



# Relationship between an Oral Health Risk Assessment Using a Salivary Multi-Test System and Woman's Subjective Oral Health Symptoms and Sleep Disorder

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Saliva is used as a diagnosis and monitoring tool for various diseases because it can maintain the balance of the oral ecosystem and reflect the physiological and pathological state of the body. Because women suffer more fatigue than men because of physiological, psychological, and social factors, individual management strategies are needed to evaluate mental health and oral diseases. Therefore, this study examined the oral health risk level from seven saliva factors using a saliva multi-test system for adult women to confirm the possibility of screening for sleep disorders. The saliva of 83 adult female participants was surveyed along with a self-reported questionnaire consisting of seven subjective oral health symptoms and three questions about sleep disorders. Seven saliva factors were evaluated using the saliva multi-test system (SiLL-Ha ST-4910) to assess the oral health risk levels. In the tooth health risk groups, the acidity was high, while the buffering capacity was low ( $p < 0.001$ ). The periodontal health risk groups showed significant differences in acidity, occult blood, leukocytes, proteins, and ammonia ( $p < 0.05$ ). The oral malodor risk group had higher levels of cariogenic bacteria, occult blood, leukocytes, and ammonia ( $p < 0.05$ ). In groups with 'irregular sleep times' and 'insomnia', the acidity was high, and the buffering capacity was low ( $p < 0.001$ ). This study confirmed the relevance of saliva factors and sleep disorder. Therefore, an evaluation using saliva was confirmed for oral health risk assessments and as an early screening tool for sleep disorders.

**Keywords:** oral health assessment; reliability; salivary multi-test; sleep disorder; subjective oral health symptoms  
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## Introduction

Dental caries and periodontitis are the most common oral diseases. If proper oral hygiene management is not maintained, the disease can deteriorate, leading to tooth loss (Renz et al. 2007). The diagnostic method of traditional dental caries and periodontal disease involves the subjective judgment of the examiner using a visual inspection and tactile sensations, so only the advanced stages of the disease can be identified (Johnson et al. 1988; Gomez et al. 2013). Therefore, there are limitations in detecting the initial stage of the lesions and the progression of the disease is unpredictable (Braga et al. 2010). Thus, non-invasive diagnostic methods are required to diagnose and monitor the progression of lesions for optimal treatment timing and good prognoses.

Saliva analysis has been used for the non-invasive diagnosis of oral diseases. Because saliva reflects the physiological and pathological conditions of the body and is easy to collect (Nunes et al. 2011; Liu and Duan 2012), it is used widely as a substitute for blood and other body fluids for screening, diagnosing, and monitoring diseases in many scientific fields, such as medicine, dentistry, and drug therapy (Kaufman and Lamster 2002).

Recently, SiLL-Ha ST-4910 (ARKRAY, Kyoto, Japan), saliva multi-test system that can measure seven saliva factors related to dental caries, periodontal disease, and oral malodor, was developed to apply a comprehensive oral health assessment with saliva (Nishinaga et al. 2015). A single saliva collection provided a risk assessment of oral diseases, such as dental caries, periodontal disease, and oral malodor by evaluating the cariogenic bacteria, acidity, buff-

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ering capacity and occult blood, leukocytes, proteins, and ammonia (Irie et al. 2018; Kuwamura et al. 2019; Takaesu et al. 2020).

Previous studies have shown that oral disease can affect an individuals' sleep and quality of life (Schmidlin et al. 2020). On the other hand, most were cross-sectional studies that investigated the effects of oral diseases that had already occurred on sleep disorders; there was a lack of a preventive approach. Considering that psychological factors, such as stress, anxiety, and sleep disorder can affect saliva factors (Obayashi 2013; Abell et al. 2016), an early clinical response would be possible if a saliva multi-test system can be used to screen for oral bacteria, acidity, buffering capacity, blood components, and inflammatory reactions.

Therefore, this study examined the oral health risk from saliva factor analysis using the saliva multi-test system to determine if an individual's sleep disorders can be predicted using this result.

## Methods

### *Statement of ethics*

This protocol for cross-sectional study was approved by the institutional Review Board of Yonsei Wonju Christian Hospital (IRB No. CR320088). All procedures were carried out in accordance with the ethical principles of human participatory medical research as set out in the Helsinki Declaration of the World Medical Association (version 2013). This study was also conducted according to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines (von Elm et al. 2007). The purpose and procedures of this study were described to all participants before commencing the study, and written consent was obtained from all subjects prior to inclusion. The general qualifications of each participant were determined using the screening procedures described below.

### *Subject population*

Adult women aged 18 or older were recruited from September to November 2020. Those with conditions that could affect saliva due to changes in hormones, such as pregnant women, taking drugs including antibiotics, and systemic diseases, were excluded.

### *Questionnaires*

A self-reported questionnaire was performed to identify the subjective oral health symptoms and sleep disorders. A survey on the subjective oral health symptoms were seven questions on 'dry mouth', 'frequent opening of the mouth', 'hypersensitivity', 'pain when the tip of the bristles touches', 'bleeding during tooth brushing', 'awareness of oral malodor', and 'not refreshing after tooth brushing'; the subjects responded with 'Yes' and 'NO' (Locker and Miller 1994). The survey on sleep disorder was configured to respond 'Yes' and 'NO' to the questions of 'excessive tension', 'insomnia', and 'irregular sleep time' as three ques-

tions.

### *Oral health risk assessment using the saliva test*

In this study, saliva multi-test system (SiLL-Ha ST-4910) was performed for assessing the oral health risk. One trained examiner measured the saliva factors associated with tooth health, periodontal health, and oral malodor using the saliva multi-test system according to the manufacturer's instructions. Each participant was instructed to rinse their mouth with 3 mL of distilled water for 10 seconds. One trained examiner dropped a 10  $\mu$ L sample on each of seven pads of a test strip to analyze the cariogenic bacteria, acidity, buffering capacity, occult blood, leukocytes, proteins, and ammonia. The color changes (i.e., changes in reflectivity) on each pad of the test strip were evaluated for the specified wavelength. The acidity, buffering capacity, occult blood, leukocytes, proteins, and ammonia were measured one minute later. The cariogenic bacteria were evaluated by measuring the changes in the reflectivity after five minutes. The reliability of the test was assessed by testing 20 samples randomly and repeating the measurement after one week.

### *Statistical analysis*

For the oral health risk assessment using the saliva multi-test system, seven items were classified as a risk if the cut-off value was higher than the set cut-off value of the adult saliva test factors, and none if the cut-off value was lower than the set cut-off value (Nishinaga et al. 2015). The risk of tooth health, periodontal health, and oral malodor was assessed. The total risk of a common sub-item was calculated and assigned as a risk group if more than one risk existed. Samples without risk were finally classified as a non-risk group. All data were analyzed using SPSS version 25 (IBM Corp, Chicago, Illinois, USA). A normality test using the Shapiro-Wilk test resulted in a non-normal distribution. Therefore, the nonparametric test Mann-Whitney *U*-test compared the difference in the values of subjective oral health symptoms, sleep disorder, and saliva factors according to the oral health risk levels to the median and quartiles. The association between the subjective oral health symptoms and level of oral health risk assessments due to sleep disorder was analyzed using a Fisher's exact test. Reliability analysis was conducted to assess the reproducibility of saliva testing. All statistical analyses were analyzed at a significance level of 0.05.

## Results

Of 89 applicants, 83 were selected as the final volunteers according to the screening test. The subjects were women with an average age of 21.7 years (100.0 %).

To evaluate the reliability of the saliva multi-test system used in this study, repeated measurements using a sample of 20 participants showed that the seven saliva factors were 0.67-0.93, which was above the moderate-level reliability (Koo and Li 2016) (Table 1).

Table 1. Reliability of the saliva test.

Factor	ICC	95% CI	Reproducibility rating
Cariogenic bacteria level	0.93	0.82-0.97	Excellent
Acidity	0.82	0.61-0.93	Good
Buffer capacity	0.85	0.66-0.94	Good
Occult blood	0.87	0.70-0.95	Good
Leukocyte level	0.70	0.38-0.87	Moderate
Protein level	0.69	0.36-0.86	Moderate
Ammonia level	0.67	0.34-0.86	Moderate

N= 20 for each factor.

ICC, intraclass correlation coefficient; CI:, confidence interval.

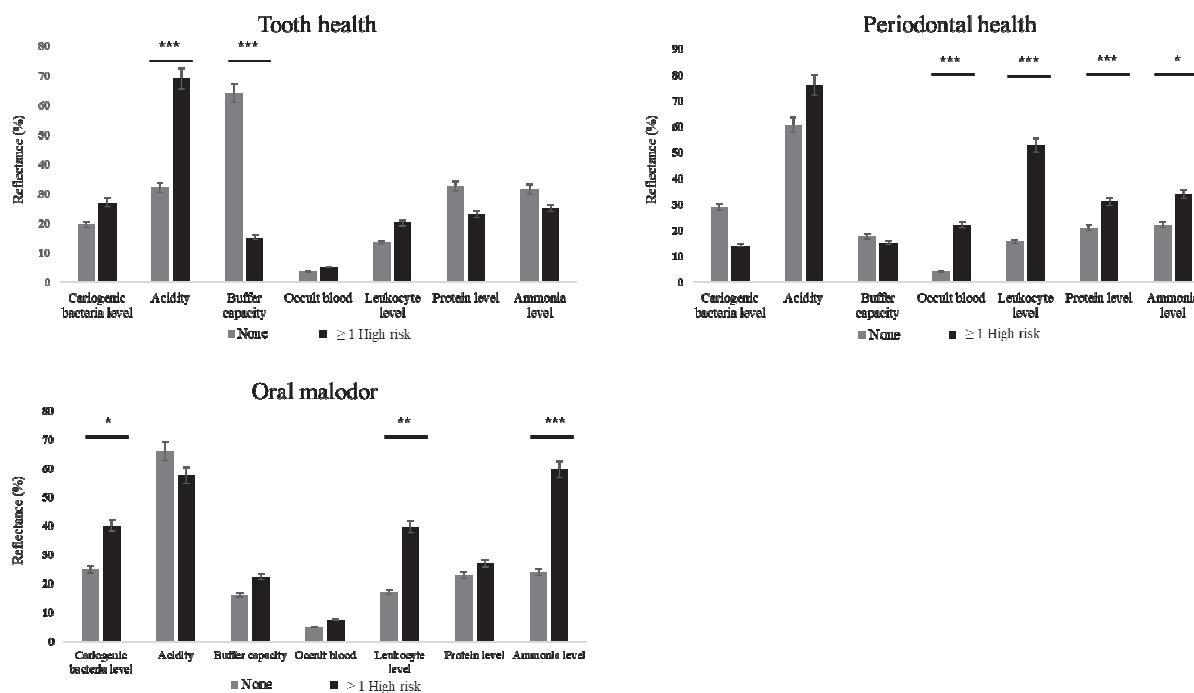


Fig. 1. Comparison of the salivary factors by the level of oral health risk assessment. *p*-values denote differences between groups determined by Mann-Whitney *U*-test. \**p* < 0.05, \*\**p* < 0.01, \*\*\**p* < 0.001.

A significant difference was noted in the results of the saliva test items of the participants after classifying them based on the cut-off value and determining the effects of having an oral health risk on seven saliva test factors (Fig. 1,  $p < 0.05$ ). In the tooth health risk group, acidity was high, while the buffering capacity was low ( $p < 0.001$ ). The periodontal health risk group showed significant differences in acidity, occult blood, leukocytes, proteins, and ammonia compared to the group that did not ( $p < 0.05$ ). The oral malodor risk group showed high levels of cariogenic bacteria, leukocytes, ammonia (Fig. 1,  $p < 0.05$ ). To check the effect of saliva changes on subjective oral health symptoms, seven saliva factors were significantly different if they complained of 'dry mouth', 'pain when the tip of the bristles touches', 'bleeding during tooth brushing', 'awareness of oral malodor', and 'not refreshing after tooth brushing' (Table 2,  $p < 0.05$ ). The group that complained of all sub-

jective oral health symptoms showed a difference in protein level among the saliva factors except for 'frequent opening of the mouth' and 'hypersensitivity' (Table 2). A common significant difference in occult blood was noted depending on the presence of symptoms, such as 'pain when the tip of the bristles touches', 'bleeding during tooth brushing', 'awareness of oral malodor', and 'not refreshing after tooth brushing' ( $p < 0.05$ ). The oral health risk assessment using the saliva multi-test system showed that subjective oral health symptoms are related to periodontal health and oral malodor (Table 3). In particular, the periodontal health and oral malodor risk showed the same high value in the group that complained of an 'awareness of oral malodor' and 'not refreshing after tooth brushing' (Table 3,  $p < 0.05$ ).

The difference in the saliva test factors according to the sleep disorder was significant among all the items examined (Table 4). When responding with 'excessive ten-

Table 2. Differences in salivary screening data according to the subjective oral health symptoms (median [IQR]).

	Cariogenic bacteria level	Acidity	Buffer capacity	Occult blood	Leukocyte level	Protein level	Ammonia level
Symptom of dry mouth							
Yes (N = 37)	25.0 (0.0 - 33.0)	75.0 (60.0 - 83.0)*	11.0 (6.0 - 16.0)*	5.0 (2.0 - 13.0)	18.0 (11.0 - 51.0)	20.0 (13.0 - 28.0)*	24.0 (9.0 - 38.0)
No (N = 46)	27.0 (4.0 - 41.0)	58.5 (49.0 - 75.0)*	26.0 (13.0 - 36.0)*	5.0 (3.0 - 9.0)	20.0 (11.0 - 31.0)	25.5 (21.0 - 36.0)*	28.0 (16.0 - 46.0)
Frequent opening of the mouth							
Yes (N = 24)	28.0 (0.0 - 37.5)	62.0 (52.5 - 78.5)	12.0 (10.5 - 25.0)	5.0 (1.5 - 7.0)	16.5 (10.5 - 33.0)	25.0 (19.5 - 33.5)	24.0 (5.0 - 45.0)
No (N = 59)	25.0 (4.0 - 40.0)	70.0 (51.5 - 79.5)	18.0 (9.0 - 30.0)	5.0 (3.0 - 16.0)	21.0 (11.0 - 43.0)	22.0 (13.0 - 32.5)	26.0 (14.5 - 43.5)
Symptom of hypersensitivity							
Yes (N = 9)	20.0 (8.0 - 33.0)	75.0 (57.0 - 82.0)	12.0 (7.0 - 20.0)	7.0 (3.0 - 13.0)	20.0 (11.0 - 26.0)	20.0 (16.0 - 28.0)	17.0 (9.0 - 26.0)
No (N = 74)	26.0 (0.0 - 40.0)	62.0 (51.0 - 79.0)	17.0 (10.0 - 29.0)	5.0 (2.0 - 13.0)	19.0 (11.0 - 37.0)	24.0 (16.0 - 34.0)	27.0 (13.0 - 46.0)
Pain when the tip of the bristles touches							
Yes (N = 19)	14.0 (0.0 - 28.0)	75.0 (58.5 - 87.0)	13.0 (8.0 - 22.5)	24.0 (18.5 - 38.0)*	43.0 (18.0 - 64.5)*	29.0 (22.0 - 46.5)*	27.0 (15.0 - 42.5)
No (N = 64)	28.0 (4.0 - 40.0)	60.5 (51.0 - 78.0)	17.0 (10.5 - 30.0)	4.5 (2.0 - 6.5)*	16.5 (9.5 - 28.0)*	22.0 (13.5 - 30.0)*	24.5 (10.5 - 44.0)
Bleeding during tooth brushing							
Yes (N = 19)	14.0 (0.0 - 28.0)	72.0 (54.5 - 87.0)	17.0 (10.5 - 25.5)	24.0 (18.5 - 38.0)*	48.0 (27.0 - 64.5)*	30.0 (23.0 - 46.5)*	29.0 (17.5 - 46.5)
No (N = 64)	28.0 (4.0 - 40.0)	62.0 (51.5 - 78.0)	16.5 (9.5 - 30.0)	4.0 (2.0 - 6.0)*	16.0 (9.5 - 27.0)*	21.5 (13.5 - 29.5)*	24.0 (9.5 - 43.5)
Awareness of oral malodor							
Yes (N = 29)	25.0 (0.0 - 40.0)	61.0 (51.0 - 80.0)	21.0 (12.0 - 31.0)	8.0 (5.0 - 19.0)*	37.0 (21.0 - 53.0)*	30.0 (22.0 - 40.0)*	48.0 (38.0 - 54.0)*
No (N = 54)	26.0 (4.0 - 38.0)	65.5 (53.0 - 79.0)	13.5 (9.0 - 26.0)	4.0 (2.0 - 7.0)*	13.0 (8.0 - 26.0)*	20.5 (13.0 - 29.0)*	18.5 (3.0 - 27.0)*
Not refreshing even after tooth brushing							
Yes (N = 23)	27.0 (5.5 - 38.5)	56.0 (44.5 - 73.5)	27.0 (10.5 - 35.0)	7.0 (4.5 - 20.5)*	29.0 (12.0 - 49.0)	29.0 (24.0 - 42.5)*	43.0 (26.0 - 52.0)*
No (N = 60)	25.0 (2.0 - 40.0)	69.5 (55.0 - 79.5)	15.5 (9.5 - 25.0)	5.0 (2.0 - 9.5)*	17.0 (9.5 - 31.5)	21.0 (13.5 - 30.0)*	21.0 (9.0 - 35.0)*

IQR, interquartile range.

\*Significant difference between the two groups by Mann-Whitney *U* test ( $p < 0.05$ ).

sleep', the levels of cariogenic bacteria, occult blood, leukocytes, and ammonia were high ( $p < 0.05$ ). In the case of insomnia and irregular sleep times, the acidity was high, buffering capacity was low, and there was a significant difference ( $p < 0.001$ ).

Furthermore, the results from the oral health risk assessment level confirmed that sleep disorders had a statistically significant association with tooth health (Table 5,  $p < 0.05$ ). Among the items of sleep disorders, 'insomnia' and 'irregular sleep time' were related to dental health, and 'excessive tension' was related to oral malodor (Table 5,  $p < 0.05$ ).

## Discussion

A sleep duration of 7-9 h is generally needed for adults to maintain optimal health (Wang et al. 2017). On the other hand, modern life is characterized by reduced sleep times and worsened sleep quality caused by changes in modern lifestyles (working late and using computers and the Internet, and watching TV late at night) (Suzuki et al. 2017). There is compelling evidence that both habitual short and long sleep durations are associated with poor health outcomes, including oral health (Bixler 2009; Huynh et al. 2014; Carra et al. 2017). Although the relationship between oral disease and sleep disorders is well known, there are no reports on the early detection of sleep disorders from oral conditions. As a method for screening systemic diseases, saliva can provide considerable information as a

biomarker (Dawes and Wong 2019). Saliva multi-test system was recently developed to analyze seven factors related to oral diseases from the saliva of a subject and present the risk level of major diseases in the oral cavity. Therefore, if the relationship between the oral health risk from saliva and sleep disorders can be confirmed, it will provide a means of screening sleep disorders early in dental clinics.

Before evaluating the relationship between oral health risk assessment and sleep disorder, 20 subjects were analyzed repeatedly to confirm the reliability of the saliva multi-test system used in this study. As a result, all seven salivary factors showed good reliability with moderate to excellent statistical test results (ICC; 0.67-0.93, CI; 0.34-0.97) (Table 1). This result confirmed that it is reasonable to evaluate the relationship between the oral health risk level, subjective oral health symptoms, and sleep disorders using saliva multi-test system. Furthermore, the results of the saliva factors of the study subjects were classified according to the cut-off values suggested in preceding studies to confirm whether it is reasonable to evaluate the risk of oral health occurrence using the saliva factor. The result confirmed that the presence or absence of the risk of oral health was related to the seven saliva test items (Fig. 1). The point to be scrutinized in this result is that the saliva characteristics of each oral disease risk group are consistent with the predictive or diagnostic means of disease suggested in previous studies (Giannobile et al. 2009; Guo and Shi 2013). Another previous study tried to utilize the

Table 3. Relationship between the subjective oral health symptoms and level of oral health risk assessment by salivary screening data.

	Tooth health		<i>p</i> -value	Periodontal health		<i>p</i> -value	Oral malodor		<i>p</i> -value
	None (n = 6)	≥ 1 High (n = 77)		None (n = 62)	≥ 1 High (n = 21)		None (n = 75)	≥ 1 High (n = 8)	
Symptom of dry mouth									
Yes (N = 37)	1 (2.7%)	36 (97.3%)	0.218	27 (73.0%)	10 (27.0%)	0.803	34 (91.9%)	3 (8.1%)	0.727
No (N = 46)	5 (10.9%)	41 (89.1%)		35 (76.1%)	11 (23.9%)		41 (89.1%)	5 (10.9%)	
Frequent opening of the mouth									
Yes (N = 24)	2 (8.3%)	22 (91.7%)	0.562	22 (91.7%)	2 (8.3%)	0.027	23 (95.8%)	1 (4.2%)	0.428
No (N = 59)	4 (6.8%)	55 (93.2%)		40 (67.8%)	19 (32.2%)		52 (88.1%)	7 (11.9%)	
Symptom of hypersensitivity									
Yes (N = 9)	1 (8.3%)	22 (91.7%)	0.509	7 (77.8%)	2 (22.2%)	0.592	9 (100.0%)	0 (0.0%)	0.589
No (N = 74)	4 (6.8%)	55 (93.2%)		55 (74.3%)	19 (25.7%)		66 (89.2%)	8 (10.8%)	
Pain when the tip of the bristles touches									
Yes (N = 19)	1 (5.3%)	18 (94.7%)	0.582	5 (26.3%)	14 (73.7%)	< 0.001	17 (89.5%)	2 (1.05%)	0.589
No (N = 64)	5 (7.8%)	59 (92.2%)		57 (89.1%)	7 (10.9%)		58 (90.6%)	6 (9.4%)	
Bleeding during tooth brushing									
Yes (N = 19)	1 (5.3%)	18 (94.7%)	0.582	4 (21.1%)	15 (78.9%)	< 0.001	17 (89.5%)	2 (1.05%)	0.589
No (N = 64)	5 (7.8%)	59 (92.2%)		58 (90.6%)	6 (9.4%)		58 (90.6%)	6 (9.4%)	
Awareness of oral malodor									
Yes (N = 29)	3 (10.3%)	26 (89.7%)	0.417	16 (55.2%)	13 (44.8%)	0.004	21 (72.4%)	8 (27.6%)	< 0.001
No (N = 54)	3 (5.6%)	51 (94.4%)		46 (85.2%)	8 (14.8%)		54 (100.0%)	0 (0.0%)	
Not refreshing even after tooth brushing									
Yes (N = 23)	3 (13.0%)	20 (87.0%)	0.340	13 (56.5%)	10 (43.5%)	0.025	17 (73.9%)	6 (26.1%)	0.005
No (N = 60)	3 (5.0%)	57 (95.0%)		49 (81.7%)	11 (18.3%)		58 (96.7%)	2 (3.3%)	

*p*-values denote differences between groups determined by Fisher's exact test.

Table 4. Differences in salivary screening data according to sleep disorder (median [IQR]).

	Cariogenic bacteria level	Acidity	Buffer capacity	Occult blood	Leukocyte level	Protein level	Ammonia level
Excessive tension							
Yes (N = 31)	33.0 (22.5 - 40.5)*	59.0 (50.5 - 75.0)	21.0 (14.5 - 30.0)	7.0 (5.0 - 13.0)*	30.0 (15.5 - 49.0)*	24.0 (17.0 - 31.0)	46.0 (29.5 - 53.5)*
No (N = 52)	14.0 (0.0 - 32.0)*	71.5 (52.5 - 82.0)	12.0 (9.0 - 26.5)	4.0 (1.5 - 8.5)*	15.5 (8.5 - 27.0)*	23.0 (14.5 - 34.0)	19.0 (3.0 - 27.0)*
Presence of insomnia							
Yes (N = 53)	25.0 (0.0 - 40.0)	75.0 (59.0 - 84.0)*	12.0 (7.0 - 17.0)*	5.0 (2.0 - 18.0)	25.0 (10.0 - 42.0)	21.0 (14.0 - 30.0)	24.0 (9.0 - 36.0)
No (N = 30)	28.0 (8.0 - 40.0)	52.0 (41.0 - 64.0)*	34.0 (24.0 - 52.0)*	5.0 (3.0 - 10.0)	16.5 (11.0 - 26.0)	27.0 (21.0 - 34.0)	31.5 (16.0 - 47.0)
Irregularity in sleep time							
Yes (N = 37)	20.0 (0.0 - 37.0)	78.0 (60.0 - 88.0)*	9.0 (5.0 - 12.0)*	6.0 (2.0 - 13.0)	17.0 (9.0 - 42.0)	22.0 (12.0 - 29.0)	21.0 (3.0 - 38.0)
No (N = 46)	28.0 (8.0 - 40.0)	57.5 (41.0 - 71.0)*	26.5 (17.0 - 38.0)*	5.0 (3.0 - 13.0)	21.0 (13.0 - 36.0)	24.5 (20.0 - 35.0)	28.0 (16.0 - 47.0)

*IQR*, interquartile range.

\*Significant difference between the two groups by Mann-Whitney *U* test ( $p < 0.05$ ).

hemoglobin level in human saliva to predict periodontal disease, and showed that saliva can be used as a biomarker of oral disease (Maeng et al. 2016). In this study, the acidity of saliva was high, and the capacity was low in the group with the possibility of developing dental caries ( $p < 0.001$ ). On the other hand, there were significant differences in acidity, occult blood, leukocytes, protein, and ammonia in the periodontal health risk group ( $p < 0.05$ ). Moreover, the level of cariogenic bacteria and ammonia, which cause oral malodor, were high in the oral malodor

risk group ( $p < 0.05$ , Fig. 1). This verified that the risk of oral health occurrence using saliva multi-test system could be evaluated, and this test is suitable for evaluating the possibility of screening for sleep disorders through the changes in saliva according to the purpose of this study.

Sleep disorders affect the rate of salivation, which may appear as subjective oral symptoms. Therefore, the objective of the saliva multi-test system and the subjective oral health symptoms were compared to determine if they can be used to select saliva testing targets. The results revealed



Table 5. Relationship between sleep disorders and level of oral health risk assessment by salivary screening data.

	Tooth health		<i>p</i> -value	Periodontal health		<i>p</i> -value	Oral malodor		<i>p</i> -value
	None (n = 6)	≥ 1 High (n = 77)		None (n = 62)	≥ 1 High (n = 21)		None (n = 75)	≥ 1 High (n = 8)	
Excessive tension									
Yes (N = 31)	2 (6.5%)	29 (93.5%)	0.601	21 (67.7%)	10 (32.3%)	0.302	23 (74.2%)	8 (25.8%)	< 0.001***
No (N = 52)	4 (7.7%)	48 (92.3%)		41 (78.8%)	11 (21.2%)		52 (100.0%)	0 (0.0%)	
Presence of insomnia									
Yes (N = 53)	0 (0.0%)	53 (100.0%)	0.002**	38 (71.7%)	15 (28.3%)	0.444	48 (90.6%)	5 (9.4%)	0.607
No (N = 30)	6 (20.0%)	24 (80.0%)		21 (80.0%)	6 (20.0%)		27 (90.0%)	3 (10.0%)	
Irregularity in sleep time									
Yes (N = 37)	0 (0.0%)	37 (100.0%)	0.031*	29 (78.4%)	8 (21.6%)	0.613	35 (94.6%)	2 (5.4%)	0.289
No (N = 46)	6 (7.2%)	77 (92.8%)		33 (71.7%)	13 (25.3%)		40 (87.0%)	6 (13.0%)	

*p*-values denote differences between groups determined by Fisher's exact test.

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

significant differences in the seven saliva factors if the subjects complained of 'dry mouth', 'pain when the tip of the bristles touches', 'bleeding during tooth brushing', 'awareness of oral malodor', and 'not refreshing after tooth brushing' (Table 2,  $p < 0.05$ ). Moreover, the subjective oral health symptoms were related to periodontal health and oral malodor in the oral health risk assessment using the saliva multi-test system (Table 4). In particular, the groups who complained of 'awareness of oral malodor' and 'not refreshing even after tooth brushing' have a high risk of periodontal health and oral malodor (Table 3,  $p < 0.05$ ). Although a direct comparison with the results of this study is difficult, previous studies have shown that sleep disorders in humans can affect the secretion of saliva in the oral cavity (Carra et al. 2017). The change in salivary flow rate affects the incidence of dental caries and periodontal disease and the rate of complaints of oral malodor. Based on these results, it is possible to consider sleep disorders as a causative factor of subjects who complain of abnormal oral health in the future and to consider changes in the oral cavity as an early detection index of sleep disorders.

There were significant differences in the saliva test items and oral health risk according to the presence or absence of sleep disorders in all groups (Table 4, 5). 'excessive tension', 'insomnia', and 'irregular sleep time' were symptoms of sleep disorders; this study analyzed how they affected the saliva factors. Changes in cariogenic bacteria, occult blood, leukocytes, and ammonia were observed in subjects who complained of 'excessive tension', and changes in oral acidity and buffering capacity were observed in subjects who complained of 'insomnia' and 'irregular sleep time'. These changes also affected each oral health risk. In the case of tooth health, it was confirmed that changes in saliva acidity and buffering capacity due to the effects of 'insomnia' and 'irregular sleep time' had a significant effect. This result is consistent with the relationship between the previously analyzed saliva factors and oral disease risk. According to previous studies, sleep

disorders affect saliva flow. Although the exact cause has not been identified, it was confirmed that a change in the saliva flow rate leads to an increase in the incidence of dental caries and periodontal disease (Lee and Lee 2017). Dental caries, periodontal disease, and oral malodor are multifactorial diseases, of which saliva factors, such as secretion rate, acidity, and buffering capacity, have a significant influence on the occurrence of the diseases (Brooks et al. 1997; Gunepin et al. 2018). Considering these points, the relationship between sleep disorders and the oral disease risk suggests that the results of an oral health risk assessment can be used to screen for the presence or absence of sleep disorders in subjects.

The possibility of early screening for sleep disorders through saliva testing will be of great clinical significance. Nevertheless, this study had some limitations. First, the oral examination data of subjects through a visual examination were not collected during the study. Therefore, it is difficult to determine how well the oral experience risk reflects the current oral condition. Future studies will be needed to determine the oral effect of the subject's sleep disorder based on oral examination data. Furthermore, this study did not adequately consider confounding factors that may affect sleep disorders, such as sex and age. There are many causes of sleep disturbances. Therefore, correlation analysis based on the objective indicators is required.

In conclusion, this study confirmed the possibility of using oral saliva changes and oral disease risk as an early screening tool for sleep disorders by confirming the relationship between the oral changes and sleep disorders. Considering the study results, it is expected that systemic changes and oral diseases can be detected more objectively.

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### Conflict of Interest

The authors declare no conflict of interest.

### References

- Abell, J.G., Shipley, M.J., Ferrie, J.E., Kivimaki, M. & Kumari, M. (2016) Recurrent short sleep, chronic insomnia symptoms and salivary cortisol: a 10-year follow-up in the Whitehall II study. *Psychoneuroendocrinology*, **68**, 91-99.
- Bixler, E. (2009) Sleep and society: an epidemiological perspective. *Sleep Med.*, **10** Suppl 1, S3-6.
- Braga, M.M., Mendes, F.M. & Ekstrand, K.R. (2010) Detection activity assessment and diagnosis of dental caries lesions. *Dent. Clin. North Am.*, **54**, 479-493.
- Brooks, S.L., Brand, J.W., Gibbs, S.J., Hollender, L., Lurie, A.G., Omnell, K.A., Westesson, P.L. & White, S.C. (1997) Imaging of the temporomandibular joint: a position paper of the American Academy of Oral and Maxillofacial Radiology. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.*, **83**, 609-618.
- Carra, M.C., Schmitt, A., Thomas, F., Danchin, N., Pannier, B. & Bouchard, P. (2017) Sleep disorders and oral health: a cross-sectional study. *Clin. Oral Investig.*, **21**, 975-983.
- Dawes, C. & Wong, D.T.W. (2019) Role of saliva and salivary diagnostics in the advancement of oral health. *J. Dent. Res.*, **98**, 133-141.
- Giannobile, W.V., Beikler, T., Kinney, J.S., Ramseier, C.A., Morelli, T. & Wong, D.T. (2009) Saliva as a diagnostic tool for periodontal disease: current state and future directions. *Periodontol. 2000*, **50**, 52-64.
- Gomez, J., Tellez, M., Pretty, I.A., Ellwood, R.P. & Ismail, A.I. (2013) Non-cavitated carious lesions detection methods: a systematic review. *Community Dent. Oral Epidemiol.*, **41**, 54-66.
- Gunepin, M., Derache, F., Trousselard, M., Salsou, B. & Risso, J.J. (2018) Impact of chronic stress on periodontal health. *J. Oral Med. Oral Surg.*, **24**, 44-50.
- Guo, L. & Shi, W. (2013) Salivary biomarkers for caries risk assessment. *J. Calif. Dent. Assoc.*, **41**, 107-109, 112-108.
- Huynh, N.T., Emami, E., Helman, J.I. & Chervin, R.D. (2014) Interactions between sleep disorders and oral diseases. *Oral Dis.*, **20**, 236-245.
- Irie, Y., Tatsukawa, N., Iwamoto, Y., Nakano, M., Ogasawara, T., Sakurai, K., Mitsuhashi, C. & Kozai, K. (2018) Investigation of pediatric specifications for the Salivary Multi Test<sup>®</sup> saliva test system. *Pediatr. Dent. J.*, **28**, 148-153.
- Johnson, N.W., Griffiths, G.S., Wilton, J.M., Maiden, M.F., Curtis, M.A., Gillett, I.R., Wilson, D.T. & Sterne, J.A. (1988) Detection of high-risk groups and individuals for periodontal diseases. Evidence for the existence of high-risk groups and individuals and approaches to their detection. *J. Clin. Periodontol.*, **15**, 276-282.
- Kaufman, E. & Lamster, I.B. (2002) The diagnostic applications of saliva--a review. *Crit. Rev. Oral Biol. Med.*, **13**, 197-212.
- Koo, T.K. & Li, M.Y. (2016) A guideline of selecting and reporting intraclass correlation coefficients for reliability research. *J. Chiropr. Med.*, **15**, 155-163.
- Kuwamura, Y., Sakamoto, E., Sumikawa, M., Matsuhisa, M., Hinode, D., Uemura, H. & Kishida, S. (2019) Assessing the oral health of in-patients with diabetes using a clinical version of the Diabetes Oral Health Assessment Tool(c) and its association with dental examinations. *J. Med. Invest.*, **66**, 328-336.
- Lee, D.H. & Lee, Y.H. (2017) Association between sleep duration, dental caries, and periodontitis in Korean adults: the Korea National Health and Nutrition Examination Survey, 2013~2014. *J. Dent. Hyg. Sci.*, **17**, 38-45.
- Liu, J. & Duan, Y. (2012) Saliva: a potential media for disease diagnostics and monitoring. *Oral Oncol.*, **48**, 569-577.
- Locker, D. & Miller, Y. (1994) Evaluation of subjective oral health status indicators. *J. Public Health Dent.*, **54**, 167-176.
- Maeng, Y.J., Kim, B.R., Jung, H.I., Jung, U.W., Kim, H.E. & Kim, B.I. (2016) Diagnostic accuracy of a combination of salivary hemoglobin levels, self-report questionnaires, and age in periodontitis screening. *J. Periodontal. Implant. Sci.*, **46**, 10-21.
- Nishinaga, E., Uchiyama, C., Maki, R., Saito, K., Fukasawa, T., Suzuki, N., Yamamoto, T., Murakoshi, M., Odera, M., Fukuta, I., Okubo, A., Fujitani, M. & Senda, A. (2015) Development of comprehensive salivary test system: - validity and reliability of a newly-developed salivary multi-test system (AL-55) compared with standard methods. *Jpn. J. Conserv. Dent.*, **58**, 321-330.
- Nunes, L.A., Brenzikofer, R. & Macedo, D.V. (2011) Reference intervals for saliva analytes collected by a standardized method in a physically active population. *Clin. Biochem.*, **44**, 1440-1444.
- Obayashi, K. (2013) Salivary mental stress proteins. *Clin. Chim. Acta*, **425**, 196-201.
- Renz, A., Ide, M., Newton, T., Robinson, P.G. & Smith, D. (2007) Psychological interventions to improve adherence to oral hygiene instructions in adults with periodontal diseases. *Cochrane Database Syst. Rev.*, CD005097.
- Schmidlin, P.R., Khademi, A. & Fagheran, O. (2020) Association between periodontal disease and non-apnea sleep disorder: a systematic review. *Clin. Oral Investig.*, **24**, 3335-3345.
- Suzuki, K., Miyamoto, M. & Hirata, K. (2017) Sleep disorders in the elderly: diagnosis and management. *J. Gen. Fam. Med.*, **18**, 61-71.
- Takaesu, Y., Suzuki, N., Naito, M., Watanabe, T., Shimazu, A., Yatabe, N., Yoneda, M., Hirofujii, T. & Hanioka, T. (2020) Novel oral biomarkers predicting oral malodor. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol.*, **130**, 667-674.
- von Elm, E., Altman, D.G., Egger, M., Pocock, S.J., Gøtzsche, P.C., Vandenbroucke, J.P. & Initiative, S. (2007) The strengthening of reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann. Intern. Med.*, **147**, 573-577.
- Wang, S., Li, B., Wu, Y., Ungvari, G.S., Ng, C.H., Fu, Y., Kou, C., Yu, Y., Sun, H.Q. & Xiang, Y.T. (2017) Relationship of sleep duration with sociodemographic characteristics, lifestyle, mental health, and chronic diseases in a large Chinese adult population. *J. Clin. Sleep Med.*, **13**, 377-384.