Received: 14 February 2024 | Revised: 16 April 2024 | Accepted: 02 May 2024
DOI: 10.48309/JMPCR.2024.448523.1144
Journal of Medicinal and Pharmaceutical Chemistry Research
SAMI

FULL PAPER

Analysis of salivary levels of lactate dehydrogenase, alkaline phosphatase and arginase as well as detection of streptococcus mutans in smoking and non-smoking periodontitis patients

Hazim Saad Jabbar Al-Maliki[a] |Rana Adnan Fayez Al-jaberi[b] |Abdul Amir H. Kadhum[c,*] | Ahmed A. Al-Amiery[d]

[a]*Department of Basic Sciences, College of Dentistry, University of Basrah, Iraq*

[b]*Department of Basic Sciences, College of Dentistry, University of Basrah, Iraq*

[c]*College of Medicine, University of Al-Ameed, Karbala, Iraq*

[d]*Department of Chemical Engineering, Faculty of Engineering, Universiti Kebangsaan Malaysia, Malaysia*

***Corresponding Author:**
Abdul Amir H. Kadhum
Email: amir1719@gmail.com
Tel.: + 9647717173582

Periodontal disease is an inflammatory disorder affecting the oral cavity, caused by *Streptococcus mutans*. Diagnostic tests for periodontal disease involve analysis of biomarkers in saliva, such as lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and arginase (AR) levels. This study aimed to assess salivary enzyme levels and isolate *S. mutans*, in smoking and non-smoking patients with periodontal disease. A sample between 18 and 60 years old who suffering from Periodontitis due to bacterial infection was involved and divided into three groups: Group A consisted of 25 smokers with periodontitis, Group B included 25 non-smokers with periodontitis, and Group C (control) comprised 25 healthy non-smokers without periodontitis. Saliva samples were collected from each participant and analysed using auto-analyser to measure enzyme levels. *S. mutans* was identified using biochemical, and polymerase reaction (PCR). Salivary enzyme levels were found higher in Groups A and B compared to the control Group C. LDH levels were 323.21 ± 22.31 UI/L (Group A), 138.02 ± 13.22 UI/L (Group B), and 104.21 ± 12.33 UI/L (Group C). ALP levels were 67.77 ± 5.34 UI/L (Group A), 53.36 ± 4.24 UI/L (Group B), and 21.88 ± 1.79 UI/L (Group C). AR levels were 21.58 ± 1.21 UI/L (Group A), 17.89 ± 1.35 UI/L (Group B), and 15.31 ± 1.45 UI/L (Group C). Approximately 80% of bacterial isolates were cultured on MSB from smokers with periodontal and approximately 60% from non-smokers with periodontal disease identified. *S. mutans* was in 90% of smokers with periodontal disease and 80% non-smokers with periodontal disease.

KEYWORDS

Arginase; periodontitis; lactate dehydrogenase; alkaline phosphatase.

Introduction

The salivary glands are exocrine organs responsible for producing saliva. Major salivary glands, including the parotid, submandibular, and sublingual glands, along with other minor salivary glands, contribute to saliva production. Saliva primarily consists of water, along with salivary proteins, electrolytes, antibodies, and other substances

important for dental health [1]. When conducting salivary testing to assess underlying issues, the entire saliva sample is typically considered. Saliva is a complex fluid, with water constituting the majority of its composition and organic and inorganic materials making up around 1% [2]. The major salivary glands account for approximately 93% of saliva volume, while the minor glands contribute the remaining 7%. Saliva's pH typically ranges from 5.3 to 7.8 [2]. Periodontitis is a chronic bacterial infection characterised by the loss of connective tissue and surrounding bone in the teeth, as well as the formation of periodontal pockets resulting from the apical migration

of the epithelial junction which leads to the formation of periodontal pockets and the loss of bone surrounding the teeth [3]. Bacteria play a significant role in the development of periodontitis, and the complex microbial community, along with the host's immune response, determines the outcome of the infection, making it challenging to define precise markers for the disease and to determine the level of infection among smokers. The virulence factors produced by bacteria directly degrade target tissues and induce the release of biological mediators from host tissue cells, leading to tissue destruction [4].

Host immune response generates cytokines, prostaglandins, and proteinases, which contribute to tissue destruction, along with various enzymes derived from bacteria such as collagenases, trypsin-like proteases, elastase-like enzymes, aminopeptidases, dipeptidyl peptidases, and other inflammatory mediators [5]. *S. mutans* is a common constituent of the human oral microbiota, alongside 25 different types of oral *streptococci* [6]. Different regions of the oral cavity harbour distinct biological characteristics, and each species has specific properties for colonizing different oral sites oral sites. *S. mutans* is predominantly found in pits and fissures, constituting 39% of the total streptococci in the oral cavity. On the buccal surface, the presence of *S. mutans* microorganisms is relatively lower, ranging from 2% to 9% [7]. Extensive research has been conducted on *S. mutans* due to its significant role in extracellular matrix production and its ability to rapidly form cariogenic biofilms in the presence of fermentable sugars in the diet. Among sugars, sucrose is considered the most cariogenic as it serves as an excellent substrate for the synthesis of extracellular (EPS) and intracellular [8].

The study involves the measurement of the enzymatic concentration at the lactate dehydrogenase, alkaline phosphatase, and arginase in saliva. The enzymes analysis in the salivary and gingival crevicular fluid can enhance the understanding of periodontitis aetiology and improve the accuracy of diagnosis. Lactate dehydrogenase (LDH) is a widely distributed enzyme found in the cytoplasm of nearly all human cells, catalysing the anaerobic reduction of pyruvate to lactate [9,10]. Alkaline phosphatase (ALP) is an intracellular glycoprotein enzyme produced throughout the periodontium and gingival crevice, secreted by polymorphonuclear neutrophils, osteoblasts, and periodontal ligament fibroblasts during bone inflammation, development, and periodontal regeneration, respectively [11,12].

Arginase (AR) is a predominantly liver-based enzyme involved in various physiological processes, such as protein and urea synthesis [13]. Cigarette smoking has been shown to cause an oxidative imbalance in the periodontium, leading to increased production of free radicals and oxidants, resulting in cell necrosis and tissue degradation. This smoking-induced tissue injury leads to the release of enzymes such as LDH, ALP, and AR, and subsequently, their elevation in saliva [10]. In addition, individuals with periodontitis have exhibited higher salivary levels of these enzymes compared to their healthy counterparts [12]. The current study aimed to assess the salivary levels of

LDH, ALP, and AR in both smokers and non-smokers, while also conducting molecular detection of *Streptococcus mutans* bacteria in patients with periodontitis. The study was conducted in the Microbiology Laboratory and the Central Unit Laboratory in the College of Medicine, as well as in the College of Dentistry.

Experimental

Study population

Seventy-five individuals, aged 18 to 60 years, participated in this study. The participants were divided into three groups: group A consisted of 25 smokers with periodontitis, group B included 25 non-smokers with periodontitis, and group C comprised 25 healthy non-smokers without periodontitis. The selection criteria for smokers included a minimum of 10 cigarettes per day for at least 10 years. Participants with chronic illnesses or recent medication use within the last 6 months were excluded. Ethical approval was obtained, and informed consent was obtained from all participants before their inclusion in the study.

Saliva sampling

Participants in the study were instructed to rinse their mouths thoroughly with water and chew on a standardized paraffin wax to stimulate saliva production. Saliva samples were collected every 60 seconds, resulting in a total volume of 1.5 ml for each sample.
A known sample size was determined using graduated test tubes. The collection process occurred at approximately 9 a.m. To remove any particulate matter, the samples were centrifuged at 15,000 g for 10 minutes at 4 °C. The samples were then stored at -20 °C until further analysis.

LDH estimation

Salivary LDH levels were assessed by measuring the conversion of reduced nicotinamide adenine dinucleotide (NADH) to oxidized NADH at 340 nm in the presence of sodium pyruvate. A 100 µL aliquot of saliva was mixed with a reaction mixture containing 0.05 M tris buffer (pH 7.4), 3.33 mM magnesium chloride, and 1.6 mM sodium pyruvate. After the addition of 100 µL of NADH (final concentration: 0.08 mM), the change in absorbance was monitored for 3 minutes using a U-2910 spectrophotometer [14-16].

ALP estimation

For the estimation of salivary ALP, ALP IFFCC mod. Kits® were utilized following the manufacturer's instructions (Huma Star Company, Germany). Salivary samples (20 µL) were mixed with the working reagent. The absorbance was recorded at regular intervals of time (e.g., every 30 seconds) at 405 nm using a colourimeter. The mean absorbance change per minute was calculated.

Estimation of AR

To determine the AR activity, the urea produced by the hydrolysis of L-arginine was measured spectrophotometrically using the Diaacetylomonoxime method. Under strongly acidic conditions, diacetylmonoxime reacts with the urea generated by arginase in the presence of ferric ion and thiosemicarbazide, resulting in the formation of a pink diacetyl complex that can be measured spectrophotometrically at 540 nm. The intensity of the colour complex was directly proportional to the arginase activity [17].

Isolation and identification of S. mutans

The saliva samples were homogenized for 2 minutes using a vortex mixer. To obtain tenfold serial dilutions, normal saline was used. Two dilutions were selected for each type of microorganism and inoculated onto Mitis Salivarius Bacitracin Agar (MSB agar), which is a selective medium for *streptococcus mutans*. From the dilutions (10^3-10^5), 0.1 mL of the sample was withdrawn and plated in duplicate

on MSB agar plates using a sterile microbiological spreader. The plates were then incubated anaerobically at 37 °C for 48 hours [18]. After quantifying the positive samples on the MSB medium, small colonies were subcultured onto the surface of blood-agar plates for further purification. These plates were incubated anaerobically at 37 °C for two days. The isolates were initially characterized based on the colony morphology on MSB agar, Gram staining, microscopic examination, catalase test, and carbohydrate fermentation.

DNA extraction and PCR analysis

To confirm the presence or absence of the 16S rRNA genes in the bacterial isolates, PCR (Polymerase Chain Reaction) was employed. Genomic DNA was extracted from *Streptococcus mutans* bacterial isolates using the Genomic DNA Mini Bacteria Kit, and specific primers for the detection of the 16S rRNA genes were designed by the Bioneer Company (Korea) as described by Miyoshi *et al.* (2005) [19]. The PCR reaction was carried out using 5µL of template DNA in a total volume of 20 µL, which included 1.5 µL of the forward primer, 1.5 µL of the reverse primer, and 12 µL of nuclease-free water. The PCR program consisted of 30 cycles of denaturation (95 °C for 30 seconds), annealing (58 °C for 30 seconds), and extension (72 °C for 1 minute), followed by a final extension step at 72 °C for 5 minutes to amplify the 16S rRNA gene. The presence of the amplified product was confirmed by running the PCR products on a 1% agarose gel using electrophoresis at 100 volts and 80 amperes for 1 hour. The PCR products were visualized using a UV transilluminator [20].

Statistical analysis

The data were expressed as mean ± standard deviation. To determine the statistical significance between the study groups and control groups, this group had healthy gums and was not infected with bacteria. The test result was performed and shows a significance level set at $p < 0.05$.

Results

The relative concentrations of salivary enzymes of groups in this study namely, Group A (smokers with periodontitis) exhibited the highest LDH level, with a mean LDH value of 323.21 ± 22.31 UI/L as presented in Table 1, which was significantly higher compared to group B (non-smokers with periodontitis) with a mean LDH value of 138.02 ± 13.22 UI/L, as well as group C (control) with a mean LDH value of 104.21 ± 12.33 UI/L ($p < 0.05$). Group B also showed significantly higher LDH values compared to group C (138.02 ± 13.22 UI/L vs. 104.21 ± 12.33 UI/L) at $p < 0.05$. Similar patterns were observed for ALP levels, where group A exhibited the highest ALP level with a mean ALP value of 67.77 ± 5.34 UI/L, significantly higher compared to group B (53.36 ± 4.24 UI/L) and group C (21.88 ± 1.79 UI/L) ($p < 0.05$). Additionally, group B showed significantly higher ALP values compared to group C (53.36 ± 4.24 UI/L vs. 21.88 ± 1.79 UI/L) at $p < 0.05$. Regarding AR levels, group A had significantly higher AR levels compared to groups B and C (21.58 ± 1.21 UI/L (group A) vs. 17.89 ± 1.35 UI/L (group B) and 21.58 ± 1.21 UI/L. (group A) vs. 15.31 ± 1.45 UI/L (group C). However, there was no statistically significant difference in AR levels between groups B and C ($p < 0.05$). The relative concentration of the salivary enzymes in the different study groups is graphically presented in Figure 1.

TABLE 1 Concentration of salivary LDH, ALP, and AR in the patients and Control Groups

	Study Groups*		
Salivary Enzymes *(UI/L)*	**Group A (mean ±SD)**	**Group B (mean ±SD)**	**Group C (mean ±SD)**
LDH	323.21 ± 22.31*#	138.02±13.22*	104.21±12.33
ALP	67.77±5.34*#	53.36±4.24*	21.88±1.79
AR	21.58±1.21*#	17.89±1.35	15.31±1.45

*indicates a statistically significant difference compared to group C (control) at $p < 0.05$

*#indicates a statistically significant difference compared to group B (non-smokers with periodontitis) at $p < 0.05$.

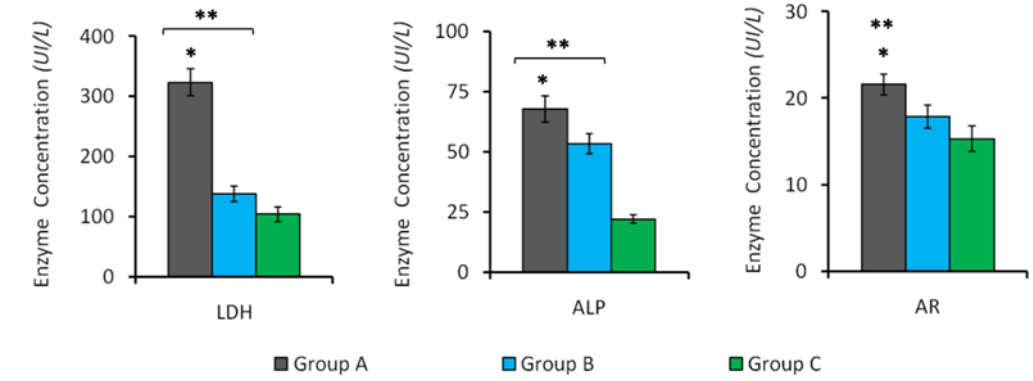

FIGURE 1 Mean concentration of salivary LDH, ALP, and AR among the study groups

**The analysis of salivary enzyme concentrations revealed that group A (smokers with periodontitis) exhibited the highest levels of LDH, ALP, and AR, followed by group B (non-smokers with periodontitis), while group C (non-smokers without periodontitis) had the lowest enzyme levels. Statistical analysis indicated that the differences between group A and group C were statistically significant ($p < 0.05$), denoted by **. Furthermore, the differences between group A and group B were also statistically significant ($p < 0.05$), indicated by*.*

In the samples collected from patients with periodontitis (group A and group B), *S. mutans* colonies were observed. These colonies exhibited colour variations, ranging from light blue, and their diameter was approximately 2-3 mm. The colonies appeared either round or irregular in shape with raised or curved surfaces, firmly attached to the medium. Some colonies displayed a rough or iridescent glass-like appearance, while others had a smooth surface. Many of the *S. mutans* colonies exhibited a depressed centre containing polysaccharide droplets, and in some cases, the entire colony was immersed in a pool of polysaccharides (for visual representation, see Figure 2). The existence of *Streptococcus mutans* on the MSB medium was taken from smokers with periodontitis.

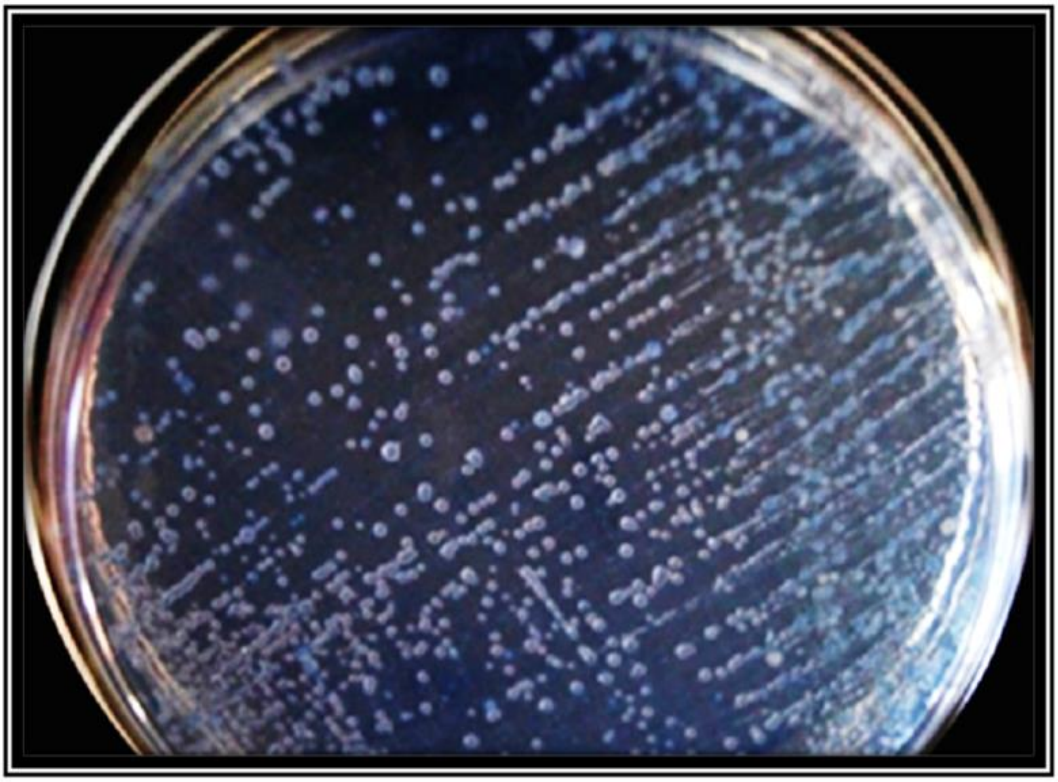

FIGURE 2 Colonies of bacteria Streptococcus mutans growth on MSB agar

As presented in Table 2, *Streptococcus mutans* was detected on the MSB medium in 20 patients (80%) in group A and 15 patients (60%) in group B. Other bacterial species were also identified in 5 patients (20%) in group A and 10 patients (40%) in group B**.**

Subsequently, DNA extraction and PCR analysis were performed on the bacteria isolated from the MSB medium to confirm the presence of the 16S rRNA gene. The results revealed the presence of a 1500 base pair (bp) band, indicating the presence of the gene, as demonstrated in Figure 3. The PCR analysis further demonstrated that 18 patients from group A (90% of the 20 patients with S. mutans in group A) and 12 patients from group B (80% of the 15 patients with S. mutans in group B) tested positive for the gene, Table 2

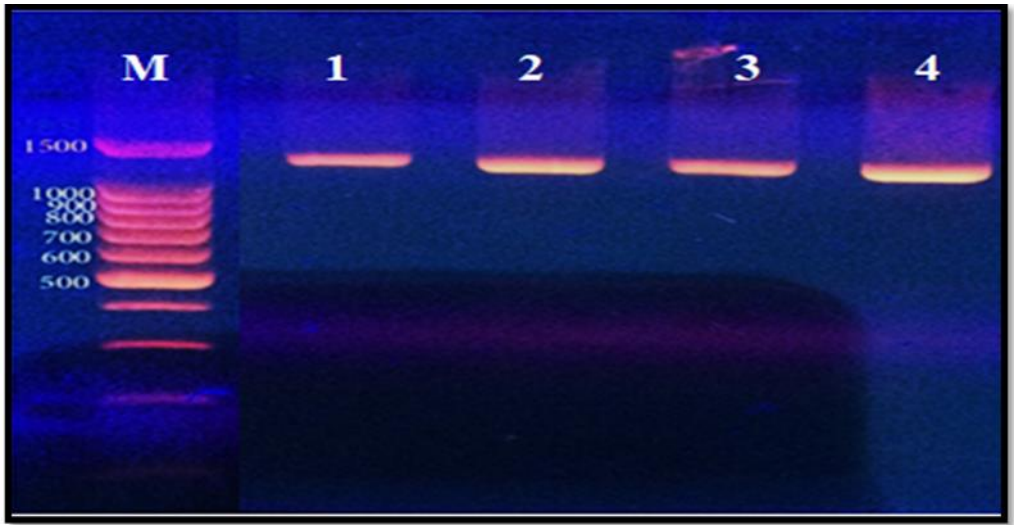

Figure 3 Agarose gel electrophoresis patterns show the amplified PCR product of 16Sr DNA. Lanes M: DNA marker (100 bp); Lanes 1-4: 16Sr DNA bands at 1500 bp

TABLE 2 Detection of Streptococcus mutans and confirmation using conventional PCR

	Group A n (%)	**Group B** n (%)
Isolated bacteria		
Streptococcus mutans	20 (80%)	15 (60%)
Others bacteria	5 (20%)	10 (40%)
Conventional PCR		
Positive PCR	18 (90%)	12 (80%)
Negative PCR	2 (10%)	3 (15%)

Discussion

The severity of periodontitis is influenced by various factors including microbial, immunological, ecological, and genetic risks, as well as age and sex. Smoking is responsible on 85% [21] a major source of risk factors that contribute to the occurrence and progression of periodontal disease. Moreover, non-smokers who are passive smokers are also more susceptible to developing oral and systemic disorders [22].

Saliva plays a crucial role in maintaining oral health. Alterations in its composition and production can hurt oral health and also enhance tooth decay. Dry mouth patients are prone to dental cavities and struggle to maintain proper oral hygiene. Interestingly, both saliva and Gingival Crevicular Fluid (GCF) have a dual role in preventing periodontal disease and promoting periodontal pathology [23]. LDH, a widely distributed enzyme, plays a significant role in clinical diagnostics for pathological processes. The high concentration of LDH in saliva indicated cellular necrosis can be utilised as a marker of the oral lesion affecting mucosa of oral, as presented in Table 1 [24]. The results showed increased salivary LDH, ALP and AR levels in smokers with periodontal and non-smokers with periodontal patients compared to healthy controls as indicated through a statistical study using SPSS assay. Our findings are consistent with a study that examined LDH activity in whole saliva of individuals with and without periodontal disease. The study revealed elevated LDH levels in smokers and passive smokers with periodontal disease compared to healthy individuals, suggesting LDH is a potential epidemiological biomarker for this condition [25].

ALP, an intracellular enzyme released from neutrophil granules, significantly increases with inflammation and plaque accumulation. The presence of periodontitis may be a consequence of destructive processes in the alveolar bone and metabolic changes in inflamed gingival tissues. High levels of ALP indicate increased cellular damage, and its concentration is elevated in both smokers and non-smokers with periodontitis [26,27]. In the present study, the levels of salivary AR were found to be elevated in both smoking and non-smoking patients with periodontitis, compared to the healthy control group. This observation supports previous research findings [28,29] that also reported increased salivary AR levels in individuals with the disease. The pathogenesis of periodontal diseases can manifest in various ways. One common cause of periodontitis is the accumulation of a bacterial biofilm on the tooth's surface, which triggers an excessive inflammatory response [30]. Dental caries and decay, on the other hand, are primarily caused by *Streptococcus mutans,* as listed in Table 2, besides some other parameters that allowed *S. mutans* to thrive [31].

The presence of sucrose promotes the production of insoluble glucans by *S. mutans*, leading to the formation of dental plaque or oral biofilm and initiating the caries process

[32]. Our findings support the hypothesis proposed by multiple authors that alterations in oral microbiota interspecies competition occur before visible tooth lesions [33]. These results were further substantiated by a local study conducted by Chayan, Sekhi *et al.* (2021) [34], which reported a higher prevalence of *S. mutans* isolates. Numerous studies have consistently identified *S. mutans* as the primary etiological bacterium responsible for human dental caries and the formation of biofilms on the hard tissues of the oral cavity [35].

Conclusion

Periodontitis is a common oral disorder that can have significant implications for both oral and overall health. Bacterial infection, particularly by *S. mutans*, has been identified as the primary cause of this disease. *S. mutans* possesses several unique virulence factors that contribute to its role in the development of periodontitis. Early detection and assessment of periodontal health are crucial for preventing disease progression. Our study findings demonstrated elevated levels of salivary flow enzymes, including LDH, ALP, and AR, in both smokers and non-smokers with periodontitis compared to healthy individuals. These enzymes serve as reliable markers for evaluating the status of periodontal health and can indicate the presence of tissue damage or defects in the oral cavity.

Acknowledgements

The authors would like to extend their thanks to the University of Basrah.

Funding

This work is not funded by any institution.

Authors' Contributions

Hazim & Rana: data collection

Abdul Amir: Article formulation and approval

Ahmed: Graphic design

Conflict of Interest

All authors declare that there is no conflict of interest in this study.

Consent for Publication

The authors consent to the publication of the article.

Orcid:

Hazim Saad:
https://orcid.org/0000-0003-0779-2023
Rana Adnan:
https://orcid.org/0000-0002-3119-3154
Abdul Amir H. Kadhum:
https://orcid.org/0000-0003-4074-9123
Ahmed A. Al-Amiery:
https://orcid.org/0000-0003-1033-4904

References

[1] S. Fatima, A. Rehman, K. Shah, M. Kamran, S. Mashal, S. Rustam, M. Sabir, A. Nayab, M. Muzammal, Composition and function of saliva: A review, *World J. Pharm. Pharm. Sci*, **2020**, *9*, 1552-1567. [Crossref], [Google Scholar], [Publisher]

[2] N.S. Abdul, S.M. AlGhannam, A.A. Almughaiseeb, F.A. Bindawoad, S.M. Alduraibi, M. Shenoy, A review on salivary constituents and their role in diagnostics, *Bioinformation*, **2022**, 18, 1021–1028. [Crossref], [Google Scholar], [Publisher]

[3] D. Plančak, L. Musić, I. Puhar, Quorum sensing of periodontal pathogens, *Acta Stomatologica Croatica*, **2015**, *49*, 234. [Crossref], [Google Scholar], [Publisher]

[4] L. Sedghi, V. DiMassa, A. Harrington, S.V. Lynch, Y.L. Kapila, The oral microbiome: Role of key organisms and complex networks in oral health and disease, *Periodontology,* **2021**, *2000*, 107-131. [Crossref], [Google Scholar], [Publisher]

[5] K. Rao, S.G. Babu, U.A. Shetty, R.L. Castelino, S.R. Shetty, Serum and salivary lactate

dehydrogenase levels as biomarkers of tissue damage among cigarette smokers, A biochemical study, *Stomatologija*, **2017**, *19*, 91-96. [Google Scholar], [Publisher]

[6] V.P. Richards, S.R. Palmer, P.D. Pavinski Bitar, X. Qin, G.M. Weinstock, S.K. Highlander, C.D. Town, R.A. Burne, M.J. Stanhope, Phylogenomics and the dynamic genome evolution of the genus Streptococcus, *Genome Biology and Evolution*, **2014**, *6*, 741-753. [Crossref], [Google Scholar], [Publisher]

[7] C.M. Bedoya-Correa, R.J.R. Rodríguez, M.T. Parada-Sanchez, Genomic and phenotypic diversity of Streptococcus mutans, *Journal of Oral Biosciences*, **2019**, *61*, 22-31. [Crossref], [Google Scholar], [Publisher]

[8] M.D. Ferrer, S. Pérez, A.L. Lopez, J.L. Sanz, M. Melo, C. Llena, A. Mira, Evaluation of clinical, biochemical and microbiological markers related to dental caries, *International Journal of Environmental Research and Public Health*, **2021**, *18*, 6049. [Crossref], [Google Scholar], [Publisher]

[9] C.K. Naresh, S.M. Rao, P.R. Shetty, V. Ranganath, A.S. Patil, A.J. Anu, Salivary antioxidant enzymes and lipid peroxidation product malondialdehyde and sialic acid levels among smokers and non-smokers with chronic periodontitis—A clinico-biochemical study, *Journal of Family Medicine and Primary Care*, **2019**, *8*, 2960-2964 [Crossref], [Google Scholar], [Publisher]

[10] A.K. Pandarathodiyil, A. Ramanathan, R. Garg, J.G. Doss, F.B. Abd Rahman, W.M.N. Ghani, S. Warnakulasuriya, Lactate dehydrogenase levels in the saliva of cigarette and E-cigarette smokers (vapers): a comparative analysis, *Asian Pacific Journal of Cancer Prevention: APJCP*, **2021**, *22*, 3227. [Crossref], [Google Scholar], [Publisher]

[11] K.H. Fathima, V.S. Harish, Evaluation of alkaline phosphatase in gingival crevicular fluid among chronic periodontitis patients with smoking habit, *Journal of Advanced Medical and Dental Sciences Research*, **2019**, *7*, 163-167. [Crossref], [Google Scholar], [Publisher]

[12] A. Jain, D. Jain, S. Jain, S. Jaiswal, R.K. Goyal, Evaluation of salivary alkaline phosphatase levels in tobacco users to determine its role as a biomarker in oral potentially malignant disorders, *International Journal of Health Sciences*, (II), **2022**, 12443-12449. [Crossref], [Google Scholar], [Publisher]

[13] T.R. Menaka, G. Vasupradha, S.S. Ravikumar, K. Dhivya, J. Dinakaran, V. Saranya, Evaluation of salivary alkaline phosphatase levels in tobacco users to determine its role as a biomarker in oral potentially malignant disorders, *Journal of Oral and Maxillofacial Pathology*, **2019**, *23*, 344-348. [Crossref], [Google Scholar], [Publisher]

[14] S. Hegde, C. Nunes, K. Harini, S. Babu, S. Kumari, V. Ajila, Estimation of salivary arginase levels in smokers and nonsmokers with chronic periodontitis: A biochemical study, *Dent Oral Craniofac Res*, **2015**, *1*, 15-8. [Crossref], [Google Scholar], [Publisher]

[15] M. Sivaramakrishnan, B. Sivapathasundharam, M. Jananni, Evaluation of lactate dehydrogenase enzyme activity in saliva and serum of oral submucous fibrosis patients, *Journal of Oral Pathology & Medicine*, **2015**, *44*, 449-452. [Crossref], [Google Scholar], [Publisher]

[16] S.A. Ali, R.L. Telgi, A. Tirth, I.Q. Tantry, A. Aleem, Lactate Dehydrogenase and β-glucuronidase as salivary biochemical markers of periodontitis among smokers and non-smokers, *Sultan Qaboos University Medical Journal*, **2018**, *18*, 318. [Crossref], [Google Scholar], [Publisher]

[17] M. Urbanowicz, K. Sadowska, A. Paziewska-Nowak, A. Sołdatowska, D.G. Pijanowska, Biosensor based on coupled enzyme reactions for determination of arginase activity, *Bioelectrochemistry*, **2022**, *146*, 108137. [Crossref], [Google Scholar], [Publisher]

[18] M.S. Hossain, S. Alam, Y.M. Nibir, T.A. Tusty, S.M. Bulbul, M. Islam, M.S. Hossain, Genotypic and phenotypic characterization of Streptococcus mutans strains isolated from patients with dental caries, *Iranian Journal of*

Microbiology, **2021**, *13*, 449. [Crossref], [Google Scholar], [Publisher]
[19] T. Miyoshi, T. Iwatsuki, T. Naganuma, Phylogenetic characterization of 16S rRNA gene clones from deep-groundwater microorganisms that pass through 0.2-micrometer-pore-size filters, *Applied and Environmental Microbiology*, **2005**, *71*, 1084-1088. [Crossref], [Google Scholar], [Publisher]
[20] S.R. Al-Farhan, A.A. AL-Abdullah, A.A. Al-Moussawi, Isolation and diagnosis of anaerobic bacteria of periodontitis by molecular methods in diabetic and non-diabetic patients in Basra Province/Iraq, *Scientific Journal of Medical Research*, **2019**, *3*, 53-63. [Google Scholar], [Publisher]
[21] P.M. Duarte, C.F.P. Nogueira, S.M. Silva, C.M. Pannuti, K.C. Schey, T.S. Miranda, Impact of smoking cessation on periodontal tissues, *International Dental Journal*, **2022**, *72*, 31-36. [Crossref], [Google Scholar], [Publisher]
[22] B. Kanmaz, G. Lamont, G. Danacı, H. Gogeneni, N. Buduneli, D.A. Scott, Microbiological and biochemical findings in relation to clinical periodontal status in active smokers, non-smokers and passive smokers, *Tobacco Induced Diseases*, **2019**, *17*, 1-6. [Crossref], [Google Scholar], [Publisher]
[23] R.M.R.D. Patel, S. VaRMa, G.R.S. SuRaGiMath, S.M.R. ZoPe, Estimation and comparison of salivary calcium, phosphorous, alkaline phosphatase and pH levels in periodontal health and disease: A cross-sectional biochemical study, *Journal of Clinical and Diagnostic Research*, **2016**, *10*, ZC58-61. [Crossref], [Google Scholar], [Publisher]
[24] B. Kumar, N. Kashyap, A. Avinash, R. Chevvuri, M.K. Sagar, K. Shrikant, The composition, function and role of saliva in maintaining oral health: A review, *Proteins*, **2017**, *220*, 140-640. [Crossref], [Google Scholar], [Publisher]
[25] K. Abdel Kader, S. Elhabbak, M. Assadawy, Evaluation of some salivary enzymes as a diagnostic biomarker for progression of periodontal disease, *Al-Azhar Journal of Dental Science*, **2021**, *24*, 175-186. [Crossref], [Google Scholar], [Publisher]
[26] A. De, R. Puttannavar, F. Rahman, A. Adak, R. Sahoo, B.R. Prakash, Estimation of salivary and serum alkaline phosphatase level as a diagnostic marker in type-2 diabetes mellitus with periodontal health and disease: a clinico-biochemical study, *Journal of Oral and Maxillofacial Pathology*, **2018**, *22*, 445. [Crossref], [Google Scholar], [Publisher]
[27] N. Risteska, B. Poposki, K. Ivanovski, K. Dirjanska, S. Ristoska, M. Saveski, Diagnostic and prognostic markers of periodontal disease, *prilozi*, **2021**, *42*, 89-95. [Crossref], [Google Scholar], [Publisher]
[28] P.G. Stathopoulou, N. Buduneli, D.F. Kinane, Systemic biomarkers for periodontitis, *Current Oral Health Reports*, **2015**, *2*, 218-226. [Google Scholar], [Publisher]
[29] S. Podzimek, L. Vondrackova, J. Duskova, T. Janatova, Z. Broukal, Salivary markers for periodontal and general diseases, *Disease Markers*, **2016**, *2016*. [Crossref], [Google Scholar], [Publisher]
]٣٠[L.K. Bakkir, The antibacterial activity of green coffee and Arabica coffee extracts on cariogenic Streptococcus mutans isolated from dental caries: An in vitro study, *University of Thi-Qar Journal of Science*, **2017**, *6*, 70-74. [Google Scholar], [Publisher]
[31] J.A. Lemos, S.R. Palmer, L. Zeng, Z.T. Wen, J.K. Kajfasz, I.A. Freires, J. Abranches, L.J. Brady, The biology of Streptococcus mutans, *Microbiology Sectrum*, **2019**, *7*, 1110-1128. [Crossref], [Google Scholar], [Publisher]
[32] Z. Saeed, B. Abbas, R. Othman, The effect of Lactobacillus plantarum on expression of biofilm genes of Streptococcus mutans isolated from dental caries, *The Medical Journal of Basrah University*, **2019**, *37*, 91-99. [Google Scholar], [Publisher]
[33] R.J. Lamont, H. Koo, G. Hajishengallis, The oral microbiota: dynamic communities and host interactions, *Nature Reviews Microbiology*, **2018**, *16*, 745-759. [Google Scholar], [Publisher]

[34] M.A. Chayan, R.J. Sekhi, A.A.N. Amiry, Molecular diagnosis of S. Mutans isolated from dental caries in Thi-Qar Province, *Annals of the Romanian Society for Cell Biology*, **2021**, 2927-2931. [Google Scholar], [Publisher]

[35] H.S. Abd Al-Zahra, M.B. Saleh, Isolation and identification of Streptococcus mutans from dental caries patients at Thi-Qar province/Iraq, *Univ Thi-Qar J Sci*, **2018**, *6*, 22-7. [Google Scholar], [Publisher]

How to cite this article: Rana Adnan Fayez Al-jaberi , Hazim Saad Jabbar Al -Maliki , Abdul Amir H. Kadhum, Ahmed A. Al-Amiery, Analysis of salivary levels of lactate dehydrogenase, alkaline phosphatase and arginase as well as detection of streptococcus mutans in smoking and non-smoking periodontitis patients. *Journal of Medicinal and Pharmaceutical Chemistry Research*, 2024, 6(10), 1547-1557. **Link:** https://jmpcr.samipubco.com/article_195510.html