	X ₂ =1.115
no	68	5	7.35	93	4	4.3	161	9	5.59	P=.0291

Many studies have proven that there is a very close relationship between the presence of calculus and gingivitis, as the accumulation of calcifications helps in the development of gingivitis into a disease called periodontitis, and the disease is accompanied by severe inflammation accompanied by bleeding gums (Newman et al., 2006). Albuquerque et al., 2011; Ibrahim and Abbas, 2012). Several studies have confirmed the high incidence of oral parasites in patients with periodontal infections and periodontal disease, which led to the conclusion that these parasites are associated with these pathogens (Ghabanchi et al., 2010). When Onyido et al. (2011) pointed out in a study they conducted on parasites Oral hygiene in Nigeria indicated that poor oral hygiene and the presence of a layer of calcifications on the teeth are important factors for the proliferation of oral parasites, and this was in agreement with our current study.

The effect of diabetes on the rate of infection with the parasite *T.tenax*

The current study showed a close relationship between the rate of infection with *T.tenax* parasite and the incidence of diabetes, as shown in Table (3-7). The

infection rate was high among people with diabetes, reaching 12.5%, compared to people without diabetes, which amounted to 6.1%.

Table (3-7) shows the percentage of infection with *T.tenax* parasite and its relationship to diabetes

diabetes	n. examin	<i>T.tenax</i>		Total	
		n. infected	rat of infection	n. examin	n. infected
Patient	40	5	12.5	33	82.5
Non Patient	246	15	6.1	117	47.56
total	286	20	6.99	150	52.45
chi-square test	P=0.001	P=0.141	df=1	$X_2=2.168$	

Effect of male smoking habit on infection rate of *T.tenax*

Table (3-8) shows a clear effect of the smoking habit of males on the infection rate of *T.tenax* parasite, as the infection rate of *T.tenax* parasite in smoking male patients was 11.68, while it was 7.77% in non-smoking males.

Table (3-8) the percentage of infection with *T.tenax* parasite and its relationship to the smoking habit of males

person status	n.examin	<i>T.tenax</i>		Total	
		n.infect ed	rat of infectio n	n.exa min	n.infect ed
Smoking	43	5	11.68	30	69.78
No smoking	90	7	7.77	38	42.22
total	133	12	9.02	68	51.13
chi-square test	df=1	df=1	P=0.469	X2=0.525	

The results of the current study agreed in terms of the superiority of infection with *T.tenax* parasite in male smokers compared to non-smokers. A study conducted by Mohammed and Alwaaly (2019) in Karbala on *Trichomonas* showed that smoking had an effect on the infection rate, which amounted to 15.7%, while the infection rate among Non-smokers 4.58%.. Bracamonte et al. (2019) also studied the parasite and infection rate in Chile and the relationship of a number of factors, including gum disease and the practice of smoking, where the incidence of people with gum disease compared to those without was 56%,44% on the following. The reason for the high incidence may be attributed to the fact that tobacco products are harmful to the health of the gums and the tissue supporting the teeth (Yamamoto et al. 2000), where the possibility of gum disease is seven times higher in smokers compared to non-smokers, as smoking negatively affects the treatment of gums and the use of cigarettes is considered Breathing from the mouth is one of the negative and unhealthy habits that weaken the ability of gum cells to repair damaged places. The process of breathing from the mouth

contributes to dry saliva, which plays an important role in protecting the teeth and gums (Feki and Molet, 1990). There are also many studies on the effect of tobacco on the immunity of smokers. Tobacco has the ability to reduce IgG, IgM immunoglobulins by plasma cells, as well as reduce the phagocytic activity and chemical response of neutrophil cells in the gums. It also weakens the immunity of the smoker against bacteria and parasites present in periodontal pockets. Which exposes him to increased infection with oral parasites and diseases of the supporting tissue (Bozner and Demes, 1991). Smoking also works to develop a suitable environment for the growth of some types of pathogens. Other studies have indicated that smoking has an important role in the incidence of severe periodontal disease and the loss of supporting ligaments of the teeth and gum tissue damage and tooth loss in a higher percentage in smokers (Person et al., 2011).

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