

Study The Effect of *Entamoeba Gingivalis* in Some variables of Blood and Serum in Males Patients with Periodontal Disease and healthy in Iraq

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Abstract

The current study was conducted on *Entamoeba gingivalis*, where only 75 male 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 1/12/2019 to 1/12/2020. Among 59 samples of males with gum disease and 16 samples from healthy males without gum disease, the total infection rate of the amoeba parasite for males was 74%, distributed among males with gum disease, where the infection rate was 81.35% 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 cortisol, as well as measuring the effectiveness of the α -amylase enzyme. An increase in the concentration of the hormone cortisol and a decrease in the effectiveness of α -amylase were observed in the group of males with chronic periodontitis compared with the rest of the groups, which include an affected group With parasite with damage to the support tissue, and a group infected with bleeding gums and infection with parasite, compared to the control group infected with parasite and normal, as shown To study an increase in the concentration of the hormone cortisol, as well as a decrease in the activity of the enzyme of α -amylase in the group with pressure and diabetes together compared with the rest of the groups, which include infection with the parasite with diabetes and infection with the parasite with pressure compared with the control group at a probability level of ($p \leq 0.005$).

Introduction

The oral cavity is one of the largest areas of the body that is in direct contact with the external environment. The air and dust inhaled through the nose and the organisms that it contains reach the oral cavity indirectly. Also, the food entering the mouth daily contains many germs. Therefore, it is a suitable habitat for many microorganisms because it has many characteristics that make it a suitable environment. For a number of bacteria, fungi, viruses, and oral protozoans (

Deng *et.al* 2017), the oral cavity contains two types of parasitic protozoa, namely *Entamoeba gingivalis* which belongs to under the Sarcodina division and *Trichomonas tenax* which belong to under the Sarcostigophara subdivision. These parasites are found in high rates in cases of gingivitis, tooth decay and permanent tissue diseases (Mark, 2018). *Entamoeba gingivalis* is endemic to the gingival pocket, between the teeth, the dental plaque, the calculus layer, and the Tonsillar crypts, where it is associated in large proportions with periodontal disease (Hussien *et.al* 2017). Also, studies conducted on gingival amoeba in Iran showed a comparison between the presence of amebiasis in healthy people and its presence in people with gum disease, which reached 0, 12%, respectively, using the direct examination method and the PCR method (Mahmoud *et.al* 2019). The parasite is present in the vegetative phase Trophozoite only if the cystic phase has not been observed for it, it belongs to the Entamoebidae family, which are small organisms with a diameter between 10-35 micrometres, possessing pseudopodia feet that help them to move, and the vegetative phase contains a circular nucleus that contains inside it a small endosome nuclear particle. Central location, the cytoplasm of the gingival amoeba is distinguished to the outer cytoplasm of the ectoplasm and the endoplasm, which contains few organelles and a number of glycogenic granules and trophic vacuoles containing red and white blood cells and epithelial cells, and the parasite is surrounded by an outer covering (Eloufir, 2014). The life cycle of the parasite is characterized by the presence of only the active phase, which is the infectious phase of the parasite, so the transmission of the parasite is through oral contact (kissing), or by using food tools, or by flying spray. The active phase feeds on the epithelial cells of the lining of the mouth, bacteria, food remnants, red blood cells, and white blood cells by pseudopodia. The life cycle of the parasite is transmitted in the vegetative phase only stage Trophozoite and the polycystic phase does not exist in the life cycle of this parasite. Therefore, reproduction takes place by simple longitudinal fission. Binary fission (Painker, 2013).

Oxidative stress results from damage to cells resulting from damage to DNA, proteins, and lipids that increase reactive oxygen species (ROS) or decrease levels of antioxidants, including glutathione reductase (GSH). Oxidative stress contributes to the development of many infections. As a result of infection with bacteria and parasites (Almokhtaret *et.al* 2019), several studies have also shown that oxidative stress is associated with the emergence of many diseases, including cancer, diabetes, cardiovascular disorders, atherosclerosis (Liguori *et.al* 2018).

The hormone cortisol is an important hormone for the body and is often secreted in response to stress and tension in the body (Anaet *et.al* 2019), the hormone cortisol is produced from the area of the adrenal cortex under the influence of the hormone Adreno Cortico Throphic Hormone (ACTH), which is secreted from the anterior lobe of the pituitary gland and stimulates the gland

to produce The hormone to the vascular system. The hormone cortisol is called the stress hormone because stress and its various types cause an increase in hormone levels in the blood, as it performs several functions, including increasing blood glucose and increasing protein breakdown within the muscle, as it is considered a strong anti-inflammatory. It is a hormone needed and used by every cell of the body, and it has an important and exaggerated role in influencing the metabolism of carbohydrates, proteins and fats (Casto *et.al*2016). Cortisol increases its level in saliva and serum with the severity of periodontal disease (Al-Hindawiet.,*al*(2018).The hormone cortisol is an important hormone for the body and is often secreted in response to stress and tension in the body (Ana *et.al*2018), the hormone cortisol is produced from the area of the adrenal cortex under the influence of the hormone Adreno Cortico Throphic Hormone (ACTH), which is secreted from the anterior lobe of the pituitary gland and stimulates the gland to produce The hormone to the vascular system. The hormone cortisol is called the stress hormone because stress and its various types cause an increase in hormone levels in the blood, as it performs several functions, including increasing blood glucose and increasing protein breakdown within the muscle, as it is considered a strong anti-inflammatory. It is a hormone needed and used by every cell of the body, and it has an important and exaggerated role in influencing the metabolism of carbohydrates, proteins and fats Cortisol increases its level in saliva and serum with the severity of periodontal disease(Hasan and Jabir 2017).

α -amylase is an enzyme secreted automatically by the human salivary glands in response to the stimulation of the neurotransmitter by the parasympathetic nerves and sympathetic nerves. This enzyme digests carbohydrates by converting carbohydrates, glycogen and sugars into small, digestible sugar molecules (glucose).), As glucose moves through the bloodstream to the rest of the body to supply it with energy, and this is done when eating foods rich in carbohydrates, as the salivary glands secrete the α - amylase enzyme, which in turn breaks down carbohydrate molecules into smaller parts until they reach the intestine. Then the process of digesting carbohydrates is completed through the α - amylase enzyme. It is secreted by the pancreas, as is the enzyme amylase in bacteria, yeasts and plants, and the high level of the enzyme indicates the occurrence of psychological stress and stress in the body (Gomina *et.,al* 2017), α - amylase is an enzyme secreted automatically from the salivary glands in the human body, which plays an important role in the initial digestion of starch and is likely to be a favorable environment for the growth of many microorganisms in the mouth and contributes to the formation of dental plaque. α amylase may act as a receptor for bacterial adhesion to the surface of the teeth. These multiple functions of amylase indicate that it plays an important role in tooth decay and the development of gum disease (Javaid *et.,al*2016).

Materials and Methods

Collect samples

75 samples were collected from males only in the form of swabs from the periodontal pocket from the people visiting the periodontal branch of the Faculty of Dentistry / University of Mosul in Iraq, during the period from 1/12/2019 to 1/12/2020, distributed between 59 samples from

males with gum disease and 16 samples from healthy males without gum disease. Samples were taken from the gum pocket where The parasites are present (Jian *et.,al*2008) by using sterile paper thread size 40 paper pointer and left for 30 seconds and then placed in tubes of 1.0 ml Pandruff containing 0.5 ml of suitable culture medium, then the samples were then transferred to the laboratory for the purpose of testing and investigating the presence of amoeba. The gingivalis and whether the result is negative or positive, and this is done by preparing direct wet smear by placing a drop of the samples taken and placed in the middle on a clean glass slide, then placing the cover of the slide and examining it with a magnification of 40 x in order to detect the gingival amoeba through the distinctive movement Her (Hersh, 1985).Collect blood samples (5) ml of blood was drawn from the veins of the arm for each individual after taking a swab from the gum pocket to check for the presence of the parasite. Then it was placed in tubes containing a semi-liquid substance Gel tube to extract the serum and left it diagonally at room temperature for 15 minutes, then it was placed and rotated with a device Centrifugation at (3000) rpm for 15 minutes, sera were collected and distributed in plastic tubes and stored for the purpose of use in the current study.Biochemical tests used Hormone Cortisol TestIn this study, a kit was used to test the human cortisol hormone, Elisa Kit, from the Bioassay technology laboratory, α -amylase testIn this study, a Human α -amylase Elisa Kit was used in this study to test the human α -amylase Elisa Kit from the Bioassay technology laboratory.

Statistical Analysis usingStatistical analysis was performed using Chi-square andUse the Duncan multi-range test below a likelihood level ($P < 0.05$)

4. Results & Discussion

Figure (3) shows that 75 samples were collected from the gingival pocket, as shown in picture (1.) 59 samples were collected from people with gum disease, 48 samples were infected with a percentage of 81.35%, and 16 samples were collected from healthy people without gum disease. 8 samples infected with 50% percent.



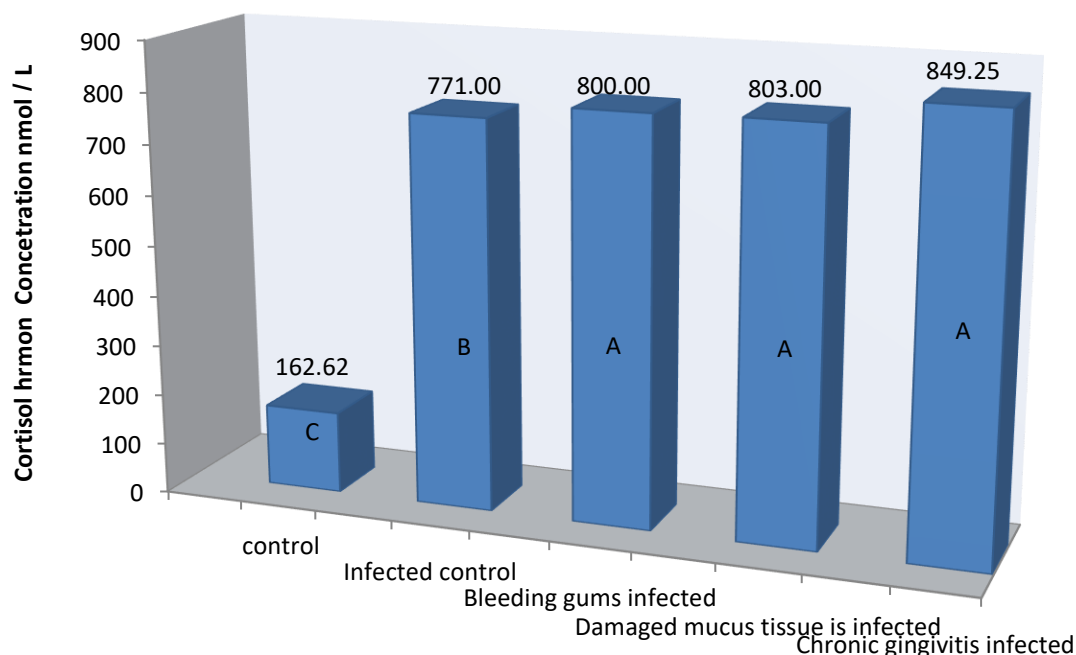
Picture(1) *Entamoeba gingivalis* in a swab taken from the gum pocket (40X)

Sex/ males	Examination N,	<i>E.gingivalis</i>	
		Infected N	Infected %
Periodontal Disease	59	48	%81.35
Healthy	16	8	%50
Total	75	56	%74
Chi-square test	Df-1 , p-value=0.30		

Figure (3) The Percentage Of Total Infection With *Entamoeba gingivalis* in Males

.In a study conducted in Baghdad on the gingival amoeba in Iraq, the percentage of infection in gingival amoeba in males with periodontal disease was 46%, where 50 samples were collected, including 23 eyes infected with the parasite (Mohammed *et.,al* 2015). In a study conducted in southern Iran, 189 samples were collected. Of males, the total infection rate with the parasite among males was 60%, and the number of males infected with the gum parasite was 30 samples (15.9%) (Sharifi et al., 2020). In another study, the percentage of males infected with gum disease was 12.5%, while the infection rate was 12.5%. (Mahmoud et al.2019).The reason for the high incidence of parasite infection in males in the current study is due to wrong social habits and unhealthy food consumption (Hamad *et.,al*2012). There are many factors that lead to differences in parasite infection rates, including age, gender, social and economic characteristics, and poor oral health conditions (Trim *et.,al*2011). Moreover, many other factors appear to be associated with parasite infestation including gum damage, poor oral health, tooth loss, tooth decay, low educational level, cigarette smoking, medication and food (Luszczak *et.,al* 2016).

From the figure (2), we note that there is a significant increase in the concentration of the hormone cortisol at the probability level ($P \leq 0.05$)) for the group of males infected with the gingival amoeba parasite with chronic gingivitis with a mean of (849.25 ± 6.96) nmol / liter compared with the rest of the groups, while the concentration of the cortisol hormone in The group of males infected with the parasite with bleeding gums (800.00 ± 5.09) nmol / l and the group of males infected with supportive tissue damage and infected with the parasite (803.00 ± 3.29) nmol / liter, while there were no significant differences showing these two groups compared with the control group for healthy males with a mean (162.62 ± 1.59) nmol / liter and the control group for males infected with gingival amoeba with a mean (771.00 ± 10.94) nmol / liter



Values are expressed as the arithmetic mean (\pm)SD

Columns associated with different letters indicate a significant difference at a probability level ($P \leq 0.05$)

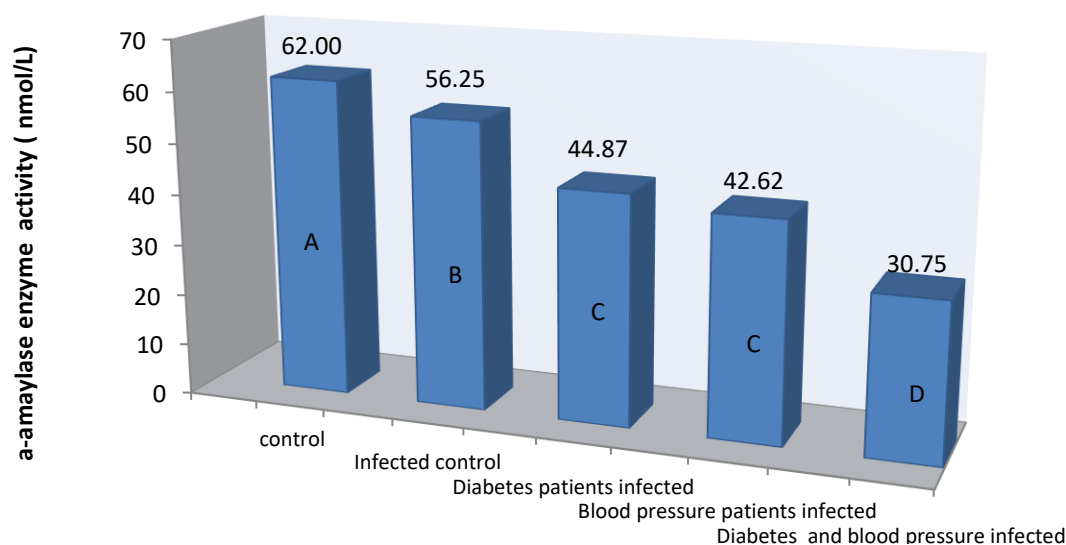
Figure (2) The concentration of the hormone cortisol (nmol / L) in the group of males infected *E.gingivalis* and those with chronic gum disease compared to the control group

The cause of the high cortisol hormone in people infected with the gingival amoeba parasite *Entamoeba gingivalis* in males and those with chronic gingivitis, it is known that parasite resistance depends on the body's immunity and its decrease, which may occur due to stress and fatigue, which in turn causes an increase in the level of steroid hormones, which are secreted by the adrenal gland as a result of stress and fear, which in turn affects the oral cavity in several ways, including the lack of saliva flow by its effect on the salivary gland (Caruso *et al*, 2018).

Recent studies have indicated a relationship between the presence of parasites and the high level of stress hormones in infected people, in addition to the occurrence of behavioral changes in patients, and studies have indicated a parasitic role in increasing the level of dopamine in the brain, which leads to an increase in the secretion of the stress hormone cortisol in addition to it is possible. The increase in cortisol is an adaptive way for the parasite to weaken the immune system and thus increase its chances of survival in the body (Garthet, *al* 2008). It is possible that the increase in the hormone cortisol has a relationship with the presence and increase in the presence of oral microorganisms (Nogueira, 2005). The effect of the presence of oral organisms correlated with chronic periodontal disease. The effect of physiological and biochemical changes

occurred, including an increase in the concentration of the hormone cortisol (stress hormone) with the presence of oral organisms and induce genetic changes to them. There are studies that have shown that the effect of the hormone cortisol on the oral microbiome, where the cause of metabolic changes, and there are races of oral microbes that respond to the stress hormone, as it works on the occurrence of genetic mutations of these microbes causing periodontal disease, steroids in the oral cavity include cortisol, which works to reduce immunity. It affects the functions of white blood cells. All these factors weaken the defense against infection by microorganisms in the gums (Ana *et al*, 2019).

Figure (3) shows a decrease in the effectiveness of α -amylase at the probability level ($P \leq 0.05$) for the group of males infected with chronic periodontitis associated with the presence of the *Entamoeba gingivalis* parasite with a mean of (30.75 ± 1.38) IU / liter compared with the rest of the groups, where the arithmetic average of one group was Control for healthy males infected with the parasite $(1.85 \pm 62.00, 56.25 \pm 1.03)$ IU / liter, respectively, while the arithmetic mean of the group of males infected with the gingival amoeba parasite with bleeding gums and receiving the supportive tissue was a mean of $(44.87 \pm 0.99, 42.62 \pm 3.46)$ IU. /Liter.



Values are expressed as the arithmetic mean (\pm)SD

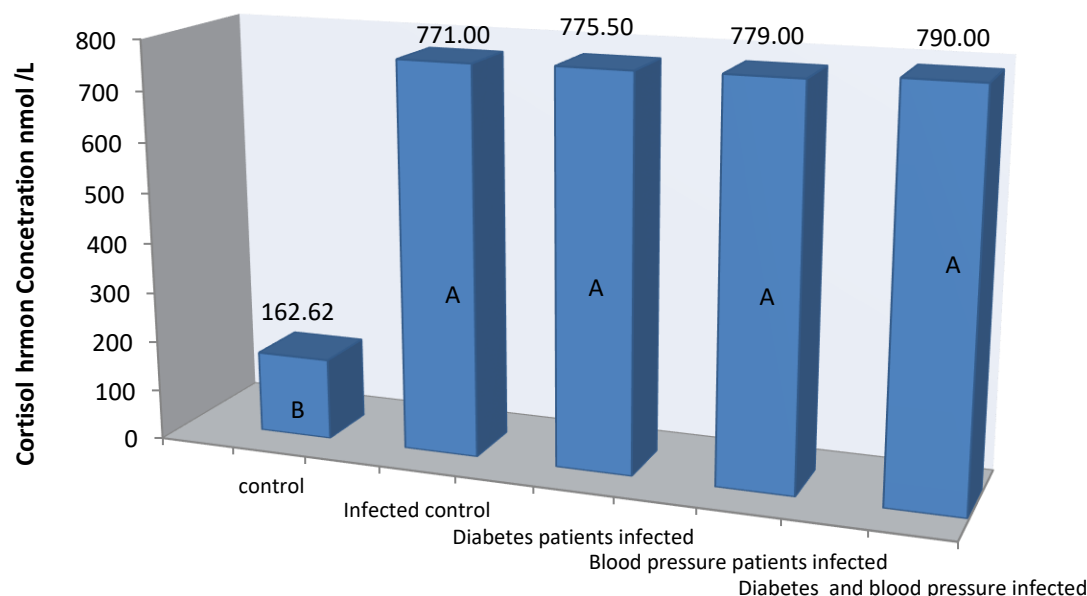
Columns associated with different letters indicate a significant difference at a probability level ($P \leq 0.05$)

Figure (3) α -amylase enzyme activity (IU / L) in group males with chronic gum disease compared to control group

Male infestation with *Entamoeba ghngivalis* with chronic periodontitis results in 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 secreted by the salivary gland and works on digesting

carbohydrates by converting carbohydrates, glycogen and sugars into small digestible molecules. (Amerongen,2007) .As this enzyme is transmitted through the bloodstream, and in fact, the studies that have been conducted in this area are few, and the interpretation of the results will depend on mere opinions or suggestions that require a lot of studies and research in order to prove or deny their validity. This enzyme, where the parasite infection associated with gum disease leads to disturbances in the secretion of the salivary glands,where the parasite produces chemicals and these substances remain inside th tissues or are excreted in the vital fluids of the body (Arhakis, 2013).

Figure (4) shows a significant increase in the level of the hormone cortisol in all treatments for males compared with the healthy control group at a probability level ($P \leq 0.05$)) with arithmetic averages that include the control group for the affected males (771.00 ± 10.94) nmol / l, the group of males with diabetes with the presence of the parasite (775.50 ± 7.42) nanomol / liter, while the mean of the group of males infected with the parasite with pressure was (779.00 ± 10.35) nmol / liter, and the mean of the group of males infected with the parasite with diabetes and pressure was (790.00 ± 47.18) nmol / liter compared with the control group. The healthy male was (162.62 ± 1.59) nmol / L



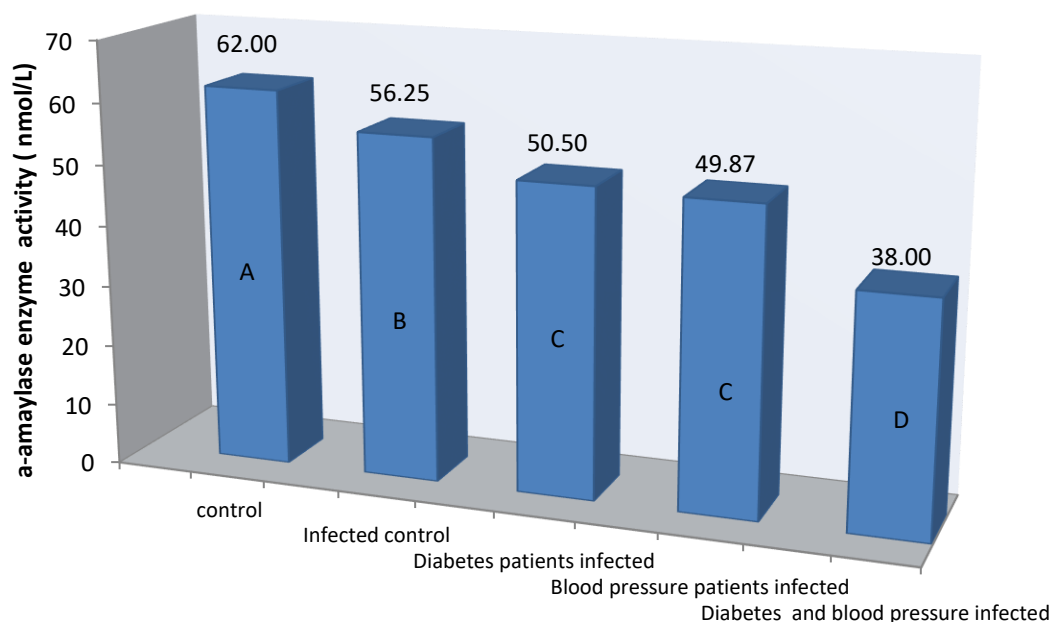
Values are expressed as the arithmetic mean (\pm)SD

Columns associated with different letters indicate a significant difference at a probability level ($P \leq 0.05$)

Figure (4) The concentration of the hormone cortisol (nmol / L) in the group of males with *E.gingivalis* and those with diabetes and hypertension compared to the control group

The infection of the male and female group with the parasite *Entamoeba gingivalis*, along with their diabetes and stress, led to an increase in the level of the hormone cortisol in all treatments compared to the control group, perhaps due to the fact that the hormone cortisol performs several mechanisms that raise the concentration of glucose in the blood, which is to activate the manufacturing process Gluconeogenesis is from non-carbohydrate sources, and cortisol stimulates the breakdown of fats in the adipose tissue, which leads to the secretion of fatty acids and their level in the blood, and this leads to the occurrence of resistance to the hormone insulin and reduce the sensitivity of tissues to it, and this in turn is a key factor for the emergence of diabetes later (Weberet.,al2002).Cortisol increases its level in saliva and serum with the severity of periodontal disease (Al-Hindawet.,al 2018)).Where studies have indicated the role of parasites in increasing the secretion of the stress hormone (cortisol) as an adaptive method by the parasite to weaken the immune system and thus increase its chances of survival in the body(Flegr,2007).

Figure (5) shows the presence of a significant decrease in the activity of the α -amylase enzyme in the group of males infected with the parasite *E.gingivalis* with diabetes and pressure at a probability level ($P \leq 0.05$) with a mean (38.00 ± 2.44) IU / liter compared with the rest of the groups, where we notice no significant differences in The two groups of males infected with the parasite and infected with blood pressure with a mean of (49.87 ± 1.642) IU / liter and the mean of the group of males infected with the gingival amoeba parasite with diabetes was (50.50 ± 1.51) IU / liter and the mean of the control group for males infected with the parasite was (56.25 ± 1.03) IU / liter (one wheel / liter) in which the arithmetic mean of a healthy male group was (62.00 ± 1.85) IU / liter.



Values are expressed as the arithmetic mean (\pm) SD

Columns associated with different letters indicate a significant difference at a probability level ($P \leq 0.05$)

Figure (5)a-amylase enzyme activity (IU / L) in the group of males with E,gingivalis and those with diabetes and hypertension compared to the control group

The amylase enzyme is considered one of the most important proteins in saliva, and the enzyme belongs to a class of hydrolysis enzymes. It works on the analysis of starch and glycogen by randomly attacking the glycosidic family (1-4) of the polysaccharide chain to produce a short chain of low-unit sugars El-Sayed *et.,al*2016). This enzyme has a major role in mucosal immunity in the apical lumen by preventing the attachment and growth of bacteria and parasites (Chitra 2008). The decrease in salivary a--mylase enzyme in the group of male and hypertensive and diabetic patients may be an indicator of an important diagnostic potential for many diseases (Della, 2017). Several studies have shown a decrease in alpha-enzyme levels in people with insulin-dependent diabetes, as parasite infection, diabetes, and stress may lead to oxidative stress and free radical buildup in the salivary gland, which reduced the activity of the alpha-salivary enzyme (Sewalt *et.,al*2018).

Conclusion

We conclude from the study that the parasite infection with chronic gum disease, blood pressure and diabetes for males caused the increase in the concentration of the hormone cortisol and the lowering of the activity of the alpha-amylase enzyme compared to the control groups.

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