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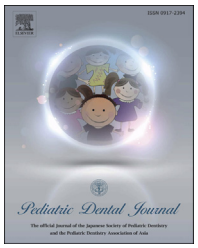

Case Report

Black pigmentation in primary dentition: Case report and literature review

Yukiko Takashima, Yuki Matsumi, Yoshie Yamasaki, Keiko Hirano, Kanako Yanagida, Kazuyo Fujita, Michiyo Matsumoto-Nakano *

Department of Pediatric Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan

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ABSTRACT

Black tooth staining is an extrinsic discoloration found in both primary and permanent dentition, and seen as dark pigmented lines extending to the gingival margin or an incomplete coalescence of dark dots that rarely extend beyond the cervical third of the crown. An association between black tooth staining and *Actinomyces* bacterial strains has been reported, while black-pigmented bacteria associated with such staining are known to be harbored in the oral cavity. *Prevotella intermedia* and *Prevotella nigrescens* are black-pigmented bacteria known to be dependent on the heme portion of hemoglobin as an iron source required for their growth. Recently, developments in molecular biological techniques have enabled rapid and easy detection of periodontopathic bacterial species using bacterial DNA extracted from oral specimens, such as plaque and saliva. Here, we report a case of black pigmentary deposition identified on all teeth of a 2-year-old girl, as well as the results of analysis of the distribution of oral bacteria in saliva and plaque specimens obtained from the patient using a molecular biological technique. In addition, a literature search found a case of disease related to the oral bacteria detected in our patient. We concluded that the bacteria detected in this case may have a strong relationship with black pigmentation, although the route of bacterial infection and cause of staining remain to be elucidated.

1. Introduction

Tooth discoloration is a common dental finding that is associated with clinical and esthetic problems [1]. Pigmentary deposition is caused by various deposits on teeth, such as coloring by exogenous food, drink, or metal; endogenous systemic disease and associated pulp necrosis; or administered medicine [2]. Black tooth staining is an extrinsic discoloration seen in both primary and permanent dentition, and

* *Corresponding author*. Department of Pediatric Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Science, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8525, Japan. Tel.: +81 86 235 6715; fax: +81 86 235 6719.
E-mail address: mnakano@cc.okayama-u.ac.jp (M. Matsumoto-Nakano).
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shown as pigmented dark lines extending to the gingival margin or incomplete coalescence of dark dots that rarely extend beyond the cervical third of the crown [3,4]. Such staining has been speculated to be a form of dental plaque different from other types based on contents that include insoluble iron salt and high levels of calcium and phosphate [4,5]. The black material is a ferric compound, most likely ferric sulfide, that arises from the interaction of hydrogen sulfide with iron in saliva or gingival fluid [4].

An association between black tooth staining and *Actinomyces* bacterial strains has been reported [6,7]. In addition, black-pigmented bacteria normally harbored in the oral cavity are associated with such black stains [8]. *Prevotella intermedia* and *Prevotella nigrescens*, black-pigmented bacteria, are dependent on the heme portion of hemoglobin as an iron source required for bacterial growth [9], and black pigmentation results from associated chemical reactions.

Developments in molecular biological techniques have enabled rapid and easy detection of periodontopathic bacterial species using bacterial DNA extracted from oral specimens, such as plaque and saliva [10]. Based on analyses of specimens collected from children and adolescents, the following 10 periodontal species are considered to be associated with periodontal diseases: *Porphyromonas gingivalis*, *Tannerella forsythia*, *Campylobacter rectus*, *Eikenella corrodens*, *Aggregatibacter actinomycetemcomitans*, *Capnocytophaga ochracea*, *Capnocytophaga sputigena*, and *Treponema denticola*, as well as the black-pigmented bacteria *P. intermedia* and *P. nigrescens*. Other studies have found that polymerase chain reaction (PCR) methods are quite sensitive and rapid for determining the prevalence of periodontal bacteria [11,12], and it has been speculated that a PCR assay system would be a useful tool for analyzing the role of the black-pigmented *Prevotella* species in the mouth [8].

Herein, we report a case of black pigmentary deposition identified on all teeth of a 2-year-old girl as well as our analysis of the distribution of related oral bacteria in saliva specimens using a molecular biological technique.

2. Case report

The protocol of the present study was approved by the Ethics Committee of Okayama University (No. 702). A girl of age 2 years 2 months visited our clinic for detailed examination of pigmentation on the teeth, initially noticed by her guardian in January 2012 when the patient was approximately 1 year 5 months of age. An intraoral examination showed that the first molar tooth had not yet emerged, with no caries lesions or periodontal problems. Wide-ranging black pigmentation was recognized on the surfaces of all teeth (Fig. 1). The medical history and dietary habits of the patient were unremarkable, there was no family history of dental diseases, and she had not taken any medicine up to that time. Based on these findings, we diagnosed the black pigmentation as foreign precipitation and decided to perform ultrasonic scaling and tooth flank polishing every 3 months to remove the pigmentation. Fig. 1B shows the appearance after treatment.

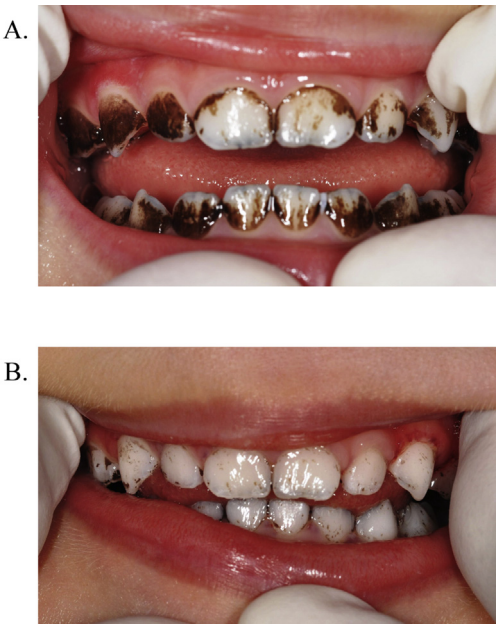

Fig. 1 – Oral photographs. (A) First visit. (B) After scaling and polishing.

3. Identification of oral bacteria in saliva specimens and review of literature

Whole saliva specimens were collected from the patient at 2 years 2 months, 2 years 11 months, and 3 years 2 months of age. Subgingival dental plaque was also collected from the buccal side of both maxillary second primary molar teeth at 3 years 2 months of age and mixed with 1 ml of sterile saline. Microbiological analyses were performed as previously described [13]. Briefly, the specimens in sterile saline were centrifuged at 15,000 rpm for 5 min to pellet the bacterial cells, and then bacterial genomic DNA was extracted from each pellet using a DNA isolation kit (QIAGEN Sciences, Germantown, MD, USA). The samples were frozen at −20 °C and stored until use. Amplification of 16S ribosomal DNA (rDNA) occurred under standardized conditions using previously published universal eubacterial primers. The primers used were PA 5′-AGA GTT TGA TCC TGG CTC AG-3′ and PD 5′-GTA TTA CCG CGG CTG CTG-3′. Using a hot-start protocol, the samples were denatured at 95 °C for 15 min, followed by amplification consisting of a denaturation step at 95 °C for 40 s, annealing at 58 °C for 1 min, and elongation at 72 °C for 1 min. A total of 30 cycles was performed, followed by a final elongation step at 72 °C for 10 min. The amplified fragments were cloned into a pGEM-T Easy vector (Promega, Madison, WI, USA) and clones from each sample were randomly chosen. Sequencing was performed by Fasmac Co., Ltd. (Atsugi, Kanagawa, Japan). For each of the 16S rDNA fragments, the sequences of all isolates were compared using a Japanese DNA database (http://www.ddbj.nig.ac.jp/index-j.html) and their identities determined.

The numbers of mutans streptococci and lactobacilli organisms in the specimens were measured using previously described methods [14,15]. Briefly, the saliva and plaque