**Research Report**

**Prevotella intermedia and Porphyromonas gingivalis in dental caries with periapical granuloma**

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**ABSTRACT**

**Background:** Dental caries with necrotic pulp is a multifactorial disease that attacks enamel involving tooth pulp. The anaerobic bacteria infection in the pulp chamber could induce the formation of periapical granuloma. However, the presence of the most frequently anaerobic bacteria identified in apical periodontitis, Porphyromonas gingivalis and Prevotella intermedia, in periapical granuloma have not been confirmed. **Purpose:** The aims of study were to determine the presence of Porphyromonas gingivalis and Prevotella intermedia in dental caries with necrotic pulp and to determine its relation to periapical granuloma. **Methods:** Thirty-six patients of dental caries with necrotic pulp in Dr. Moewardi General Hospital in Surakarta, Indonesia were involved and classified into two groups, the group of patients with periapical granuloma and the group of patients without periapical granuloma. The caries tooth was extracted, and the chronic periapical tissue was swabbed and cultured on blood agar medium in anaerobic condition. The bacterial DNA was extracted from the positive cultures and subjected for Polymerase Chain Reaction (PCR). **Results:** Periapical granuloma was more likely found in women (OR 5.5, 95% CI= 1.277-23.693; RR 2.5, 95% CI= 1.025-6.100). Black colonies bacteria were associated with periapical granuloma (OR 2.2, 95% CI=0.517-9.594; RR 1.5, 95% CI=0.655-3.623). Porphyromonas gingivalis and Prevotella intermedia were detected in group with or without periapical granuloma, however, only Prevotella intermedia was associated with periapical granuloma (OR 1.6, 95% CI=0.418-5.903; RR 1.3, 95% CI=0.653-2.393). **Conclusion:** The presence of Porphyromonas gingivalis and Prevotella intermedia in periapical granuloma were confirmed, however, only Prevotella intermedia were associated with periapical granuloma.

**Key words:** Dental caries, necrotic pulp, periapical granuloma, Prevotella intermedia, Porphyromonas gingivalis

**ABSTRAK**

**Latar belakang:** Karies gigi dengan pulpa nekrosis adalah penyakit multifaktorial yang menyerang enamel hingga ruang pulpa gigi. Infeksi bakteri anaerob dalam pulpa nekrosis dapat menginduksi pembentukan granuloma periapikal. Namun, keberadaan bakteri anaerob yang paling banyak ditemukan di periodontitis apikal, Porphyromonas gingivalis dan Prevotella intermedia, di granuloma periapikal masih perlu dieteliti. **Tujuan:** Penelitian ini bertujuan meneliti keberadaan bakteri Porphyromonas gingivalis dan Prevotella intermedia, di karies gigi dengan pulpa nekrosis dan menganalisis kaitannya dengan granuloma periapikal. **Metode:** Tiga puluh enam pasien karies gigi dengan pulpa nekrosis di Rumah Sakit Umum Dr. Moewardi di Surakarta Indonesia dibatasi dan diklasifikasikan dalam dua kelompok yaitu kelompok pasien dengan granuloma periapikal dan kelompok pasien tanpa granuloma periapikal. Gigi karies diekstraksi dan jaringan periapikal krisis diasap dan dikultur di media agar darah dalam kondisi anaerob. DNA bakteri diekstrak dari kultur yang positif dan dilakukan pemeriksaan dengan Polymerase Chain Reaction (PCR). **Hasil:** Granuloma periapikal lebih banyak ditemukan pada wanita (OR 5,5, 95% CI= 1,277-23,693; RR 2,5, 95% CI= 1,025-6,100). Koloni bakteri berwarna hitam diasosiasikan dengan granuloma periapikal (OR 2,2, 95% CI= 0,517-9,594; RR 1,5, 95% CI= 0,655-3,623). Porphyromonas gingivalis dan Prevotella
INTRODUCTION

Dental caries is characterized by the progressive demineralization of enamel, following acid metabolism by the bacteria.\textsuperscript{1} The Indonesian population in range of age 15 years old or older, 71.2\% had caries and 52.3\% cases are untreated.\textsuperscript{2} The untreated dental caries could lead into necrotic pulp, and infection of the root canal system resulting a disruption at the apical.\textsuperscript{3-5} In some case, the tooth become sensitive that is caused by hyperemia, edema and inflammation of the apical periodontal.\textsuperscript{6}

Endodontic and periodontal infections are commonly found preceded by caries process and associated with anaerobic bacteria, including that of the black-pigmented bacteria,\textit{Porphyromonas spp.} and\textit{Prevotella spp.}.\textsuperscript{7,9} The most frequently identified in acute and chronic apical periodontitis are\textit{Porphyromonas gingivalis} and\textit{Prevotella intermedia}.\textsuperscript{9,10} As gram-negative bacteria, both bacteria have lipopolysaccharide (LPS) in the cell wall, which can induce macrophage to release pro-inflammatory cytokines such as interleukin-8 (IL-8), IL-1β, interferon gamma (IFN-γ) and tumor necrosis factor alpha (TNF-α), causing tissue inflammation and bone resorption.\textsuperscript{7,11} The bacteria also have exopolysaccharides (EPS) which can enhance their virulence.\textsuperscript{12}\textit{Porphyromonas gingivalis} (\textit{P. gingivalis}) has occurred between family members\textsuperscript{13} and the bacteria presentation in periodontitis tissue also indicative of alveolar bone loss.\textsuperscript{14}\textit{Prevotella intermedia} (\textit{P. intermedia}) has ability to invade and evade the host innate response, so the possibility of infection will be increased.\textsuperscript{14} However, the role of both bacteria in periapical granuloma have not been elucidated.

The presence of periodontal pathogen may affected by ethnic and geography.\textsuperscript{13} In Indonesia, the presence of\textit{P. gingivalis} and\textit{P. intermedia} are quite high in oral cavity, but there is lack information concerning the bacteria of chronic periapical tissue due to dental caries. The aims of study were to determine the presence of\textit{Porphyromonas gingivalis} and\textit{Prevotella intermedia} in dental caries with necrotic pulp and to determine its relation to periapical granuloma.

MATERIALS AND METHODS

Chronic periapical tissue of permanent teeth from dental caries patients aged 17-57 years old with normal albumin levels and no anemia at Dr. Moewardi General Hospital, in Surakarta, Indonesia were assayed in the study. All patients had no antibiotic and or immunosuppressant therapy prior the tooth extraction. None patient had systemic disease. The sample size was 9 in every group, calculated as previously published.\textsuperscript{16} The sample used was doubled for each group. Finally, the total sample used was 36, were classified into two groups, with and without periapical granuloma, based on following dental radiographic criteria: well-circumscribed radiolucent periapical lesion attached to the root apex and measured less than 1 cm\textsuperscript{6,17} (Figure 1A and 1B).

![Figure 1](image1.png)

Figure 1. The radiographic of tooth (A) without periapical granuloma, (B) with periapical granuloma (radiolucent).

Kata kunci: Karies gigi, pulpa nekrosis, granuloma periapikal, \textit{Prevotella intermedia}, \textit{Porphyromonas gingivalis}

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Table 1. Age, gender, dental element and radiographic of dental caries patients in General Hospital Dr. Moewardi in Surakarta.

<table>
<thead>
<tr>
<th>Age</th>
<th>With periapical granuloma (n=18)</th>
<th>Without periapical granuloma (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
</tr>
<tr>
<td>&lt;21</td>
<td>5</td>
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<td>&gt;50</td>
<td>2</td>
<td>11.1</td>
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<tr>
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</tr>
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<td>0</td>
</tr>
<tr>
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<td>7</td>
<td>38.9</td>
</tr>
<tr>
<td>Quadrant 4</td>
<td>7</td>
<td>38.9</td>
</tr>
</tbody>
</table>

Figure 2. A sample of electrophoresis result from PCR assay to detect the presentation of *P. gingivalis*’s DNA. PCR results from sample no. 19-25 were shown. M= VC 100bp Plus DNA Ladder (Vivantis, Selangor, Malaysia). *P. gingivalis*’s DNA was detected in sample no. 19-22 and 24-25.

Figure 3. A sample of electrophoresis result from PCR assay to detect the presentation of *P. intermedia*’s DNA. PCR results from sample no. 19-25 were shown. M= VC 100bp Plus DNA Ladder (Vivantis). *P. intermedia*’s DNA was detected in sample no. 19-22 and 24-25.
Approval was obtained from institutional ethical committee review boards of the Faculty of Medicine of Universitas Sebelas Maret and Dr. Moewardi General Hospital in Surakarta, Indonesia. Informed consent was obtained from all patients involved in the study. Data including age, gender, dental elements and dental radiographic were obtained from all patients.

The caries tooth from each patient was extracted and the chronic periapical tissue was swabbed. The swab was then smeared on blood agar medium and incorporated into anaerobic jar with Gas generating kit (Thermo Scientific, Hampshire, United Kingdom) at 37° C for 7-14 days. The present study was focused on P. gingivalis and P. intermedia, so only the black-pigmented bacteria colonies’ DNA were extracted using a High Pure PCR Template Preparation Kit (Roche Molecular Diagnostics, Mannheim, Germany), according to the manufacturer’s instructions. The P. gingivalis’ DNA was detected using 5’ - AGG CTT CAG GCC ATA CTG CG - 3’ and 5’ - ACT GTT AGC AAC TAC CGA TGT - 3’ set primer. Briefly, the PCR reaction was performed by initial denaturation at 94°C for 5 minutes, 40 cycles of 94°C for 45 seconds, 66°C for 1 minute and 72°C for 2 minutes, followed by a final extension at 72°C for 10 minutes. The P. intermedia’s DNA was detected using 5’ - TTT GTT GGG GGG GAG TAA AGC - 3’ and 5’ - TCA ACA TCT CTG TAT CCT GCG T’ - 3’ set primer. The PCR reaction was performed by initial denaturation at 94°C for 5 minutes, 40 cycles of 94°C for 45 seconds, 62°C for 1 minute and 72°C for 2 minutes followed by a final extension at 72°C for 10 minutes. All PCR reaction was performed using Ready To Go PCR Bead (Promega, Buckinghamshire, United Kingdom). The PCR products were then subjected to electrophoresis in 1% agarose gels, stained with Ethidium Bromide, and visualized under ultraviolet illumination. The data was analyzed by SPSS version 16 software (SPSS, Chicago, IL). A 95% confidence interval (CI) was used for all data analysis.

RESULTS

Thirty-six dental caries patients (19 women and 17 men) with necrotic pulp were agreed to participate. The mean age of the patients studied was 35.7 years old (in range of 17 to 57 years old) and the most frequent element was derived from 4th quadrant (38.9%, 14/36). Periapical granuloma was found in 77.8% (14/18) women (OR 5.5, 95% CI= 1.277-23.693; RR 2.5, 95% CI= 1.025-6.100) and 22.2% (4/18) men (OR 0.2; 95% CI= 0.042-0.783; RR 0.40; 95% CI= 0.164-0.976) (Table 1).

Twenty-five of the 36 (69.4%) samples had black-pigmented bacteria. These bacteria were identified in 77.8% (14/18) periapical granuloma’s samples (OR 2.2, 95% CI= 0.517-9.594; RR 1.3, 95% CI=0.817-1.983) and 61.1% (11/18) of non periapical granuloma’s samples (OR 0.4, 95% CI= 0.104-1.934; RR 0.8, 95% CI= 0.504-1.224). P. gingivalis’s DNA was detected (Figure 2) in 64.3% (9/14) of black colonies from periapical granuloma’s samples and 63.6% (7/11) of black colonies from non periapical granuloma’s samples (p= 1.00) and had no association with periapical granuloma formation (OR 1.0, 95% CI= 0.271-3.694). P. intermedia’s DNA was detected (Figure 3) in 57.1% (8/14) of black colonies from periapical granuloma’s samples (OR 1.6, 95% CI= 0.418-5.903; RR 1.3, 95% CI=0.653-2.393). Co-infection of P. gingivalis and P. intermedia was detected only in 33.3% (6/18) periapical granuloma’s samples (OR 1.0, 95% CI= 0.250-3.999; RR 1.0, 95% CI= 0.500-1.999).

DISCUSSION

Dental caries with necrotic pulp is a multi-causal disease that attacks the enamel and reach tooth pulp chamber, causing destruction structure of the tooth, and open the door to bacterial infection.1,4 The bacterial infection can activate host immune response to isolate and eradicate microorganisms or chronic irritants, causing periapical granuloma, an inflammatory reaction in the apex of non-vital tooth. The inflammatory tissue of periapical granuloma (containing macrophages, polymorphonuclear leukocytes and lymphocytes) is covered by epithelial cells and ultimately results in destruction of the alveolar bone surrounding the tooth.7,8

The P. gingivalis and P. intermedia already known dominant in primary endodontic infection due to its content, LPS.8 LPS induces pulp fibroblast and osteoblast to produce IL-8. The IL-8 then attracts and activates polymorphonuclear leukocytes to surround and kills the bacteria, stimulates osteoclast activity and causes pain symptom in periapical lesion. The LPS also activates macrophages through CD14 receptor to produce pro-inflammatory mediators, IL-1 and TNF-α. IL-1 then induces the production of IL-12, TNF-α, IFN-γ and IL-1 itself. In human periapical lesion, IL-1β, one variant of IL-1, is predominant and stimulates T-lymphocytes; enhances bone resorption and inhibits bone formation. The macrophages and T-lymphocytes in the inflammatory tissue also produce TNF-α. The TNF-α activates the macrophages, T-lymphocytes and natural killer cells and also stimulates bone resorption. However, if the bacteria (P. gingivalis and or P. intermedia) are difficult to be eliminated, T-lymphocyte would lead B-lymphocyte activation, to synthesize the antibody to bacterial antigen. The bacterial-antibody formation will attract activated-macrophages to surround and phagocyte the formation. Finally, all reaction will causes periapical granuloma formation in the root of the tooth.7,16-20

P. gingivalis and P. intermedia are frequently detected in intraradicular15,16 and the numbers of these bacteria are increase in the disease site compared with healthy site.9 In the present study, P. gingivalis and P. intermedia could be isolated from dental caries tissue, both of with and without periapical granuloma, consistent with previous reports,7,8,21
however, only *P. intermedia* was associated with periapical granuloma.

In the present study, periapical granuloma was more likely found in women than in men. LPS of bacteria induces production of IL-1β and TNF-α. These pro-inflammatory cytokines could be influenced by sex hormone. Sex hormone, especially estrogen, already known significantly reduces *P. gingivalis* bacteria compared with *P. intermedia*. Testosterone also could reduce the expression of TNF-α and IL-1β, so reduce the inflammation process. The study revealed that the presence of *P. gingivalis* and *P. intermedia* were confirmed in periapical granuloma and could be isolated in dental caries tissue; however, only *P. intermedia* had association with periapical granuloma. Further studies are needed to confirm and extend our findings.

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