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ORIGINAL ARTICLE

Salivary lactate dehydrogenase and salivary total protein as potential biomarkers for screening periodontal disease

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Abstract *Background:* Timely diagnosis of periodontal disease is crucial for restoring healthy periodontal tissue and improving patients' prognosis. There is a growing interest in using salivary biomarkers as a noninvasive screening tool for periodontal disease. This study aimed to investigate the diagnostic efficacy of two salivary biomarkers, lactate dehydrogenase (LDH) and total protein, for periodontal disease by assessing their sensitivity in relation to clinical periodontal parameters. Furthermore, the study aimed to explore the impact of systemic disease, age, and sex on the accuracy of these biomarkers in the diagnosis of periodontal health.

Materials and methods: A total of 145 participants were categorized into three groups based on their basic periodontal examination index, with 20 in the periodontally healthy group, 50 in the gingivitis group, and 75 in the periodontitis group. Salivary LDH was measured using the rate of nicotinamide adenine dinucleotide (NADH) oxidation, to measure the kinetics of LDH activity, while total protein was measured using the Lowry method. Descriptive and analytical statistical analyses were performed to examine the associations between the variables and biomarkers.

Results: The results of the study demonstrated that salivary LDH was 72% sensitive, while salivary total protein was 78% sensitive in correlation to clinical periodontal parameters. The accuracy of the test was not influenced by sex, but age had a significant effect on both biomarkers, particularly LDH. Systemic disease was another factor that significantly affected the accuracy of the test.

Conclusions: Although salivary LDH and total protein show promise as biomarkers for screening periodontal disease, their interpretation may be impacted by age and systemic disease. To

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enhance the accuracy of periodontal disease detection, the study suggests combining these biomarkers with more specific indicators and diagnostic tools.

1. Introduction

Periodontal disease is one of the most prevalent oral diseases among the middle-aged and elderly populations (Gul et al., 2020). With advances in clinical knowledge and techniques, early detection can prevent the progression of periodontal disease. Therefore, periodontal screening may help prevent periodontal disease and improve oral health-related quality of life (Kim et al., 2013). Periodontal disease involves both bacterial and host inflammatory mediators. Pathogens induce the production of biologically active molecules and stimulate host inflammatory mediators leading to tissue and bone destruction (Isola, 2022).

In recent years, there has been a trend toward screening for systemic diseases using human body fluids, such as blood, cerebrospinal fluid, saliva, urine, sputum, peritoneal fluid, and pleural fluid, which have been approved for definitive diagnosis and disease state maintenance(Giannobile, 2012). Saliva is one of the body's fluids secreted by the major and minor salivary glands. In periodontal diseases, the marginal and sulcular gingivae act as a frontline area where the interaction between bacteria and the host immune response occurs by aggregation, adherence, cell-killing, and inhibition of microbial metabolism (Zin et al., 2021). These interactions may be maintained under oral ecological conditions (Scannapieco, 1994). It is hypothesized that quantitative variations in the salivary protein concentration affect the prevalence of oral diseases(Zin et al., 2021).

Albumin acts as a serum ultrafiltrate in the oral cavity (Shaila et al., 2013). Salivary protein concentrations can serve as markers of plasma protein leakage resulting from inflammation (Shaila et al., 2013). Lactate dehydrogenase (LDH), a stable cytoplasmic enzyme found in all cells, is another salivary biomarker in addition to total protein. LDH is rapidly released into the cell culture supernatant when the plasma membrane is damaged, a key feature of cells undergoing apoptosis, necrosis, or other forms of cellular damage (Miyoshi et al., 2018). LDH activity can be easily quantified using nicotinamide adenine dinucleotide (NADH) produced during the conversion of lactate to pyruvate. The amount of LDH in saliva is directly proportional to the number of dead or damaged cells (Azizi et al., 2011).

This study aimed to investigate the diagnostic efficacy of salivary lactate dehydrogenase (LDH) and total protein, for periodontal disease and the effect of systemic disease, age, and sex on the accuracy of these biomarkers.

2. Materials and methods

The study enrolled 145 individuals with an age range of 19–73 years and a mean age of 37 years Standard deviation (SD) = 14.109. The sex distribution was 55.2% female and 44.8% male. The participants were classified into three groups based on their periodontal health status: periodontally healthy control group (n = 20), gingivitis group (n = 50), and periodontitis group (n = 75), using the basic periodontal examination index (BPE) as defined by the new classification system (Chapple et al., 2018).

This observational case-control study was conducted among patients attending public health centers between February 2022 and July 2022. Inclusion criteria were as follows: participants who had not used antibiotics or anti-inflammatory drugs in the past month. Exclusion criteria included the following: individuals who had eaten, drank, brushed their teeth, or gargled within 1–2 h before saliva collection, because these factors could impact the results. Participants undergoing periodontal treatment, pregnant or lactating women, and smokers were also excluded from the study.

Prior to clinical evaluation, saliva samples were collected from the patients between 8:00 a.m. and 11:00 a.m. (Segawa et al., 2019). Each participant provided 3–5 ml of unstimulated saliva by spitting into a sterile container, which took approximately 5–10 min.

The samples were stored in a cooler box and assigned code numbers for analysis. Clinical examination was performed using the BPE index (Chapple et al., 2018). Probing depth (PD) and bleeding on probing were measured using World Health Organization (WHO)-standard probes.

The following BPE codes were used to diagnose periodontal disease:

Code 0: Periodontally healthy.
Codes 1 and 2: Gingivitis.
Codes 3 and 4: Periodontitis.

The saliva samples were centrifuged for 10 min at 1000 rpm. One milliliter of supernatant saliva was stored at −20 °C for total protein determination, and 1 ml was stored at 4 °C for LDH determination within the same week of collection (de la Peña et al., 2004; Zin et al., 2021).

LDH levels were determined using the method suggested by the French Society of Clinical Biology (SFBC) (Giuliani et al., 2019). This technique measures LDH activity kinetics by assessing the rate of NADH oxidation.

Commercial kits (Spinreact's Quantitative LDH in vitro diagnostic (IVD) kits) were used to conduct the LDH test. The working reagent was prepared and combined with 1 ml of the saliva supernatant. After incubation for 1 min, the mixture was transferred to a 1 cm light path cuvette, and the absorbance was measured at 340 nm. Mathematical calculations followed the manufacturer's guidelines, and the results were reported as U/L.

Lowry method, which relies on the Folin phenol reagent for protein measurement, was employed for total protein determination(Waterborg, 2009). Spinreact's Quantitative determination of total urinary and cerebrospinal (CSF) protein IVD kits were used. The colorimetric method was utilized: 200 µl of saliva sample was first mixed with 1 ml of the working reagent. After a 10-min incubation, the mixture was

transferred to a 1 cm light path cuvette, and the absorbance was measured at 598 nm. The protein concentration was calculated using the manufacturer's guidelines, and the results were expressed as mg/mL.

Sample size calculations were performed using G*Power 3.1.9.2 software, assuming equal SD in the three groups according to a study by Nomura et al., 2006 considering one-way analysis of variance (ANOVA) with an effect size of 0.25, statistical power of 80%, and a significance level of 95% ($\alpha < 0.05$). Based on these parameters, a minimum of 20 participants were required for each group.

Data analysis was performed using the SPSS statistical software (version 21.0; SPSS Inc., Chicago, IL, USA). Descriptive statistics, such as mean and SD, were used to describe variables, including sex, age group, systemic health status, and the distribution of LDH and total protein among the healthy, gingivitis, and periodontitis groups.

To assess the differences between the three groups, one-way ANOVA tests were conducted. Independent t-tests were used to examine the correlation between LDH or total protein levels and sex. ANOVA was employed to compare LDH and total protein levels across age groups and health status. Pairwise comparisons between age groups were conducted using Scheffe's test to identify significant differences.

Receiver operating characteristic (ROC) analyses were performed to evaluate the performance of LDH and total protein levels. The sensitivities of LDH and total protein were estimated using the area under the curve (AUC). Statistical significance was defined as $p < 0.05$.

3. Results

A total of 145 participants were included in this study. The demographic characteristics of the participants are shown in Table 1.

A comparison of the groups in terms of age showed a statistically significant difference among the periodontally healthy, gingivitis, and periodontitis groups ($p < 0.05$), with the periodontitis group having a higher mean age than the healthy periodontal and gingivitis groups. However, there was no significant difference in sex distribution among the groups ($p > 0.05$), indicating a relatively equal representation of males and females across all the three groups. Of the participants, 118 had no medical history of systemic diseases, while 27 had medical conditions and were undergoing treatment. The most common categories of medical conditions were diabetes and hypertension (12), followed by hypertension alone (10), diabetes (4), and thalassemia (1).

The one-way ANOVA analysis of LDH and total protein indicated a significant difference among the three groups for LDH ($F(2, 142) =$ F-value 53.562, $p < 0.05$) and total protein ($F(2, 142) =$ F-value, 24.548, $p < 0.05$); Figs. 1A and 1B show the mean levels for each group. For both biomarkers, post-hoc pairwise comparisons revealed significant differences between the periodontally healthy and gingivitis groups ($p < 0.05$), as well as between the periodontally healthy and periodontitis groups ($p < 0.05$). Additionally, a significant difference was observed between the gingivitis and periodontitis groups ($p < 0.05$).

There was no evidence to suggest that sex influenced LDH or total protein levels, as listed in Table 2. However, age had a strong effect on both the biomarkers (Table 3). Post-hoc analysis revealed a significant association between age group and salivary biomarkers ($p < 0.05$). In pairwise comparisons, older individuals had significantly higher LDH levels than young ($p < 0.05$) and middle-aged adults ($p < 0.05$). Additionally, total protein levels increased significantly with age, with older participants exhibiting significantly higher levels compared to young adults ($p < 0.05$).

This study also investigated the effect of systemic disease on both LDH and total protein levels. A significant correlation was found between systemic disease and these salivary biomarkers, as shown in Table 4.

Using the ROC curve, the sensitivity of LDH as a diagnostic test was found to be 72%, whereas the sensitivity of the total protein test was 78%.

4. Discussion

Saliva, an easily collected and noninvasive sample, contains valuable markers of periodontal disease derived from systemic and local sources (Saliem, 2016). Biomarkers, including inorganic and immunological substances, play crucial roles in oral cavity defense and can be used for screening, diagnosis, and disease monitoring (Mohammed et al., 2022).

In this study, LDH and total protein levels were assessed using straightforward colorimetric biochemical techniques that are widely used in various scientific fields because of their versatility, quantitative capabilities, speed, cost-effectiveness, and ease of use. However, they have limitations, such as low sensitivity, inability to analyze colorless compounds, and potential errors from interfering materials with similar colors (Hosseini et al., 2021).

Numerous studies have extensively explored the presence of LDH in saliva, covering various aspects. Studies investigated its association with oral squamous cell carcinoma (Kallalli et al., 2016; Merza et al., 2010). Another study investigated the relationship between LDH levels and gingivitis in patients with diabetes(Chidzhavadze et al., 2006). Studies have further investigated the impact of smoking and the presence of LDH on periodontal health(Ali et al., 2018; Risteska et al., 2021). Moreover, a previous study evaluated the health of the peri-

Table 1 Demographic variables of the study groups.

Groups	Number of participants	Age (years)		Sex	
		Range	**Mean**	**Male**	**Female**
Periodontally healthy	20	19–50	31.28	7	13
Gingivitis	50	18–50	29.28	25	25
Periodontitis	75	25–73	47.94	33	42

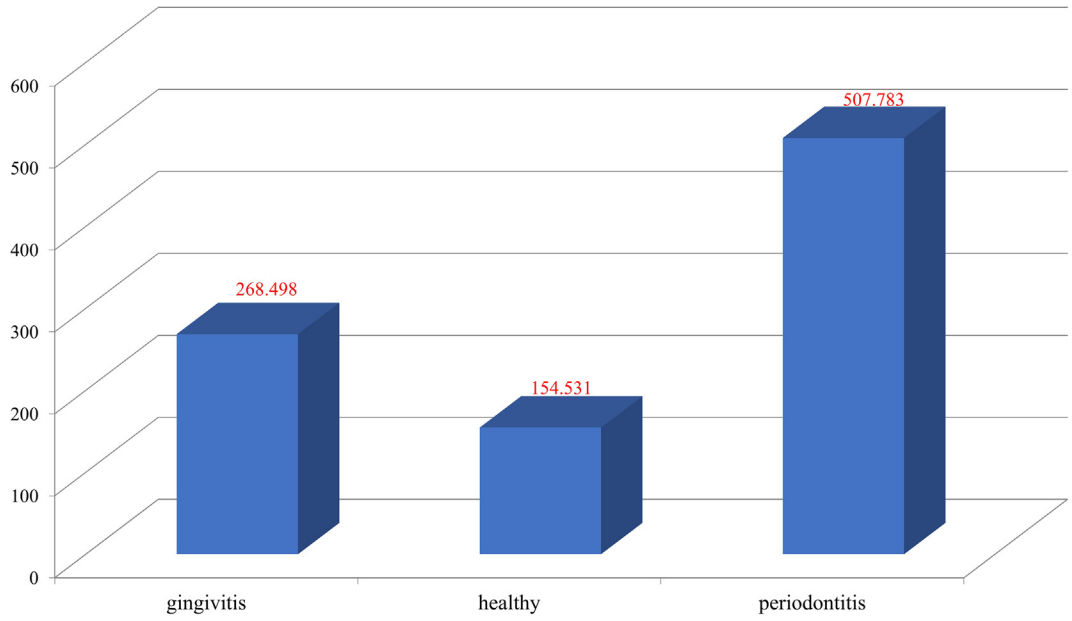

Fig. 1A The mean levels of lactate dehydrogenase (LDH) in saliva among participants categorized into three groups: periodontally healthy, gingivitis, and periodontitis groups.

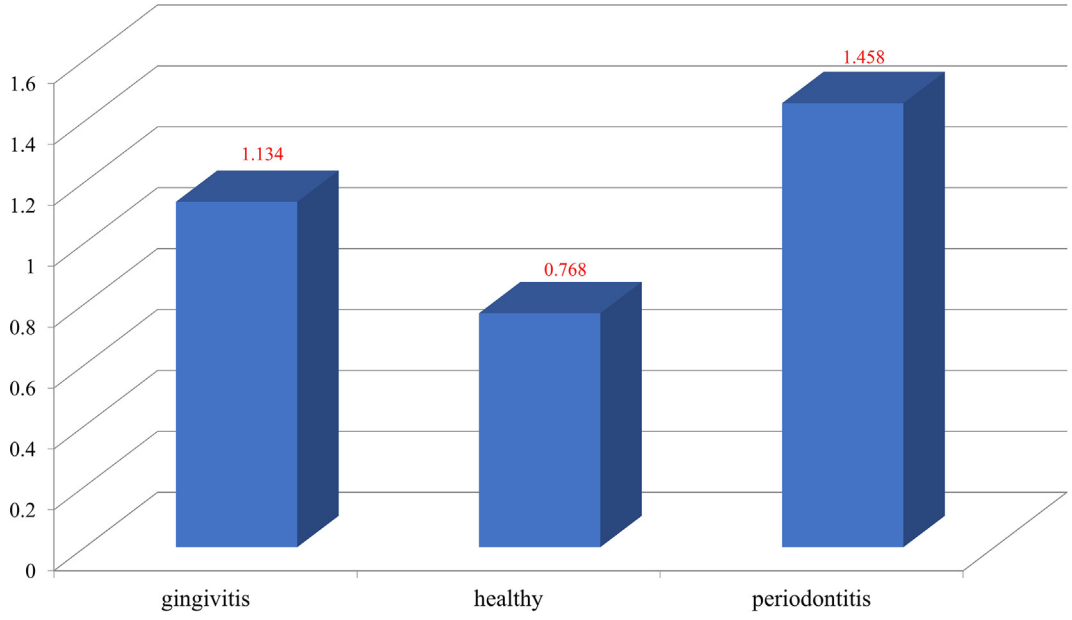

Fig. 1B The mean levels of total protein in saliva among participants categorized into three groups: periodontally healthy, gingivitis, and periodontitis groups.

Table 2 The effect of sex on LDH and total protein levels.

	Sex	N	Mean	Standard Deviation	T-value	p-value
LDH level	Male	63	378.522	218.935	0.155	0.877
	Female	82	372.969	217.463		
Total protein level	Male	63	1.328	0.488	1.748000	0.082
	Female	82	1.189	0.483		

Table 3 The effect of age on LDH and total protein levels.

	Age (years)	N	Mean	Standard Deviation	F-value	p-value
LDH level	15–29	43	287.21734	152.841614	25.969000	0.000*
	30–44	50	300.97148	164.328681		
	45–59	34	444.86903	221.150110		
	≥ 60	18	677.11689	176.667434		
	Total	145	375.37539	217.385099		
Total protein level	15–29	43	1.12066	0.424973	4.138000	0.008*
	30–44	50	1.20488	0.426490		
	45–59	34	1.32743	0.482605		
	≥ 60	18	1.55622	0.669275		
	Total	145	1.24925	0.488388		

* The mean difference is significant at the 0.05 level.

Table 4 The effect of systemic diseases on LDH and total protein levels.

		N	Mean	Standard Deviation	F-value	p-value
LDHlevel	Diabetes	4	428.664	255.447	13.472000	0.000*
	Hypertension	10	665.178	271.201		
	Hypertension and diabetes	12	625.626	123.083		
	Uninfected	118	325.582	183.523		
	Thalassemia	1	385.792			
	Total	145	375.375	217.385		
Total protein level	Diabetes	4	1.410	0.407	3.675000	0.007*
	Hypertension	10	1.286	0.506		
	Hypertension and diabetes	12	1.711	0.684		
	Uninfected	118	1.192	0.447		
	Thalassemia	1	1.692			
	Total	145	1.249	0.488		

* The mean difference is significant at the 0.05 level.

odontium by measuring total protein levels in saliva without considering the potential factors that could affect protein levels(Zin et al., 2021). However, the current study sets itself apart by examining the combined effect of cofactors on the levels of LDH and total protein in saliva. Furthermore, we investigated how these cofactors influence the diagnostic potential of the biomarkers under investigation.

The present study supports the use of salivary LDH as a screening biomarker for periodontal health, which is consistent with previous studies (Ansari Moghadam et al., 2022; Nomura et al., 2016 Miyoshi et al., 2018). Based on the ROC curves, we determined that AUC for LDH was 0.72. This finding indicates that LDH holds promise as a potential biomarker for periodontal disease. Our study results confirmed the influence of age as a cofactor affecting salivary LDH levels. This finding is consistent with that of a recent study, which indicated that LDH levels vary with age (Farhana and Lappin, 2022). These observations may be attributed to natural changes in salivary glands and saliva composition that occur with age, potentially affecting the levels of enzymes such as LDH in saliva.

Another significant finding of this study was the impact of systemic diseases on salivary LDH levels. This result can probably be attributed to the increased levels of LDH in response to tissue damage or disease (De La Peña et al., 2007). Systemic conditions can influence LDH levels in saliva, potentially indicating oral cavity damage or systemic disorders affecting salivary gland function (De La Peña et al., 2007). Interestingly, this finding aligns with those of previous studies that proposed different LDH levels in patients with diabetes (Chidzhavadze et al., 2006) and that identified LDH as a diagnostic marker for oral squamous carcinoma (Kallalli et al., 2016).

This study revealed a significant correlation between periodontal health and salivary total protein level, the AUC for total protein was determined to be 0.78, indicating a high level of accuracy. This suggests that total protein has the potential to serve as a biomarker for periodontal disease. The increase in salivary total protein is primarily due to the immune response and inflammatory processes that occur in periodontal tissues (Shaila et al., 2013). Several factors contribute to the higher levels of salivary total protein in individuals with periodontitis, gingival inflammation increases vascular permeability, and protein exudates from tissue breakdown products can contribute to an overall increase in salivary total protein levels (Zin et al., 2021).

These findings are consistent with those of a study conducted by Wakde et al., 2018 who also observed significantly higher salivary total protein levels in the gingivitis and periodontitis groups compared to periodontally healthy individuals. Furthermore, our results are consistent with those obtained by Shaila et al., 2013, Karthiga et al., 2017, and Henskens et al., 1993. Additionally, a study by Zin et al., 2021 found that patients with chronic gingivitis and chronic periodontitis had total protein contents that were 1.6 and 4.2 times higher, respectively, than periodontally healthy controls. However, it is worth noting that the salivary total protein levels reported in previous studies, including ours, vary

considerably. This variation may be influenced by factors such as protein detection techniques, reagents used, racial differences, and genetic variations in the salivary glands.

Age is an important factor that influences salivary total protein levels. The current study sheds light on the significant relationship between salivary total protein and age and its implications for assessing periodontal health. Several factors could explain this observation. Age-related changes in salivary glands, saliva flow rate, and oral microbial flora are potential cofactors affecting salivary total protein(Xu et al., 2019).

Additionally, salivary total protein exhibits a significant relationship with systemic diseases such as diabetes, cardiovascular disease, and autoimmune disorders (Hu et al., 2007). Elevated levels of salivary total protein in individuals with systemic diseases suggest potential tissue damage or inflammation, possibly due to plasma protein leakage caused by cellular damage (Lawrence, 2002).

One limitation of this study was the small sample size for each systemic disease, which may not have been sufficient to accurately assess the effect of each systemic disease on biomarkers in relation to periodontal health status.

5. Conclusions

The salivary biomarkers LDH and total protein show promise as screening biomarkers for periodontal disease. However, it is important to note that these biomarkers should be utilized alongside other salivary biomarkers. This is because their levels can be influenced by various cofactors such as age and systemic disease, which can impact their reliability, as evidenced in this study. Therefore, further research is recommended.

Ethical statement

This research was authorized by the Medical Ethical Committee of the University of Baghdad's College of Dentistry, with Ref. number: 641, date 4/8/2022, project No. (641622). Informed consent was obtained from patients for participating in the study.

CRediT authorship contribution statement

Rasha Rawdhah: Methodology, Data curation, Writing - original draft. **Maha Sh Mahmood:** Project administration, Supervision, Writing – review & editing.

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