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Isolation and diagnosis Porphyromonas gingivitis isolated from some periodontal disease patients in Misan governorate center

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Abstract: *There is anaerobic bacterium in the mouth called Porphyromonas gingivalis, and it causes periodontal inflammatory illnesses. The oral anaerobe is gram-negative. One of the main causes of periodontal diseases is P. gingivalis. Multiple studies have linked P. gingivalis to periodontal disease and found that it is an important factor in the development of periodontal problems. . In the present study aim, isolation and identification of Porphyromonas gingivalis anaerobic bacteria by conventional phenotype, Vitek 2 Compact System, and molecular methods found 16SrRNA gene sequencing. Fifty patients and fifty healthy controls were gathered. Periodontal evaluations were performed on all individuals. The following clinical indicators were noted in order to assess the periodontal health of the patients: pocket depth, bleeding on probe (BOP), and approximate plaque index (API). Using the PCR technique, the presence of P. gingivalis was determined in samples from periodontal pockets (PP) develop instead of a healthy gingival sulcus periodontal pocket. This is accomplished after pathological alveolar bone loss and migration of the supracrestal attachment tissues apically, because of their mucosal wall, it is called in this case, gum pockets.*

.**Keywords:** *Porphyromonas gingivalis , periodontal diseases gum pockets., bleeding on probe (BOP), plaque index (API).*

1.Introduction

Oral Community Population thought to contain at least 700 different bacterial species, the gingival sulcus is the area of the oral mucosa that has been the subject of the most research. The gingival sulcus (the space between the gingiva and the hard surface of the teeth) is home to microbial communities that interact with the mucosal epithelial cells [1] Periodontal disease is a long-lasting inflammatory condition caused by bacteria that interact with host cells and destroy the tissues that support the teeth. A growing number of bacteria pathogens in the dental plaque have been thought to be the cause of the inflammation. *Porphyromonas gingivalis*, the main culprit, is a Gram-negative, non-motile, asaccharolytic anaerobe. The virulence factors that *P. gingivalis* contains include fimbriae, lipopolysaccharide, outer membrane vesicles (OMVs), and gingipains. The gingipains are trypsin-like cysteine proteases that account for 85% of the total proteolytic activity and are crucial to the pathogenicity of the organism [2],[3]. *P. gingivalis* fimbriae, a stringy cell

surface protrusion, facilitate adhesion of other bacteria and host cells. With the aid of its fimbriae, *P. gingivalis* may cling to early bacterial colonies and helps biofilms grow. The two different types of fimbriae are I (major) type fimbriae, known as fimbrillin or FimA, encoded by the fimA gene, and II (minor) type fimbriae, known as Mfa subunit protein, encoded by the mfa1 gene. [4] The particular role of fimbriae in the experimental periodontitis paradigm is to promote bonedamage[5].

2.Methodology

Bacterial strains and growth conditions

100 adult patients with periodontitis had subgingival plaque samples taken. Patients were older than 10 years old and had periodontal pockets that measured more than 5 mm and showed bleeding when probed. In the three months prior, the patients had not taken any antibiotics. Using sterile paper points, samples were taken from the deepest periodontal pocket in each quadrant of the dentition. 1.5 cc of decreased transport fluid . Pocket formation starts as an inflammatory change in the connective tissue wall of the gingival sulcus, was used to combine the samples. On blood agar plates, 100 of the dilutions were plated and incubated at 37 °C for 7 to 14 days [6]. Gram staining, anaerobic growth, the inability to ferment glucose, the formation of indole, and other factors were used to identify *P. gingivalis.* [7].

Isolation of DNA from plaque samples and bacterial cultures.

The reference strain utilized was *P. gingivalis* strain W83 [8]. After cultivating the bacteria for two to three days in brain heart infusion, they were injected on blood agar plates to determine the number of CFU of the *P. gingivalis* suspension

PCR primers .

The bacterial isolates was identified by using PCR to amplify universal 16S rDNA primers F 5′ - GTACAGTTGCTTCAGGACGTATC-3 and R 5′ - GGT TAC CTT GTT ACG AC TT-3′ as well as, two specific primers used to identification bacterial isolates *p.gingivalis* F1 5′AGGCAGCTTGCCATACTGCG′3 R1 3′CTGTTAGCAACTACCGATGT′5 Agarose Gel Electrophoresis [9]. PCRs were carried out utilizing a matrix of forward, reverse, and probe concentrations to find the concentration that produced the lowest threshold cycle values and, thus, the maximum amplification efficiency.

3.RESULTS

Morphological and microscopic diagnosis:

They were then raised on blood agar at 37 °C in an anaerobic environment. After 7 days of incubation, colonies showed dark pigmentation. When gingivalis is grown on blood agar plates, the colonies have a black color and a rod shape. According to research on *Porphyromonas gingivalis*, the accumulation of hemin produced by erythrocytes on a surface and inside bacterial cells is what gives colonies their dark color. The majority of the bacteria analyzed could grow on blood agar plates.

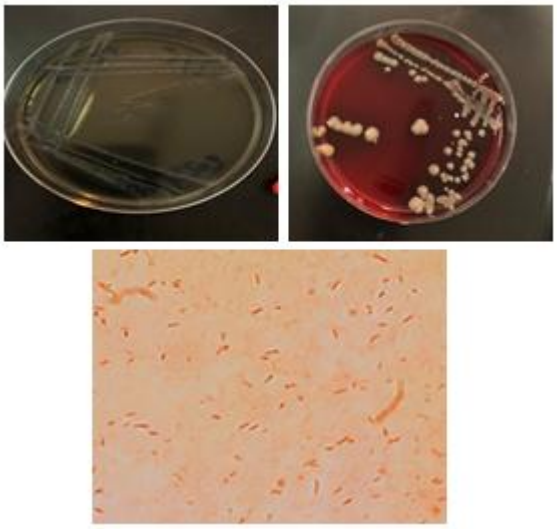

Figure (1): A Colony of *Porphyromonas gingivalis* on Blood Agar After 7 Days of Incubation 37°c , B Colony of *P. gingivalis* on Blood Agar After 14 Days of Incubation 37°c , C. Important Oral Bacteria *P. gingivalis* Observed by Microscopy 1000 x G-ve and coccobacilli, belongs to patients groups.

Amplification of 16S rDNA and special primer

Two types of primers were used the general primer F5-GTACAGTTGCTTCAGGACGTATC-3 and R5-GGTTACCTTGTTACGACTT-3 and the special primer F5′ -AGGCAGCTTGCCATACTGCG -3′ R5′ -ACGTTCGATTTCATCACGTTG-3 The amplified 16S rDNA gene was located using gel electrophoresis; in contrast to the conventional molecular DNA marker (50-2000 bp), the distinct gene bands were separated by 1500 bp show in Figure (2)

The collected DNA was used in the PCR process to amplify particular primers. All 10 of the discovered microorganisms could be amplified using a specific primer. The amplified versions of particular primers were identified using gel electrophoresis. Compared to the normal molecular DNA marker (100-300 bp), the distinct gene bands could be differentiated by 197 bp show in Figure (3)

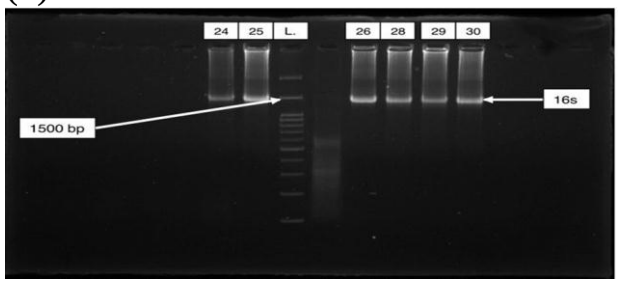

Figure (2):PCR Products Amplified of 16Sr DNA Can Be Seen on Agarose Gel Electrophoresis, L: Ladder. belongs to patients groups

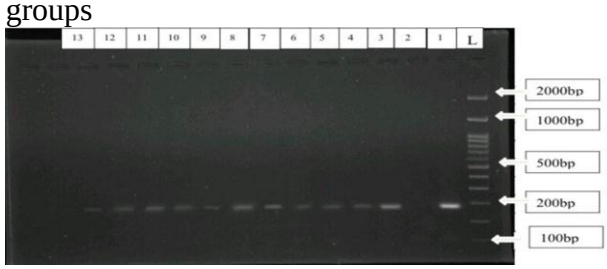

Figure (3): PCR Products Amplified of specific primers Can Be Seen on Agarose Gel Electrophoresis, L: Ladder. belongs to patients groups

4. Discussion

The aliquot that will be used for culture was divided up, added to with hemin, and then plated on Blood Agar. The plates were preserved anaerobically for seven days in an anaerobic jar using a specialized gas pack system. They manifest in the blood agar as clusters of black pigment because they help in the transfer of iron using the. She is supported by Hemin as she moves, and as a result, she creates a black dye. This could help to explain why those who take in large amounts of iron are more likely to develop gingivitis. Hemin, which P.gingivalis bacteria use as a source of iron for development, builds up and produces a black pigment as a result. and tissue swelling in

the mouth and gums. After the incubation period, plates were checked to see if any coccobacilli colonies, black-pigmented colonies, or mucoid colonies were present [Figure1]. A study by [10]. revealed the importance of recognizing and quantifying P. gingivalis and other periodontal infections using various techniques in plaque samples. These colonies are small, glossy, black-pigmented, and mucoid, with or without hemolysis.

Results of the analysis of the 16S rDNA gene and specific primer sequence.

The reason of periodontitis is varied because endodontic bacteria vary from person to person. Using molecular detection methods like species-specific PCR assays, endodontic samples can aid in the discovery of species that are difficult to culture. Bacterial 16S rRNA contains nine hypervariable regions that can be utilized to distinguish between distinct bacterial species since they exhibit uniform sequence diversity. The PCR method is more sensitive than traditional culture approaches for microbiological identification in refractory cases [11]. is a method to only find the target bacterium's DNA; it is not suitable for a qualitative analysis of many different unidentified bacterial species. According to various studies, when using conventional culture techniques on individuals with periapical periodontal disease, obligatory anaerobes are infrequently identified in the root canal. The 16S rRNA gene assay based on PCR is helpful for identifying a variety of anaerobic bacteria that are challenging to grow using conventional culture techniques. similar outcomes to what was previously reported [12].

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