An Analysis of the Periodontopathic Bacteria Involved in Extrinsic Discoloration of Teeth

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Abstract

Recent dental esthetics are paying attention to whitening. Many people take an interest in tooth color, and wish to remove extrinsic discoloration. While a relationship between tooth discoloration and periodontopathic bacterial prevalence has been suggested, such a relationship has not been clearly elucidated. The aim of the present study was to clarify the relationship between the periodontopathic bacteria present in plaque and tooth discoloration. Plaque samples were taken from 54 subjects whose tooth color was evaluated using a shade guide. The subjects were then divided into a white group (30 women) and a discolored group (24 women). Using bacterial DNA prepared from the plaque samples as a template, polymerase chain reaction was performed with specific primers to identify the presence of five periodontopathic bacteria (Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Tannerella forsythensis, Treponema denticola and Prevotella intermedia). The numbers of the bacterial species were counted and the percentage of the subjects that were carriers of each bacterium was calculated. In addition, the mean number (± SE) of periodontopathic bacterial species per person was calculated as 1.23 (± 0.15) and 2.04 (± 0.21) in the white and discolored groups, respectively, and there was a significant difference between these two groups (p<0.01). We thus found a positive relationship between superficial tooth color and the number of periodontopathic bacterial species.

Keywords: Teeth color, Extrinsic discoloration, Shade guide, Periodontopathic bacterial species, Polymerase chain reaction.

Introduction

The cause of tooth discoloration can be intrinsic or extrinsic [1]. Intrinsic tooth discoloration is the result of aging, exposure to tetracycline antibiotics, excessive fluoride intake during the enamel formation period, or other factors. Extrinsic discoloration is known to be the result of various factors including food and drink, salivary components, smoking, and bacteria. While a relationship between tooth discoloration and periodontopathic bacterial prevalence has been suggested, its exact mechanism has not yet been well clarified. In this study, we focused on the relationship between tooth discoloration and periodontopathic bacteria by examining five types of periodontopathic bacteria involved in the formation of biofilm [2,3].

The aim of the present study was to clarify the relationship between the periodontopathic bacteria present within intraoral plaque and tooth discoloration by investigating tooth color and periodontopathic bacterial prevalence in healthy, young adult women. On the basis of this examination, the relationship found between tooth color and bacterial prevalence was reported here.

Material and methods

The subjects were 54 students of the Nippon Dental University College at Niigata (aged 18–22 years, mean age: 18.98 ± 1.11 years, all female and healthy). This study was conducted following review and approval by the ethical review board of the Nippon Dental University, College at Niigata (NDUC-58) in full accordance with ethical principles including the World Medical Association Declaration of Helsinki. The subjects provided written informed consent and agreed to provide plaque samples and to be examined for intraoral findings (tooth coloration). Tooth color was measured in the present study using a composite resin shade guide (Clearfil AP-X Accessory, Kuraray Noritake Dental Inc., Tokyo, Japan) (Figure 1), which is a convenient method for tooth color matching [4]. A typical shade guide was used in the present study as a screening test to measure tooth color. The teeth color of the subjects were estimated by the shade guides (Figure 1). The colors shown, “XL” indicates clear white; shades A1 to A3 are reddish-browns where the color darkens in the order of A1 to A2 to A3; shades B2 and B3 are reddish-yellows, where B3 is a darker shade than B2. Shades may differ slightly depending on the manufacturer and the same manufacturer may adopt a variety of composite resin systems. These shade guides are widely available and easy to use, formed essentially of a layer of ivory porcelain and a layer of enamel porcelain. Thus, it is difficult
PCR product size, as shown in Figure 2. Provided by Dr. Mikami M.) was performed, and provided each DNA size of the PCR product was confirmed using 2% agarose gel electrophoresis. Positive control in each plaque sample, the DNA size of the PCR product was described [7,8]. To identify the periodontopathic bacteria in plaque [7,8]. PCR conditions were as previously described [7,8]. To identify the periodontopathic bacteria (Table 1) to identify the periodontopathic bacteria, we investigated periodontopathic bacterial species in the dental plaques of all subjects using polymerase chain reaction (PCR) of periodontopathic bacterial DNA prepared from sampled plaques of the subjects, with primers specific to the five bacteria [Aggregatibacter actinomycetemcomitans (A. a.), Porphyromonas gingivalis (P. g.), Tannerella forsythensis (T. f.), Treponema denticola (T. d.) and Prevotella intermedia (P. i.)](Table 1)]. Plaque sampling and DNA extraction were performed in accordance with the methods described by Shimomura-Kuroki et al [7,8]. The DNA templates were prepared from the plaque samples, and used for the PCR with PCR SuperMix (Invitrogen, Carlsbad, CA, USA) using specific primers to each of the five bacteria [Aggregatibacter actinomycetemcomitans (A. a.), Porphyromonas gingivalis (P. g.), Tannerella forsythensis (T. f.), Treponema denticola (T. d.) and Prevotella intermedia (P. i.)](Table 1)].

Results and Discussion

The subject numbers of the teeth color based on the shade guides are shown in Figure 3. The subjects were thus divided accordingly into a white group (XL, A1, and A2; n = 30) and a discolored group (A3, B2, and B3; n = 24), which were then used for analysis.

The percentages of the 54 subjects that were carriers for each bacterium were as follows: P. g., 92.6% (50 carriers); T. d., 29.5% (16 carriers); T. f., 16.7% (9 carriers); P. i., 9.3% (5 carriers); and A. a., 7.4% (4 carriers), as shown in Table 2. Fifty subjects (92.6%) had at least one type of periodontopathic bacteria, while four subjects had none of the five analyzed periodontopathic bacteria. These four subjects were therefore not P. g. carriers. In addition, to investigate a relationship between tooth color and the presence of periodontopathic bacteria, the percentage of carriers of each bacterium was calculated for each of the white and the discolored groups (Figure 4). The percentage of P. g. carriers in the white group was more than 90%, and was similar to that of the discolored group. However, the prevalence of four other bacteria (A. a., T. f., T. d. and P. i.) in the discolored group


Table 1: Species-specific primers for the detection of bacteria by PCR

<table>
<thead>
<tr>
<th>Primer pairs (5’-3’)</th>
<th>Base Position (bp)</th>
</tr>
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<tbody>
<tr>
<td>Aggregatibacter actinomycetemcomitans (A. a.)</td>
<td>478-1,034 (557)</td>
</tr>
<tr>
<td>AAA CCC ATC TCT GAG TTT TTC TTC</td>
<td></td>
</tr>
<tr>
<td>ATG CCA ACT TGA CGT TAA AT</td>
<td></td>
</tr>
<tr>
<td>Porphyromonas gingivalis (P. g.)</td>
<td>729-1,132 (404)</td>
</tr>
<tr>
<td>AGG CAG CTT GCC ATA CTG CG</td>
<td></td>
</tr>
<tr>
<td>ACT GTT AGC AAC TAC CGA TGT</td>
<td></td>
</tr>
<tr>
<td>Tannerella forsythensis (T. f.)</td>
<td>120-760 (641)</td>
</tr>
<tr>
<td>GCG TAT GTA ACC TGC CCG CA</td>
<td></td>
</tr>
<tr>
<td>TGC TTC AGT GTC AGT TAT ACC T</td>
<td></td>
</tr>
<tr>
<td>Treponema denticola (T. d.)</td>
<td>193-508 (316)</td>
</tr>
<tr>
<td>TAA TAC CGA ATG TGC TTA TCA TCA</td>
<td></td>
</tr>
<tr>
<td>TCA AAG AAG CAT TCC TTC TCC TTA</td>
<td></td>
</tr>
<tr>
<td>Prevotella intermedia (P. i.)</td>
<td>458-1,032 (575)</td>
</tr>
<tr>
<td>TTT GTT GGG GAG TAA AGC GGG</td>
<td></td>
</tr>
<tr>
<td>TCA ACA TCT CGT TAT CCT GGG T</td>
<td></td>
</tr>
</tbody>
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PCR, polymerase chain reaction

Figure 1: Tooth shade guides were used in this investigation to select natural teeth color using [5,6]. For accurate shade evaluation, discolored teeth and natural teeth need to be analyzed using precise equipment [5,6]. However, in the present study we used a shade guide because of the large subject sample and the simplicity of the tooth color evaluation. We also considered that we should clarify whether tooth discoloration is related to the prevalence of periodontopathic bacteria by roughly dividing subjects into white and discolored tooth groups based primarily on tooth brightness.

Next, to estimate the percentage of carriers for each periodontopathic bacteria, we investigated periodontopathic bacterial species in the dental plaques of all subjects using polymerase chain reaction (PCR) of periodontopathic bacterial DNA prepared from sampled plaques of the subjects, with primers specific to the five bacteria [Aggregatibacter actinomycetemcomitans (A. a.), Porphyromonas gingivalis (P. g.), Tannerella forsythensis (T. f.), Treponema denticola (T. d.) and Prevotella intermedia (P. i.)](Table 1)].

Figure 2: Agarose-electrophoresis of polymerase chain reaction (PCR) products from periodontopathic bacteria. Single DNA bands of the predicted size were obtained by PCR using specific primers as described in Materials and methods. Lane 1, 100 base pair (bp) DNA ladder; lane 2, Aggregatibacter actinomycetemcomitans (A. a.), 557 bp; lane 3, Porphyromonas gingivalis (P. g.), 404 bp; lane 4, Tannerella forsythensis (T. f.). 641 bp; lane 5, Treponema denticola (T. d.), 316 bp; lane 6, Prevotella intermedia (P. i.), 575 bp. Molecular weights are shown on the left.

Figure 3: The number of subjects grouped by tooth color according to the shade guide. The subjects were divided into white (30 women) and discolored (24 women) groups.
was approximately 2-5 times that in the white group, as shown in Figure 4. These results suggested an absolute requirement for the presence of other periodontopathic bacteria in subjects for teeth discoloration. Finally, we calculated the average number of periodontopathic bacterial species per person (p.p.) in the two color groups. The mean (± SE) value of the white group was 1.23 (± 0.13) types p.p. and that of the discolored group was 2.04 (± 0.21) types p.p. A t-test that was used to compare the white and discolored groups revealed a significant difference (p < 0.01) between them (Figure 5). The finding that there was a significantly greater number of species in the discolored compared to the white group, suggested that tooth discoloration and the number of different periodontopathic bacterial species present are related. Figure 4 clearly showed that the prevalence of four bacteria (A. a., T. f., T. d. and P. i.) in the discolored group was more than that in the white group. In particular, XL and A1 subjects in the white group was never the carriers of A. a., T. f. and P. i., as shown in Table 2. This result suggested A. a., T. f. and P. i. may induce the discoloration. However, tooth discoloration is the result of not only of bacteria but also of various other known factors including food, drink, and salivary components. In the present study, for example, four subjects were found to have A. a., three of whom were in the discolored group and presented with tea stains, which are caused by components of tea or foods (data not shown). Black stain, which is a form of bacterial discoloration, is reportedly caused by iron ions and A. a., which, as mentioned above, is a type of periodontopathic bacterium [2]. The results of the present study also indicated the possibility that A. a. is involved in food-related discoloration of teeth. It is likely that teeth discoloration is caused by T. f., T. d. and P. i. together with other factors, including other bacterial species. Further investigation is necessary to clarify the cause-and-effect relation of many factors such as food, drink, salivary components, and periodontopathic bacteria to teeth discoloration. A previous report of mechanical tooth cleaning and other procedures in young adults with mild periodontopathic bacterium-related gingivitis or normal gingiva found that intraoral test values and periodontopathic bacterial species numbers decreased following such treatment [8,9]. The combined results of that previous report and of the present study suggest that periodontal disease prevention and bacterial discoloration prevention can be achieved by maintaining a clean intraoral environment through routine checkups and precautions at dental clinics, as well as by daily oral hygiene.

Conclusion

The present study clarified the relationship between tooth
discoloration and the prevalence of periodontopathic bacterial species. The results of the study indicated that maintaining a clean intraoral environment may prevent both periodontal disease and tooth discoloration.

**Acknowledgment**

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**References**