Effect of chlorhexidine varnish application on mutans streptococci counts in orthodontic patients

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Introduction: Enamel demineralization occurs frequently around orthodontic brackets. High levels of mutans streptococci (MS) increase the risk of caries. The hypotheses tested in this study were that high-frequency application of 40% chlorhexidine varnish (40%CHX) results in lower levels of MS in plaque of orthodontic patients compared with low-frequency application, and that bimonthly application of 40%CHX results in lower levels of MS in plaque compared with 1% chlorhexidine/1% thymol varnish (1%CHX). Methods: Eighty adolescent patients were randomly allocated to 4 treatment groups: monthly, bimonthly, and quarterly 40%CHX application, and bimonthly application of 1%CHX. Plaque samples were collected monthly and processed for MS counts. Twenty patients dropped out before the evaluation time at 1 month. Data from the remaining 60 adolescents were analyzed by using linear regression models with the plaque bacterial ratio (PBRx) as the dependent variable. The trial was ended after 5 months because of a high number of dropouts. Results: High-frequency application of 40%CHX did not provide lower mean PBRx than low-frequency application. Mean PBRx after 40%CHX application was lower than 1%CHX after 1 month (p < .002). This reduction was undiscernible 2 months after the application. Conclusions: 40%CHX application results in greater reduction of MS in plaque than 1%CHX a month after application. A follow-up study with a larger study population is warranted to test the first hypothesis. (Am J Orthod Dentofacial Orthop 2008;133:435-9)

Enamel demineralization occurs frequently around orthodontic brackets.1 Fixed orthodontic appliances create extra retention sites, leading to more mutans streptococci (MS) soon after the start of treatment2,3 and thus to increased risk for caries development.4

MS are particularly sensitive to chlorhexidine (CHX).5 The most effective mode of CHX application appears to be the varnish in a concentration of 40%,6,7 Applications of 40% CHX and 1% CHX /1% thymol varnish suppressed the MS for several months in nonorthodontic patients.2,9 In orthodontic patients, however, CHX varnish might be less effective, because the increase in plaque retention sites makes it more difficult to suppress MS and hastens the recolonization of the dentition.5

The effect of 1% CHX /1% thymol varnish (Cervitec) in orthodontic patients was studied10-13 and showed decreased levels of MS. The application of 40% CHX varnish in orthodontic patients, however, has not yet been studied thoroughly.

The research hypotheses tested in this pilot randomized clinical trial were that high-frequency application of 40% CHX varnish results in lower levels of MS in plaque around orthodontic brackets than low-frequency application over a 6-month period, and that bimonthly application of 40% CHX varnish results in lower levels of MS in plaque around the bracket base than bimonthly application of 1% CHX/1% thymol varnish over a 6-month period.

MATERIAL AND METHODS

The study was approved by the ethical committee of the Radboud University Nijmegen Medical Centre (protocol number 2003/170). Informed consent was signed by both participants and guardians. The inclusion criteria were healthy patients 12 to 15 years of age with full fixed appliances for at least 3 months. Standard oral hygiene instructions (rinsing with 0.05%
sodium fluoride mouth rinse daily and brushing with fluoride toothpaste 3 times a day) were given.

Four groups were formed (Fig 1). Monthly (group A), bimonthly (group B), and quarterly (group C) application of 40% CHX varnish, and bimonthly application of 1% CHX/1% thymol varnish (group D). Before the start of the study, the initial MS counts were calculated from 1 mL of stimulated saliva.

By using a validated computer program (Trial Balance, Nestle, Switzerland), the 80 participants were randomly allocated to 4 groups according to 3 stratification criteria: sex, initial MS counts in saliva, and mean decayed/filled/surface (DFS) scores at the start of orthodontic treatment.

Twenty participants dropped out before the evaluation time at 1 month, resulting in a final sample of 60 participants (38 girls, 22 boys).

The CHX varnishes used were EC40 (Biodent, Arnhem, Netherlands) and Cervitec (Ivoclar Vivadent, Schaar, Lichtenstein). EC40 consists of 40% CHX, 36% sandarac, and 24% ethanol (40%CHX). Cervitec consists of 1% CHX, 1% thymol, 10% polyvinyl butyral, ethanol, and ethyl acetate (1%CHX). A thin layer of varnish was applied around the brackets on the enamel surface of the teeth. The manufacturers’ instructions were followed, except that the 40%CHX was not removed. The patients were instructed to refrain from any oral hygiene activity for 3 hours.

Plaque samples were collected cervically from the bracket on the incisors and the canines in both jaws at all evaluation times (Fig 1) by using 2 sterile metal pins. The samples were homogenized by vortex mixing for 60 seconds, diluted in PBS, and plated on selective MS plates. The samples were plated for total bacteria counts on nonselective blood agar plates.

**Statistical analysis**

The analyses were based on the per protocol subject method. At T5, the number of participants eligible for analysis dropped from 60 to 27, necessitating stopping the trial at T5.

Since plaque collection was unweighted, MS scores were divided by the total bacteria score of the sample. For each subject at each time point, this resulted in the plaque bacterial ratio (PBRx):

\[
\text{plaque bacterial ratio } T_x = \frac{\text{MS score } T_x}{\text{total bacterial score } T_x}
\]

For all groups, paired \( t \) tests were used to describe the longitudinal changes in the PBRx, comparing the measurements from T1 until T4 with T0. All comparisons of the experimental groups were based on linear regression analysis, with indicator variables to denote the membership of a group as the independent variable. The dependent variable was the transformed PBRx. The transformed baseline PBR0 was included as a covariate.

To assess the effect of the application frequencies of 40%CHX, groups A, B, and C were included in the regression model, with group C as the reference. To analyze the difference between 40%CHX and 1%CHX varnish, regression models were formulated including groups B and D only. At T1, all groups had received 1 application. Therefore, at this evaluation, the combined groups A, B, and C could be compared with group D.

**RESULTS**

Forty-two patients (70%) had more than \( 10^6 \) MS colony-forming units (CFU) per milliliter of saliva at the start of the trial. Nine patients (15%) had scores
between $10^5$ and $10^6$ CFU per milliliter, and 9 patients (15%) had scores lower than $10^5$ MS CFU per milliliter in saliva.

Table I. Mean PBRx and standard errors of monthly (A), bimonthly (B), and quarterly (C) applications of 40%CHX, and bimonthly application of 1%CHX (D) by evaluation moments T0, T1, T2, T3, T4 (n = number of participants)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
<th>n</th>
<th>Mean</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>15</td>
<td>0.40</td>
<td>0.07</td>
<td>14</td>
<td>0.42</td>
<td>0.06</td>
<td>14</td>
<td>0.36</td>
<td>0.05</td>
<td>13</td>
<td>0.35</td>
<td>0.05</td>
</tr>
<tr>
<td>B</td>
<td>15</td>
<td>0.55</td>
<td>0.07</td>
<td>13</td>
<td>0.34</td>
<td>0.05</td>
<td>12</td>
<td>0.45</td>
<td>0.06</td>
<td>8</td>
<td>0.29</td>
<td>0.06</td>
</tr>
<tr>
<td>C</td>
<td>13</td>
<td>0.62</td>
<td>0.09</td>
<td>12</td>
<td>0.35</td>
<td>0.06</td>
<td>11</td>
<td>0.41</td>
<td>0.07</td>
<td>9</td>
<td>0.55</td>
<td>0.07</td>
</tr>
<tr>
<td>D</td>
<td>17</td>
<td>0.54</td>
<td>0.07</td>
<td>17</td>
<td>0.58</td>
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<td>0.6</td>
<td>0.07</td>
<td>14</td>
<td>0.54</td>
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Fig 2. Changes in mean PBRx (Tx-T0) and standard errors (SE) of monthly (A), bimonthly (B), and quarterly (C) applications of 40%CHX, and bimonthly application of 1%CHX (D) by evaluation time (T1, T2, T3, T4). Paintbrush, application of 40%CHX; small brush, application of 1%CHX.

The mean PBRx and standard errors over time are shown in Table I and Figure 2, and the changes in mean PBRx over time are given in Table II. When groups A, B, and C were combined into 1 group at T1, an insignificant reduction in mean PBRx compared with T0 ($P = .07$, 95% CI for reduction = [37%; -1%]) was seen.

The effects of the application frequency of 40%CHX are shown in Table III. Group C was used as the reference group. At T3, the mean PBRx in groups A ($P = .049$) and B ($P = .008$) was statistically significant lower compared with group C.
Statistically significant differences were found in the reduction of the mean PBRx between groups B (40%CHX) and D (1%CHX) at T1 (P = 0.03) and T3 (P = 0.005), showing a lower mean PBRx in group B compared with group D. At T1, group B had a 34% higher reduction in mean PBRx compared with group D (95% CI for reduction = [4%; 54%]). At T3, group B had a 44% higher reduction compared with group D (95% CI for reduction = [18%; 62%]). No statistically significant differences were found at T2 (P = 0.47) and T4 (P = 0.12).

Groups A, B, and C, having received 1 application of 40%CHX, were combined and compared with group D (1%CHX) at T1. The reduction in mean PBRx for the combined group was 37% and was statistically significant (P = 0.002) (95% CI for reduction = [16%; 52%]).

**DISCUSSION**

Seventy percent of the participants had more than $10^6$ MS CFU per milliliter of saliva, denoting a strongly elevated caries risk at the start of the trial.

Collection of standardized plaque samples was extremely difficult. No attention to this problem has been given in previous studies. Saliva samples can be standardized more easily. However, for CHX varnish application, bacterial growth should be monitored in a site-specific way, since the reduction of MS in plaque is not adequately reflected in the whole saliva sample.\(^1\)\(^1\)\(^3\)\(^1\)\(^4\) Therefore, the MS counts were divided by the total bacterial counts of the same plaque sample, resulting in the PBRx.

The first hypothesis was rejected. It could not be concluded that high-frequency application of 40%CHX resulted in a greater suppression of MS than low-frequency application. No decrease in the mean PBRx was found in group A, possibly because the mean PBRx in group A at the start of the application was lower than the beginning values in groups B and C, despite the stratification of MS in saliva. Both groups B and C showed an insignificant reduction in mean PBRx a month after application (Fig 2). These groups seemed to show a tendency for lower mean PBRx after application, with higher mean PBRx at the next application but not exceeding the beginning value. Another reason for the rejection of the first hypothesis was the many dropouts.

The second hypothesis was accepted but only over a 4-month period. At T1 and T3, 40%CHX application was more effective in reducing MS counts than the 1%CHX application. In group D, the mean PBRx did not decrease after the first and second application of 1%CHX, although the group started with a high level of MS (Fig 2). The fact that 40%CHX performed better is in line with the observations of Schaeken et al\(^6\) and Attin et al.\(^8\) However, in our study, the varnish effect was indiscernible 2 months after application. In contrast, Jenatschke et al\(^15\) observed a significant reduction in MS after bimonthly application of 40%CHX in

| Table II. Effect of application on mean PBRx (Tx-T0) in percentages (Eff%), 95% CI, and P values (P%) of monthly (A), bimonthly (B), and quarterly (C) applications of 40%CHX, and bimonthly application of 1%CHX (D) by evaluation moments T1, T2, T3, T4; positive effect means increase in mean PBRx; negative effect means decrease in mean PBRx compared with T0 |
|---|---|---|---|---|---|---|---|---|---|
| Group | T1 | T2 | T3 | T4 |
| A | 52.1 | +13 | [–25...72] | 66.3 | +8 | [–28...63] | 57.4 | +9 | [–22...54] |
| B | 6.7 | –30 | [–52...3] | 29.4 | –13 | [–35...15] | 5.7 | –35 | [–59...2] |
| C | 5.4 | –39 | [–63...1] | 30.4 | –24 | [–56...33] | 99.0 | 0 | [–48...90] |
| D | 34.4 | +17 | [–17...66] | 50.2 | +16 | [–27...85] | 87.7 | +2 | [–30...51] |

| Table III. Effect (Eff%) of different frequencies of 40%CHX application on the mean PBRx, 95% CI, and P values (P%), with group C as the reference group (group A, monthly application; group B, bimonthly application; group C, quarterly application) |
|---|---|---|---|---|---|---|---|---|---|
| Group | T1 | T2 | T3 | T4 |
| A | 23.0 | +28 | [–15...92] | 90.2 | –21 | [–34...45] | 4.9 | –35 | [–58...0] |
| B | 97.1 | 0 | [–32...5] | 54.7 | +13 | [–24...66] | 0.8 | –46 | [–65...–16] |
| C | 94.2 | +1.5 | [–33...54] | 56.1 | +12 | [–25...67] |
orthodontic patients after 4 months. At debonding, the MS scores were not significantly different from those at baseline. This difference in results might have occurred because, in the study of Jenatschke et al,15 MS were scored in saliva.

CONCLUSIONS

A significantly greater reduction in MS in the plaque of patients with fixed appliances was found 1 month after 40%CHX varnish application compared with 1%CHX application. However, the varnish effect was indiscernible 2 months later. Due to the large interindividual variation, a high drop-out rate, and a low initial number of MS in the group with the highest application frequency, it was not possible to demonstrate the optimal application frequency of 40%CHX. A follow-up study is warranted to take into account these deficiencies.

REFERENCES