

ORIGINAL ARTICLE

ORAL PROTOZOA *ENTAMOEBEA GINGIVALIS* AND *TRICHOMONAS TENAX* AMONG PERIODONTITIS AND GINGIVITIS PATIENTS IN PERIODONTOLOGY DEPARTMENT DENTAL SECTION QUETTA

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Background: A group of eukaryotic organisms that are either free-living or parasitic and feed on organic matter, such as microorganisms or organic detritus. This group is referred to as a polyphyletic classification. They can be found in a wide variety of patterns and sizes, ranging from an amoeba, which can vary its shape, to a paramecium, which has a fixed shape and a complicated structure. The objective was to find out the occurrence of *Entamoeba gingivalis* and *Trichomonas* among Dental Patients visiting the Periodontology Department Dental Section, Sandeman, Provincial Hospital Quetta. **Methods:** The study design was randomized cross-sectional and conducted in the Department of Periodontology Dental Section, Sandeman Provincial Hospital Quetta, from March 2022 to February 2023. A total of 110 known cases of periodontitis and gingivitis were recruited in this study, and further to laboratory work the bacterial biofilm samples were collected from both gingivitis and periodontitis patients. The dental plaque was placed on individual glass microscope slides and a drop of saline and mixed and covered with a coverslip. The wet smear was examined immediately under 40X, and the objective for the presence and absence of motile amoebae or flagellated protozoa was recorded. **Results:** out of 110 known cases of periodontitis and gingivitis 91 (83%) patients were males and 19 (17%) were females among 110 patients, 40 (36%) patients were periodontitis and 70 (64%) patients were gingivitis. Among 40 (36%) periodontitis patients 3(7.5%) were *Entamoeba gingivalis* and 4(10%) were *Trichomonas Tenax* while 70 (64%) cases of Gingivitis 20 (28%) of *Entamoeba gingivalis* and 1(1%) *Trichomonas tenax* was recorded. No patient had both species of protozoa were seen in this study. **Conclusion:** The results of this study revealed the frequency of occurrence of *E. gingivalis* and *T. tenax* and the state of periodontitis and gingivitis. The prevalence of *E. gingivalis* was higher than *T. tenax*. We recommended further research with a higher number of patients and may use more advanced and reliable laboratory techniques such as PCR and electron microscopy.

Keywords: *Entamoeba gingivalis*; *Trichomonas tenax*; Gingivitis; Periodontitis

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INTRODUCTION

When we talk about proto-zoa, we are referring to a group of eukaryotic organisms that are either free-living or parasitic and feed on organic matter, such as microorganisms or organic detritus. This group is referred to as a polyphyletic classification. They can be found in a wide variety of patterns and sizes, ranging from an amoeba, which can vary its shape, to a paramecium, which has a fixed shape and a complicated structure. Parasites are organisms that live in other organisms, such as plants and animals, including humans and are responsible for the transmission of diseases. Depending on the species and strain of the parasite, as well as the body's resistance to the infection, infections can range from being asymptomatic to being potentially fatal. Protozoa are eukaryotic organisms that are tiny and

unicellular. They possess a highly sophisticated internal structure and are capable of carrying out multifaceted metabolic processes. Some protozoa have structures that allow them to move in a variety of ways, including propulsion. As of right now, six phyla are recognised based on the morphology of protozoa observed under optical and electron microscopes. The majority of organisms that infect humans belong to the phyla *Sacromastigophora* and *Apicomplexa*. Trophozoites are the term used to describe the stages of parasitic protozoa that actively feed and reproduce.¹

The usual home of microorganisms such as viruses, protozoa, fungi, and parasites are the oral cavity. The relationship between these pathogenic microorganisms, the human defence system, and additional exacerbating variables leads to a variety of diseases in the oral cavity, such as growth and

periodontal infections, ulcerations, and upper respiratory disorders. Normal gingiva has a coral-pink appearance, is firm and well-supported, does not lose its bone attachments, and has very little to no periodontal disease. Examining the gums for bleeding when probing or swelling indicates the presence of plaque-induced gingivitis. In terms of infection or inflammation, periodontal diseases are treatable with early intervention and appropriate maintenance of good dental hygiene. Individuals with periodontitis—a severe loss of bone and tissue—also have poor dental hygiene. In individuals with periodontitis, poor dental hygiene, extensive gingival pockets, and decaying teeth, parasites such as T-Tenax and *Entamoeba gingivalis* are frequently detected.² Protozoans known as *E. gingivalis*, which can endure in the lack of oxygen, are commonly found in the oral cavity. The majority of these sporozoans range in size from 10 to 35 µm, which is an extremely small size. Owing to their small diameter, these parasites can spread easily from person to person through contact with the lips or oral mucosa. They can also spread indirectly through the use of common kitchen tools like knives, forks, and spoons as well as contaminated food, picks, and candy bars. The majority of these parasite species are opportunistic, meaning they live in the oral cavity and are found in the areas surrounding the teeth, gingiva, interdental gaps, gingival pocketing, dental calculi, and dentition. Anaerobic protozoa like *Trichomonas tenax* can easily spread to other people via contaminated food, water, or contact with the saliva of an infected individual. Because *T. tenax* has the potential to infiltrate the respiratory system and produce catastrophic conditions like bronchopulmonary trichomoniasis, oral infections are now thought to be very valuable.³

In the human mouth, *E. gingivalis* and *T. tenax* were initially identified in 1849 and 1850, respectively. Both species parasitize the oral cavity, according to studies conducted all around the world.⁴ Alveolar bone deterioration and eventual tooth loss are symptoms of periodontitis. Within the designated World Health Organization (WHO) regions, periodontitis affects approximately one in two adults between the ages of 35 and 44. As people age, this frequency increases. Gingivitis that goes untreated and unchecked develops into periodontitis, which is characterized by an anaerobic environment, bone deterioration, collagen fibre degradation, and a plasma cell-based inflammatory infiltrate. Clinical attachment loss, or the deeper location of the tooth root's cementum and junction epithelium, is the outcome of periodontitis. When there is less than 3 mm between the free gingival edge and the epithelial attachment, it is considered that there is periodontitis, which is supported by bleeding upon probing and inflammation

(redness, swelling, and soreness). Oral fissures were home to sporozoan species such as *Entamoeba gingivalis* and *Trichomonas tenax*. This parasite lives in soft tissue surrounding immature teeth as well as in cavities in teeth that have decayed. It also inhabits the area between teeth. In particular, when pustular inflammation is present, and they are aggressively growing without free oxygen.⁵ Few researchers believe that these protozoa are opportunistic because periodontal and gingival diseases change the gingival habitat and these sporozoa can reproduce in these changed conditions.⁶ In 1849, Gross discovered *E. gingivalis*, the first parasitic amoeba ever recovered from a human being, from tooth tartar. It belongs to the following taxa: genus *Entamoeba*, class Archamoebae, phylum Protozoa, subphylum Sarcodina, and species *E. gingivalis*. This protozoa contains a 2 to 4µm spherical nucleus and is between 10 to 35 µm in length. It also has pseudopods that enable it to move swiftly. Its incidence has always been linked to poor oral hygiene practices, advanced age, the presence of plaque and calculus, and periodontal diseases. For these oral protozoa to proliferate, oral epithelial cells, food particles, bacteria, erythrocytes, and leukocytes acted as food sources.⁷

Due to their minimal environmental resilience, oral sporozoa such as *Entamoeba gingivalis* and *Trichomonas tenax* are most frequently spread from person to person among close contacts. Patients with advanced periodontitis often have T-Tenax detected. Pus pocket near the base of teeth; tonsillitis; sinusitis; oesophageal malignancy; abscess in the jaw. larger lymph nodes in the upper cervical region, infecting the submaxillary gland. *E. gingivalis* was found in saliva, tonsils, crevicular fluid, tooth surface plaque interproximal gaps, and cyst depth. According to some writers, this parasite plays a part in the development of tooth decay.⁸

Flagellated *E. gingivalis* is a member of the tubulinae sub-order and family, with a size range of 5 to 35 µm. Based on documented research, we conclude that these oral sporozoa are a significant cause of gingivitis, palatal sores, bad breath odour, exhaustion, and excruciating migraines.⁹ Both *T. tenax* and *E. gingivalis* are found in the oral cavity, although *T. tenax* is more active than the other. The transmission pathways of these trophozoites are identical; however, *T. tenax* survives in water for several hours to several days¹⁰. Common protozoa in the human oral cavity include *E. gingivalis* and *T. tenax*, which have been detected in swabs from deep dental pockets, plaque, and the periodontium's surface¹¹. The prevalence of *E. gingivalis* and *T. tenax* in the mouth has been observed to vary between 4% and 53% in the rest of the world.^{12,13}

For almost a century, investigations using light microscopy have also shown that people with dental diseases, such as periodontitis, have a high frequency of protozoan parasites like *E. gingivalis*. This has given rise to conjecture that it may also play a role in the development of periodontal disease. Periodontitis is thought to be the most frequent oral infection due to its unique clinical appearance. Between 5% and 20% of the population experienced severe gingival infections that caused damage to both soft and hard tissues. This inflammatory condition is linked to numerous bacterial infections brought on by Gram-negative, anaerobic bacteria.¹⁴

Research from throughout the globe has demonstrated that both organisms parasitize both healthy mouths and oral cavities that have been altered by inflammation.^{15,16} The oral cavity has long been disregarded in investigations on parasite infections because it is a complex ecology. Very few investigations on oral parasites have been carried out in a few different nations. These investigations demonstrated the presence of two parasitic protozoa in the oral cavity, named *E. gingivalis* and *T. tenax*.¹⁷ Research on the oral cavity, dental tissue, and common oral parasite diseases, however, seems to be quite important. The purpose of this study is to ascertain how frequently patients with periodontitis and gingivitis who visit the periodontology clinic dental section at Sandeman Provincial Hospital Quetta have oral cavity protozoa from dental plaque, periodontal pockets, and decayed teeth.

The objective of the study was to determine the frequency of oral cavity protozoa among the dental patients visiting in periodontology clinic dental section of Sandeman Provincial Hospital Quetta. It was a Randomized, Cross-sectional study among Dental patients visiting in periodontology clinic dental section of Sandeman Provincial Hospital Quetta.

Outcome measures will be to determine the frequency of oral cavity protozoa from dental plaque, periodontal pockets and decay teeth among the dental patients visiting in periodontology clinic dental section of Sandeman Provincial Hospital Quetta.

Inclusion criteria

Included those patients having more than 20 teeth with identification of gum diseases for the first time and periodontal attachment loss of more than 2mm and no record of any type of conventional periodontal therapy or deep cleaning.

Exclusion criteria

As for exclusion criteria, the patients were excluded from those who had been using systemic antibiotics in the past two months, history of pregnancy, had past periodontal treatment, presence of dental implants, taking orthodontics treatment, and using immunosuppressive drugs.

MATERIAL AND METHODS

Gingivitis and periodontitis patients were selected from the outpatient clinics of the Department of Oral Medicine and Periodontology Dental Section Sandeman Provincial Hospital Quetta. In subjects with periodontal disease confirmed by radiographs and clinically the collection of the specimens is done by scratching the space with a septic periodontal curette and placed in a petri dish. A temporary laboratory set up will be in the Oral Medicine/Periodontology Department, Dental Section Sandeman Provincial Hospital Quetta. The questionnaire will be filled out by the patients who attend the oral medicine clinic and are willing to participate this study included in this study. A prepared chart will be used to record socio-demographic data including information about age, gender, underlying diseases like blood pressure, cardiac problems, diabetes mellitus, and usage of any type of recreational drugs. smoking habits, teeth cleaning, presence or absence of bacterial dental plaque, sub-gingival and supra-gingival calculus and gingival bleeding. Patients will be interviewed to obtain information about the instrument used to clean their teeth (toothbrush, chewing stick (*Miswak*) or both) and the frequency of cleaning teeth (once a day, more than once, sometimes).

Scrapings of plaque will be taken from the upper and lower anterior and posterior teeth with different surfaces near the gingival margins, periodontal pockets and grossly carious teeth by curesttes. The plaque will be placed on individual glass microscope slides and a drop of saline, will mix with the plaque and be covered with a coverslip. The wet smears will be examined immediately under a 40X, objective for the presence of motile amoebae or flagellates and their presence or absence will be recorded. The parasites of *Entamoeba gingivalis* were recognised by their appearance relying upon the shaping and extension of pseudopode while *Trichomonas tenax* was detected by its flagella and typical motivity.

RESULTS

The research was conducted to look over the presence of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque among the patients who visited the Dental Section of Sandeman Provincial Hospital Quetta. A total of 110 numbers of samples were examined of patients with different dental problems. The results revealed that out of 110 samples, 23 were *Entamoeba gingivalis*. While 5 cases of *Trichomonas tenax* were recorded. Among 23 positive samples, 9 were female and 14 male cases were recorded, with mean ages of 30 and 34 years respectively. In 5 cases of *trichomonas tenax* 4 were recorded in males with a

mean age is 40 years while only one is noted in females whose age is 50 years. According to our results prevalence of oral protozoa among the dental section of SPH Quetta is about 0.25%.

A total of 70 patients who are suffered from gingivitis was examined and samples collected from them for the Detection of oral protozoa, among them 20 were found positive for *Entamoeba gingivalis* and only 1 case of *Trichomonas tenax* was noted. 40 patients with Periodontitis were considered in this study and we found 3 cases of *Entamoeba gingivalis* and 4 cases of *Trichomonas tenax* were recorded

A total of 110 samples were collected for the Detection of oral protozoa among them 91 patients were males, and we recorded 14 cases of *Entamoeba gingivalis* and 4 Positive cases of *Trichomonas tenax*. Out of 110 Total 19 female patients were considered among them 9 cases of *Entamoeba gingivalis* were recorded while only one case of *Trichomonas tenax* was noted. Different age groups of patients were considered in this study for the Detection of oral protozoa. Patients' ages ranged from 11 to 20, 3 cases of *Entamoeba gingivalis* were noted, while No case of *Trichomonas tenax* was recorded. In patients among 21 to 30, 6 positive cases of *Entamoeba gingivalis*

were observed and no finding of *Trichomonas tenax*. For patients with age ranging from 31 to 40, 3 cases of *Entamoeba gingivalis* were seen and 1 case of *Trichomonas tenax* was noted. For patients between 41 to 50, 3 cases of *Entamoeba gingivalis* and 3 cases of *Trichomonas tenax* were noted. Patients among 51 to 60 No case of *Entamoeba gingivalis* is found while only one case of *Trichomonas tenax* was recorded.

A total of 25 patients were smokers among them 2 were found positive for *Entamoeba gingivalis* and only one case of *Trichomonas tenax* was recorded. 5 samples were collected from patients who use Betel quid among them only one case of *Entamoeba gingivalis* was noted and no detection of *Trichomonas tenax* was recorded. In this study, 10 patients were Diabetic while among them 8 cases of *Entamoeba gingivalis* were noted, and only one case of *Trichomonas tenax* was found positive. During this research 20 samples were taken from hypertensive patients, it was found that 3 cases of *Entamoeba gingivalis* were positive and no case of *Trichomonas tenax* was noted. During this research 20 samples were taken from hypertensive patients, it was found that 3 cases of *Entamoeba gingivalis* were positive and no case of *Trichomonas tenax* was noted.



Figure-1: *Entamoeba gingivalis*



Figure-2 *Trichomonas tenax*

Table-1: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque according to the periodontal status of the patients.

Clinical status	No. of samples	<i>Entamoeba gingivalis</i> (%)	<i>Trichomonas tenax</i> (%)
Gingivitis	70	(20) 28%	(1) 1%
Periodontitis	40	(3) 7.5%	(4) 10%
Total	110	(23) 21%	(5) 4.5%

Table-2: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque according to the gender of the patients.

Sex	No. of samples		<i>Entamoeba gingivalis</i>		<i>Trichomonas tenax</i>	
	No.	%	No.	%	No.	%
Male	91	14	15.4		4	4.4
Female	19	9	47.4		1	5.3
Total	110	23	21		5	4.5

Table-3: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque according to the patient

Age (years)	Total		<i>Entamoeba gingivalis</i>		<i>Trichomonas tenax</i>	
	No.	%	No.	%	No.	%
11-20	5	3	60	0	0	0
21-30	33	6	18	0	0	0
31-40	32	11	34.4	1	3.1	3.1
41-50	32	3	9.3	3	9.3	9.3
51-60	8	0	0	1	12.5	12.5
Total	110	23	21	5	4.5	4.5

Table-4: Detection rate of *Entamoeba gingivalis* and *Trichomonas tenax* in dental plaque according to the risk factors of patients

Risk factor	No. sample	<i>Entamoeba gingivalis</i>		<i>Trichomonas tenax</i>		Negative sample
		No.	%	No.	%	
Smoking	25	2	8	1	4	22
Betel quid	5	1	20	0	0	4
Diabetic	10	8	32	1	0.25	1
B. pressure	20	3	15	0	0	17
Total	60	14	18.6	2	2.6	44

DISCUSSION

Free-living, transferable amoebas, such as *Entamoeba gingivalis* and *Trichomonas tenax*, are thought to be non-pathogenic to humans but may have unintended consequences. However, the parasites may develop into cunning pathogens or only rarely turn hostile.¹⁸ To determine the frequency of *E. gingivalis* and *T. tenax* among the patients of the dental department of the municipal hospital Quetta, the current study was created.

According to the findings of the current study, patients with periodontitis and gingivitis had a 21 percent chance of having nematodes. *E. gingivalis* was more common than *Trichomonas tenax*, which was present in 4.5% of cases. A study conducted at Baghdad University's teaching hospital yielded very specific results, including an approximate incidence rate of 35% for *Entamoeba gingivalis*.¹⁹ Our study is distinct from that of EL Hayawan, who was able to isolate *E. gingivalis* from patients' dental cavities who had gingival infections, or periodontitis and gingivitis, but was unable to identify *T. tenax* in these patients' mouths.²⁰ According to the current study, we found that patients with gingivitis had 28% of *Entamoeba gingivalis* and 1% of *Trichomonas tenax*, whereas patients with periodontitis had 7.5% of *Entamoeba gingivalis* and 10% of *Trichomonas tenax*. Therefore, we observed both kinds of protozoa in individuals with periodontal disorders. During their research on oral hygiene in Nigeria, Onyido and his colleague mentioned that the presence of calculus and plaque are the main factors that contribute to the growth of oral protozoa, and their findings are consistent with the current study.²¹ Prior research in Kayseri found that 2.8% and 35.5% of participants had *Entamoeba gingivalis* and *Trichomonas tenax* assembled in their bacterial plaque, respectively. Additionally, 1.9% of

patients had simultaneous infection by distinct pathogens such as *Entamoeba gingivalis* and *Trichomonas tenax*. Our recent work differs from it in a term that we do not find a case in which both *E. gingivalis* and *T. tenax* were recorded. It was previously shown that these sporozoans might infect the oral cavity in cases of marginal chronic periodontitis when there was a drop in immunity or when immunosuppressive medications were taken.²²

Regarding the demographic data, there are disagreements concerning the distribution of infection with oral protozoa concerning gender. In the present study, the ratio of occurrence of infection in females was higher than in males. Our study revealed 47.4% of *Entamoeba gingivalis* and 5.3% of *Trichomonas tenax* were noted in females which is similar to the study done by El Hayawan and Bayoumy who isolated *E. gingivalis* from patients with periodontal diseases with higher percentage, especially in females. However, we disagree with their approach in that they were unable to separate *T. tenax* from them.²³ We discovered both protozoa in females in this latest investigation. In contrast to numerous earlier research that claimed that men are more likely than women to have oral protozoa, we found that 15.4% of *Entamoeba gingivalis* and 4.4% of *Trichomonas tenax* in males.²⁴ Similar to research by A. Najar and Adnan, who found that men were more often than women to have *E. gingivalis* and *T. tenax*, with percentages of 38.4% and 28.5%, respectively.²⁵ Our most recent findings are consistent with a research by Salah A. Ali Muhammad and his colleague on the prevalence of *T. tenax* in Karbala patients. They also observed a higher ratio of female parasite infection, around 11.8%, compared to the overall male proportion of 6.79%.²⁶ The reason for a high incidence of infection in females may be due to a lack of awareness and attention to oral

and dental hygiene, in addition to the fact that females may have some habits like talking for extended periods and close quarters, which also contribute to the spread of infection, even though the precise cause of the difference may not be mentioned.²⁷

We took into consideration participants in this study who were between the ages of 20 and 60. The rate of incidence of *E. gingivalis* in males and females with mean ages of approximately 34 and 30 years, respectively, was determined based on their age. While the mean age of males was 40 years noted for the incidence of *T. tenax* only one case of *T. tenax* was recorded in a female patient whose age is 50 years. In this present work patients, age range from 11 to 20 years we recorded 3 cases of *Entamoeba gingivalis* and no case of *Trichomonas tenax* was found, patients whose ages ranged from 21 to 30 years we recorded 6 cases of *E. gingivalis* and no case of *T. tenax* was noted, when we consider patients whose age range between 31 to 40 years we found 11 cases of *E. gingivalis* and only one case of *T. tenax* was noted, to check the prevalence of *T. tenax* and *E. gingivalis* we consider patients whose age range between 41 to 50 years we recorded 3 of *E. gingivalis* and 3 cases of *T. tenax*, and those patients whose age range between 51 to 60 years we recorded only one case of *T. tenax* but no case of *E. gingivalis* was noted. We concur with the findings of Gharavi and his colleagues, who observed that the incidence of oral protozoa is higher in persons older than 20 years of age. On the other hand, we disagree with Albuquerque, Maybodi, and his colleague who claimed that there was no connection between the age of patients and *E. gingivalis* colonization. In this current work, 110 patients were considered, and we did not record any occurrence of *E. gingivalis* and *T. tenax* in subjects younger than 16 years of age. Therefore, we found that the frequency of infection with oral protozoa increased with age. This finding is consistent with the Vrablic et al work, which states that oral protozoa, including *E. gingivalis* and *T. tenax*, does not occur in toddlers. Additionally, Vrablic discovered that persons with certain periodontal diseases—primarily gingivitis and periodontitis—had a greater rate of occurrence, which is connected to the findings of the current study.²⁸ Recently, eight research focused on juveniles have revealed the role and prevalence of *Trichomonas tenax* in periodontal illnesses. It was reported that the bacteria's proportion was quite low, ranging from 0–4%.²⁹ Some writers, such as Vrablic and Tomovas, have backed the theory that the incidence of *T. tenax* rises as people age. We back up this theory because, in our current patient population, the incidence of *T. tenax* similarly rises with patients aged.³⁰

In this investigation, we took 25 samples from smokers and found that 4% of the samples had

Trichomonas tenax and 8% had *Entamoeba gingivalis*. Diabetes, hypertension, and smoking are among the factors that raise the risk of periodontal diseases. This is a result of their immune systems being weakened by these chronic illnesses. Smoking can significantly lower the host's defence by inhibiting the production of IgG and IgM by plasma cells as well as the phagocytic activity and chemotactic responsiveness of gingival neutrophils.³¹ Ismail and his colleague discovered that smoking, vaping, or puffing still pose a serious risk of gingival infection even after controlling for variables including age, gender, dental hygiene, and socioeconomic position.³² It seems that smoking affects host defence through two main mechanisms: on the one hand, smoking causes systemic changes that affect the immune system.³³ Conversely, it affects fibroblast and vascular response locally using cytotoxic metabolites and vasoactive substances released upon combustion of the latter.³⁴ This was confirmed by many studies so the result of our research matches them and shows that there is an effect of a smoking factor in the incidence of oral protozoa including the study of Ibrahim and Abbas in Baghdad and work done by Segovia and his co-worker in Croatia and the study of Matheu in France. The reason for this is that smokers have a greater rate of gum disease and tooth decay than non-smokers because they frequently disregard their oral hygiene, avoid brushing their teeth, or use mouthwash seldom.³⁵ Due to weakened immune systems, diabetics are more susceptible to infectious diseases because their bodies react less severely to infections. One chemical that is intimately linked to dental disease is glucose, which makes plaque stickier to the surface of teeth³⁶. The literature on diabetes contains conflicting results; some studies claim that the prevalence of these protozoa is modest, while others report a high incidence of almost 74%.³⁷ In this present work, we collect 10 samples from diabetic patients out of which we recorded *Entamoeba gingivalis* 32% and 0.25% of *Trichomonas tenax*. When compared to healthy individuals, the frequency of oral parasite occurrence in diabetics is five times higher.³⁸ We contrast with the research conducted by Mendoza and colleagues, who found 74% of these parasites in diabetic patients a significantly greater percentage than what we observed.³⁹ We agree with the work done by Nocito et al who found that there was a higher rate of *E. gingivalis* in diabetic patients, as in our study out of 10 patients we recorded 8 positive cases of *E. gingivalis*. In this present work, we have collected samples from a total of 20 hypertensive patients we found 15% of *Entamoeba gingivalis* while no case of *Trichomonas tenax* was noted. We concur with the findings of Fadhil Ali Malaa S and colleagues, who observed 16% of *E. gingivalis* in hypertensive

individuals.⁴⁰ The majority of periodontal infections including inflammation and periodontitis together with some chronic diseases like hypertension along with a group of metabolic diseases as well as some bad habits such as the use of betel quid and smoking are strongly associated with the existence of oral protozoa. Except rare circumstances in which these parasites affect internal organs like the bronchial tubes and pulmo may involve and result in parasitic infection, oral protozoa often do not cause serious complications. One should stop using drugs and commit to maintaining proper oral hygiene, which includes brushing, using mouthwash, and using dental floss, to have a healthier mouth. The main goal of this activity is to raise awareness among the general public and inspire them to maintain a healthy, aseptic mouth. In light of our findings, we can conclude that *Trichomonas tenax* and *Entamoeba gingivalis* play a part in the aetiology of periodontal disorders. To better understand the causal connection between parasites and periodontal disorders, more research is required.

CONCLUSION

While *Trichomonas tenax* and *Entamoeba gingivalis* are not generally regarded as hazardous protozoa, their presence in the oral cavity may be a sign of some oral illnesses, including gingivitis and oral trichomoniasis. The results of our most recent research showed that patients with periodontal illnesses such as gingivitis and periodontitis are more likely to have symptoms of *E. gingivalis* and *T. tenax* infections. However, further research is necessary to determine the precise nature of these oral protozoa and their link to other risk factors, as they may be the primary cause of a variety of oral illnesses. Furthermore, we recommend increasing the quantity of samples and recommending other diagnosis kinds using more sophisticated and dependable biological techniques like PCR and electron microscopy.

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AUTHORS' CONTRIBUTION

ASG: literature review and conceptualization of study design. MM: Data collection, analysis and interpretation. MTH: Write up. DG: Proofreading and review

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