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Granulicatella Adiacens Bacteria Isolation from Perodontitical Patients with Polymerase Chain Reaction Techniques

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ABSTRACT

Background: Periodontitis is an inflammatory disease of dental support tissue caused by certain groups of microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone, with pocket formation, recession, or both[1]. Destruction of periodontal caused by the host defence.

Objective: Granulicatella adiacens is a small Gram-positive, non-motile, non-spore-forming, facultatively anaerobic coccus. As a member of Nutritionally Variant Streptococci, genus Granulicatella, it is a part of the normal microbiota in the oral cavity, urogenital, and intestinal tract. It may also participate in periodontitis, caries, and endodontic infections. Our aim in this study was to investigate the presence of Granulicatella adiacens in periodontal disease

Methods: A total 18 samples from patients with chronic periodontitis was collected, by using the conventional PCR technique for checking the presence of bacterial bands, followed by using DNA sequence methods, to complete the examination of bacteria with Conventional PCR which results are used as a basis for nucleotide examination on the NCBI Gene Bank using BLAST analysis.

Result: In this study, Granulicatella adiancense was found in 13 samples from 18 solated samples from patients with chronic periodontitis and in periodontal pocket depth more than 7 mm.

Conclusion: Granulicatella adiancense is a normal flora bacteria in the oral cavity and chronic periodontitis conditions, the presence of this bacteria is detected in the deeper periodontal pocket depth.

Keywords: Chronic periodontitis, DNA squence, Granulicatella adiacens

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INTRODUCTION

Periodontitis is an inflammatory disease ofdental support tissue caused by certain groups of microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone, with pocket formation, recession, or both[1]. Destruction of periodontal caused by the host defence. Clinically periodontitis is characterized by plaque accumulation, calculus and pocket formation, periodontal tissue inflammation and alveolar bone loss, and gum bleeding. Pathogenic bacteria are suspected of causing an inflammatory response, gingival and periodontal damage[2]. Periodontal disease can be treated primarily with debridement supragingiva and subgingiva. The combination of surgical or mechanical treatments and proper oral hygiene can maintain a healthy periodontal condition[3]. Periodontal disease is no longer solely caused by the presence of specific bacteria but by ecological changes in the biofilm community and the population level of bacterial species in the oral microbiota[4]. Bacteria in the phylum firmicutes are lactic acid bacteria, which is commensal bacteria in the oral cavity. However, due to a change from symbiosis to dysbiosis, this bacteria becomes a pathogen. Lactic acid bacteria have the ability to ferment sugar into lactic acid which can further cause demineralization and bone resorption.[5]Nutritionally variant streptococcus (NVS) were originally described by Frenkel and Hirsch (1961) as a new type of streptococcus exhibiting satellitism around colonies of other bacteria. Isolates have been recovered from blood, abscesses, oral ulcers, and urethral samples. Because of both difficulties in culturing these organisms and the variety of appearances that they present on primary detection, such strains have caused major diagnostic difficulties. [6]

The condition of normal flora is where microorganisms that are usually in certain locations and under normal circumstances do not cause disease[6]. Granulicatella adiacens in the form of small Gram-positive, non-motile, non-spore coccus, oxidase, and catalase-negative, facultative anaerobes. The nutritional requirements of Granulicatella adiacensare complex and require pyridoxal or L-cysteine in growth media as essential nutrition for the normal growth of this bacteria[7]. Granulicatella adiacenshas a normal commensal relationship on most mucosal surfaces which allows it to affect the mucosal area of the human body but although these bacteria can infect all these areas, these bacteria rarely cause disease species of this genus found in the oral cavity, intestine and urinary tract[8]. Because of complex nutritional growth requirements, Granulicatella adiacenscan most often cause infections in nutrient-rich tissue, such as the endocardium or the eye, causing endocarditis or endophthalmitis, in addition to causing intravascular infections. Infection in this area causes endovascular infections, the central nervous system, ocular, jawbones and joints, and joint and urinary tract infections[9].

Granulicatella infection is quite alarming for several reasons. First, this infection, especially endocarditis, has been associated with greater morbidity and mortality compared with other infections caused by another streptococcus. Second, Granulicatella infection has antibiotic resistance and the recurrence rate is higher than other streptococcus[10]. The present study was carried out to determine the presence of Granulicaella adiacens bacteria in chronic periodontitis patients from the subgingival plaque of these patients.

MATERIAL AND METHODS

This study was approved by the ethical committee of the Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia. The study included a total of 18 subjects (8 Male and 10 female) undergoing periodontal treatment. Medical histories from the participants were obtained and thorough clinical and radiographic examinations of their teeth were conducted after written informed consent. Patients with a pocket depth of at least 4 mm; aged 35-50 years systemic disease; OHI-S Moderate-severe; patients do not have a systemic disease; were inclusion criteria and the exclusion criteria were bacteria does not obtain from the sample and bacterial nucleotides do not exist in Gene Bank.

Assessment of periodontal status was performed by a single trained examiner, the status was means of clinical attachment loss (CAL), probing pocket depths (PPD), which was measured more than four millimeters per tooth by using a WHO periodontal probe. Oral hygiene indices such as papillary bleeding index (PBI), plaque index (PI), and gingival index (GI) were also assessed.

Sample Collection: The sample was collected from the gingival area of buccal tooth surfaces using sterile curettes onto the swab, then the swab put into sterile transport media.

Microbiological Analysis: The swab is inserted into a tube containing the BHIB (Brain Heart Infusion Broth) medium then incubated for at least 8 hours at 370 C. The bacteria culture transferred to Blood Agar medium and incubated for 24 hours at 370 C.

DNA preparation: The basic principle of DNA extraction is a series of processes to separate DNA from other components such as proteins and others. This extraction procedure includes the process of Sample Preparation, Cell Lysis, DNA Binding, Wash, Elution. The liquid contained in the Eppendorf tube is a DNA product that is ready for PCR.

Running PCR: Cycle 1 for the Pre-Denaturation process, Cycle 2 for the process of denaturation, annealing, extension. Cycle 3 for the final extension process.

Gel Electrophoresis: A total of 8 µl amplicons from the PCR (Positive Control, Negative Control, sample) were inserted into 10 µl gel wells and in the first hole add 10 ul of 100 bp DNA ladder into the well near the positive control. Electrophoresis is turned on and run from a negative charge (cathode) to a positive charge (anode) at 100 A and 60 minutes. After electrophoresis, the band is formed. The positive result if the band is aligned with the positive control, continued by DNA Sequence examination to obtain the nucleotide sequence which was then performed a BLAST (Basic Local Alignment Search Tool) Nucleotide sequence on NCBI Gene Bank data (National Center for Biotechnology Information)

RESULT

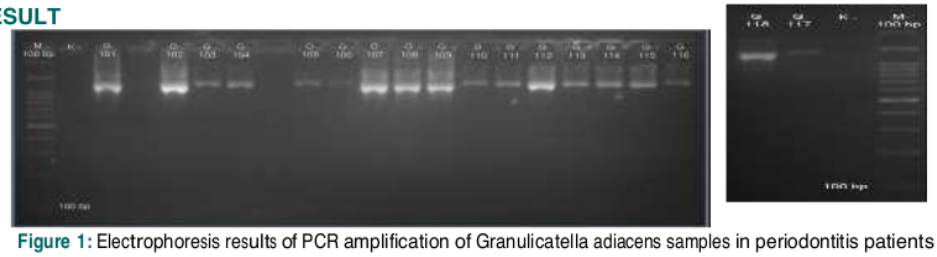

Figure 1: Electrophoresis results of PCR amplification of Granulicatella adiacens samples in periodontitis patients

The study used PCR targeting the 16S rRNA gene for the detection of bacterial DNA. From the PCR amplification results obtained DNA band fragments of 100 bp in all isolation samples. Then proceed with the DNA sequence and the results obtained in the form of nucleotide fasta, which then processed the data based on the order of nucleotides in NCBI Gene Bank data, using the BLAST analysis.

Table 1: Blast NCBI analysis for **Granulicatella adiancense**

Sample	Tooth number	Pocket Periodontal Depth (mm)	Data NCBI
1	21	10	+
2	11	11	+
3	24	9	+
4	31	7	-

5	12	5	-
6	27	6	+
7	21	5	-
8	16	7	+
9	23	11	+
10	31	8	+
11	17	6	-
12	27	9	+
13	16	5	+
14	16	7	+
15	34	5	-
16	31	8	+
17	12	9	+
18	36	8	+

DNA sequence results in 18 gram-positive bacterial isolation samples obtained by the Nucleotide sequence were then adjusted to the NCBI Gene Bank so that 13 samples were obtained with the DNA of the bacterium Granulicatella adiacens.

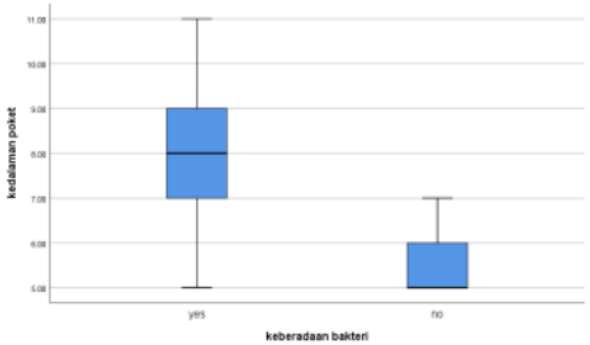

Figure 2: Granulicatella adiacens distribution based on periodontal depth

In figure 2 there were Granulicatella adiacens found on the periodontal pocket with 7-9 mm depth (SD±2mm), while no Granulicatella adiacens were found on 5 to 6 mm periodontal pocket depth (SD±1mm). From 18 bacterial isolation samples, 3 samples collect from pocket periodontal with more then 10 mm depth, 1 sample is collected from 6 mm depth and 9 samples from 7 to 9 mm pocket depth, all of this 13 samples were found obtained with the DNA of the bacterium Granulicatella adiacens. The remain samples is no Granulicatella adiacens found.

Based on the Kruskal-Wallis Test, the results showed that there is a difference between the depth of the pocket with a shallow pocket against the presence of the bacterium Granulicatella adiacens. Through observation, a significant difference (0.008) was obtained at different pocket depth levels, this difference was in the presence or absence of Granulicatella adiacens found at the depth of the periodontal pocket. From the results above that the bacterium Granulicatella adiacens was found at a depth of more than 7 mm.

DISCUSSION

The conventional PCR technique was chosen for bacterial identification because of its accuracy in checking the presence of bacterial bands, we also using DNA sequence methods, where this method aims to complete the examination of bacteria with Conventional PCR which results are used as a basis for nucleotide examination on the NCBI Gene Bank using BLAST analysis. In conventional PCR examination, a 16S rRNA universal primer is used, however, the data produced does not show the type of bacteria, but the quality of the bacteria through the presence of a bacterial band. On examination using the DNA sequence method, the type of bacteria can be clearly identified, both at the level of genus, species and even subspecies of bacteria.

The bacteria are the predominant group of organisms in the oral cavity, and there are probably some 500 to 700 common oral species or phylotypes which is only 50% to 60% are cultivable[11]. In periodontitis condition, the discoveryof streptococcus species and their variants in large numbers, this indicates the possibility of a shift in bacterial species from healthy periodontal to periodontitis[12]. Hajishengallis

(2013) revealed that periodontal disease is no longer solely caused by the presence of specific bacteria but by ecological changes in the biofilm community and bacterial species population level.[4]Colonization by the main pathogen is aided by additional pathogens causing impaired host defense and increasing inflammation[13].

Based on the results of the study it was found that there was a relationship between the depth of the periodontal pocket with the Granulicatella adiacens bacteria, where the deeper the periodontal pocket the bacteria could be found. This is consistent with bacterial morphology in the form of small Gram-positive, non-motile, non-spore coccus, oxidase, and catalase-negative, facultative anaerobes[14]. The anaerobic traits of the facultative bacterial causes these bacteria to develop in deep periodontal pockets, in additional it was bacteria in the phylum firmicutes that classified into lactic acid bacteria, commensal bacteria in the oral cavity. Which has the ability of demineralization and bone resorption[15]

Granulicatella adiacens is part of a normal microbiota in the oral cavity, urogenital, and intestinal tract. Protein secretion can help bacteria in normal growth and physiology, otherwise, it has a function as a virulence mechanism in host colonization or by modulating the host immune response.[16] There are more than 20 putative virulence- related proteins for Granulicatella adiacens survival and cause infection, bacterial colonization, and virulence[17]. Protein secretion helps bacteria in their normal growth and also function as a virulence mechanism in host colonization or by modulating host immune responses[18]. Bacteria have devised dedicated secretory systems for protein secretion[19], Gram-positive species secrete mainly via general secretory system[20]. Virulence potential of the Granulicatella adiacens secretome was assessed by searching for their associations with virulence activities in other species. Only a view study is available for the protein secretion of Granulicatella species. Recently, a close phylogenetic relative of Granulicatella adiacens, Granulicatella elegans, was shown to secrete arginine deiminase, which through citrullination inhibits proliferation of human peripheral blood mononuclear cells in vitro and may associate with the pathogenesis of periodontitis and certain systemic[1621].

Putative virulence-associated proteins in Granulicatella adiacens secretome such as serine protease, thioredoxin, superoxide dismutase, phosphor carrier, and acyl carrier proteins[22]. Superoxide dismutase converts superoxide anions into oxygen and hydrogen peroxide, it is required not only for H_2O_2 resistance in S. mutans, but also is needed for coexistence with S. sanguinis[23]. Serine proteases cause cytopathic effects and exhibit enterotoxin activity, it degrades mucins, resulting in an advantage for mucosal colonization and immune modulation[24]. In Salmonella enterica, thioredoxin helps the bacterium in intracellular replication and virulence[25][27][29]. Granulicatella adiacens secretome consisted of several moonlighting proteins shown to play a role in bacterial virulence. The glycolytic enzymes, phosphoglycerate kinase, triose-phosphate isomerase, aldolase, and enolase possess moonlighting properties, they function as adhesins, receptors for transferrin, neutrophil evasion proteins immunomodulators and participate in extracellular polysaccharide synthesis. Oral bacteria express a number of molecular chaperones found in Granulicatella adiacens secretome. They express on the cell surface to use them as adhesins and can release them into the extracellular milieu to act as signaling virulence factors[17].

CONCLUSION

Granulicatella adiancens is a normal flora bacteria in the oral cavity and in chronic periodontitis conditions, the presence of this bacteria is detected. There was a relationship between the depth of the periodontal pocket with the Granulicatella adiacens bacteria, where the deeper of the periodontal pocket the bacteria could be found.

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CONFLICT OF INTERESTS

There are no conflict of interests in this study

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