Detection of *Entamoeba gingivalis* by PCR Technology and its Association with Oral Diseases

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**Abstract**

This study included collecting 100 samples in the form of gum swabs, gum pockets, saliva, and extracted teeth, and PCR technology was used to determine the incidence of *Entamoeba gingivalis* in northern Salah El-Din governorate for healthy and people with oral diseases and those suffering from diabetes of the first and second types. The overall injury rate was 52%. It was found that the incidence of people with oral diseases was 60%, and the rate was 33.3% in healthy subjects. And that the rate of infection in persons with gingivitis is the highest rate of infection among oral diseases, as the rate reached 80% and the lowest incidence of 30% for those with tooth decay disease. The incidence of oral diseases and those suffering from diabetes is 61%, and the incidence of persons with oral diseases and those suffering from type 1 diabetes is the highest incidence of persons with oral diseases and those suffering from diabetes type II, as the rate reached 66.6 , 55.5%, respectively. And the incidence of smokers and those with oral diseases is higher than that of persons with oral diseases and non-smokers, as the rate was 78.5 and 47.6%, respectively.

1. **INTRODUCTION**

The human body is home to trillions of microbes, and the oral cavity is one of the largest sources of microbes, there are about 700 to 1000 types of microbes that colonize the human mouth, the occurrence and development of oral diseases such as tooth decay, gum disease and oral cancer are closely related to microorganisms in the mouth(1, 2 ,3), *E.gingivalis* is a widely distributed anaerobic protozoan amoebic organism widely distributed in the human oral cavity (4), the *E. gingivalis* parasite is usually found in the oral cavity and is frequently observed in the gingival sinuses of humans and pets(5), It was also isolated from the womb ,and from the cervix ,and from the tonsils it was also found in a pulmonary abscess (6, 7, 8, 9), this parasite has a simple life cycle characterized by the presence of only one phase,
trophozoite, which can be diagnosed according to its amoebic form and misses the cystic phase (10), the *E. gingivalis* parasite is mainly transmitted by kissing or indirectly through food contaminated with trophozoites, toothpicks, or other utensils and 95% of individuals infected with *E. gingivalis* have an unhealthy mouth (11), reproduction is done by means of binary fission (12), this parasite contributes to the initiation, and development of gingivitis and periodontitis, and these stomatitis cases in turn facilitate the reproduction of the gingival mutant, this endless loop may explain the presence of *E. gingivalis* in saliva and dental plaque for patients with gingivitis and periodontitis (13, 14), in addition, it causes itching, bad breath, severe headache, inflammation of the palate and a breakdown in gum tissue (15), periodontal disease is spread all over the world and its prevalence appears to increase with age, periodontal disease includes gingivitis and periodontitis, gingivitis is associated with an inflammatory response in the gums only, while periodontitis includes the destruction of the supporting tissues of the teeth (16), studies in animals and humans agree that both forms of diabetes increase inflammatory events in gum tissue (17), a clear association between prevalence of periodontal disease and smoking has been reported (18), tobacco smoking increases the development of gum disease and has a significant damaging effect on gum tissue (19).

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTION

100 samples (50 males and 50 females) were collected for people visiting Shirquat Hospital, Al-Baiji Hospital, and some outpatient clinics, with the help of specialist doctors, these samples were divided into two parts, the first section (30) samples for healthy people and the second section (70) samples for the sick persons, who were distributed in groups according to the disease condition (such as bleeding gums, wet sinuses, dry sinuses, periodontitis, gingivitis, tooth decay, abscesses, and periodontics). Samples were placed in numbered tubes containing sterile physiological saline solution, samples were kept at -20°C for the purpose of performing a PCR examination, this technique was performed in the central laboratory at Tikrit University.

2.2. MOLECULAR STUDY

DNA was extracted from the samples under study using the Quick-DNATM Minidprep Kit equipped by ZYMO RESEARCH (USA) according to the manufacturer's instructions, the polymerase chain reaction was conducted in laboratory conditions, sterilized by using primers supplied by (Bioneer- Koria) for the diagnostic gene (Bioneer-Koria) for the 18S-SSU rDNA diagnostic genes with a molecular size of 250bp and its gene sequence (F_GAATAGGCGCGCATTTGAACAGG, R_
TCCCCACTAGTAAGGTACTACTC) and taking this gene from (20) which was designed for this study, designed for this study, while the interaction program for each gene was approved as shown in TABLE 1.

**TABLE 1. PCR device program**

| Steps | The operation |
|-------|---------------|
| 1-    | One cycle 5-minute cycle at 95°C for initial template DNA mutilation |
|       | 40 cycles included : |
| 2-    | A  30 seconds at 95°C to scan template DNA |
|       | B  30 seconds at 55°C to attach the primers to the mold |

3. **RESULTS AND DISCUSSION**

Amplification of 18S-SSUrDNA as shown Fig.1. revealed that the overall *E. gingivali* prevalence was 52%, it was distributed between 60% and 33.3% in patients and healthy controls, respectively, as shown Fig. 1.
FIGURE 1. Electrophoresis of DNA polymerase chain reaction results using a 16srRNA primer on mucinous gel medium at a concentration of 1.5% M: volumetric index 100 bp. Abbreviations_ Per: periodontitis_D.car : Dental caries_ Non.p: Healthy people_ Gin: Gingivitis_Poc.w: Periodontal pocket wet.

FIGURE 2. The rate of infection with E.gingivalis parasite in healthy people and those with oral diseases for both sexes by the PCR technique. $X^2 = 5.983$ P-Value = 0.014**

3.1. RELATIONSHIP OF THE E. GINGIVALIS WITH ORAL DISEASES

The rate of infection in males with oral diseases was higher than that of females with the same diseases, as the percentage was 65.7 and 54.2%, respectively, and the rate of infection in persons with gingivitis was the highest rate of infection among oral diseases, as the rate reached 80%, and the lowest incidence is 30% of people with tooth decay as shown TABLE 2. The gingivitis disease provides a suitable and nutrient-rich environment for microorganisms where there is a positive relationship between gingivitis and the increase in the number of pathogenic microorganisms (21), when an abnormality occurs in the epithelial barrier of the gums, the E. gingivalis parasite invades the gingival tissue, where it moves and feeds on the host cells (22).

TABLE 2. The prevalence of E. gingivalis in different oral illness according to sex. $X^2 = 6.947$ P-Value = 0.036*

| oral illness | Males | Female | Total Number of cases |
|--------------|-------|--------|-----------------------|
|               | Positive | No  | Positive | No  | Positive | No  |

| 60 | 65.7 | 54.2 | 33.3 | 40 | 26.6 |
3.2. THE RELATIONSHIP OF E. GINGIVALIS TO ORAL DISEASE IN SUBJECTS WITH TYPE 1 AND TYPE 2 DIABETES

The rate of infection in persons with oral diseases and those suffering from diabetes was 61%, the rate of infection in persons with oral diseases and those suffering from diabetes type 1 is the highest incidence of persons with oral diseases and those suffering from diabetes type II as it reached The ratio is 66.6%, and 55.5% respectively as shown Fig. 3. Hyperglycemia enhances expression of pathogen receptors, which enhances host response to microbes (23), in addition, the proportion of patients with periodontitis is more than double that of people with T1DM compared to individuals without diabetes (24), the inability of the body to absorb blood sugar negatively affects the health of the gums, which makes them vulnerable to many infections and provides opportunities for many types of bacteria and parasites to attack the gums as well as slow healing of wounds and thus increase gum disease (25), patients with type 1 diabetes suffer from gum disease and tooth loss five times more than healthy people, and he attributed the reason for this to the fact that diabetes affects the regulation of intercellular materials, disruption of oxygen diffusion and the excretion of metabolites, as well as damage to the migration of white blood cells and that all these factors increase periodontal disease in diabetic patients (26). In addition, diabetes
weakens the immune system's defense mechanisms, making diabetic patients not only more susceptible to infection, but also the manifestations of that infection are more severe compared to patients without diabetes (27)

![Figure 3](image)

**FIGURE 3.** The incidence of *E. gingivalis* is according to diabetes in persons with oral diseases, for both sexes. $X^2 = 6.616$ P-Value $= 0.033^*$

### 3.3. THE RELATIONSHIP OF *E. GINGIVALIS* TO ORAL DISEASE IN SMOKERS

The incidence of smokers and those with oral diseases is the highest rate of non-smokers, as the rate was 78.5% and 47.6%, respectively, and the highest rate of infection among smokers and people with oral diseases was 68.7% as shown Fig. 4. The reason for the high infection rate in smokers may be attributed to the negative effect of cigarettes and their components of tobacco products, as tobacco affects the immune system because it reduces immunoglobulins IgG and IgM by the plasma cells as well as the reduction of the phagocytic activity and the chemical response of the neutrophil cells in the gums, which leads to a weakening of immunity. Smokers are against the microorganisms that attack the gums, making them more susceptible to infection with oral parasites(28), Smoking creates a favorable environment for the growth of some types of pathogens and increases the destructive effect of the support tissue by weakening the natural defenses of the host and contributes to many oral diseases and disorders such as oral and pharyngeal cancer and dental caries (29,30), smokers tend to have lower levels of oral hygiene and more plaque deposition (tartar) than non-smokers and may cause narrowing of the blood vessels in the vessels supporting the tooth, and in this way may lead to colonization of anaerobic bacteria such as *P.*
gingivitis, vasoconstriction caused by work adrenaline stimulated by nicotine and adrenaline rushes (31). However, the total microbes in smokers may transfer to a more pathogenic level (32).

![Figure 4](image)

**FIGURE 4.** The incidence of *E. gingivalis* is according to smoking in persons with oral diseases, for both sexes. X² = 6.706  P-Value = 0.010**

4. CONCLUSIONS

Our study revealed that there is a relationship between infection with the parasite *E. gingivalis* and oral diseases, and it showed that people with oral diseases and those suffering from type 1 ,and 2 diabetes have an increased incidence of infection, and it also confirmed the high incidence of infection in oral diseases and those who practice smoking habit.

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