Abstract: The aim of this study was to investigate the association between the presence of gingivitis estimated using the salivary level of lactate dehydrogenase (LD) and related factors in young Japanese adults. Data from 1,915 participants (21.4 ± 2.5 years) were analyzed. Unstimulated saliva was collected from each participant and the salivary LD level was evaluated using a commercially available test kit with an integer scale ranging from 1 to 10. Gingivitis was defined as the LD level of ≥8. The number of permanent teeth, the simplified oral hygiene index (OHI-S), the presence of partially erupted molars and body mass index were recorded. Additionally, participants answered a questionnaire. The percentage of male participants, the number of permanent teeth, the OHI-S and the presence of partially erupted molars were higher, whereas the proportion receiving dental check-ups was lower in the gingivitis group (n = 88, 4.6%) than in the healthy group. Logistic regression analysis showed that gingivitis was significantly associated with OHI-S (OR: 2.68, 95% CI: 1.94-3.69) and receiving dental checkups (OR: 0.31, 95% CI: 0.10-0.99). The present findings indicated that the OHI-S and receiving dental checkups were significantly associated with gingivitis, as assessed by the salivary LD level, in this cohort.

Keywords: lactate dehydrogenase; gingivitis; screening; young adults; cross-sectional study.

Introduction

Periodontal disease is a highly prevalent and major cause of tooth loss among middle-aged and elderly adults. However, because periodontal disease has minimal symptoms, individuals cannot recognize their deteriorating periodontal condition (1-3). The early stage of periodontal disease, especially gingivitis, is readily reversible by appropriate oral hygiene care (4). Therefore, early detection of gingivitis is a critical public health issue.

Periodontal disease, including periodontitis and gingivitis, has been traditionally diagnosed by clinical and radiographic examinations. However, these examinations are not suitable for screening periodontal patients in...
large-scale epidemiological studies because they require technical skill and are laborious and expensive. The community periodontal index (CPI), developed by the World Health Organization (5), has been used to screen for periodontal disease. However, there are some limitations associated with this method, including the need for probing by well-trained dental experts, pain during the examination, and risk of bacteremia (6). Therefore, other noninvasive screening methods, such as salivary examinations, have been suggested (7-10).

The salivary level of lactate dehydrogenase (LD) has been investigated as a tool for gingivitis screening among young adults (11). LD is an enzyme that is present in extracellular fluid following tissue breakdown (12). Some studies have suggested that the salivary LD level is useful for periodontal disease screening (11-16). A new screening test kit capable of estimating the salivary LD level within 1 min has been developed. It is a color-changing sheet with an integer scale ranging from 1 to 10. At a cut-off LD level of 8, the sensitivity and specificity for detection of gingivitis, defined as bleeding on probing (BOP) at ≥20% of sites (six sites per tooth), a pocket depth of ≥5 mm and a clinical attachment level of ≥2 mm at all sites (17), were quite high (0.89 and 0.98, respectively). It has been suggested that measurement of LD level using this test kit would be useful for screening young populations for the presence of gingivitis (11). Therefore, the next step for validating the kit is to apply it in a large-scale study and determine its utility.

The aim of the present study was to investigate the association between the presence of gingivitis, estimated according to the salivary LD level, and related factors in young Japanese adults, based on the hypothesis that this association would be affected by oral conditions and oral health behavior, as assessed with the CPI.

Materials and Methods

Participants
During general health examinations at the Health Service Center of Okayama University in April 2014, a total of 2,051 university students voluntarily participated in the present study. Participants who had provided incomplete responses in the questionnaire, had systemic disease or medications that would impact on the salivary LD level or gingivitis, or who had undergone orthodontic treatment, were excluded. The study was approved by the Ethics Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences (No. 808). Verbal informed consent was obtained from each participant.

Measurement of salivary LD level
The salivary LD level was measured using a commercially available kit that consists of a reagent strip (PD-1, Nagata Corp., Shiso, Japan). The strip includes 3.347 mg/mL nicotinamide adenine dinucleotide, 500 U/mL diaphorase, 5.0 mg/mL nitroblue tetrazolium, 12 mg/mL Tris buffer, 40 mg/mL lithium lactate and 10 mg/mL bovine serum albumin. In the presence of LD, formazan (purple color) is produced from nitroblue tetrazolium (faint yellow) (11). Before oral examination, more than 0.5 mL of unstimulated whole saliva was collected from each participant and immediately applied to the reagent strip in accordance with the manufacturer’s protocol. The color change, which indicates the salivary LD level, was recorded after 1 min using the test kit’s scale guide (scale, 1-10). Gingivitis was defined as the salivary LD level of ≥8 and a healthy gingival condition was defined as the salivary LD level of <8 (11).

Oral examination
Trained dentists examined the participants’ oral health status after measurement of the salivary LD level. The examiners counted the number of permanent teeth, and oral hygiene status was evaluated using the simplified oral hygiene index (OHI-S) (18). The presence of partially erupted molars, which might cause pericoronitis and thus affect the salivary LD level (19), was also assessed. When part, but not all, of a tooth crown was erupted, the tooth was defined as a partially erupted molar. Participants who underwent orthodontic treatment were recorded.

Medical examination
Public nurses measured the participants’ height and body weight using a Tanita body fat analyzer (Model No, BF-220; Tanita Corp., Tokyo, Japan) during the general health examinations. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (20,21). For this analysis, BMI was categorized into three levels: underweight (<18.5 kg/m²), normal weight (18.5-24.9 kg/m²) and overweight/obese (≥25.0 kg/m²) (Obesity: preventing and managing the global epidemic. Report of a WHO consultation, 2000).

Questionnaire
The questionnaire included the following items: age, sex, systemic disease, medication, smoking habit, and oral health behavior (daily frequency of tooth brushing, use of dental floss and receiving dental checkups during the past year) (22,23). To inquire about systemic disease and medication, a free description-type questionnaire was
used. Systemic diseases were classified according to the International Statistical Classification of Diseases and Related Health Problems (ICD) 10 (http://www.dis.h.u-tokyo.ac.jp/byomei/icd10/). The association between LD/gingivitis and systemic diseases/medication was investigated.

Sample size estimation
Sample size was estimated based on 13 variables for multivariate logistic regression analysis using statistical software (SamplePower ver. 3.0, IBM, Tokyo, Japan). Based on the relationship between a predictor variable (overweight) and periodontal disease (event rate, 0.254 vs. 0.579) by reference to a previous study (21), it was necessary to include 218 participants for a power of 80% and a two-sided significance level of 5%.

Statistical analysis
First, differences between male and female participants were evaluated with the unpaired t test or chi-squared test. Second, the unpaired t test, chi-squared test or Fisher’s exact test were used to compare parameters between the healthy group and the periodontal disease group. Finally, using logistic regression analysis, both odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. The presence of gingivitis was used as a dependent variable. Sex, the number of permanent teeth, the OHI-S, the presence of partially erupted molars and receiving dental checkups, which differed significantly between the healthy group and the periodontal disease group, were included as independent variables in the multivariate analysis. Spearman’s rank correlation coefficient (ρ) was used to confirm the correlation between the salivary LD level and the other parameters.

All data analyses were performed using the Statistical Package for the Social Sciences (SPSS ver. 22, SPSS Japan, Tokyo, Japan). The level of significance was set at \( P < 0.05 \).

Results
Participants who had provided incomplete responses in the questionnaire (\( n = 129 \)), had systemic disease or medication that impacted on the salivary LD level or gingivitis (\( n = 1 \)), or had undergone orthodontic treatment (\( n = 6 \)) were excluded. Finally, data from 1,915 students (981 males, 934 females; mean age ± standard deviation, 21.4 ± 2.5 years) were subjected to analysis. There were 88 participants (4.6%) with the salivary LD level of ≥8.

Table 1 Differences in characteristics between male and female participants

| variable                              | male \( n = 981 \) | females \( n = 934 \) | total \( n = 1,915 \) | \( P \) value\(^‡\) |
|---------------------------------------|-------------------|----------------------|-----------------------|-------------------|
| age (years)                           | 21.4 ± 2.4*       | 21.3 ± 2.5           | 21.4 ± 2.5            | 0.493             |
| gingivitis (LD ≥ 8)                   | yes               | 56 (5.7)\(^†\)      | 32 (3.4)              | 88 (4.6)          | 0.017             |
| number of permanent teeth             | 29.5 ± 1.7        | 28.8 ± 1.6           | 29.1 ± 1.7            | <0.001            |
| OHI-S                                 | 0.56 ± 0.55       | 0.39 ± 0.44          | 0.48 ± 0.51           | <0.001            |
| presence of partially erupted molars | yes               | 536 (54.6)           | 418 (44.8)            | 954 (49.8)        | <0.001            |
| BMI                                   | underweight       | 131 (13.4)           | 154 (16.5)            | 285 (14.9)        | <0.001            |
|                                       | normal             | 743 (75.7)           | 737 (78.9)            | 1,480 (77.5)      |                   |
|                                       | overweight/obese   | 107 (10.9)           | 43 (4.6)              | 150 (7.8)         |                   |
| systemic disease                      | yes               | 38 (3.9)             | 37 (4.0)              | 75 (3.9)          | 0.921             |
| medication                            | yes               | 115 (11.7)           | 140 (15.0)            | 255 (13.3)        | 0.035             |
| smoking habit                         | yes               | 66 (6.7)             | 7 (0.7)               | 73 (3.8)          | <0.001            |
| daily frequency of tooth brushing     | ≥2 times           | 705 (71.9)           | 844 (90.4)            | 1,549 (80.9)      | <0.001            |
| use of dental floss                   | yes               | 137 (14.0)           | 166 (17.8)            | 303 (15.8)        | 0.022             |
| receiving dental check-ups            | yes               | 99 (10.1)            | 160 (17.1)            | 259 (13.5)        | <0.001            |

LD, lactate dehydrogenase; OHI-S, simplified oral hygiene index; BMI, body mass index.

* mean ± standard deviation.

† number (%).

‡ unpaired t test or chi-squared test.
medicine, and contraceptives, which were not related to the salivary LD level or gingivitis.

Table 2 compares characteristics between the healthy group and the gingivitis group. The percentage of male participants, the number of permanent teeth, the OHI-S and the presence of partially erupted molars were higher, whereas the proportion receiving dental check-ups was lower in the gingivitis group than in the healthy group (\( P < 0.05 \) for all).

Logistic regression analyses showed that the presence of gingivitis was significantly associated with the OHI-S (OR: 2.68, 95\% CI: 1.94-3.69) and receiving dental check-ups (OR: 0.31, 95\% CI: 0.10-0.99) (Table 3).

In addition, the salivary LD level was confirmed positive correlation with only the OHI-S score (Spearman’s rank correlation coefficient, \( \rho = 0.22, P < 0.001 \)).

### Table 2 Differences in characteristics between the healthy group and the gingivitis group

| variable                                | healthy (LD < 8) | gingivitis (LD ≥ 8) | \( P \) value‡ |
|-----------------------------------------|------------------|---------------------|----------------|
| age (years)                             | 21.4 ± 2.5*      | 21.4 ± 1.8          | 0.927          |
| sex                                     |                  |                     |                |
| male                                    | 925 (50.6)†      | 56 (63.6)           | 0.017          |
| number of permanent teeth               | 29.1 ± 1.7       | 29.6 ± 1.8          | 0.013          |
| OHI-S                                   | 0.46 ± 0.50      | 0.87 ± 0.62         | <0.001         |
| presence of partially erupted molars    | yes              |                     |                |
| BMI                                     |                  |                     |                |
| underweight                             | 273 (14.9)       | 12 (13.6)           | 0.246          |
| normal                                  | 1,415 (77.4)     | 65 (73.9)           |                |
| overweight/obese                        | 139 (7.6)        | 11 (12.5)           |                |
| systemic disease                        | yes              | 70 (3.8)            | 0.382          |
| medication                              | yes              | 247 (13.5)          | 0.232          |
| smoking habit                           | yes              | 67 (3.7)            | 0.132          |
| daily frequency of tooth brushing       | ≥2 times         | 1,481 (81.1)        | 0.377          |
| use of dental floss                     | yes              | 288 (15.8)          | 0.748          |
| receiving dental check-ups              | yes              | 256 (14.0)          | 0.004          |

LD, lactate dehydrogenase; OHI-S: simplified oral hygiene index; BMI, body mass index.
* mean ± standard deviation.
† number (%).
‡ unpaired \( t \) test, chi-squared test or Fisher’s exact test.

### Table 3 Logistic regression analysis with gingivitis as the dependent variable

| variable                                | adjusted OR | 95\% CI | \( P \) value |
|-----------------------------------------|-------------|---------|--------------|
| sex                                     | female      | 1 (reference) |             |
|                                         | male        | 1.22    | 0.76-1.94, 0.413 |
| number of permanent teeth               |             | 1.09    | 0.94-1.27, 0.259 |
| OHI-S                                   |             | 2.68    | 1.94-3.69, <0.001 |
| presence of partially erupted molars    | no          | 1 (reference) |             |
|                                         | yes         | 1.17    | 0.70-1.97, 0.548 |
| receiving dental check-ups              | no          | 1 (reference) |             |
|                                         | yes         | 0.31    | 0.10-0.99, 0.048 |

OR, odds ratio; CI, confidence interval; OHI-S, simplified oral hygiene index.
Hosmer and Lemeshow test, chi-squared test, 8.37 (\( P = 0.398 \)).

**Discussion**

This appears to have been the first study to estimate the salivary level of LD using a commercially available test kit to screen for gingivitis in a large population. The test kit used in this study has certain advantages, with a sensitivity and specificity of 0.89 and 0.98, respectively (11). Another salivary LD test kit was shown to have a relatively lower sensitivity and specificity for periodontitis (0.66 and 0.67, respectively) (13). Therefore, the validity of this test kit is satisfactory. The kit does not require any special equipment and costs 200 Japanese yen (1.89 US dollars using the average exchange rate in 2014). In addition, the result of the test kit can be obtained within 1 min, allowing both the subjects and examiners to know the result at the chairside. Because of its simplicity, rapidity and cost-effectiveness, the new test kit is recommended as a useful tool for gingivitis screening in both
the public health sector and school health examinations.

Logistic regression analysis revealed a positive association between OHI-S and gingivitis diagnosed on the basis of the salivary LD level. There was also a positive correlation between the salivary LD level as an ordinal variable and the OHI-S score. Gingivitis is caused by bacterial biofilms that accumulate on teeth adjacent to the gingiva (4). A previous study reported an association between OHI-S and gingivitis assessed using the PMA index (Takagi et al. Koku Eisei Gakkai Zasshi 21, 78-89, 1971).

An association between the plaque control record and salivary LD level was also found previously (11). The present findings are in agreement with these results and confirm the association between dental hygiene and gingivitis.

There was also a negative association between receiving dental checkups and gingivitis as diagnosed using the salivary LD level, suggesting that receiving dental checkups was associated with better gingival condition. Some previous studies have also reported that participants who visit dentists regularly have a better gingival condition (22,23). These findings suggest the importance of dental checkups for maintaining gingival health.

In the present study, 88 participants (4.6%) were defined as having gingivitis. Previously, 48.2% of university students were reported to have at least one site with BOP (Furuta et al. Koku Eisei Gakkai Zasshi 59, 165-172, 2009.). A national survey also showed that 42.9% of participants aged 20-24 years had BOP (http://www.mhlw.go.jp/toukei/list/62-28.html). Those previous studies reported the prevalence of participants who had at least one BOP-positive site. However, in the present study, the gingivitis group may refer to BOP ≥20% based on six site of each teeth, because the salivary LD level of ≥8 referred to BOP ≥20% in the previous study (11). The differences in the results between the present study and others might be explained by the difference in the BOP cut-off value.

In the present study, there was no evident association between the presence of gingivitis assessed by the salivary LD level and age or sex. These factors have been associated with gingival inflammation previously (22,23). Because of the small deviation in the age of participants (standard deviation = 2.5 years) in the present study, it was not possible to determine the association between age and the presence of disease as defined by the salivary LD level. However, it is unclear why there was no evident association between sex and the presence of gingivitis in this study. Further investigations will be required to confirm these associations.

Some studies have reported that the salivary LD level is associated with various systemic diseases, including type 1 or 2 diabetes (24), pneumonia (25), epilepsy (26), complex regional pain syndrome (27), Sjögren’s syndrome (28), and oral cancer (29-34). These conditions might affect the salivary LD level. However, none of the participants in this study had these conditions or had received related medication, except for type 1 diabetes.

The salivary LD level may be affected by smoking. Cigarette smoke exposure directly causes a 34% reduction in the salivary LD level (35). Thus, the salivary LD level may be lower in smokers than in non-smokers. However, there was no significant difference in smoking habit between the gingivitis group and the healthy group in the present study. The effects of smoking on the salivary LD level appeared to be similar between the two groups.

There were some limitations associated with this study. First, a causal association could not be shown because the study was cross-sectional. Additional studies will be needed to determine whether oral hygiene condition or attending dental checkups affects the salivary LD level. Second, the participants were students recruited from Okayama University. This may have limited the possibility of extrapolating these findings to the general young population. Third, the gingivitis group might have included students with periodontitis. Finally, it was not possible to consider other possible confounders that might be associated with periodontal disease, such as psychological stress (36), oral health literacy (37), and genetic factors (38).

In conclusion, the OHI-S and receiving dental checkups were significantly associated with the presence of gingivitis assessed by the salivary LD test kit in Japanese university students.

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Conflict of Interest
The authors have no conflict of interest to declare.

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