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The relationship between turbidity of mouth-rinsed water and oral health status

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Abstract

Objectives. The purpose of this study was to examine the relationship between turbidity of mouth rinsed water and oral health status such as dental and periodontal conditions, oral hygiene status, flow rate of saliva and oral bacteria. ***Materials and methods.*** Subjects were 165 patients who visited the Dental Hospital, Tokyo Medical and Dental University. Oral health status, including dental and periodontal conditions, oral hygiene status and flow rate of saliva, was clinically examined. The turbidity was measured with a turbidimeter. Quantification of *Fusobacterium* spp, *Porphyromonas gingivalis, Tannerella forsythia, Treponema denticola* and total bacteria levels was performed using real-time PCR. The Pearson correlation and multiple regression analysis were used to explore the associations between the turbidity and oral health parameters. ***Results.*** The turbidity showed significant correlations with the number of decayed teeth and deep pockets, the plaque index, extent of tongue coating and *Fusobacterium* spp, *P. gingivalis, T. forsythia, T. denticola* and total bacteria levels. In a multiple regression model, the turbidity was negatively associated with the flow rate of saliva and positively associated with the total number of bacteria ($p < 0.001$). ***Conclusion.*** Current findings suggested that turbidity of mouth rinsed water could be used as an indicator to evaluate oral health condition and the amount of bacteria in the oral cavity. In addition, the turbiditimeter appeared as a simple and objective device for screening abnormality of oral health condition at chair side as well as community-based research.

Key Words: *oral health status, oral bacteria, turbidity*

Introduction

Dental caries and periodontal diseases are the main dental diseases both in developing countries and developed countries and are the chief reasons for patients visiting a dentist. In Japan, according to a national survey conducted in 2005, the prevalence of dental caries and gum diseases among 30–69-year-olds were 38 and 80%, respectively [1]. With these high prevalences of unfavorable dental-caries and gingival conditions observed in the population, it would be useful to establish an easy and simple method for screening oral health status for early intervention to prevent dental disease.

It is well known that dental caries and periodontal diseases are caused by a number of factors in the oral cavity. Bacteria in dental plaque or the condition of the saliva are the main factors. Previous studies have examined the effectiveness of biological tests (e.g. Dentocult SM®, benzoyl-DL-arginine-naphthylamide (BANA) and Perioscreen®) for estimating the risk for oral diseases [2–4]. However, these screening tests have some disadvantages. Dentocult SM® takes 48 h of incubation to obtain results, the BANAtest is expensive and the Perioscreen® test is difficult to apply in patients with xerostomia.

It would be preferable for the screening tool to be simple and objective, produce immediate results and be used easily even by unskilled examiners. Clinically, the oral hygiene level of a subject is evaluated based on the oral hygiene index, plaque index, plaque control index, etc. These evaluations, however, depend on the

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subjective opinion of the examiners to some degree. Thus, a simpler and more objective method to measure oral health status would be desirable for predicting risk for oral diseases, such as dental caries or periodontal diseases.

The turbidity of mouth-rinsed water, measured using a turbidimeter, might be a promising method for screening oral health status. In a previous study, Hakuta et al. [5] estimated oral hygiene status of study subjects by visually evaluating the murkiness of mouth-rinsed water. Turbidity quantifies the murkiness of a fluid sample, being defined as an expression of the optical property that causes light to be scattered and absorbed rather than transmitted in straight lines through a fluid. Thus, turbidity of mouth-rinsed water might be a useful indicator of the hygiene level of the oral cavity, reflecting the amount of dental plaque, tongue coating and food debris. Therefore, the purpose of this study was to examine the relationship between the turbidity of mouth-rinsed water and oral health status (i.e. dental and periodontal conditions, oral hygiene status, flow rate of saliva and oral bacteria levels).

Materials and methods

Subjects

Subjects were 182 patients, who visited the Dental Hospital at Tokyo Medical and Dental University from April 2009 to January 2010. Subjects who were edetulous (n = 4) or had missing data on study variables (n = 13) were excluded from the study [6]. A total of 165 subjects (47 males and 118 females; mean age 49.3 ± 14.1 years) were finally recruited for this study. All participants received verbal and written information about the study and signed an informed consent form. The study protocol was approved by the Ethics Committee for Human Research, Tokyo Medical and Dental University (No. 132).

Collection of saliva

Resting whole saliva samples were collected by asking subjects to spit resting saliva into pre-weighed paper cups for 5 min. Saliva flow rates were determined gravimetrically and calculated as milliliter per minute (mL/min).

Turbidity measurement

Subjects were asked to swish 20 mL of distilled water (OTSUKA DISTILLED WATER, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan) around their mouth, transferring the water from left to light 20-times, and spit it into the paper cup. This mouth-rinsed water was transferred into a 20 mm optical path-length cell and the turbidity was measured by absorbance at 660 nm, using a turbidimeter (WA1®; Nippon Denshoku, Tokyo, Japan). Turbidity was calibrated with polystyrene standard turbidity liquid (Nippon Denshoku, Tokyo, Japan) and the cell was washed with distilled water before each measurement. The result was displayed in a built-in monitor and printed in a few seconds. The rest of the sample was used for the quantification of bacteria levels.

Oral examination

All subjects had an oral examination, including dental and periodontal conditions, oral hygiene and tongue coating status. Assessment of the dental condition included the number of present and decayed teeth. Periodontal status was evaluated by exploring all teeth circumferentially using a periodontal probe (PCP UNC 15 Hu-Friedy: Hu-Friedy Mfg. Co., Inc., Chicago IL), and the deepest pocket depth was recorded for each tooth. Gingival bleeding was assessed as presence or absence 30 s after the pocket depth measurement [7]. Oral hygiene status was evaluated by the Plaque Index of Silness & Löe [8] criteria. The thickness of the tongue coating was evaluated by a modification of the Oho et al. [9] criteria. The thickness of the tongue coating was recorded with a 0–3 score, where a score of 0 = no coating, 1 = a thin coating, 2 = a medium coating and 3 = a thick coating.

Quantification of bacteria

Assessment of bacteria and growth conditions. Fusobacterium nucleatum FDC1436, *Porphyromonas gingivalis* ATCC33277, *Tannerella forsythia* ATCC43037 and *Treponema denticola* ATCC35405 were used in this study. The first two bacteria species were cultured at 37°C under anaerobic conditions (80% N_2, 10% CO_2, 10% H_2) in Todd-Hewitt Broth (Becton, Dickinson and Company, Sparks, MD) supplemented with 5 μg/mL hemin and 1 μg/mL menadione (THBHM). *T. forsythia* was cultured in THBHM supplemented with 0.001% *N*-Acetylmuramic acid (SIGMA-ALDRICH Corp, St. Louis, MO). *T. denticola* was cultured in TYGVS medium [10]. All bacterial strains were cultured to late-log phase and a 0.5 mL culture was used for determination of the number of colony-forming units (CFU), with isolation of genomic DNAas quantitative PCR standards. Bacterial counting was performed by measuring each colony in its culture medium using the serial dilution method except for *T. denticola*. For *T. denticola* a bacterial counting board (Kayagaki Corp, Tokyo, Japan) was used under the microscope. Standard curves were generated by plotting the threshold cycle (C_T) values against the number of CFU values.

DNAisolation from mouth-rinsed water and bacterial culture. Aliquots (500 μL) of each specimen were centrifuged at 14,000 rpm for 3 min. Total bacterial DNAwas isolated from the pellet using the Genomic DNAIsolation Kit for Bacteria (nexttec™ GmbH, Leverkusen, Germany) according to the manufacturer's instructions. The genomic DNAwas stored at −40°C until PCR assay.

Quantitative real-time PCR. For quantitative identification of bacteria, PCR amplifications were performed in a total volume of 25 μL using the Premix Ex Taq™ (Takara Biomedicals, Shiga, Japan), containing 1 μl of template DNAand 200 nM of each primer. All PCR amplification and detection was performed with an Opticon™ 2 System (Bio-Rad Laboratories, Hercules, CA) and C_T values were determined with the Opticon monitor™ Ver.3.1 software supplied by Bio-Rad Laboratories. Quantification of the individual target bacteria levels and total bacteria from the clinical specimens were calculated using each standard curve described below. To estimate the total number of bacteria, quantitative PCR was carried out by using primers and probes sets and *P. gingivalis* DNAas the standard [11]. Specific probes for *Fusobacterium* spp., *Tannerella forsythia*, *Treponella denticola* and conserved regions of the bacterial gene were used for Quantitative real-time PCR [11–13].

Statistical analysis

The Chi-square test and the independent *t*-test were used to detect the distributional or mean differences by gender. Because of the non-normal distribution of turbidity and amount of bacteria, a logarithmic transformation was applied to these variables before analyses. The distribution of values after logarithmic transformation was diagnosed as normal with the Kolmogorov-Smirnov test. Pearson correlation coefficients were used to detect the association of turbidity with oral health and bacterial parameters. A multiple linear regression analysis was performed to assess the association of turbidity with oral health and bacterial parameters, in which turbidity was the dependent variable and age, gender, oral health and bacterial parameters were independent variables. Data were analyzed using SPSS 15.0 (SPSS Japan Inc., Tokyo, Japan) and a *p*-value of less than 0.05 was considered statistically significant.

Results

Turbidity of mouth-rinsed water and oral health parameters

The mean turbidity of mouth-rinsed water was 116.9 ± 95.3 (mean ± SD). Males (131.3 ± 101.6) tended to have higher turbidity than females (111.2 ± 92.5), but no significant difference was detected between the sexes.

The oral health parameters of subjects are presented in Table I. The number of teeth present, the number of decayed teeth, the number of teeth with periodontal pockets ≥ 4 mm and the number of teeth with gingival bleeding were 25.9 ± 4.5, 0.2 ± 0.8, 1.3 ± 2.6 and 4.0 ± 4.9, respectively. Males had a significantly higher number of teeth present (27.0 ± 3.9) than females (25.5 ± 4.7) ($p = 0.045$). The percentage of subjects with tongue coating scores of 0, 1, 2 and 3 were 1.8%, 49.1%, 38.8 and 10.3%, respectively. Males had significantly thicker mean tongue coatings (1.83 ± 0.76) than females (1.47 ± 0.65) ($p = 0.003$). A significantly higher salivary flow rate was observed

Table I. Oral health parameters of subjects ($n = 165$).

Parameters		Total Mean ± SD	Males ($n = 47$) Mean ± SD	Females ($n = 118$) Mean ± SD	*p*-value
Number of teeth present		25.9 ± 4.5	27.0 ± 3.9	25.5 ± 4.7	0.045
Number of decayed teeth		0.2 ± 0.8	0.2 ± 0.5	0.2 ± 0.9	0.925
Number of teeth with periodontal pocket ≥ 4 mm		1.3 ± 2.6	2.1 ± 3.9	1.0 ± 1.8	0.067
Number of teeth with bleeding		4.0 ± 4.9	4.2 ± 4.3	4.0 ± 5.1	0.774
Plaque Index		0.5 ± 0.5	0.7 ± 0.6	0.5 ± 0.4	0.051
Tongue coating		1.6 ± 0.7	1.8 ± 0.8	1.5 ± 0.7	0.003
Flow rate of saliva	(mL/min)	0.3 ± 0.3	0.5 ± 0.4	0.3 ± 0.2	0.006
Bacterium					
Fusobacterium spp	(CFU/mL)	$8.5 \times 10^5 \pm 1.1 \times 10^6$	$1.1 \times 10^6 \pm 1.5 \times 10^6$	$7.3 \times 10^5 \pm 9.0 \times 10^5$	0.084
Porphyromonas gingivalis	(CFU/mL)	$6.1 \times 10^4 \pm 2.4 \times 10^5$	$6.5 \times 10^4 \pm 2.5 \times 10^5$	$6.0 \times 10^4 \pm 2.4 \times 10^5$	0.893
Tannerella forsythia	(CFU/mL)	$1.5 \times 10^5 \pm 2.6 \times 10^5$	$2.0 \times 10^5 \pm 3.1 \times 10^5$	$1.3 \times 10^5 \pm 2.4 \times 10^5$	0.169
Treponema denticola	(CFU/mL)	$1.8 \times 10^5 \pm 4.8 \times 10^5$	$3.7 \times 10^5 \pm 8.3 \times 10^5$	$1.1 \times 10^5 \pm 1.9 \times 10^5$	0.039
Total bacteria	(CFU/mL)	$1.4 \times 10^8 \pm 1.3 \times 10^8$	$1.7 \times 10^8 \pm 1.5 \times 10^8$	$1.3 \times 10^8 \pm 1.3 \times 10^8$	0.039

in males (0.5 ± 0.4) than in females (0.3 ± 0.2) ($p = 0.006$).

Bacterial counts in mouth-rinsed water

The mean numbers of *Fusobacterium* spp, *P. gingivalis*, *T. forsythia*, *T. denticola* and total bacteria were $8.5 \times 10^5 \pm 1.1 \times 10^6$, $6.1 \times 10^4 \pm 2.4 \times 10^5$, $1.5 \times 10^5 \pm 2.6 \times 10^5$, $1.8 \times 10^5 \pm 4.8 \times 10^5$ and $1.4 \times 10^8 \pm 1.3 \times 10^8$ CFU/mL, respectively. There were no significant differences in the mean numbers of *Fusobacterium* spp, *P. gingivalis* or *T. forsythia* between the sexes. However, males had significantly higher numbers of total bacteria and *T. denticola* than females ($p = 0.039$).

Correlation between turbidity and oral health parameters

Turbidity was significantly correlated with the number of decayed teeth ($r = 0.16$), the number of teeth with periodontal pockets ≥ 4 mm ($r = 0.19$), the plaque index ($r = 0.22$), tongue coating thickness ($r = 0.23$) and total number of bacteria ($r = 0.84$) (Table II). Turbidity showed the highest correlation with *Fusobacterium* spp ($r = 0.66$), followed by *T. forsythia* ($r = 0.35$), *T. denticola* ($r = 0.27$) and *P. gingivalis* ($r = 0.16$).

Multiple linear regression analysis of the turbidity

Turbidity was significantly associated with salivary flow rate and total bacteria level ($F = 44.79$, $p < 0.001$, Adj $R^2 = 0.73$) (Table III). Subjects with a lower salivary flow rate or with a higher amount of total bacteria in the mouth-rinsed water were more likely to have higher turbidity. No significant associations between turbidity and age, gender, the number of decayed teeth, the number of teeth with periodontal pockets ≥ 4 mm, the number of teeth with gingival bleeding, the plaque index or tongue coating thickness were detected in the regression analysis model.

Table II. The correlation between turbidity and oral health parameters.

Parameters	Correlation coefficient	*p*-value
Number of teeth present	−0.12	0.135
Number of decayed teeth	0.16	0.036
Number of teeth with periodontal pocket ≥ 4 mm	0.19	0.016
Number of teeth with bleeding	0.01	0.944
Plaque Index	0.22	0.002
Tongue coating	0.23	0.004
Flow rate of saliva	−0.09	0.238
Bacteria		
Fusobacterium spp	0.66	< 0.001
Porphyromonas gingivalis	0.16	0.044
Tannerella forsythia	0.35	< 0.001
Treponema denticola	0.27	< 0.001
Total bacteria	0.84	< 0.001

Table III. The multiple linear regression analysis of turbidity.

	β	t	*p*-value
Gender	−0.01	−0.30	0.76
Age	0.07	1.29	0.20
Number of teeth present	0.03	0.55	0.59
Number of decayed teeth	0.01	0.21	0.83
Number of teeth with periodontal pocket ≥ 4 mm	0.00	0.04	0.97
Number of teeth with bleeding	0.02	0.47	0.64
Plaque index	0.07	1.33	0.18
Tongue coating	−0.02	−0.34	0.73
Flow rate of saliva (mL/min)	−0.14	−3.24	< 0.001
Total bacteria	0.82	17.65	< 0.001

Discussion

To date few studies have examined the relationship between turbidity and oral health status, including teeth and periodontal conditions, salivary flow rate and bacteria levels. Our study indicated that the turbidity of mouth-rinsed water reflects salivary flow rate and the amount of total bacteria in the oral cavity. Therefore, turbidity could be used as an indicator to evaluate oral health status. We calculated the tertile of turbidity to provide a simple scale for prediction of oral bacterial loads. According to the tertile, 33.3 percentile and 66.6 percentile of turbidity were 61.3 and 133.0, respectively. Hence, the following scales of turbidity could be regarded as : green (OK); <60, amber (caution); 61–130, red (referral/intervention); >131.The turbiditimeters used for testing the water quality of rivers, lakes, seas and at the drinking water treatment plant [14] appear to be simple and objective devices that could be used for estimating oral hygiene status and for screening oral health conditions.

A decreased salivary flow rate is known to affect general and oral health, because such a condition causes a decrease of the natural cleaning action, resulting in greater accumulation of debris, plaque and bacteria [15,16]. A reduced salivary flow rate is also considered to be one of the factors influencing the formation of a tongue coating. Koshimune et al. [17] reported that a lower salivary flow rate was significantly related to greater accumulation of tongue coating.

The observed significant relationship between turbidity of mouth-rinsed water and total oral bacteria

suggests that measurement of turbidity could be used to estimate oral bacteria levels. Using a different device, Ishikawa et al. [18] also demonstrated that turbidity was positively correlated with total oral bacteria in mouth-rinsed water. They concluded that turbidity might be a useful indicator of oral hygiene status and risk for periodontal disease.

Our bivariate analysis indicated positive correlations between turbidity and dental plaque and tongue coating levels. Dental plaque consists mostly of bacterial cells and a matrix composed mostly of salivary proteins and large molecular weight bacterial products. Tongue coating is a solid interactive phase in the oral cavity [19] composed of large amounts of desquamated epithelial cells, bacteria and blood components. The morphology of the tongue dorsum is characterized by fissures, grooves and papillae that facilitate the accumulation of micro-organisms. Approximately one-third of the bacteria in the oral cavity are found on the surface of the tongue [20]. The saliva of subjects with more abundant dental plaque, tongue coating or both is thought to have more bacteria and to contribute to higher turbidity, as indicated by the multiple regression analysis.

F. nucleatum is an important Gram-negative bacterium in the development of complex dental plaque biofilms. This bacterium is reported as a 'bridge bacterium' because it mediates multiple co-aggregation interactions with other bacteria [21]. *F. nucleatum* is also believed to support the growth of other anaerobes of dental plaque [22]. The amount of *Fusobacterium* spp had the strongest correlation with turbidity. Therefore, the amount of *Fusobacterium* spp has a greater influence on the turbidity of mouth-rinsed water compared to other bacterial species. The proportion of *F. nucleatum* in saliva, tongue coating and subgingiva was reported to be higher compared to that of other bacteria [23]. In addition, the bacterial composition in saliva is highly similar to that in the tongue coating [19]. Because most study subjects presented with some degrees of tongue coating, such conditions might also contribute to the highest amount of *Fusobacterium* spp in the mouth-rinsed water.

Three Gram-negative anaerobic bacteria *P. gingivalis*, *T. forsythia* and *T. denticola* are particularly periodontopathic and have been considered 'the red complex', i.e. the most important bacteria in causation and progress of periodontitis [24]. *T. forsythia* has been identified along with *P. gingivalis* in subgingival plaque and saliva samples of severe periodontitis subjects [25,26]. In this study, subjects had, on average, 1–3 teeth with a periodontal pocket ≥ 4 mm, i.e. their periodontal conditions were not so severe. This may explain why detected amounts of *P. gingivalis*, *T. forsythia* or *T. denticola* were low. Kurata et al. [27] also reported that the prevalence of *P. gingivalis*, *T. forsythia* and *T. denticola* in saliva was related to periodontal health and was low in subjects without periodontal disease [27]. In addition, because *P. gingivalis*, *T. forsythia* and *T. denticola* reside in deep pockets, the detection of these bacteria in a mouth-rinsed water sample might be limited. We did not explore any specific cariogenic bacteria such as *Streptococcus mutans*. It is probable that not only periodontopathogenic bacteria but also cariogenic bacteria contribute to the turbidity. Future research that includes the assessment of cariogenic bacteria would be required to clarify this relationship.

This study had some limitations. The study samples were patients who visited a dental hospital. Therefore, it would not be possible to apply current results to the general population. Further research using a representative sample of the general population would be necessary to confirm current findings. It is also necessary to decide on standardized values of turbidity. In spite of these limitations, this study provided new evidence that the turbidity of mouth-rinsed water can reflect oral health conditions such as salivary flow and bacterial level.

Conclusion

The findings in this study suggest that the turbidity of mouth-rinsed water can be used to estimate oral health conditions, such as salivary flow and bacterial levels. Its advantages include low cost and a short measurement time. The turbidimeter is a simple and objective device for screening oral health status at chair side as well as in community-based research.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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