Incidence of *Entamoeba Gingivalis* and *Trichomonas Tenax* in the Oral Cavity of Periodontal and Patients Under Chemotherapy, Confirmed with *in Vivo* Study

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Abstract

Aim: The study aimed to evaluate the impact of occurrences *Entamoeba gingivalis*, and *Trichomonas tenax* with periodontal in one hand and patients receiving chemotherapy on the other hand and compared to normal subjects. And evaluate the pathogenecity of these parasites *In Vivo*.

Materials and Method: A total of 80 patients, 30 with Periodontitis attending AL-Karama dentistry center, and 20 patients under chemotherapy referred to the national center of hematology- Mustanasiriyah University, Iraq, were involved in this study. And the last 30 subjects were considered as control with good oral condition. Scrapings of gums were taken and examined immediately microscopically,once by wet-mount smear, and the other stained with Giemsa-Romanovesky. The positive samples were cultured on special cultures for the experimental part and then spread on the gingival margins of three groups of rats (for each parasite), the first was given immunosuppressive drug, while the other two groups were not given it.

Results: The results shows that the higher frequency of *Entamoeba gingivalis* in patients with Periodontitis 36.7%. Patients receiving chemotherapy showed higher frequency of *E. gingivalis* and *Trichomonas tenax* comparing with the control group 30% and 10%, 15% and 3.333% respectively. All rats which were deal with *T. tenax* still a life and with healthy gingiva, in the other hand, all rats in the 1st group that deal with *E. gingivalis* have been showed periodontal ulcers and two of them died after 10 days, while one rat of the 2nd group has been showed mild gingival inflammation with no death, and no ulceration, inflammation or death have been occurred in the control group.

Conclusion: These findings suggest that: 1-Periodontal patients showed higher frequencies of *E. gingivalis*. 2-Patients receiving chemotherapy showed higher frequencies than the normal control. 3- *In Vivo Entamoeba gingivalis* may become pathogenic in individuals under chemotherapy.

Keywords: *Oral cavity; toxicity; patients; chemotherapy; Iv Vivo; health.*

Introduction

Though the saliva contains low nutrient concentrations and antimicrobial defense systems[(1)], the human oral cavity is still as a home to numerous microorganisms[(2)], oral hygiene alone has little effect on subgingival microflora in deep pockets [(3)], Periodontitis is a handicapping disease, WHO (world health organization) calculated that 0.132% of the world wide are disability-adjusted life years, a measure of disease burden as the loss of healthy life years (DALYs)[(4)] .Risk factors for this disease have been identified: alcohol consumption[(5)] poor mouth hygiene[(6)] and smoking[(7)]

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Trichomonas tenax and *Entamoeba gingivalis* are human buccal protozoan parasites, *T. tenax* is an anaerobic, commensal, and can live only in the mouth, they are most abundant between the teeth and gums and in pus pockets, tooth cavities and crypts of the tonsils. Transmission, then, is direct usually through kissing or common use of eating or drinking utensils[8]. There are studies that relate to its prevalence in patients with marginal chronic Periodontitis.[9]

Entamoeba gingivalis lives on the surface of teeth and gums, in gingival pockets near the base of teeth,and sometimes in the crypts of tonsils. Because no cyst is formed, transmission most be direct from one person to another by kissing, by droplet spray,or by sharing eating utensils[8]. It has been noted in publication that the increased prevalence of *E. gingivalis* is connected with diseases of the oral cavity, and particular with periodontal diseases[10]. Chomize et al[11] have,however,demonstrated the pathogenecity of *E. gingivalis* in the following groups of patients: those receiving immunosuppressive, with genetic diseases and with lowered body immunity.

Materials and Method

Survey Part: Study subjects : The present sample consists of 80 individuals aged 20-60 year, and comprised 35 females and 45 males. No of the study subjects were completely edentulous[12] .Thirty of patients were with advanced Periodontitis who referred to AL-Karama dentistry center, and 20 patient who are under chemotherapy were selected from the national center of hematology/Mustanasiriyah university/Baghdad, Iraq. The rest 30 subjects were considered as control group with no systemic disease and with good oral condition.

Samples Collection: A temporary laboratory was set up in both sites of the study, identification of *E. gingivalis* and *T. tenax* are made by the finding of trophozoites in scrapings the gums and teeth with sterile swab rubbed . Samples are taken from deep pockets surrounding the teeth of patient.

Microscopic Examination: The deposit was suspended in a drop of physiological saline solution,and two slides were performed for each sample,light microscope examination by original wet-mount smear is performed immediately for presence of *T. tenax,*due to its motility[13]. And the other was stained with Giemsa-Romanovsky[14] after the samples were fixed examined immediately under light microscope (40 x)[15] to detect the *E. gingivalis*. The parasite of *T. tenax* was identified by their flagella and characteristic locomotion, while E. *gingivalis* was identified by its shape depending on the expansion of the pseudopodia formation and presence of vacuoles[16] .

Experimental Study:

Parasites Culturing:

1. **Entamoeba Gingivalis:** After the isolation of *E. gingivalis* from periodontal patients, the organism cultured on (Cleveland and collier medium) for 72 hour, filtered, washed with physiological normal saline for 3 times[17]
2. **Trichomonas Tenax:** The samples that positive of *T. tenax* were inoculated in the broth selective Kupferberg, used for the growth of *T. tenax* (Kupferberg Trichomonas Broth), to which 0.1g of chloramphenicol was added to prevent the growth of bacteria and other microorganisms. Seeded culture media were taken to the oven at 37c^{o} for 72 hour.[18]

The Animals: Two suspensions of *Entamoeba gingivalis* and *Trichomonas tenax* were prepared for experimental application by using 24 rats about (200-250 g/rat) provided by animal house of the Biological Chemical Pharmaceutical Control, the rats had normal teeth and periodontal tissue.[17]

For each parasite, 12 rats are divided into three groups (4 rats for each). The first group injected with prednisolone acetate (0.25 mg/rat) daily for one week before animal test[19], while the other two groups of rats are not given immunosuppressive drug. Parasites suspension spreaded on the gingival margin of the rats (first and second groups only), the third group is the control in which their gingival margins are spreaded by normal saline free from parasites[17]

Results and Discussion

Cross sectional study designed of 80 subjects were selected as a study samples, 30 with advanced Periodontitis, 20 under chemotherapy, and the latest 30 were controll group with no systemic disease and with good oral condition. Out of 30 Periodontal examined patients, 11(36.7%) were infected with *E. gingivalis*, while 3(10%) were harbored *T. tenax* as illustrated in table (1). In addition to that, results shows that occurrence of *E. gingivalis* and *T. tenax* in the 20 patients under chemotherapy are accounted 6(30%) and 3 (15%)

respectively, while their occurrence in the controlled group were 3(10%) and 1 (3.33%) respectively fig. (1).

Comparisons significant shows that with periodontitis group in light of positive *E. gingivalis* outcomes compared with controlled group recorded significant different at P<0.05, with more than three times occurrences (Cohort=3.375)[20]. In addition to that, with respect to *T. tenax* positive outcomes compared with controlled group, no significant different at P>0.05 was obtained, but it's more informative for that result to be reported rather than simply that p-value was not achieved to 0.05, as well as periodontitis group with respect to *T. tenax* parasite are reported three times (Cohort=3.00) of a positive outcomes compared with controlled group. The high incidence of *E. gingivalis* in patients with periodontal disease in the present study, suggests that, this parasite might has an important role in the etiology of this condition. And that is in agreement with[21] and[22] who find that *E. gingivalis* might elaborate a proteolytic enzyme that could result in the pathogenesis of periodontal disease.

Comparisons significant shows that with under chemotherapy group in light of positive *E. gingivalis* outcomes compared with controlled group recorded highly significant different at P<0.01, with more than fourteen times occurred (Cohort=14.5). In addition to that, with respect to *T. tenax* positive outcomes compared with controlled group, no significant different at P>0.05 was obtained, but it's more informative for that result to be reported rather than simply that p-value was not achieved to 0.05, as well as under chemotherapy group with respect to *T. tenax* parasite are reported more than four times (Cohort=4.5) of positive outcomes compared with controlled group. And these results are the same as the findings of [23], who revealed that when the immunity level decreases or when immunosuppressive drug is used the infection may occur in the oral cavity .

Finally, comparisons significant shows that with periodontitis and under chemotherapy groups in light of positive *E. gingivalis* outcomes, had no significant different at P>0.05, with a similar times occurred (Cohort=1.22). In addition to that, with respect to *T. tenax* positive outcomes, no significant different at P>0.05 was obtained, but according to related ratios (Cohort=0.667) of positive outcomes, it could be conclude that under chemotherapy group had one and a half times of reported positive outcomes more than those reported with Periodontitis group. Figure (1) represent graphically by cluster bar charts distribution of *Entamoeba gingivalis* and *Trichomonas tenax* sensitive to positive outcomes among studied groups (periodontitis, under chemotherapy, and control) groups.

Table (1): Frequencies of *Entamoeba gingivalis* and *Trichomonas tenax* in the studied groups

Groups	Total number in each groups	E. gingivalis		T. tenax	
		+ve%	+ve	+ve%	+ve
Periodontitis	30	36.7%	11	10%	3
Under Chemotherapy	20	30%	6	15%	3
Control	30	10%	3	3.33%	1
C.S.(*) P-value	Periodontitis X Control	P=0.016 (S) For cohort *E. gingivalis* = +ve (3.375)		P=0.071 (NS) For cohort *T. tenax* = +ve (3.00)	
	Under Chemotherapy X Control	P=0.002 (HS) For cohort *E. gingivalis* = +ve (14.5)		P=0.136 (NS) For cohort *T. tenax* = +ve (4.50)	
	Periodontitis X Under Chemotherapy	P=0.626 (NS) For cohort ***E. gingivalis* = +ve (1.222)**		P=0.594 (NS) For cohort *T. tenax* = +ve (0.667)	

(*)HS: Highly Sig. at P<0.01; S: Sig. at P<0.05; NS: Non Sig. at P>0.05; Testing based on Contingency Coefficient test and measuring ratio related rates (Cohort +ve)

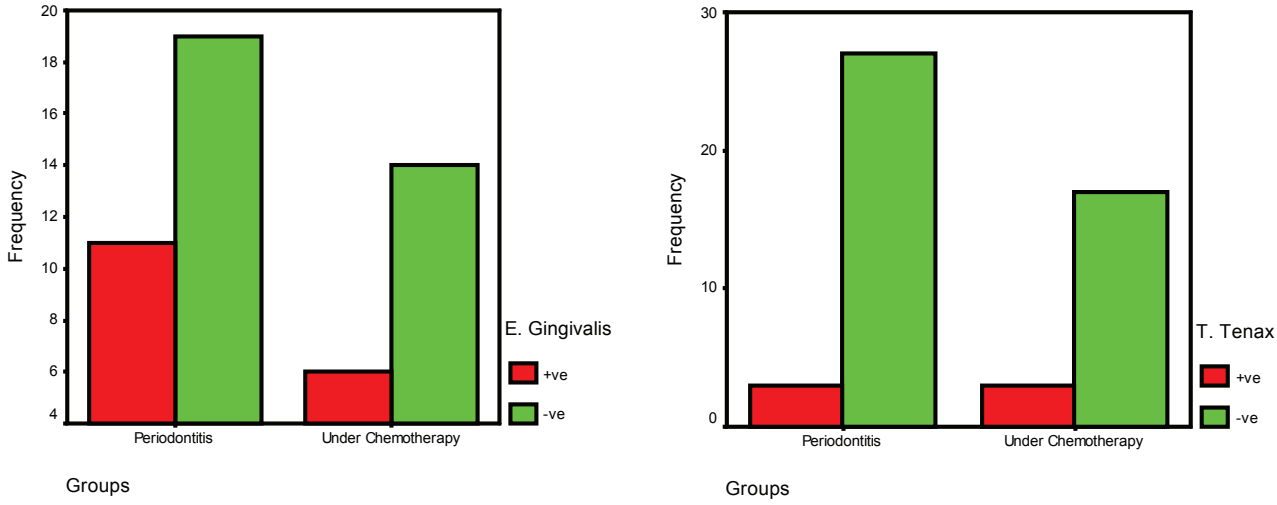

Figure (1): Distribution of *Entamoeba gingivalis* and *Trichomonas tenax* sensitive at the studied groups

According to experimental part of this study, table (2) explains that, each of the twelve rats which were deals with *Trichomonas tenax*, and *Entamoeba gingivalis* reported the following results:

***Trichomonas tenax*:** Dealing with *Trichomonas tenax*, twelve rats stilled a life and with healthy gingival, and that's remained with the same responses in view of 2nd and 3rd groups.

***Entamoeba gingivalis*:** Dealing with *Entamoeba gingivalis*, twelve rats had reversed behavior with their a life and with healthy gingival, and that's given in each groups, as follows:

First group: All rats injected with immunosuppressive drug have been shown periodontal ulcers after 7 days of dealing with *E. gingivalis,* and two of them died after 10 days of dealing.

Second group: One of the rats has been shown Gingival Erythmatous and swollen after 6 days of dealing with *E. gingivalis.* No periodontal ulcers and no death occurs to the rats.

Third group: All the 4 rats were still healthy,and no death occurs to them.

In addition to that, statistically, and comparing between *Trichomonas tenax*, and *Entamoeba gingivalis*, a significant different at P<0.05 are accounted according to test distribution of multiple ratio's infections (+ve) in contrasts of different groups, and it could be indicating that *Entamoeba gingivalis* had reported the worst type of studied parasite resulted either with number of infection, or with dead status of rates.And these results are in agreement with [17].

Table (2): Frequency of *Trichomonas tenax* and *Entamoeba gingivalis* in studied rats.

Parasites	No. of group	No. of rats	+ve Infection	+ve %	Dead rats
Trichomonas tenax	1st With Chemotherapy and Parasite	4	0	0	0
	2nd With Parasite	4	0	0	0
	3rd Without Chemotherapy and Parasite	4	0	0	0
Entamoeba gingivalis	1st With Chemotherapy and Parasite	4	4	100%	2 50%
	2nd With Parasite	4	1	25%	0 0.0%
	3rd Without Chemotherapy and Parasite	4	0	0%	0 0.0%
	C.S.(*) P-value				MZ=8.914 P<0.05 (S)

(*) S: Sig. at P<0.05; Testing based on Multiple Z-test.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq.

Conflict of Interest: Non

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