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# The relationship of *Entamoeba gingivalis* with cortisol hormone and alpha-amylase in females with gingivitis

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**A B S T R A C T**

The current study was conducted on the Entamoeba gingivalis parasite, where 81 samples were collected from females in the form of gingival pocket swabs from visitors to the Periodontal Department of the Teaching Hospital at the College of Dentistry / University of Mosul during the period from December 1, 2019, to January 12, 202. It was distributed among 65 samples from females with gum disease and 16 samples from healthy females without gum disease. The total infection rate with the amoeba parasite was 69.14%. It was distributed among females with gum disease, where the infection rate reached 73.84%, and among females without gum disease, which amounted to 50. %. A number of biochemical tests were conducted on people with gum disease, some of whom had high blood pressure and diabetes, and included measuring the concentration of the hormone cortisol, as well as measuring the activity of the alpha-amylase enzyme. An increase in the concentration of the hormone cortisol and a decrease in the activity of the alpha-amylase enzyme were observed in the group of females suffering from chronic gingivitis compared to the rest of the groups, as well as a decrease in the activity of the enzyme. Amylase in both the group of females with high blood pressure and diabetes compared to the control group at the probability level ($p \leq 0.005$).The study also found an increase in the percentages of white blood cells, lymphocytes, and neutrophils in women infected with the parasite Entamoeba gingivalis at a probability level ($p < 0.005$).

## Introduction

Microorganisms are found on human epithelial tissues such as the skin, oral cavity, respiratory system, digestive system, urinary system and reproductive system. The number and type of these microbes varies with age, diet and levels of personal hygiene, and they are referred to as normal microorganisms in the human body [1]. Oral organisms are diverse due to the constant contact of the oral cavity with the external environment. Temperature, ecosystem, pH and feeding habits are important factors that contribute to the formation of the oral microbiota [2]. The oral cavity contains about half of what is present of the symbiotic bacteria in the human body, that is, approximately 6 billion microbes, and they represent (30-50) genera in the human mouth [3]. As the mouth is a suitable environment for the presence of many bacteria such as *Lactobacillas , Steptococcus* and the parasites *Entamoeba gingivalis*, *Trichomonas tenax* [4, 5] . *Entamoeba gingivalis* is a genus of *Entamoeba* and its life cycle includes only one phase, the vegetative phase, where it lacks the cystic phase. The parasite has been found in the gums, especially in the gum pocket, where the level of oxygen decreases, which is important in the survival of the vegetative phase. The parasite is transmitted directly from one person to another. By kissing or using contaminated utensils or aerosols [6]. Trophozoite is characterized by containing an inner cytoplasm called endoplasm and an outer cytoplasm called ectoplasm. The endoplasm contains a centrosome nucleus surrounded by regular chromatin granules [7]. It was previously believed that the parasite *Entamoeba gingivalis* has an unsatisfactory effect, but with many studies that have proven its association with many periodontal diseases, which show the parasite's ability to attack and destroy red blood cells and white blood cells, and it has been observed that a number of parasites attack the White blood Cells continuously, as it enters in the form of finger structures inside the cell membrane, and works to release a substance that leads to the release of the enzyme Elastase in large quantities, which is one of the main enzymes that cause the destruction of the supporting tissue and it is one of the most important causes of gums hemorrhagic and liberating large amounts of erythrocytes [8]. There are studies that diagnosed the parasite *Entamoeba gingivalis* in HIV-infected persons [9]. Metronidazole is the most common drug used to treat parasites [10]. Oxidative stress in the biological system is defined as an imbalance between antioxidants and oxidation products. Many studies have proven that oxidative stress is associated with the emergence of many diseases, including infections caused by bacteria and parasites.11, 12]. High or low cortisol is evidence of the occurrence of oxidative stress [13], as well as the activity of the enzyme α-amylase less or more than the normal limit in the case of stress and psychological stress [14]. Cortisol hormone is called the stress hormone because it is secreted in case of fatigue and low blood sugar and works to increase the level of sugar in the body and suppresses the immune system. It is secreted in response to physical and psychological stress that is secreted from the anterior lobe of the pituitary gland and stimulates the gland to release the hormone to the vascular system [15]. It was indicated Studies to the role of parasites in increasing the secretion of the stress hormone (cortisol) as an adaptive means by the parasite to weaken the immune system and thus increase the chances of survival in the body [16]. There are studies that have demonstrated the effect of the hormone cortisol on the oral microbiome, as it causes metabolic changes. Periodontal disease, steroids in the oral cavity, and including cortisol, are reduced immunity and affects the function of white blood cells. All of these factors weaken the defense against infection by microorganisms in the gums [17]. Alpha amylase is an enzyme that is automatically secreted from the salivary glands in the human body. It has an important role in the initial digestion of starch and is likely to be a suitable environment for the growth of many microorganisms in the mouth and contributes to the formation of dental plaque. Alpha amylase may act as a receptor for bacteria to adhere to the surface of the teeth. These multiple functions of the enzyme indicate that it plays an important role in dental caries and the development of periodontal disease [18].The immune system is one of the functional systems that play a very important role for the human body. It consists of many immune cells that are formed and are located in the lymphoid organs such as the spleen, lymph nodes and tonsils. These immune cells resist foreign bodies that attack the human and animal body, so the immune system is an important mechanism. One of the mechanisms of internal balance in the body as it resists foreign bodies such as parasites, germs and viruses that penetrate the body. Therefore its main function is to identify what is foreign in the body and then eliminate it by implementing appropriate defense mechanisms. The aim of the study is to investigate the presence of the parasite *Entamoeba gingivalis* in people with gum disease, in addition to conducting a number of tests and knowing the changes that occur and their association with infection with the parasite for people with gum disease, high blood pressure, and diabetes compared to healthy people. The tests included (estimating the concentration of the hormone cortisol, estimating the effectiveness of an enzyme, Alpha amylase, percentage of lymphocytes, and percentage of neutrophil white blood cells).

## Materials and methods

**Sample collection**

Eighty-one samples were collected from females that included (65) samples taken in the form of swabs from the gingival pocket, during the period 1,2019 to December 1,2020 from females with periodontal disease, and 16 samples were taken from healthy people, they were distributed within the age groups ranging from (16-75). Samples were taken from persons attending the Department of Periodontics of the Teaching Hospital at the College of Dentistry / University of Mosul and some outpatient clinics for dentists in Nineveh Governorate in order to detect the presence of the gingival amoeba parasite. Using sterile paper pointer size 40 threads, left for 30 seconds and then placed in Eppendorf tubes of 1.0 ml containing 0.5 ml in the appropriate culture medium for the parasite [19]. Then the samples were transferred to the laboratory for the purpose of examination and to determine whether the result was negative or positive [20, 21].

**Sample examination**

The method includes taking samples from the mouth from the gum pocket area, which is soaked with saliva, then attaching the sample directly to the glass slide and then examining it with an optical microscope under both small and high powers (10X, 40X), which enables us to observe the amoeboid movement of the parasite, which we can distinguish from other organisms in the mouth.

**Sample dyeing method**

This method is done after preparing wet slides and ensuring the presence of oral parasites. It is dried at room temperature, and then the sample is stained using giemsa stain. These dyes distinguish the cytoplasm, nucleus, and flagella of the parasite, and then it is washed with distilled water. To dilute the dye, it is then examined under a microscope [22].

**Biochemical examinations**

Hormone Cortisol Test, a kit was used to test the human cortisol hormone, Elisa Kit, from the Bioassy technology laboratory. α- amylase test , a Human α-amylase Elisa Kit was used from the Bioassy technology laboratory.

The percentage of white blood cells, lymphocytes and neutrophils, was estimated for the drawn blood samples for females placed in tubes containing an anticoagulant substance EDTA, using the Swelab alfa plus device from a Swedish origin, for taking tests for a complete blood picture [23, 24, 25, 26].

**Data Analysis**

Statistical analysis was performed using software SPSS and tests included chi-square and Duncan's multiple range test under the probability level ($P < 0.05$).

**Results and Discussion**

From Table No.1, we note that the total percentage of infection with the parasite *Entamoeba gingivalis* and after its diagnosis, as noted in Figure No. 1, amounted to 69.14%, represented by 73.84% among women with gum disease. This percentage is higher than what was recorded in Baghdad, where the percentages were in studies conducted by researchers (27, 21), (24%, 30%), respectively. Also higher than what was recorded in Iran, and the percentages were (11, 7 %, 28%) according to the researchers [28, 29].

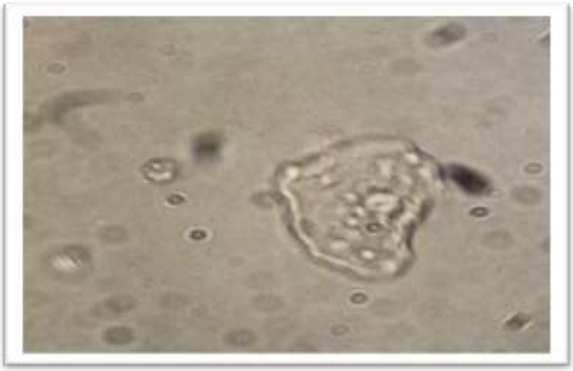
**Table 1.**The percentage of total infection with *Entamoeba gingivalis* in female

| Sex/ Female | *Examination(N)* | infected with *E.gingivalis* (N) | (%) |
|---|---|---|---|
| **Periodontal Disease** | **65** | **48** | **73.84%** |
| **Healthy** | 16 | 8 | **50%** |
| **Total** | **81** | 56 | **69.14%** |
| **Chi-square test** | **Df- 1, p-value=0.4639** | | |

As for healthy females, the rate of 50% was recorded in this study, which is higher than what the researcher (30) recorded in Najaf, which was 37%, and higher than what the researcher (30) recorded in Iran, which was 33%, while in Egypt, the researcher recorded (31) 17%. One of the most important reasons that leads to a higher incidence of parasites in people with gum disease compared to healthy people is neglecting to brush the teeth after eating, which leads to the formation of what is called the dental plaque layer, which forms very quickly, less than an hour or so, and its thickness increases, deposits occur in it, and it becomes softer. The longer it is left, the more this layer contains a large number of germs and parasitic protozoa. These germs feed on food

debris found between the teeth, and their growth results in the secretion of large amounts of acids and secretions that affect the mouth, causing an unpleasant odor, a change in tooth color, and tooth decay [32].

Figure (1*) Entamoeba gingivalis* in a smear is taken from the periodontal pocket (400X).



**Cortisol concentration in female blood serum test**

As shown in Table (2), there was a significant increase in the level of cortisol hormone for the group of females affected by chronic gingivitis with the presence of the parasite *Entamoeba gingivalis* compared with the rest of the groups with a mean of 830.75 ± 13.78) nmol/L at the probability level (P ≥ 0.05). The mean of cortisol for the control group of healthy and infected females was 10.67 ± 741.00 3.69 ± 164.75) nmol/L, respectively. Whereas the mean of cortisol for the group of females infected with Entamoeba gingivalis parasite with chronic gum bleeding was (7.40 ± 783.00) nmol/L, while the mean Arithmetic for the group of females affected by damage to the supporting tissue with the parasite for the hormone cortisol (6.14 ± 808.50) nmol/L**.**

**Table 2.** The concentration of Cortisol hormone (nmol/L) in the group of females infected with *Entamoeba gingivalis* and those with chronic periodontal diseases compared to the control group

| **The group** | **concentration of Cortisol nmol/L** |
|---|---|
| control | 146.75<br>E |
| Infected control | 741<br>D |
| Bleeding gums infected | 783<br>C |
| Damaged mucus tissue is infected | 808.5<br>B |
| Chronic gingivitis infected | 830.75<br>A |

The reason for high cortisol in people infected with the female *Entamoeba gingivalis* parasite and with chronic gingivitis is that cortisol levels may raise in saliva and serum with the severity of periodontal disease [33]. The role of parasites in increasing the secretion of the stress hormone (cortisol) as an adaptive means used by the parasite to weaken the immune system and thus increase the chances of survival in the body [15]. Cortisol reduces the body's immunity and affects the function of white blood cells. All of these factors weaken the body's resistance. In defense against microorganisms, including those found in the oral cavity [16-20]. There is a study conducted by Al-Hindawi et al. to determine and compare cortisol levels in people with hypothyroidism with or without gingivitis. The result was an increase in cortisol in people with gum disease compared to people without gum disease [34].

**Activity of α-amylase enzyme in saliva test**

Table (3) shows a significant decrease in the activity of the α-amylase enzyme for a group of females infected with the *E.gingivalis* parasite with chronic periodontitis with an arithmetic mean. (49.37 ± 3.06) IU/L at the level of probability (P ≥ 0.05) compared with the rest of the groups, where the arithmetic mean of the control group for healthy females was (1.68 ± 63.62) IU/L, while the mean of the α-amylase enzyme activity was for the control group for the affected females. The arithmetic mean of groups of females infected with the parasite with bleeding gums and damage to the supporting tissue reached (53.75 ± 2.71, 53.00 ± 2.77) international units / liter.

**Table 3.** The activity of α-amylase enzyme (international units/liter) in the group of females infected with Entamoeba gingivalis and those with chronic gum diseases compared to the control group

| The group | The activity of α-amylase enzyme |
|---|---|
| control | 63.625<br>A |
| Infected control | 55.5<br>B |
| Bleeding gums infected | 53.75<br>B |
| Damaged mucus tissue is infected | 53<br>B |
| Chronic gingivitis infected | 49.375<br>C |

Infection of females with the parasite *Entamoeba gingivalis* with chronic gingivitis has led to a decrease in the activity of the α-amylase enzyme for these groups compared to the rest of the groups. This enzyme is present in the mouth and is automatically secreted from the salivary glands in the human body and has an important role in the initial digestion of starch to be a suitable environment for the growth of many microorganisms in the mouth and contribute to the formation of dental plaque. α- amylase may act as a receptor for bacterial attachment to the surface of the teeth. These multiple functions of the enzyme indicate that it plays an important role in dental caries and the development of periodontal disease [18].

**The percentage of white blood cells lymphocytes in the blood of females**

It was clear from Table (**4**) that there was a significant increase in the percentage of white blood lymphocytes at the probability level ($P<0.05$) for the group of females infected with the parasite associated with chronic gingivitis with a mean of (42.00 ± 7.83%) compared with the rest of the groups, while it was the arithmetic mean of the control groups for the healthy females infected with the parasite was (4.53 ± 33.00, %) 5.50 ± 35.00, respectively, while the mean percentage of white blood lymphocytes for the group of females infected with the parasite with bleeding gums was (37.00 ± 6.36) and for the group of females with damage to the supporting tissue and infected with a *E. gingivalis*, the arithmetic mean was (8.81 ± 39.00).

**Table 4.** The percentage of lymphocytes (%) in the group of females infected with *Entamoeba gingivalis* and those with chronic gum diseases compared to the control group

| The group | The percentage of lymphocytes (%) |
|---|---|
| Control | 33<br>C |
| Infected control | 35<br>BC |
| Bleeding gums infected | 37<br>ABC |
| Damaged mucus tissue is infected | 39<br>AB |
| Chronic gingivitis infected | 42<br>A |

The results of our study agree with a first study conducted in Iraq in the city of Kirkuk by Lazar et al. (2020) to find out the effect of the presence of the parasite *Entamoeba gingivalis* associated with the germs that cause gingivitis on the number of white blood cells between sick and healthy people. The numbers of the parasite *Entamoeba gingivalis* are increased in the sick people, reaching 7.58 ± 1.633 than their numbers in the healthy people, which amounted to 5.94 ± 0.585. 2.5 ± 0.458, while the numbers of white blood lymphocytes were low in healthy people, reaching 1.9 ± 0.36, with significant differences at the probability level ($p\leq0.05$),[35]. These results agreed with Nicu et al. (2009) in Amsterdam in terms of increasing the number of cells White blood with the presence of bacteria that cause diseases and gingivitis [36]. Also, it is agreed with a study conducted by Buhlin et al. (2003) in Sweden, which confirmed the increase in the number of white blood cells with periodontal disease [37]. Compared with healthy people, they found an increase in the total number of white blood cells in people with gingivitis and teeth and as gum disease that affects the bones and its supporting tissues called periodontitis and is characterized by the formation of spaces between the teeth and gums, and it

develops into chronic diseases that may lead to tooth loss, and it rises WBCs are a consequence of chronic periodontal inflammation [38].

Therefore, this study showed that people suffering from periodontal disease and dental diseases associated with the presence of the parasite *E. gingivalis* caused an increase in the percentage of white blood lymphocytes in people with chronic periodontitis compared to healthy people. The reason for the high numbers of white blood lymphocytes may be attributed to the activation of the bone marrow to produce cells to provide protection for the body through activating the vegetative phase trophozoite by forming a barrier that prevents the spread of the parasite to different areas of the body. White blood cells are among the cells that reach the site of infection first when the occurrence of the inflammatory response [39]. There are many studies that prove an increase in the percentage of white blood cells and lymphocytes in people infected with parasites. [40]

**The percentage of neutrophilic white blood cells in female blood**

As shown in Table (5) there was a significant increase in the percentage of neutrophilic white blood cells at the probability level ($0.05 \geq P$) in the group of females infected with gingivitis and infected with the parasite with a mean) of $56.00 \pm 7.30$ (%) and the group of females infected with the parasite with bleeding gums With a mean of ($8.03 \pm 54.00$)%, and there were significant differences with the healthy control group with a mean of $50.00 \pm 9.63$ %) and the control group for females infected with the parasite with a mean of ($9.21 \pm 52.00$)%.

**Table 5.** Percentage of neutrophilic (%) in the group of females infected with *Entamoeba gingivalis* and those with chronic periodontal diseases compared to the control group

| The group | The percentage of neutrophilic (%) |
|---|---|
| control | 50<br>C |
| Infected control | 52<br>BC |
| Bleeding gums infected | 54<br>BA |
| Damaged mucus tissue is infected | 55<br>A |
| Chronic gingivitis infected | 56<br>A |

The study indicates an increase in female neutrophilic white blood cells of groups infected with the parasite *Entamoeba gingivalis* with chronic periodontitis, whereby white blood cells are considered one of the body's defense mechanisms. So disturbances occur as a result of cellular filtration of immune cells in areas of infection. [41 ,42] The reason for this is due to the breakdown of blood cells, including white blood cells, as a result of the presence of the parasite, or the reason for this rise may be due to the fact that neutrophils have the role of defending the body against protozoa, as they digest and phagocytize them by the process of phagocytosis. The number of neutrophils is increased in the case of bacterial or parasitic infections as an immune function because it contains some lysosome enzymes that kill pathogenic germs and the process of their digestion, thus reducing the total number of them [39]. Lysosome enzymes possessed **[**43].

**Conclusion**

The study shows a high incidence of infection with the *Entamoeba gingivalis* parasite in females with gum disease, while the infection rate is decreased in females without gum disease. After conducting a number of biochemical tests on people with gum disease, an increase in the concentration of the hormone cortisol and a decrease in the activity of the thyroid gland were noted. The presence of an enzyme was noted. Alpha amylase in the group of females suffering from chronic gingivitis compared to the rest of the groups and a decrease in enzyme activity. In both the group of females suffering from high blood pressure and diabetes compared to the control group and an increase in the percentages of white blood cells**.**

## References


[1] Berger, G., Bitterman, R.and Azzam, ZS. (2015).The human microbiota: the rise of an empire‖. Rambam Maimonides Med J., 6: 1–5.

[2] Neetu, S., Sonu, B., Abhinashi, S., and Navneet, B.(2018). Oral microbiome And Health. Aims Microbiol J., 4(1): 42–66.

[3] Rose, L. R., Genco, R. J., Gohen, D. W. and Mealey, B. L.(2000). Periodental Medicine. London. Saint Louis. Pp2-3.

[4] White, D.J., K.M. Kozak, R. Gibb, J. Dunavent, M. Klukowska and P.A. Sagel (2006). A 24-hour dental plaque prevention study with a stannous fluoride dentifrice containing hexametaphosphate. J. Contemp. Dent. Pract., 7(3): 1- 11.

[5] Rosier, B. T., Marsh, P. D., and Mira, A. (2018). Resilience of the oral microbiota in health: mechanisms that prevent dysbiosis. J. Dent. Res. 97(4): 371–30.

[6] Bonner, M., Amard,V., Bar-Pinatel. C., Charpentier, F.and Chatard, J.M.(2014). Detection of the amoeba Entamoeba gingivalis in periodontal pockets. Parasite, 21: 30.

[7] Eloufir, F., Khelaifia, S., Aboudharam, G., & Drancourt, M. (2014). In vitro activity of metronidazole against Entamoeba gingivalis. J Infect Dis Ther, 2, 170.

[8] Lyon, T., Scholten, T. N., Palmer, J. C. and Stanfield, E. (1983). Oral Amoebiasis role of Entamoeba gingivalis in periodontal disease. Quintessence Inte., 12: 1245 – 1248.

[9] Wantland، W. and Lauer ,D. (1970).Correlation of some oral hygiene variables with age, sex, and incidence of oral Protozoa. J Dent Res; 49:293-297.

[10] Salih, A. A., Obaid, H. M., & Jasim, W. M. (2022). An in vitro study of zinc effect on Trichomonas vaginalis isolated from infected women. NTU Journal of Pure Sciences, 1(4), 44-53.

[11] Tunez I., Sanchez-Lopez, F.,.Aguera, E., Fernandez, R., Sanchez, F. and Tasset, I..(2011). Important role of oxidative stress biomarkers in Huntington's disease. J Med Chem. 54( 15) 5602-5606.

[12] Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D. and Abete,P.(2018). Oxidative stress, aging, and diseases. J.C.l I.I. A,, 13, 757.

[13] Casto, K. V., and Edwards, D. A. (2016). Before, during, and after: How phases of competition differentially affect testosterone ,cortisol, and estradiol levels in women athletes.Adaptive Human Behavior and Physiology, 2(1), 11–25.

[14] Sakac, N., Karnaš, M., and Grcic, M. (2015). Direct potentiometric method for human stress determination. Chemical and Biochemical Engineering Quarterly, 29(3): 315-321.

[15] Stratakis C. A. (2012).Cushing's syndrome in pediatrics, Endocrinol Metab Clin North Am., 37(6):1313-1332.

[16] Djuric, Z., Bird, C. E., Furumoto-Dawson, A., Rauscher, G. H., Ruffin IV, M. T., Stowe, R. P., ... & Masi, C. M. (2008). Biomarkers of psychological stress in health disparities research. The open biomarkers journal, 1, 7.

[17] Duran-Pinedo, A. E., Solbiati, J., and Frias-Lopez, J. (2018). The effect of the stress hormone cortisol on the met transcriptome of the oral microbiome.NPJ biofilms and microbiomes, 4(1), 1-4.

[18[ Scannapieco, F. A., Torres, G. and Levine, M. J. (1993). Salivary a-Amylase: Role in Dental Plaque and Caries Formation Critical Reviews in Oral Biology and Medicine, 4(3):301-307.

[19] Jian, B., Kolansky, A. S.,. Baloach, Z. W. and Prabodh K, G. (2008). Entamoeba gingivalis pulmonary abscess Diagnosed by fine needle aspiration. Cyto Journal. J.,5(12):12-34.

[20] Nuaimi, Ban-Nzar (2017). Epidemiology of oral parasites in patients with periodontal disease and the effect of extracts of arak, tannins and cloves on Entamoeba gingivalis in glass. Master's thesis, College of Science, University of Mosul.

[21] Nuaimi, B.N.,AL-kattan M.M,AL-Taee,A.F (2021).Study The Effect Of Entamoeaba gingivalis Some variables of Blood and Serum in Males Patients with Periodontal Disease and healthy in Iraq .Annals of The Roomanian Society for cell Biology 25 (3)2915-2926.

[22]Bafghi,A.F.,Aflatoonian,A.,Barzegar,B., Ghafourzadeh,M. and Nabipour, S. (2009). Frequency distribution of trichomoniasis in pregnant women referred to health centers of Ardakan, Meibod and Yazd, Iran. Jundishapur J. Microbiol.,2(4): 132-139.

[23] Nuaimi, Ban-Nzar (2021). Molecular Diagnosis of Entamoeba gingivalis and It's effect on Some Physiological and Biochemical Parameters In human. PD. Thesis Biology/ Zoology, College of Science, University of Mosul.

[24] Nuaimi, B.N., AL-Taee,A.F.,AL-kattan M.M, (2021).Conventional and Moiecular Identification Of Entamoeaba gingivalis From Periodontitis Patients in Nineveh Governorate / Iraq .Annals of The Roomanian Society for cell Biology 25 (4)1293-1306.

[25] Mohammed, S. T., Sulaiman, N. M., & Kamal, S. B. (2015). Prevalence Of Entamoeba gingivalis Among Students Of Collage Of Science In Ai-Mustasyria University, Baghdad, Iraq.

[26 ]Nuaimi, B. N., Al-Taee, A. F., & AL-Kattan, M. M. (2022). The effect of Entamoeba gingivalis infection in patients with diabetes and high pressure on the level of Malonaldehyde and Glutathione. International Journal of Health Sciences, 6(S3), 6143–6152

[27] Ibrahim, S., and Abbas, R. (2012). Evaluation of Entamoeba gingivalis and Trichomonas tenax in patients with periodontitis and gingivitis and its correlation with some risk factors. J Bagh Coll Dentistry, 24(3): 158-62.

[28] Gharavi, M. J., Hekmat, S., Ebrahimi, A., and Jahani, M. R. (2006). Buccal cavity protozoa in patients referred to the faculty of dentistry in Tehran, I. I. J. O. P., 43-46.

[29] Rahdar, M., Abolfazli-Karizi,S. and Hootan Pedram(2019). The Comparison of Entamoeba gingivalis Presence in Healthy and Periodontitis Patients by Using Direct Examination and PCR Methods. Jundishapur J. Health Sci11(1): 60-66.

[30] Hammadi, Haitham and Obaid, Samira Jaber (2008). Prevalence of the parasites Entamoeba gingivalis and Trichomonas tenax in Al-Najaf Governorate. University of Kufa Journal of Medical 11(2):1-8.

[31] Hassan, S., Madkour, G., Henin, R., Gad, S., and Abd El-Aal, A. (2019). Is Entamoeba gingivalis a risk factor for periodontal diseases? A case- control study. Journal of Periodontology, 3: 18-28.

[32]Hayawni, J. A. and Bayoumy, M. M. (1992). The prevalence of Entamoeba gingivalis and Trichomon tenax periodontal disease J. E_Son. Parasitol.22(11):101-5.

[33] Rai, B., Kaur, J., Anand, S. C. and Jacobs, R. (2011).Salivary stress markers, stress, and periodontitis: a pilot study. J. Periodontal . 82, 287–292.

[34] Al-Hindawi,S.H.,Al-Ghurabei,B. H.and Luaibi,N. M.(2018).Cortisol Hormone On Hypothyroid Patients With And Without Periodontitis>international J.O.A .B. R.7 (2): 335-338.

[35] Lazar, L. T. Y., Al-Ammash, M. S. J., Shakir, O. M., and Abass, K. S. (2020). Study the effect of some microbiota in oral cavity on some parameters of blood. EurAsian Journal of Bio Sciences, 14(1), 2307-2311.

[36] Nicu,E.A.,Vander Velden,U.,Nieuwland,R., Everts, V., and Loos, B. G.(2009). Elevated platelet and leukocyte response to oral bacteria in periodontitis. Journal of Thrombosis and Hemostasis, 7(1), 162-170.

[37] Buhlin, K., Gustafsson, A., Pockley, A. G., Frostegård, J., and Klinge, B. (2003).Risk factors for cardiovascular disease in patients with periodontitis. European heart journal, 24(23), 2099-2107.

[38] Ghabanchi, J., Zibaei. M., Afkar, M.and Sarbazie, A. (2010).Prevalence of oral Entamoeba gingivalis and Trichomonas tenax in patients with periodontal disease and healthy population in Shiraz, southern Iran .Indian Dent Res. 21(1): 89-91.

[39] Chavez-Tapia, N. C., Hernandez-Calleros, J., Tellez-Avila, F. I., Torre, A., & Uribe, M. (2009). Image-guided percutaneous procedure plus metronidazole versus metronidazole alone for uncomplicated amoebic liver abscess. The Cochrane database of systematic reviews, (1), CD004886. https://doi.org/10.1002/14651858.CD004886.pub2

[40] Al-Hoot, A., and Taha, S.(2017). Effect Of Cutaneous Leishmaniasis on some haematological and immunological parameters in patients from areas of north Baghdad /iraq. bulletin of faculty of science, Zagazig University, 354-366.

[41] Ahmed, A. O., Ali, A. A., & Mardan, H. J. (2023). Hematological change in type II diabetic mellitus (TIIDM) patient infected with intestinal parasites in Kirkuk/Iraq. NTU Journal of Pure Sciences, 2(1).

[42] Playfair,, J. (2007). Living with Germs: In health and disease: OUP Oxford.

[43] Muraro, A., Lemanske Jr, R. F., Hellings, P. W., Akdis, C. A., Bieber, T., Casale, T. B. & Agache, I. (2016). Precision medicine in patients with allergic diseases: airway diseases and atopic dermatitis—PRACTALL document of the European Academy of Allergy and Clinical Immunology and the American Academy of Allergy, Asthma & Immunology. Journal of allergy and clinical immunology, 137(5), 1347-1358.