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Accuracy of a salivary examination kit for the screening of periodontal disease in a group medical check-up (Japanese-specific health check-up)

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Abstract

The purpose of the present study was to investigate the accuracy of a screening method using salivary tests to screen for periodontal disease.

In total, 1888 individuals older than 30 years in 2017 and 2296 in 2018 who underwent medical check-ups for metabolic syndrome agreed to participate and simultaneously underwent a dental examination by dentists and salivary tests. Salivary occult blood, protein, and ammonia levels and white blood cell counts were evaluated in salivary tests using commercially available kits. The relationship between the results of the salivary tests and dental examination was examined and classification performance was analyzed.

The prevalence of periodontal disease was 69.9% in 2017 and 66.8% in 2018. Salivary ammonia showed the highest classification performance in both years (sensitivity 83.5 and 83.1%, precision 73.0 and 69.3%, F-measure 0.779 and 0.756). Occult blood, which was assessed using a monoclonal antibody to human hemoglobin, also showed good performance (sensitivity 69.5%, precision 70.6%, F-measure 0.701). Questions regarding self-reported gingival bleeding were not sufficient to screen for periodontitis. The present results suggest that screening tests using salivary samples may detect periodontal disease in approximately 70% to 80% of subjects in a large population.

Conclusion: Salivary ammonia and hemoglobin have potential as salivary markers in the screening of periodontal disease.

Abbreviations: CAL = clinical attachment loss, CPI = Community Periodontal Index, DM = diabetes mellitus, MetS = Metabolic syndrome, PD = probing depth, SMT = Salivary Multi Test, WBC = white blood cell count.

Keywords: ammonia, occult blood, periodontitis, saliva, salivary hemoglobin

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Formal consent was not required for this type of study.

Compliance with Ethical Standards

The authors have no conflicts of interests to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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1. Introduction

Periodontitis, a pathological infection-driven inflammatory disease that causes the destruction of periodontal tissues, is the one of the most common oral diseases in adults.[1,2] According to the Japanese Survey of Dental Diseases conducted in 2016, the number of individuals with more than 20 teeth has increased; however, the proportion of those with a periodontal probing depth (PD) ≥4 mm was high in all age groups, particularly the elderly.[3] Periodontal disease is one of the major causes of permanent tooth extraction, with a high rate of extraction being reported in individuals aged 30 to 60 years.[4] Regular periodontal maintenance therapy is important for preventing the onset and progression of periodontal disease. In Japan, screening for periodontitis has been conducted as part of the health services for individuals aged 40 to 50 years by the Japanese Ministry of Health since 1995, and was extended to those aged 60 to 70 years in 2005.[5] However, the percentage of individuals undergoing screening is low.

Metabolic syndrome (MetS) is a disorder complex of components including visceral fat-type obesity, hypertension, and abnormal glucose and lipid metabolism. Individuals with MetS are at an increased risk of developing cardiovascular diseases and type 2 diabetes mellitus (DM). The prevalence of MetS in a middle-aged Japanese population was previously reported to be 14.9%. [6] A strong relationship exists between

MetS/MetS components and periodontal disease.[6–21] Individuals with MetS were found to have poor periodontal conditions and a higher prevalence of more severe and prolonged periodontitis.[21] A comprehensive health examination revealed a correlation between Mets and deep PD with severe clinical attachment loss (CAL) or moderate PD with moderate CAL.[6] An interactive causal relationship has been suggested between these parameters. Risk factors for MetS include obesity, physical inactivity, insulin resistance, aging, hormonal imbalances, and a genetic predisposition,[7,8] while those for periodontitis may include being overweight, obesity, weight gain, and increased waist circumference.[18] A previous study suggested that many chronic diseases, including periodontitis, hypertension, and DM, are influenced by common risk factors such as a poor diet, smoking, alcohol, lack of exercise, and stress.[22] Therefore, investigations and health public policies that include MetS and periodontitis are important for promoting public health.

Basic guidelines by the Ministry of Health, Labor, and Welfare in Japan propose an obligatory specific health check-up (a group medical examination) that focuses on visceral fat obesity by medical insurers of the insured and their dependents aged older than 40 years.[23] However, this health check-up does not include a dental check-up because of the associated costs and manpower needed to conduct dental examinations. Therefore, a simple and low-cost method that effectively diagnoses periodontal disease is needed. Previous studies reported the clinical significance of simple and non-invasive screening methods using saliva to screen for periodontal disease.[5,24–28] In this study, the results of salivary screening tests and the dental examination were compared. The purpose of the present study was to investigate the accuracy of screening methods using saliva in dental check-ups that may be included in the specific health check-up in the future.

2. Materials and methods

The protocol of the present study was approved by the Committee on Medical Research of Shinshu University (♯2775).

Between 2017 and 2018, a dental check-up was simultaneously conducted on individuals who underwent the specific health check-up in Azumino and Shiojiri cities, Nagano Prefecture, Japan. All individuals were insured by the national health insurance system (including self-employed workers, farmers, and the elderly) and were aged 30 years and older. They all provided written informed consent to participate as subjects in this study.

All subjects underwent a dental examination and salivary tests during the specific health check-up. The dental examination included assessments of dental and periodontal conditions by 5 well-trained dentists (all authors). All dentists were trained with models before dental examination for calibration. The grade of periodontal disease was assessed according to the World Health Organization (WHO) Community Periodontal Index (CPI) criteria.[29] PD was measured using standard WHO probes. Periodontal disease was diagnosed according to the CPI code: Code 0 (healthy periodontal condition) was judged as healthy, Codes 1 and 2 (with gingival bleeding on probing, BOP) as gingivitis, and Codes 3 and 4 (PD $\geq$ 4 mm) as periodontitis. In 2017, a self-reported questionnaire that included the question "Have your gums bled recently?" was completed, and the presence or absence of gingival bleeding was confirmed based on the answer provided.

A salivary sample as stimulated saliva was collected with 3 ml of mouthwash before the dental examination and immediately subjected to salivary tests. Salivary screening testes were performed using the following commercially available test kits: Salivary Multi Test (SMT) (LION Dental Products Co., Ltd., Tokyo, Japan) and Perioscreen (Sunstar, Osaka, Japan). SMT was utilized in both years (2017 and 2018) and Perioscreen was added in 2018. Salivary screening tests with SMT and Perioscreen were performed according to the manufacturers' protocols. SMT evaluated 4 items: the levels of occult blood, protein, and ammonia and the white blood cell count (WBC). Measurement results were expressed as percentages and classified into 3 ranks (much, average, or little) according to the criteria established in a previous study.[30] Occult blood was assessed using Perioscreen, which is an immunochromatographic strip that measures a monoclonal antibody reaction against human hemoglobin. In Perioscreen, occult blood was judged as being positive or negative according to the manufacturer's reference. Subjects were asked to refrain from eating and drinking, brushing their teeth, or gargling for 2 hours prior to salivary tests because these factors may cause bleeding and affect the results obtained.[5,30]

The results of salivary screening tests and the dental examination were compared, and the accuracy of salivary screening tests was assessed. The statistical analysis between the results of salivary screening tests and the dental examination were performed with chi-squared test, Spearman's rank correlation coefficient, and one- way analysis of variance. The F-measure was calculated to test validity.[31] Statistical analyses were performed using JMP ver.13 (SAS Institute Inc., North Carolina, USA). *P* values <.05 were considered to be significant.

3. Results

Among subjects who underwent the specific health check-up, 1888 (24.1%) out of 7848 in 2017 and 2296 (32.4%) out of 7,084 in 2018 provided consent to and underwent the dental examination and salivary tests. There were 875 (46.3%) men and 1013 (53.7%) women with a mean age of 64.8 $\pm$ 12.9 years in 2017, and 1125 men (49.0%) and 1,171 (51.0%) women with a mean age of 67.7 $\pm$ 11.7 years in 2018. The results of the dental examination for periodontal disease were shown in Table 1. In 2017, 489 subjects were diagnosed with gingivitis and 831 with periodontitis, and the prevalence of periodontal disease was 69.9%. In 2018, 457 subjects were diagnosed with gingivitis and 1076 with periodontitis, and the prevalence of periodontal disease was 66.8%.

The results of salivary tests were also shown in Table 1. Some samples were not available/analyzed due to subject or examiner errors (including the absence of samples and technical errors). Therefore, 1887 samples in 2017 and 2253 in 2018 were obtained for testing using SMT. Regarding occult blood, 49.9% of subjects were classified as "much" in 2017 and 54.8% in 2018. In the assessment of WBC, 55.6% of subjects were classified as "much" in 2017 and 54.9% in 2018. Concerning protein levels, 66.4% of subjects were classified as "much" in 2017 and 64.1% in 2018. In the evaluation of ammonia levels, 81.7% of subjects were classified as "much" in 2017 and 81.4% in 2018. On the other hand, the results of Perioscreen showed that 1525 out of 2296 salivary samples (66.4%) were positive for occult blood. In the self-reported questionnaire, 179 out of 1887 subjects (9.5%) reported the presence of gingival bleeding.

Table 1

Results of dental and salivary examinations.

	2017 Number (%)	2018 Number (%)
Number of subjects who underwent the specific health check-up	7848	7084
Number of subjects who underwent the dental check-up	1888 (24.1)	2296 (32.4)
Sex		
Male	875 (46.3)	1125 (49.0)
Female	1013 (53.7)	1171 (51.0)
Age		
Average ± SD	64.8±12.9	66.0±12.6
Range	25–95	29–94
Results of the dental check-up for periodontal disease	1888 (24.1)	2296 (32.4)
Healthy (CPI=0)	528 (28.0)	725 (31.6)
Gingivitis (CPI=1, 2)	489 (25.9)	457 (19.9)
Periodontitis (CPI=3, 4)	831 (44.0)	1076 (46.9)
Unmeasurable	40 (2.1)	38 (1.7)
Results of the salivary examination using SMT*	1887 (24.0)	2253 (31.8)
Occult blood		
Little	350 (18.5)	331 (14.7)
Average	596 (31.6)	687 (30.5)
Much	941 (49.9)	1,253 (54.8)
White blood cell count		
Little	291 (15.4)	315 (14.0)
Average	546 (28.9)	700 (31.1)
Much	1,050 (55.6)	1238 (54.9)
Protein		
Little	239 (12.7)	285 (12.6)
Average	395 (20.9)	524 (23.3)
Much	1253 (66.4)	1444 (64.1)
Ammonia		
Little	93 (4.9)	108 (4.8)
Average	253 (13.4)	310 (13.8)
Much	1541 (81.7)	1835 (81.4)
Results of the salivary examination using Perioscreen	–	2296 (32.4)
Positive	–	1525 (66.4)
Negative	–	771 (33.6)
Results of the questionnaire survey	1887 (24.0)	–
Presence of gingival bleeding	179 (9.5)	–
Absence of gingival bleeding	1708 (95.0)	–

* Some samples were not available/analyzed due to subject or examiner reasons.
CPI = Community Periodontal Index.

The relationship between the results of SMT and the dental examination for periodontal disease was shown in Table 2. In the classification performance of each item of SMT in the screening of periodontal disease (gingivitis and periodontitis), "Much" was defined as positive for periodontal disease in each item.

The classification performance of each item of SMT in the screening of periodontal disease (gingivitis and periodontitis) was summarized in Table 3. "Much" was defined as positive for periodontal disease in each item. Ammonia showed the highest sensitivity (83.5% in 2017 and 83.1% in 2018), but low specificity (22.9% in 2017 and 21.6% in 2018). Specificity was the highest for occult blood at 66.9% in 2017 and 57.6% in 2018 with moderate sensitivity (57.5% in 2017 and 61.5% in 2018). Precision was the highest for occult blood (81.3% in 2017 and 75.6%), and was also high for ammonia (73.0% in 2017 and 69.3% in 2018). The F-measure was the highest for ammonia (0.779 in 2017 and 0.756 in 2018), followed by protein (0.746 in 2017 and 0.702 in 2018). The significant correlation wad detected between ammonia concentration and bleeding on probing (one- way analysis of variance: $P<.05$) (Fig. 1). The pocket depth also correlated with ammonia concentration significantly (one- way analysis of variance: $P<.05$)(Fig. 2).

The classification performance of each item of SMT for the screening of periodontitis was also assessed (Table 4). "Much" in each item was defined as positive for periodontitis. Ammonia showed the highest sensitivity (87.7% in 2017 and 83.3% in 2018), but low specificity (23.3% in 2017 and 20.0% in 2018). Occult blood had the highest specificity at 60.0% in 2017 and 53.9% in 2018 with moderate sensitivity (53.5% in 2017 and 65.6% in 2018). Precision was the highest for occult blood (56.4% in 2017 and 56.6% in 2018) and the lowest for ammonia (48.3% in 2017 and 48.9% in 2018). The highest F-measure was obtained for protein (0.633) in 2017 and ammonia (0.616) in 2018.

The classification performance of Perioscreen in the screening of periodontal disease and the relationship between the results of Perioscreen and the dental examination were shown in Table 5. A correlation was observed between the results of Perioscreen and the dental examination (Spearman's rank correlation; $r=0.13$, $P<.01$). In the screening of periodontal disease (periodontitis

Table 2

Relationships between salivary test results using the Salivary Multi Test (SMT) and the diagnosis of periodontal diseases in the dental examination.

	2017					2018				
Items in SMT	**Periodontitis**	**Gingivitis**	**Healthy**	**Unmeasurable***	***Spearman's rank correlation***	**Periodontitis**	**Gingivitis**	**Healthy**	**Unmeasurable***	***Spearman's rank correlation***
Occult blood										
Much (n)	527	232	175	7	$r=0.28$	695	232	300	8	$r=0.21$
Average (n)	229	160	195	12	$P<.01$	263	141	269	14	$P<.01$
Little (n)	74	97	158	21		102	75	138	16	
White blood cell count										
Much (n)	537	268	239	6	$r=0.19$	653	240	334	11	$r=0.14$
Average (n)	227	134	177	8	$P<.01$	304	151	237	8	$P<.01$
Little (n)	66	87	112	26		103	57	136	19	
Protein										
Much (n)	654	299	284	16	$r=0.23$	761	268	394	21	$r=0.16$
Average (n)	113	121	146	15	$P<.01$	206	113	195	10	$P<.01$
Little (n)	63	69	98	9		93	67	118	7	
Ammonia										
Much (n)	728	373	407	33	$r=0.13$	883	370	554	28	$r=0.05$
Average (n)	80	87	80	6	$P<.01$	132	56	116	6	$P<.05$
Little (n)	22	29	41	1		45	22	37	4	

* No dentition.

and gingivitis), sensitivity was 69.5% and precision was 70.6%, with an F-measure of 0.701. In the screening of periodontitis, sensitivity was 73.8% and precision was 52.6%, with an F-measure of 0.614.

Regarding the use of self-reported gingival bleeding as a marker of periodontal disease, awareness of gingival bleeding correlated with the prevalence of periodontal disease; however, the correlation coefficient was very low (Spearman's rank correlation; $r=0.07$, $P<.01$)(Table 6). In the classification performance of screening for periodontal disease, specificity was very high (93.8%), whereas sensitivity was very low (11.0%). The resulting F-measure was also low (0.195). In the screening of periodontitis, sensitivity was 11.2% and precision was 52.2%, with an F-measure of 0.185.

4. Discussion

The effectiveness of the addition of a dental check-up to the specific health check-up performed in Japan has not yet been established. However, a correlation has been reported between

Table 3

Classification performance of each item of SMT for the screening of periodontal disease (gingivitis and periodontitis).

2017 Items in SMT		Periodontal disease	Healthy		2018 Items in SMT	Items in SMT	Periodontal disease	Healthy	
Occult blood	Much (n)	759	175	$P<.01$*	Occult blood	Much (n)	927	300	$P<.01$*
	Average/Little (n)	560	353			Average/Little (n)	581	407	
	Sensitivity	57.5%	Accuracy	60.2%		Sensitivity	61.5%	Accuracy	60.2%
	Specificity	66.9%	Precision	81.3%		Specificity	57.6%	Precision	75.6%
	F-measure	0.674				F-measure	0.678		
White blood cell count	Much (n)	805	239	$P<.01$*	White blood cell count	Much (n)	893	334	$P<.01$*
	Average/Little (n)	514	289			Average/Little (n)	615	373	
	Sensitivity	61.0%	Accuracy	59.2%		Sensitivity	59.2%	Accuracy	57.2%
	Specificity	54.7%	Precision	77.1%		Specificity	52.8%	Precision	72.8%
	F-measure	0.681				F-measure	0.653		
Protein	Much (n)	953	284	$P<.01$*	Protein	Much (n)	1,029	394	$P<.01$*
	Average/Little (n)	366	244			Average/Little (n)	479	313	
	Sensitivity	72.3%	Accuracy	64.8%		Sensitivity	68.2%	Accuracy	60.6%
	Specificity	46.2%	Precision	77.0%		Specificity	44.3%	Precision	72.3%
	F-measure	0.746				F-measure	0.702		
Ammonia	Much (n)	1,101	407	$P<.01$*	Ammonia	Much (n)	1,253	554	$P<.01$*
	Average/Little (n)	218	121			Average/Little (n)	255	153	
	Sensitivity	83.5%	Accuracy	66.2%		Sensitivity	83.1%	Accuracy	63.5%
	Specificity	22.9%	Precision	73.0%		Specificity	21.6%	Precision	69.3%
	F-measure	0.779				F-measure	0.756		

* Chi-Squared test.

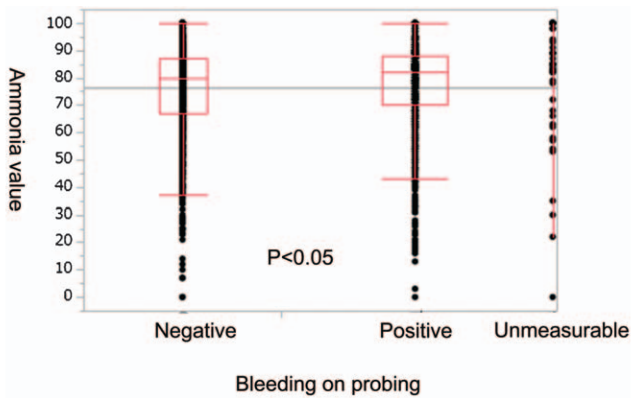

Figure 1. The correlation between salivary ammonia and bleeding on probing. There was a significant correlation between salivary ammonia and bleeding on probing.

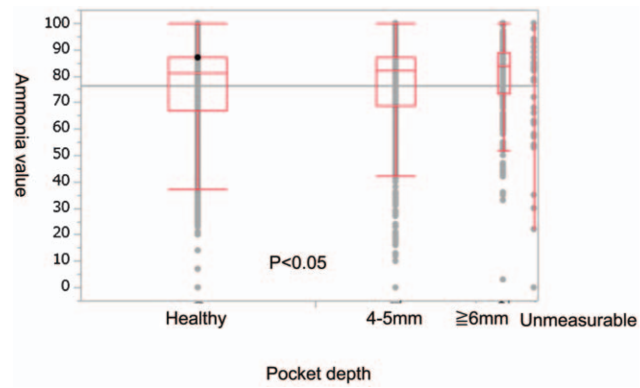

Figure 2. The correlation between salivary ammonia and pocket depth. There was a significant correlation between salivary ammonia and pocket depth.

MetS and periodontitis.[6–21] In our longitudinal study,[20] the prevalence of subjects with more positive MetS components was higher in those with persistent/progressive periodontitis than in those with no/improved periodontitis; therefore, periodontitis appears to be an important factor in the prevention of pre-MetS and MetS. Based on the concept of the common risk factor approach, the early detection and treatment of periodontitis may effectively suppress or prevent the development of pre-MetS and MetS.[28] However, difficulties are associated with performing accurate periodontal examinations on large-scale populations, such as in the specific health check-up. The accuracy of the CPI code was previously reported to vary with the extent of training conducted by dentists, which may have affected the findings obtained.[32–34] Furthermore, the associated costs and manpower needed to perform dental check-ups by trained dentists are prohibitive. Screening tests using saliva were previously shown to be useful for the diagnosis of periodontitis because they are non-invasive and simple to perform without the need for a direct examination of periodontal tissue by skilled professionals.[5,24–28] Therefore, in the present study, the efficacy of salivary screening tests was investigated in the specific health check-up.

A number of salivary biomarkers for periodontitis, including β-glucuronidase, lactate dehydrogenase, alkaline phosphatase, and occult blood, were employed in previous studies. Salivary β-glucuronidase activity ≥100 was reported with an odds ratio of 3.77 in at least 4 sites with PD ≥5 mm.[24] In another study, the activities of lactate dehydrogenase and alkaline phosphatase and level of occult blood were used in the screening of periodontitis in pregnant women (sensitivity; 0.90, specificity; 0.62, positive predictive value; 0.18, negative predictive value; 0.98).[25] In the present study, SMT and Perioscreen, commercially available salivary test kits in Japan, were used to screen for periodontal disease. SMT measures 3 items (occult blood, WBC, and protein)

Table 4

Classification performance of each item of SMT for the screening of periodontitis.

2017					2018				
Items in SMT		**Periodontitis**	**Gingivitis/ Healthy**		**Items in SMT**	**Items in SMT**	**Periodontitis**	**Gingivitis/ Healthy**	
Occult blood	Much (n)	527	407	$P<.01^*$	Occult blood	Much (n)	695	532	$P<.01^*$
	Average/Little (n)	303	610			Average/Little (n)	365	623	
	Sensitivity	63.5%	Accuracy	61.6%		Sensitivity	65.6%	Accuracy	59.5%
	Specificity	60.0%	Precision	56.4%		Specificity	53.9%	Precision	56.6%
	F-measure	0.598				F-measure	0.608		
White blood cell count	Much (n)	537	507	$P<.01^*$	White blood cell count	Much (n)	653	574	$P<.01^*$
	Average/Little (n)	293	510			Average/Little (n)	407	581	
	Sensitivity	64.7%	Accuracy	56.7%		Sensitivity	61.6%	Accuracy	55.7%
	Specificity	50.1%	Precision	51.4%		Specificity	50.3%	Precision	53.2%
	F-measure	0.573				F-measure	0.571		
Protein	Much (n)	654	583	$P<.01^*$	Protein	Much (n)	761	662	$P<.01^*$
	Average/Little (n)	176	434			Average/Little (n)	299	493	
	Sensitivity	78.8%	Accuracy	58.9%		Sensitivity	71.8%	Accuracy	56.6%
	Specificity	42.7%	Precision	52.9%		Specificity	42.7%	Precision	53.5%
	F-measure	0.633				F-measure	0.613		
Ammonia	Much (n)	728	780	$P<.01^*$	Ammonia	Much (n)	883	924	$P<.01^*$
	Average/Little (n)	102	237			Average/Little (n)	177	231	
	Sensitivity	87.7%	Accuracy	52.2%		Sensitivity	83.3%	Accuracy	50.3%
	Specificity	23.3%	Precision	48.3%		Specificity	20.0%	Precision	48.9%
	F-measure	0.623				F-measure	0.616		

* Chi-Squared test.

Table 5

Classification performance of Perioscreen in the screening of periodontal disease and periodontitis and the relationship between results of Perioscreen and the dental examination.

A: Relationship between results of Perioscreen and the dental examination					
Results of Perioscreen	**Periodontitis**	**Gingivitis**	**Healthy**	**Unmeasurable***	***Spearman's rank correlation***
Positive (n)	794	272	443	16	$r=0.13$
Negative (n)	282	185	282	22	$P<.01$

B: Classification performance in the screening of periodontal disease			
Results of Perioscreen	Periodontal disease	Healthy	
Positive (n)	1,066	443	
Negative (n)	467	282	
Sensitivity	69.5%	Accuracy	59.7%
Specificity	38.9%	Precision	70.6%
F-measure	0.701		

C: Classification performance in the screening of periodontitis			
Results of Perioscreen	Periodontitis	Gingivitis/ Healthy	
Positive (n)	794	715	
Negative (n)	282	467	
Sensitivity	73.8%	Accuracy	55.8%
Specificity	39.5%	Precision	52.6%
F-measure	0.614		

* No dentition.

as markers of periodontal disease and 1 item (ammonia) for oral cleanliness. A previous study showed that PD, BOP, and CPI correlated with occult blood and protein levels as well as WBC measured by SMT.[35] The results of the present study were consistent with these findings. The present results also showed that the total number of bacteria correlated with ammonia levels measured by SMT. The level of oral ammonia is considered to reflect oral cleanliness. Occult blood was also evaluated using Perioscreen. Salivary occult blood was measured using peroxidase activity in SMT and by a monoclonal antibody reaction against human hemoglobin in Perioscreen.

The results of the present study showed that the level of salivary ammonia correlated with periodontal disease, and that the salivary ammonia test was the most accurate marker (F-measure, 0.779) in the screening of periodontal disease (gingivitis and periodontitis) with high sensitivity (83.1–83.5%) and precision (69.3–73.0%); however, its specificity was low (21.6–22.9%). This is the first study to demonstrate the usefulness of salivary ammonia in the screening of periodontal disease. Additionally, salivary ammonia correlated significantly with bleeding on probing and pocket depth. Previous studies reported a correlation between salivary ammonia and the oral bacterial count; ammonia in mixed oral saliva was derived by the bacterial hydrolysis of urea within the mouth.[36–41] Therefore, an increase in the level of salivary ammonia may be used as an indicator of oral hygiene, reflected by the total number of salivary

Table 6

Classification performance of self-reported gingival bleeding in the screening of periodontal disease and the relationship between results of the questionnaire and dental examination.

A: Relationship between results of the questionnaire and dental examination					
Self-reported gingival bleeding	**Periodontitis**	**Gingivitis**	**Healthy**	**missing data**	***Spearman's rank correlation***
Presence (n)	93	52	33	1	$r=0.07$
Absence (n)	737	437	495	39	$P<.01$

B: Classification performance in the screening of periodontal disease			
Self-reported gingival bleeding	Periodontal disease	Healthy	
Presence (n)	145	33	
Absence (n)	1,174	495	
Sensitivity	11.0%	Accuracy	34.7%
Specificity	93.8%	Precision	81.5%
F-measure	0.194		

C: Classification performance in the screening of periodontitis			
Self-reported gingival bleeding	Periodontitis	Gingivitis/ Healthy	
Presence (n)	93	85	
Absence (n)	737	932	
Sensitivity	11.2%	Accuracy	55.5%
Specificity	91.6%	Precision	52.2%
F-measure	0.185		

* No dentition.

bacteria. Ishikawa et al. examined ammonia production by oral and opportunistic microorganisms in 40 standard microbial strains. Among 23 species and 31 strains of oral bacteria, 21 species and 26 strains produced ammonia and the most active bacteria was *Porphyromonas gingivalis*, a Gram-negative oral anaerobe that is considered to be the main etiological bacteria in periodontal disease.[37] Although salivary ammonia levels appear to be the most accurate marker in the screening of periodontal disease and periodontitis in large populations, they were previously shown to be affected by systemic disease/conditions due to the translocation of ammonia from blood to saliva by *Helicobacter pylori* infection.[42] Furthermore, the correlation between the grade of periodontal disease and level of ammonia was weak ($r=0.13$ in 2017 and $r=0.05$ in 2018) due to the difficulties associated with distinguishing between gingivitis and periodontitis.

Periodontal disease includes 2 stages, gingivitis and periodontitis. Gingivitis is inflammation of the gingiva caused by bacteria in dental plaque, which may be reversed by the control of dental plaque. However, when gingivitis is left untreated, it may progress to a more severe infection, namely, periodontitis, in which the periodontium (soft and bone tissues responsible for firmly anchoring teeth) is irreversibly destroyed. Therefore, the progression of gingivitis to periodontitis needs to be prevented. In the present study, the classification performance of salivary tests in the screening of periodontitis from gingivitis/healthy gums was also examined. In all salivary tests used in the present study, their performance at classifying periodontitis (periodontitis vs gingivitis and healthy gingiva) was lower than that for periodontal disease (periodontitis and gingivitis vs healthy gingiva). A correlation was observed between the grade of periodontal disease and the results of each salivary test. The salivary tests employed in the present study did not accurately distinguish between periodontitis and gingivitis. Salivary occult blood, protein, and ammonia levels as well as the WBC count are markers of inflammation or bacterial counts as well as tissue destruction. Inflammation and infection exist in both stages of periodontal disease, while tissue destruction only occurs in the advanced stage (periodontitis). Therefore, the items examined in the present study reflected the inflammation/infection of gingival tissue and have potential as markers to screen for periodontal disease.

The screening test of salivary occult blood was previously reported to be useful in the screening of subjects with periodontitis.[5,25–28] Salivary occult blood levels correlated with the proportion of teeth that bled following probing and with PD≥4 mm.[26] Detection methods for salivary occult blood measured salivary hemoglobin using colorimetric methods or mono- or polyclonal antibody reactions. [5,25–28,43–46] The monoclonal antibody method was previously demonstrated to be useful and adequate for the screening for periodontitis.[5,26–28] In the present study, occult blood was measured by 2 different methods. The method using the monoclonal antibody reaction (Perioscreen) showed higher sensitivity (0.695) and F-measure (0.701) than that using the peroxide reaction (SMT). The percentages of subjects with periodontitis were 44.0% in 2017 and 46.9% in 2018, and these results were consistent with the percentage of subjects older than 30 years with periodontitis (PD of ≥4 mm) reported by the Survey of Dental Diseases in 2016[3]. In the present study, the sensitivity, specificity, and accuracy of Perioscreen were 69.5, 38.9, and 59.7%, respectively. These percentages were consistent with previous findings.[26,47,48] Therefore, the present results support the use of Perioscreen as a screening tool for periodontal disease in a large population, such as in the specific health check-up.

The main symptom of periodontal disease is swollen and bleeding gingiva. Gingivitis and periodontitis are suspected when gingiva bleed easily when eating, brushing, flossing, and probing. Self-reporting is a widely accepted technique for assessing the occurrence of many diseases in population surveys. In the present study, a question on gingival bleeding was included in the questionnaire and the accuracy of self-reported gingival bleeding in the screening of periodontal disease was assessed. The results obtained showed that the accuracy of self-reported gingival bleeding was low (accuracy 34.7%, sensitivity 11.0%, and specificity 93.8%). These results were not consistent with previous findings because of differences in the questions asked, methodologies (periodontal examination and definition of periodontitis), and sample characteristics (age, socioeconomic characteristics, and access to dental services). The validity of self-reported gingival bleeding was previously examined in adult population-based studies and was found to have low sensitivity and high specificity, which is in accordance with the present results.[49,50] Therefore, questions solely on self-reported gingival bleeding are not sufficient to screen for periodontitis in an adult-based medical check-up. Previous studies advocated the usefulness of a set of questions.[51,52] Further studies are needed to assess self-reported measures in the screening of periodontal disease.

The present study is the first to demonstrate the useful of SMT and Perioscreen in the screening of subjects for periodontal disease and periodontitis in a large population study, such as a dental check-up in the Japanese-specific health check-up. However, there was some imitations that need to be addressed. Differences in diagnostic criteria for periodontitis make comparisons with previous studies difficult. The dentist calibration also affects the results of the dental examination. Therefore, the dentists that participated in the present study were trained with the model for the dentist calibration.[34] Another limitation was the cut-off values of the test items in SMT. In SMT, occult blood, WBC, protein, and ammonia was classified and graded into 3 grades. Further studies are necessary to set the cut-off values of these items.

In conclusion, the validity of salivary screening tests for the screening of periodontal disease in a large population (in the Japanese-specific health check-up) was investigated in the present study. The results obtained indicate that salivary ammonia is a useful salivary test with high accuracy and precision. Occult blood detected by the monoclonal antibody to human hemoglobin was also useful. Screening tests using salivary samples may detect periodontal disease in approximately 70% to 80% of subjects. A correlation has been reported between salivary occult blood and BMI, DM, and brushing frequency[5]. In future studies, we intend to examine the relationship between the results of salivary tests and Mets.

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