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Comparison of Whole Salivary Lactate Dehydrogenase Level in Patients With and Without Periodontal Disease

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Abstract

Background: Tracking various biomarkers in serum, gingival crevicular fluid (GCF), and saliva has been introduced as a diagnostic tool for periodontal disease detection.
Objectives: The aim of this study was to compare salivary lactate dehydrogenase (LDH) levels in subjects with periodontal disease and levels in subjects without periodontal disease.
Materials and Methods: In this case-control study, 170 patients at Hamadan faculty of Dentistry, including patients with periodontal disease and patients with normal periodontium, were selected and divided into test and control groups. Unstimulated saliva was collected in the same situation from the test and control groups. Each saliva sample was analyzed to measure salivary LDH level on the day of collection, by using commercially available kits according to the manufacturers' instructions. A statistical T-test was employed to evaluate significant differences among groups.
Results: The mean LDH levels in the test and control groups were 1071.67 ± 731.004 and 550.91 ± 217.215, respectively. As the level of statistical significance was set at $P < 0.05$, data analysis showed a significant difference between the LDH enzymatic level in the test and control groups ($P = 0.000$). Comparison of the LDH enzymatic level in subjects with different genders in the test and control groups showed no significant differences ($P = 0.340$).
Conclusions: Salivary LDH levels can be used as marker of periodontal disease for screening periodontitis in large populations.

Keywords: Periodontal Disease Biomarkers, Lactate Dehydrogenase, Diagnostic Indicator, Periodontal Diagnosis, Periodontal Diseases, Periodontitis, Saliva

1. Background

Periodontal disease is a chronic, infectious, and multifactorial disease, which is caused by an interaction of microbial invasion and host responses, resulting in irreversible destruction of the supporting tissues of the teeth, progressive attachment loss, and bone loss. Due to the negative impact of the disease, which can lead to tooth mobility and subsequent tooth loss, early detection is important (1-3). Accurate diagnosis also can distinguish the borderline conditions of periodontal diseases so as to avoid the unnecessary treatment for patients. The conventional diagnosis of periodontal disease is determined through clinical measurements such as probing pocket depth, bleeding during probing, clinical attachment loss, plaque index, and radiographs (3-7). The disadvantages of these conventional methods of diagnosis include the need for skilled clinicians and significant time, and the inability to diagnose present disease activity (4-6). To address these problems, new diagnostic methods with respect to biomarkers of periodontal disease are being developed (4, 6-10). Since they don't need to be done by a trained clinician and can be done quickly, these methods are appropriate for screening periodontal disease in large populations.

In recent years, several biomarkers of periodontal disease have been introduced (4-6). Among these biomarkers, lactate dehydrogenase is one of the first biomarkers which has been used for periodontal diagnosis (11). Since lactate dehydrogenase is a cytoplasmic enzyme that can be found in the cells of almost all body tissues, it could be released in the extracellular environment from necrotic cells, as a result of periodontal destruction leading to cell injury and cell death. From there, it could get into the GCF and saliva. Therefore, lactate dehydrogenase may be used as a marker for the diagnosis of periodontal disease (6, 9, 10, 12). Periodontal biomarkers could be detected in serum, GCF, or saliva. Among the resources available for obtaining biomarkers, saliva is an important biological substance. It contains biomarkers excreted from serum, gingival crevicular fluid, and mucosal transudate that could be helpful in

detecting and explaining the pathogenesis of several systemic diseases, including periodontal disease (4, 13-15). The advantages of using this source of biomarkers include its ease of collection, the low cost of the test, and the fact that the test is not technique sensitive or invasive (4, 5, 13, 16).

Many previous studies have been done on detecting biomarkers of periodontal disease and to establish an appropriate source for sampling (17, 18). Even though LDH is known as a biomarker of periodontal disease, given the low sensitivity and specificity of LDH detection, there is still a need for more studies (10). Furthermore, though saliva is an appropriate and beneficial source for sampling, it could be affected by several local and systemic factors that could lead to incorrect test results (13). So, in order to find out the effectiveness of detecting the LDH level of saliva for diagnosis of periodontal disease in a broad population, this study was conducted.

2. Objectives

The aim of this study was to evaluate the differences in the lactate dehydrogenase levels of saliva between patients with and without periodontal disease.

3. Materials and Methods

From the patients aged 20 to 70 years who were visited at Hamadan faculty of dentistry a total of 85 subjects with periodontitis (the test group) and 85 subjects without periodontitis (the control group) were selected to participate in this case control study. Patients with systemic problems, pregnant patients, those who had received antibiotic and/or anti-inflammatory therapy within the previous 3 months or periodontal treatment within the previous 6 months, those with any abnormal inflammation or local ulcers that were not related to periodontal disease, and those with a history of alcohol, tobacco, or drug abuse were excluded. This study was approved by the ethics committee of Hamadan University of Medical Sciences. Written informed consent was obtained from patients before their enrollment in the study. Patients were diagnosed with periodontitis according to the following criteria: The subjects with chronic periodontitis had at least 20 teeth, and had at least eight sites with PD > 4 mm and attachment level > 2 mm. The periodontally healthy subjects had at least 24 natural teeth, no probing depth (PD) > 3 mm, and no attachment level > 2 mm (19). All the patients selected in each group were examined and approved by two periodontists.

3.1. Saliva Sampling

To collect unstimulated whole saliva samples from the test and control groups, patients were asked to avoid eating or drinking for at least two hours prior to saliva collection. The samples were obtained between 9:00 AM and 12:00 PM. Before sampling, patients were given oral irrigation with water for one minute (6). Then, their mouths were examined to ensure the absence of blood and debris. About 3ml of saliva was collected in a sterile microtube and kept at 4°C. Laboratory analysis of each saliva sample was done on the day of collection. Hence, the samples were transferred immediately to the biochemistry lab of Hamadan University of Medical Sciences, and the LDH levels were measured using commercially available kits (Pars Azmoon; Iran) according to the manufacturer's instructions (10).

3.2. Statistical Analysis

Descriptive statistics, including means and standard deviations, were calculated to describe the data according to participants' sex, age, and LDH enzymatic levels, among subjects with and without periodontitis. Results were analyzed by a statistical t-test to detect significant differences between the case and control groups and also between different sex groups. The statistical significance level was set at $P < 0.05$.

4. Results

A total of 170 patients (104 women, 66 men) with a mean age of 31.07 years (ranging from 28 to 67) were included in this study (Table 1). Descriptive statistics on the age of the patients are given in Table 2. Mean values for LDH enzymatic levels are shown in Table 3. The mean LDH levels in the test and control groups were 1071.67 ± 731.004 and 550.91 ± 217.215, respectively.

Data analysis with a T-test showed a significant difference between the LDH enzymatic levels in the test and control groups ($P = 0.000$) (Table 4). Comparison of the LDH enzymatic levels between male and female patients in each group didn't show significant differences ($P = 0.340$) (Table 5). So, there was no correlation shown between LDH enzymatic level and subject gender.

5. Discussion

The use of biomarkers as a diagnostic tool during check-up dental examinations could be helpful in early diagnosis of periodontal disease. Additionally, screening periodontal disease in large populations by the means of biomarkers could be done more quickly and easily than

Table 1. Number of Patients and Sex Distribution in Both Groups

Sex	Test	Control	Total
Male			
Per group	32	34	66
Percentage	18.8	20	38.8
Female			
Per group	51	53	104
Percentage	30	31.2	61.2
Total			
Per group	85	85	170
Percentage	50	50	100

conventional methods of periodontal diagnosis. Therefore, during recent decades detecting biomarkers for diagnosis of periodontal disease has attracted attention (4, 5, 10). Periodontal disease biomarkers are substances that could be produced during the host's defensive responses against bacterial invasion, reflect inflammation, or be released from cell death as a result of tissue destruction due to periodontal disease (4, 14).

The diagnostic efficacy of various biomarkers of periodontal disease has been demonstrated in previous studies (20-22). It is believed that blood is the gold standard source for detecting biomarkers (5). Many studies have shown that detecting periodontal biomarkers in GCF is a reliable approach for periodontal disease diagnosis (19, 23, 24). However, GCF sampling is difficult, costly, and as it is a site-specific method, it is time consuming. Therefore, in order to solve problems regarding blood and GCF sampling, saliva has been proposed as a source of biomarkers (6, 23, 24). Previous studies have shown that these biomarkers could be measured in saliva (13, 24, 25). In this study, LDH salivary level was compared between subjects with periodontitis and subjects without periodontitis. In a previous study, oral epithelium was demonstrated as the major source for saliva LDH. So, LDH salivary level could be used as a biomarker of periodontal disease (26).

The results of this study indicated that LDH salivary level was significantly higher in subjects with periodontitis. LDH is an intracellular enzyme that is released from dead cells as a result of periodontal destruction. LDH level can be measured in saliva (4, 6, 23). Therefore, in the test group that included periodontitis patients, a higher salivary LDH level was shown. But gender and age was not correlated with salivary LDH level. The result of this study is in agreement with the results of Azizi et al. (27). They compared salivary LDH and aspartate aminotransferase level between chronic periodontitis, aggressive periodontitis, and healthy subjects, and concluded that salivary enzymes in periodontal patients were higher, so these enzymes can be used as markers for determining the amount of destruction of periodontal tissues (27). Rehab et al. (28) similarly assessed LDH in ischemic heart disease patients and chronic periodontitis patients, and showed the LDH level of serum is a significant marker in IHD patients, while salivary LDH increases with chronic periodontitis progression (28). Kugahara et al. (29) used salivary LDH as a screening test to detect the presence of periodontitis in pregnant women (29).

Nomura et al. (23) evaluated salivary LDH and the total count of Porphyromonas gingivalis and Prevotella intermedia, concluding that salivary LDH level is an indicator of inflammation and destruction of periodontal tissue and a clinically useful marker following periodontal therapy (23). Todorovic et al. (30) examined various salivary enzymes such as LDH from patients with periodontal disease before and after periodontal treatment, and suggested that the activity of these enzymes in saliva may be useful in diagnosis, prognosis, and evaluation of therapy effects in periodontal disease (30). Rai et al. (31) concluded that salivary AST, ALT, and LDH levels indicated inflammation and destruction of periodontal tissues, and so could be used as clinically useful markers (31). The results of all these studies showed higher LDH salivary level in periodontitis than subjects with normal periodontium. Therefore according to these results, LDH salivary level could be used as a marker of periodontal destruction for diagnosing periodontal disease. Determination of salivary LDH levels can thus be used as a marker of periodontal disease for screening periodontitis in large populations.

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Footnotes

Authors' Contribution: Janet Moradi Haghgoo developed the original idea and the protocol, abstracted and analyzed the data, and is the guarantor. Sara Soheilifar, Mohsen Bidgoli, Neda Rastegarfard, Maral Saremi, and Samare Kafilzade contributed to the development of the protocol and abstracted data. Neda Rastegarfard prepared the manuscript.

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Table 2. Descriptive Statistics of Age

	Mean ± Standard Deviation	m	Min	Max
Test	35.98 ± 11.138	35	15	67
Control	26.16 ± 4.284	25	20	38
Total	31.07 ± 9.746	28	15	67

Table 3. Descriptive Statistics of Lactate Dehydrogenase Levels

Enzyme Level	Mean ± Standard Deviation	m	Min	Max
Test	1071.67 ± 731.004	881	65	2969
Control	550.91 ± 217.215	383	111	2390
Total	811.29 ± 683.203	580	65	2969

Table 4. Comparison of Enzyme Levels Between Test and Control Groups

Enzyme Level	Mean ± Standard Deviation	T Value	Degrees of Freedom	P Value
Test	1071.67 ± 731.004	5.362	168	0.000
Control	550.91 ± 217.215			

Table 5. Comparison of Enzyme Levels Between Male and Female Patients

Enzyme Level	Mean ± Standard Deviation	T Value	Degrees of Freedom	P Value
Male	748.35 ± 672.508	0.957	168	0.340
Female	851.23 ± 690.144			

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