Correlations between the properties of saliva and metabolic syndrome

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Running title: Usefulness of a saliva test kit for screening for metabolic syndrome

Abstract

Saliva tests, which are easy to perform and non-invasive, can be used to monitor both oral disease (especially periodontal disease) and physical conditions, including metabolic syndrome (MetS). Therefore, in the present study the associations between saliva test results and MetS were investigated based on medical health check-up data for a large population. In total, 1,888 and 2,296 individuals underwent medical check-ups for MetS and simultaneous saliva tests in 2017 and 2018, respectively. The saliva tests were performed using a commercially available test kit, which could be used to evaluate the buffer capacity of saliva; salivary pH; the salivary white blood cell count; the number of cariogenic bacteria in saliva; and salivary occult blood, protein, and ammonia levels. The relationships between the results of the saliva tests and MetS components were examined in cross-sectional and longitudinal multivariate analyses. Significant relationships were detected between salivary protein levels and serum HbA1c levels or blood pressure levels and between the buffer capacity of saliva and serum triglyceride levels. In addition, salivary pH was increased irreversibly by impaired renal function. This study suggested that saliva tests conducted during health check-ups of large populations might be a useful screening tool for periodontal disease and MetS/MetS components.

Keywords: saliva test, metabolic syndrome, medical check-up, blood pressure, screening

Abbreviations: Metabolic syndrome (MetS), diabetes mellitus (DM), high-density lipoprotein cholesterol (HDL-C), Salivary Multi Test (SMT), white blood cell (WBC), chronic kidney disease (CKD)

Introduction

Metabolic syndrome (MetS) is a complex medical disorder, which is defined as the presence of three out of five interrelated conditions attributed to visceral fat-type obesity, including hypertension and abnormal glucose and lipid metabolism[1, 2]. MetS was reported to increase the risk of cardiovascular disease, including atherosclerotic cardiovascular disease, and type 2 diabetes mellitus (DM)[3, 4]. The prevalence of MetS has increased worldwide[5]. In 2011–2012, the estimated prevalence of MetS in the USA was 34.7% and increased with age; i.e., it was 18.3% in adults aged 20–39 years and 46.7% in those aged ≥60 years[6]. In middle-aged Japanese individuals, the prevalence of MetS was reported to be 14.9%[7].

Periodontitis is a pathological infectious inflammatory disease, which causes the destruction of periodontal tissue and can lead to tooth loss[8]. In previous studies[7, 9–11], a close correlation was detected between periodontitis and MetS, and individuals with MetS have been reported to present with a worse periodontal status, including a higher prevalence of periodontitis, more severe periodontitis, and more wide-ranging periodontitis[10]. Many chronic diseases, including periodontitis, hypertension, and DM, are influenced by common risk factors including diet, smoking, alcohol, a lack of exercise, and stress (Figure 1)[12, 13]. It has been reported that chronic systemic inflammation might predispose individuals with periodontal disease to develop components of MetS or vise versa[14]. Therefore, investigations and health public policies targeting MetS and periodontitis are important for promoting public health.

Saliva tests are easy to conduct and non-invasive, and it has been reported that such tests can produce clinically significant information relating to both systemic and oral disease[15–21]. Many researchers have reported that saliva-based screening tests are useful for diagnosing periodontitis[15–21]. As stated above, periodontitis and MetS are closely related and influenced by the same common risk factors. Previously, we reported the effectiveness of incorporating dental check-ups into health check-ups and detected a significant association between periodontitis and MetS[11]. These results suggested that saliva tests could be used to monitor not only periodontal conditions, but also physical conditions related to MetS. Therefore, the purpose of the present study was to investigate the associations between the results of saliva tests and MetS based on medical health check-up data for a large population.

Materials and methods

The protocol of the present study was approved by the Committee on Medical Research of Shinshu University (♯2775). Individuals who underwent specific health check-ups (health check-ups for MetS)

in the Japanese cities Azumino and Shiojiri between 2017 and 2018 were invited to participate in the study. All of the subjects, which included self-employed workers, farmers, and the elderly, were insured by the Japanese national health insurance system and were aged ≥25 years. They all provided written informed consent before participating in this study. The subjects underwent saliva tests during their health check-ups. The health check-ups were conducted according to the standard program provided by the Ministry of Health, Labour and Welfare of Japan (2013)[22]. They included an interview on lifestyle and systemic disease treatment status (including on recent smoking habits and whether the patient was taking medication for hypertension, lipid abnormalities, or hyperglycemia); height, weight, abdominal circumference, and blood pressure measurements; and blood tests (of triglyceride, high-density lipoprotein cholesterol [HDL-C], blood sugar, hemoglobin A1c [HbA1c], and creatinine levels).

Regarding the saliva tests, each saliva sample was collected with 3 ml of mouthwash and was immediately evaluated using a commercially available test kit (Salivary Multi Test [SMT]; LION Dental Products Co., Ltd., Tokyo, Japan). The saliva tests were performed according to the manufacturer's protocols and were used to evaluate the buffer capacity of saliva; the number of cariogenic bacteria present in saliva; salivary pH; salivary occult blood, protein, and ammonia levels; and the salivary white blood cell (WBC) count. The test kit consisted of test strips and a measuring device. In this test, the color changes that occur in each pad of the test strip are assessed by measuring reflectance at a specific wavelength. Specifically, the number of cariogenic bacteria present in saliva is evaluated based on the reduction of resazurin sodium by Gram-positive bacteria, pH is assessed based on the color change exhibited by a pH indicator, buffer capacity is determined based on the color change exhibited a compound pH indicator in the presence of a fixed quantity of acid, the salivary occult blood level is assessed by measuring pseudo-peroxidase activity in hemoglobin, the WBC count is evaluated by measuring leukocyte esterase activity, the salivary protein level is determined based on the "protein error of indicators" phenomenon, and the salivary ammonia level is assessed based the color change seen after the addition of bromocresol green. The principles underlying the measurement of each parameter are summarized in Table 1. The results of the saliva tests are expressed as percentages (0–100) and were classified into 3 categories (high, moderate, and low), according to the values established by the manufacturer[23]. Individuals who had been eating/drinking, had brushed their teeth, or had gargled within 2 hours before the salivary test were excluded from the study because these might have affected the test results.

Table 1. Detection principles of the Salivary Multi Test		
Number (%)	Number (%)	
Test item	**Measurement principle**	**Detection range**
No. of cariogenic bacteria	Resazurin —(Reduction by bacteria)→ Resorufin(magenta)	10^6-10^8 cfu/mL
pH	H^+ + pH indicator → Color change of pH indicator (yellow-blue)	pH 6.0-8.0
Buffer capacity	H^+ + Combined pH indicator → Color change of combined pH indicator (yellow -blue)	pH 2.8-6.0
Occult blood level	CHP+TMBZ —(Hemoglobin)→ H_2O+Cumene+Oxidized TMBZ(blue)	0-0.50 mg/dL
WBC count	TAI —(Leukocyte esterase, Hydrolysis)→ Indoxyl Indoxyl+MMB —(Coupling reaction)→ Azo dye(purple)	0-200 U/L
Protein level	Protein+TCTIF(light pink) —(Acid)→ Complex formation(red)	0-60 mg/dL
Ammonia level	Ammonium ion —(Alkaline)→ Ammonia gas Ammonia gas+BCG → Color change of BCG(blue)	1-10,000 (as nitrogen) μg/dL

Table 1. Detection principles of the Salivary Multi Test

_____: Detected Substance □:Component of strip

The results of the saliva test were compared with the results of the health check-up in the cross-sectional analysis. In addition, in the longitudinal analysis the relationships between the changes in the saliva test results and the changes in the health check-up results were analyzed in the individuals who underwent examinations in both 2017 and 2018. In this study, the interyear changes in the saliva test results that occurred between 2017 and 2018 were classified into the four following categories:
Remained high: “high” in both 2017 and 2018
Increased: “moderate/low” in 2017 and “high” in 2018
Decreased: “high” in 2017 and “moderate/low” in 2018
Remained low: “moderate/low” in both 2017 and 2018

Statistical analyses were performed using JMP ver.13 (SAS Institute Inc., North Carolina, USA). In the cross-sectional analysis, the correlations between the results of the saliva test and the health check-up results were examined using univariate analyses (Spearman’s rank correlation coefficient) and multivariate analysis involving common risk (confounding) factors. In the longitudinal analysis, the correlations between the interyear changes in the results of the saliva test and the interyear changes in the health check-up parameters (the value obtained in 2018 minus the value obtained in 2017) were evaluated using univariate analyses (involving the Tukey-Kramer HSD test) and multivariate analysis of common risk factors (sex, age in 2017, change in BMI, and change in smoking habits). P-values of <0.05 were considered to indicate statistical significance.

Results

Among the individuals who underwent the health check-up, 1,887 (24.0%) out of the 7,848 individuals who underwent the health check-up in 2017 and 2,279 (32.2%) out of the 7,084 individuals who underwent the health check-up in 2018 consented to saliva tests and participated in the study. The subjects’ characteristics and the results of the saliva tests are summarized in Table 2.

The results of the cross-sectional analysis

The correlations between systolic or diastolic blood pressure and the results of the saliva test are shown in Tables 3 and 4. This analysis included the data from the subjects who were not taking antihypertensive medication (n=1,374). Although in the univariate analyses weak but significant correlations were observed between systolic or diastolic blood pressure and the buffer capacity of saliva (diastolic blood pressure: $p<0.05$) or the salivary levels of occult blood (systolic blood pressure: $p<0.05$; diastolic blood pressure: $p<0.05$), protein (systolic blood pressure; $p<0.01$; diastolic blood pressure: $p<0.05$), or ammonia (systolic blood pressure: $p<0.01$; diastolic blood pressure: $p<0.01$), the multivariate analysis did not reveal any significant correlations between these parameters. The only significant correlation found in the multivariate analysis was between systolic blood pressure and the number of cariogenic bacteria in saliva ($p<0.05$), even though no such correlation was detected in the

univariate analysis.

The correlations between serum triglyceride or HDL-C levels and the results of the saliva test are shown in Tables 5 and 6. This analysis included the data for the subjects who were not taking antihyperlipidemic medication (n=1,545). Although weak but significant or nearly significant correlations were observed between serum triglyceride or HDL-C levels and salivary buffer capacity (serum HDL-C level: $p<0.05$), the salivary levels of occult blood (serum triglyceride level: $p<0.05$; serum HDL-C level: $p<0.01$) or protein (serum triglyceride level: $p<0.01$; serum HDL-C level: $p<0.01$), or the salivary WBC count (serum triglyceride level: $p<0.05$; serum HDL-C level: $p=0.058$) in the univariate analyses, the multivariate analysis only showed nearly significant correlations between the serum triglyceride ($p=0.053$) or HDL-C ($p=0.091$) level and the salivary WBC count. In addition, the multivariate analysis revealed significant correlations between the serum triglyceride level and salivary buffer capacity ($p<0.05$) and between the serum HDL-C level and salivary pH ($p<0.05$) or the salivary ammonia level ($p<0.01$); however, no significant correlations were observed between these parameters in the univariate analyses.

The correlations between the serum HbA1C level and the results of the saliva test are shown in Table 7. This analysis included the data for the subjects who were not taking antidiabetic medication (n=1,769). A significant correlation was found between the serum HbA1C level and salivary buffer capacity in both the univariate and multivariate analyses (univariate analysis: $p<0.01$; multivariate analysis: $p<0.05$). In addition, a significant correlation between the serum HbA1C level and the salivary protein level was detected in the univariate analyses, and a nearly significant correlation between these parameters was found in the multivariate analysis ($p=0.060$). While the serum HbA1C level exhibited significant correlations with the salivary occult blood level, WBC count, and ammonia level in the univariate analyses, no such correlations were found in the multivariate analysis.

The correlations between the serum creatinine level and the results of the saliva test are shown in Table 8. Significant correlations were found between the serum creatinine level and salivary pH or buffer capacity in both the univariate and multivariate analyses (pH: univariate analysis, $p<0.01$, multivariate analysis, $p<0.01$; buffer capacity: univariate analysis, $p<0.01$, and multivariate analysis, $p<0.01$). Although weak but significant correlations were observed between the serum creatinine level and the number of cariogenic bacteria in saliva ($p<0.05$), the salivary occult blood level ($p<0.01$), the salivary protein level ($p<0.01$), and the salivary ammonia level ($p<0.01$) in the univariate analyses, no such correlations between these parameters were detected in the multivariate analysis.

	2017	2018
	Number (%)	Number (%)
No. of subjects that underwent health check-ups	7,848	7,084
No. of subjects that underwent saliva tests	1,887 (24.0)	2,279 (32.2)
Sex		
Male	875 (46.3)	1,119 (49.1)
Female	1,012 (53.7)	1,160 (50.9)
Age		
Mean ± SD	64.8 ± 12.9	67.6 ± 11.7
Range	25–95	29–96
Results of the salivary tests performed using the SMT	1,887	2,279
No. of cariogenic bacteria		
High	994 (52.7%)	1,051 (46.1%)
Moderate	495 (26.2%)	542 (23.8%)
Low	399 (21.1%)	686 (30.1%)
pH		
High	1,239 (65.3%)	1,571 (69.9%)
Moderate	430 (22.8%)	485 (21.3%)
Low	219 (11.6%)	223 (9.8%)
Buffer capacity		
High	757 (40.1%)	921 (40.4%)
Moderate	640 (33.9%)	788 (34.6%)
Low	491 (26.0%)	570 (25.0%)
Occult blood level		
High	941 (49.9)	1,253 (55.0)
Moderate	596 (31.6)	693 (30.4)
Low	350 (18.5)	333 (14.6)
WBC count		
High	1,050 (55.6)	1,253 (55.0)
Moderate	546 (28.9)	708 (31.1)
Low	291 (15.4)	318 (14.0)
Protein level		
High	1,253 (66.4)	1,463 (64.2)
Moderate	395 (20.9)	528 (23.2)
Low	239 (12.7)	288 (12.6)
Ammonia level		
High	1,541 (81.7)	1,858 (81.5)
Moderate	253 (13.4)	312 (13.7)
Low	93 (4.9)	109 (4.8)

Table 2. Characteristics of study subjects

			Univariate analysis							Multivariate analysis				
Number (%)	Number (%)		Systolic blood pressure					Spearman's rank correlation						
	Level	n	Mean	SE	95%CI			r	p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacteria	High	703	122.0	0.62	120.8	–	123.2	-0.005	0.853	**No. of cariogenic bacteri**	-1.041	0.506	-2.06	<0.05
	Moderate	362	122.2	0.86	120.5	–	123.9			Sex (female/male)	-0.465	0.432	-1.08	0.283
	Low	309	122.6	0.93	120.8	–	124.4			Age (years)	0.379	0.031	12.17	<0.01
										BMI (kg/m^2)	1.246	0.130	9.55	<0.01
										Smoker (no/yes)	-0.893	0.747	-1.2	0.232
pH	High	912	121.9	0.5	120.9	–	123.0	-0.026	0.342	**pH**	0.182	0.603	0.3	0.763
	Moderate	310	122.6	0.9	120.7	–	124.4			Sex (female/male)	-0.485	0.436	-1.11	0.266
	Low	152	123.1	1.3	120.5	–	125.7			Age (years)	0.372	0.031	11.98	<0.01
										BMI (kg/m^2)	1.240	0.131	9.5	<0.01
										Smoker (no/yes)	-0.855	0.752	-1.14	0.256
Buffer capacity	High	508	123.5	0.7	122.1	–	125.0	-0.026	0.342	**Buffer capacity**	-0.606	0.541	-1.12	0.263
	Moderate	471	122.6	0.8	121.1	–	124.1			Sex (female/male)	-0.550	0.439	-1.25	0.211
	Low	395	120.0	0.8	118.4	–	121.6			Age (years)	0.383	0.033	11.75	<0.01
										BMI (kg/m^2)	1.244	0.131	9.52	<0.01
										Smoker (no/yes)	-0.835	0.749	-1.12	0.265
Occult blood level	High	638	123.5	0.6	122.2	–	124.8	0.117	<0.01	**Occult blood level**	0.071	0.544	0.13	0.897
	Moderate	460	122.1	0.8	120.6	–	123.6			Sex (female/male)	-0.469	0.433	-1.08	0.280
	Low	276	119.4	1.0	117.5	–	121.3			Age (years)	0.370	0.032	11.67	<0.01
										BMI (kg/m^2)	1.239	0.131	9.45	<0.01
										Smoker (no/yes)	-0.871	0.750	-1.16	0.246
Protein level	High	854	123.4	0.6	122.3	–	124.5	0.111	<0.01	**Protein level**	-0.306	0.593	-0.52	0.606
	Moderate	321	121.2	0.9	119.4	–	122.9			Sex (female/male)	-0.473	0.433	-1.09	0.275
	Low	199	118.6	1.2	116.4	–	120.9			Age (years)	0.377	0.033	11.45	<0.01
										BMI (kg/m^2)	1.245	0.131	9.51	<0.01
										Smoker (no/yes)	-0.868	0.748	-1.16	0.246
WBC count	High	734	122.5	0.6	121.3	–	123.7	0.031	0.252	**WBC count**	-0.435	0.549	-0.79	0.428
	Moderate	411	122.3	0.8	120.7	–	123.9			Sex (female/male)	-0.453	0.434	-1.05	0.296
	Low	229	121.0	1.1	118.9	–	123.1			Age (years)	0.375	0.031	11.97	<0.01
										BMI (kg/m^2)	1.243	0.131	9.51	<0.01
										Smoker (no/yes)	-0.885	0.748	-1.18	0.237
Ammonia level	High	1088	123.1	0.5	122.1	–	124.0	0.111	<0.01	**Ammonia level**	0.598	0.770	0.78	0.438
	Moderate	209	119.9	1.1	117.7	–	122.1			Sex (female/male)	-0.442	0.435	-1.02	0.309
	Low	77	116.2	1.9	112.6	–	119.8			Age (years)	0.364	0.032	11.31	<0.01
										BMI (kg/m^2)	1.239	0.131	9.48	<0.01
										Smoker (no/yes)	-0.896	0.748	-1.2	0.231

Table 3. Correlation between systolic blood pressure and results of salivary multi test in those who had no antihypertensive medication （n = 1,374）

			Univariate analysis							Multivariate analysis				
Number (%)	Number (%)		Diastolic blood pressure					Spearman's rank correlation						
	Level	n	Mean	SE	95%CI			r	p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacteri	High	703	73.9	0.41	73.1	–	74.7	-0.004	0.880	**No. of cariogenic bacte**	-0.529	0.340	-1.56	0.120
	Moderate	362	74.1	0.57	73.0	–	75.3			Sex (female/male)	-1.726	0.290	-5.94	<0.01
	Low	309	74.3	0.62	73.1	–	75.5			Age (years)	0.154	0.021	7.36	<0.01
										BMI (kg/m^2)	0.798	0.088	9.11	<0.01
										Smoker (no/yes)	-0.908	0.502	-1.81	0.071
pH	High	912	73.9	0.36	73.2	–	74.6	-0.024	0.380	**pH**	0.119	0.405	0.29	0.769
	Moderate	310	74.2	0.62	73.0	–	75.4			Sex (female/male)	-1.739	0.293	-5.94	<0.01
	Low	152	74.8	0.88	73.0	–	76.5			Age (years)	0.151	0.021	7.23	<0.01
										BMI (kg/m^2)	0.795	0.088	9.07	<0.01
										Smoker (no/yes)	-0.885	0.504	-1.76	0.080
Buffer capacity	High	508	74.8	0.48	73.8	–	75.7	0.06	<0.05	**Buffer capacity**	-0.377	0.363	-1.04	0.299
	Moderate	471	74.3	0.50	73.3	–	75.2			Sex (female/male)	-1.778	0.295	-6.04	<0.01
	Low	395	72.9	0.55	71.8	–	74.0			Age (years)	0.157	0.022	7.19	<0.01
										BMI (kg/m^2)	0.797	0.088	9.1	<0.01
										Smoker (no/yes)	-0.873	0.502	-1.74	0.082
Occult blood level	High	638	74.6	0.43	73.7	–	75.4	0.065	<0.05	**Occult blood level**	-0.048	0.365	-0.13	0.895
	Moderate	460	74.4	0.51	73.4	–	75.4			Sex (female/male)	-1.729	0.291	-5.95	<0.01
	Low	276	72.4	0.65	71.1	–	73.7			Age (years)	0.151	0.021	7.07	<0.01
										BMI (kg/m^2)	0.796	0.088	9.05	<0.01
										Smoker (no/yes)	-0.904	0.503	-1.8	0.073
Protein level	High	854	74.5	0.37	73.8	–	75.2	0.069	<0.05	**Protein level**	-0.221	0.398	-0.56	0.578
	Moderate	321	74.0	0.61	72.8	–	75.2			Sex (female/male)	-1.731	0.291	-5.96	<0.01
	Low	199	72.3	0.77	70.8	–	73.8			Age (years)	0.154	0.022	6.98	<0.01
										BMI (kg/m^2)	0.798	0.088	9.09	<0.01
										Smoker (no/yes)	-0.893	0.502	-1.78	0.076
WBC count	High	734	74.4	0.40	73.6	–	75.1	0.044	0.106	**WBC count**	0.269	0.369	0.73	0.465
	Moderate	411	74.1	0.54	73.1	–	75.2			Sex (female/male)	-1.738	0.291	-5.97	<0.01
	Low	229	73.0	0.72	71.5	–	74.4			Age (years)	0.148	0.021	7.01	<0.01
										BMI (kg/m^2)	0.794	0.088	9.06	<0.01
										Smoker (no/yes)	-0.895	0.502	-1.78	0.075
Ammonia level	High	1088	74.5	0.33	73.9	–	75.2	0.086	<0.01	**Ammonia level**	0.553	0.517	1.07	0.2845
	Moderate	209	72.9	0.75	71.4	–	74.3			Sex (female/male)	-1.703	0.292	-5.84	<0.01
	Low	77	70.5	1.23	68.1	–	72.9			Age (years)	0.144	0.022	6.65	<0.01
										BMI (kg/m^2)	0.793	0.088	9.05	<0.01
										Smoker (no/yes)	-0.917	0.502	-1.83	0.068

Table 4. Correlation between diastolic blood pressure and results of salivary multi test in those who had no antihypertensive medication （n = 1,374）

			Univariate analysis							Multivariate analysis				
Number (%)	Number (%)		Serum triglyceride level					Spearman's rank correlation						
	Level	n	Mean	SE	95%CI			r	p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacteria	High	797	110.3	2.51	105.4	–	115.2	-0.011	0.680	**No. of cariogenic bacteria**	-4.037	2.123	-1.9	0.058
	Moderate	414	116.0	3.48	109.2	–	122.9			Sex (female/male)	-6.484	1.788	-3.63	<0.01
	Low	334	114.6	3.88	107.0	–	122.2			Age (years)	0.453	0.131	3.47	<0.01
										BMI (kg/m^2)	6.359	0.533	11.92	<0.01
										Smoker (no/yes)	-10.438	3.078	-3.39	<0.01
pH	High	1033	113.9	2.21	109.6	–	118.3	0.01	0.696	**pH**	3.945	2.480	1.59	0.112
	Moderate	336	110.9	3.87	103.4	–	118.5			Sex (female/male)	-6.782	1.799	-3.77	<0.01
	Low	176	109.3	5.35	98.8	–	119.8			Age (years)	0.437	0.130	3.36	<0.01
										BMI (kg/m^2)	6.332	0.534	11.87	<0.01
										Smoker (no/yes)	-9.965	3.091	-3.22	0.001
Buffer capacity	High	596	113.3	2.91	107.6	–	119.0	0.016	0.527	**Buffer capacity**	-5.276	2.247	-2.35	<0.05
	Moderate	517	111.8	3.12	105.7	–	117.9			Sex (female/male)	-7.226	1.816	-3.98	<0.01
	Low	432	113.2	3.41	106.5	–	119.9			Age (years)	0.525	0.137	3.84	<0.01
										BMI (kg/m^2)	6.335	0.533	11.89	<0.01
										Smoker (no/yes)	-10.233	3.077	-3.33	<0.01
Occult blood level	High	755	117.5	2.57	112.5	–	122.5	0.087	<0.01	**Occult blood level**	2.817	2.263	1.24	0.213
	Moderate	488	113.1	3.20	106.8	–	119.4			Sex (female/male)	-6.395	1.791	-3.57	<0.01
	Low	302	100.4	4.07	92.4	–	108.4			Age (years)	0.385	0.133	2.89	<0.01
										BMI (kg/m^2)	6.286	0.536	11.74	<0.01
										Smoker (no/yes)	-10.157	3.086	-3.29	<0.01
Protein level	High	992	116.8	2.25	112.4	–	121.2	0.083	<0.01	**Protein level**	4.173	2.472	1.69	0.092
	Moderate	334	107.8	3.87	100.3	–	115.4			Sex (female/male)	-6.367	1.790	-3.56	<0.01
	Low	219	102.2	4.78	92.8	–	111.5			Age (years)	0.344	0.138	2.5	<0.05
										BMI (kg/m^2)	6.317	0.534	11.84	<0.01
										Smoker (no/yes)	-10.403	3.079	-3.38	<0.01
WBC count	High	842	116.1	2.44	111.3	–	120.9	0.054	<0.05	**WBC count**	4.409	2.281	1.93	0.0534
	Moderate	449	111.4	3.34	104.8	–	117.9			Sex (female/male)	-6.560	1.789	-3.67	<0.01
	Low	254	104.1	4.44	95.4	–	112.9			Age (years)	0.382	0.131	2.91	<0.01
										BMI (kg/m^2)	6.318	0.533	11.84	<0.01
										Smoker (no/yes)	-10.386	3.078	-3.37	<0.01
Ammonia level	High	1235	113.9	2.02	110.0	–	117.9	0.036	0.156	**Ammonia level**	0.585	3.241	0.18	0.8569
	Moderate	226	111.0	4.71	101.8	–	120.3			Sex (female/male)	-6.452	1.796	-3.59	<0.01
	Low	84	100.1	7.73	84.9	–	115.2			Age (years)	0.417	0.135	3.09	<0.01
										BMI (kg/m^2)	6.340	0.534	11.87	<0.01
										Smoker (no/yes)	-10.402	3.083	-3.37	<0.01

Table 5. Correlation between triglyceride and results of salivary multi test in those who had no antihyperlipidemic medication (n = 1,545)

			Univariate analysis							Multivariate analysis				
Number (%)	Number (%)		Serum HDL-C level					Spearman's rank correlation						
	Level	n	Mean	SE	95%CI			r	p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacteri	High	797	63.6	0.58	62.4	–	64.7	-0.014	0.580	**No. of cariogenic bacter**	0.174	0.460	0.38	0.706
	Moderate	414	63.8	0.80	62.2	–	65.4			Sex (female/male)	4.260	0.387	11	<0.01
	Low	334	63.8	0.89	62.1	–	65.6			Age (years)	-0.057	0.028	-2.03	<0.05
										BMI (kg/m^2)	-1.577	0.115	-13.66	<0.01
										Smoker (no/yes)	0.912	0.666	1.37	0.171
pH	High	1033	63.5	0.51	62.5	–	64.5	-0.009	0.723	**pH**	-1.096	0.536	-2.04	<0.05
	Moderate	336	64.2	0.89	62.5	–	66.0			Sex (female/male)	4.344	0.389	11.17	<0.01
	Low	176	63.9	1.22	61.5	–	66.3			Age (years)	-0.060	0.028	-2.13	<0.05
										BMI (kg/m^2)	-1.574	0.115	-13.65	<0.01
										Smoker (no/yes)	0.792	0.668	1.19	0.236
Buffer capacity	High	596	62.7	0.66	61.4	–	64.0	-0.062	<0.05	**Buffer capacity**	0.563	0.487	1.16	0.247
	Moderate	517	63.8	0.71	62.4	–	65.2			Sex (female/male)	4.339	0.393	11.04	<0.01
	Low	432	64.8	0.78	63.3	–	66.4			Age (years)	-0.067	0.030	-2.26	<0.05
										BMI (kg/m^2)	-1.576	0.115	-13.66	<0.01
										Smoker (no/yes)	0.893	0.666	1.34	0.180
Occult blood level	High	755	62.2	0.59	61.1	–	63.4	-0.107	<0.01	**Occult blood level**	-0.785	0.489	-1.61	0.109
	Moderate	488	64.3	0.73	62.8	–	65.7			Sex (female/male)	4.236	0.387	10.94	<0.01
	Low	302	66.4	0.93	64.6	–	68.3			Age (years)	-0.045	0.029	-1.58	0.115
										BMI (kg/m^2)	-1.561	0.116	-13.49	<0.01
										Smoker (no/yes)	0.845	0.667	1.27	0.205
Protein level	High	992	62.7	0.51	61.7	–	63.7	-0.082	<0.01	**Protein level**	-0.711	0.535	-1.33	0.184
	Moderate	334	65.4	0.89	63.7	–	67.2			Sex (female/male)	4.240	0.387	10.95	<0.01
	Low	219	65.3	1.09	63.2	–	67.5			Age (years)	-0.043	0.030	-1.43	0.153
										BMI (kg/m^2)	-1.573	0.115	-13.62	<0.01
										Smoker (no/yes)	0.912	0.666	1.37	0.171
WBC count	High	842	63.1	0.56	62.0	–	64.2	-0.048	0.058	**WBC count**	-0.834	0.493	-1.69	0.091
	Moderate	449	63.9	0.77	62.4	–	65.4			Sex (female/male)	4.275	0.387	11.05	<0.01
	Low	254	65.3	1.02	63.3	–	67.2			Age (years)	-0.048	0.028	-1.7	0.089
										BMI (kg/m^2)	-1.572	0.115	-13.63	<0.01
										Smoker (no/yes)	0.909	0.666	1.37	0.172
Ammonia level	High	1235	63.8	0.46	62.9	–	64.7	0.008	0.766	**Ammonia level**	1.984	0.699	2.84	<0.01
	Moderate	226	63.6	1.08	61.4	–	65.7			Sex (female/male)	4.347	0.387	11.22	<0.01
	Low	84	62.8	1.77	59.3	–	66.3			Age (years)	-0.079	0.029	-2.71	<0.01
										BMI (kg/m^2)	-1.581	0.115	-13.73	<0.01
										Smoker (no/yes)	0.868	0.665	1.31	0.192

Table 6. Correlation between HDL-cholesterol and results of salivary multi test in those who had no antihyperlipidemic medication (n = 1545)

			Univariate analysis								Multivariate analysis				
Number (%)	Number (%)		Serum HbA1c level					Spearman's rank correlation							
	Level	n	Mean	SE	95%CI			r	p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacter	High	926	5.72	0.02	5.69	–	5.75	0.017	0.483	**No. of cariogenic bacteri**	0.000	0.001	0.13	0.895
	Moderate	467	5.71	0.02	5.67	–	5.75			Sex (female/male)	0.024	0.015	1.63	0.103
	Low	376	5.71	0.02	5.66	–	5.76			Age (years)	0.011	0.001	9.73	<0.01
										BMI (kg/m^2)	0.033	0.004	7.61	<0.01
										Smoker (no/yes)	-0.019	0.026	-0.71	0.475
pH	High	1156	5.72	0.01	5.69	–	5.74	-0.018	0.451	**pH**	0.021	0.015	1.38	0.168
	Moderate	405	5.72	0.02	5.67	–	5.76			Sex (female/male)	0.023	0.011	2.03	<0.05
	Low	208	5.69	0.03	5.63	–	5.76			Age (years)	0.010	0.001	11.39	<0.01
										BMI (kg/m^2)	0.027	0.003	8.24	<0.01
										Smoker (no/yes)	0.003	0.021	0.17	0.869
Buffer capacity	High	697	5.75	0.02	5.72	–	5.79	0.129	<0.01	**Buffer capacity**	0.026	0.011	2.29	<0.05
	Moderate	603	5.73	0.02	5.69	–	5.77			Sex (female/male)	0.009	0.001	10.54	<0.01
	Low	469	5.63	0.02	5.59	–	5.68			Age (years)	0.027	0.003	8.26	<0.01
										BMI (kg/m^2)	0.001	0.020	0.03	0.974
										Smoker (no/yes)	0.009	0.014	0.64	0.522
Occult blood level	High	870	5.74	0.02	5.71	–	5.77	0.079	<0.01	**Occult blood level**	0.004	0.014	0.31	0.758
	Moderate	562	5.72	0.02	5.68	–	5.76			Sex (female/male)	0.025	0.011	2.22	<0.05
	Low	337	5.63	0.03	5.58	–	5.68			Age (years)	0.009	0.001	10.95	<0.01
										BMI (kg/m^2)	0.027	0.003	8.21	<0.01
										Smoker (no/yes)	0.001	0.020	0.07	0.943
Protein level	High	1427	5.74	0.01	5.71	–	5.76	0.157	<0.01	**Protein level**	0.030	0.016	1.88	0.060
	Moderate	249	5.66	0.03	5.60	–	5.72			Sex (female/male)	0.025	0.011	2.25	<0.05
	Low	93	5.51	0.05	5.42	–	5.61			Age (years)	0.009	0.001	10.02	<0.01
										BMI (kg/m^2)	0.027	0.003	8.2	<0.01
										Smoker (no/yes)	0.001	0.020	0.05	0.963
WBC count	High	980	5.74	0.02	5.71	–	5.77	0.061	<0.05	**WBC count**	0.014	0.015	0.96	0.339
	Moderate	512	5.70	0.02	5.66	–	5.74			Sex (female/male)	0.024	0.011	2.17	<0.05
	Low	277	5.65	0.03	5.59	–	5.71			Age (years)	0.009	0.001	11.01	<0.01
										BMI (kg/m^2)	0.027	0.003	8.26	<0.01
										Smoker (no/yes)	0.001	0.020	0.06	0.955
Ammonia level	High	1427	5.74	0.01	5.71	–	5.76	0.135	<0.01	**Ammonia level**	0.034	0.021	1.65	0.098
	Moderate	249	5.66	0.03	5.60	–	5.72			Sex (female/male)	0.026	0.011	2.33	<0.05
	Low	93	5.51	0.05	5.42	–	5.61			Age (years)	0.009	0.001	10.47	<0.01
										BMI (kg/m^2)	0.027	0.003	8.23	<0.01
										Smoker (no/yes)	0.001	0.020	0.03	0.980

Table 7. Correlation between HbA1c and results of salivary multi test in those who had no antidiabetic medication (n = 1,769)

Number (%)	Number (%)		Univariate analysis: Serum creatinine level					Spearman's rank correlation		Multivariate analysis				
	Level	n	Mean	SE	95%CI			r	p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacte	High	994	0.75	0.01	0.73	–	0.76	0.053	<0.05	**No. of cariogenic bacteri**	0.011	0.006	1.70	0.089
	Moderate	495	0.72	0.01	0.70	–	0.74			Sex (female/male)	-0.113	0.005	-21.62	<0.01
	Low	399	0.72	0.01	0.69	–	0.74			Age (years)	0.002	0.000	4.17	<0.01
										BMI (kg/m^2)	0.004	0.002	2.84	<0.01
										Smoker (no/yes)	-0.015	0.009	-1.64	0.100
pH	High	1239	0.71	0.01	0.69	–	0.72	-0.160	<0.01	**pH**	-0.044	0.007	-6.09	<0.01
	Moderate	430	0.74	0.01	0.72	–	0.76			Sex (female/male)	-0.109	0.005	-20.90	<0.01
	Low	219	0.85	0.02	0.82	–	0.88			Age (years)	0.002	0.000	4.02	<0.01
										BMI (kg/m^2)	0.005	0.002	3.05	<0.01
										Smoker (no/yes)	-0.020	0.009	-2.17	0.030
Buffer capacity	High	757	0.78	0.01	0.76	–	0.80	0.209	<0.01	**Buffer capacity**	0.020	0.007	3.01	<0.01
	Moderate	640	0.71	0.01	0.69	–	0.73			Sex (female/male)	-0.110	0.005	-20.79	<0.01
	Low	491	0.68	0.01	0.66	–	0.70			Age (years)	0.001	0.000	3.27	<0.01
										BMI (kg/m^2)	0.004	0.002	2.82	<0.01
										Smoker (no/yes)	-0.016	0.009	-1.75	0.081
Occult blood level	High	940	0.75	0.01	0.73	–	0.76	0.080	<0.01	**Occult blood level**	0.003	0.007	0.40	0.692
	Moderate	597	0.72	0.01	0.70	–	0.74			Sex (female/male)	-0.113	0.005	-21.59	<0.01
	Low	351	0.71	0.01	0.68	–	0.73			Age (years)	0.002	0.000	4.19	<0.01
										BMI (kg/m^2)	0.004	0.002	2.84	<0.01
										Smoker (no/yes)	-0.015	0.009	-1.63	0.104
Protein level	High	1254	0.74	0.01	0.73	–	0.76	0.067	<0.01	**Protein level**	0.004	0.007	0.58	0.564
	Moderate	395	0.72	0.01	0.69	–	0.74			Sex (female/male)	-0.113	0.005	-21.59	<0.01
	Low	239	0.70	0.02	0.67	–	0.73			Age (years)	0.002	0.000	3.94	<0.01
										BMI (kg/m^2)	0.004	0.002	2.87	<0.01
										Smoker (no/yes)	-0.016	0.009	-1.66	0.098
WBC count	High	1050	0.73	0.01	0.71	–	0.74	0.001	0.959	**WBC count**	-0.002	0.007	-0.30	0.765
	Moderate	545	0.74	0.01	0.72	–	0.76			Sex (female/male)	-0.113	0.005	-21.59	<0.01
	Low	293	0.73	0.01	0.70	–	0.76			Age (years)	0.002	0.000	4.39	<0.01
										BMI (kg/m^2)	0.004	0.002	2.90	<0.01
										Smoker (no/yes)	-0.015	0.009	-1.65	0.099
Ammonia level	High	1539	0.74	0.01	0.73	–	0.75	0.114	<0.01	**Ammonia level**	0.013	0.010	1.30	0.1954
	Moderate	255	0.69	0.02	0.66	–	0.72			Sex (female/male)	-0.112	0.005	-21.45	<0.01
	Low	94	0.66	0.03	0.61	–	0.71			Age (years)	0.002	0.000	3.90	<0.01
										BMI (kg/m^2)	0.004	0.002	2.84	<0.01
										Smoker (no/yes)	-0.016	0.009	-1.67	0.096

Table 8. Correlation between serum creatinine and results of salivary multi test (n = 1,888)

The results of the longitudinal analysis

The correlations between the interyear changes in systolic and diastolic blood pressure and the interyear changes in the saliva test results are shown in Tables 9 and 10. This analysis included the data for the subjects who were not taking antihypertensive medication in either 2017 or 2018 (n=539). The interyear change in systolic blood pressure was significantly correlated with the interyear changes in the salivary protein level ($p<0.01$) and WBC count ($p<0.01$), whereas diastolic blood pressure was significantly correlated with the interyear change in the salivary protein level ($p<0.01$). The subjects that exhibited high salivary protein levels and WBC counts in both 2017 and 2018 had elevated blood pressure, while those with low salivary protein levels and WBC counts displayed decreased blood pressure in both years.

The correlations between the interyear changes in the serum levels of triglycerides or HDL-C and the interyear changes in the saliva test results are shown in Tables 11 and 12. This analysis included the data for the subjects who were not taking antihyperlipidemic medication in either 2017 or 2018 (n=608). A significant inverse correlation was found between the interyear change in the serum

triglyceride level and the interyear change in the buffer capacity of saliva in the multivariate analysis ($p<0.05$), even though no significant correlation between these parameters was detected in the univariate analysis.

The correlations between the interyear change in the serum HbA1C level and the interyear changes in the saliva test results are shown in Table 13. This analysis included the data for the subjects who were not taking antidiabetic medication in either 2017 or 2018 (n=728). The interyear change in the serum HbA1C level was shown to be significantly correlated with the interyear change in the salivary protein level in the univariate analyses ($p<0.05$), and the correlation between these parameters was found to be nearly significant in the multivariate analysis ($p=0.052$). Increased serum HbA1C levels were seen in the subjects who had high salivary protein levels in both 2017 and 2018, while decreased serum HbA1C levels were observed in those that displayed low salivary protein levels in both years.

The correlations between the interyear change in the serum creatinine level and the interyear changes in the saliva test results are shown in Table 14. No significant correlations were found between these parameters.

		Univariate analysis							Multivariate analysis				
Number (%)	Number (%)		Interyear change in systolic blood pressure					Tukey-Kramer HSD					
		n	Mean	SE	95%CI			p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacte	Remained high	136	1.169	1.079	-0.950	–	3.288	NS	**Change in No. of cariogenic bact**	0.435	0.462	0.94	0.347
	Increased	88	1.477	1.341	-1.157	–	4.112		Sex (female/male)	0.168	0.549	0.31	0.760
	Decreased	130	1.092	1.103	-1.075	–	3.260		Age (2017)	0.029	0.044	0.65	0.515
	Remained low	185	-0.524	0.925	-2.341	–	1.293		Change in BMI	0.868	0.358	2.42	<0.05
									Change in smoking habits	1.264	0.803	1.57	0.116
pH	Remained high	266	1.008	0.772	-0.509	–	2.524	NS	**Change in pH**	0.321	0.475	0.68	0.500
	Increased	99	0.748	1.265	-1.738	–	3.233		Sex (female/male)	0.126	0.552	0.23	0.819
	Decreased	76	-1.013	1.444	-3.850	–	1.824		Age (2017)	0.041	0.044	0.93	0.352
	Remained low	98	0.704	1.272	-1.794	–	3.203		Change in BMI	0.882	0.358	2.46	<0.05
									Change in smoking habits	1.298	0.802	1.62	0.106
Buffer capacity	Remained high	77	-0.558	1.435	-3.378	–	2.261	NS	**Change in buffer capacity**	-0.433	0.499	-0.87	0.385
	Increased	75	0.587	1.454	-2.270	–	3.444		Sex (female/male)	0.214	0.551	0.39	0.698
	Decreased	83	0.145	1.383	-2.571	–	2.860		Age (2017)	0.026	0.045	0.59	0.555
	Remained low	304	1.056	0.722	-0.363	–	2.475		Change in BMI	0.881	0.358	2.46	<0.05
									Change in smoking habits	1.337	0.800	1.67	0.095
Occult blood level	Remained high	168	0.881	0.968	-1.021	–	2.783	NS	**Change in occult blood level**	0.551	0.431	1.28	0.202
	Increased	108	2.787	1.208	0.415	–	5.159		Sex (female/male)	0.175	0.548	0.32	0.749
	Decreased	59	-0.458	1.634	-3.667	–	2.752		Age (2017)	0.024	0.044	0.55	0.583
	Remained low	204	-0.431	0.879	-2.157	–	1.295		Change in BMI	0.879	0.358	2.46	<0.05
									Change in smoking habits	1.297	0.800	1.62	0.106
Protein level	Remained high	233	2.498	0.815	0.898	–	4.098		**Change in protein level**	1.658	0.435	3.81	<0.01
	Increased	65	2.092	1.542	-0.937	–	5.122	<0.01	Sex (female/male)	0.104	0.542	0.19	0.848
	Decreased	74	-0.176	1.445	-3.015	–	2.663		Age (2017)	-0.024	0.046	-0.52	0.600
	Remained low	167	-2.222	0.962	-4.111	–	-0.332		Change in BMI	0.869	0.354	2.46	<0.05
									Change in smoking habits	1.328	0.790	1.68	0.093
WBC count	Remained high	194	2.083	0.897	0.320	–	3.845		**Change in WBC count**	1.180	0.433	2.73	<0.01
	Increased	93	1.538	1.296	-1.008	–	4.083	<0.05	Sex (female/male)	0.024	0.548	0.04	0.965
	Decreased	85	1.082	1.355	-1.580	–	3.745		Age (2017)	0.017	0.044	0.39	0.698
	Remained low	167	-1.826	0.967	-3.726	–	0.073		Change in BMI	0.869	0.356	2.44	<0.05
									Change in smoking habits	1.224	0.796	1.54	0.125
Ammonia level	Remained high	362	0.950	0.662	-0.350	–	2.251	NS	**Change in ammonia level**	0.501	0.549	0.91	0.362
	Increased	57	-0.105	1.668	-3.382	–	3.172		Sex (female/male)	0.228	0.553	0.41	0.681
	Decreased	64	0.750	1.574	-2.342	–	3.842		Age (2017)	0.023	0.046	0.51	0.609
	Remained low	56	-0.929	1.683	-4.234	–	2.377		Change in BMI	0.881	0.358	2.46	<0.05
									Change in smoking habits	1.387	0.802	1.73	0.085

Table 9. Correlation between the interval change of systolic blood pressure and that of salivary multi test in those who had no antihypertensive medication （n = 539）

	Univariate analysis								Multivariate analysis				
Number (%)	Number (%)	nteryear change in diastolic blood pressure						Tukey-Kramer HSD					
		n	Mean	SE	95%CI			p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacter	Remained high	136	0.066	0.713	-1.334	–	1.466	NS	**Change in No. of cariogenic bac**	0.444	0.304	1.46	0.144
	Increased	88	-0.352	0.886	-2.092	–	1.388		Sex (female/male)	0.612	0.361	1.70	0.090
	Decreased	130	0.092	0.729	-1.339	–	1.524		Age (2017)	0.002	0.029	0.08	0.934
	Remained low	185	-1.616	0.611	-2.816	–	-0.416		Change in BMI	0.693	0.236	2.94	<0.01
									Change in smoking habits	0.868	0.528	1.64	0.101
pH	Remained high	266	-0.004	0.510	-1.006	–	0.998	NS	**Change in pH**	0.244	0.313	0.78	0.435
	Increased	99	-1.404	0.836	-3.047	–	0.239		Sex (female/male)	0.580	0.364	1.60	0.111
	Decreased	76	-1.526	0.954	-3.401	–	0.349		Age (2017)	0.014	0.029	0.47	0.637
	Remained low	98	-0.541	0.841	-2.192	–	1.110		Change in BMI	0.707	0.236	2.99	<0.01
									Change in smoking habits	0.912	0.528	1.73	0.085
Buffer capacity	Remained high	77	-1.844	0.949	-3.708	–	0.020	NS	**Change in buffer capacity**	-0.272	0.328	-0.83	0.408
	Increased	75	0.253	0.961	-1.635	–	2.142		Sex (female/male)	0.641	0.363	1.77	0.078
	Decreased	83	-0.241	0.914	-2.036	–	1.554		Age (2017)	0.004	0.030	0.13	0.900
	Remained low	304	-0.546	0.477	-1.484	–	0.392		Change in BMI	0.706	0.236	2.99	<0.01
									Change in smoking habits	0.941	0.527	1.78	0.075
Occult blood level	Remained high	168	-0.524	0.644	-1.788	–	0.741	NS	**Change in occult blood level**	0.082	0.284	0.29	0.772
	Increased	108	-0.269	0.803	-1.846	–	1.309		Sex (female/male)	0.613	0.362	1.69	0.091
	Decreased	59	-0.644	1.086	-2.778	–	1.490		Age (2017)	0.008	0.029	0.27	0.789
	Remained low	204	-0.755	0.584	-1.902	–	0.393		Change in BMI	0.705	0.236	2.99	<0.01
									Change in smoking habits	0.933	0.528	1.77	0.078
Protein level	Remained high	233	0.142	0.542	-0.923	–	1.206		**Change in protein level**	0.763	0.288	2.65	<0.01
	Increased	65	0.092	1.026	-1.923	–	2.108	<0.05	Sex (female/male)	0.583	0.359	1.62	0.106
	Decreased	74	0.311	0.962	-1.578	–	2.200		Age (2017)	-0.018	0.030	-0.59	0.555
	Remained low	167	-2.222	0.640	-3.479	–	-0.964		Change in BMI	0.700	0.235	2.98	<0.01
									Change in smoking habits	0.936	0.524	1.79	0.075
WBC count	Remained high	194	-0.278	0.599	-1.455	–	0.898	NS	**Change in WBC count**	0.167	0.287	0.58	0.561
	Increased	93	-0.366	0.865	-2.064	–	1.333		Sex (female/male)	0.591	0.363	1.63	0.104
	Decreased	85	-0.577	0.905	-2.353	–	1.200		Age (2017)	0.007	0.029	0.24	0.813
	Remained low	167	-1.030	0.645	-2.298	–	0.238		Change in BMI	0.703	0.236	2.98	<0.01
									Change in smoking habits	0.923	0.528	1.75	0.081
Ammonia level	Remained high	362	-0.365	0.438	-1.225	–	0.496	NS	**Change in ammonia level**	0.459	0.361	1.270	0.205
	Increased	57	-0.842	1.104	-3.012	–	1.327		Sex (female/male)	0.667	0.364	1.83	0.067
	Decreased	64	-0.672	1.042	-2.719	–	1.375		Age (2017)	-0.002	0.030	-0.06	0.952
	Remained low	56	-1.536	1.114	-3.724	–	0.653		Change in BMI	0.706	0.236	2.99	<0.01
									Change in smoking habits	0.987	0.528	1.87	0.062

Table 10. Correlation between the interval change of diastolic blood pressure and that of salivary multi test in those who had no antihypertensive medication (n = 539)

	Univariate analysis								Multivariate analysis				
Number (%)	Number (%)	nteryear change in serum triglyceride levels						Tukey-Kramer HSD					
		n	Mean	SE	95%CI			p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacteri	Remained hig	157	3.02	4.50	-5.82	–	11.85	NS	**Change in No. of cariogenic bac**	1.288	1.863	0.69	0.490
	Increased	100	2.34	5.64	-8.73	–	13.41		Sex (female/male)	-0.865	2.217	-0.39	0.697
	Decreased	150	8.43	4.60	-0.61	–	17.47		Age (2017)	-0.448	0.180	-2.49	<0.05
	Remained low	201	-1.33	3.98	-9.14	–	6.47		Change in BMI	9.685	1.196	8.10	<0.01
									Change in smoking habits	-4.117	3.333	-1.24	0.217
pH	Remained hig	300	3.24	3.26	-3.16	–	9.64	NS	**Change in pH**	-0.490	1.900	-0.260	0.797
	Increased	111	0.84	5.36	-9.69	–	11.36		Sex (female/male)	-0.864	2.232	-0.39	0.699
	Decreased	86	6.44	6.09	-5.51	–	18.40		Age (2017)	-0.434	0.180	-2.41	<0.05
	Remained low	111	0.77	5.36	-9.76	–	11.29		Change in BMI	9.719	1.195	8.13	<0.01
									Change in smoking habits	-3.997	3.338	-1.20	0.232
Buffer capacity	Remained hig	85	-8.35	6.10	-20.34	–	3.63	NS	**Change in buffer capacity**	-4.479	2.010	-2.23	<0.05
	Increased	83	6.40	6.17	-5.73	–	18.52		Sex (female/male)	-0.345	2.222	-0.16	0.877
	Decreased	95	-0.73	5.77	-12.06	–	10.61		Age (2017)	-0.516	0.181	-2.85	<0.01
	Remained low	345	5.66	3.03	-0.29	–	11.61		Change in BMI	9.663	1.190	8.12	<0.01
									Change in smoking habits	-3.932	3.320	-1.18	0.237
Occult blood level	Remained hig	214	1.21	3.84	-6.34	–	8.76	NS	**Change in occult blood level**	-0.516	1.709	-0.30	0.763
	Increased	106	-1.80	5.46	-12.53	–	8.93		Sex (female/male)	-0.957	2.217	-0.43	0.666
	Decreased	68	17.21	6.82	3.81	–	30.60		Age (2017)	-0.416	0.181	-2.30	<0.05
	Remained low	220	2.12	3.79	-5.32	–	9.57		Change in BMI	9.724	1.195	8.14	<0.01
									Change in smoking habits	-4.026	3.333	-1.21	0.228
Protein level	Remained hig	287	3.42	3.33	-3.12	–	9.96	NS	**Change in protein level**	1.968	1.749	1.13	0.261
	Increased	60	7.27	7.28	-7.03	–	21.56		Sex (female/male)	-0.970	2.214	-0.44	0.661
	Decreased	77	7.42	6.43	-5.21	–	20.04		Age (2017)	-0.498	0.188	-2.64	<0.01
	Remained low	184	-1.54	4.16	-9.70	–	6.63		Change in BMI	9.635	1.196	8.06	<0.01
									Change in smoking habits	-4.070	3.330	-1.22	0.222
WBC count	Remained hig	228	4.04	3.73	-3.30	–	11.37	NS	**Change in WBC count**	2.276	1.748	1.30	0.193
	Increased	99	7.80	5.67	-3.33	–	18.93		Sex (female/male)	-1.161	2.219	-0.52	0.601
	Decreased	99	3.11	5.67	-8.02	–	14.24		Age (2017)	-0.464	0.180	-2.59	<0.05
	Remained low	182	-1.63	4.18	-9.84	–	6.58		Change in BMI	9.680	1.194	8.11	<0.01
									Change in smoking habits	-4.324	3.335	-1.30	0.195
Ammonia level	Remained hig	416	3.71	2.77	-1.72	–	9.15	NS	**Change in ammonia level**	2.472	2.232	1.110	0.269
	Increased	65	0.12	7.00	-13.63	–	13.88		Sex (female/male)	-0.629	2.231	-0.28	0.778
	Decreased	65	3.12	7.00	-10.63	–	16.88		Age (2017)	-0.484	0.185	-2.62	<0.01
	Remained low	62	-0.82	7.17	-14.90	–	13.26		Change in BMI	9.692	1.194	8.12	<0.01
									Change in smoking habits	-3.714	3.343	-1.11	0.267

Table 11. Correlation between the interval change of triglyceride and that of salivary multi test in those who had no antihyperlipidemic medication (n = 608)

	Univariate analysis								Multivariate analysis				
Number (%)	Number (%)	nteryear change in serum HDL-C level						Tukey-Kramer HSD					
		n	Mean	SE	95%CI			p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacter	Remained high	157	-0.09	0.89	-1.83	–	1.65	NS	**Change in No. of cariogenic bac**	-0.290	0.358	-0.81	0.418
	Increased	100	-0.62	1.11	-2.80	–	1.56		Sex (female/male)	-0.749	0.426	-1.76	0.079
	Decreased	150	-0.56	0.91	-2.34	–	1.22		Age (2017)	0.006	0.035	0.18	0.854
	Remained low	201	1.03	0.78	-0.51	–	2.57		Change in BMI	-2.283	0.230	-9.93	<0.01
									Change in smoking habits	-0.296	0.641	-0.46	0.645
pH	Remained high	300	0.36	0.64	-0.90	–	1.62	NS	**Change in pH**	0.437	0.365	1.20	0.232
	Increased	111	0.08	1.06	-1.99	–	2.16		Sex (female/male)	-0.797	0.429	-1.86	0.064
	Decreased	86	-0.33	1.20	-2.68	–	2.03		Age (2017)	0.008	0.035	0.23	0.822
	Remained low	111	-0.37	1.06	-2.45	–	1.71		Change in BMI	-2.292	0.230	-9.98	<0.01
									Change in smoking habits	-0.354	0.641	-0.55	0.581
Buffer capacity	Remained high	85	0.11	1.21	-2.27	–	2.48	NS	**Change in buffer capacity**	0.131	0.388	0.34	0.736
	Increased	83	0.10	1.22	-2.30	–	2.50		Sex (female/male)	-0.751	0.429	-1.75	0.081
	Decreased	95	0.56	1.14	-1.69	–	2.80		Age (2017)	0.004	0.035	0.12	0.907
	Remained low	345	-0.06	0.60	-1.24	–	1.11		Change in BMI	-2.288	0.230	-9.95	<0.01
									Change in smoking habits	-0.316	0.641	-0.49	0.623
Occult blood level	Remained high	214	0.49	0.76	-1.00	–	1.98	NS	**Change in occult blood level**	0.059	0.329	0.18	0.858
	Increased	106	-0.84	1.08	-2.96	–	1.28		Sex (female/male)	-0.731	0.426	-1.71	0.087
	Decreased	68	-0.51	1.35	-3.16	–	2.14		Age (2017)	0.000	0.035	0.01	0.994
	Remained low	220	0.30	0.75	-1.17	–	1.78		Change in BMI	-2.291	0.230	-9.96	<0.01
									Change in smoking habits	-0.314	0.641	-0.49	0.624
Protein level	Remained high	287	-0.36	0.65	-1.64	–	0.93	NS	**Change in protein level**	0.087	0.337	0.26	0.797
	Increased	60	0.58	1.43	-2.23	–	3.40		Sex (female/male)	-0.735	0.426	-1.73	0.085
	Decreased	77	2.58	1.26	0.10	–	5.07		Age (2017)	-0.002	0.036	-0.05	0.963
	Remained low	184	-0.46	0.82	-2.06	–	1.15		Change in BMI	-2.294	0.230	-9.96	<0.01
									Change in smoking habits	-0.314	0.641	-0.49	0.625
WBC count	Remained high	228	0.00	0.74	-1.45	–	1.44	NS	**Change in WBC count**	0.065	0.337	0.19	0.847
	Increased	99	-0.45	1.12	-2.65	–	1.74		Sex (female/male)	-0.740	0.427	-1.73	0.084
	Decreased	99	0.90	1.12	-1.30	–	3.10		Age (2017)	0.000	0.035	0.01	0.992
	Remained low	182	0.03	0.83	-1.59	–	1.65		Change in BMI	-2.291	0.230	-9.96	<0.01
									Change in smoking habits	-0.320	0.642	-0.50	0.618
Ammonia level	Remained high	416	-0.32	0.54	-1.39	–	0.75	NS	**Change in ammonia level**	-0.260	0.430	-0.610	0.545
	Increased	65	3.37	1.37	0.67	–	6.07		Sex (female/male)	-0.766	0.429	-1.78	0.075
	Decreased	65	-0.48	1.37	-3.18	–	2.22		Age (2017)	0.008	0.036	0.21	0.832
	Remained low	62	-0.11	1.41	-2.88	–	2.65		Change in BMI	-2.287	0.230	-9.95	<0.01
									Change in smoking habits	-0.347	0.643	-0.54	0.590

Table 12. Correlation between the interval change of HDL-cholesterol and that of salivary multi test in those who had no antihyperlipidemic medication (n = 608)

	Univariate analysis									Multivariate analysis				
Number (%)	Number (%)	eryear change in serum HbA1C leve						Tukey-Kramer HSD						
		n	Mean	SE	95%CI			p-value			Estimate	SE	t-value	p-value
No. of cariogenic bacter	Remained hig	194	0.05	0.02	0.01	–	0.09	NS		**Change in No. of cariogenic bact**	0.012	0.009	1.45	0.147
	Increased	117	0.00	0.03	-0.05	–	0.05			Sex (female/male)	-0.010	0.010	-0.99	0.325
	Decreased	183	0.01	0.02	-0.03	–	0.05			Age (2017)	0.001	0.001	1.48	0.139
	Remained low	234	-0.01	0.02	-0.04	–	0.03			Change in BMI	0.039	0.005	7.36	<0.01
										Change in smoking habits	-0.018	0.015	-1.14	0.256
pH	Remained hig	359	0.01	0.01	-0.02	–	0.04	NS		**Change in pH**	0.000	0.009	-0.04	0.968
	Increased	135	-0.01	0.02	-0.06	–	0.04			Sex (female/male)	-0.011	0.010	-1.05	0.295
	Decreased	95	0.05	0.03	-0.01	–	0.10			Age (2017)	0.001	0.001	1.74	0.082
	Remained low	139	0.02	0.02	-0.03	–	0.07			Change in BMI	0.039	0.005	7.38	<0.01
										Change in smoking habits	-0.016	0.015	-1.03	0.301
Buffer capacity	Remained hig	91	-0.02	0.03	-0.08	–	0.03	NS		**Change in buffer capacity**	-0.007	0.010	-0.74	0.457
	Increased	102	0.03	0.03	-0.02	–	0.09			Sex (female/male)	-0.010	0.010	-0.95	0.341
	Decreased	114	-0.03	0.03	-0.08	–	0.02			Age (2017)	0.001	0.001	1.54	0.124
	Remained low	421	0.03	0.01	0.00	–	0.05			Change in BMI	0.039	0.005	7.37	<0.01
										Change in smoking habits	-0.016	0.015	-1.05	0.294
Occult blood level	Remained hig	250	0.04	0.02	0.01	–	0.08	<0.05		**Change in occult blood level**	0.006	0.008	0.74	0.457
	Increased	132	-0.04	0.02	-0.08	–	0.01			Sex (female/male)	-0.010	0.010	-1.03	0.305
	Decreased	79	0.03	0.03	-0.03	–	0.09			Age (2017)	0.001	0.001	1.61	0.108
	Remained low	267	0.00	0.02	-0.03	–	0.04			Change in BMI	0.039	0.005	7.37	<0.01
										Change in smoking habits	-0.017	0.015	-1.07	0.284
Protein level	Remained hig	346	0.04	0.01	0.01	–	0.07			**Change in protein level**	0.016	0.008	1.95	0.052
	Increased	74	0.00	0.03	-0.07	–	0.06	<0.05		Sex (female/male)	-0.011	0.010	-1.06	0.291
	Decreased	98	0.03	0.03	-0.03	–	0.08			Age (2017)	0.001	0.001	1.03	0.303
	Remained low	210	-0.03	0.02	-0.07	–	0.01			Change in BMI	0.039	0.005	7.36	<0.01
										Change in smoking habits	-0.017	0.015	-1.07	0.284
WBC count	Remained hig	273	0.03	0.02	0.00	–	0.07	NS		**Change in WBC count**	0.013	0.008	1.65	0.098
	Increased	124	0.02	0.03	-0.03	–	0.07			Sex (female/male)	-0.012	0.010	-1.22	0.224
	Decreased	119	0.00	0.03	-0.05	–	0.05			Age (2017)	0.001	0.001	1.50	0.135
	Remained low	212	-0.01	0.02	-0.05	–	0.03			Change in BMI	0.039	0.005	7.37	<0.01
										Change in smoking habits	-0.018	0.015	-1.15	0.251
Ammonia level	Remained hig	497	0.03	0.01	0.01	–	0.06	NS		**Change in ammonia level**	0.016	0.010	1.580	0.116
	Increased	80	-0.05	0.03	-0.11	–	0.02			Sex (female/male)	-0.009	0.010	-0.86	0.389
	Decreased	83	-0.03	0.03	-0.09	–	0.03			Age (2017)	0.001	0.001	1.29	0.196
	Remained low	68	-0.01	0.03	-0.07	–	0.06			Change in BMI	0.039	0.005	7.39	<0.01
										Change in smoking habits	-0.015	0.015	-0.96	0.340

Table 13. Correlation between the interval change of HbA1C and that of salivary multi test in those who had no antidiabetic medication (n = 728)

	Univariate analysis								Multivariate analysis				
Number (%)	Number (%)	eryear change in serum creatinine lev						Tukey-Kramer HSD					
		n	Mean	SE	95%CI			p-value		Estimate	SE	t-value	p-value
No. of cariogenic bacter	Remained high	207	0.002	0.008	-0.013	–	0.017	NS	**Change in No. of cariogenic bact**	-0.006	0.003	-1.80	0.073
	Increased	127	0.013	0.010	-0.006	–	0.033		Sex (female/male)	0.009	0.004	2.33	<0.05
	Decreased	195	0.023	0.008	0.007	–	0.039		Age (2017)	0.000	0.000	0.38	0.704
	Remained low	252	0.020	0.007	0.006	–	0.034		Change in BMI	0.017	0.002	8.64	<0.01
									Change in smoking habits	-0.004	0.006	-0.62	0.534
pH	Remained high	389	0.013	0.006	0.002	–	0.024	NS	**Change in pH**	-0.002	0.003	-0.73	0.468
	Increased	141	0.013	0.009	-0.005	–	0.032		Sex (female/male)	0.010	0.004	2.46	<0.05
	Decreased	103	0.017	0.011	-0.004	–	0.039		Age (2017)	0.000	0.000	-0.04	0.965
	Remained low	148	0.020	0.009	0.002	–	0.038		Change in BMI	0.017	0.002	8.59	<0.01
									Change in smoking habits	-0.004	0.006	-0.67	0.504
Buffer capacity	Remained high	95	0.015	0.011	-0.008	–	0.037	NS	**Change in buffer capacity**	-0.003	0.004	-0.78	0.437
	Increased	104	0.003	0.011	-0.018	–	0.025		Sex (female/male)	0.010	0.004	2.48	<0.05
	Decreased	121	0.019	0.010	-0.001	–	0.039		Age (2017)	0.000	0.000	-0.13	0.895
	Remained low	461	0.017	0.005	0.006	–	0.027		Change in BMI	0.017	0.002	8.58	<0.01
									Change in smoking habits	-0.004	0.006	-0.74	0.457
Occult blood level	Remained high	277	0.019	0.007	0.006	–	0.032	NS	**Change in occult blood level**	0.001	0.003	0.42	0.674
	Increased	139	0.001	0.009	-0.018	–	0.019		Sex (female/male)	0.009	0.004	2.41	<0.05
	Decreased	82	0.029	0.012	0.004	–	0.053		Age (2017)	0.000	0.000	-0.02	0.984
	Remained low	283	0.014	0.007	0.001	–	0.027		Change in BMI	0.017	0.002	8.61	<0.01
									Change in smoking habits	-0.004	0.006	-0.73	0.463
Protein level	Remained high	382	0.017	0.006	0.006	–	0.029	NS	**Change in protein level**	0.001	0.003	0.27	0.784
	Increased	79	-0.005	0.013	-0.029	–	0.020		Sex (female/male)	0.009	0.004	2.40	<0.05
	Decreased	102	0.025	0.011	0.004	–	0.047		Age (2017)	0.000	0.000	-0.04	0.971
	Remained low	218	0.013	0.008	-0.002	–	0.027		Change in BMI	0.017	0.002	8.61	<0.01
									Change in smoking habits	-0.004	0.006	-0.73	0.466
WBC count	Remained high	294	0.009	0.007	-0.004	–	0.022	NS	**Change in WBC count**	-0.005	0.003	-1.60	0.110
	Increased	129	0.007	0.010	-0.012	–	0.026		Sex (female/male)	0.010	0.004	2.56	<0.05
	Decreased	132	0.029	0.010	0.010	–	0.048		Age (2017)	0.000	0.000	0.30	0.763
	Remained low	226	0.019	0.007	0.004	–	0.033		Change in BMI	0.017	0.002	8.59	<0.01
									Change in smoking habits	-0.004	0.006	-0.66	0.511
Ammonia level	Remained high	543	0.016	0.005	0.007	–	0.026	NS	**Change in ammonia level**	0.003	0.004	0.77	0.441
	Increased	83	0.008	0.012	-0.016	–	0.033		Sex (female/male)	0.010	0.004	2.47	<0.05
	Decreased	87	0.023	0.012	-0.001	–	0.047		Age (2017)	0.000	0.000	-0.16	0.876
	Remained low	68	0.002	0.014	-0.025	–	0.028		Change in BMI	0.017	0.002	8.60	<0.01
									Change in smoking habits	-0.004	0.006	-0.68	0.495

Table 14. Correlation between the interval change of creatinine and that of salivary multi test (n = 781)

Discussion

Saliva is widely used for diagnostic purposes, monitoring systemic disease status, and predicting disease progression[24]. The purpose of this study was to investigate the associations between the results of saliva tests and MetS based on medical health check-up data for a large population.

Both the longitudinal and cross-sectional studies showed a significant relationship between salivary protein levels and serum HbA1c levels. The subjects with higher serum HbA1c levels had higher salivary protein levels. The SMT was used to measure three items (the salivary levels of occult blood and protein and the salivary WBC count) as markers of periodontal disease. In a study involving the SMT, periodontal pocket depth, bleeding on probing, and the Community Periodontal Index were reported to be correlated with salivary occult blood and protein levels as well as the salivary WBC count[25]. Salivary occult blood and protein levels and the salivary WBC count are considered to be markers of inflammation in periodontal tissue. Salivary protein composition was also reported to be affected by the development of periodontitis[26]. In addition, many investigators have suggested that a two-way relationship exists between DM and periodontal disease[27, 28]. Previously, it was reported that

salivary protein concentration was higher in DM patients with HbA1c levels of >0.7% than in those with HbA1c levels of <0.7%[29]. It was also stated that the increase in the salivary protein concentration was due to a reduction in salivary secretion and inflammatory oral conditions, including periodontitis, in individuals with DM[29]. These results suggested that the protein content of saliva increases in DM patients because of periodontal disease and hyposalivation, and therefore, the salivary protein level could be a useful marker of both periodontal disease and hyperglycemia.

In this study, the longitudinal analysis revealed significant correlations between the interyear change in systolic blood pressure and the interyear changes in the salivary protein level and WBC count, and between the interyear change in diastolic blood pressure and the interyear change in the salivary protein level. In the cross-sectional analysis, significant relationships were observed between the salivary levels of protein or occult blood and blood pressure in the univariate analyses. These findings suggested that a causal relationship exists between higher salivary protein levels and increased blood pressure/hypertension. As stated above, the salivary protein level is a marker of periodontal disease. A few previous studies have investigated the associations among hypertension, blood pressure, and periodontal disease[30–32]. In a prospective Japanese cohort study conducted over 3 years, it was suggested that the progression of periodontal disease might be associated with blood pressure[32]. In another 4-year longitudinal study involving Japanese employees, the worsening of hypertension was also reported to be correlated with the presence of periodontal pockets[30]. On the other hand, it was reported that there was no association between periodontal measurements and hypertension in a cohort study of middle-aged health-professionals[31]. Although the precise mechanism responsible for the association between hypertension and periodontal disease remains uncertain, increased levels of C-reactive protein, which are seen in patients with hypertension, coronary arterial heart disease, and periodontal disease, might contribute to it[18, 33–36]. In a randomized controlled trial, the intensive periodontal treatment group exhibited lower diastolic and systolic blood pressure and markedly smaller endothelial microparticles than the control group, as well as parallel improvements in periodontal status[37]. These findings suggested that there might be a relationship between periodontal disease and hypertension. Furthermore, the salivary protein level, which reflects periodontal tissue inflammation, could be a useful marker of both periodontal disease and hypertension. In addition to the salivary protein level, the salivary occult blood level and WBC count are also markers of periodontal tissue inflammation. The results of this study suggested that the salivary protein level displayed a stronger relationship with periodontal inflammation than the salivary occult blood level or WBC count. The critical reason why the salivary protein level exhibited the strongest relationship with periodontal inflammation was unclear although the measurement methods and the detection range of the test kit employed in this study (the SMT) might have contributed to it.

A significant relationship was also observed between salivary buffer capacity and the serum triglyceride level in both the cross-sectional and longitudinal analyses. The buffer capacity of saliva

was lower in the subjects with higher levels of triglycerides/hyperlipidemia. Tremblay et al. investigated the association between salivary pH and MetS in females and reported that mean salivary pH levels decreased as the number of MetS components increased and that salivary pH was correlated with markers of MetS components, such as triglyceride levels[38]. Our results were consistent with the latter report. Salivary cholesterol concentrations were reported to reflect serum cholesterol concentrations to some extent[39]. The buffer capacity and pH of saliva are important and are affected by enzymes and the levels of bicarbonate, urea, and amphoteric proteins[40, 41]. In particular, bicarbonate affects the buffering system, and the pH of saliva is dependent on the bicarbonate concentration. The salivary bicarbonate concentration decreases with the salivary flow rate, resulting in a reduction in the pH of saliva[42]. In hyperlipidemic patients with xerostomia, there a close relationship was detected between salivary gland swelling, salivary gland hypofunction, and serum lipid levels[43]. These results indicate that associations exist between serum triglyceride levels and the salivary flow rate/salivary pH.

In the present study, the cross-sectional analysis (multivariate analysis) revealed a significant relationship between the serum creatinine level and the pH or buffer capacity of saliva. Both salivary pH and salivary buffer capacity were higher in the subjects with higher serum creatinine levels/decreased renal function. Previous studies have assessed salivary flow, pH, and buffer capacity in chronic kidney disease (CKD) patients. In one study, the CKD patients exhibited hyposalivation and increased salivary pH and buffer capacity[44]. Our results were consistent with the latter study. In CKD patients, the blood tends to become acidic (due to metabolic acidosis) as renal function degrades, and metabolic acidosis is a common finding[45]. Therefore, we speculated that the salivary pH might decrease as the serum creatinine level increases. However, our results showed the opposite, as was demonstrated in previous studies. A significant association between salivary and serum urea levels was reported to exist in pre-dialysis patients[46]. The hydrolysis of nitrogen compounds by bacterial urease has been reported to result in the production of carbon dioxide and ammonium ions, leading to increased alkalizing potential[47]. Impaired renal function might also affect salivary flow and salivary properties, which can result in saliva becoming alkaline.

The present study, which was based on health check-up data for a large population, is the first to demonstrate the utility of saliva tests for screening individuals for MetS/MetS components as well as periodontal disease. However, it had some limitations. For example, we used a commercially available saliva test kit. The test kit had a limited analytical ability and limited ranges of detection for salivary components. Another limitation was the cut-off values used for each test item in the SMT. In the SMT, the salivary WBC count and the salivary levels of occult blood, protein, and ammonia were classified into three grades. Further studies involving more sophisticated methods are required.

In conclusion, correlations between the results of saliva tests and the results of health check-ups for MetS were revealed in a large population study. A longitudinal study revealed significant

correlations between salivary protein levels and serum HbA1c levels or blood pressure. In addition, a significant correlation was detected between the buffer capacity of saliva and the serum triglyceride level. Salivary pH increased irreversibly in subjects with impaired renal function. Therefore, saliva tests might be a useful tool for screening for not only periodontal disease but also MetS/MetS components in health check-ups of large populations.

Compliance with Ethical Standards

Conflicts of Interest: The authors declare that they have no conflicts of interest.

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Ethical Approval: The study protocol was approved by the ethics committee of Shinshu University School of Medicine (No. 2775).

Informed Consent: Formal consent was not required for this type of study.

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