The relationship between interleukin 1-B and calprotecin in periodontal Disease

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ABSTRACT
Backgrounds: One of the most common diseases affecting the oral cavity in humans is periodontitis. Researchers looked at the effects of calprotectin and IL-1B in human gingival Among the cytokines involved in the pathogenesis of periodontal disease, IL-1β, an inflammatory cytokine, can be highlighted for its contribution to stimulating the recruitment and differentiation of osteoblasts in tissues. Calprotectin is a heterodimer protein consisting of two calcium-bindinglinkages, belonging to the S-100 family.Materials and method: Ninety samples (patient and control) were obtained for the study between July 2023 and December 2024. Of them, sixty samples came from patients who saw periodontists in outpatient clinics and at Balad General Hospital. A sterile cotton swab (transport cotton swab) was used to obtain the sample. Using a syringe and a 5-milliliter Slim syringe, blood was drawn for 79 medical devices and then put into gelatinous gel tubes. The blood was then allowed to clot for fifteen minutes, sediment on the central computer at 3500 for one minute, and the serum was separated and kept in Eppendorf tubes with a 2-milliliter top at room temperature. The bacterial isolates were identified using physiological and biochemical assays, and the sample was held for 20 AD before being employed in the decimal filter. immunological examination The levels of immunological proteins calprotectin and human interleukin (IL-1β) were measured using ELISA technique. Results: IL-1B level in patients with Periodontitis recorded that (50.3±26.7) in comparison with control group that were (49.8±21.8,9.85±4.28) .While calprotectin level in patients with Periodontitis recorded that (10.79±2.69) in comparison with control group that were (9.85±4.28). Conclusion: Most common causes of Periodontitis was Prophyromonas gingivalis ,Staph. Aureus, Strep. Pyogenes. functional polymorphisms in the IL-1 B were not related to the calprotectin in development in Periodontitis prevalent diseases.

1. Introduction

Between 20 and 50 percent of people worldwide suffer from periodontal disorders, which are common in both industrialized and developing nations [1]. Because periodontal disease affects adults, adolescents, and the elderly more frequently than other age groups, it is a public health concern. Periodontal disorders are associated with a number of risk factors, including stress, age, genetics, diabetes, smoking, and poor oral hygiene. [2]. In periodontal disease, bacteria at the sub-gingival biofilm cause the gingival epithelium to produce cytokines and chemokines, which severely harms the gingiva, periodontal ligaments, and alveolar bone. [3] Many studies have shown that the worsening condition of gum disease leads to a number of systemic diseases such as cardiovascular disease and arthritis [4]. The body's immune system is not able to identify gingivitis infections and develop immunity against them over time, and in the case of In the absence of appropriate treatment, soft tissue infection develops [5]. As a beta-hemolytic streptococcus, Streptococcus pyogenes (S. pyogenes) is categorized as a Lancefield group A streptococcus (GAS). For centuries, S. pyogenes has continued to be a significant human pathogen. It can cause anything from minor upper respiratory tract infections and skin infections to serious, sometimes fatal illnesses like septicemia, pneumonia, necrotizing fasciitis, and streptococcal toxic shock syndrome in people [6]. Enterococcus faecalis bacteria are known for their importance in dentistry due to their association with primary tooth root canal infections and secondary treatment failure. E. faecalis bacteria were rarely found in primary root canal infections, as they were present side by side with anaerobic bacteria. In contrast, E. faecalis [7] was the most frequently isolated species from secondary root canal infections. Traditional cultural methods were used to investigate Microorganisms that cause infection of tooth root canals, and it was confirmed that Enterococcus faecalis bacteria are the most common types [8].

The pleiotropic, multifunctional cytokine interleukin-1 beta (IL-1β) is secreted by B cells, macrophages, natural killers, and monocytes. According to reports, IL-1β promotes blood flow at the site of inflammation by accumulating leukocytes and infiltrating neutrophils [9]. According to studies, IL-1β promotes the production of matrix metalloproteinases and collagenolytic enzymes, which deteriorates the matrix and destroys bone and tissue. Furthermore, it has been documented that the RANK/RANKL/OPG pathway connected to bone metabolism is impacted by IL-1β. [10]. The author

came to the conclusion that patients with diabetic periodontitis had a two-fold rise in the protein level when compared to those with chronic periodontitis based on the levels of calprotectin in periodontitis and periodontitis individuals. [11] Kido et al. showed that the lipopolysaccharide of Porphyromonas gingivalis bacteria induced neutrophils to release more calprotectin. Calprotectin chelates zinc to display broad antibacterial action. Calprotectin was discovered to be expressed in the spinous layers of the gingival epithelium in people with healthy periodontal tissues. In a study on calprotectin's effects on human gingival fibroblasts, Nishikawa et al. showed that the inductive effect of monocyte chemotactic [12].

Aim of study: Isolate and identify aerobic and anaerobic bacteria from patients with gingivitis and measurement of some immunological parameters IL-1B and calcium binding protein**.**

2. Materials and method

Study design

Ninety samples (patient and control) were collected for the study, of which sixty came from patients who saw a peredontist at Ballad General Hospital and outpatient clinics.The sample was taken between July 2023 and December 2024, spanning both sexes and a range of ages. A sterile cotton swab (transport cotton swab) was used to obtain the sample. The patient's name, age, gender, and other details were included in the questionnaire. Using the plotting approach, samples were directly grown on culture media that were conducive to bacterial growth, such as blood agar, MacConkey agar, mannitol salt agar, MSB agar, and chromo agar. For 18–24 days, the plates were incubated upside down at 37°C. They were planted once more on fresh plates using the same growing media an hour later. Before being employed for diagnostic testing, the pure and isolated colonies were moved to Brain Heart Infusion Agar Slant media and incubated for 24 hours at 37°C. The approaches were followed in making the diagnosis..

Biochemical Tests

Catalase test

This test was carried out by transplanting bacteria to examine their susceptibility to the development of the catalase enzyme. Using a sterile wooden stick, a portion of a young colony developing on Nutrient agar medium was transferred to a clean glass slide, and a drop of 3% hydrogen peroxide was added. Within seconds, the formation of oxygen gas bubbles indicates a successful outcome.

Bacitracin susceptible test

This test serves as a discriminant between organisms that are susceptible to the antibiotic bacitracin and those that are not. While Streptococcus agalactia is resistant to antibiotics, this test was conducted using Streptococcus pyogen bacteria, which are susceptible to them. The experiment involved spreading the bacterial solution over 5% sheep blood agar, covering the agar surface with a Bacitracin disc, and then incubating the dish for 24 hours at 37°C in optional anaerobic conditions with a concentration of 5–10% carbon dioxide (CO2).

Optochin susceptible test

Using this technique, optochin-sensitive Streptococcus pneumoniae is differentiated from optochin-resistant a-hemolytic streptococci. The test was then carried out by putting Optochin on the agar disk's surface, spreading the bacterial suspension over the medium of blood agar 5 sheep blood agar, and incubating the ears in anaerobic conditions with facultative anaerobes at a concentration

Investigating hemolysin production

The goal of this experiment was to identify the kind of lysis that bacteria on blood agar cause. Plotting on a blood agar plate was used to inoculate the isolates, which were then cultured for 48 hours at 37 degrees C. Following incubation, the kind of lysis (gamma beta alpha) is observed.

Immunological test

The immunological proteins human interleukin (IL-1β) and calprotectin were measured using ELISA technology in the serum of study participants. Afterwards, the evaluation was carried out by multiple SunLong Biotech is the manufacturer of the pre-assembled kit.

Statistical methods

Version 19 of the statistical package for social sciences (SPSS) was used for data analysis. A t-test sample was used for the analysis of the findings. The data was represented in an appropriate table.P-values less than 0.05 were regarded as statistically significant..

3.Results and discussion

3.1 Isolation & identification of bacteria from periodontitis patients

A total of 100clinical samples were obtained from patient's Periodontitis. After collecting the samples all samples were grown on blood agar and MacConkey agar, mannitol salt agar and Chromogenic Medium and other simple media plates and incubated aerobically at 37 °C for 24 hours.

About (60%) showed positive bacterial growth which were *Prophyromonas gingivalis* (30%), *Staph. aureus* (16.7%), *Strep. pneumonia* (11.7%), *Staph. epidermidis* (10%), *Strep. pyogenes*, *Strep. Viridans*, *Strep. mutans* (8.3% %) for them, and (6.7%) for *Enterococcus fecalis* that was diagnosed based on biochemical tests, and the diagnosis of some bacterial species was confirmed with the VITEK device., whereas (40%) showed no growth, which might be attributed to antibiotic treatment or the presence of other types of causative agents which may need specialized diagnostic tests

Table (1): Bacterial percentage in periodontitis

Bacteria	No. of isolate	Percentage
Strep. pyogenes	5	8.3%
Strep. Viridans	5	8.3%
Strep. pneumonia	7	11.7%
Strep. mutans	5	8.3%
Staph. aureus	10	16.7%
Staph. epidermidis	6	10%
Prophyromonas gingivalis	18	30%
Enterococcus fecalis	4	6.7%
Total	60	100%

Isolation and Identification of aerobic and anaerobic bacteria according to source of isolation

Identification of *Streptococcus spp.* (gram positive)

Streptococcus isolation agar media supplemented with blood. This technique allows the detection of β-hemolysis of *Streptococcus pyogenes*, *Streptococcus pneumonia* is alpha hemolytic and *Streptococcus mutans* is normally alpha- or green gamma-hemolytic on blood agar plates.

Swab from gums surface are taken from Patients with periodontitis resulting from orthodontic and placement from medical clinics the samples were cultured on primary media (blood agar and Azide blood agar), the appearance of growth, the shape of the developing colonies, their size, and type of hemolysis were monitored. Bacterial species were diagnosed using microscopic examination. It was found that the stained bacterial smears are gram positive cells figure (1). Five bacterial isolate (8.3%)

belong to *Streptococcus pyogenes* were obtained, 7 (11.7%) *Strep. pneumonia* and 5 (8.3%) isolate belong to *Strep. mutans* were obtained. depending on cultural and microscopic characteristics. Beta hemolysis (due to complete hemolysis of RBCs in the medium) surrounded the colonies of *strep. pyogenes* were observed after 24 hours of incubation.

Table (2): Differential tests used for differentiation of *Streptococcus pyogenes* group from other Streptococci

Isolates	Biochemical and physiological tests					
	Bacitracin	**Catalase**	**CAMP test**	**β-hemolysis**	**Capsule test**	**Oxidase test**
Streptococcus pyogenes	**S**	-	-	+	+	-

Identification of *Enterococcus faecalis* (gram positive)

Typically, Enterococcus feacalis is cultured aerobically at 35 (±2)0C. In a microbiology lab, E. faecalis is frequently primarily isolated and characterized using blood agar medium. Small, turquoise-colored colonies of Enterococcus faecalis/faecium are produced when other media, such as MacConkey Agar and Chromogenic Medium, are used for their isolation and identification. Under greater magnification, E. faecalis, which are Gram-positive cocci, have a slight oval shape. The cells have an oval form and are usually 0.5 to 2 μm in diameter. Under certain development conditions, they can elongate up to 0.6 to 2.5 μm.

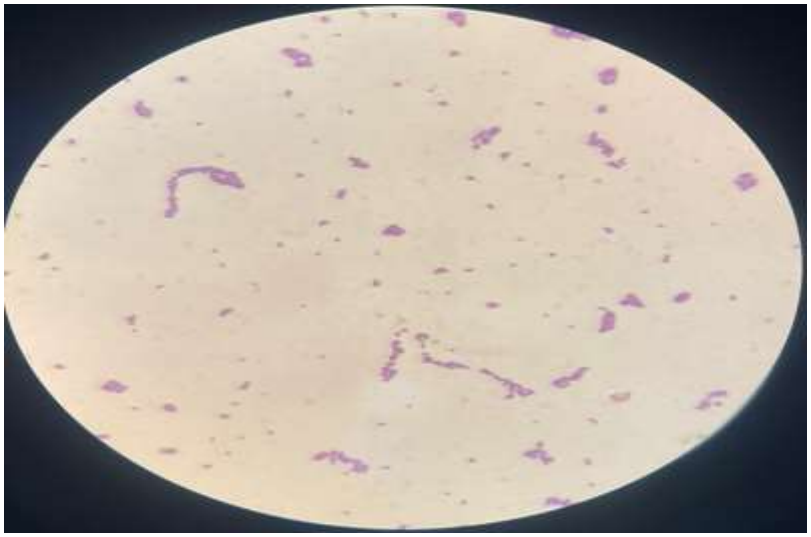

Figure (1): Diagnosis *Enterococcus spp*. under microscope

Study the means of some immunological parameters in Periodontitis patients compared with control group

In the present study, there were no significant differences in IL-1B and A8/A9(calcium binding protein) level in patients with Periodontitis(50.3±26.7, 10.79±2.69) in comparison with control group that were (49.8±21.8,9.85±4.28), respectively , as shown in Table (3).

Table (3): Comparison between Periodontitis patients and healthy controls in regarding to Mean± SD of cortisol

Transactions	**IL-1B (pg/ml)**	**calcium binding protein**
Patients	**50.3±26.7**	**10.79±2.69**
control	**49.8±21.8**	**9.85±4.28**
p-value	**0.10**	

When viewed under a microscope, they have an oval form and can be found alone, in pairs, or in chains.

They are not pigment-producing, spore-forming, or motile bacteria. They are able to produce pili and biofilm, which aid in their ability to colonize and endure in hostile environments. [13]. Together with additional tests, the results in table (2) demonstrated that the isolates from the Streptococcal group were all β-hemolytic gram positive cocci with chains that varied in length and formed micro-colonies. [14]. A catalase test was conducted for the 17 isolates suspected of being *Streptococci* spp. to distinguish *Streptococci* from *Staphylococci*, and all of them (100%) showed a negative result for this test, figure (4-1D) as no oxygen gas bubbles formed on the colonies when a reagent was added. CAMP test was used to differentiate between β-hemolytic group B *Strep. agalactiae* (positive) and *Strep. pyogenes* (group A) (CAMP negative) group. Also the results of bacitracin sensitivity test were used to exclude Streptococci group A *Strep. pyogenes* (bacitracin positive) and Optochin test gave positive result for *Strep pneumonia*, [15]. A biological process known as the oxidase test is used to determine whether cytochrome oxidase is present. When a bacteria is oxydase positive, it signifies that it has cytochrome c oxidase and can use oxygen to produce energy through an electron transfer chain. Oxidase negative is GAS [16]. By employing capsule stain preparations for negative staining, the capsule of Strep. pyogenes has been studied. Under a light microscope, the capsule appeared as bright spots, indicating that it was present in all of the isolates under investigation. One important modulator of the inflammatory response is the cytokine interleukin-1β (IL-1β). Not only is it necessary for the host's defense against infections and response to them, but it also makes acute and chronic tissue damage worse. Although many different cell types can make and secrete it, the majority of research has been on how innate immune system cells like macrophages and monocytes produce it. [17]. Increased risk for periodontitis has been associated with variations in the IL-1 gene cluster. Patients with chronic periodontitis have higher amounts of IL-1β in their periodontal tissues when they have IL-1B [18]. The multiprotein complex known as the inflammasome is an oligomeric assembly that is necessary for the maturation of IL-1β and its subsequent secretion. [19]. de Alencar *et al.* [20] showed high level of IL-1B in periodontitis patients. IL-1B polymorphisms are associated with a higher severity of periodontitis in study of Auerkari *et al.* [21], these results were in-compatible with present results. According to George et al., calprotectin in gingival perivascular fluid or saliva may be a potential biomarker of periodontal inflammation and granulocyte activity. It can be concluded that calprotectin and its constituent homodimers have an altered expression pattern in periodontal disorders. [22].

Calprotectin, which is unique in that it is not produced in the liver and is quite stable in comparison to other linear groups, is newly thought to be an acute-phase protein. Additionally, the protein is expressed in serum, GCF, and saliva from the affected patient's samples, making it a more visible and accessible diagnostic marker to forecast the staging and grading of the progression of periodontal disease [23]. In contrast to people with gingivitis, patients with aggressive and chronic periodontitis had much higher amounts of the protein, according to a study by Que et al. that assessed the protein during the early stages of experimental gingivitis and periodontitis. [24] Zheng et al. discovered that, in contrast to controls, the GCF of aggressive periodontitis had higher expression levels of calprotectin. [25] Kaner et al. discovered that in individuals with advanced periodontitis, calprotectin levels gradually dropped following first non-surgical therapy in an interventional longitudinal trial. the author found that patients with diabetic periodontitis had a two-fold rise in the protein level when compared to those with chronic periodontitis after comparing the levels of calprotectin in periodontitis and periodontitis individuals. [12] Kido et al. showed that the lipopolysaccharide of Porphyromonas gingivalis bacteria induced neutrophils to release more calprotectin.

Conclusions

Most common causes of Periodontitis was *Prophyromonas gingivalis ,Staph. Aureus, Strep. Pyogenes.* functional polymorphisms in the *IL-1* were not related to the calcium binding protein in development in Periodontitis prevalent diseases.

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