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Changes of Periodontopathic Bacteria According to Gingival Health, Dental Plaque Control and Toothbrushing Behavior in Adults

Hyun-Ja Jeong[1], Hyejin Kim[2]

[1]Department of Dental Hygiene, Daegu Health College, Republic of Korea; [2]Department of Dental Hygiene, Dongeui University, Gaya 1-dong, Busanjin-gu, Busan, Republic of Korea

ABSTRACT

Background/Objectives: This study targeted 30 subjects who visited D university from March 15th to June 30th of 2018 for oral care management program, and agreed to participate in study.

Method/Statistical Analysis: The collected data were analyzed using SPSS statistics v.23. One sample t-test was conducted for the oral health environment condition, toothbrushing habits and oral hygiene management. Then, paired t-test was conducted for the changes in the distribution of periodontopathic bacteria depending on toothbrushing habits and oral care behaviors. As for all the statistics, the level of significance was set as $p<0.05$.

Findings: In regard to the changes in periodontopathic bacteria depending on toothbrushing habits, Aa & Red complex of a group that brushes twice or more in a day was 39.12 pre-education and 24.78 post-education compared to the group that brushes once a day or does not brush. In regard to brushing timing, the group that brushes more than three times daily showed Aa & Red complex of 54.85 pre-education and 29.31 post-education, which showed significant reduction. In regard to the duration of toothbrushing, the group that brushes for more than 3 minutes showed Aa & Red complex of 51.46 pre-education, and 27.61 post-education which showed significant reduction. As for the change of periodontopathic bacteria according to the subjects' oral care behavior, for interdental cleaning, Orange Complex significantly decreased in the group of persons who were using dental floss or interdental brush post-education (89.87) than pre-education (97.94). For tongue cleaning, Aa & Red Complex significantly decreased in the group of persons who cleaned the tongue every day post-education (26.06) than pre-education (47.18).

Improvements/Applications: The overall analysis of research findings showed differences in BOP, CAL and O'Leary Index depending on the gender and marital status. Also, it was shown that periodontopathic bacteria depending on toothbrushing habits and oral care behaviors gets reduced after education, compared to before education. It is considered that toothbrushing education using disclosing agents can motivate the experimenters, since they can directly observe the changes in dental plaque and O'Leary Index.

Keywords: *Dental hygiene care, Oral hygiene state, Oral health behavior, Oral microorganisms,*

Introduction

Today, as an interest in the quality of life appears as a social issue with the society's acceleration to become an aging society, people's interest in health continues to grow. The weight of oral health in the domain of whole-body health gradually increases[1], and as a factor of oral health affecting whole-body health, as they get older, tooth loss[2,3,4,5], due to various different internal and external causes, brings about digestive dysfunction,

Corresponding Author:
Hyejin Kim
Department of Dental Hygiene,
Dongeui University, Gaya 1-dong, Busanjin-gu,
Busan, Republic of Korea
Email: khj1126@deu.ac.kr

inaccurate pronunciation and deformation of the face because of masticatory function inconvenience, which also causes inconvenience in interpersonal relations.

The concept of oral health is experienced from preschool children to adults through various educational experiences and promotions, and dental medical institutions are assisting the oral care with the latest medical techniques and treatment methods[6]. Toothbrushing, which is the base of oral care, is not an exception, and proper care starting from childhood can promote oral health in adulthood. Toothbrushing behavior is mostly conducted from dental health education program experiences. In order to maintain clean oral environments, proper toothbrushing is the most effective. In addition, reducing dental plaque and tongue fur with appropriate oral hygiene supplementary goods[7,8], is also effective. Various oral microbes exist in dental plaques and saliva, including *'Aggregatibacter actinomycetemcomitans', 'Porphyromonas gingivalis', 'Prevotella intermedia', 'Tannerella forsythia', 'Fusobacterium nucleatum', 'Capnocytophaga species', 'Campylobacter rectus'* and etc[9,10], which are closely related to gingivitis and periodontal diseases in adults[11,12]. Toothbrushing method, interdental brushing, dental flossing, mouth rinsing and other methods that have been introduced to the South Korean public for promoting oral health are really helpful when it comes good oral hygiene. Various individual factors such as eating habits, living habits, oral care habits, social factors and systemic factors affect the oral environment conditions, but fundamentally increasing the rate of oral care practice will be effective. Therefore, this study aimed to verify the effect of adults' oral care behaviors and toothbrushing habits on the changes in oral microbes that are related to oral gingivitis and periodontal disease. Individually, in dental plaque control, the toothbrushing method is the most fundamental, which may help prevent oral diseases. In the oral environmental conditions, various oral and external factors act, such as individual eating habits, lifestyle habits, oral care habits, social factors, and universal factors, and basically, as local factors, intraoral factors affect the occurrence of oral diseases. Thus, this study would check oral microbial changes after the education according to gum health status or toothbrushing behavior in the process of the oral care program with adults and utilize them as the basic data for the importance of dental health education and self-care (toothbrushing).

Materials and Method

Study Subjects: This study originally targeted research subjects who visited D university in B city from March 15th to October 30th of 2018 to benefit from the oral health care program. After considering ethical aspects, the purpose and method of the research were explained and the consent for participating in the research was obtained, and a total of 30 research subject who finally completed the program were selected as the research subjects.

The program for dental health care consisted of assessment, judgment, planning, performance, and evaluation. General characteristics included sex, age, and marital status. For gingival health conditions, bleeding on probing (BOP) and clinical attachment level (CAL), and dental plaque index (O'Leary Index) were inspected. For toothbrushing behaviors, the number of times, frequency, intensity, time, and method of toothbrushing were investigated. Concerning oral hygiene care, interdental cleaning and tongue cleaning were investigated.

As an analysis of periodontopathic bacteria, a quantitative analysis was conducted by real-time PCR. Using NEXprep™Cell/TissueGenomic DNA Kit, genomic DNA was extracted according to the manufacturer's instructions. For a quantitative analysis of oral bacteria, PerioGen™PerioReal-TimePCRKit (Microis, Korea) was employed. For the detection of the whole microorganisms existing in the oral cavity, 16s rDNA fragments were amplified, and for the detection of each microorganism, each of about 200 bp DNA fragments were amplified by producing specific primer from functional genes (rgp, waa, and gtf). As the probes used in multiplex real-time PCR, three species (FAM, Cy5, HEX) were selected, of which the wavelength is not overlapped, considering the interference of each other. Four kinds of the panel were constructed by binding three species together respectively from the target bacterial species for the analysis in each reaction, which was shown in Table 1. For real-time PCR reaction solution, the extracted total DNA 2 μL was mixed with each of primer set 10 pmol, probe and buffer solution, and 1 unit Hot-start Taq DNA polymerase (GeneAll, Korea) to produce 20 μL in total. It was seeded in 96-well plates, and then, a quantitative analysis was conducted, using ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). Based on the characteristics, correlation, dyeing

reaction, formation of colonies, generation of pigment and clinical indicator of causative bacteria that cause periodontal diseases, they are classified into five by color, including Red Complex, Orange Complex, Green Complex, Yellow Complex, and Purple Complex[9], while this study classified them into two groups, including Red Complex and Orange Complex. Of the subgingival bacteria with a correlation with periodontal diseases, *P. gingivalis*, *T. forsythia*, and *T. denticola* were classified as Red Complex, and it was reported that this complex has strong correlations with the indices of periodontal diseases, like periodontal pocket depth and bleeding while probing[9]. *P. intermedia*, *F. nucleaum*, *P. micra*, and *C. rectun* are Orange Complex, deeply associated with periodontal diseases.

Table 1: The bacteria strains used in this study

Division	Target strain
A Panel	*Aggregatibacter actinomycetemcomitans* KCTC3698 *Porphyromonas gingivalis* KCTC 5352 *Tannerella forsythia* KCTC 5666
B Panel	*Treponema denticola* KCTC 15104 *Prevotella intermedia* KCTC 5692 *Fusobacterium nucleatum* KCTC 2640
C Panel	*Parvimonas micra* ATCC 33270 *Campylobacter rectus* KCTC 5636 *Eikenella corrodens* KCTC 15198
D Panel	*Eubacterium codatum* KCTC 15015 *Prevotella nigrescens* KCTC 5690

Study Methods: The collected data were analyzed using SPSS statistics v.23. One sample t-test was conducted for the oral health environment condition(BOP, CAL, O'Leary Index), toothbrushing habits(toothbrushing timing, frequency, intensity, duration and method) and oral hygiene management(interdental and tongue cleaning). Then, paired t-test was conducted for the changes in the distribution of periodontopathic bacteria depending on toothbrushing habits and oral care behaviors. As for all the statistics, the level of significance was set as $p<0.05$.

Result and Discussion

BOP, CAL, O'Leary Index, and the number of periodontopathic bacteria (Aa & Red Complex and Orange Complex) according to the subjects' general characteristics: As a result of an analysis of the average of BOP, CAL, O'Leary Index, and the number of periodontopathic bacteria (Aa & Red Complex, Orange Complex) according to the general characteristics, BOP was higher in men (24.02%) than in women, and by age, it was higher in those over 30 (23.33%) than in those under 29. CAL was more in men (34.28%) than in women, and it was a little more in those over 30 (68.77%) than in those under 29. O'Leary Index was a little higher in women (51.31 points) than in men, and it was higher in those over 30 (54.46 points than in those under 29. Aa & Red Complex was more in men (36.69) than in women, and Orange Complex, too, was more in men (94.29) than in women. By age, Aa & Red Complex was a little more in those under 29 (36.80) than in those over 30, and Orange Complex was a little more in those over 30 (100.87) than in those under 29. It was interpreted that BOP, CAL, O'Leary Index, and the number of periodontopathic bacteria (Aa & Red Complex, Orange Complex) according to the general characteristics were homogeneous since there were no differences in them in Table 2.

Table 2: General characteristics BOP, CAL, O'Leary Index, and the number of periodontopathic bacteria (Aa & Red Complex and Orange Complex) according to the subjects' general characteristics[1)]

Division		N	BOP	CAL	O'Leary Index	Aa & Red complex	Orange complex
			Mean	Mean	Mean	Mean	Mean
Sex	Male	19	24.02	34.28	48.69	36.69	94.29
	Female	11	16.71	26.71	51.31	28.47	80.23
Age	<29	21	20.94	63.17	39.15	36.80	87.74
	30≤	9	23.33	68.77	56.46	24.71	100.87
Married	Married	18	23.41	65.39	37.52	26.95	116.48
	Single	12	20.80	71.73	42.03	37.25	80.87

[1)]: BOP, CAL, O'Leary Index, the number of periodontopathic bacteria (Aa & Red Complex and Orange Complex) are Base values and indicated as the mean (t-test)

Distribution of periodontopathic bacteria according to the subjects' oral hygiene care after the education: Changes in the periodontopathic bacteria depending on the subject's toothbrushing habits are shown in Table 3. For the group that brushes teeth twice a day or more than twice a day(2pt), Aa & Red complex was 39.12 before education and 24.78 after education, and orange complex was 99.82 before education and 87.77 after education($p<.05$). As for the group that brushes more than three times a day, after dinner time and before sleeping(4pt), Aa & Red complex was 54.85 before education, and 29.31 after education($p<.01$), and orange complex was 106.16 before education, and 92.71 after education($p<.05$). As for the group that brushes for more than 3 minutes(2pt), Aa & Red complex was 51.46 before education, and 27.61 after education($p<.001$), and orange complex was 104.34 before education and 94.92 after education. For interdental cleaning, in the group of people who used dental floss or interdental brush more than once a day (2pt), Aa & Red Complex was 22.03 before the education and 10.39 after. Orange Complex was 97.94 before the education and 89.87 after($p<.05$). For tongue cleaning, in those who cleaned the tongue every day (2pt), Aa & Red Complex was 47.18 before the education and 26.06 after($p<.01$). Orange Complex was 96.10 before the education and 85.26 after. In those who brushed the tongue occasionally(1pt), Aa & Red Complex was 17.15 before the education and 16.53 after. Orange Complex was 106.75 before the education and 84.49 after($p<.05$).

Table 3: Distribution of periodontopathic bacteria according to the subjects' oral hygiene care after the education

Division		Score	Aa & Red complex			Orange complex		
			Pre-edu	Post-edu	p	Pre-edu	Post-edu	p
Frequency of brushing	Twice a day, or more than twice a day	2 point	39.12	24.78	.015	99.82	87.77	.035
	once a day	1 point	29.80	19.66	.382	113.74	89.20	.409
	Not wiping daily	0 point	-	-	-	61.44	57.27	-
When do you brushing it?	More than three times a day(after dinner)	4 point	54.85	29.31	.002	106.16	92.71	.031
	Twice a day	3 point	36.00	23.01	.320	107.70	100.42	.629
	once a day	2 point	7.83	15.23	.414	82.45	67.72	.429
	Self-defense	1 point	22.35	14.75	.325	107.35	84.90	.263
	At any time, not self-defense	0 point	28.77	30.01	-	29.28	27.89	-
Brushing strength	Smooth	2 point	42.37	30.01	.076	107.90	101.26	.425
	Smooth + give me strength.	1 point	37.71	24.92	.145	105.82	89.26	.108
	Give me strength.	0 point	26.41	10.81	.211	78.61	62.69	.118
Brushing time	More than 3 minutes	2 point	51.46	27.61	.001	104.34	94.92	.190
	2-3 minutes	1 point	22.60	17.81	.541	89.12	76.23	.105
	Less than 1 minute	0 point	30.69	29.50	.760	140.78	104.59	.495
Brushing method	Bath method Other recommended usage	2 point	61.10	28.89	-	139.29	127.23	-
	Horizontal motion or blending method	1 point	32.25	23.67	.106	102.59	91.83	.082
	Vertical motion or rotational motion	0 point	44.07	21.30	.087	87.94	68.54	.162
Interdental cleaning	Use floss and toothbrush once a day	2 point	22.03	10.39	.368	97.94	89.87	.050
	Do interdental cleaning but not daily	1 point	45.80	24.75	.071	92.12	86.97	.722
	No interdental cleaning	0 point	36.88	24.94	.066	101.51	96.18	.062
Tongue cleaning	Daily Wipes	2 point	47.18	26.06	.007	96.10	85.26	.145
	Occasional wipes	1 point	17.15	16.53	.910	106.75	84.49	.024
	Not wiping at all	0 point	44.02	32.18	.598	96.32	107.02	.535

Conclusion

The average of BOP and CAL according to the general characteristics of the subjects was higher in men than in women. The average of BOP was higher in married people than in singles by marital status. The average of CAL was higher in married people than in singles. The average of O'Leary Index was higher in women than in men. By marital status, it was higher in married people than in singles. As for the BOP and O'Leary Index of the frequency of toothbrushing according to the subjects' toothbrushing habits, the score was the highest in those who did not brush teeth every day, and the score of CAL was the highest in those who brushed teeth twice a day, or more than twice a day. In the time of toothbrushing, the BOP score was the highest in those brushed teeth before sleeping. The score of CAL was the highest in those who brushed teeth more than three times a day, before dinner or sleeping. The score of O'Leary Index was the highest in those who brushed teeth anytime, not before sleeping. The BOP of interdental cleaning according to the subjects' oral care behavior was the highest in those who never did interdental cleaning. The scores of CAL and O'Leary Index were the highest in those who did interdental cleaning but not every day. For tongue cleaning, the scores of BOP and CAL were the highest in those who never cleaned the tongue while that of O'Leary Index was the highest in those who did not clean the tongue occasionally. As for changes in the distribution of periodontopathic bacteria in Aa & Red Complex and Orange Complex according to toothbrushing behaviors, all of the frequency of toothbrushing, the number of times, intensity, time and method decreased after the education than before the education. As for changes in the distribution of periodontopathic bacteria in Aa & Red Complex and Orange Complex according to oral care behaviors, interdental cleaning and tongue cleaning decreased after the education than before the education. Overall, Aa & Red Complex and Orange Complex tended to decrease after the education than before, and especially, in toothbrushing behaviors, when they brushed teeth more frequently, e.g. Twice or more than twice a day, and when they brushed teeth for more than three minutes, they significantly decreased, and also, in oral hygiene care, they significantly decreased when they did interdental cleaning or tongue cleaning than when they did not. According to Ha and Choi[13], the better the health perceived by oneself, the higher the interest in health becomes, and the higher the interest, the higher the practice of the behavior becomes, which are similar to the results of this study. When the oral care program is applied, individually customized dental health education has a very great potential of motivation, and it is judged that it had an effect on the reduction of Aa & Red Complex and Orange Complex. The overall analysis of research findings showed differences in BOP, CAL(clinical attachment level) and O'Leary Index depending on the gender and marital status. Also, it was shown that periodontopathic bacteria depending on toothbrushing habits and oral care behaviors gets reduced after education, compared to before education. Also, according to a precedent study[14], toothbrushing education using[15] disclosing agents allows direct observation of dental plaque, and therefore motivates experimenters, and fluctuations in O'Leary Index was informed to the patients to allow them to understand the current level of care and how erroneous their toothbrushing habits are. It is considered that toothbrushing education using disclosing agents can motivate the experimenters, since they can directly observe the changes in dental plaque and O'Leary Index.

Therefore, it would be effective if the right method was recognized and applied in toothbrushing behavior and oral hygiene care, and it would be necessary to look for a method that could increase subjects' interest in oral health. There are various toothbrushing methods, and each method has a different effect on the removal of dental plaque[16,17]. When the effect of transformed Bass method on the removal of dental plaque was compared to that of other toothbrushing methods in Park[18], there was a limitation in interpretation with a statistical method. However, Bass method is effective for removing oral physiotherapy on the lingual region where the dental plaque is easily accumulated and gingivitis is likely to occur, and in this study, too, the score of O'Leary index was the highest when the Bass method and other recommended usages were used. Basically, the rotating method is recommended for people who do not have gum diseases or have clean oral conditions, but the Bass method was more effective for people with gingivitis accompanied by intermittent inflammation. However, there is a limitation to interpret the effects only with the consequences of toothbrushing behaviors. In the future, it would be necessary to make efforts to understand oral care problems by understanding various age groups, whole-body health, and the characteristics of the oral cavity and consistently investigating changes in bacteria according to oral health behaviors or environmental conditions.

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Conflict of Interest: Nil

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