Journal of Oral Science, Vol. 61, No. 1, 133-139, 2019

Original

The level of salivary lactate dehydrogenase as an indicator of the association between gingivitis and related factors in Japanese university students

Ayano Taniguchi-Tabata[1)], Daisuke Ekuni[1,2)], Tetsuji Azuma[3)], Toshiki Yoneda[1)], Mayu Yamane-Takeuchi[1)], Kota Kataoka[1)], Hirofumi Mizuno[1)], Hisataka Miyai[1)], Yoshiaki Iwasaki[4)], and Manabu Morita[1)]

[1)]Department of Preventive Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan
[2)]Advanced Research Center for Oral and Craniofacial Sciences, Okayama University Dental School, Okayama, Japan
[3)]Department of Community Oral Health, Asahi University School of Dentistry, Mizuho, Japan
[4)]Health Service Center, Okayama University, Okayama, Japan

(Received January 31, 2018; Accepted April 3, 2018)

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

Correspondence to Dr. Daisuke Ekuni, Department of Preventive Dentistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
Fax: +81-86-235-6714 E-mail: dekuni7@md.okayama-u.ac.jp

J-STAGE Advance Publication: February 26, 2019
doi.org/10.2334/josnusd.18-0038
DN/JST.JSTAGE/josnusd/18-0038

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/m^2), normal weight (18.5-24.9 kg/m^2) and overweight/obese (≥25.0 kg/m^2) (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

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 (77.3)	
	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.

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 gingivitis 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 shows the differences in characteristics between male and female participants. Significant male-female differences in the presence of gingivitis, the number of permanent teeth, the OHI-S, the presence of partially erupted molars, BMI, medication, smoking habit and oral health behavior were observed ($P < 0.05$ for all). Female participants tended to have better oral conditions and oral health behavior than male participants. The systemic diseases according to the ICD10 were as follows: code L (n = 19), J (n = 12), K (n = 6), N (n = 5), K (n = 6), D, F (n = 2), G, I, M, R (n = 1), and blank (n = 22). The participants were taking antiallergic medicine, stomach

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

variable		healthy (LD < 8) n = 1,827	gingivitis (LD ≥ 8) n = 88	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	900 (49.3)	54 (61.4)	0.027
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)	5 (5.7)	0.382
medication	yes	247 (13.5)	8 (9.1)	0.232
smoking habit	yes	67 (3.7)	6 (6.8)	0.132
daily frequency of tooth brushing	≥2 times	1,481 (81.1)	68 (77.3)	0.377
use of dental floss	yes	288 (15.8)	15 (17.0)	0.748
receiving dental check-ups	yes	256 (14.0)	3 (3.4)	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).

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 checkups (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, ρ = 0.22, $P < 0.001$).

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 $\geq$20% based on six site of each teeth, because the salivary LD level of $\geq$8 referred to BOP $\geq$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.

Acknowledgments

The authors are grateful to Prof. Takaaki Tomofuji (Department of Community Oral Health, Asahi University School of Dentistry, Mizuho, Japan), Dr. Shinsuke Mizutani (Section of Geriatric Dentistry and Perioperative Medicine in Dentistry, Division of Maxillofacial Diagnostic and Surgical Sciences, Faculty of Dental Science, and OBT Research Center, Faculty of Dental Science, Kyushu University, Fukuoka, Japan), Dr. Tatsuya Machida (Grace Dental Clinic, Okayama, Japan) and Dr. Yuya Kawabata (Kawabata Dental Clinic, Hiroshima, Japan) for their assistance with data entry and technical suggestions. This work was supported by JSPS KAKENHI (No. 15K11415) .

Conflict of Interest

The authors have no conflict of interest to declare.

References

1. Vered Y, Sgan-Cohen HD (2003) Self-perceived and clinically diagnosed dental and periodontal health status among young adults and their implications for epidemiological surveys. BMC Oral Health, doi.org/10.1186/1472-6831-3-3.
2. Ueno M, Zaitsu T, Ohara S, Wright C, Kawaguchi Y (2011) Factors influencing perceived oral health of Japanese middle-aged adults. Asia-Pacific J Public Heal, doi.org/10.1177/1010539511428352.
3. Kojima A, Ekuni D, Mizutani S, Furuta M, Irie K, Azuma T et al. (2013) Relationships between self-rated oral health, subjective symptoms, oral health behavior and clinical conditions in Japanese university students: a cross-sectional survey at Okayama University. BMC Oral Health, doi.org/10.1186/1472-6831-13-62.
4. Pihlstrom BL, Michalowicz BS, Johnson NW (2005) Periodontal diseases. Lancet 366, 1809-1820.
5. Ainamo J, Barmes D, Beagrie G, Cutress T, Martin J, Sardo-Infirri J (1982) Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). Int Dent J 32, 281-291.
6. Daly CG, Mitchell DH, Highfield JE, Grossberg DE, Stewart D (2001) Bacteremia due to periodontal probing a clinical and microbiological investigation. J Periodontol 72, 210-214.
7. Kaufman E, Lamster IB (2000) Analysis of saliva for periodontal diagnosis--a review. J Clin Periodontol 27, 453-465.
8. Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT (2009) Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol 2000 50, 52-64.
9. Liu J, Duan Y (2012) Saliva: a potential media for disease diagnostics and monitoring. Oral Oncol 48, 569-577.
10. Salminen A, Gursoy UK, Paju S, Hyvärinen K, Mäntylä P, Buhlin K et al. (2014) Salivary biomarkers of bacterial burden, inflammatory response, and tissue destruction in periodontitis. J Clin Periodontol 41, 442-450.
11. Ekuni D, Yamane-Takeuchi M, Kataoka K, Yokoi A, Taniguchi-Tabata A, Mizuno H et al. (2017) Validity of a new kit measuring salivary lactate dehydrogenase level for screening gingivitis. Dis Markers, doi.org/10.1155/2017/9547956.
12. De La Peña VA, Diz Dios P, Tojo Sierra R (2007) Relationship between lactate dehydrogenase activity in saliva and oral health status. Arch Oral Biol 52, 911-915.
13. Nomura Y, Tamaki Y, Tanaka T, Arakawa H, Tsurumoto A, Kirimura K et al. (2006) Screening of periodontitis with salivary enzyme tests. J Oral Sci 48, 177-183.
14. Kugahara T, Shosenji Y, Ohashi K (2008) Screening for periodontitis in pregnant women with salivary enzymes. J Obstet Gynaecol Res 34, 40-46.
15. Nomura Y, Shimada Y, Hanada N, Numabe Y, Kamoi K, Sato T et al. (2012) Salivary biomarkers for predicting the progression of chronic periodontitis. Arch Oral Biol 57, 413-420.
16. Nomura Y, Okada A, Kakuta E, Gunji T, Kajiura S, Hanada N (2016) A new screening method for periodontitis: an alternative to the community periodontal index. BMC Oral Health, doi.org/10.1186/s12903-016-0216-x.
17. Syndergaard B, Al-Sabbagh M, Kryscio RJ, Xi J, Ding X, Ebersole JL et al. (2014) Salivary biomarkers associated with gingivitis and response to therapy. J Periodontol 85, e295-303.
18. Greene JG, Vermillion JR (1964) The simplified oral hygiene index. J Am Dent Assoc 68, 7-13.
19. Yamalık K, Bozkaya S (2008) The predictivity of mandibular third molar position as a risk indicator for pericoronitis. Clin Oral Investig 12, 9-14.
20. Ekuni D, Yamamoto T, Koyama R, Tsuneishi M, Naito K, Tobe K (2008) Relationship between body mass index and periodontitis in young Japanese adults. J Periodontal Res 43, 417-421.
21. Ekuni D, Mizutani S, Kojima A, Tomofuji T, Irie K, Azuma T et al. (2014) Relationship between increases in BMI and changes in periodontal status: a prospective cohort study. J Clin Periodontol 41, 772-778.
22. Lang WP, Farghaly MM, Ronis DL (1994) The relation of preventive dental behaviors to periodontal health status. J Clin Periodontol 21, 194-198.
23. Mizutani S, Ekuni D, Furuta M, Tomofuji T, Irie K, Azuma T et al. (2012) Effects of self-efficacy on oral health behaviours and gingival health in university students aged 18- or 19-years-old. J Clin Periodontol 39, 844-849.
24. Malicka B, Skoskiewicz-Malinowska K, Kaczmarek U (2016) Salivary lactate dehydrogenase and aminotransferases in diabetic patients. Medicine (Baltimore) 95, e5211.
25. Klein Kremer A, Kuzminsky E, Bentur L, Nagler RM (2014) Salivary and serum analysis in children diagnosed with pneumonia. Pediatr Pulmonol 49, 569-573.
26. Shahar E, Attias U, Savulescu D, Genizin J, Gavish M, Nagler R (2014) Oxidative stress, metalloproteinase and LDH in children with intractable and non-intractable epilepsy as reflected in salivary analysis. Epilepsy Res 108, 117-124.
27. Eisenberg E, Shtahl S, Geller R, Reznick AZ, Sharf O, Ravbinovich M et al. (2008) Serum and salivary oxidative analysis in complex regional pain syndrome. Pain 138, 226-232.
28. Ryo K, Yamada H, Nakagawa Y, Tai Y, Obara K, Inoue H et al. (2007) Possible involvement of oxidative stress in salivary gland of patients with Sjögren's syndrome. Pathobiology 73, 252-260.
29. Shetty SR, Chadha R, Babu S, Kumari S, Bhat S, Achalli S (2012) Salivary lactate dehydrogenase levels in oral leukoplakia and oral squamous cell carcinoma: a biochemical and clinicopathological study. J Cancer Res Ther 8 Suppl 1, S123-125.
30. Joshi PS, Golgire S (2014) A study of salivary lactate dehydrogenase isoenzyme levels in patients with oral leukoplakia and squamous cell carcinoma by gel electrophoresis method.

J Oral Maxillofac Pathol 18, S39-44.
31. Patel S, Metgud R (2015) Estimation of salivary lactate dehydrogenase in oral leukoplakia and oral squamous cell carcinoma: a biochemical study. J Cancer Res Ther 11, 119-223.
32. Kallalli BN, Rawson K, Muzammil, Singh A, Awati MA, Shivhare P (2016) Lactate dehydrogenase as a biomarker in oral cancer and oral submucous fibrosis. J Oral Pathol Med 45, 687-690.
33. Lokesh K, Kannabiran J, Rao MD (2016) Salivary lactate dehydrogenase (LDH)- a novel technique in oral cancer detection and diagnosis. J Clin Diagn Res 10, ZC34-37.
34. Saluja TS, Spadigam A, Dhupar A, Syed S (2016) Equating salivary lactate dehydrogenase (LDH) with LDH-5 expression in patients with oral squamous cell carcinoma: an insight into metabolic reprogramming of cancer cell as a predictor of aggressive phenotype. Tumour Biol 37, 5609-5620.
35. Avezov K, Reznick AZ, Aizenbud D (2014) LDH enzyme activity in human saliva: the effect of exposure to cigarette smoke and its different components. Arch Oral Biol 59, 142-148.
36. Peruzzo DC, Benatti BB, Ambrosano GMB, Nogueira-Filho GR, Sallum EA, Casati MZ et al. (2007) A systematic review of stress and psychological factors as possible risk factors for periodontal disease. J Periodontol 78, 1491-1504.
37. Wehmeyer MMH, Corwin CL, Guthmiller JM, Lee JY (2014) The impact of oral health literacy on periodontal health status. J Public Health Dent 74, 80-87.
38. Meng H, Ren X, Tian Y, Feng X, Xu L, Zhang L et al. (2011) Genetic study of families affected with aggressive periodontitis. Periodontol 2000 56, 87-101.