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A Study on the Correlation of Oral Microorganisms of Children and Mothers in Day Care Centers

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Abstract

Background/Objectives:This study examined the correlation of oral microorganisms using the salivary caries-related tests in children in the early stage of baby teeth erupting and their mothers.

Methods/Statistical analysis:This study was done in 15 childrens and 15 mothers. The research uses the quantitative method to examine their oral condition and the degree of their understanding of oral-health. To analyze oral microorganisms, Denomix™ Oral Bacteria Diagnosis PCR kit (Microis, Korea) was used for quantitative analysis of oral microorganisms.

Findings:This study found not only S.mitis, Smutans, Lc which are microorganisms causing dental decay in the children's oral cavity, but also Aa, PG, Tf, Fn, Pi, Pn which are periodontal diseases causing bacteria. This study found the same oral microbial composition between mothers and children as a result of comparing the oral microbial composition between mothers and childrenbased on a one-to-one match.

Improvements/Applications: Oral health care education needs to be considered carried out not only by children but also by parents. An institutional approach will be needed to provide continuous oral health education for improving oral hygiene.

Keywords: Oral Microorganisms, PCR, Infections in mothers and children, Children, oral health, the bacteria associated with dental caries

1. Introduction

The oral cavity of children is rapidly exposed from an aseptic amniotic environment to external environment upon birth, and becomes the most active place for interaction with the external environment[1]. Over time, a variety of microorganisms invade the mouth and gradually settle down[2]. Named the period of 19 to 31months as 'the window of infection' as the period of oral microorganism infection, and around 18 months as S.Mutans's colony initiative period related to the first primary molar eruption. The incidence rate of dental caries has decreased in recent years, in some high-risk groups incidence rates are still high and showing an unbalanced distribution[3]. Recent studies show that behavior factors, dietary factors, children's' medical history in their prenatal and birth period, nutritional status and microbiological factors can be the risk factor for dental caries in children[4]. Behavior factors and dietary factors can include: breastfeeding, a type of snack, frequency of food intake, and a caregiver's effort to maintain good oral health. Where a prenatal medical condition such as premature birth and low birth weight can cause enamel hypoplasia and is a recognized risk factor for early childhood caries. As a microbiological factor, *streptococci mutans(SM)* is known as a major causative agent that induces caries and the infection caused by a mother[4,5]. Many previous studies have already shown that a mothers' habits and values toward oral health are closely related to children's oral health [6,7,8, 22-24]. In addition, the person who transmits the microorganism to the newborns after birth is most likely a mother. And children and mothers form a very close relationship microbiologically because the first microorganism settles down first in oral cavity to secure an ecologically advantageous position[9]. Therefore, there is a high possibility of having a microbiological correlation between mother and a children's oral cavity. This correlation will raise the importance of maternal and child health care. The previous studies related to parental infection and oral microorganisms were limited to only some strains such as s.mutans, also were not examined based on a one-to-one match.

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The purpose of this study is toidentify the types of microorganisms in an oral cavity using the salivary caries-related tests in children in the early stages of baby teeth erupting and their mothers, and to analyze the oral microorganism infection between a mother and children based on one-to-one match.

2. Materials and Methods

2.1 Materials

This study was conducted from May 1st to May 30th, 2020 by visiting the 00 Daycare Center in Y City, Gyeongsangnam-do, and explaining the intention and purpose of the study to the director of the daycare center, nursery teachers, and the mother of the children and toddler. This study selected 15 childrens under 48 months old and 15 mothers and excluded respondents answering randomly or not paying sufficient attention to the questions.

2.2 Methods

2.2.1. Survey tools

A questionnaire developed by Song [5] was used to examine the oral condition and the degree of understanding of oral health of children and their parents. This questionnaire consisted of 4 questions on children and 5 questions on parents about their general background information, 6 oral health questions, 30 questions on their knowledge of oral health, 7 oral health-related behavior questions, and 66 verbal oral health literacy questions.

2.2.2 The salivary caries-related tests

In the case of children, after putting the gargle solution in the cornical tube, the researchers directly collected the plaque using the cotton swab and put it the gargle solution.In the case of parents, 30 seconds of gargling is done and then spit in the Cornical Tube. Samples were refrigerated prior to inspection request. Denomix™ Oral Bacteria Diagnosis PCR kit (Microis, Korea) was used for the quantitative analysis of oral microorganisms. Kit consists of gargle 10cc and 50cc of cornical tube.

2.2.3 Multiplex Real-time PCR

Denomix™ Oral Bacteria Diagnosis PCR kit (Microis, Korea) was used for the quantitative analysis of oral microorganisms. For strain detection, specific primers are prepared from functional genes to amplify DNA fragments of about 200 bp each. For DNA chain separation, real-time PCR undergoes the steps of thermal denaturation, binding of the DNA synthesis. For the initial denaturation, 10 minutes at 95°C and then 45 cycles were performed at 95°C for 15 seconds, 55°C for 15 seconds, and 72°C for 30 seconds. In this study, the numerical value with a large unit is briefly presented as a value converted to a natural logarithmic function to facilitate comparison of differences.

2.3 Analysis Method

The statistical software IBM SPSS statistics (ver.23.0 for windows, Chicago, IL. USA) was used for the analyses of the data. For each analysis, the threshold for statistical significance was at $p<0.05$.The method used for statistical analysis for the general background information and oral care behavior were presented in frequency (N) and percentage (%) by conducting a frequency analysis (category type). The oral microbial composition in the oral cavity depends on the number of baby months was presented as frequency (N) and percentage (%) by conducting a frequency analysis and cross-analysis.

The number of microorganisms in the oral cavity was converted to a natural logarithmic function, and an average analysis was performed with the converted value to show the mean value(M).

3. Results

3.1. General characteristics of mother

In the general background of the mother, the majority (80 percent) were between the ages of 30 to 39 years old, 13.3 percent were over 40 years old, 6.7 percent were under 29 years old. For the educational background 86.7% were college graduates, and for employment category 66.7% were full time housewives. Regarding oral health, 86.7% of respondents answered ‘high interest’[Table 1].

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Table 1.General background information of mother

Group		N(%)
Age	Under 29 years old	1(6.7)
	Between 30and 39 years old	12(80.0)
	Older than40years old	2(13.3)
Education background	High school and below	2(13.3)
	University and above	13(86.7)
Income	Lower than 2 million KRW	2(13.3)
	Between 2 million KRW and 3 million KRW	3(20.0)
	Between 3 million KRW and 4 million KRW	4(26.7)
	Between 4 million KRW and 5 million KRW	5(33.3)
	Higher than 5million KRW	1(6.7)
employment	Full time housewives	10(66.7)
	employee	5(33.3)
Oral health interest	High interest	13(86.7)
	Average interest	2(13.3)

3.2. General characteristics of children

According to the analysis of the study, the general background of the children was 73.3% male and 26.7% female. For under 18 months it was 13.3%, 33.3% for between 18 months and 32 months, 53.3% for older than 32 months. For the family hierarchy, the second oldest child 53.3%, the first oldest child 26.7%, and the third oldest child 20.0%. 60.0% of the children's primary dentition were completed and 40.0% were incomplete, and 93.3% of the children hadn't had oral health education. 46.7% of children had dental treatment experience where 53.3% of them had no experience related to dental treatment. Candies and chips were 100% for their snack, followed by fruit 73.3% and milk 26.7%.86.7% of family answered not sharing the dishes between the family members, where 13.3% of family shared the dishes[Table 2].

Table 2.General background information of children

Group		N(%)
Gender	Male	11(73.3)
	Female	4(26.7)
Age	Under 18 months	2(13.3)
	Between 18 months and 24 months	5(33.3)
	Older than 32 months	8(53.3)
Family hierarchy	First oldest	4(26.7)
	Second oldest	8(53.3)
	Third oldest	3(20.0)
Primary dentition	Complete	9(60.0)
	Incomplete	6(40.0)
Experience of oral health education	Yes	1(6.7)
	No	14(93.3)
Experience of dental treatment	Yes	7(46.7)
	No	8(53.3)
Types of snack*	Candies and chips	15(100.0)
	Fruit	11(73.3)
	Milk	4(26.7)
Dish sharing between family members	Yes	2(13.3)

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	No	13(86.7)

*multiple responses.

3.3. Oral health related behavior

As a result of the analysis of a mother and child's oral health-related behavior, brushing teeth more than 3 times a day was the biggest percentage among mothers at 66.7% and 86.7% for children. Brushing after meal was biggest percentage in mothers and children 93.3%, 100%, and for brushing method, 93.3% of children and 100.0% of mother answered 'mixed method'. Brushing duration in the children, 'between 1 min and 2 mins' was highest percentage at 53.3%, 'between 2 mins and 3 mins' 20.0%, 'more than 3 mins' and 'less than 1 min' were both 13.3%. Brushing duration for the mother, 'between 2 mins and 3 mins' was highest at 53.3%, 'more than 3 mins' 26.7%, , 'between 1 min and 2 mins' 20.2%. 100% of mothers cleans their tongue where 66.7% children clean their tongue when they brush. 26.7% children use toothpaste with fluoride and 73.3% uses non-fluoride tooth paste. 93.3% of mothers use fluoride toothpaste and 6.7% use non-fluoride tooth paste[Table 3].

Table 3. Oral health-related behavior

Variable		Children	Mother
		N(%)	N(%)
Brushing (per a day)	1time	1(6.7)	-
	2times	4(26.7)	2(13.3)
	Moe than 3 times	10(66.7)	13(86.7)
Brushing time*	Before meal	0(0.0)	1(6.7)
	After meal	14(93.3)	15(100.0)
	After snacks	3(20.0)	2(13.3)
	Before bed time	13(86.7)	14(93.3)
Brushing method	Vertical method	1(6.7)	-
	Mixed method	14(93.3)	15(100.0)
Brushing duration	Less than 1 min	2(13.3)	-
	between 1 min and 2 mins	8(53.3)	2(20.0)
	between 2 min and 3 mins	3(20.0)	8(53.3)
	More than 3 mins	2(13.3)	4(26.7)
Tongue Cleaning	Yes	10(66.7)	15(100.0)
	No	5(33.3)	-
Fluoride toothpaste	Yes	4(26.7)	14(93.3)
	no	11(73.3)	1(6.7)
Amount of toothpaste	Full toothbrush head size	0(0.0)	1(6.7)
	1/2 of toothbrush head size	5(33.3)	3(20.0)
	1/3 of toothbrush head size	10(66.7)	11(73.3)

*multiple responses.

3.4. The oral microbial composition

The analysis results of the oral microbial composition in the oral cavity are shown in Tables 4, 5, and 6. In children, 100% of Fn, S.mitis, and S. mutans were observed in all samples, followed by Pn 33.3%, PI and Lc 20.0%, Aa, Tf, Td respectively 6.7%.In the mother, 100% of Fn, Pn, S.mitis, and S. mutans were observed in all samples, followed by Pg and Pi in 66.7%, Tf 53.3%, Lc 33.3%, Td 26.7%, Aa 13.3%.For children, the average quantitative value of microorganisms in the oral cavity was S.mitis 7.45%, S.mutans 7.41%. For mothers, the value was S.mutans 6.25% as the highest percentage and Pm 5.89% [Table 4],[Table 5],[Table 6].

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Table 4. The oral microbial composition of mother and children

Variable	Aa	Pg	Tf	Td	Fn	Pi	Pn	S.mitis	S.mutans	Lc
A	○	○	○		○	○	○	○	○	
A'	○				○		○	○	○	○
B		○	○		○	○	○	○	○	○
B'					○		○	○	○	○
C		○	○	○	○	○	○	○	○	○
C'					○			○	○	
D					○		○	○	○	
D'					○	○	○	○	○	
E		○			○	○	○	○	○	
E'					○			○	○	
F		○			○		○	○	○	
F'					○			○	○	○
G			○	○	○	○	○	○	○	
G'		○			○			○	○	
H		○			○		○	○	○	
H'					○			○	○	
I					○	○	○	○	○	
I'					○			○	○	
J		○	○		○	○	○	○	○	
J'					○		○	○	○	
K	○	○	○	○	○	○	○	○	○	
K'		○	○		○	○		○	○	
L		○			○	○	○	○	○	
L'				○	○			○	○	○
M					○		○	○	○	
M'		○			○	○	○	○	○	○
N		○	○	○	○	○	○	○	○	
N'					○			○	○	
O					○		○	○	○	
O'					○			○	○	

Aggregatibacteractinomycetemcomitans(Aa), Porphyromonasgingivalis(Pg), Tannerellaforsythia(Tf), Treponemadenticola(Td), Fusobacteriumnucleatum(Fn) Prevotellaintermedia(Pi),, Prevotellanigrescens(Pn) Streptococcus mitis(S.mitis), Streptococcus mutans(S.mutans) Lactobacillus casei(Lc)

A~O :Children, A'~O' : Mother

Table5. Oral microbial composition concordance rate in a match between a mother and child

Oral microbial composition	N(%)
Aggregatibacter actinomycetemcomitans(Aa)	1(6.7)
Porphyromonas gingivalis(Pg)	1(6.7)
Tannerella forsythia(Tf)	1(6.7)
Treponema denticola(Td)	-
Fusobacterium nucleatum(Fn)	15(100.0)
Prevotella intermedia(Pi)	1(6.7)
Prevotella nigrescens(Pn)	5(33.3)
Streptococcus mitis(S.mitis)	15(100.0)
Streptococcus mutans(S.mutans)	15(100.0)

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Lactobacillus casei(Lc)	2(13.3)
Total	15(100.0)

Table 6. Number and distribution of oral microorganism in a match between a mother and child

Oral microbial composition	Child		Mother	
	N(%)	Mean ±SD*	N(%)	Mean±SD*
Aggregatibacter actinomycetemcomitans(Aa)	1(6.7)	4.69±0.00	2(13.3)	4.76±0.80
Porphyromonas gingivalis(Pg)	3(20.0)	3.40±0.25	10(66.7)	4.58±0.89
Tannerella forsythia(Tf)	1(6.7)	4.78±0.00	8(53.3)	4.72±0.45
Treponema denticola(Td)	1(6.7)	3.43±0.00	4(26.7)	4.17±1.85
Fusobacterium nucleatum(Fn)	15(100.0)	5.94±0.65	15(100.0)	5.28±0.75
Prevotella intermedia(Pi)	3(20.0)	3.63±0.45	10(66.7)	5.11±0.90
Prevotella nigrescens(Pn)	5(33.3)	4.87±0.59	15(100.0)	5.89±0.59
Streptococcus mitis(S.mitis)	15(100.0)	7.45±0.80	15(100.0)	5.58±1.13
Streptococcus mutans(S.mutans)	15(100.0)	7.41±0.37	15(100.0)	6.25±1.08
Lactobacillus casei(Lc)	3(20.0)	3.75±1.44	5(33.3)	2.92±0.72
Total	15(100.0)	4.94±0.46	15(100.0)	4.92±0.92

*: The numerical value converted to a natural logarithmic function

3.5. Oral microbial composition changes with increasing months

The results of analyzing the oral microbial composition in the oral cavity of children depends on the number of months as shown in Table 7 below. Among oral microorganism, Fn, S.mitis, and S. mutans tend to increase to 13.3% for less than 18 months, 33.3% between 18 months and 31 months, and 53.3% for more than 32 months as the number of months increases[Table 7].

Table 7.The oral microbial composition changes with increasing months

Oral microbial composition	Under 17 mos.	Between 18mos. and 31 mos.	Higher 32mos.
Aggregatibacter actinomycetemcomitans(Aa)	-	-	1(6.7)
Porphyromonas gingivalis(Pg)	-	1(6.7)	2(13.3)
Tannerella forsythia(Tf)	-	0(0.0)	1(6.7)
Treponema denticola(Td)	-	1(6.7)	-
Fusobacterium nucleatum(Fn)	2(13.3)	5(33.3)	8(53.3)
Prevotella intermedia(Pi)	0(0.0)	-	3(20.0)
Prevotella nigrescens(Pn)	0(0.0)	-	5(33.3)
Streptococcus mitis(S.mitis)	2(13.3)	5(33.3)	8(53.3)
Streptococcus mutans(S.mutans)	2(13.3)	5(33.3)	8(53.3)
Lactobacillus casei(Lc)	0(0.0)	1(6.7)	2(13.3)
Total	15(100.0)	15(100.0)	15(100.0)

4. Discussion

As the number of single-child families increases, parents become more conscious about oral health [10]. In this way, the DMF rate among 5 years old children with primary dentition in Korea had been decreasing from 83.3% in 2000 to 67.7% in 2006 and 61.5% in 2010. But it went up to 68.5% in 2018[11].The list of risk factors include: behavior factors, dietary factors, microbiological factors. Among the behavior and dietary factors includes the caregiver's effort to maintain good oral health. In particular, the eruption period of the first primary molar is the initiation period of S. mutans, which is around

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18 months after birth[4]. This period is a very important time for caries prevention because of the diet changes that can develop caries and worsen it. In addition, this period is an efficient time to motivate parents because the first primary molar erupted, the parents need the education for oral health-related information[6,9]. The childcare facilities become responsible party as the oral health care provider for the children due to the advancement of childcare support policies and women's social participance. However mothers still take an important role because the children stay at home in the critical period[12]. In this way, children's oral health care is often dependent on their parents, especially mothers who take a very important role for oral health in the family's daily life[13]. Many previous studies have shown that the level of a mother's understanding of oral health has an effect on children's oral health[12,13]. However, the health statistical yearbook published by the national health insurance service in Korea for a recent 5years(2012-2016), the regular oral examination rate for children has gradually increased 22.7%in 2012 to 39.4% in 2016. However, compared to the 2016 children health checkup rate of 71.9%, it is very low[14,15]. In this study, only 46.7% of respondents had an experience visiting a dentist, and only 6.7% of the mothers had experience in oral health education. Previous studies have shown similar results with this study[12]. It is necessary to actively promote the regular oral health examination of children and for parents to be active participants. The regular oral examination for children can provide early detection of oral-related diseases and a chance to have oral health-related education and consultation. In addition, the effort to improve the quality of education provided during a regular oral health examination is also necessary. As previous studies have shown, S.mutans infection in children mainly caused from mother to child[16]. It has been reported that the number of bacteria in the maternal saliva is closely related to the number of bacteria in the children's saliva and dental caries, suggesting the possibility of metastasis from the maternal body[17-20]. Precedent research suggested the metastasis through maternal saliva in a study of bacterioc in typing and a profound association with the number of S.mutans between mother and child[19,21]. This study found not only S.mitis, Smutans, Lc which are microorganisms causing dental decay in children's oral cavity, but also Aa, PG, Tf, Fn, Pi, Pn which is a periodontal disease causing bacteria. This study found the same oral microbial composition between mothers and children as a result of comparing the oral microbial composition between mothers and children based on a one-to-one match. Likewise, the infection of dental caries between a mother and child as suggested in previous studies. This result shows that periodontal disease bacteria can also be influenced by the lifestyle of parents and children. As a result of the above, oral microorganisms can be a useful factor in predicting the possibility of oral disease in the future, and more awareness is required to reduce the amount of pathogenic microorganisms in the oral cavity of children and mothers. This will enable to early action and preventive treatment by predicting various risk factors related to the progress of an oral disease in the future. Therefore, it is important to improve oral hygiene and daily habits of all family members, including children's caregivers. Oral health care education needs to be considered carried out not only by children but also by parents as well.

5. Conclusion

As a result of a comprehensive analysis of the results of this study, not only S.mitis, Smutans, Lc which are microorganisms causing dental decay in the children's oral cavity, but also Aa, PG, Tf, Fn, Pi, Pn which are periodontal diseases causing bacteria. This study found the oral microbial composition between mothers and children was the same, as a result of comparing the oral microbial composition between mothers and children based on a one-to-one match. As in many previous studies, the infection of intraoral microorganisms can be caused due to daily habits of a mother and child. As a result of the above, oral microorganisms can act as a useful factor in predicting the possibility of oral diseases in the future, and enable early action and preventive treatment by predicting various risk factors related to the progress of oral diseases. Therefore, it is important to improve oral hygiene and the lifestyle habits of all family members, including children's caregivers. Oral health care education also needs to be considered carried out not only by children but also by parents as well.

There are several limitations in our study. Expanded sample and follow-up studies will be necessary in the future.

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