Sultan Qaboos University Med J, August 2018, Vol. 18, Iss. 3, pp. e318–323, Epub. 19 Dec 18
Submitted 8 Feb 18
Revision Req. 28 Feb 18; Revision Recd. 27 Mar 18
Accepted 12 Apr 18
doi: 10.18295/squmj.2018.18.03.009

CLINICAL & BASIC RESEARCH

Lactate Dehydrogenase and β-Glucuronidase as Salivary Biochemical Markers of Periodontitis Among Smokers and Non-Smokers

*Syed A. Ali,[1] Ravi L. Telgi,[1] Amit Tirth,[1] Irfan Q. Tantry,[2] Abdul Aleem[3]

نازعة هيدروجين اللاكتات وبيتا غلوكورونيداز كواسمات لعاب حيوية كيميائية في التهاب دواعم السن عند المدخنين وغير المدخنين

سيد أمان علي، رافي لينجيشا تيلجي، أميت تيرث، عرفان قادر تنتري، عبدالعليم

ABSTRACT: ***Objectives:*** This study aimed to establish lactate dehydrogenase (LDH) and β-glucuronidase as salivary biomarkers of periodontitis among smokers and non-smokers. ***Methods:*** This cross-sectional case-control study was conducted at the Aligarh Muslim University, Aligarh, India, between January and June 2017. A total of 200 participants were divided into four groups based on their periodontal and smoking statuses. Unstimulated mixed saliva samples were collected to estimate LDH and β-glucuronidase levels. In addition, total protein was estimated using Lowry's method. ***Results:*** There was a significant increase in enzyme activity in the periodontitis groups compared to the non-periodontitis groups (P <0.001). However, significantly lower enzyme activity was observed among smokers, irrespective of periodontal status (P <0.001). Nevertheless, a receiver operating characteristic curve analysis indicated the diagnostic potential of both enzymes to be fair-to-excellent. ***Conclusion:*** Although smoking was found to significantly alter enzyme activity, LDH and β-glucuronidase were reliable salivary biomarkers of periodontitis among both smokers and non-smokers.

Keywords: Periodontitis; Biomarkers; Saliva; Lactate Dehydrogenase; beta-Glucuronidase; Smoking; Tobacco Use; India.

الملخص: الهدف: تهدف هذه الدراسة إلى التثبت من نازعة هيدروجين اللاكتات وبيتا غلوكورونيداز كواسمات لعاب حيوية كيميائية في التهاب دواعم السن عند المدخنين وغير المدخنين. **الطريقة:** أجريت هذه الدراسة لحالات الشواهد المقطعية في جامعة أليجاره الإسلامية، أليجاره، الهند، في الفترة ما بين يناير ويونيو 2017. تم تقسيم ما مجموعه 200 مشارك إلى أربع مجموعات على أساس حالة اللثة والتدخين. تم جمع عينات اللعاب المختلط وغير المحفز لتقدير مستويات نازعة هيدروجين اللاكتات وبيتا غلوكورونيداز. بالإضافة إلى ذلك، تم تقدير البروتين الكلي باستخدام طريقة لأوري. **النتائج:** كان هناك زيادة معنوية في نشاط الإنزيم في مجموعات التهاب اللثة بالمقارنة بمجموعات بدون التهاب اللثة (P <0.001). ومع ذلك، لوحظ وجود نشاط أنزيم أقل بشكل ملحوظ بين المدخنين، بغض النظر عن حالة اللثة (P <0.001). ومع ذلك، أشار تحليل منحنى خصائص التشغيل المتلقي إلى إمكانات التشخيص لكل الإنزيمات لتكون عادلة إلى ممتازة. **الخلاصة:** على الرغم من أن التدخين يغير نشاط الإنزيم بشكل كبير، كانت نازعة هيدروجين اللاكتات وبيتا غلوكورونيداز مؤشرات حيوية لعابية موثوقة لالتهاب اللثة بين المدخنين وغير المدخنين على حد سواء.

الكلمات المفتاحية: التهاب دواعم السن؛ واسمات بيولوجية؛ لعاب؛ نازعة هيدروجين اللاكتات؛ بيتا غلوكورونيداز؛ تدخين؛ استخدام التبغ؛ الهند.

Advances in Knowledge

- *This study rules out the effect of tobacco smoke on the diagnostic ability of two salivary enzymes—lactate dehydrogenase (LDH) and β-glucuronidase—in risk profiling for periodontal disease.*

Application to Patient Care

- *The current findings support the use of these salivary enzymes as biomarkers for periodontal disease.*

Periodontal disease is a common inflammatory disease caused by the interaction between certain Gram-negative bacterial species and components of the host immune response.[1] Chronic periodontal infections trigger the release of a myriad of metabolic byproducts, destructive cellular enzymes and other mediators of tissue destruction at the interface between the tooth and the periodontal pocket.[2] As a result, the normal histological architecture of the periodontium is disturbed, with persistent inflammation associated with the irreversible loss of mineralised and non-mineralised tissues.[1]

[1]*Department of Public Health Dentistry, Kothiwal Dental College & Research Centre, Moradabad, Uttar Pradesh, India; Departments of* [2]*Biochemistry and* [3]*Periodontology, Aligarh Muslim University, Aligarh, Uttar Pradesh, India*

**Corresponding Author's e-mail: aliamaan29@gmail.com*

Chronic periodontitis may advance without causing severe oral discomfort; as such, subjects often seek professional care only after the periodontal tissue is considerably damaged.[3] Thus, there is an urgent need to diagnose this disease in its initial stages so as to initiate early intervention. Ideally, a diagnostic marker should be highly specific and sensitive and should indicate the presence of a disease process prior to the occurrence of extensive clinical damage.[4] At present, a diagnosis of periodontitis is usually made via imaging and clinical assessment of probing pocket depth, bleeding upon probing and clinical attachment level.[5] However, the utility of such measurements is limited because these findings may display evidence of previous rather than current disease activity.[6]

The saliva contains a wide and unique variety of proteins and enzymes with important oral biological functions. The pathogenesis of periodontitis has been linked to alterations in various salivary enzymes, including lactate dehydrogenase (LDH), alkaline phosphatase, matrix metalloproteinases 8 and 1, aminotransferases, amylase, β-glucuronidases, arginase, chitinases and dipeptidyl peptidase.[7] Metabolic LDH plays a key role in anaerobic glycolysis and its extracellular presence is always related to cell necrosis and tissue breakdown.[8] In contrast, β-glucuronidase is a neutrophil-derived lysosomal acid hydrolase stored in the azurophilic granules. It is active in the degradation of proteoglycans and the ground substance and is considered a marker for azurophilic granule release by polymorphonuclear leukocyte lysosomes.[9] Under these considerations, salivary LDH and β-glucuronidase may play a potential role as salivary biomarkers of periodontal disease.[7]

Unfortunately, the diagnostic use of these enzymes in periodontitis cases has been hampered as current understanding of the biomolecules present in saliva and their relevance to disease aetiology is still limited. In addition, various factors may cause enzymatic alterations, such as temperature, pH, and enzyme and substrate concentrations of inhibitors or activators.[10] For example, tobacco smoke compounds have been found to impair salivary enzyme activities at the molecular level.[11] Therefore, this study aimed to attempt to establish LDH and β-glucuronidase as salivary biochemical markers for periodontitis and to assess their diagnostic potential among both smokers and non-smokers.

Methods

This cross-sectional case-control study was conducted at the Department of Periodontology of the Aligarh Muslim University, Aligarh, Uttar Pradesh, India, between January and June 2017. The required sample size was calculated using G*Power Software, Version 3.0.10 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany), based on a calculated effect size of 0.565 as per the results of a pilot study involving 20 subjects, with a 5% level of precision, 95% confidence interval and 80% statistical power. The minimum sample size was calculated to be 200 subjects. Patients who had received antiseptic, professional hygiene or periodontal therapies or treatment with anti-inflammatory drugs, immunosuppressants or corticosteroids in the preceding six months were excluded from the study. In addition, individuals undergoing orthodontic treatment or other dental procedures, subjects with acute oral mucosal lesions and/or suspected oral malignancies, those with adverse behavioural habits such as tobacco/*paan* chewing or alcohol abuse, subjects suffering from any systemic diseases and pregnant, lactating or post-menopausal women were also excluded.

A total of 1,306 patients aged 30–50 years old presenting to the Department of Periodontology, Dr. Ziauddin Ahmad Dental College & Hospital, Aligarh, Uttar Pradesh, were screened. A simple random sampling method was used to select 200 of these individuals for inclusion in the study. The participants were divided into four groups of 50 subjects each, including non-smokers with no/mild gingivitis, non-smokers with no/mild gingivitis, smokers with chronic generalised periodontitis and smokers with chronic generalised periodontitis [Figure 1]. Unfortunately, it was logistically difficult to find sufficient cases with no gingival inflammation; as such, cases with no/mild gingivitis were considered to constitute the control groups in the study design. Mild gingivitis was diagnosed based on the Silness-Löe gingival index.[12] Chronic generalised periodontitis was defined as a probing pocket depth of ≥5 mm, clinical attachment loss of ≥3 mm and moderate, severe or generalised disease progression involving >30% of the mouth. Patients were categorised as smokers or non-smokers based on the updated definition of the Centers for Disease Control and Prevention.[13] All smokers reported currently smoking at least five times per day and had a history of smoking of at least five years. Former smokers were not included in the study.

A sample of approximately 5 mL of unstimulated mixed saliva was aseptically collected via aspiration from each participant while seated in an upright position. The collection was performed by a single trained expert between 9 and 11 AM, five minutes after the mouth was rinsed with 15 mL of water to wash out any exfoliated cells. Eating, drinking and smoking were restricted for at least two hours prior to collection. The saliva was collected in sterile test tubes capped with sterile tinfoil. The samples were transferred to a refrigerated container at 4 °C and then centrifuged for 10 minutes at 1,500 revolutions per minute. Subsequently, estimations of LDH and β-glucuronidase activity were carried out

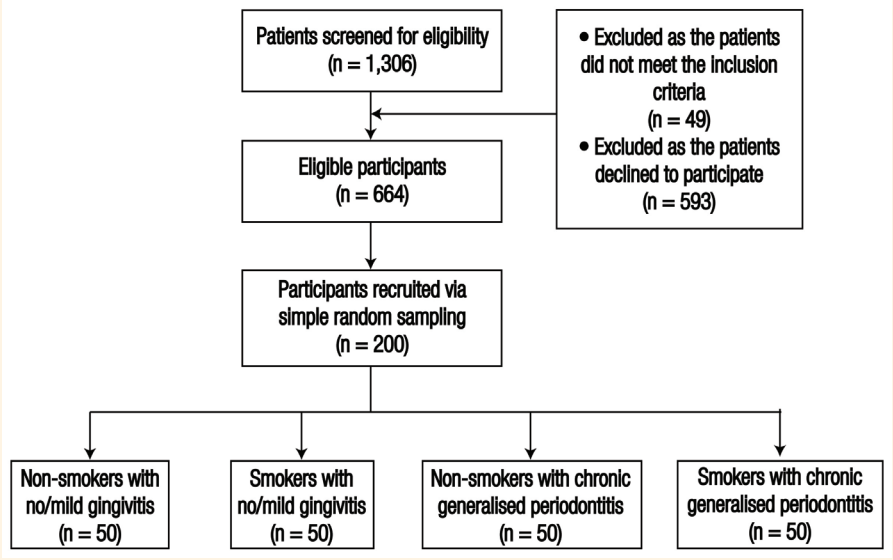

Figure 1: Diagram showing the protocol and design used in the current study.

immediately by a single trained biochemist who was blinded to the study design.

The biochemical analysis of LDH was performed following strict protocols for salivary enzyme estimation.[14] LDH was assayed following the conversion of reduced nicotinamide adenine dinucleotide (NADH) to oxidised NADH at 340 nm in the presence of sodium pyruvate. Briefly, 100 µL of saliva was added to a 2.8 mL reaction mixture consisting of 0.05 M tris buffer at a pH of 7.4, 3.33 mM of magnesium chloride and 1.6 mM of sodium pyruvate. Then, 100 µL of NADH (at a final concentrate of 0.08 mM) was added just before the change in absorbance observed after 3 minutes using a U-2910 spectrophotometer (Hitachi High-Technologies Corp., Tokyo, Japan).[14] The results were expressed in IU/L.

For the analysis of salivary β-glucuronidase, 50 µL of 0.9% saline solution (as the control), 50 µL of standard 4-methylumbelliferone (Sigma-Aldrich Corp., St. Louis, Missouri, USA) and 50 µL of the salivary samples were separated in designated tubes. In each tube, 100 µL of methylumbelliferyl β-D-glucuronide containing 0.001% bovine serum albumin (BSA) was added and the contents were mixed and incubated at 37 °C for 15 minutes. The reaction was stopped with 2 mL of 0.2 M glycine buffer at a pH of 11.7 and containing 0.2% sodium dodecyl sulphate. After the solution was mixed for 60 minutes, the fluorescence was measured at an excitation wavelength of 360 nm and an emission wavelength of 459 nm. For the standard, the fluorescence of 0.008 mM and 0.016 mM of 4-methylumbelliferone was measured using an RF-5301 spectrofluorophotometer (Shimadzu Corp., Kyoto, Japan), as the fluorescence of 0.008 mM of 4-methylumbelliferone is equivalent to 1 IU of β-glucuronidase.[15] The total protein in the saliva was estimated by means of Lowry's method using BSA to derive standard and specific enzyme activity.[16]

Data were analysed using the Statistical Package for the Social Sciences (SPSS), Version 21.0 (IBM Corp., Armonk, New York, USA). Parametric tests (i.e. an unpaired t-test) and a one-way analysis of variance were used to compare the mean enzyme activities of the four independent groups using an F distribution, followed by a post-*hoc* Tukey's analysis. A receiver operating characteristic (ROC) curve was used to determine the diagnostic ability of the enzymes as their discrimination thresholds varied. Binomial logistic regression was then conducted to estimate the frequency of periodontitis among the patients. A *P* value of <0.050 was considered statistically significant.

This study was reviewed and approved by the institutional ethical committee of the Kothiwal Dental College & Research Centre (#KDCRC/IERB/11/2015/34). Written informed consent from the participants was obtained using a pre-designed *proforma*. All participation in the study was voluntary in nature.

Results

The patients ranged in age between 30–35 years old (mean: 32.5 years). There were very few female smokers (20.5%). The standard and specific enzyme activity of LDH and β-glucuronidase in each group is presented in Table 1. There was a significant increase in LDH and β-glucuronidase enzyme activity among participants with periodontitis compared to those without periodontitis (*P* <0.001) [Table 2]. However, significantly lower enzyme activity was noted among the smokers, irrespective of periodontal status (*P* <0.001) [Table 3]. Overall, there was a 23.8% and 25% reduction in LDH

Table 1: Enzyme activity of salivary lactate dehydrogenase and β-glucuronidase according to smoking and periodontal status (N = 200)

Group	Mean LDH activity ± SD		Mean β-glucuronidase activity ± SD	
	Standard activity in IU/L	Specific activity in nmol/min/mg of total protein	Standard activity in IU/L	Specific activity in nmol/min/mg of total protein
Non-smokers with no/mild gingivitis	285.34 ± 35.76	237.78 ± 32.46	30.29 ± 9.43	25.24 ± 6.32
Smokers with no/mild gingivitis	213.92 ± 34.70	178.26 ± 32.34	24.81 ± 7.19	20.67 ± 6.56
Non-smokers with chronic generalised periodontitis	1,075.88 ± 253.76	896.56 ± 264.14	91.76 ± 12.05	76.46 ± 10.43
Smokers with chronic generalised periodontitis	819.10 ± 315.59	682.58 ± 274.12	85.53 ± 13.21	71.27 ± 12.71

LDH = lactate dehydrogenase; SD = standard deviation; min = minute.

Table 2: Comparison of specific enzyme activity of lactate dehydrogenase and β-glucuronidase according to periodontal status (N = 200)

Enzyme	Mean specific activity in nmol/min/mg of total protein ± SD		*P* value*
	No/mild gingivitis	Chronic generalised periodontitis	
LDH	208.02 ± 22.95	789.57 ± 73.87	<0.001
β-glucuronidase	32.4 ± 6.44	269.13 ± 11.57	<0.001

min = minute; SD = standard deviation; LDH = lactate dehydrogenase.
**Using an independent t-test.*

Table 3: Comparison of specific enzyme activity of lactase dehydrogenase and β-glucuronidase according to smoking and periodontal status (N = 200)

Enzyme	Mean specific activity in nmol/min/mg of total protein ± SD				*P* value*	Post *hoc* Tukey value
	Non-smokers with no/mild gingivitis	Smokers with no/mild gingivitis	Non-smokers with chronic generalised periodontitis	Smokers with chronic generalised periodontitis		
LDH	237.78 ± 32.46	178.26 ± 32.34	896.56 ± 264.14	682.58 ± 274.12	<0.001	4>3>1>2
β-glucuronidase	25.24 ± 6.23	20.67 ± 6.56	76.46 ± 10.43	71.27 ± 12.71	<0.001	4>3>1>2

min = minute; SD = standard deviation; LDH = lactate dehydrogenase.
**Using a one-way analysis of variance.*

Table 4: Logistic regression model of lactate dehydrogenase and β-glucuronidase as possible predictors of periodontitis among smokers and non-smokers (N = 200)

Predictor	B	SE	Wald	OR (95% CI)	*P* value
LDH	0.007	0.005	2.109	1.007 (0.998–1.016	0.046
β-glucuronidase	0.394	0.153	6.617	1.482 (1.098–2.001)	0.010
Constant	–19.35	6.746	8.227	-	0.004

SE = standard error; OR = odds ratio; CI = confidence interval; LDH = lactate dehydrogenase.

enzyme activity among smokers with periodontitis and smokers with no/mild gingivitis, respectively. Similarly, there was a 6.8% and 18.1% reduction in β-glucuronidase activity, respectively.

Nevertheless, logistic modelling highlighted a strong linear association between LDH and β-glucuronidase activity and periodontitis, regardless of smoking status (P = 0.046 and 0.010, respectively) [Table 4]. Furthermore, the sensitivity and specificity of the enzymes were established at the 25th, 50th and 75th quartiles. According to the ROC curve analysis, the diagnostic potential of LDH at the 25th and 75th quartiles indicated that this biomarker had fair-to-good potential in predicting periodontitis, with the 50th quartile showing excellent potential.

Table 5: Sensitivity, specificity and area under receiver operating characteristic curve of salivary lactate dehydrogenase and β-glucuronidase alterations among periodontitis patients (N = 200)

Quartile	Percentile value	Sensitivity in %	Specificity in %	Area under ROC curve*
LDH				
25th	206.75	63.3	90	0.774
50th	298.50	90	90	0.948
75th	841.50	100	66.7	0.819
β-glucuronidase				
25th	21.95	98	98	0.980
50th	43.52	98	98	0.980
75th	72.35	100	66.7	0.750

ROC = receiver operating characteristic; LDH = lactate dehydrogenase.

**The diagnostic accuracy of the enzymes was interpreted as follows: 1.0–0.91 = excellent; 0.90–0.81 = good; 0.80–0.71 = fair; 0.70–0.61 = poor; 0.60–0.51 = failure.*

For β-glucuronidase, the 25th and 50th percentiles indicated excellent predictive potential, while the 75th percentile indicated fair potential [Table 5].

Discussion

The pathogenesis of tissue destruction in periodontitis is due to host-bacteria interactions which cause the host cells (mainly polymorphonuclear leukocyte lysosomes) to release granular enzymes which are capable of invading extracellular matrix components.[17] Thus, the extracellular presence of certain enzymes seems to play an important role in connective tissue damage. The quantification of enzyme activity in saliva, such as LDH and β-glucuronidase, can therefore provide important information and contribute to the timely diagnosis of periodontal disease.[18] Combining these two markers into a risk profile for periodontal disease may offer improved accuracy in identifying susceptible patients.

In the current study, significantly higher LDH and β-glucuronidase activity was noted in the periodontitis groups compared to the non-periodontitis groups. However, Lamster *et al*. observed variations in β-glucuronidase enzyme activity among subjects with periodontitis.[19] This accentuates the fact that identical clinical conditions may reflect different host responses. Therefore, while increased extracellular enzyme activity may have a positive correlation with disease activity, other factors could be involved in the aetiopathology of periodontitis.[19] Such factors may also influence treatment planning.

In the current study, relatively low enzyme activity was noted among smokers, including both those with no/mild gingival inflammation as well as those with chronic generalised periodontitis. Such findings are likely related to salivary changes resulting from exposure to inhaled cigarette smoke. Cigarette smoke contains over 4,000 chemicals as well as oxygen free radicals and volatile aldehydes that can cause damage to biomolecules.[20] Nagler *et al*. examined the effect of the *in vitro* exposure of saliva to the gas phase of cigarette smoke; exposing saliva to cigarette smoke for three hours caused a 41% reduction in LDH activity.[21] Furthermore, Avezov *et al*. reported a 34% reduction in LDH activity when salivary samples were exposed to different levels of cigarette smoke.[22]

Nevertheless, although tobacco smoke significantly altered enzyme activity in the current study, these changes did not invalidate the predictive value of salivary LDH and β-glucuronidase as diagnostic biomarkers. Therefore, enzyme-based salivary diagnosis can be deemed an effective option in periodontitis cases, as influence of smoking on the biomarker status of LDH and β-glucuronidase can be ruled out. With further studies, changes in enzyme activity may eventually form the basis of a convenient point-of-care diagnostic tool in routine oral health monitoring.

This study was subject to certain limitations. First, the analysis focused on the estimation of LDH and β-glucuronidase in the saliva rather than the gingival crevicular fluid, which would better reflect enzyme activity. Second, the methodology did not analyse the utility of these enzymes as markers of disease severity or response to treatment and, ultimately, disease prognosis. Further studies are necessary to evaluate the reliability of these parameters in this regard. Third, gender matching was not possible due to the unequal distribution of the sample. However, previous research has confirmed that gender does not significantly affect LDH levels, either among patients with periodontal disease or those with normal periodontia.[23] Finally, the current study was designed with strict inclusion criteria; thus, the exclusion of potential subjects may have hampered the generalisability of the results.

Conclusion

The current study found that salivary LDH and β-glucuronidase enzyme activity significantly increased among patients with periodontal disease in comparison to those with no/mild gingival inflammation. While smoking significantly influenced enzyme activity, these changes were within acceptable limits and did not rule out the use of these enzymes as diagnostic biomarkers of periodontitis. However, further research is needed to identify other variables which may influence LDH and β-glucuronidase enzyme activity.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

FUNDING

No funding was received for this study.

References

1. Guthmiller JM, Novak KF. Periodontal diseases. In: Brogden KA, Guthmiller JM, Eds. Polymicrobial Diseases. From: www.ncbi.nlm.nih.gov/books/NBK2496/ Accessed: Mar 2018.
2. Giannobile WV. Salivary diagnostics for periodontal diseases. J Am Dent Assoc 2012; 143:6S–11S. doi: 10.14219/jada.archive.2012.0341.
3. Ji S, Choi Y. Point-of-care diagnosis of periodontitis using saliva: Technically feasible but still a challenge. Front Cell Infect Microbiol 2015; 5:65. doi: 10.3389/fcimb.2015.00065.
4. Barros SP, Williams R, Offenbacher S, Morelli T. Gingival crevicular fluid as a source of biomarkers for periodontitis. Periodontol 2000 2016; 70:53–64. doi: 10.1111/prd.12107.
5. Gursoy UK, Könönen E. Editorial: Use of saliva in diagnosis of periodontitis - Cumulative use of bacterial and host-derived biomarkers. Front Cell Infect Microbiol 2016; 6:196. doi: 10.3389/fcimb.2016.00196.
6. Srivastava N, Nayak PA, Rana S. Point of care: A novel approach to periodontal diagnosis - A review. J Clin Diagn Res 2017; 11:ZE01–6. doi: 10.7860/JCDR/2017/26626.10411.
7. Khiste SV, Ranganath V, Nichani AS, Rajani V. Critical analysis of biomarkers in the current periodontal practice. J Indian Soc Periodontol 2011; 15:104–10. doi: 10.4103/0972-124X.84376.
8. AlMoharib HS, AlMubarak A, AlRowis R, Geevarghese A, Preethanath RS, Anil S. Oral fluid based biomarkers in periodontal disease: Part 1 - Saliva. J Int Oral Health 2014; 6:95–103.
9. Prabhahar CS, Niazi KT, Prakash R, Yuvaraj A, Goud S, Ravishekar P. Estimation of salivary β-glucuronidase activity as a marker of periodontal disease: A case control study. J Int Soc Prev Community Dent 2014; 4:S193–8. doi: 10.4103/2231-0762.149039.
10. Bisswanger H. Enzyme assays. Perspect Sci (Neth) 2014; 1:41–55. doi: 10.1016/j.pisc.2014.02.005.
11. Zappacosta B, Persichilli S, Mordente A, Minucci A, Lazzaro D, Meucci E, et al. Inhibition of salivary enzymes by cigarette smoke and the protective role of glutathione. Hum Exp Toxicol 2002; 21:7–11. doi: 10.1191/0960327102ht202oa.
12. Löe H. The Gingival Index, the Plaque Index and the Retention Index systems. J Periodontol 1967; 38:610–16. doi: 10.1902/jop.1967.38.6.610.
13. Schoenborg CA, Adams PE. Health behaviors of adults: United States, 2005-2007. Vital Health Stat 10 2010; 245:1–132.
14. Alonso de la Peña V, Diz Dios P, Lojo Rocamonde S, Tojo Sierra R, Rodríguez-Segade S. A standardised protocol for the quantification of lactate dehydrogenase activity in saliva. Arch Oral Biol 2004; 49:23–7. doi: 10.1016/S0003-9969(03)00196-1.
15. Lamster IB, Kaufman E, Grbic JT, Winston LJ, Singer RE. Beta-glucuronidase activity in saliva: Relationship to clinical periodontal parameters. J Periodontol 2003; 74:353–9. doi: 10.1902/jop.2003.74.3.353.
16. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265–75.
17. Pöllänen MT, Laine MA, Ihalin R, Uitto VJ. Host-bacteria cross-talk at the dentogingival junction. Int J Dent 2012; 2012:821383. doi: 10.1155/2012/821383.
18. Dabra S, China K, Kaushik A. Salivary enzymes as diagnostic markers for detection of gingival/periodontal disease and their correlation with the severity of the disease. J Indian Soc Periodontol 2012; 16:358–64. doi: 10.4103/0972-124X.100911.
19. Lamster IB, Holmes LG, Gross KB, Oshrain RL, Cohen DW, Rose LF, et al. The relationship of beta-glucuronidase activity in crevicular fluid to clinical parameters of periodontal disease: Findings from a multicenter study. J Clin Periodontol 1994; 21:118–27. doi: 10.1111/j.1600-051X.1994.tb00289.x.
20. Yeh CC, Barr RG, Powell CA, Mesia-Vela S, Wang Y, Hamade NK, et al. No effect of cigarette smoking dose on oxidized plasma proteins. Environ Res 2008; 106:219–25. doi: 10.1016/j.envres.2007.09.008.
21. Nagler RM, Lischinsky S, Diamond E, Klein I, Reznick AZ. New insights into salivary lactate dehydrogenase of human subjects. J Lab Clin Med 2001; 137:363–9. doi: 10.1067/mlc.2001.114710.
22. Avezov K, Reznick AZ, Aizenbud D. LDH enzyme activity in human saliva: The effect of exposure to cigarette smoke and its different components. Arch Oral Biol 2014; 59:142–8. doi: 10.1016/j.archoralbio.2013.11.003.
23. Haghgoo JM, Soheilifar S, Bidgoli M, Rastegarfard N, Saremi M, Kafilzade S. Comparison of whole salivary lactate dehydrogenase level in patients with and without periodontal disease. Avicenna J Dent Res 2016; 8:e29026. doi: 10.17795/ajdr-29026.