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## **The effect of Entamoeba gingivalis infection in patients with diabetes and high pressure on the level of Malonaldehyde and Glutathione**

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**Abstract**--The current study was conducted on the Entamoeba gingivalis parasite, where only 65 females samples were collected in the form of swabs from the periodontal pocket from the subjects visiting the periodontal branch of the Teaching Hospital of the Faculty of Dentistry of the College of Dentistry / University of Mosul during the period from 12/1/2019 to 1/12/2020. Among 59 samples of females with gum disease and 16 samples from healthy female without gum disease, the total infection rate of the amoeba parasite for males was 69.14%, distributed among males with gum disease, where the infection rate was 73.84% and among males without gum disease 50%. A number of biochemical tests were performed on males with gum disease and some of them had hypertension and diabetes, and included measuring the concentration of glutathione (GSH), as well as measuring the of Malone didehyde (MDA) in the serum of females. An decrease in the level of the glutathione and a increase in the level of MDA were observed in the group of females with chronic periodontitis compared with the rest of the groups, as well as increase in the level of MDA in both the hypertensive and diabetic group compared to the control group at 0.05 (p) likelihood level.  $p \leq 0.05$ .

**Keywords**---entamoeba gingivalis, glutathione, Malone didehyde, GSH, MDA.

## **Introduction**

The oral cavity contains different genera of microorganisms, but bacterial infection occurs when these organisms penetrate into tissues with cases of immunodeficiency (Loesche et al., 2007). The oral cavity contains approximately 700 or more different types of bacteria that live in the mouth and it is important to preserve oral health, the mouth is a suitable environment for the presence of many microbes such as *Lactobacillus*, *Streptococcus* and parasites, namely *Entamoeba gingivalis*, *Trichomonas tenax* (White et al., 2006; Rosier et al., 2018). *Entamoeba gingivalis* is a protozoan that lives in areas of the upper digestive tract such as the oral cavity, around the teeth and on the gum edges. The prevalence of infection in the parasite increases in people who suffer from problems in the hygiene of the mouth, teeth and gums (Lyon et al., 1983). *Entamoeba gingivalis* can cause infection in the pharynx, where the infection rate increases in persons with immunocompromised patients (Bhaijee and Bell, 2011; Cembranelli; et al., 2013). Studies show that in addition to tooth decay and gum disease, microorganisms can have negative effects on other aspects of human health (Jabuk et al. 2015).

The immune system is an important mechanism of the internal balance mechanisms in the body, as it resists foreign bodies such as parasites, germs and viruses that penetrate the body, and therefore its main function is to identify what is foreign in the body and then eliminate it by implementing appropriate defense mechanisms and therefore it is necessary to identify and eliminate the foreign body (Janway et al., 2003). The resistance to infection with parasites depends on the body's cellular immunity, whose decrease is accompanied by an increase in the level of steroid hormones, which helps the parasite to remain in the body and the continuation of the infection. and personality among these patients (Raquel et al. 2003). Oxidative stress is an important factor for the development of various diseases, so it is important to find indicators to follow up on oxidative stress, including the deficiency in glutathione (Nicola, 2018) and the level of malondialdehyde, which is the final product of lipid peroxide in certain tissues, where the level of malondialdehyde in plasma and urine is measured as an indicator for stress in the body (Tin et al., 2016).

## **Materials and methods**

### **Investigation of the parasite**

#### **Physical examination**

This is done by observing the color and texture of the gums, the layer of calcifications on the surface of the teeth, and the state of the teeth loosening, under the supervision of the specialist doctor Carranza (2002).

### **Sample collection**

#### **Collecting samples from the periodontal pocket**

Sixty-five (65) samples were taken in the form of swabs from the gingival pocket, during the period from 1/12/2019 to 1/12/2020, from females with periodontal disease, and 16 samples were taken from healthy people, they were distributed

and the samples were 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. The parasite (Jian et al., 2008) using sterile paper pointer size 40 threads and left for 30 seconds and then placed in Eppendorf tubes of 1.0 ml and containing 0.5 ml in the appropriate culture medium for the parasite, then the samples were then transferred to the laboratory for the purpose of examination and to determine whether The result was negative or positive (Al-Nuaimi, 2017).

### **Blood sample collection**

(4) ml of blood was taken and placed in tubes containing a semi-liquid gel tube with a red cap to extract the serum and left tilted at room temperature for 15 minutes and then placed and rotated in a centrifuge at 3000 revolutions per minute for 15 minutes The sera were collected and distributed in plastic Ependorf tubes and tagged, where the sera were used directly in conducting tests for glutathione, malondialdehyde and cortisol.

### **Biochemical examinations**

#### **Glutathione Test**

In the study, a ready-made glutathione analysis kit was used to test glutathione in humans. The Human Glutathione Elisa Kit from the Bioassy technology laboratory company

#### **Malondialdehyde Test (MDA)**

In this study, a ready-made assay kit was used to examine the human Malondialdehyde Elisa Ki.

### **Results and Discussion**

Study of the prevalence of the parasite *Entamoeba gingivalis* in females: After examining the samples using a microscope, the parasite was identified and detected in different forms, which were distinguished in irregular shapes and with dimensions ranging between (10-30) microns and with a nucleus containing a central endosome surrounded by organized and rough chromatin granules, in addition to the phantom foot and the distinctive movement of the parasite, as shown in the figure (3-1)

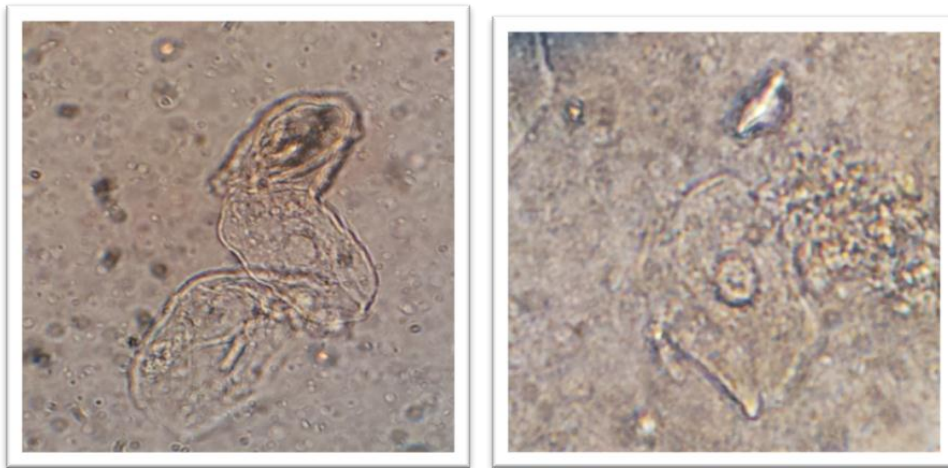


Figure 3-1. Forms of the parasite *Entamoeba gingivalis* in a smear taken from the periodontal pocket (400X)

It is evident from the table below (3-1) that the total incidence of females, which reached 73.84, was distributed among the females suffering from periodontal disease. % and among healthy subjects, as it reached 50%, and after performing the statistical analysis and using the Chi-Square test, it was noticed that there were no significant differences between the percentage of transmissible infection for females with gum disease and the healthy ones at the probability level ( $p \leq 0.05$ ).

Figure 3-2. The Percentage Of Total Infection With *Entamoeba gingivalis* in females

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

The rates of total infection with the amoeba gingiva parasite in females varied for the studies conducted on the infection rate in our current study, including a study conducted in Baghdad, the total infection rate in females was 30% (Mohammed et al., 2015). In a study presented by Ibrahim and Abbas (2012) in Baghdad was The infection rate in females was 24%, and a study was also conducted in Iran, where the infection rate in females was 28%, respectively (Gharavi et al., 2006). In a study conducted by Rahdar et al. (2019) in Iran, the infection rate of *Entamoeba gingivalis* was 11.7 in females, and another study was conducted in southern Iran, 5.7% in females (Sharifi et al., 2020).

Biochemical study of the parasite *Entamoeba gingivalis* in subjects with periodontal disease associated with bleeding gums, periodontal tissue damage, chronic gingivitis and healthy subjects without periodontal disease, female.

### Determination of glutathione concentration in human blood serum

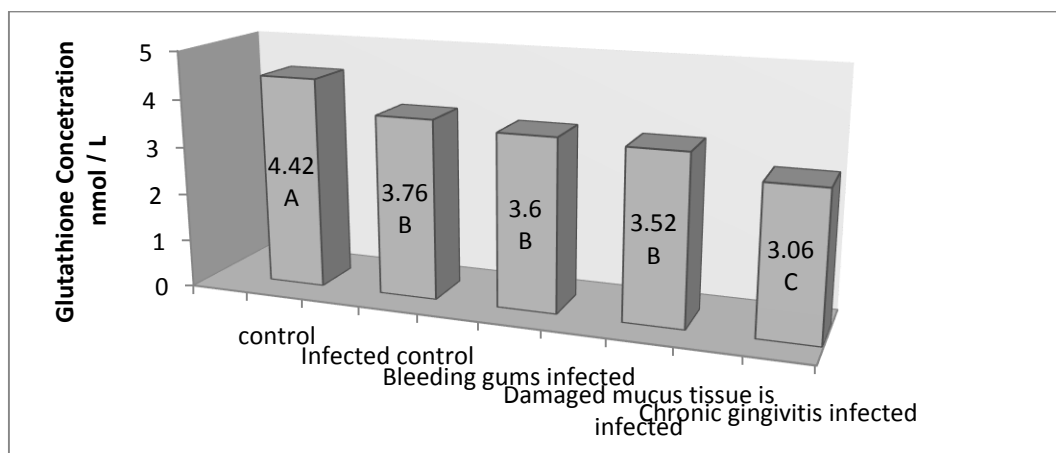


Figure 3-3. The concentration of glutathione (nmol/L) in the group of females infected with the parasite *Entamoeba gingivalis* and those with chronic periodontal diseases compared to the control group

The values are expressed as the arithmetic mean ( $\pm$ ) standard deviation, number of females / group = 8. The columns associated with different letters indicate a significant difference at the P . 0.05 probability level

Figure (3-3) shows a significant decrease in the level of glutathione for the female group infected with the parasite with gingivitis compared to the rest of the groups with a mean of  $(3.06 \pm 0.33)$  nmol/L at a probability level ( $P \leq 0.05$ ), while the arithmetic mean of glutathione for the control group was for females The healthy group was  $(4.42 \pm 0.08)$  nmol/L, while the mean was for the control group for females infected with the parasite *Entamoeba gingivalis* was  $0.14 \pm 3.76$  nmol/l, and the mean was for the females infected with the parasite with bleeding gums  $(3.60 \pm 0.25)$  nmol/l, while the mean was Arithmetic for females infected with the parasite with damage to the supporting tissue  $(3.52 \pm 0.24)$  nmol/L.

The reason for the decrease in the level of GSH in the blood serum of males and females of groups infected with the parasite *Entamoeba gingivslis* with chronic gingivitis compared with the rest of the groups may be due to increased destruction or decreased synthesis and that the decrease in its level is an indication of the occurrence of oxidative stress and sometimes its transformation into the form of disulfide (Hudson). , 1999) Or perhaps there is a change in the defense mechanisms against oxidative stress and this was reflected in a decrease in the level of glutathione in the blood serum, or perhaps the reason for the decrease in its level in the blood serum was a deficiency in the essential amino acids for its manufacture as a peptide consisting of (3) essential amino acids. They are glycine, cysteine, and glutamic, which are necessary raw materials for its construction during oxidative stress or deficiency in the enzymatic facilities of

NAPDH resulting from the pentaphosphate sugar pathway, which are the catalysts for the action of the enzyme glutathione reductase, which works to restore the active form of glutathione from its inactive form (Weiji et al., 2004).

### Determination of the concentration of Malone didehyde in the serum of females

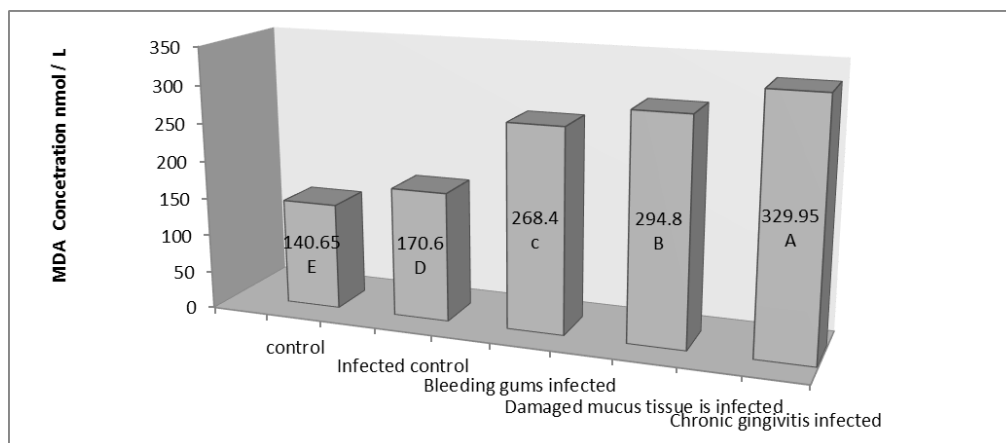


Figure 3-4. The concentration of MDA (nmol/L) in the group of females infected with the parasite *Entamoeba gingivalis* and those with chronic periodontal diseases compared to the control group

The values are expressed as the arithmetic mean ( $\pm$ ) standard deviation number of females / group = 8. The columns associated with different letters indicate a significant difference at the P . 0.05 probability level

Figure 3-4 shows a significant increase in the level of MDA for the group of females infected with the parasite *Entamoeba gingivalis* with chronic gingivitis with a mean ( $25.09 \pm 329.95$ ) nmol/L compared to the healthy and infected control group with a mean of ( $140.65 \pm 2.50$ ,  $170.60 \pm 7.84$ ). nmol/L, respectively. While the mean of the group of females infected with the parasite with bleeding gums was ( $268.40 \pm 45.07$ ) nmol/L, while the MDA level of the group of females infected with the parasite with damage to the supporting tissue was ( $294.80 \pm 5.28$ ).

The high level of MDA in the serum of males and females of groups infected with *E. gingivalis* with chronic periodontitis compared with the rest of the groups may be due to the stimulation of the enzyme (Flatty Acyl \_COA Oxidasase) that stimulates the oxidation of fatty acids that leads to an increase in the production of endogenous free radicals Which contribute to the production of lipid peroxides, and MDA is considered the final product of it, or perhaps the reason for the high MDA is due to the parasite's resistance to the process of phagocytosis by neutrophils, which leads to an increase in the production of free radicals, which helped in the occurrence of various complications in the body (Al-Alaf, 2004; Al-Tae and AL Hassan, 2012).

**Biochemical study in people with periodontal disease associated with those with diabetes and stress and healthy people without periodontal disease, male and female**

**Determination of glutathione concentration in female blood serum**

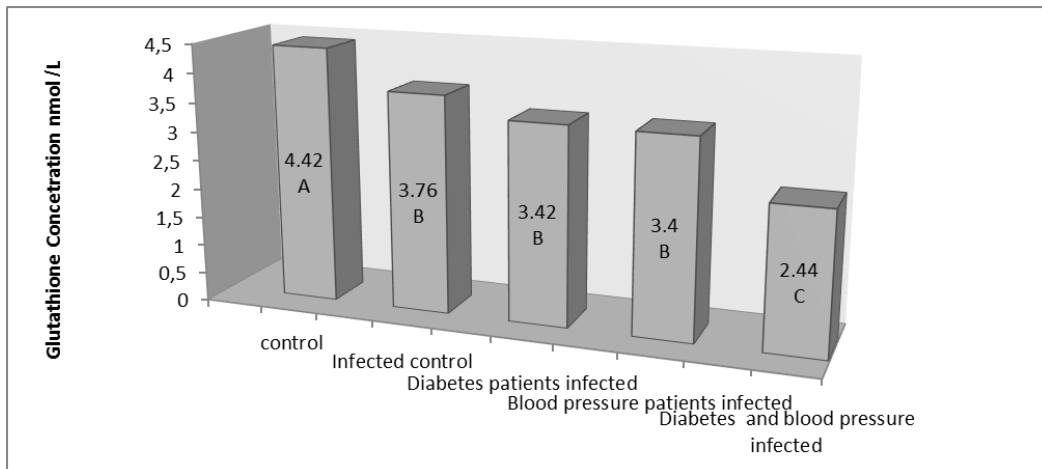


Figure 3-5. The concentration of glutathione (nmol/L) in the group of females infected with the parasite *Entamoeba gingivalis* and those with diabetes and hypertension compared to the control group

The values are expressed as the arithmetic mean ( $\pm$ ) standard deviation, number of females / group = 8. The columns associated with different letters indicate a significant difference at the  $P \geq 0.05$  probability level.

Figure (3-5) shows a significant decrease in the concentration of glutathione at the probability level ( $P \geq 0.05$ ) for people with gum disease for the female group with diabetes and blood pressure with the presence of the parasite compared with the rest of the groups with a mean ( $0.16 \pm 2.44$ ) micromol/L, while the highest concentration of the healthy female group was with a mean ( $0.08 \pm 4.42$ ) micromole/ The arithmetic mean of glutathione for the group of infected females of the non-gum disease group was ( $0.14 \pm 3.76$ ) micromol / liter and the arithmetic mean of the group of females infected with the parasite and diabetes was ( $0.17 \pm 3.42$ ) micromol / liter, and the arithmetic mean of the group of females infected with the parasite with blood pressure ( $0.20 \pm 3.40$ )  $\mu\text{mol/L}$ . The reason for the low level of GSH in the group of females infected with the parasite *Entamoeba gingivalis* and patients with periodontal disease, as well as diabetes and stress, as it is one of the most powerful non-enzymatic antioxidants in biological systems. Moreover, glutathione is one of the most powerful factors that control lipid peroxides, and the decrease in GSH in the case of parasite infection with males and females with diabetes and stress may This happened due to a decrease in the conversion of the penta sugar (Pentose Shunt), as the activity of the enzyme Glucose 6 Phosphate Dihydrogenase (G6 PHD) decreases, and this enzyme is necessary in the conversion of the penta sugar, and thus the defect in the formation of the reducing agent (NADPH) enzymatic accompaniment, and thus the enzyme cannot Glutathione Reduction (GSH-RD) Reducing the oxidized form of glutathione leads to a decrease in the concentration of reductive glutathione

(Nicola et al., 2018) or the decrease in the concentration of glutathione in the serum of patients infected with amoeba parasite with diabetes and stress may be due to the occurrence of Metabolic disorders as a result of parasite infection, followed by a decrease in the level of the hormone insulin.

There is a recent study on the effects of diabetes on the oral cavity, in which the parasite *E. gingivalis* is widely present, and the occurrence of other complications such as gum disease associated with bad breath, burning in the mouth and dysgeusia. The study proved that people with diabetes are also more susceptible For fungal and bacterial infections and soft tissue lesions in the mouth, tooth decay and tooth loss in particular (Miller and Ouanounou, 2020), and there are studies that have proven the close relationship between gum disease and many other diseases such as diabetes, cardiovascular disease and premature birth. Genco and Grossi, 2005; Jeftha and Holmes, 2013; Moodley et al.,2013; Sitholimela and Shangase, 2013).

### Determination of the concentration of Malone didehyde in the serum of females

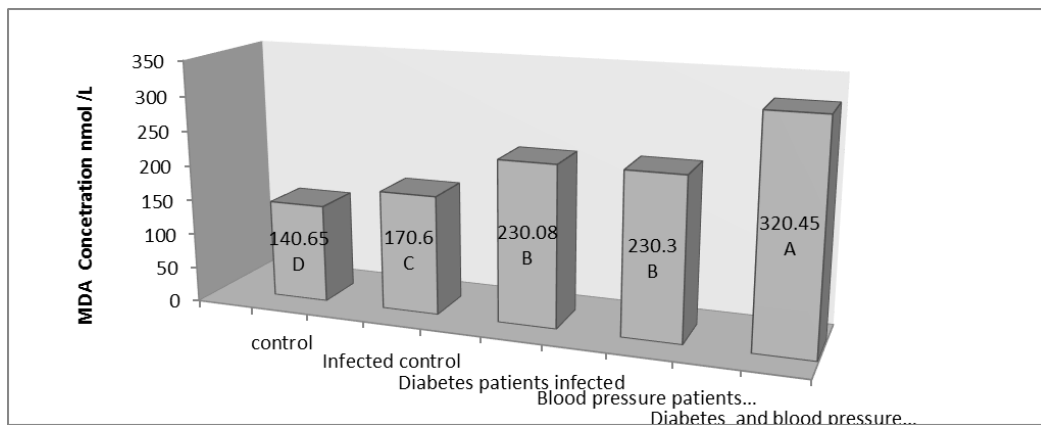


Figure 3-6. The concentration of MDA (nmol/L) in the group of females infected with the parasite *Entamoeba gingivalis* and those with diabetes and hypertension compared to the control group

The values are expressed as the mean ( $\pm$ ) standard deviation

The columns associated with different letters indicate a significant difference at the  $P \geq 0.05$  probability level.

Figure (3-6) shows a significant increase in the level of MDA in the group of females infected with the parasite with pressure and diabetes with an average of  $(320.45 \pm 15.94)$  nmol/L, and the lowest mean of MDA for the healthy control group was for females with a mean of  $(140.65 \pm 2.50)$  nmol/l. Liter at the probability level of  $P \leq 0.05$ , and the arithmetic mean of the control group for the parasite-infected females was  $(170.60 \pm 7.84)$  nmol/L, and the arithmetic mean of the parasite-infected females with pressure was  $(230.30 \pm 11.99)$  nanomol / liter). Among the possible reasons that led to the high level of MDA in females infected with the amoeba gingivalis parasite *E.gingivalis* in the two groups affected by diabetes and stress, compared to the rest of the groups, it may be considered

one of the main indicators of high lipid peroxidation, as the parasite is likely to have oxidative effects that oxidize acids Unsaturated amino acids phosphorylated in cell membranes, which leads to the production of a number of toxic compounds, including MDA (Sevda, 2015, Ayse)

Diabetes caused an increase in the level of malondialdehyde, and the reason for this was the occurrence of oxidative stress in the case of diabetes and an increase in the level of free radicals, causing damage to the cell membranes of the cells in them, as well as a lack of lipid peroxidation in the antioxidants, and thus an increase in the level of malon didehyde Or, the cause may be a disturbance in cellular metabolism and a high level of sugar due to hyperglycemia, and then hyperglycemia itself leads to oxidative stress and an increase in the level of reactive oxygen species that attack cells, causing an increase in the level of malondialdehyde, since MDA is a byproduct. lipid peroxide after exposure to active oxygen species and free radicals, or used as an indicator of damage to cell membranes (Al-Jubouri et al., 2018).

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