Prevalence of *Entamoeba gingivalis* and *Trichomonas tenax* among Patients suffering from Chronic Systemic Diseases in Egypt

Eman M.H. Meabed, Ramy W. Henin
Department of Parasitology., Faculty of Medicine, Fayoum University.

Corresponding Author
Ramy W. Henin

Mobile:
01225808475
01014814087

E mail:
Ramyharoun@gmail.com

Key words:
Diabetic, Entamoeba, Hepatic, Renal, Trichomonas.

Background and study aim: The Oral cavity harbors many microorganisms and their balance is essential for its health. *Entamoeba gingivalis* and *Trichomonas tenax* are the most commonly found oral protozoa. This study aims to explore their prevalence in patients with chronic systemic diseases and the associated risk factors..

Materials and Methods: This case-control study included 150 chronic patients from Fayoum Governorate, Egypt, designated into three groups (50 each). These are; a group of diabetic patients, a group of chronic renal failure on regular hemodialysis, and a group of chronic liver diseases with Child-Pugh Class B and C. In addition to 50 healthy volunteer subjects were enrolled as a control group. A specialized dentist collected dental plaques and saliva samples from all subjects. A designed questionnaire was taken for personal, demographic data, oral risk factors as regular oral hygiene, smoking, halitosis, and history of chronic or recurrent gum and teeth complaints. Oral samples were examined using direct microscopy, saline wet mount, and Giemsa staining. Each sample was cultured on Diamond's medium [TYM] for detection of *T. tenax*, and on Locke's-egg medium for detection of *E. gingivalis*. HbA1c as indicator for diabetic control was measured in sera drawn from cases and controls.

Results: The prevalence of oral protozoa was significantly increased in chronic diseases, as *E. gingivalis* was reported in 80%, 76, and 74% of diabetic, renal, and hepatic groups of patients respectively compared to 20% in the control healthy group. While *T. tenax* was reported in 70%, 62%, and 64% respectively compared to 16 % in the control group.

Conclusion: This high prevalence in chronic systemic disease needs more investigations concerning its pathogenesis, immunological mediators that may affect systemic diseases and the interplay between them .

INTRODUCTION

The oral cavity is the second largest gathering of microorganisms after the colon, it shelters 700 species **[1].** Many bacteria, fungi, and protozoa inhabit the mouth cavity in equilibrium. Disruption of this balance can lead to various diseases such as oral thrush, dental caries, gingivitis, and periodontitis.

Entamoeba gingivalis and *Trichomonas tenax* are the most important protozoa that inhabit the oral cavity of humans, living in the gingival tissues near the base of the teeth without the cyst stage. Some researchers consider them as commensals. However, they are more predominant in persons with oral infections such as gingivitis, and periodontitis as they flourish with conditions of bad oral hygiene, and inflammatory anaerobic and suppurative conditions. Transmission occurs by saliva, droplet spray, close contact, and kissing or sharing eating utensils **[2].**

Known risk factors for these parasites are identified as smoking, alcohol consumption, diabetes, bad oral hygiene, aging, genetic predisposition, and systemic diseases **[3].**

Chronic non-communicable diseases such as diabetes, chronic liver, and kidney diseases are major public health problems causing 60% of global deaths and accounts for 82% of all mortality rate in Egypt, and 67% of premature deaths **[4].**

Chronic liver diseases especially viral hepatitis, and its consequences as cirrhosis are global health problems that affect hundreds of millions of people worldwide. In Egypt, over 6 million people are chronically infected with about 150,000 new cases every year. Other common chronic liver conditions as non-alcoholic fatty liver disease (NAFLD), and non-alcoholic steatohepatitis (NASH) **[5].** Recent studies have linked these conditions to intestinal dysbiosis. A significant increase in the phylum *Proteobacteria* was detected in these patients compared to the healthy controls, indicating that there is an association between intestinal microbiota, and hepatic pathology. Interestingly germ-free mice colonized with gut microbes from NAFLD mice developed severe fatty liver, and cirrhosis supporting that gut dysbiosis has a causal effect on liver diseases **[6].**

This interplay between mouth microbiota causing periodontal diseases, and systemic diseases was investigated regarding stroke, diabetes, atherosclerosis, coronary heart diseases, and chronic kidney diseases as it seems that there is a certain microbiota signature to be associated with the development of these diseases. However, whether these changes are causal factors or effects is still under debate. **[7,8].**

Diabetes is the most common metabolic disease worldwide and affects 10.7 % of the population in the Middle East and 15.2% in Egypt **[9].** Many studies have investigated oral dysbiosis as a contributing factor to distal organ damage caused by diabetes through stimulation of innate immune response which relies on pathogen-associated molecular patterns (PAMPs) leading to the release of proinflammatory mediators that increase the insulin resistance and contribute to distal organ pathology **[10].**

Renal failure or end-stage renal disease is the fifth leading cause of death in Egypt with a prevalence of 10.6%. The associated uremia, dietary modification, metabolic acidosis, anemia, and electrolyte imbalance contribute to oral dysbiosis with a higher prevalence of gingival hyperplasia, chronic periodontitis, xerostomia, and root canal calcification **[11].**

Increasing data are accumulating regarding the bacterial component of oral microbiota but few studies discussed the oral protozoa. Its relation to systemic diseases and general health should be investigated and highlighted. The present study aimed to investigate the prevalence of two oral protozoa *E. gingivalis* and *T. tenax* in some common systemic diseases found in Egypt and to study the associated risk factors.

SUBJECTS AND METHODS

Subjects:

The sample size was calculated using OpenEpi. The hypothetical power of the study was 80%, and the ratio of cases to control was 1:1. Patients were attendee of Fayoum general hospital and Fayoum health insurance hospital. Samples were collected in the period from July 2022 to October 2022. They were selected after their approval to participate in the study, by careful studying of their hospital case file for assessment of their systemic condition. This included previous recent clinical, imaging, and laboratory examination within the same week of oral sampling. On the other hand, the normal control subjects were selected from healthy University employees and their relatives. They underwent general clinical examination and routine laboratory examination as glycated HB, liver enzymes, and renal function tests to exclude any general diseases.

Exclusion criteria: patients receiving anti-parasitic or antibiotic treatment for 1 month before the study, Subjects with multiple chronic diseases were excluded.

Study design

This is a case-control study. The study included 200 subjects designated into 3 groups suffering from chronic diseases (50 patients each). These were; a group of diabetic cases, a group of chronic renal failure on regular hemodialysis, and a group of chronic liver diseases with Child-Pugh Class B and C. In addition, a fourth group of 50 systemically healthy volunteer subjects was enrolled as a control group.

Methods:

A questionnaire sheet was designed to include personal, demographic data as age, sex, residence, and literacy or educational level, where high education means a university degree, any degree between that and illiterate is considered moderate education. The socioeconomic status (SES) of cases was evaluated in relation to the education and occupational levels according to Revised Kuppuswamy's SES Scale **[12].**

Oral risk factors a regular oral hygiene (subjects gave history about the rate and the regularity of using oral brush per day), smoking, bad oral smell (halitosis), and history of chronic or recurrent gum complaints such as gingivitis, loose teeth, caries, and the use of dental prosthesis .

A blood sample was drawn from each subject and underwent chemical analysis for the level of glycated hemoglobin (HbA1c), using kits (Human Inc.), according to the manufacturer's instructions. HbA1c indicates the average blood glucose level for the last two to three months and measure of glycaemic control in different subjects.

Oral sampling and diagnosis of oral protozoa by staining and culture techniques:

Dental plaques and calculus with saliva were collected by a specialized dentist using a curette in the morning without prior washing or after at least 3 hours after the last oral hygiene and placed in 2 sterile Eppendorf tubes according to **[13].** One sample was used for direct microscopic examination and the other was used immediately for cultures.

The 1st sample was preserved in 0.5 ml saline 0.9% and used for wet mount microscopy and Giemsa staining **[14].** Microscopic examination were made three times under dry magnification (400x) and then the specimens were fixed with methyl alcohol for 5 minutes and stained with Giemsa stain for 20 minutes at a dilution of 1:20. The preparations were examined at 1000x to detect violet, pear-shaped trophozoites with characteristic morphologic features. *T. tenax* was identified as a flagellated trophozoite, pear-shaped sized 5-16 µ, and with characteristic movement. *Entamoeba gingivalis* was differentiated by its size (10-20µ), the presence of a single nucleus with a small central karyosome, prominent pseudopodia, and sluggish movement. Micrometry was done to confirm the size.

For the cultivation of *T. tenax,* a part from the oral sample was inoculated immediately into Diamond's medium (TYM) **[14]**, TYM is a nutrient broth of trypticase, yeast, and iron to which inactivated horse serum and antibiotics are added] in standard culture tubes (16 by 125 mm) and incubated vertically at 37°C.

For the cultivation of *E. gingivalis*, another part of oral sample was immediately inoculated on diphasic Locke's-egg Medium (modified Boeck and Drbohlav's medium) **[15].** After 24 h incubation, A drop of culture media was examined daily for 72 hours by light microscope using a 400X.

Statistical Analysis:

All Data are tabulated and analyzed using SPSS software version 22.0. The sensitivity, specificity, and diagnostic accuracy were calculated. The comparison between each diseased group and the control group was performed using Chi-square test. Comparison between more than 2 groups was done using Kruskal Wallis test. *P* value ≤ 0.05 is considered significant.

RESULTS

The demographic data of examined groups are shown in table1. The table shows the distribution of cases according their age, sex, residence, educational level, and the SES**.** The table also shows the related oral risk factors as smoking habit, halitosis, the mouth hygiene level, presence of dental prosthesis, the history of chronic gum inflammation. The level of HbA1c was measured indicating the average blood glucose level for the last two to three months and measure of glycemic control in different groups.

Table 2 illustrates that *E. gingivalis* was found in 80%, 76, and 74% of diabetic, renal, and hepatic groups respectively compared to 20% in control group while *T. tenax* was found in 70%, 62%, and 64% respectively compared to 16 % in control group.

Table (3) compares wet mount and culture in detection of oral protozoa, wet mount microscopy detected 38.5% of *E. gingivalis* and 31% of *T. tenax* cases compared to 62.5% and 53% for culture. In addition, when taking culture as a confirmatory test, the wet mount had a sensitivity of only 58.5% - 61.6% and 100% specificity. Figure (1) shows some of the detected organisms during examinations**.**

Table (4) shows the distribution of cases of diagnosed E. gingivalis in the different studied groups and the relation to different risk factors. The table shows that 80% of the diabetic groups, 76% in the renal group, and 74% in the hepatic group were positive for *E. gingivalis* compared to 20% in the control group. *E. gingivalis was* significantly detected in cases with age range

from 41-60 and >60 group in diseased and control group [p value <0.05]. It was significantly detected in renal cases who had rural residence and illiteracy. The parasite was significantly detected in hepatic and renal cases with bad oral hygiene. *E. gingivalis was* significantly detected in diabetic and renal cases with HbA1c >7.0, who gave history of halitosis and chronic gum inflammation. Factors as literacy, SES, mouth hygiene, having dental prosthesis , halitosis, and history of gum inflammation are significantly associated with infection in all groups.

Table (5) shows the prevalence of *T. tenax* in different groups and in relation to different risk factors. *T. tenax* was detected in 70% of diabetic group, 62% of renal group, 64% of hepatic group and16 of control group. In diabetic group, the parasite was significantly associated with HbA1c >7.0, in relation to age more than 40 years old, rural residence, being smoker, halitosis and chronic gum inflammation, and having dental prosthesis . Diagnosed cases in renal group showed significant risk factors of rural residence, and halitosis. Diagnosed cases in hepatic group showed significant risk factors of being smoker, halitosis, and having dental prosthesis.

Table (1): Demographic data and some studied risk factors among the study groups.

Studied factor	Study groups	Control		Diabetic		Renal		Hepatic		*p*-value*	Total	
		N	%	N	%	N	%	N	%		N	%
Age	20 – 40	15	30	15	30	10	20	5	10	0.340	45	22.5
	41- 60	17	34	22	44	25	50	20	40		84	42
	>61	18	36	13	26	15	30	25	50		71	35.5
Sex	Male	24	48	23	46	34	68	30	60	0.288	71	55.5
	female	26	52	27	54	16	32	20	40		89	44.5
Residence	Rural	20	40	22	44	10	20	37	74	0.231	89	44.5
	Urban	30	60	28	56	40	80	13	26		111	55.5
Literacy (education)	Illiterate	10	20	20	40	11	22	31	62	0.121	72	36
	Moderate	28	56	19	38	23	46	12	24		82	41
	Higher	12	24	11	22	16	32	7	14		46	23
Socioeconomic status	Low	14	28	23	46	11	22	30	60	0.112	78	39
	Moderate	25	50	16	32	27	54	12	24		80	40
	Higher	11	22	11	22	12	24	8	16		42	21
Regular tooth brushing (at least once daily)	Good hygiene	20	40	15	30	18	36	16	32	0.727	69	34.5
	Bad hygiene	30	60	35	70	32	64	34	88		131	60.5
Smoking (current or in last one year)	Smoker	23	46	34	68	6	12	4	8	0.001	67	33.5
	Non- smoker	27	54	16	32	44	88	46	92		133	66.5
Dental Prosthesis	Yes	29	58	34	68	32	64	30	60	0.738	125	62.5
	No	21	42	16	32	18	36	20	40		75	37.5
HbA1c	<7.0	50	100	19	38	50	100	50	100	0.001	169	84.5
	>7.0	0	0	31	62	0	0	0	0		31	15.5
Halitosis	Yes	34	68	46	92	44	88	42	84	0.001	166	83
	No	16	32	4	8	6	12	8	16		34	17
Chronic Gum inflammation	Yes	33	66	37	74	34	68	33	66	0.001	121	60.5
	No	17	34	13	26	16	32	17	34		79	39.5

*Significant differences at $p < 0.05$, using Kruskal Wallis test.

Table (2): Prevalence of oral protozoa in the study groups.

Studied factor	Study groups	Control		Diabetic		Renal		Hepatic		Total Diseased		total	
		N	%	N	%	N	%	N	%	N	%	N	%
E. gingivalis	Positive	10	20	40	80	38	76	37	74	115	76.7	125	62.5
	*P value**			0.001		0.001		0.001		0.001			
T. tenax	Positive	8	16	35	70	31	62	32	64	98	65.3	106	53
	*P value**			0.001		0.001		0.001		0.002			
Mixed infections		2	4	15	30	7	14	8	16	30	20	32	16
	P value**	0.046*											

* comparing diseased group with control group using Chi-Square test, Significant difference at $p < 0.05$.
** Comparing the four groups using Kruskal Wallis Test, Significant difference at $p < 0.05$

Table (3): Comparison between wet mount and culture in detection of oral protozoa among cases and controls.

	Wet mount		Culture	
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)
E. gingivalis	77(38.5)	123(61.5)	125(62.5)	75(37.5)
	Sensitivity 61.60% Specificity100.00% Positive Predictive Value 100.00% Negative Predictive Value 87.29% Accuracy 89.44%			
T. tenax	62 (31)	138(69)	106 (53)	94(47)
	Sensitivity 58.49% Specificity 100.00% Positive Predictive Value 100.00% Negative Predictive Value 86.4% Accuracy 88.58%			

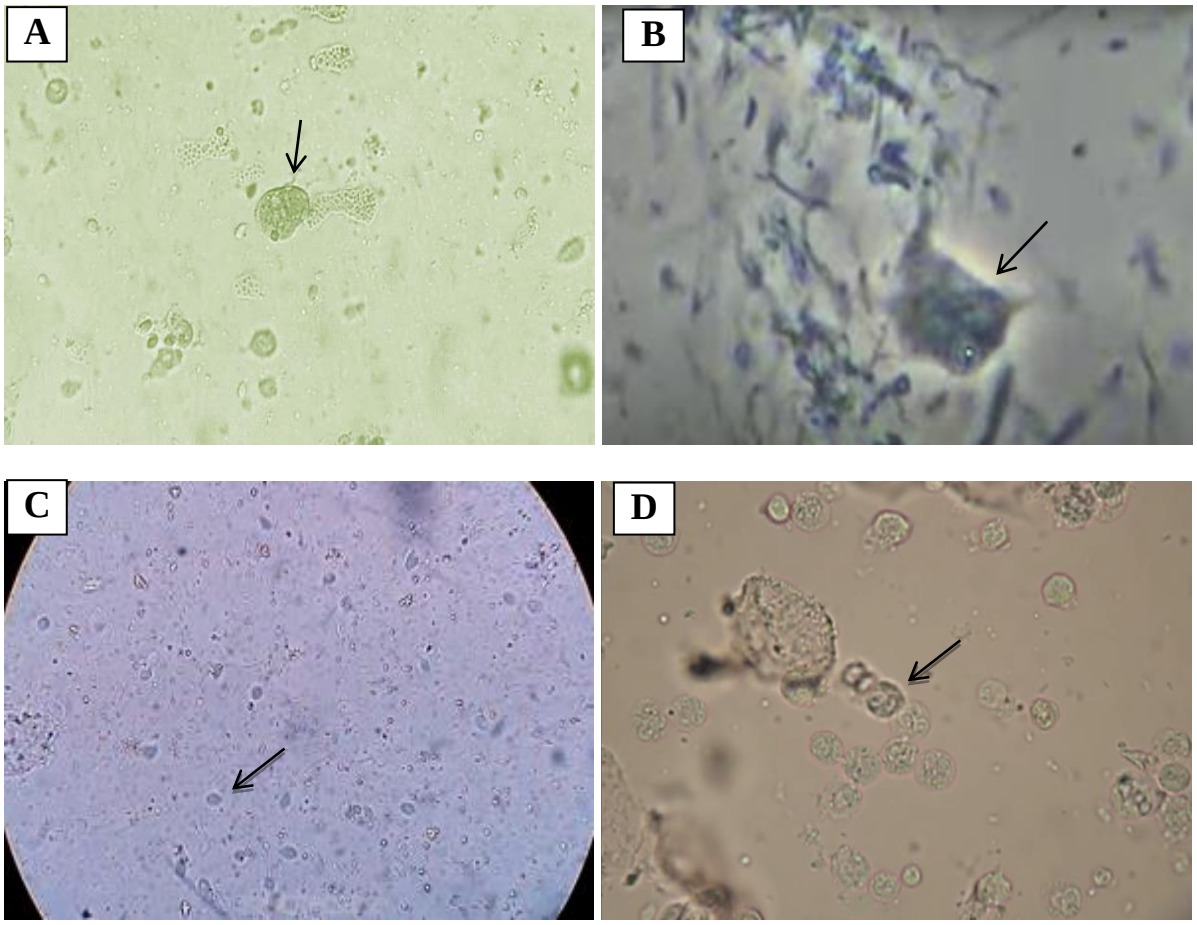

Figure (1):

A) *E. gingivalis* from culture 1000X
B) *E. gingivalis* troph. Wet mount 1000X
C) *T. tenax* culture 400X)
D) *T. tenax* wet mount Saliva 1000X

Table (4): Prevalence of *E. gingivalis* in relation to risk factor.

Characteristic	Study group	Control group			Diabetic group			Renal group			Hepatic group			Total diseased		
	E.gingivalis	Positive N	%	P value	Positive N	%	P value	Positive N	%	P value	Positive N	%	P value	Positive N	%	P value
Age	20 - 40	1	6.3		6	40.0		3	30.0		1	20.0		10	22.2	
	41- 60	5	18.5	**0.048**	21	95.5	**0.001**	20	80.0	**0.023**	16	80.0	**0.045**	62	73.8	**0.038**
	>60	4	57.1		13	100.0		15	100.0		20	80.0		52	73.2	
Sex	Male	6	25	0.489	19	82.6	0.372	24	70.6	0.162	19	63.3	0.548	62	87.3	**0.006**
	Female	4	25.4		21	77.8		14	87.5		18	90.0		53	59.6	
Residence	Rural	7	35	0.067	22	100.0	0.125	10	100.0	**0.001**	30	81.1	0.499	62	69.7	**0.001**
	Urban	3	10		18	64.3		28	70.0		7	53.8		53	47.7	
Literacy	Illiterate	6	60		20	100.0		10	90.9		27	87.1		57	79.2	
	Moderate	3	10.7	**0.041**	14	73.7	**0.047**	19	60.9	**0.025**	8	66.7	**0.046**	36	43.9	**0.001**
	Higher	1	8.3		6	54.5		9	25.0		2	28.6		12	26.1	
ocioeconomic status	Low	4	28.6		20	87.0		10	90.9		30	100.0		60	76.9	
	Moderate	5	20	**0.049**	15	93.8	**0.035**	23	85.2	**0.037**	5	41.7	**0.039**	43	53.8	**0.004**
	Higher	1	9.1		5	45.5		5	41.7		2	25.0		12	28.6	
Regular tooth brushing	Good hygiene	0	0	**0.003**	12	80.0	**0.037**	6	33.3	**0.001**	3	18.8	**0.004**	21	30.4	**0.001**
	Bad hygiene	10	33.3		28	80.0		32	100.0		34	100.0		104	79.4	
Smoking	Smoker	7	30.4		27	79.4		6	100.0		4	100.0		37	55.2	
	Non-smoker	3	11.1	0.154	13	81.3	**0.028**	32	72.7	0.620	33	71.7	0.107	78	58.6	0.124
Dental Prosthesis	Yes	٧	٢٤,١	**0.023**	30	88.2	**0.04**	30	93.8	**0.032**	29	96.7	**0.027**	89	69.0	**0.030**
	No	3	14.3		10	62.5		8	44.4		8	40.0		26	40.0	
HbA1c	<7.0	10	20		10	52.6	**0.018**	38	76.0		37	74.0		85	50.3	**0.001**
	>7.0	0	0		30	96.8		0						30	96.8	
Halitosis	Yes	9	26.5	**0.038**	38	82.6	**0.001**	38	86.4	**0.004**	37	88.1	**0.001**	113	68.1	**0.001**
	No	1	6.3		2	50.0		0	0.0		0	0.0		2	5.9	
Gum inflammation	Yes	8	24.2	**0.076**	31	83.8	**0.027**	34	100.0	**0.001**	33	100.0	**0.007**	98	81.0	**0.001**
	No	2	11.7		9	69.2		4	25.0		4	23.5		17	21.5	
Total		**10**	**20**		**40**	**80**		**38**	**76**		**37**	**74**		**115**	**57.5**	

Comparing each diseased group with control group using Chi-square test Significant differences at $p < 0.05$.

Table (5): Prevalence of *T. tenax* in relation to risk factors.

Characteristic	Study group	Control group			Diabetic group			Renal group			Hepatic group			Total diseased		
	T. tenax	Positive N	%	P value	Positive N	%	P value	Positive N	%	P value	Positive N	%	P value	Positive N	%	P value
Age	20 - 40	0	0.0		4	26.7		1	10.0		1	20.0		6	13.3	
	41- 60	4	11.8	0.244	18	81.8	**0.001**	15	60.0	**0.002**	11	55.0	**0.007**	44	52.4	**0.016**
	>60	4	11.1		13	100.0		15	100.0		20	80.0		48	67.6	
Sex	Male	4	8.3	0.590	17	73.9	0.324	21	61.8	0.544	16	53.3	0.061	54	76.1	0.840
	Female	4	7.7		18	66.7		10	62.5		16	80.0		44	49.4	
Residence	Rural	5	12.5	0.098	1۸	81.8	**0.014**	10	100.0	**0.001**	28	75.7	**0.019**	56	62.9	**0.033**
	Urban	3	5.0		17	60.7		21	52.5		4	30.8		42	37.8	
Literacy	Illiterate	6	30.0		20	100.0		10	90.9		24	77.4		54	75.0	
	Moderate	1	1.8	**0.027**	12	63.2	**0.023**	15	65.2	0.286	7	58.3	**0.013**	34	41.5	**0.030**
	Higher	1	4.2		3	27.3		6	37.5		1	14.3		10	21.7	
Socioeconomic status	Low	7	25.0		20	87.0		10	90.9		28	93.3		58	74.4	
	Moderate	1	2.0	**0.047**	12	75.0	**0.002**	17	63.0	0.051	4	33.3	**0.019**	33	41.3	**0.011**
	Higher	0	0.0		3	27.3		4	33.3		0	0.0		7	16.7	
Regular tooth brushing	Good hygiene	0	0.0	**0.001**	8	53.3	**0.036**	3	16.7	0.658	0	0.0	**0.009**	11	15.9	**0.003**
	Bad hygiene	8	13.3		27	77.1		28	87.5		32	94.1		87	66.4	
Smoking	Smoker	7	15.2	**0.046**	27	79.4		6	100.0		2	50.0		35	52.2	
	Non smoker	1	1.9		8	50.0	**0.020**	25	56.8	0.106	30	65.2	**0.452**	63	47.4	0.057
Dental prosthesis	Yes	5	17.2	**0.043**	29	73.5	**0.036**	30	93.8	0.656	24	80.0	0.410	83	66.4	**0.001**
	No	3	7.1		7	68.8		1	5.6		8	40.0		16	21.3	
HbA1c	<7.0	10	10.0	0.244	5	26.3	**0.001**	31	62.0		32	64.0		68	40.2	**0.001**
	>7.0	0	0		30	96.8		0			0			30	96.8	
Halitosis	Yes	8	11.8	0.034	35	76.1	**0.001**	31	70.5	**0.001**	32	76.2	**0.004**	96	59.0	**0.000**
	No	1	4.0		٠	0.0		0	0.0		1	3.7		2	0.0	
Gum inflammation	Yes	7	10.6	0.028	33	89.2	**0.038**	27	79.4	0.117	27	81.8	0.016	87	71.9	**0.003**
	No	1	2.9		2	15.4		4	25.0		5	29.4		11	13.9	
Total		**8**	**16**		**35**	**70**		**31**	**62**		**32**	**64**		**98**	**94**	

Comparing each diseased group with control group using Chi-square test Significant differences at $p < 0.05$.

DISCUSSION

This study aimed to investigate the prevalence of oral protozoa in the most common systemic disease found in Egypt compared to a healthy group and to assess the relationship between these organisms and different risk factors. The bacterial component of the oral microbiome has been extensively reviewed but the protozoal members and their relation to systemic diseases are rarely investigated.

Table (1) shows there is no significant association regarding age, sex, residency, literacy, SES, and oral hygiene but the other factors as HbA1c, halitosis and gum inflammation showed significant distribution, partially because of the specific groups selected in this study.

The present study showed that 80% of the diabetic groups, 76% in the renal group, and 74% in the hepatic group were positive for *E. gingivalis* compared to 20% in the control group (Table 2). For *T. tenax* 70%, 62%, 64% and 16% were detected in the studied groups respectively.

Most previous studies were concentrating on the prevalence among oral diseases such as gingivitis and periodontitis **[16,17].** A Previous study reported the prevalence of *E. gingivalis* as 47.9% among healthy subjects compared to 88.9% among patients with periodontitis, the prevalence was lower for *T. tenax* 25.6% in the periodontitis group and 3.2% in the healthy group **[18].** Another study reported that the prevalence of *E. gingivalis* in the periodontitis group ranges from 30% and 80% **[19].** Recently, a study that compared the prevalence in patients

with chronic diseases attending the university dental clinic in Iraq, found that the prevalence of *E. gingivalis* and *T.tenax* was 20%, 18.9%, respectively among diabetics, and 3.3% and 5.4% among patients with renal disease **[7].** This wide range may be due to the location of each study, the sample selection, and prior use of antibiotics.

In the present study, wet mount microscopy detected 38.5% of *E. gingivalis* and 31% of *T. tenax* cases compared to 62.5% and 53% for culture. Wet mount has 100% specificity and a sensitivity rate of only 58.4% to 61.6 %, which is slightly lower than previous study that reported its sensitivity 64.28% and specificity 97.2%, compared to PCR as a gold standard **[14].** For culture, the same authors reported a sensitivity rate of (85.7%), and specificity (97.2%). They reported that wet mount microscopy is the cheapest, quickest, and the most specific when done on a fresh sample for detection of the motile trophozoite **[14]**. However, wet mount depends on the examiner's expertise and the immediate transfer to the laboratory and processing the sample. Lugol's iodine was not used in wet preparation as it kills the trophozoite and causes shape distortion so motile organisms cannot be seen **[20].**

Culturing is a reliable method; however, it can be affected by many factors as the disintegration of the trophozoite before culturing, the low number of organisms in the sample, absence or presence of chemicals that is essential or harmful to the organisms. **A previous study** reported that less than 6 trophozoites led to negative culture despite positive PCR **[21].**

Results of this study showed a significant relationship regarding age for both protozoa with more prevalence in the older group with 67.5% (> 61 Y.) and 52.4% in (41-60 Y.). This is in agreement with previous studies that the prevalence of these protozoa increases with age as 35% (<40 years) to reach 85% in older subjects (>60Y) **[22]. A study** performed on diabetic patients concluded that prevalence increases with age with maximum prevalence in (61 – 70 age group) **[7].** There was no significant association regarding sex as described by **[23,24].**

This study found a significant association of parasitic prevalence in the diabetic group with 80% of diabetics having *E. gingivalis* and 70% having *T. tenax,* Also infection is significantly associated with HbA1c, Mixed infection increased significantly in the diabetic group (30%) compared to other diseased groups.

Results of this study showed that the prevalence of oral protozoa was significantly associated with HbA1c >7 as shown in previous studies **[25,26].** There is a mutual relationship between these parasites and diabetes. Uncontrolled diabetes creates a good environment for microbes in part due to decreased mucosal membrane immunity, poor repair, impaired secretion of saliva or xerostomia, decreased capillary perfusion, and peripheral neuropathy. In addition, increased sugar concentration in saliva makes the dental plaque thicker and sticky enough to allow more organisms to flourish. So oral infections as gingivitis and periodontitis are more prevalent in uncontrolled diabetics as the present study confirmed. On the other hand, the presence of these protozoa can aggravate glycemic control as the associated inflammation can contribute to insulin resistance and distal organ damage. It was found that healthy subjects with oral inflammatory conditions have higher HbA1c so have an increased risk to develop diabetes, also diabetic patients with periodontitis have less efficient diabetic control and more incidence of complications than healthy diabetics **[27].**

The present study showed a significant association between infection and having dental prosthesis for 69% of *E. gingivalis* and 66.4% for *T. tenax*. This finding comes in agreement with other studies that reported a prevalence of 70.37% and 64.81%, respectively. The two recently emergent diseases Peri-implant mucositis and peri-implantitis which represent gingivitis and periodontitis for natural teeth, these conditions affect 80% and 56% respectively of patients baring dental implants**.**

The present study showed a significant association between infection and smoking in the diabetic group only. Smoking is a well-known risk factor for oral and systemic diseases, as it leads to disruption of oral symbiosis through mucosal immunosuppression, decreases salivation, and decreased oxygen and biofilm formation. Most previous studies reported an increased prevalence of oral protozoa as well as oral inflammatory conditions among smokers **[29].** Diabetic smokers are at high risk for poor periodontal prognosis than non-smokers **[30].** On the contrary, few studies showed no significant association related to smoking. **[31]**

Halitosis is a common problem affecting 20 – 60 of the population. This study showed a significant association with both *E. gingivalis* and *T. tenax* in all tested groups. Oral causes account for 80 to 85% of all cases, etiology includes overgrowth of microorganisms, and oral diseases such as periodontitis, alveolitis, deep carious lesions, tongue biofilm, and candidiasis. Extra-oral causes such as gastroesophageal reflux disease, gastritis, sinusitis, or systemic diseases as diabetes, renal and hepatic patients **[32].** Aerobic and anaerobic bacteria were studied as etiological factors, these microorganisms tend to produce volatile sulfur compounds (VSCs) that are responsible for bad odor **[33].** Conversion of methionine to VSCs was discovered in *E. histolytica* and *T. vaginalis* as they own the gene encoding methionine-γ-lyase (MGL) **[34].** Whether oral protozoa can Produce VSCs or not is not yet investigated.

In the present study, *E. gingivalis* was detected in 76%, and *T. tenax* was detected in 62% in the renal group. Chronic kidney disease (CKD) is a global public health problem, affecting 10% of the population worldwide **[35].** Patients with chronic renal insufficiency and those on dialysis are more prone to periodontitis and other oral pathology, Studies have shown that chronic gingivitis can adversely affect renal diseases through oxidative stresses and inflammatory cytokines [**36].**

Among renal group a significant association was found regarding residence, literacy, and oral hygiene. Common findings in this group include dry mouth, taste disturbance, uremic odor, whitish discoloration of the tongue, parotid gland dysfunction, and mucosal inflammation.

Recently, several studies have reported the link between non-alcoholic fatty liver diseases (NAFLD), non-alcoholic steatohepatitis (NASH), and chronic periodontal diseases. It was suggested that 3 months of periodontal treatments in patients with NAFLD led to improvement in liver enzymes **[37]**. It is thought that chronic periodontitis and the related pathogens release endotoxins and lipopolysaccharides, bacterial DNA, interleukin 1alpha, and various cytokines that lead to systemic inflammatory response aggravating liver diseases (steatosis, fibrosis, and hepatocellular carcinoma) **[38,39].** Most fingers point at *Porphyromonas gingivalis*, a major pathogenic bacteria in periodontitis **[40].**

CONCLUSION

E. gingivalis was found in 76.7% in chronic disease groups compared to 20% in the control group. *T. tenax* was found in 65.3% in the study groups compared to 16% in the control group. There is a significant association between oral protozoa and HbA1c level, gum inflammation, halitosis, and dental prosthesis. The most important risk factors are residence, socioeconomic status, literacy, and oral hygiene. This high prevalence in systemic diseases needs to be investigated concerning its pathogenesis, immunological mediators that affect systemic diseases, and the interplay between them.

Acknowledgment: Special thanks go to all hospital staff members and patients who agreed to participate in this study. Great thanks to Dr. Maged Fahmy, a consultant of Dentistry who helped in collecting the samples.

Funding: None. Author funded

Conflicts of interest: None.

Ethical consideration:

This work has been done according to the declaration of Helsinki and the sound practice. The present study was approved by the Ethics Committee of the Faculty of Medicine, Fayoum University. Informed consent was taken from each patient prior included in this study and after an explanation of the purpose of the study.

HIGHLIGHTS

- The study detected significant increase in prevalence of E. gingivalis, T. tenax in patients with chronic diseases.
- There is significant association of oral protozoa and HbA1c level, gum inflammation, halitosis and dental Prosthesis.
- The most important risk factors are residence, socioeconomic status, literacy, and oral hygiene.

REFERENCES

1. Deo PN, Deshmukh R. Oral microbiome: Unveiling the fundamentals. *J Oral Maxillofac Pathol*. 2019; 23(1):122-128.
2. Limeres Posse J, Diz Dios P, Scully C. Infection Transmission by Saliva and the Paradoxical Protective Role of Saliva. *Saliva Protection and Transmissible Diseases*. 2017 Jul; 31:1–18.

3. Azadbakht K, Baharvand P, Artemes P, Niazi M, Mahmoudvand H. Prevalence and risk factors of oral cavity parasites in pregnant women in Western Iran. *Parasite Epidemiol Control*. 2022 Sep 22; 12: 3 - 12
4. Boutayeb A, Boutayeb S, Boutayeb W. Multi-morbidity of non communicable diseases and equity in WHO Eastern Mediterranean countries. *Int J Equity Health*. 2013 Aug 20;12:11 - 17.
5. Fairfield B, Schnabl B. Gut dysbiosis as a driver in alcohol-induced liver injury, *JHEP Rep*. 2020 Dec 10;3(2):100220.
6. Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology*. 2018 Jan;67(1):123-133.
7. Fadhil Ali Malaa S, Abd Ali Abd Aun Jwad B, Khalis Al-Masoudi H. Assessment of *Entamoeba gingivalis* and *Trichomonas tenax* in Patients with Chronic Diseases and its Correlation with Some Risk Factors. *Arch Razi Inst.* 2022; 77(1):87-93.
8. Beck JD, Moss KL, Morelli T, Offenbacher S. Periodontal profile class is associated with prevalent diabetes, coronary heart disease, stroke, and systemic markers of C-reactive protein and interleukin-6. *J Periodontol.* 2018 Feb; 89(2):157-165.
9. El-Kebbi, Imad M. Epidemiology of type 2 diabetes in the Middle East and North Africa: Challenges and call for action. *World journal of diabetes.* 2021; 12(9):1401-1425.
10. Belkaid, Yasmine, and Timothy W Hand. Role of the microbiota in immunity and inflammation. *Cell.* 2014;157(1):121-141
11. Honarmand M, Farhad-Mollashahi L, Nakhaee A, Sargolzaie F. Oral manifestation and salivary changes in renal patients undergoing hemodialysis. *J. Clin. Exp. Dent*. 2017; 9(2): e207–e210.
12. Sharma, Rahul. Revised Kuppuswamy's Socioeconomic Status Scale: Explained and Updated. *Indian pediatrics.* 2017;54(10):867-870.
13. Dubar M, Zaffino ML, Remen T, Thilly N, Cunat L, Machouart MC, Bisson C. Protozoans in subgingival biofilm clinical and bacterial associated factors and impact of scaling and root planing treatment. *J Oral Microbiol.* 2019;12(1):1693222.
14. El-Sayed, Nagwa & Méabed, Eman. Detection of *Trichomonas tenax* in patients with periodontitis using microscopy and culture compared to PCR. *Egypt J Medical Sciences*. 2008; 291(2):537-550.
15. Clark CG, Diamond LS. Methods for cultivation of luminal parasitic protists of clinical importance. *Clin Microbiol Rev*. 2012; 15(3):329–341.
16. Graziani F, Gennai S, Solini A, Petrini M. A systematic review and meta-analysis of epidemiologic observational evidence on the effect of periodontitis on diabetes An update of the EFP-AAP review. *J Clin Periodontol.* 2018 Feb; 45(2):167-187.
17. Ibrahim S, Abbas RS. Evaluation of *Entamoeba gingivalis* and *Trichomonas tenax* in patients with periodontitis and gingivitis and its correlation with some risk factors. *J Baghdad Coll Dent*. 2012; 24(3):158-162.
18. Yaseen A, Mahafzah A, Dababseh D, Taim D, Hamdan AA, Al-Fraihat E, Hassona Y, Şahin GÖ, Santi-Rocca J, Sallam M. Oral Colonization by *Entamoeba gingivalis* and *Trichomonas tenax*: A PCR-Based Study in Health, Gingivitis, and Periodontitis. *Front Cell Infect Microbiol*. 2021 Dec 7; 6(11):782805.
19. Garcia G, Ramos F, Maldonado J. Prevalence of two *Entamoeba gingivalis* ST1 and ST2-kamaktli subtypes in the human oral cavity under various conditions. *Parasitol Res.* 2018;117(9):2941–2948.
20. Proctor, E. M. Laboratory diagnosis of amebiasis," *Clinics in laboratory medicine* 1991; 11(4), 829-859.
21. Mahmoud S, Rahman, GA. Pulmonary trichomoniasis : improved diagnosis by using polymerase chain reaction targeting *Trichomonas tenax* 18S rRNA gene in sputum specimens. *J Egypt Soc Parasitol.* 2004;34(1):197-211.
22. Oladokun, Adenike, Opeodu, Olanrewaju, Lawal Ahmed, and Falade Mofolusho. *Entamoeba gingivalis* and *Trichomonas tenax* in Periodontal Disease. *Microbiology Research Journal International.* 2021;31(4): 61-72.
23. Eke, Paul I et al. Periodontitis prevalence in adults ≥ 65 years of age, in the USA. *Periodontology 2000*. 2016; 72(1):76-95.
24. Ioannidou, Effie, The Sex and Gender Intersection in Chronic Periodontitis. *Frontiers in public health*.2017 Aug 4;5(189).
25. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, and Taylor R. Periodontitis and diabetes: a two-way relationship. *Diabetologia.* 2012 Jan; 55(1):21-31.
26. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Res Clin Pract.* 2011; 94(3):311-321.

27. Barutta F, Bellini S, Durazzo M, Gruden G. Novel Insight into the Mechanisms of the Bidirectional Relationship between Diabetes and Periodontitis. *Biomedicines.* 2022 Jan 16; 10(1):178- 187.

28. Arpağ OF, Kaya ÖM. Presence of *Trichomonas tenax* and *Entamoeba gingivalis* in peri-implantitis lesions. *Quintessence Int.* 2020; 51(3):212-218.

29. Obradović R, Kesić LJ, Gasić J, Petrović M, Zivković N. Role of smoking in periodontal disease among diabetic patients. *West Indian Med J*. 2012 Jan;61(1):98-101.

30. Jia YJ, Liao Y, He YQ, Zheng MQ, Tong XT, Xue WQ, Zhang JB, Yuan LL, Zhang WL, Jia WH. Association Between Oral Microbiota and Cigarette Smoking in the Chinese Population. *Front Cell Infect Microbiol*. 2021 May 28; 11:658203. doi: 10.3389/fcimb.2021.658203.

31. Emilia Ollikainen, Tuomas Saxlin, Tellervo Tervonen, Anna Liisa Suominen, Matti Knuuttila, Antti Jula, Pekka Ylöstalo, Association between periodontal condition and blood pressure is confounded by smoking. *Acta Odontologica Scandinavica* 2022; 8(6):457-464.

32. Porter, S. R., and C. Scully. Oral malodour (halitosis). Bmj 2006; 632-635.

33. Hampelska K, Jaworska MM, Babalska ZŁ, Karpiński TM. The Role of Oral Microbiota in Intra-Oral Halitosis. *J Clin Med*. 2020 Aug 2; 9(8):2484.

34. Suganya K, Govindan K, Prabha P, and Murugan M. An extensive review on L-methioninase and its potential applications. *Biocatalysis and Agricultural Biotechnolog* 2017; 12(23):104-115.

35. Honarmand M, Farhad-Mollashahi L, Nakhaee A, Sargolzaie F. Oral manifestation and salivary changes in renal patients undergoing hemodialysis. *J Clin Exp Dent* 2017; 72:431-432.

36. Costantinides F, Castronovo G, Vettori E, et al. Dental Care for Patients with End-Stage Renal Disease and Undergoing Hemodialysis. *Int J Dent.* 2018 Nov; 9610892. doi:10.1155/2018/9610892

37. Yoneda M, Naka S, Nakano K, Wada K, Endo H, Mawatari H, et al. Involvement of a periodontal pathogen, Porphyromonas gingivalis on the pathogenesis of non-alcoholic fatty liver disease. *BMC Gastroenterol.* 2012;12:16-22.

38. Beck JD, Moss KL, Morelli T, Offenbacher S. Periodontal profile class is associated with prevalent diabetes, coronary heart disease, stroke, and systemic markers of C-reactive protein and interleukin-6, *J Periodontol* 2018 Feb;89(2):157-165.

39. Ladegaard Grønkjær P, Holmstrup P, Jepsen H. The impact of oral diseases in cirrhosis on complications and mortality. *JGH Open*. 2021;5(2):294-300.

40. Bainbridge BW, Hirano T, Grieshaber N, Davey ME. Deletion of a 77-base-pair inverted repeat element alters the synthesis of surface polysaccharides in *Porphyromonas gingivalis*. *J Bacteriol*. 2015 Apr; 197(7):1208-1220.