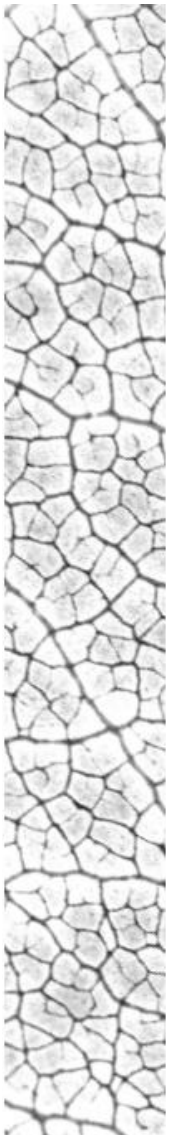

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Study the effect of some microbiota in oral cavity on some parameters of blood

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Abstract

The current study was conducted in the period from March 2018 to June 2018 to investigate the infection of the protozoan parasite *Entamoeba gingivalis* and its relationship to bacteria in the mouth and its effect on Total white blood cells count, Lymphocytes and Granular leukocytes.The number of examined samples reached 50 samples of gum smears for the age group of (18-28) years collected from students of the University of Kirkuk and for both sexes. The samples were examined by direct wet mount, and the swabs from parasite-infected samples used for culture on Blood agar in the Microbiology Laboratory / College of Nursing. Blood tests were performed in external laboratories in Kirkuk governorate. The blood tests included total white blood cell count, Lymphocytes and Granular leukocytes. The results of the current study of blood samples taken showed that the total number of white blood cells increased significantly with a level ($p < 0.05$) among those infected with the parasite compared with the control group as it reached (7.58 and 5.94), respectively. The results of bacterial culture showed the presence of *Streptococcus mutans*, which is one of the most important factors causing tooth decay associated with oral parasites, as its presence was 41% in the examined samples.

Keywords: *Entamoeba gingivalis*, *Streptococcus mutans*, Blood parameters, WBC count

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INTRODUCTION

Tooth decay, periodontitis and gingivitis are one of the most common problems in the world regardless of age, gender (Arweiler et al., 2016). The oral cavity is a constantly changing environment, where its susceptibility to infection with many organisms such as parasites, bacteria and fungi and is very necessary to know the main diseases that affect the oral cavity and the methods for its diagnosis and prevention (Philip et al., 2018). Immediately after birth, the sterile oral cavity is affected by the external environment, and after several days the characteristics of Microbiota and the first bacteria to be isolated are *Streptococcus mutans* (Rosenblatt et al., 2015), as it is considered the main cause of this disease and is often called “Cariogenic bacteria in addition to many Microorganisms”. Others also have several factors such as genetic or environmental factors such as smoking, diabetes, and immunological imbalance in Interleukin-1 (Ismail et al., 2017). Also, unbalanced food has a major impact on Homeostasis, which leads to satisfactory changes. Microbiota of the oral cavity is unstable, as it depends on the physiological and anatomical conditions, as it differs in the holes in the salivary glands and on the surface of the teeth.

Likewise in Sulcus gingivalis and on the tongue as well as tonsils and oral mucosa, as the growth of microorganisms depends on temperature and pH, reductive oxidative stress, the presence of water and nutrients, saliva flow and the presence of Antimicrobial components all of these factors control the oral Ecosystem and help to maintain a balance between the groups of microorganisms (Kõll et al., 2008). Among the types of bacteria that cause gingivitis and teeth are: *Streptococcus mutans, Propionibacterium acnes, Lactobacillus casei, Peptostreptococcus micros*. The symptoms of gingivitis with the appearance of bleeding from the gingival incision, especially when brushing the teeth, and the gums appear in a red, swollen color. In the end, they may cause tooth loss. The most important parasites that cause gingivitis are: *Entameoba gingivalis* and *Tirchomonas tenax* (Ghabanchi et al., 2010).

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Therefore, the aim of this study is to detect the presence of oral parasites, especially *Entamoeba gingivalis* and the effect of infection on the gum and teeth tissue and some blood parameters such as the total white blood cell count and the presence of bacteria that cause gingivitis and teeth associated with the infection of the oral parasites.

MATERIALS AND METHODS

Collecting Samples

During the period from March 2018 to June 2018, 50 swab samples from the gums of both sexes were examined for different age groups from (18-28) years collected from students of the University of Kirkuk and the sample was taken from people who suffer from tooth decay or gingivitis or who have by taking their teeth off. The culture was prepared by cultivating swabs taken to reveal the bacteria present in the mouth. Likewise, blood was drawn from the injured and uninfected to see the effect of *Entamoeba gingivalis* on some blood parameters.

Examination Methods

Macroscopic examination

This is done by observing the color and texture of the gums and Tar Tar layer (greenish layer) in the gum tissue, as well as the white layer covering the surface of the teeth (Carranza, 2002).

Microscopy examination

The direct wet mount done by taken samples from the mouth either from the gums directly and taken from the part that is greenish color when the gums are inflamed and if they are intact from the nearby part of the tooth decay using Swap then fix the sample on the glass slide by adding a drop of the physiological saline solution Normal saline or iodine stain is mixed with it and then scan by light microscopy under the forces (100 X, 400X), observing the movement of parasites and examining samples during a maximum period of 30 minutes from the collection period (Bafghi, 2009). The specimen soaked with saliva taken from the mouth of a patient with oral disease is placed on a clean and sterile glass slide and mixed well with saliva, then we put the slide cover on it and examine it under a microscope with the power of magnification of the forces (100X, 400X) to detect the parasite *E. gingivalis* (Luszczak, 2016).

Checking blood samples

Complete blood picture: The blood was examined by an Automation automatic analysis of blood (Swelab, Sweden), where the blood sample is placed by means of a strip that exits the apparatus and the results appear on the screen

Procedure:

1- The device has been activated by delivering the electrical current to the device, pressing the on button, and the appearance of the screen indicating the start of the calibration of the device.

2- The tube containing blood was taken and placed at the beginning of the portal mouth of the device and it is used to enter the tube to take a drop of blood as it consists from the bottom a square shape with a white color inside which has a ring circular to place the tube inside and not move it, i.e., it forms a seat for the tube, and from the top there is a long column. It is inserted inside the tube to take a drop of blood for the purpose of examination.

3- After that, the tube is removed from the place designated for it and waiting until it starts reading. Then reading was done on the device screen and printed by the attached printer.

4- Culture of smears

After the samples taken from the oral cavity were examined using a direct wet mount, they were grown on the blood agar medium.

Preparation of the culture medium

As 28 g of blood agar powder was taken in 1 liter of distilled water then heated the mixture until all the contents melted and then the culture medium in Autoclave was sterilized at 121 ℃ for 15 minutes and then left to cool a little until its temperature reached 45-50℃. But before it hardens, 5% blood was added at room temperature, mixing the ingredients together and pouring it into the plates until it hardens. Then we inoculated the culture medium with swab taken from the oral cavity of the infected people. Then, elevated convex and opaque colonies were found causing α-hemolysis (Levinson and Jawetz.1994).

Selective media was used in this study is called Mitis Salivarius agar (MS), as it is considered one of the most important culture media to isolate *Streptococcus mutans* due to the distinctive colonies shape on this medium with high efficiently (Kimmel and Tinanoff, 1991). After the inoculation by swab taken from the oral cavity and incubated 37℃ for 24-48 hours and raised, blue convex colonies with rough margins and frosted glass appearance were observed (Jabbarifar et al., 2015).

Detection of bacterial colonies

The developing bacteria were detected using the Gram staining method and examined with an OPTIKA type microscope at a magnification force of 100 X. The result was cocci Gram + arranged in chains of the *Streptococcus mutans*, which is a compulsory pathogen living in the oral cavity (Cornejo, 2012).

Statistical analysis

Statistical analysis was performed using (t-test) under a significant level (0.05) (Al-Rawi, 1989).

RESULTS AND DISCUSSION

The Results of Blood Tests

Effect of *Entamoeba gingivalis* on total white blood count

Table 1. Total WBCs count of infected people with Entamoeba gingivalis

Groups	Number of samples	Total white blood cells 10^3 X cells / mm^3 Mean± SD	*P* value
Infected with the parasite	10	7.58 ± 1.633	a
Control	10	5.94 ± 0.585	b

Table 2. Lymphocyte count of infected people with Entamoeba gingivalis

Groups	Number of samples	Total white blood cells 10^3 X cells / mm^3 Mean± SD	*P* value
Infected with the parasite	10	2.5 ± 0.458	a
Control	10	1.9 ± 0.36	a

Table 3. The number of granulated blood cells of infected people with Entamoeba gingivalis

Groups	Number of samples	Total white blood cells 10^3 X cells / mm^3 Mean± SD	*P* value
Infected with the parasite	10	4.525 ± 0.575	a
Control	10	3.76 ± 0.488	a

The results of the current study of blood samples taken from people with the *E. gingivalis* parasite showed an increase in Total WBCs in patients compared to healthy subjects as shown in **Table 1**.

Similar English letters are an indication of non significant differences at (P<0.05) level comparison between groups.

These results were compatible with Monteiro et al. (2009) and Buhlin et al. (2003) and Sahingur et al. (2003), as they found an increase in the total white blood cell count in people with gingivitis and teeth. As periodontal diseases that affect the bones and tissues supporting them, they are called periodontitis, and they are characterized by the formation of distances between the teeth and gums, and this develops into chronic diseases, which may lead to tooth loss, and WBC rises due to the nature of chronic inflammation (Ghabanchi, 2010), since the parasite *E. gingivalis* is linked and exists In periodontal disease, it increases rapidly with increased food availability in the mouth. More than 95% of people with unhygienic mouth develop this parasite (Geraled and Larry, 2005). The results also showed an increase in lymphocytes among patients with the *E. gingivalis* parasite compared to the healthy ones, with no significant differences at the level of probability (P<0. 05) as shown in **Table 2**.

Similar English letters are an indication of non significant differences at (P<0.05) level comparison between groups While **Table 3**, showing the granular leukocytes population, the results showed that there were no significant differences between those with the E. gingivalis parasite and the non-infected at a probability level (P<0. 05).

Leukocytes are the major cellular elements in infections and immune reactions in living organisms. The normal Leukocytes number ranges between 4000-11000 cell / mm^3 and a change in Leukocytes numbers is the result of an imbalance between their bone marrow formation and the disinfection due to Phagocytosis (Nicu et al., 2009). Also, the factors that affect its composition are many and different, as is the case with Tumors. Or for benign reasons due to a normal bone marrow reaction towards Inflammatory Infections or Infection or other causes such as taking some medications such as Corticosteroids as they are associated with many risk factors such as weight gain, alcohol and smoking use (Rasheed, 2012) are all factors that increase the WBC preparation.

WBC present in the circulatory system are Granulocytes, Lymphocytes and Monocytes and are considered the first line of defense against pathogens that enter the body such as bacteria, viruses, parasites and fungi.

Periodontal inflammation leads to Biofilm formation due to inflammatory bacteria such as *Streptococcus mutans*, and persistent inflammation leads to the production of pre-inflammatory cytokines and that Antibody specialized for Oral cavity is produced in peripheral blood, and the acute immune response is activated by C-Reactive Protein (CRP) and Complement, Fibrinogen and these proteins exacerbate the local inflammatory response due to toxic metabolites Toxic metabolites produced by bacteria such as Organic acids and Hydrogen sulphate and other metabolites affect gum tissue and stimulate the immune response leads to an increase in WBC that reflects inflammatory activity (Dave, 2004).

Likewise, Lymphocytes are endemic in three regions (lymph nodes, spleen, mucosa-associated lymphoid tissue that lines the respiratory tract and digestive tract in humans. In these areas, microbial antigens will be caught and presented. To T and B Lymphocytes, and that antigen is associated with B-cell and produces large quantities of Immunoglobulin to aid the Opsonization process, thus increasing the number of Lymphocytes in bacterial and parasitic infections (Kini, 2009; Admas, 2016).Therefore, this study showed that people with gum and dental diseases caused by bacteria are accompanied by *E gingivalis* have an increase in Total WBCs, Lymphocytes, and Granular Leukocytes compared to healthy people due to the nature of chronic inflammation and this is consistent with (Sambashivaiah et al., 2010) and (Al-Rasheed, 2012).

The presence of *E. gingivalis* in the oral cavity is a sign of decreased interest in oral hygiene, dental care and gum disease, and the method of spread by kissing, volatile spray, or joint use of eating and drinking tools. Many studies indicate that poor oral hygiene, calcifications, and loss of connective fiber gingival tissue are preferred factors for the proliferation of *E. gingivalis* and that the poor state of the mouth increases the

prevalence of oral parasites (Onyido et al., 2011) and (Jawad, 2011)

The Results of the Cultural Study

The current study showed the presence of *Streptococcus mutans* in bacteria taken from the oral cavity, if the bacterial presence was 41% after it was cultivated on the blood agar media and Mitis Salivarius with Bacitracin, and the reason for this is due to the human oral cavity is the natural home of *Streptococcus mutans,* especially in the case of tooth decay, where the bacteria settle down, forming Biofilms that form on the teeth (Cornejo, 2012).

In addition, the oral environment suffers from large and rapid fluctuations in the PH, the presence of nutrients, the oxygen potential in addition to temperature, all of which contribute to the growth of bacteria and tooth decay (Baron, 1996). As the dental plaque consists of Microorganisms that produce complex matrix which is Extracellular products and Salivary components and the bacteria that are isolated are Gram positive and Facultative anaerobic mainly due to the genus *Streptococcus* SPP (Jakubovics, 2015). The virulence of bacteria depends on the formation of glucan through the ability of bacteria to fermentation of carbohydrates and glycan, which contributes to the adhesion of bacteria on the surfaces of the teeth. (Lemos et al., 2005) In addition to other metabolic processes of bacteria causing tooth decay, an imbalance occurs between bacteria naturally present in the mouth which results in many diseases in oral cavity, so these Microorganisms may appear under certain conditions only such as Reduced body immunity, therefore it is referred to as facultative or opportunistic pathogens (Papaioannou et al., 2009). *Streptococcus mutans*, which are among the most important bacterial species, are isolated in addition to the presence of Protozoa such as *Entamoeba gingivalis* which considered the most important types of Microorganisms in sulcus gingivalis (Zaura, 2014) and this is consistent with other studies T in Baghdad, such as (Flayyih, 2016), as has been isolated from *Streptococcus mutans* patients suffering from tooth decay, as well as with (Patil, 2010) as toothpaste effect was observed on the type of bacteria isolated from *Streptococcus mutans* patients suffering from tooth decay.

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