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The Evaluation of Streptococcus Mutans Colonization in Private School Nursery-Kindergarten Children's Toothbrush#

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Research Article

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History

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ABSTRACT

Aim: Disinfection of the toothbrush is an essential parameter for oral hygiene. As a result, especially children cannot clean their toothbrushes sufficiently after brushing their teeth. The purpose of this study was to evaluate the accumulation level of *Streptococcus mutans* colonization at private school nursery-kindergarten children's toothbrushes and evaluate the effectiveness of chlorhexidine solution in four weeks for disinfection.
Methods: This study was approved by the local ethics committee. Two hundred thirty-four private school nursery-kindergarten children were selected for this study, who's aged between 24-72 months. These selected children for this study did not have any dental, antibiotic, antimycotic treatment in the last three months. Before the study, a survey was conducted about parents' education, employment, the income. A pediatric dentist made an oral examination of these children, dental caries, fillings, and missing teeth were determined. Children were divided into two groups; toothbrushes, toothpaste, and practical solutions distilled water and %0.12 chlorhexidine gluconate solution was given in a bottle for four weeks. In addition, the toothbrushes of children were collected every week. After incubation, *Streptococcus mutans* colonization was evaluated under stereomicroscope. All the collected data were statistically evaluated.
Results: The DMF-T scores were statistically significant when evaluated with parents' education and income, but there was no statistical relationship between parents' employment and DMF-T scores. *Streptococcus mutans* colony reduction at the Chlorhexidine group was statistically significant.
Conclusion: From the first brushing, toothbrushes can be contaminated with bacteria. If the toothbrushes are not clean sufficiently, the bacteria taken from the oral environment have a chance to form a colony on the toothbrushes with the effect of moisture. In addition, Streptococcus Mutans is a bacteria that can stay on toothbrushes and cause re-infection. This study shows that toothbrush disinfection is essential to prevent bacteria from re-infection and contamination of oral flora.

Key Words: Oral Health, Disinfection, Toothbrush, Chlorhexidine, *Streptococcus Mutans*.

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Introduction

Between 24-72 months, the nursery-kindergarten period is a period in which children begin to socialize, increase their contact with the environment, and display cognitive and sensory development. As a result, children's dental needs and many of their other needs change during this period. With the developing physical and psycho-emotional structure, children participate in various activities, making them vulnerable to diseases that can be transferred from the environment.[1] During this period, children's nutritional habits also changed and accordingly, they faced many nutrition-related problems, especially dental caries. In this period, their tendency to essential is vital in acquiring tooth brushing habits.[2-4] Toothbrushes are of various qualities and features, and after fabrication, toothbrushes are sterile after packaging.[5,6] Bacteria, viruses and fungi accumulate on the surface of the brush bristles after brushing for the first 30 seconds to 4 minutes after the toothbrush is turned on. [5-10] These microorganisms are bacteria found both in the external environment and in the oral cavity. Some microorganisms can live on toothbrushes between 24 hours and 7 days.[5,6,11,12] As a result of routine use of toothbrushes, these microorganisms spread to the oral cavity in the same individual or different individuals.[13,14] Generally, toothbrushes are stored in the bathroom with other individuals from the same family. Contamination occurs as a result of contact with toothbrushes belonging to different individuals. Thus, it can be seen that microorganisms spread from one individual to another individuals. Brushing teeth at the same sink as their friends, storing toothbrushes in school lockers, or carrying them in their backpacks may all be sources of contamination in nursery and kindergarten children. Microorganisms can be transmitted from one person to another if more than one child uses the same toothbrush or changes it among themselves. For this reason, disinfection of toothbrushes is essential in terms of

preventive medicine. Studies on this subject are very few regarding the importance of the issue. [5,6,8,12,15]

This study aims to examine the caries rates and tooth brushing habits of 234 children selected among 1005 kindergarten children aged between 24-72 months, taking into account their parents' education and income level. It also evaluates the response of microbiological agents accumulated in children's toothbrushes to disinfectant chlorhexidine solution.

Material and Methods

This study was accepted by the Dicle University Faculty of Medicine Ethics Committee with the number 25.11.2011-312. 234 children (120 controls, 114 hexidines) who had not received any antibiotic, antimitotic, or dental treatment in the last three months, selected from 1005 private nursery-kindergarten children aged between 24-72 months, were included in this study. Attention was paid to the fact that the children included in the study had completed the primary dentition period. The distribution of these children into groups was made randomly. After the doctors were given information about the survey to parents, the pediatric dentist gave tooth brushing training to the children and parents. After a questionnaire was made for the parents' education, employment, and income status, the oral examination was performed by a pediatric dentist. Care has been taken to ensure that the brushes given to children are travel type, soft and without any extra features, with a closed box. The first microbiological cultures were taken with a brush; after brushing the first dispensed toothbrushes for at least 2 minutes using only water, without using any paste, under the guidance of a dentist. After the first brushing, the samples were placed in the storage container of the toothbrush without any washing. Toothbrushes and the solutions they will use (distilled water and hexidine) were given to the children to be collected every week. Children were provided to brush their teeth for 2 minutes at least twice a day. Spraying the solution on all surfaces of the brushes and brushing control were given to both parents and kindergarten teachers. As a result of evaluating the samples taken in the first week, the samples taken for four weeks were also assessed. The colonization values of S. mutans bacteria were examined. The first samples were given to the children once a week for four weeks, and the data obtained from the brushes taken back the following week were tried to be compared. Spray bottles (50cc) given to the patients were numbered. According to numbers throughout the study, distilled water and 0.12%, Chlorhexidine Gluconate solution was left weekly.

To examine the numbers of Streptococcus Mutans colonies accumulated on the brushes, the samples taken from the brushes are based on the scoring system used in the study of Filho et al. in 2006: [5]

- Score 0: no colony/biofilm.
- Score 1: 1-50 colonies/biofilm
- Score 2: 51-100 colonies/biophilia
- Score 3: Over 100 colonies/biofilm

For Streptococcus Mutans analysis, Bacitracin-Succurose broth enriched with Jensen and Brattall's modification was incubated and kept at 37˚C for four days. The bristles were washed, the planktonic microbiota was removed, and the remaining mushroom-shaped or spike-shaped colonies were counted under a stereomicroscope.

IBM SPSS 21.0 for windows statistical package program was used for the statistical evaluation of our research data. Measured variables were presented as mean ± standard deviation (SD), and categorical variables were presented as numbers and percentages (%). Chi-square ($\chi 2$) test and ANOVA analysis were used for the intergroup comparison of qualitative variables. In addition, Spearman correlation analysis was performed for the relationship between the variables. The hypotheses were two-sided, and a statistically significant result was accepted if $p \leq 0.05$.

Results

In this study, 234 patients with a mean age of 50.87± 9.56 months in the Control group and 50.28± 9.74 months in the Hexidine group, aged between 24-72 months, were included obtaining parental and ethical approval (Table 1). The dmf index of the children participating in our study was 4.09±2.89 for 120 patients in the control group and 3.86 ± 2.78 for 114 patients in the Hexidine group, and 3.98 ± 2.83 for a total of 234 patients (Table 2). No significant difference was found between the dmf index of girls and boys, regardless of the study groups ($p=0.106$ $p \geq 0.05$) (Table 3).

Table 1. The average age of the groups

AGE			
Group	Mean	n	Std. Deviation
Control	50.8750	120	9.56935
Heksidine	50.2807	114	9.74589
Total	50.5855	234	9.63960

Table 2. Dmf scores for groups

DMF			
Group	Mean	n	Std. Deviation
Control	4.0917	120	2.89884
Hexidine	3.8684	114	2.78273
Total	3.9829	234	2.83898

Table 3. Dmf indexes among girls and boys independent of study groups

DMF				
Gender	n	Mean	Std. Deviation	SEM
Girl	113	3.6726	2.82034	.26532
Boy	121	4.2727	2.83725	.25793

SEM: Std. Error Mean

Table 4. Dmf evaluation according to the mother and father study

DMF			
Working	Mean	n	Std. Deviation
Father	4.3548	124	3.04223
Mother	5.0000	1	.
Mother and Father	3.5505	109	2.54764
Total	3.9829	234	2.83898

Table 5. Education levels of the mother

DMF			
Education Mother	Mean	n	Std. Deviation
Out Of School	2.6000	5	3.28634
Primary School	4.7692	13	2.52170
Middle	6.2308	26	3.61450
High School	3.8247	97	2.79139
University	3.6585	82	2.26743
Academic	2.1818	11	2.67650
Total	3.9829	234	2.83898

Table 6. Education levels of the father

DMF			
Education Father	Mean	N	Std. Deviation
Out of School	4.0000	2	5.65685
Primary School	8.0000	1	.
Middle	5.5660	53	3.30215
High School	3.6266	158	2.43249
University	2.4000	20	2.62378
Academic	3.9829	234	2.83898

Table 7. Relationship between income level and dmf.

DMF			
Income	Mean	n	Std. Deviation
Minimum Wage	4.0000	2	5.65685
3000-6000 TL	5.8718	39	3.27012
6000-10000 TL	3.8000	80	2.88339
10000-20000 TL	3.5484	93	2.23858
20000 TL And Above	3.0500	20	2.76205
Total	3.9829	234	2.83898

Table 8. Correlation evaluation of colony distribution of Streptococcus Mutans accumulated in the samples taken from brushes.

	Control	Hexidine	p	r
0. week	120/%51.3	114/%48.7	1	-0.002
1. week	120/%51.3	114/%48.7	0	-0.619
2. week	120/%51.3	114/%48.7	0	-0.509
3. week	120/%51.3	114/%48.7	0	-0.572
4. week	120/%51.3	114/%48.7	0	-0.673

According to the mother and father working rate, no significant difference was observed between the groups in the dmf evaluation (p=0.91 p≥0.05) (Table 4). It has been observed that there is a significant difference between the education levels of the mother and father and the dmf index. It was observed that as the education levels of the parents increased, and the dmf level decreased (p=0.00 and p≤ 0.05) (Table 5,6). It was found that there is a significant relationship between income level and dmf index (p=0.00 and p≤0.05) (Table 7). According to the four-week distribution chart of Streptococcus mutans colonies in the Control and Hexidin groups, significant reductions in the number of colonies were observed in the Hexidin group.

In the statistical evaluation, it was observed that the decrease in the Hexidine group by weeks was significant (0 week p=1,00 p≥0.05 r=-0,002, 1th week p=0,0 and p≤0.05 r=-0,619, 2nd week p=0,0 and p≤0.05 r=-0,509, 3rd week p=0,0 and p≤0.05 r= -0,572, 4th week p=0,0 and p≤0.05 r= -0.673) (Table 8).

Discussion

This study aims to give the children participating in the study the habit of daily brushing. It has been reported that the incidence of caries is lower in individuals who acquire the habit of brushing their teeth at an early age.[16,17] Furthermore, the effectiveness of the education given to families and children can also be monitored by the weekly change of bacteria in the samples taken from toothbrushes. Therefore, it is essential to acquire hygiene education as a habit against various pathogens coming from the external environment and changing nutritional habits, socialization and environmental conditions.[1,2] In some studies conducted in Europe, it has been observed that it plays a significant role in the early detection and prevention of childhood caries in children who receive family assistance for tooth brushing.[4,18] In contrast, it does not play a significant role in studies conducted in the USA.[19]

The mean dmf-t index of the children participating in this study was found to be 3,9829 in 234 children, which was found to be higher than other studies in this age group. In the study performed by Chu et al. in 2012, the mean dmf-t for 764 pre-school children was reported as 2.2.[16] According to 2003 data in the United Kingdom, this rate was 1.6 in children aged five years.[7] In a study conducted in Australia, the mean dmf-t score was 1.5.[20] In a study conducted in Brazil in 2012, the dmf-t index of pre-school children was found to be 1.53±2.6.[21] In our study, there was statistical significance between the parents' income levels and the caries levels of the children. Different results were obtained in previous studies. According to Schroder et al. and Panino et al., there were no significant difference between income level and dmf.[4,18] These results can make it difficult to reach dental protective products because of purchasing power. In this study, it was observed that the level of caries in children with the low educational level of parents was lower than children with parents who received university and academic education. In the study of Chu et al., it was reported that the rate of caries is high in children of families with low socio-economic and educational levels.[2,16,22] However, in our study, the variability between education level and caries may be due to the high-income level of private nursery and kindergarten children. We expect this outcome to be different in general school studies.

In this study, *Streptococcus Mutans* colonies were found in the samples taken after 2 minutes of brushing in

week zero, regardless of the study and control groups. This shows parallelism with other studies.[5,6,12,13] A new toothbrush should be disinfected to prevent the formation of a bacterial biofilm layer on the brush bristles after the first tooth brushing.[5,24] However, disinfection of toothbrushes should be continued daily and replaced with a new toothbrush every 3-4 months.[5,24,25] Various methods have been used for the disinfection of toothbrushes in many studies; microwave, boiled water, ultraviolet light and chemical agents, especially chlorhexidine gluconate solutions, are preferred. Some researchers have tried to create disinfection by covering the toothbrush surfaces with chlorhexidine or disinfectant substances during the production phase.[26-28] In this study, the number of Streptococcus Mutans colonies in the group given chlorhexidine gluconate spray (0.12%) decreased significantly compared to the control group. This result shows parallelism with the studies of Filho et al. in 2000 and 2006.[5,6] This is due to both regular brushing and the effectiveness of chlorhexidine gluconate. Similar results were obtained in the study of Nascimento et al. in 2010 with a commercial solution containing 0.12% chlorhexidine (9). We think that the decrease in the colonization of other bacterial species is due to the effectiveness of clorhexidine. We believe that a reduction in *Streptococcus Mutans* colonies in the oral environment can be achieved at certain rates with tooth brushing.

Conclusions

While the number of bacterial colonization was very high in the first samples taken from private nursery-kindergarten children aged 24-72 months, the decrease in these rates independent of the income-education levels of the parents after regular brushing showed that tooth brushing is essential in this age group. A significant reduction in the amount of *Streptococcus Mutans* colonies accumulated on toothbrushes was observed using chlorhexidine gluconate solution (0.12%) in the disinfection of toothbrushes. To protect children's oral health in this age group, we think that brushing teeth more effectively and microbiological protection of toothbrushes will be precious in terms of public health. New studies should be done on this subject.

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Conflicts of Interest

The named authors have no conflict of interest, financial or otherwise.

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