

Measuring levels of Alpha-amylase ,TNF alpha and IL4 in diabetic patients with Periodontitis

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Abstract: The current study included collecting samples for the period from February 10, 2023 to June 5, 2023, where 50 samples were collected, 30 samples for patients infected with the *E. gingivalis* parasite who also had type 2 diabetes, and 20 samples for healthy people selected as a control group from Samarra General Hospital and dental clinics. The levels of tumor necrosis factor alpha, interleukin 4, and alpha amylase were measured in the blood serum using the radioactive enzyme assay technique. The final results of the present research have shown an essential increase in tumor necrosis factor alpha 273.0 ± 24.04 pg/ml in the patient group when compared with control group 58.5 ± 13.18 pg/ml, as well as an increase in alpha amylase enzyme levels in the patient group 50.826 ± 6.214 pg/ml when compared with control group 30.054 ± 8.424 . In relation, the results have given the decreasing indication in the rate of interleukin-4 in the patient group 0.37 ± 0.108 when compared with control group 0.58 ± 0.107 . We have concluded from these results that the uppermost indications of inflammatory factors have a critical role in detecting the ratio of disease progression.

Keywords: Diabetic patient 1; TNF alph 2; alpha amylase 3; IL4 4;

1. Introduction

The human oral cavity represents a safe habitat to a huge number of microorganisms. This area of the body possesses a reasonable number of characteristics that give it the opportunity to become a unique microbial environment.[1] *E. gingivalis* is a parasite that is found in tooth pits, festering gum tissue, and tonsil pits, and many studies indicate that there is a relationship between its presence and infections Periodontitis .[2], *E. gingivalis* belongs to the family Entamoebidae and the phylum Amoebozoa. Sarcodina .[3] Some researchers have noted that this parasite is opportunistic due to its presence in the oral cavity of healthy people, but it has the ability to reproduce in the oral environment infected with periodontal disease [4]. The researcher referred to Ghabanchi *et al* (2010). In his study, infection with oral parasites is common among people with periodontal disease. The parasite is transmitted from one person to another through kissing, spray, saliva, or sharing eating utensils, and that 95% of individuals with an unhealthy mouth are infected with *E. gingivalis* [5]. *E. gingivalis* has been found in the healthy oral cavity as well, but discrepancies remain regarding the true impact on gum disease [6] [7]. Researchers suggest that this organism causes periodontitis, While others note that *E. gingivalis* is only an opportunistic survivor that works



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synergistically with symbiotic bacteria to cause periodontal disease in immunocompromised hosts [4]. However, the parasite prefers gingival pockets, indicating a favorable niche either for parasite survival, or that *E. gingivalis* causes subtle molecular changes that lead to these conditions [8]. Serum beta -amylase is influenced by many factors under both physiological and pathological conditions. Dysfunction of the amylase-producing glands might alter enzymatic activities, which can be important in different clinical conditions [9] [10]. Indeed, serum amylase is a frequently requested test in patients presenting with acute abdominal pain in emergency wards. Therefore, the serum amylase level is the most widely used biochemical marker in the diagnosis of many diseases. High serum amylase is detected in several diseases such as acute pancreatitis, pancreatic cancer, ectopic amylase-producing tumors, abdominal trauma, kidney dysfunction, severe burns, hyperlipidemia, and diabetes mellitus [11] [12]. Although tumor necrosis factor-alpha (TNF-alpha and IL4) is one of the best-characterized inflammatory cytokines causing insulin resistance, several studies have reported that interleukin-6 also causes insulin resistance.[13], TNF-alpha and IL4 are an adipocytokine involved in systemic inflammation and stimulates the acute phase reaction.[14] It is primarily secreted by macrophages and also by a broad variety of other cells including adipocytes.[15] Disturbance in the TNF-alpha metabolism may affect the onset of type 2 DM and the progression of the disease.[16,17] The inflamed periodontium is highly vascular and may serve as an endocrine-like source for TNF-alpha and other inflammatory mediators.[18]

2. Materials and Methods

Collect of sample

First, the researcher worked on collecting blood samples for the forementioned patients who had diagnosed by the aspect of the parasite and DM2 by withdrawing 5 ml of venous blood using a medical syringe with a capacity of (5 ml), then placed in tubes containing gel to separate the blood, then separated at a speed of 3000 rpm for 5 minutes for the purpose of separating the serum. The blood was separated and the serum was taken, and the sera were distributed in Eppendorf tubes with a capacity of 1.5 ml. After that, the research procedures were to store the samples at a certain temperature of a stable level in -20 C until ELISA tests was performed. The samples were submitted to a division of two main groups: The first group was named as the the control group, which consists of 20 samples. On the other hand, the second group was specified to be named as the patient group, which consists of 30 samples

Laboratory diagnosis

Enzyme-linked immunosorbent assay

ELISA kit is the suitable procedure to be used to measure IL-4 ,TNF as well as alpha amylase concentration in serum quantitatively. In fact, the mentioned kit was urged by the providing process of Bioassay Technology Laboratory (Nanhu Dist, Jiaxing, Zhejiang, China).

Statistical Analysis : using statistical analysis was performed carefully through the employment of Chi-square and Use the Duncan multi-range tests intentionally below a certain likelihood gauge this means $P < 0.05$.

3. Results and dissection

-Levels of IL4 in study groups

Table (1): Average levels of interleukin 4 among patients with *E. gingivitis* and compare with the control group pd/ml.

Interleukin 4	IL4
	S.D ± Mean
Patient group	0.37±0.108
Control group	0.58±0.107

Commenting on the statistics of the above table, periodontitis and DM are obviously immune illnesses to inflammatory where leukocyte permeation and cytokines stimulate alveolar bone deprivation, synovitis, and joint destruction interleukin-4, generated from T-helper 2 cells, is an essential cytokine for the process of growth and the reproduction of the B type of lymphocytes. IL-4 shows an anti-inflammatory influence this is due to its naturally efficient restrain of the productions of proinflammatory cytokines, examples about this is the tumor necrosis factor-alpha (TNF- α), IL-1 α , IL-1 β , IL-6, as well as IL-8 by monocytes/macrophages [19]

-Levels of α -amylase in study groups

Table (2): Average of α -amylase values and average of *E.gingivitis* infection and compare it with the control group in pg/ml.

Interleukin 4	IL4
	S.D ± Mean
Patient group	50.826±6.214
Control group	30.054±8.424

the males constant infestation with *Entamoeba gingivalis* with the persistent periodontitis leads to the result of a decreasing state in the activation of the α -amylase crucial enzyme for the mentioned groups while compared to the other various participants of the groups. This enzyme is seen to be present directly in the mouth area and secreted gradually by the activation of the salivary glands. After that, it works on the digestion process of the carbohydrates as an auxiliary by the conversion of carbohydrates as well as glycogen. In addition, it works on the digestion of sugars into

small digestible molecules. [20, As this enzyme is transmitted through the bloodstream, and of course , the previous research projects that have been established in this area are considered to be limited to a reasonable number. Not only this, but the objective interpretation of the achieved results may rely on a variety of absolute opinions or suggestions. This requires a wider number of statistical projects and research with the aim to demonstrate an approval or denial to their validity and credibility. In fact, this enzyme, where the parasite infection is related to gum diseases may lead to a critical disturbance to the process of secretion by the salivary glands. In return, the parasite reacts with the production of chemicals and the substances are naturally meant to remain inside the tissues. In other cases, they may be excreted in the vital fluids of the patient's body. [21]

-The level of TNF-alpha in study groups

Table (3): Average of α -amylase values and average of *E.gingivalis* infection and compare it with the control group in pg/ml.

Tumor necrosis factor alpha	TNF-α Mean \pm SD
Patients	273.0 \pm 24.04 pg/ml
Controls	58.5 \pm 13.18 pg/ml

They showed that TNF- α is one of the inflammatory mechanisms that cause fever, which is an immune response to pathogens, including parasites, especially entamoeba gingivitis, as TNF- α works with some inflammatory cytokines such as INF- α and NK (Natural kill cells) against parasites, as it is an active factor against the activity of microbes in general and parasites including them [22] and the secretion of TNF- α from macrophages and monocytes is an immune response against the parasite because some studies have found that a decrease in the level of TNF- α in the blood or its absence may lead to an increase in the activity of the parasite.[23] and this is evidence that TNF- α works to limit the spread of the parasite, and the reason for the increase in tumor necrosis factor alpha in the blood and during infection with the parasite is the result of an immune response against it, as this cytokine participates directly in limiting the growth of the parasite in the brain and body by stimulating some other cytokines such as IL-10, INF- α , IL-8 and other inflammatory cytokines. These cytokines work to limit the spread and growth of the parasite inside the body, and they also play a major role in stimulating natural killer cells, which act as a defense against the parasite.[24]

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