

## Detection of *Entamoeba gingivalis* in Diseased and Healthy Periodontal Sites among Cancer Patients in Thi-Qar Province

Raed Adnan Shayal <sup>\*1a</sup> and Bassad A. Al-Aboody <sup>1b</sup>

<sup>1</sup>Department of Biology, College of Science, University of Thi-Qar, Thi-Qar, Iraq.

<sup>b</sup>E-mail: [bassadalabooddy\\_bio@sci.utq.edu.iq](mailto:bassadalabooddy_bio@sci.utq.edu.iq)

<sup>a\*</sup>Corresponding author: [raed.adnan@utq.edu.iq](mailto:raed.adnan@utq.edu.iq)

Received: 2024-03-18, Revised: 2024-04-20, Accepted: 2024-04-26, Published: 2024-12-05

**Abstract**—Periodontitis, being one of the most common illnesses in the world represents a significant public health concern. The aims of this study are to determine *Entamoeba gingivalis* prevalence in cancer patients compared to healthy individuals (as a control), study some of the factors (such as diabetes and smoking) affecting the presence of this parasite, in cancer patients and control in the Thi-Qar Province of Iraq. For detection of *Entamoeba gingivalis* samples, were collected from cancer patients (different types of cancer) and control, including individuals with and without periodontitis, from Al-Haboubi Teaching Hospital during the period from August 2023 to December 2023. The current study showed the overall infection rate of *E. gingivalis* was 46.33% in all study samples. The results also showed that the presence of Trophozoite and the cyst-like trophozoite of *E. gingivalis* was found in 7.19% of positive samples (n = 10/139). This study indicated that there are significant differences (p<0.05) in the percentage of *E. gingivalis* infections in individuals with periodontitis, diabetes, or smoking ; these have a higher infection rate than those who without, in both the cancer patients and control. No significant differences p<0.05 were seen in the infection rate of *E. gingivalis* between males and females in both groups. The results showed that the infection rate of *E. gingivalis* was highest in the age group  $\geq 61$  and lowest in the age group  $\leq 30$  for both cancer patients and the control group, regardless of whether they had periodontitis or not

**Keywords**— *Entamoeba gingivalis*, Periodontitis, cancer patients, parasites, Thi-Qar province.

### I. INTRODUCTION

The mouth cavity contains the second-largest population of microorganisms after the colon, with over 700 species. In a balanced state, many species of fungi bacteria, and protozoa reside in the oral cavity. However, an imbalance in this system can lead to different diseases, including, dental caries, gingivitis, thrush of the mouth, and periodontitis [1]. *Entamoeba gingivalis* and *Trichomonas tenax* are protozoan parasites that reside in the gingival tissues near the base of human teeth. Some studies consider them commensals, meaning they can live in the mouth without causing harm. However, individuals with inflammatory conditions like gingivitis and periodontitis and Lack of proper dental care are more likely to contain these parasites. This is because they thrive in anaerobic environments [2,3].

*Entamoeba gingivalis* exists only in the trophozoite stage, which ranges in size from 10 to 35 $\mu$ m and are spread either directly via kissing or indirectly by chewing gum, food, or toothpicks, or other cutlery contaminated with trophozoites because they do not have a cyst in their life cycle [4]. This opportunistic organism lives in the gingiva, the area around teeth, the gingival edges of the gums, dental tartar, the spaces between tooth cavities, and the necrotic mucosa surrounding the teeth [5].

Owing to *E. gingivalis* detrimental effects on a population's health, periodontitis was deemed a handicapping illness by the World Health Organization (WHO, 2016). While parasitic trophozoites of *E. gingivalis* are consistently found in oral cavities on a regular basis, opinions regarding the relationship between this protozoan and periodontitis vary widely, ranging from vehement denial to outright accusation [6].

Several risk factors are recognized for these parasites, including age, genetic susceptibility, systemic disorders, cancer, smoking, alcohol consumption, diabetes, poor dental hygiene, and improper oral hygiene [7].

### II. METHODS

#### A. Collection of Samples

This study investigated the prevalence of *Entamoeba gingivalis*. A total of 300 swab samples were collected: 100 each from cancer patients with diseased and healthy periodontal sites who visited the Thi-Qar Oncology Center at Al-Haboubi Teaching Hospital and received chemotherapy. Furthermore, 100 swab samples were collected from healthy individuals, with 50 having diseased periodontal sites and 50 with healthy periodontal sites, as a control group. The study was from August 2023 to December 2023. With informed consent from all participants, an information sheet was prepared based on a questionnaire covering demographics such as sex, type of cancer, and number of chemotherapy treatments.

#### B. Laboratory Examination

For *Entamoeba gingivalis* detection, samples were applied directly onto slides and stabilized using methanol.



Then, they were stained with giemsa stain for a duration of 15 to 20 minutes.

C. Statistical Analysis:

Statistical package of social science, version 26, was used to perform the statistical analysis of the study's data, which was based on the use of both non-parametric and descriptive Chi-Square, the significance level for all tests was set at  $p < 0.05$  [8].

III. RESULTS

A. Microscopic Diagnosis of *Entamoeba gingivalis*

The microscopical examination of smear-positive samples containing trophozoites revealed that the trophozoites are in amoebic form, following giemsa staining. Food vacuoles containing RBCs, WBCs, and ingested bacteria are seen in the cytoplasm. As seen in fig.1, the nucleus is round and has a blue center karyosome.

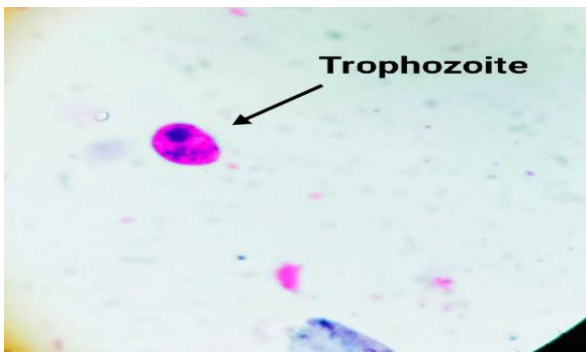


Fig.1: Trophozoite of *Entamoeba gingivalis* in direct smear from oral cavity of cancer patients, staining with Giemsa stain (100 X objective).

The results showed the presence of cyst-like trophozoite of *E. gingivalis*, as in Figure 2, in 7.19% of positive samples ( $n = 10/139$ ) as in Figure 3.

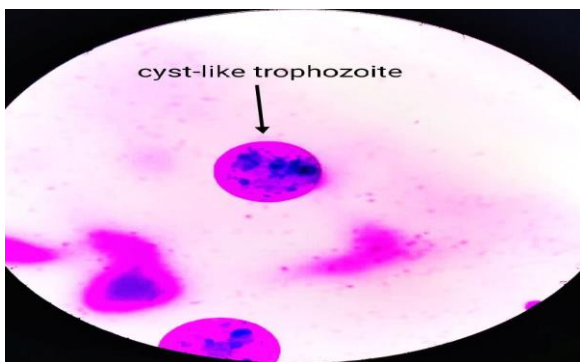


Fig. 2 : Cyst-like Trophozoite of *Entamoeba gingivalis* in direct smear from the oral cavity of cancer patients, staining with giemsa stain (100 X objective).

B. Percentage of Infection with *Entamoeba gingivalis*

The research results demonstrated that cancer patients with unhealthy periodontal sites had a higher prevalence of *E. gingivalis* compared to cancer patients with healthy

periodontal sites, and the rate of infection was 62% and 34%, respectively. As well as the control group was 56 % in individuals with diseased periodontal sites, and 30 % in individuals with healthy periodontal sites.

Table 1 presents the statistical analysis indicating significant differences ( $p < 0.05$ ) between them.

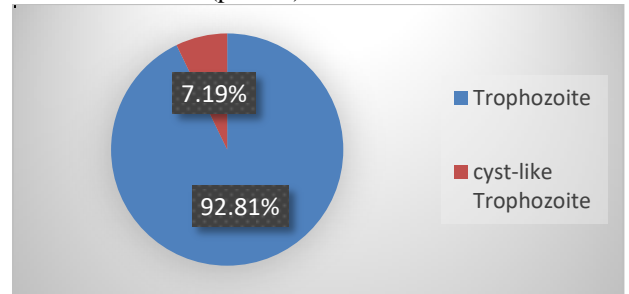


Fig. 3: Percentage of trophozoite and cyst-like Trophozoite of *Entamoeba gingivalis*

Table 1 : Percentage of infection with *Entamoeba gingivalis* in cancer patients and control group

Groups	Subgroups	Positive		Negative		Total	
		No.	%	No.	%	No.	%
Cancer patients	Periodontitis	62	62.0	38	38.0	100	33.33
	Non-Periodontitis	34	34.0	66	66.0	100	33.33
Control group	Periodontitis	28	56.0	22	44.0	50	16.66
	Non-Periodontitis	15	30.0	35	70.0	50	16.66
Total	Periodontitis	90	60.0	60	40.0	150	50.0
	Non-Periodontitis	49	32.67	101	67.33	150	50.0
Overall Total		139	46.33	161	53.67	300	100
Cancer patients		CalX <sup>2</sup> = 15.7		TabX <sup>2</sup> =3.84		DF= 1 p. value < 0.001**	
Control group		CalX <sup>2</sup> = 13.7		TabX <sup>2</sup> =3.84		DF= 1 p. value < 0.001**	
Patients vs. control		CalX <sup>2</sup> = 29.4		TabX <sup>2</sup> =7.81		DF= 3 p. value < 0.001**	

Table 2: The infection rate of *Entamoeba gingivalis* in cancer patients and control according to diabetes

Groups	Subgroups	Positive		Negative		Total	
		No.	%	No.	%	No.	%
Cancer patients	Diabetic	26	72.22	10	27.77	36	12.0
	Non-diabetic	70	42.68	94	57.31	164	54.66
Control group	Diabetic	15	68.18	7	46.66	22	7.33
	Non-diabetic	28	35.89	50	64.10	78	26.0
Total	Diabetic	41	70.69	17	29.31	58	19.33
	Non-diabetic	98	40.50	144	59.50	242	80.67
Overall Total		139	46.33	161	53.67	300	100
Cancer patients		CalX <sup>2</sup> = 17.2		TabX <sup>2</sup> =3.84		DF= 1 p. value < 0.001**	
Control group		CalX <sup>2</sup> = 11.4		TabX <sup>2</sup> =3.84		DF= 1 p. value 0.001**	
Patients vs. control		CalX <sup>2</sup> = 29.1		TabX <sup>2</sup> =7.81		DF= 3 p. value < 0.001**	

The results demonstrated that there exist significant differences ( $p < 0.05$ ) in the rate of infection with *E. gingivalis* between cancer patients with diabetes and cancer patients without diabetes, where the infection rates were 72.22 and 42.68, respectively. Furthermore in control were 68.18 % and 35, respectively, as seen in Table 2.

Table 3 showed that there were significant differences ( $p < 0.05$ ) in the infection rate with *E. gingivalis* based on smoking, as the prevalence of *E. gingivalis* in cancer patients smokers were greater than not smokers were 61.90 % and 44.30 %, respectively, while in control were 58.62 % and 36.61% respectively.

Table 3: The rate of infection of *Entamoeba gingivalis* in cancer patients and control according to smoking.

Groups	Smoking Subgroups	Positive		Negative		Total	
		No.	%	No.	%	No.	%
Cancer patients	Smoker	26	61.90	16	38.08	42	14.0
	Non-smoker	70	44.30	88	55.69	158	52.66
Control group	Smoker	17	58.62	12	41.37	29	9.66
	Non-smoker	26	36.61	45	63.38	71	23.66
Total	Smoker	43	60.56	28	39.44	71	23.67
	Non-smoker	96	41.92	133	58.08	229	76.34
Overall Total		139	46.33	161	53.67	300	100
Cancer patients		CalX <sup>2</sup> = 6.50		TabX <sup>2</sup> = 3.84		DF = 1	p. value 0.011*
Control group		CalX <sup>2</sup> = 9.69		TabX <sup>2</sup> = 3.84		DF = 1	p. value 0.002**
Patients vs. control		CalX <sup>2</sup> = 16.1		TabX <sup>2</sup> = 7.81		DF = 3	p. value 0.001**

The results demonstrated that there were no significant differences ( $p < 0.05$ ) in the rate of infection with *E. gingivalis* between males and females among cancer patients, as it was 48.33 % and 47.50 % respectively. Also in control were 43.33 % and 42.50 respectively, as included in Table 4.

Table 4 : Percentage of *Entamoeba gingivalis* infection in cancer patients and control based on sex.

Groups	Sex	Positive		Negative		Total	
		No.	%	No.	%	No.	%
Cancer patients	Male	58	48.33	62	51.67	120	40.0
	Female	38	47.50	42	52.50	80	26.67
Control group	Male	26	43.33	34	56.67	60	20.0
	Female	17	42.50	23	57.50	40	13.33
Total	Male	84	46.67	96	53.33	180	60.0
	Female	55	45.33	65	54.67	120	40.0
Overall Total		139	46.33	161	53.67	300	100
Cancer patients		CalX <sup>2</sup> = 0.005		TabX <sup>2</sup> = 3.84		DF = 1	p. value < 0.946 <sup>NS</sup>
Control group		CalX <sup>2</sup> = 0.004		TabX <sup>2</sup> = 3.84		DF = 1	p. value < 0.951 <sup>NS</sup>
Patients vs. control		CalX <sup>2</sup> = 0.008		TabX <sup>2</sup> = 7.81		DF = 3	p. value 1.00 <sup>NS</sup>

According to Table 5, there were notable significant differences ( $p < 0.05$ ) in the *E. gingivalis* infection rate by age group., as it was observed that the age group  $\geq 61$  had the highest infection rate., and the lowest rate of infection in the age group  $\leq 30$  in each of the cancer patients with periodontitis , cancer patients without periodontitis and control without the presence of periodontitis , as for control with periodontitis there were no significant differences, but also the highest infection rate was in the age group  $\geq 61$ , and the lowest rate of infection was in the age group  $\geq 30$ .

Table 5: The infection rate of *Entamoeba gingivalis* in cancer patients and control according to age.

Groups	Age group	Cancer patients				Control group				All samples (Cancer patients and Control group)			
		Positive		Negative		Positive		Negative		Positive		Negative	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Diseased Periodontal sites	$\leq 30$	11	50.0	11	50.00	4	44.44	5	55.56	15	48.39	16	51.61
	31-40	10	62.5	6	37.50	7	63.64	4	36.36	17	62.96	10	37.04
	41-50	16	57.14	12	42.86	5	55.56	4	44.44	21	56.76	16	43.24
	51-60	13	72.22	5	27.78	8	57.14	6	42.86	21	65.62	11	34.38
	$\geq 60$	12	75.0	4	25.00	4	57.14	3	42.86	16	69.57	7	30.43
	Total	62	62	38	38	28	56	22	44	90	60	60	40
	Total	4	20.0	16	80.0	2	22.22	7	77.78	6	20.69	23	79.31
Healthy Periodontal sites	$\leq 30$	4	20.0	16	80.0	2	22.22	7	77.78	6	20.69	23	79.31
	31-40	10	35.71	18	64.29	3	27.27	8	72.73	13	33.33	26	66.67
	41-50	5	27.78	13	72.22	3	27.27	8	72.73	8	27.59	21	72.41
	51-60	4	25.0	12	75.0	3	30.0	7	70.00	7	26.92	19	73.08
	$\geq 60$	11	61.11	7	38.89	4	44.44	5	55.56	15	55.56	12	44.44
	Total	34	34	66	66	15	30	35	70	49	32.67	101	67.33
	Total	4	20.0	16	80.0	2	22.22	7	77.78	6	20.69	23	79.31
Cancer patients with periodontitis		CalX <sup>2</sup> = 18.5		TabX <sup>2</sup> = 9.48		DF = 4	p. value 0.001**						
Cancer patients without periodontitis		CalX <sup>2</sup> = 46.6		TabX <sup>2</sup> = 9.48		DF = 4	p. value < 0.001**						
Healthy with periodontitis		CalX <sup>2</sup> = 8.47		TabX <sup>2</sup> = 9.48		DF = 4	p. value 0.076 <sup>NS</sup>						
Healthy without periodontitis		CalX <sup>2</sup> = 13.2		TabX <sup>2</sup> = 9.48		DF = 4	p. value 0.010*						
Patient vs. control with periodontitis		CalX <sup>2</sup> = 26.9		TabX <sup>2</sup> = 21.02		DF = 12	p. value 0.008**						
Patient vs. control without periodontitis		CalX <sup>2</sup> = 59.8		TabX <sup>2</sup> = 21.02		DF = 12	p. value < 0.001**						

#### IV. DISCUSSION

It has frequently been demonstrated that *E. gingivalis* parasite trophozoites are present in the oral cavity; nevertheless, the association between this parasite and periodontitis remains for debate, with viewpoints ranging from categorically negative to accusatory [9] . The current study indicated the prevalence of infection with *E. gingivalis* was 46.33% , and this result was in agreement with a previous study by [10] which the infection rate was 44%. The risk of developing periodontitis and gingivitis illness varies based on a number of factors, such as age groups, oral hygiene, diabetes, blood pressure, oral pH, smoking status, asthma, arthritis, and of antibiotic use [11].

The current study showed the presence of cyst-like Trophozoite of *E. gingivalis* in (7.19%) of positive samples (n = 10/139), and our study is consistent with [12] who

found the *E. gingivalis* cyst-like structures formed from trophozoites in response to antibiotic treatment, the reason for the formation of cyst-like trophozoites is the individuals maybe taking some antibiotics such as amoxicillin and metronidazole as suggested by [12], this transformation may Permit this infection to endure periodontal treatment. and recolonize periodontal or implant pockets. Cyst-like Trophozoite might influence how illnesses spread and between individuals [12].

The results of our current study revealed that individuals with diseased periodontitis sites had a higher prevalence of *E. gingivalis* than individuals without periodontitis, in both groups (cancer patients and control), this result is consistent with [13] who found that the rate was (77%) of inflamed periodontal sites and (22%) of healthy sites, [14] where was found that 73.84% of inflamed periodontal sites and 50% of healthy periodontal sites exhibited this phenomenon, additionally, the rate of *E. gingivalis* infection increases in individuals with periodontitis due to changes in the oral environment and the accumulation of bacteria, which makes it more suitable for the growth and reproduction of *E. gingivalis*. One of the most important causes of periodontitis is poor oral hygiene, which is the same reason for increased parasite infection. Therefore, we notice that the rate of *E. gingivalis* infection increases in individuals with periodontitis.

The results showed significant differences in the rate of infection with *E. gingivalis* according to diabetes, as the infection rate in individuals with diabetes higher than non-diabetic individuals, within both the cancer patient and control groups, where our study was identical to the study [15] in which the incidence rate was in *E. gingivalis* in diabetics it is (80%), also consistent with [16] Where it was (80%) in diabetics and (20%) in non- diabetics. Diabetes may affect the immune system and thus weaken the ability to resist infection and heal wounds. The rate of tartar accumulation on the teeth in diabetic patients is greater, which increases the chance of periodontitis and, thus, *E. gingivalis* infection (This study also demonstrated that the rate of *E. gingivalis* infection increases with periodontitis).

The current study indicated that there are significant differences in the rate of infection with *E. gingivalis* depending on smoking, as the incidence of *E. gingivalis* in individual smokers was higher than non-smokers, in both groups (cancer patients and control), this finding aligns with previous research by [17], where the incidence rate of *E. gingivalis* among smokers was higher than that of non-smokers, where it was (78.5%) and (47.6%), respectively, compatible with [18] where the infection rate of *E. gingivalis* among smokers was higher than that of non-smokers, where it was (78.5%) and (47.6%), respectively. Smoking reduces blood flow to the gums, making them less able to heal and resist infection. It also negatively affects the immune system, making the body less able to resist infections. Smoking may also increase the accumulation of deposits on the teeth and gums, which increases gum irritation, tartar formation, and infections, in which provides a suitable environment for the growth of *E. gingivalis* as we mentioned previously.

Our study found no significant difference in the rate of infection with *E. gingivalis* between males and females, in either the cancer patient or control group. The results were

comparable to those mentioned by [6], who found that the percentage of *E. gingivalis* isolated higher (53.8%) in males vs. (46.2 %) in females, according to [19], who discovered that (12%) of patients had isolated *E. gingivalis*. in males vs. (11.7 %) in females, while it did not correspond to [20], for which there were significant differences between each other, as the percentage of females was higher than that of males, where they were 56.7% and 34.5%, respectively. This is a feature found in scientific research findings because of several factors, such as the timing of sample collection, the type and conditions of the samples, and the type and intensity of social, cultural, and economic factors in the society. One such study was conducted by [21], which demonstrated that the sex factor had no bearing on the prevalence of any specific parasite infection and that the differences in infection rates between males and females were not statistically significant in all cases examined.

The result showed that there are significant differences ( $p < 0.05$ ) in the age group have an impact on the rate of *E. gingivalis* infection; the age group  $\geq 61$  had the greatest infection rate, according to the findings, and the lowest rate of infection in the age group  $\leq 30$  in each of the cancer patients with periodontitis, cancer patients without the presence of periodontitis and control without periodontitis, as for control with periodontitis there were no significant differences, but also the greatest rate of infection was in the age group  $\geq 61$ , and the lowest rate of infection was in the age group  $\geq 30$ . The study did not consistent with [22] as the infection rate was higher in the age group 38 – 47 years, and the lowest infection rate in the age group 78 – 87 years, it was 25.5 %, 1.9 %, respectively. Also, it did not consistent with [10] was the infection rate was higher in the age group 46 - 55 years, and the lowest infection rate in the age group 26 - 35 years, it was 66.66%, 28.57%, respectively. The explanation for the increase in the infection rate as age increases may be due to the health condition of elderly individuals, which negatively affects their psychological state and thus a lack of attention to personal hygiene, including oral hygiene. This disparity may have arisen from variations in sociocultural elements that this investigation may have taken into account. Perhaps more research in this area is necessary. According to certain research, patients with periodontal disease may be more susceptible to oral parasite infection as they age.

## V. CONCLUSIONS

*Entamoeba gingivalis*, it is one of the parasites found in the oral cavity among cancer patients in Thi-Qar. It is higher in individuals with periodontitis than individuals with healthy periodontal sites, and the microscopic examination using a giemsa stain is better than the direct smear for the diagnosis of *Entamoeba gingivalis*.

## ACKNOWLEDGMENT

Thanks to everybody who supported us and made it possible for us to gather data for our study.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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