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Relationship Between Oral Microorganisms and Dental Caries

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Objectives: The purpose of this study was to investigate the relationship between the incidences of dental caries and the amount and motility of oral microorganisms in inter proximal dental plaque. **Methods:** Oral microorganism samples from 240 volunteers in six different age groups were classified according to morphology using phase contrast microscopy. We observed the amount and motility of the microorganisms and examined for relationships to the incidences of dental caries. **Results:** There was a statistically significant difference among the age groups ($p < 0.05$) in the motility of cocci and bacilli and in the amount of spirilla. The decayed tooth (DT), filled tooth (FT), and decayed, missing, filled tooth (DMFT) index for all age groups were calculated as 7.5 ± 4.5, 1.9 ± 3.0, and 14.8 ± 8.1. There was weak, positive relationship between the amount of cocci and bacilli and caries incidence, regardless of age ($p < 0.01$). **Conclusion:** Determining the amount and motility of oral microorganisms is an effective method of evaluating and controlling the status of oral health.

Keywords: Microbiology, Microscopy, Phase-Contrast, Dental Caries

Introduction

Oral microorganisms, which are abundant in the oral cavity, are known as one of the main and important causative factors of oral diseases, and dental plaque is considered to be the cause of dental caries and periodontal diseases [1-2]. Oral microorganisms may increase with poor salivary flow, deficient chewing, oral hygiene, diet, or general health [3]. Microorganisms in a healthy person create balance, remaining at an equilibrium, but change in environment can cause an imbalance and lead to certain dental diseases.

Microorganisms such as bacteria, viruses, and fungi can be observed by the microscope. Such organisms can also be classified morphologically into the following types: cocci, bacilli,

filaments, and spirilla. Numerous studies conducted to identify the equilibrium of oral microorganisms have shown that some coccous and bacillary oral microorganisms are related to the occurrence of dental caries and gram-negative microorganisms, while some filamentous and spirillar microorganisms, such as anaerobic bacterias, can cause periodontal diseases [1, 6].

In order to predict the risk of caries and determine disease activity, a variety of methods have been suggested, but unfortunately, there is no single test available that can fully predict or explain the disease [7-9]. In clinical practice, effective methods of directly determining the amount and motility of oral microorganisms would aid in establishing plans to prevent specific diseases and maintain dental health [1, 5, 6, 8]. Though formerly limited to academic research use, a powerful tool at a clinical level may be phase contrast microscopy. Phase contrast microscopy does not require the specimen to be dyed and is able to effectively distinguish the different types of microorganisms, as well as the amount and motility of the live, oral microorganisms [1, 4, 5].

Results from latest studies in Mongolia, where our study was conducted, reveal that dental caries and periodontal disease are spreading at a more rapid rate in Mongolia, leading to deterioration of health and a greater financial burden for those who are affected. This significant social issue requires early identification of risk factors, monitoring of the disease, and a more effective tool is to examine microorganisms in the oral cavities. Currently, there is a limited amount of analysis regarding the use of phase contrast microscopy to determine the amount and motility of oral microorganisms [10-15].

Thus, the aim of this study was to use phase contrast microscopy to determine the amount and motility of oral microorganisms in dental plaque, as well as determine dental caries incidence within different age groups, to examine the relationship between oral microorganisms and dental caries incidence.

Materials and Methods

1. Sampling

Tooth plaque samples were collected from 240 consenting volunteers and observed with phase contrast microscopy from July to August 2016, at the Department of Pediatric and Preventive Dentistry in School of Dentistry at the Mongolian National University of Medical Sciences. Volunteers were divided into six age groups: preschool age (3 - 5 years old), school age (6 - 13 years old), adolescence (14 - 19 years old), young adulthood (20 - 34 years old), middle age (35 - 64 years old), and aged (65 years and above). Each age group had 40 participants, composed of 20 males and 20 females.

2. Methods

1. Sample collection

Inter proximal dental plaques were collected with the micro brush at the lingual side, using the smearing technique. All samples were taken between 10 AM to 12 PM or 3 PM to 5 PM, two hours after meal time. The sample was smeared on a slide glass with one drop of saline and covered with a cover glass.

1.1. Observation of the image

A phase contrast microscope (OMAX 40X-2500X, United States)

Table 1. Chang's score for standardization of the amount and motility of oral microorganisms observed by phase contrast microscopy and monitor

Type	Cocci		Bacilli		Filaments		Spirilla	
Score	Count	Estimation per 1mm^2	Count	Estimation per 1mm^2	Count	Estimation per 1mm^2	Count	Estimation per 1mm^2
0	0	0	0	0	0	0	0	0
1	1-7	100-1000	1-7	100-1000	1-7	100-1000	1-3	100-500
2	8-70	1000-10,0000	8-30	1000-5000	8-30	1000-5000	4-8	500-1000
3	>70	>10,000	>30	>5000	>30	>5000	>8	>1000

Table 2. Motility of oral microorganisms

Score	Motility
0	no mobility
1	Less than 10 μm²/sec
2	10 μm²/sec- 30 μm²/sec
3	more than 30 μm²/sec

was used in this study at X400 magnification. Using Toup View Ink, a computer application program, an image was captured for 10 seconds at a site where motility was visible.

1.2. Standardization for observation

Four types of microorganisms, cocci, bacilli, filaments, and spirilla, were observed for amount and motility according to Chang's score [1] (Table 1, 2).

2. Dental caries incidence

Direct oral examinations were performed, and evaluations of former dental caries incidences were conducted per the guidelines of World Health Organization (2013). Decayed tooth (DT), filled tooth (FT), and decayed, missing, filled tooth (DMFT) of both primary and permanent teeth were estimated for each of the six age groups.

3. Statistical analysis

For each age group and gender, a one way ANOVA test was used to calculate the mean Chang's score for the amount and motility of oral microorganisms. Pearson's correlation coefficient was used to identify the relationship between the amount and motility of oral microorganisms and caries incidence by comparing each age group to the find the statistical differences. The analysis was done using SPSS version 23.0. All data was recorded as mean with standard deviation.

Results

1. Oral microorganisms examination

Among the age groups, there was a statistically significant difference ($p < 0.05$) only in the motility of cocci and bacilli and in the amount of spirilla (Table 3).

Between genders, the amount and motility of oral microorganisms in dental plaque had no statistically significant difference ($p > 0.05$), except for the amount of spirillar microorganisms ($p = 0.05$) (Table 4).

Table 3. The mean ± standard deviation of the Chang's score for the amount and motility of oral microorganisms, compared among age groups

Group (mean±S.D)	Cocci		Bacilli		Filaments		Spirilla	
	amount	motile	amount	motile	amount	motile	amount	motile
preschool age	1.30 ± 0.46	1.18 ± 0.55	1.03 ± 0.28	1.33 ± 0.66	0.78 ± 0.53	0.30 ± 0.52	0.08 ± 0.27	
school age	1.33 ± 0.53	1.05 ± 0.55	0.98 ± 0.28	1.60 ± 0.96	0.68 ± 0.47	0.35 ± 0.53	0.35 ± 0.48	0.15 ± 0.36
adolescence	1.48 ± 0.55	1.10 ± 0.63	1.00 ± 0.39	1.43 ± 0.96	0.60 ± 0.55	0.25 ± 0.54	0.43 ± 0.64	0.08 ± 0.27
young adulthood	1.30 ± 0.46	1.05 ± 0.60	1.08 ± 0.35	1.55 ± 0.81	0.78 ± 0.42	0.30 ± 0.61	0.30 ± 0.46	0.15 ± 0.36
middle age	1.40 ± 0.50	1.13 ± 0.52	1.08 ± 0.27	1.53 ± 0.91	0.80 ± 0.69	0.45 ± 0.78	0.28 ± 0.55	0.13 ± 0.40
aged	1.50 ± 0.51	0.78 ± 0.58	1.13 ± 0.40	1.03 ± 0.73	0.75 ± 0.71	0.13 ± 0.33	0.20 ± 0.41	
total	1.38 ± 0.50	1.05 ± 0.58	1.05 ± 0.33	1.41 ± 0.86	0.73 ± 0.57	0.30 ± 0.57	0.27 ± 0.49	0.13 ± 0.35
p-value	0.285	0.036	0.349	0.030	0.610	0.212	0.028	0.751

p-value calculated with one-way ANOVA for age, types of oral microorganism

Table 4. Gender differences in amount and motility of oral microorganisms

Types (Mean ± S.D)		Total	male	female	p-value
cocci	amount	1.4 ± 0.5	1.4 ± 0.5	1.4 ± 0.5	0.798
	motile	1.1 ± 0.6	1.1 ± 0.6	1.1 ± 0.6	0.912
bacilli	amount	1.1 ± 0.3	1.1 ± 0.4	1.0 ± 0.3	0.333
	motile	1.4 ± 0.9	1.5 ± 0.9	1.3 ± 0.8	0.23
filaments	amount	0.7 ± 0.6	0.7 ± 0.6	0.7 ± 0.6	0.734
	motile	0.3 ± 0.6	0.3 ± 0.6	0.3 ± 0.5	0.43
spirilla	amount	0.3 ± 0.5	0.4 ± 0.6	0.2 ± 0.4	0.005
	motile	0.1 ± 0.3	0.1 ± 0.3	0.1 ± 0.3	0.377

p-value calculated with independent t-test for gender

In all age groups, there was a weak correlation between the amount and motility of oral microorganisms ($p<0.01$), except for between the coccous and spirillar microorganisms ($p>0.05$). There was moderate relationship between the amount and

Table 5. Correlation coefficient between the amount and the motility of oral microorganisms in all age groups

Types		Cocci		Bacilli		Filaments		Spirilla	
		amount	motile	amount	motile	amount	motile	amount	motile
Cocci	amount	1							
	motile	0.05	1						
Bacilli	amount	0.19**	0.05	1					
	motile	-0.02	0.21**	0.08	1				
Filaments	amount	0.22**	0.15*	0.40**	0.05	1			
	motile	0.01	0.19**	-0.05	0.21**	0.26**	1		
Spirilla	amount	0.00	0.09	0.00	0.16*	0.05	0.10	1	
	motile	-0.05	0.13	0.00	0.13*	-0.07	0.20**	0.49**	1

p-value calculated with Pearson's correlation coefficient and statistical significance is denoted as * = $p<0.05$, ** = $p<0.01$

Table 6. The dental caries experience of Mongolians according to the age groups

Age (mean±S.D)	Decayed tooth	Filled tooth	Missing tooth	Caries incidence
3 - 5	8.9 ± 3.9	0.7 ± 1.1	0.11 ± 0.3	9.6 ± 4.0
6 - 13	7.1 ± 4.2	1.4 ± 1.8	0.5 ± 1.1	9.0 ± 4.1
14 - 19	8.2 ± 5.2	2.0 ± 2.9	0.6 ± 1.1	10.8 ± 4.8
20 - 34	8.5 ± 3.3	3.4 ± 4.6	1.4 ± 2.0	13.3 ± 4.5
35 - 64	8.0 ± 4.2	3.0 ± 3.4	9.6 ± 7.9	20.6 ± 6.4
65 +	4.5 ± 4.6	0.7 ± 1.5	20.8 ± 9.6	26.1 ± 6.0
Total	7.5 ± 4.5	1.9 ± 3.0	5.5 ± 9.2	14.8 ± 8.1
p-value	0.0001	0.0001	0.0001	0.0001

p-value calculated with one-way ANOVA test

motility in spirillar microorganisms ($p < 0.01$) (Table 5).

2. Caries experience

There were significant differences for the caries experience DT, FT, MT and DMFT among all age groups ($p < 0.0001$). DMFT mean rate was high in the preschool aged (3-5 years) due to primary decayed teeth (8.9 ± 3.9), in young adulthood (20-34 years) due to permanent filled teeth (3.4 ± 4.6), and in the aged (65+ years) due to permanent missing teeth (20.8 ± 9.6) (Table 6).

3. Relationship between the oral microorganism examination and caries incidence

There was weak, positive relationship between caries incidence and amount of both cocci and bacilli microorganisms ($p < 0.01$). There was negative relationship between total decayed tooth and caries incidence ($p > 0.05$) (Table 7).

Table 7. Relationship between the oral microorganisms and caries experiences

	Caries incidence	Total decayed tooth	Amount of cocci	Amount of bacilli	Motile of cocci	Motile of bacilii
Caries incidence	1					
Total decayed tooth	-0.01	1.00				
Amount of cocci	0.19**	0.09	1.00			
Amount of bacilli	0.20**	0.01	0.19**	1.00		
Motile of cocci	-0.19**	0.07	0.05	0.05	1.00	
Motile of bacilii	-0.05	0.15*	-0.02	0.08	0.21**	1.00

p-value calculated with Pearson's correlation coefficient and statistical significance is denoted as * = $p < 0.05$, ** = $p < 0.01$

Discussion

Childhood oral disease, if untreated, leads to pain, development of dentofacial anomalies, and other serious health problems, such as severe toothaches, dental abscesses, destruction of bones, and spread of infection via the bloodstream. Tooth decay or early loss of teeth may lead to malnutrition and other health problems [10]. Recent studies have shown that dental caries prevalence and severity among Mongolians is high and increases with age [10, 16]. One of the important causes of oral diseases are microorganisms, and specific imbalances in microorganisms can be related to specific diseases [17].

Light microscopes, polarizing microscopes, dark field microscopes, phase contrast microscopes, and scanning electron microscopes were introduced to aid in the process of observation, including the examination of oral microorganisms. Phase contrast microscopy has advantages in examining oral microorganisms due to the lack of staining and ability to observe microflora in its living and motile state.

Caries incidence and caries activity has been determined by methods other than phase contrast microscopy [10-15]. However, in order to impact the control and prevention of oral disease, Yu Jeong Lee has stated that phase contrast microscopy should be used more widely in clinical settings [6].

According to a survey report on oral health knowledge, attitudes, and practice among school children and adults living in the ger districts of Ulaanbaatar, Mongolia, 52.2% of children and 30.6% of adults did not know that tooth decay can be caused by bacteria transmitted from mother to child by kissing or sharing feeding utensils. According to the WHO guidelines, the prevalence and incidence of dental caries in Mongolia is high and has been increasing over the years [11, 13-16, 18]. Thus, research is necessary to improve oral health knowledge and oral health prevention and promotion programs in Mongolia.

Though our study was limited in data processing ability, our study generates important findings in the field of dentistry in Mongolia and in international preventive dentistry. In order to promote oral health by controlling the oral microorganisms, it is necessary to account for morphological types of oral microorganisms. We examined the relationship between oral microorganisms and dental caries experience in six age groups: preschool children, school children, adolescent, young adult, middle age, and aged.

Our study collected oral microorganism samples from inter proximal dental plaque. This particular area was chosen because there are more oral microorganisms in dental plaque than in saliva, and there is a positive relationship between the amount and the motility of microorganisms in plaque and in the whole oral cavity [2, 6]. In our study, cocci averaged 1.38 to 1.88 points in amount and 1.05-1.63 in motility; in bacilli, 1.05- 1.38 in amount and 1.41-2.27 in motility; in laments, 0.73- 1.3 in amount and 0.30-0.87 in motility; in spirilla, 0.27-0.76 in amount and 0.13-0.48 in motility. In comparison to these points, our results show a higher amount and motility of coccous, bacillary, and filamentous microorganisms than previous study [1].

The amount and motility of oral microorganisms were revealed to be at a similar percentage, or to have no statistically significant difference ($p > 0.05$), for all age groups, except for the motility of cocci ($p=0.036$) and bacilli ($p = 0.03$) and amount of spirilla ($p = 0.028$). Thus, most participants had increased amounts and motility of coccous and bacillary microorganisms, perhaps explaining why oral diseases can occur regardless of age and gender.

There was an increased amount and motility of cocci, bacilli and spirilla in preschool, school aged children, adolescent subjects than any other age group. Furthermore, there was a particularly greater amount of spirrillar microorganisms in school aged and adolescence groups, a finding associated with gum bleeding or periodontitis.

Like previous findings, we found caries experiences increase with age. In this study, the DT, FT, and DMFT index of all age groups were calculated as 7.5 ± 4.5, 1.9 ± 3.0 and 14.8 ± 8.1, as high in caries incidence without treatment [10, 12, 13, 16, 19]. Decayed tooth was more common in those below 35 years old, while more tooth loss was prevalent for those above 35 years old. This is due to lack of preventive measures, proper dental care, and necessary treatments, which results in serious dental issues with age and eventually, tooth loss, a fairly common occurrence in Mongolia. The past several reports from the Mongolian national survey suggests that advanced periodontal symptoms such as as Community Periodontal Index (CPI) 3 and CPI 4 were rare and mainly found (19.7%) in people aged 65-74 years, because periodontal disease commenced in adolescence and progressed to become severe [10].

In addition, it is well reported that both amounts and motility of bacillary, filamentous, and spirrillar oral microorganisms would be increased in presence of periodontal disease, and the amount and motility of coccous would be increased in the presence of dental caries [6, 12, 17]. Our preliminary finding indicated that coccous and bacillary microorganisms are the most prominent indicators for measuring the status of oral and general health [17]. In our study, there was weak, positive relationship between the amount of cocci and bacilli and caries incidence in all age groups ($p < 0.01$). Additionally, while caries incidence was influenced positively by the amount of coccous and bacillary microorganisms, it was influenced negatively by the motility of coccous microorganisms. Lastly, in our study, there was a positive relationship both in the amount and in motility of coccous and bacillary microorganisms, especially in the preschool aged and young adulthood groups.

In addition, school aged and middle aged groups had high activity with more amounts of filamentous and spirrillar microorganisms, perhaps explaining why there is more gingivitis and periodontitis in the adolescence, while young adults and older are more impacted by periodontal diseases due to poor oral hygiene and higher caries intensity in Mongolia.

In conclusion, the amount and motility of oral microorganisms were similar in all age groups, and these organisms, particularly cocci and bacilli, have been related to oral diseases such as dental caries and periodontal diseases. Microorganisms could be the main indicators of oral health, and thus, oral microorganisms examination is an effective and useful method to predict the status of oral health. As mentioned by others including Jung et al. and Lee et al., because proper control of coccous and bacillary microorganisms may help prevent oral diseases, the amount and motility of microorganisms should be manipulated in clinical settings to adjust the equilibrium state of oral microorganisms in dental patients [2, 17]. Lastly, we recommend further study on the correlation between oral disease risk factors standardly used to analyze oral health and oral microorganisms.

Conflict of Interest

The authors state no conflict of interest.

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