

Molecular and Microscopic Investigation of *Entamoeba gingivalis* Among Patients with Cancer

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ABSTRACT

Background: *Entamoeba gingivalis* is a non-pathogenic amoeba that inhabits the human oral cavity and occasionally other sites. *Entamoeba gingivalis* may become pathogenic in cancer patients under chemotherapy. The aim of this study is to investigate *Entamoeba gingivalis* in cancer patients compared to healthy individuals (as a control), in the Thi-Qar Province of Iraq.

Methods: Samples were collected from individuals with diseased periodontal sites and from individuals with healthy periodontal sites from cancer patients and control groups in a hospital Al-Haboubi Teaching Hospital for the period from August 2023 to December 2023, for detection of *E. gingivalis*. Samples were diagnosed by microscopic staining with Giemsa stain, and the recovered positive isolates were subjected to molecular analysis.

Results: Amplification of DNA extracted from *E. gingivalis* of both cancer patients and healthy people using 18 rDNA revealed PCR products with 204 pb size for both diseased and healthy people. This study indicated that there are significant differences ($p < 0.05$) in the percentage of *E. gingivalis* infections among cancer patients with periodontitis these have a higher infection rate (62%) than those without (56%), as well as control group was 56% in individuals with periodontitis, and 30% in individuals without. The current study showed the overall rate of infection of *E. gingivalis* by PCR technique was 66.13%.

Conclusion: This study showed the sequence was identical and there was no difference in sequence between all isolates.

Keywords: *Entamoeba gingivalis*, periodontitis, cancer patients, Thi-Qar province

INTRODUCTION

Entamoeba gingivalis is a protozoan microorganism that is mostly found in the human oral cavity, and it has also been detected in the genitourinary tract. Transmission occurs through contaminated food or oral equipment, mouth droplets, and kissing (1). *E. gingivalis* has a cosmopolitan distribution with a worldwide prevalence of 37% (2). Some studies proposed an association between *E. gingivalis* and periodontal diseases (2,3).

Periodontal disease is an inflammatory condition affecting the gingiva, due to localized inflammation of the periodontium on exposure to dental plaque (4); Dental plaque build-up results in gingivitis, though reversible when oral hygiene measures are introduced. However, if left untreated, it

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may involve the underlying periodontal tissue (5). Cancer is a serious problem affecting the health of all human societies (6), it is a state in which cell proliferation occurs abnormally when it becomes uncontrollable and able to invade other cells. Additionally, the number of cancer patients has been clearly rising in recent years (7), Chronic infection or toxins production, immune evasion, and immunological suppression are all important mechanisms that can lead to cancer (8). This study was designed to investigate microscopic and molecular of *Entamoeba gingivalis* in oral cavity of cancer patients with periodontitis and compare with healthy oral persons.

MATERIALS AND METHODS

Collection of Samples

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. During the period from August 2023 to December 2023.

Microscopic Examination

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 and examined by microscope under (100X).

Molecular Examination

Genomic DNA Extraction

Genomic DNA was extracted from oral wash samples (saliva and dental plaque) by using gSYNCTM DNA Extraction Kit (Geneaid Taiwan) done according to company instruction. The PCR primers for detection *Entamoeba gingivalis* based on small subunit ribosomal RNA gene, The primers used, SSU (F-AGGAATGAACG-

GAACGTACA) and rDNA (R-CCATTTCTTCTTC-TATTGTTTCAC) (9).

PCR Reaction Compounds

The compounds that used for PCR reaction were master mix (13 µl), primer forward (1 µl), primer reverse (1 µl), DNA template ((30-100ng)) (4 µl), and free water (6 µl), totally give volume of 25 µl.

Protocol of PCR Amplification

The protocol used in this study is shown in a *table 1*.

Sequencing and Alignment of Genes

The PCR products amplified with two primers were sequenced by Macrogen Company (South Korea). NCBI BLAST tool was used to check the results of this program, and to detect any DNA alterations in the sequences of the gene.

Statistical Analysis

The data of the present study were statistically analysis by using SPSS (Statistical Package of Social Science version 26) based in using both descriptive and non-parametric Chi-Square at p. value < 0.05 (10).

RESULTS

Microscopic Examination

The current 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 control group was 56 % in individuals with diseased periodontal sites, and 30 % in individuals with healthy periodontal sites, as shown in *table 2*.

Molecular Examination

Forty-eight samples were taken from positive

Step	Temperature	Time	Cycle
Initial Denaturation	95	5.00 min	1 time
Denaturation	95	0.30 sec.	
Cyclic	Annealing	60	0.30 sec.
	Extension	72	1.00 min
Final Extension	72	10.00 min	1 time

Table 1 - Protocol of PCR amplification.

Table 2 - Percentage of infection with *Entamoeba gingivalis* in cancer patients and control group by microscopic examination.

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: $CalX^2 = 15.7$, $TabX^2 = 3.84$, $DF = 1$, $p < 0.001^{**}$
 Control group: $CalX^2 = 13.7$, $TabX^2 = 3.84$, $DF = 1$, $p < 0.001^{**}$
 Patients vs. control: $CalX^2 = 29.4$, $TabX^2 = 7.81$, $DF = 3$, $p < 0.001^{**}$

Table 3 - The percentage of *Entamoeba gingivalis* infection among cancer patients based on type of diagnostic method.

No. of Sample	Microscopic				PCR technique			
	Positive		Negative		Positive		Negative	
	No.	%	No.	%	No.	%	No.	%
48	48	100	0	0.00	40	83.33	8	16.67
14	0	0.00	14	100	1	7.14	13	92.86
62	48	77.42	14	22.58	41	66.13	21	33.87

$CalX^2 = 35.42$, $TabX^2 = 12.5$, $DF = 6$, $p < 0.001^{**}$

microscopic examination, only 40 (83.33%) were positive after PCR examination, as well as 14 samples from negative microscopic examination, only 1(7.14%) were positive after by pcr examination. The overall rate of infection of *E. gingivalis* by PCR technique (figs. 1, 2) was 66.13%, as shown in table 3.

Sequencing

The sequence was identical and there was no

difference in sequence between all isolates (each of the cancer patients with periodontitis, cancer patients without periodontitis, control with periodontitis and control without periodontitis). In this study: The isolate No.1 (Accession No. = LC804957; size= 204bp) identified 100% with specie Accession No. = OR405086. The isolate No.2 (Accession No. = LC804968; size= 204bp) identified 100% with specie Accession No. = OR405085. The isolate No.3 (Accession No. = LC804969; size= 204bp) identified

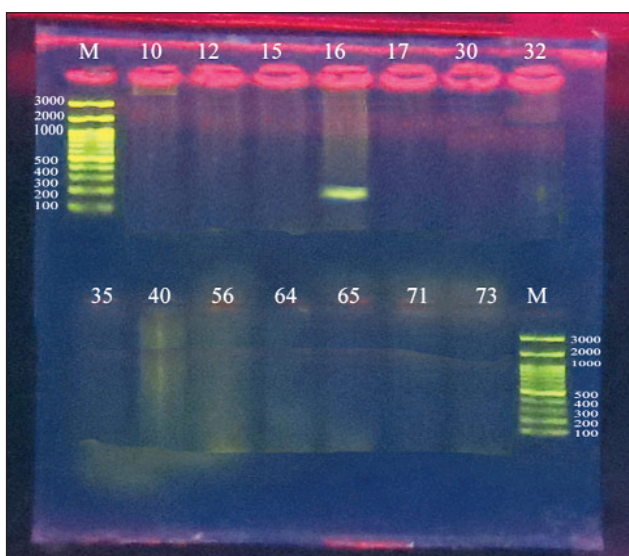


Figure 1 - Electrophoresis of a PCR product of *Entamoeba gingivalis* with a size of 204 bp, using agarose gel at a concentration of 1.5%, at a voltage of 70 V and 85 mA, for 45 minutes. For positive microscopy.

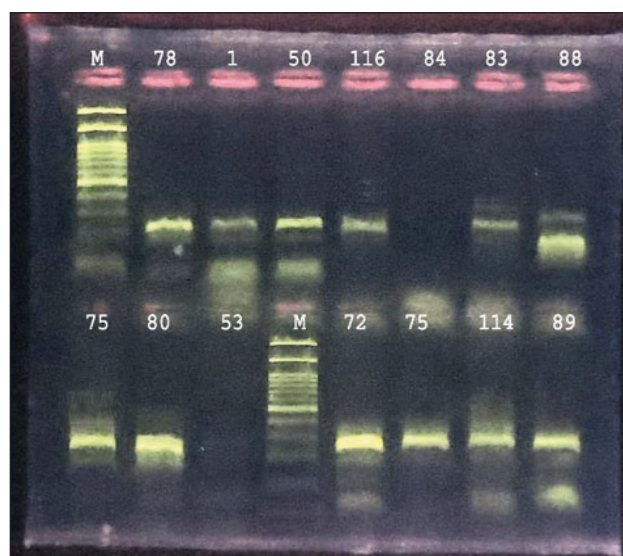


Figure 2 - Electrophoresis of a PCR product of *Entamoeba gingivalis* with a size of 204 bp, using agarose gel at a concentration of 1.5%, at a voltage of 70 V and 85 mA, for 45 minutes. For negative microscopy.

100% with specie Accession No. = OQ225457. The isolate No.4 (Accession No. = LC804970; size= 204bp) identified 100% with specie Accession No. = PP335301. The isolate No.50 (Accession No. = LC804958; size= 204bp) identified 100% with specie Accession No. = OR405085. The isolate No.72 (Accession No. = LC804964; size= 204bp) identified 100% with specie Accession No. = PP335301. The isolate No.74 (Accession No. = LC804962; size= 204bp) identified 100% with specie Accession No. = OR405085. The isolate No.75 (Accession No. = LC804965; size= 204bp) identified 100% with specie Accession No. = KX061779. The isolate No.78 (Accession No. = LC804956; size= 204bp) identified 100% with specie Accession No. = KX061779. The iso-

late No.80 (Accession No. = LC804963; size= 204bp) identified 100% with specie Accession No. = OQ225457. The isolate No.83 (Accession No. = LC804960; size= 204bp) identified 100% with specie Accession No. = PP335301. The isolate No.88 (Accession No. = LC804961; size= 204bp) identified 100% with specie Accession No. = KX061779. The isolate No.89 (Accession No. = LC804967; size= 204bp) identified 100% with specie Accession No. = KX061779. The isolate No. 114 (Accession No. = LC804966; size= 204bp) identified 100% with specie Accession No. = KX061779. The isolate No. 116 (Accession No. = LC804959; size= 204bp) identified 100% with specie Accession No. = OQ225457, (figs. 3, 4).

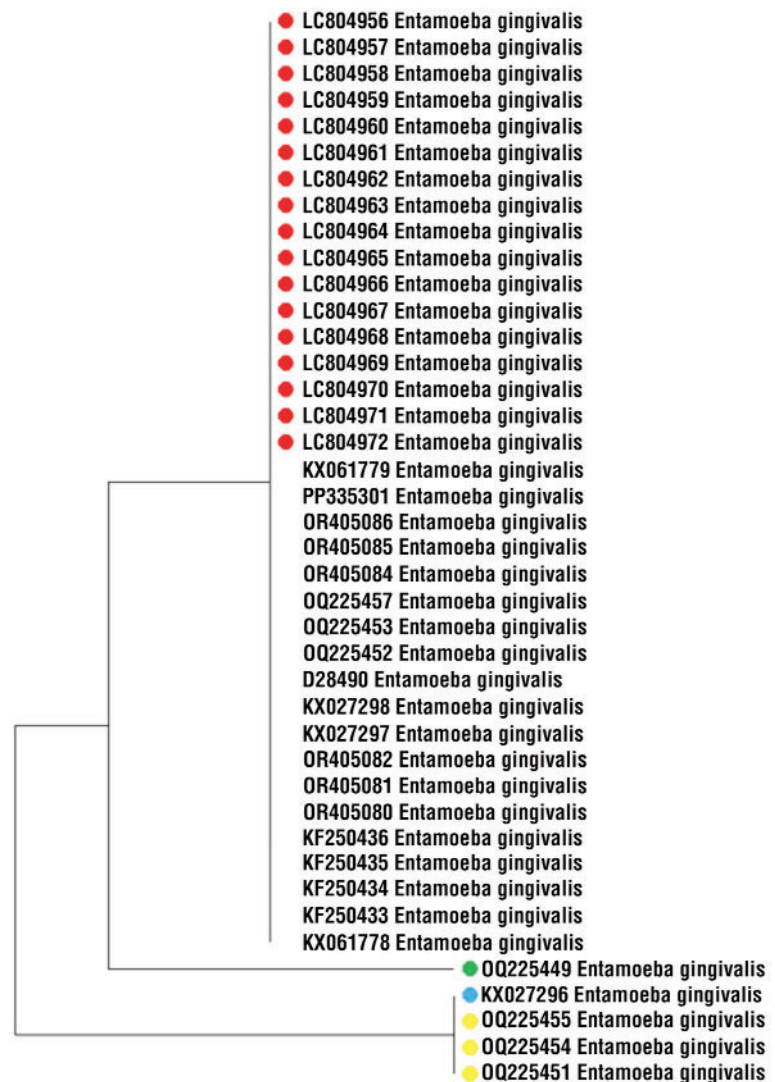


Figure 3 - The Phylogenetic tree of Entamoeba gingivalis Isolates between our isolates & reference copies in NCBI

Iraq. This study showed the sequence was identical and there was no difference in sequence between all isolates.

Conflicts of Interest

The authors declare no conflict of interest.

Informed Consent Statement

The informed consent was obtained.

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