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Prevalence of *Entamoeba gingivalis* in gingivitis patients and evaluate the level of IL1α, IL6 and IFN-Y in patient groups

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Abstract

Entamoeba gingivalis parasite has been associated with gingivitis. Infection rate for parasite *Entamoeba gingivialis* in Kanaan city and its village, Diyala, Iraq, from August 1, 2023, until September 29, 2023. The swabs were examined by studying 250 persons and 90 blood samples that were collected from gingivitis patients from visitors to private dental clinics. Percentage spread of *Entamoeba gingivialis* was higher in males (%61.3) than in females (%38.7), it showed that the smokers (%69.3) is higher than non-smokers (%30.7). It appeared that villages (%68) was higher than the city (%32). Also the study included Some immunological parameters in the serum of the infected persons and the control group. These parameters included interleukin-1 alpha, interleukin-6, and interferon-gamma. Results notice no significant differences in level IL-1α and IFN-γ ($P>0.05$) while IL-6 increased significantly ($P<0.05$).

Introduction:

Entamoeba gingivialis is non-pathogenic parasitic protozoa of the oral cavity and often found in the gingival tissue around the teeth, gums and sometimes the tonsils [1]. The trophozoite stage is the only form of the parasite and its only means of transmission. Transmission of the parasite occurs through direct methods such as spraying droplets, kissing, shared eating dishes or poor oral hygiene, causing itchy gums, bad breath, severe headaches, inflammation of the palate, and damage to the gum tissue and teeth [2]. It is characterized by the presence of pseudopods that help it move and a central circular nucleus. The peripheral cytoplasm is characterized by not containing organelles or granules and contains a nuclear particle in its center, which consists of small organelles and several nutritional vacuoles that contain red blood cells, white blood cells and bacteria, which makes it similar to *Entamoeba coli* [3]. Gingivitis is an inflammatory condition that affects the gum tissue and characterized by symptoms such as swelling, redness, pain and bleeding gums [4]. Aging, poor oral hygiene, smoking, vitamin C deficiency, hormonal changes especially in pregnant women, medications, systemic diseases such as kidney patients and diabetics, viral infections such as *herpes* and *HIV* are among the factors that cause gingivitis and increase its risk [5]. Periodontal pathogens from the oral biofilm can trigger an inflammatory response by releasing cytokines and inflammatory mediators including interleukins, the most important of which are interleukin-1α,

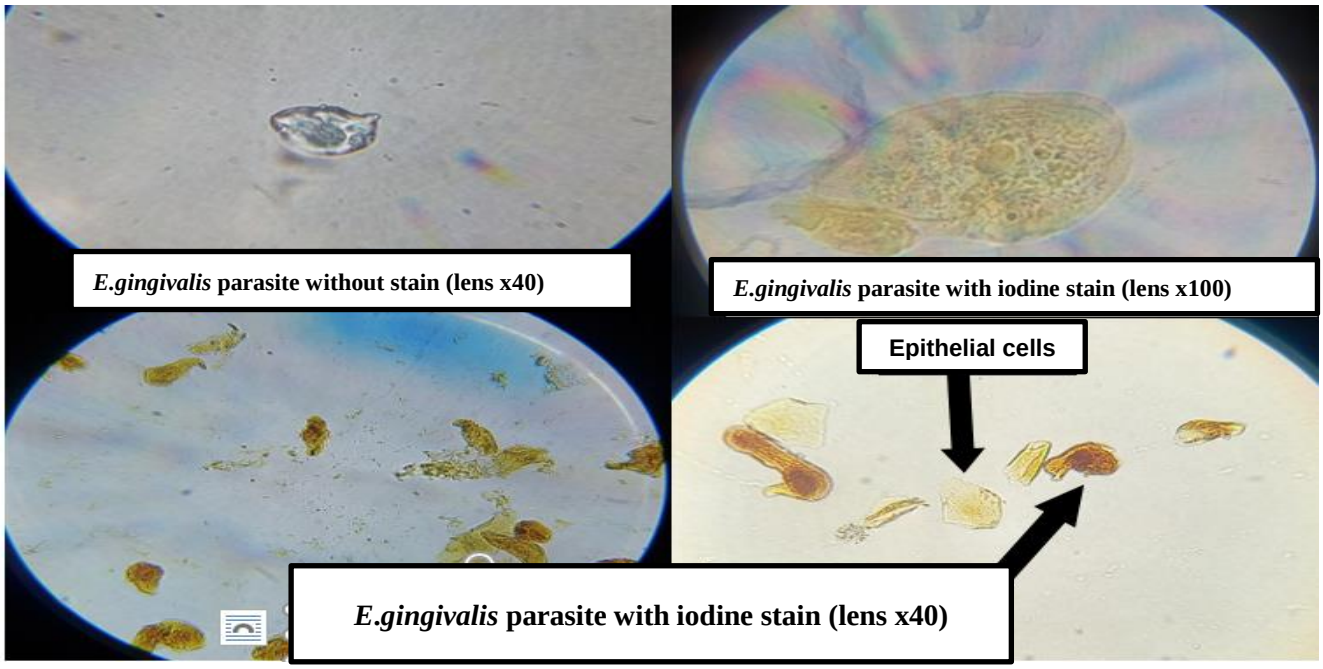
interleukin -6, interferon-gamma and tumor necrosis factor alpha, which contribute to the destruction of tooth-supporting structures [6]. The immune system releases what are known as interleukins such as IL-1, IL-6 and INF-gamma; Both forms of interleukin-1α (precursor of IL-1α and mature IL-1α) are is similar in its biological activity stimulating IL-6 and TNF-α and inflammatory cytokines which interferes with inflammatory process and accelerate the bone resorption activity of erosion leading to alveolar bone destruction [7]. It has effects on adaptive immunity, such as enhancing CD4+ T cells via IL-21 production and enhancing T cell differentiation toward Th2 T cells, the reason for using IL-6 as a biomarker is its central role in activating the inflammatory response IFN-γ [8]. Macrophages stimulated by IFN-γ show antimicrobial activity, IFN-γ activates macrophage and makes them more capable of mounting effective immunity, such as enhanced antigen processing and enhance production of Reactive oxygen species ROS that also stimulate autophagy to remove pathogens within cells, and presentation through upregulation of Major histocompatibility complex class II and increase the secretion and migration of leukocytes. IFN-γ can also deplete tryptophan, which is described as an effective anti-parasitic mechanism in humans [9].

Materials and method

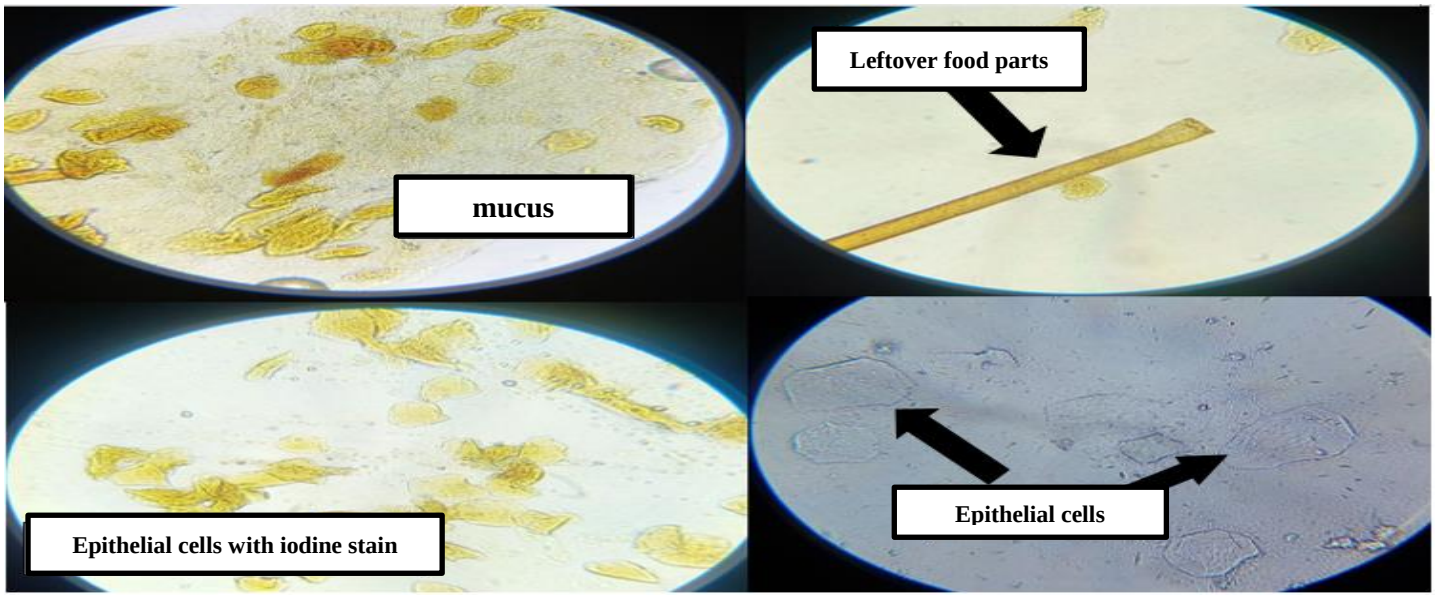
Study Design :The delegation divided the study groups into three groups: (gingivitis with *E.gingivalis*, gingivitis without *E.gingivalis,* and a control group), where the concentration in the blood serum was measured, The study contained prevalence rate for *E.gingivalis* parasite from 250 gingival swabs depending on gender, smoking and location.

Smears and Microscopic examination:

gingival smears were examined under a direct microscope without staining and other swab examined using iodine stain and diagnosis by microscope, excluding people under 18 years old, pregnant women, or taking oral medications, making sure not to eat or use a toothbrush and mouthwash for a period of not less than an hour. Shape(1) and (2).



Shape (1) Entamoeba gingivalis under microscope.



Shape (2) Swabs under microscope ((lens x40)**.**

Blood samples and Immunological tests:

Collecting blood samples for immunological tests by means of after and after blood separation through the Central Retirement Device to store the treated blood sample at a temperature of-20°C. The enzyme-linked immunosorbent assay (ELISA)was used with IL-1α, IL-6 and IFN-Y measured by private laboratories ELISA kits for each and the result analyzed by ELISA reader device.

Statistical analysis:

Data were analyzed by Minitab program system Ver.17 and ANOVA test were applied. The means compared by Duncan's Multiple Range under the level of significantly 0.05.

Results and Discussion

In table 1. The epidemic rate is shown depending on gender. It showed that the prevalence rate is higher in males 92(%61.3) compared to females 58(%38.7) out of 150 swab samples infected with *E. gingivalis* parasite. This is consistent with study in Iraq which the highest rate of infection with the *E.gingivalis* parasite in infected males was 46% [10] and a study in Iran the highest percentage of males infected with the *E.gingivalis* parasite reached 60% out of 189 samples [11]. the reason for the increase in infection with *E. gingivalis* in males is due to male personal habits such as smoking, wrong social habits and unhealthy food [12].

In table 2. The epidemiological rate is shown based on smoking, that the rate of smokers 104(%69.3) is higher compared to non-smokers 46(%30.7) out of 150 swab samples infected with *E. gingivalis* parasite.. That agree with research published up to August 2020, a total of 52 studies estimated the overall prevalence of the *E. gingivalis* parasite that show the prevalence of infection in smokers was 31% lower than non-smokers. [13]. Smoking change the contents of saliva because it contains substances and chemical compounds that may change the environment of the mouth and saliva Which reduces the level of plaque in mouth [14].

In Table 3. The epidemic rate is shown depending on the housing location. It showed that the rate in the villages 102(%68) is higher compared to the city 48(%32). It is natural that the spread of parasites depends on the complex interaction between parasites, environmental factors, nutritional nature and the immune response of the host, where the infection can become more dangerous [15].

Table 1: The percentage of infection depends on gender.

Gender / *E.gingivalis*	N(%)	Male	Female	Total
Yes	N(%)	92(%61.3)	58(%38.6)	150
No	N(%)	56(%56)	44(%44)	100
Total	N	148	102	250

Table 2: The percentage of infection depends on smoking.

Smoker / *E.gingivalis*	N(%)	Smokers	Non-smokers	Total
Yes	N(%)	104(%69.3)	46(%30.7)	150
No	N(%)	20(%20)	80(%80)	100
Total	N	124	126	250

Table 3: The percentage of infection depends on housing location.

Location / *E.gingivalis*	N(%)	City	Village	Total
Yes	N(%)	48(%32)	102(%68)	150
No	N(%)	56(%56)	44(%44)	100
Total	N	104	146	250

In Table 4. Results of the study when assessing level of IL-1α showed no significant difference (P>0.05) between gingivitis with *E.gingivalis* group (2.373±0.975) ng/L, gingivitis without *E.gingivalis* group (1.972±0.740) ng/L compare to control group(1.972±0.740) ng/L . This results agreed with [16] which they didn't find association between positive genotype and risk of periodontal disease was found for the IL-1α or IL-1β genes and in study [17] there was no significant increase in the level of IL-1α in the first days, but on day 21 it increased significantly and then decreased significantly on day 28 of infection. While it differs with study [18] on IL-1α genes showed a distinct difference between gingivitis and healthy controls. There is no difference in the level of IL-1α, as it increases after a period of time after infection to activate the level of immunity against gingivitis [17].

In Table 4. The level of IL-6 in increase in the patient serum with significant difference (P<0.05) between gingivitis with *E.gingivalis* group (13.340±4.773) ng/L, gingivitis without *E.gingivalis* group (14.001±5.344) ng/L compare to control group(9.243±3.760) ng/L. This is consistent with study [19] the levels of both IL-6 and TNF-α were high in patients with gingivitis compared to healthy controls and study [20] using ELISA, the average levels of IL-6 in patients were much higher compared to healthy people with statistically significant (P<0.001). While it differs with [21] which show a weak correlation between the amounts of IL-6 in the presence of gingivitis.

The intensity duration and condition of inflammation depend on the change in the balance between the activities of proinflammatory and anti-inflammatory cytokines during the inflammatory process in periodontitis as IL-6 has multiple biological activities[22], early IL-6 production during infection and through the control of leukocyte recruitment, activation, and apoptosis has emerged as one of a network of mediators directing this switch from the innate to the adaptive immune response [23].

In Table 4. the level of IFN-γ in the serum increase with gingivitis without *E.gingivalis* (28.99±3.08) ng/mL compare to control group (22.90±3.50) ng/mL ,while gingivitis with *E.gingivalis* group (26.12±3.90) ng/mL with no significant difference (P>0.05) between study

groups. This is consistent with [24] that aimed to determine whether the levels of 10 cytokines IL(1β,2,5,6,8,10), IFN-Y and TNF-α in saliva and shown that these cytokines cannot distinguish between gingival health and gingivitis. while it differs with study [25] that evaluate IFN-γ levels and expression of its receptor IFN-γR in gingival tissue biopsies from periodontitis patients. Where IFN-γ concentrations in gum tissue were much higher in patients with. The immune regulatory activities of IFN-γ may depend on constitutively expressed IL-1α and may serve to sensitize IFN-γ cells to T cells [26], The increase in IFN-γ within the normal level indicated the stability of the gingiva, that the lesions favored the response of Th2 cells, and that the increased destruction of the gingiva in the absence of neutrophils turned attention again to the role of lymphocytes, and in particular to the regulatory role of T cells [27].

Table 4. level of Immunological Parameters in the serum of study groups.

Immunological Parameters	Mean ± S.D			P-value
	Gingivitis with *E.gingivalis*	Gingivitis without *E.gingivalis*	Control	
IL-1α (ng/L)	2.373±0.975	1.972±0.740	1.972±0.740	0.102
IL-6 (ng/L)	13.340±4.773	14.001±5.344	9.243±3.760	0.0004
IFN-Y(ng/mL)	26.12±3.90	28.99±3.08	22.90±3.50	0.142

***Conclusion**:* Results of the epidemiological study showed that the males are higher than females, the percentage of smokers higher than non- smoking and the village is higher than in the cities. While immunological results showed no effect on the level of IL-1α, among the results there was an effect on the level of IFN-Y, while level of IL- 6 increase in Patients.

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انتشار *Entamoeba gingivalis* في مرضى التهاب اللثة وتقييم مستوى IL1α و IL6 و IFN-Yفي مجموعات المرضى

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الخلاصة:
يرتبط طفيلي *Entamoeba gingivalis* بالتهاب اللثة. بلغ معدل الإصابة بطفيلي *Entamoeba gingivialis* في مدينة كنعان وريفها، ديالى، العراق والتي امتدت من 1 يوليو 2023 ولغاية 29 اغسطس 2023. تم فحص المسحات من خلال دراسة 250 مسحة و90 عينة دم تم جمعها من المرضى الذين اشتكوا من التهاب اللثة وأمراض الأسنان للتحليل المناعي. من زوار عيادات الأسنان الخاصة. بلغت نسبة الإصابة أعلى عند الذكور (61.3%) واقلها عند الإناث (38.7%)، وتبين أن المدخنين (69.3%) أعلى من غير المدخنين (30.7%). بينما اظهر الريف (68%) أعلى إصابة بالطفيلي من المدينة (32%). كما شملت الدراسة بعض المعايير المناعية في مصل الدم للأشخاص المصابين والمجموعة الضابطة. وشملت هذه المعلمات Interleukin-1α (IL-1α)، Interleukin-6 (IL-6) و Interferon-gamma (IFN-Y) وأظهرت النتائج عدم وجود فروق معنوية في مستوى IL-1 و IFN-γ (P>0.05) بينما ارتفع مستوى IL-6 معنوياً (P<0.05).