



## Comparative Assessment of Lactate Dehydrogenase (LDH) Enzyme Levels in Saliva and Serum among Patients with Periodontal Disease

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### Abstract

Periodontal disease is a significant ailment that should be prioritized in community health check-up programs given its asymptomatic nature, early detection of the illness would be optimal, may be even before the patient exhibits any symptoms. Lactate dehydrogenase, often known as LDH, is an enzyme that can be found in the cytoplasm of almost every cell in the human body. When a cell dies, LDH is released into the extracellular space. The present study aims to compare the concentrations of lactate dehydrogenase (LDH) in saliva and serum samples of individuals with periodontal diseases. The objective is to identify any differences in LDH levels between these two bodily fluids. The case-control research included 200 people aged between 25 and 50 years old. The participants were categorized into two cohorts: 150 individuals with chronic periodontitis, and 50 control subjects who had excellent overall health and exhibited a healthy gum condition. The blood and saliva samples were collected simultaneously from the individuals after a comprehensive periodontal examination to assess the levels of lactate dehydrogenase enzyme. The analysis indicated that men with chronic periodontitis had a mean age of 40 years, whereas females in the control group with healthy periodontium had 27 years. CP patients had higher mean values for clinical periodontal indices PLI, GI, BOP, PPD, and CAL: 2.37, 2.12, 33.23, 4.25, and 4.55. LDH characteristic results showed that the CP group had a substantially higher mean blood enzyme level of 250 than the control group in saliva (123). The comparison showed no significant difference in LDH levels between saliva and serum in both the CP group and the control group. LDH levels in both saliva and serum rose as periodontal disease progressed. There was no statistically significant difference between saliva and serum LDH levels. Salivary LDH estimation may be utilized as a risk predictor in patients with periodontal disease.

**Introduction:**

Periodontal diseases in both types (gingivitis and periodontitis) are one of the most common oral conditions affecting people in their middle and advanced years. With the development of clinical knowledge and methods, periodontal disease can be stopped in its tracks by early detection<sup>(1)</sup>. The inflammatory condition of the tooth's supporting structure, which is brought on by a lingering microbial infection, and the ongoing inflammatory process causes the periodontal tissues to be destroyed<sup>(2)</sup>. The disease's symptomatology is entirely trivial, which is why individuals with this disease seek therapy when it is too late. As a result, it is critical to detect periodontal disease at an early stage utilizing simple, reliable, and easily available diagnostic techniques<sup>(3)</sup>. The Diagnostic measures like as clinical measurements (measuring of periodontal pocket depth, determination of bleeding on probing, and measurement of clinical loss of periodontal attachment) and radiography are utilized in routine practice, as advised by Salvi and Lindhe<sup>(4)</sup> these conventional diagnostic procedures are time-consuming, but they also provide limited information because they only show historical disease activity and little about the inflammatory- detrimental mechanisms of periodontal disease at the moment of their identification. Another disadvantage is that they cannot forecast how the disease will proceed in the future, particularly in individuals who are at risk. Another problem is that they do not predict future disease prognosis, particularly in at-risk individuals<sup>(5)</sup>.

Biomarkers like enzymes, cytokines, receptors, and other proteins have been studied as potential biomarkers for periodontal disease. These biochemical markers are present in various biological fluids, such as gingival crevicular fluid (GCF), which contains local biomarkers and can potentially offer site-specific information; blood, serum, or plasma, which contain systemic biomarkers and can potentially offer patient-level information; and saliva, which contains markers derived from both local and systemic sources and can potentially offer

patient-level information<sup>(6)</sup>. Periodontitis is the result of the presence of periodontal bacteria, which trigger both an innate and humoral immune response. Antibodies against these pathogens have been detected both in the gingival tissue and in the bloodstream. Blood is not able to offer precise information about particular sites in the body. Collecting blood samples is normally more intrusive compared to collecting saliva samples. However, blood sampling is a straightforward and rapid procedure that may be done outside of the dentist office as part of a regular general diagnostic check-up. It is also possible to collect blood samples with little invasiveness using a finger stick. Salivary and blood diagnostic tests might potentially be used for diagnosing and monitoring periodontal disease in patients owing to their convenient, rapid, and generally non-intrusive collection manner<sup>(7)</sup>. Human saliva characteristic is a transparent, mildly acidic (pH = 6.0-7.0) biological liquid that includes secretions from many salivary glands located under the mouth mucosa, including the parotid and submandibular glands, sublingual, and other small glands, as well as gingival crevice fluid<sup>(8)</sup>. This complex oral fluid performs a variety of physiologic tasks, including oral digestion, the process of breaking down food in the mouth, swallowing and tasting, and the lubricating of tissues, tooth integrity preserving, and protection against germs and viruses. Aside from its critical role in regulating the balance of the oral cavity system, saliva is an ideal medium for health and illness surveillance. Saliva and blood, is a complex fluid that contains enzymes, hormones, antibodies, antimicrobial components, and cytokines. Saliva may be introduced into the bloodstream via several routes, such as transcellular and paracellular pathways. This can occur by processes including passive intracellular diffusion, active transport, or extracellular ultrafiltration inside the salivary glands or the gingival sulcus<sup>(9)</sup>. Saliva may serve as an indicator of several physiological changes in the body, such as emotional, endocrine, nutritional, and metabolic states. Consequently, this fluid may be used to assess the condition of both the

oral cavity and the whole body. This serves as the basis for our aspiration to develop disease diagnoses and enhance the surveillance and monitoring of human health via the analysis of saliva, thereby warranting consideration. Saliva is a very effective biological fluid that may be used as a diagnostic tool for evaluating periodontal disease. Saliva collection is a straightforward, non-invasive, and safe procedures<sup>(10)</sup>. Lactate dehydrogenase (LDH) is a consistently active intracellular enzyme that is present in all cells of the human body. Disruption of the plasma membrane leads to rapid release of LDH into the cell culture supernatant, which is a key characteristic of cells undergoing apoptosis. (cell turn over), necrosis, or other forms of cellular destruction. LDH activity can be easily measured using nicotinamide adenine dinucleotide (NADH), which is created during the lactate-pyruvate conversion process<sup>(11)</sup>. LDH levels in saliva are directly related to the number of dead or damaged cells. The level of LDH is increased in both serum and saliva in response to tissue destruction, so can be used as active biomarker for monitoring the prognosis of periodontal disease and many systemic disorders in different stage. Miyoshi et al. (2018) investigated the connection correlation exists between salivary LDH levels and systemic inflammation in Japan. Their findings revealed a clear positive connection between salivary LDH and periodontal state, with elevated LDH levels are correlated with increased levels of systemic inflammatory markers<sup>(12)</sup>. Moradi Haghgoo et al. (2016) evaluated the LDH levels of periodontitis patients and healthy individuals at Hamadan Dental School in Hamadan, Iran. Their findings revealed that the periodontitis group's mean LDH level was substantially greater than the control group's (P 0.05)<sup>(13)</sup>. Research done in Japan by Nomura et al. (2016) examined the diagnostic accuracy of salivary biomarkers, such as LDH and Hb, as novel approaches for detecting periodontitis. These biomarkers were explored as potential substitutes for the periodontal index. Their research revealed that periodontitis patients had significantly higher levels of LDH and Hb compared to

healthy persons. Nevertheless, there was no notable correlation seen between the periodontal index and salivary levels of LDH and Hb in either group<sup>(14)</sup>. The current investigation seeks to conduct a comparative analysis of lactate dehydrogenase (LDH) concentrations within the salivary and serum samples of individuals afflicted with periodontal diseases, with the objective of elucidating potential disparities in LDH levels across these distinct bodily fluids.

## Materials and Methods

Two hundred subjects were selected for this case-control study divided into two groups (150 patients with chronic periodontitis group (CP), and 50 subjects with control group that had healthy periodontium). Patients' age 25-50 years old would be selected from a specialized center in Tikrit and from collage of dentistry university of Tikrit in the period from May to August 2023. Questionnaire case sheet was be filled out for each person that involved in the study which include (name, age, education, past dental history, past medical history, full intraoral examination, and extraoral examination). All the participants signed on specially designed informed consent. Exclusion aspects or criteria concerned, patients had taken antibiotic or anti-inflammatory drug in the past three month, undergone periodontal treatment, had any systemic diseases that may be effect on the LDH level, pregnant women, lactating women, smokers, and alcoholism. Inclusion criteria involved in study patients with chronic periodontitis with good general health and subjects with clinically healthy periodontium and systemically healthy. Resting Saliva and serum sampling had done at the same times from all participants prior to periodontal clinical examination. 3-5ml of whole unstimulated saliva (resting) was collected in sterile test tube after patients spitting on it that take about 2-5min. the period of saliva collection from 8-11 a.m.<sup>(15)</sup>. Tubes containing saliva stored in cooler box and the samples numbering for each patients, after that saliva samples were centrifuged at 3000 rpm for 10 min to collect about

1ml of supernatant saliva that stored at 4°C for LDH leveling. 5ml of blood sample had collected from the vein in the antecubital area for all the individuals for LDH determination, then the blood collects in special tubes contain Ethylene diamine tetraacetic acid (EDTA). The tubes centrifuged for 10 min at 2500 rpm and stored at -4°C after plasma sample was obtained. LDH level in saliva and serum determined using (Human Gesellschaft für Biochemica und Diagnostika mbH (Germany))<sup>(16)</sup>.

After salivary and serum sampling, clinical periodontal examination was done for all the teeth except third molar using a standard periodontal probe to evaluate the periodontal health status according to specific periodontal parameters as: PLI, GI, BOP score 1, PPD and CAL). The statistical analysis and examination of my data were performed using the SPSS V. 26 software program. The research used many variables, such as the mean, mean percentage, dependent t-test, and standard deviation (SD), to perform a descriptive statistical analysis. Significance levels (S) were utilized in statistical analysis to ascertain the statistical significance of outcomes. Non-significant (NS) results were indicated when the p-value (P) was more than 0.05. Highly significant (HS) results were indicated when the p-value was less than 0.01. Results with a p-value between 0.05 and 0.01 were regarded moderately significant.

## Result

The results that were given in the inquiry are summed up in each of the four tables. According to Table (1), men who suffer from chronic periodontitis have a mean age of 40 years, while females who are considered to have healthy periodontium (the control group) demonstrate a significantly younger mean age of 27 years. In Table (2), increased mean values for clinical periodontal parameters—PLI, GI, BOP, PPD, and CAL—are solely detected in the chronic periodontitis (CP) group, with respective values of 2.37, 2.12, 33.23, 4.25, and 4.55. This is the only group that exhibits these elevated mean values for clinical periodontal

parameters. Table (3) explains the properties of lactate dehydrogenase (LDH), which shows that the CP group has a higher mean LDH value in serum (250), whereas the control group has a lower mean LDH value in saliva (123). These results may be seen by comparing the two tables. The levels of LDH enzyme that are found in saliva are compared to those found in blood in Table (4), which uses an independent t-test to make its comparisons. When comparing LDH levels in saliva and serum, the results indicate that there are no substantial disparities in LDH levels between the chronic periodontitis group and the control group. On the other hand, among the group of those who have chronic periodontitis, a considerable gap may be seen between the sexes.

## Discussion

The findings indicate that the male participants in the chronic periodontitis group had a higher average age, suggesting that periodontitis is more likely to arise in older individuals. Several factors may contribute to this susceptibility, including alterations in food, heightened susceptibility to preexisting chronic systemic diseases, and age-related changes in the body's fundamental systems. Periodontitis is more prone to occur as a result of aging. According to Effie Ioannidou (2017)<sup>(17,18)</sup>, Periodontitis is more commonly found in males than females due to various factors. The development of periodontitis involves the potential influence of sex dimorphism on altering the bacterial biofilm and the immune response of the host, consequently impacting the microbial cause of the disease<sup>(17,18)</sup>. Previous research has provided limited evidence indicating that males had a much greater likelihood of carrying salivary and subgingival periodontal infections, such as *Prevotella intermedia*, compared to women, also found that *Bacteroides* have a higher prevalence in males compared to women<sup>(19,20)</sup>. Our study also revealed that the high mean values of clinical periodontal parameters is showed in CP group compared to control group that

responsible for this outcome that the presence of germs in tooth plaque is the key factor that leads to periodontitis. Plaque, which is a mixture of bacteria, mucus, and other particles, develops and settles on and around the teeth. Plaque comprises a mixture of these elements. An effort is made by the immune system to eliminate the bacteria, which may result in inflammation in the region and has the potential to cause damage to the gums, periodontal ligament, alveolar bone, and cementum<sup>(21)</sup>. The result appeared that the level of salivary and serum LDH enzyme is showed high mean in CP group when compared with control group. In the process of early identification of periodontal disease, the use of c biomarkers serve as a diagnostic tool in the context of checkup dental exams may prove to be very beneficial. Serum tests are used to diagnose several systemic illnesses<sup>(22)</sup>. Saliva has gained attention as a diagnostic fluid that may be used to detect both oral and systemic illnesses without the need for intrusive procedures. Collecting it is simple, inexpensive, and does not need skilled medical personnel. Due to its composition of hormones, growth factors, enzymes, antibodies, and bacteria with their byproducts, it has the potential to be valuable in the early identification of systemic illnesses such as the mentioned conditions include cancer, autoimmune illnesses, cardiovascular disease, diabetes, and virus-related diseases<sup>(23)</sup>. However, clinical parameters (PLI, GI, BOP, PD) and radiographic (alveolar bone loss) characteristics are used to diagnose periodontal disease. While these tests may help find signs of periodontal disease or verify that a patient is healthy, they don't tell you much about individuals or areas that might be at risk for periodontal breakdown in the future. It has been suggested that a variety of indicators found in saliva and serum might be used as diagnostic tests for periodontal disease<sup>(24,25)</sup>.

Previous studies revealed that the level of LDH significantly increase in CP patients when its compared with control group and this enzyme is considered a good biological fluid for screening the periodontal diseases<sup>(26-28)</sup> Santhosh Kumar Caliaperoumal et al,2021 found that significant differences in the level of LDH enzyme between gingivitis and periodontitis group<sup>(28)</sup>

Higher mean value of LDH in male with CP than female is agreed with<sup>(29)</sup> where the activity of LDH enzyme is reached maximum activity in male more than in female<sup>(30)</sup>, also males appear to be more susceptible than females to destructive periodontal disease<sup>(31,32)</sup>. Comparison the level of LDH enzyme between saliva and serum revealed a non-significant difference and this is agreed with<sup>(33)</sup> Where the previous study found that Salivary and serum LDH levels rose in tandem with the development of periodontal disease. Saliva and serum LDH levels were not significantly different. Risk prediction in patients with periodontal disorders may be achieved by estimating salivary LDH.

### **Conclusion**

Can be concluded that LDH levels in both saliva and serum rose as the illness progressed, from healthy controls to those with chronic periodontitis. When comparing the amounts of LDH in saliva and serum, no significant changes were found. Salivary LDH levels are therefore a practical and helpful biochemical indicator of the periodontal tissues' functional state. New avenues for precise and effective illness detection and therapy may open up as a result.

**Table (1): Participants' ages and sexes were analyzed statistically across the two studies.**

Groups	Gender	No.	%	Mean	±SD
<b>Chronic periodontitis (CP)</b>	Male	90	60%	40	±3.194
	Female	60	40%	48	±2.784
<b>Control group (healthy periodontium)</b>	Male	15	30%	26	±1.310
	Female	35	70%	27	±0.977

No.=number

% =percentage

SD=standard deviation

CP=chronic periodontitis

**Table (2): Statistical analysis providing a descriptive profile of clinical periodontal parameters for each of the research groups.**

Groups	PLI		GI		BOP score1		PPD		CAL	
	Mean	±SD	Mean	±SD	Mean%	±SD	Mean	±SD	Mean	±SD
<b>CP</b>	2.37	±0.102	2.12	±0.130	33.23	±0.015	4.25	±1.203	4.55	±1.521
<b>Control group</b>	0.31	±0.012	-	-	-	-	-	-	-	-

PLI=plaque index

GI=gingival index

BOP=bleeding on probing

PPD=probing pocket depth

CAL=clinical attachment loss

**Table (3): Descriptive statistics were used to evaluate the levels of LDH (U/L) in saliva and serum among individuals with CP, the control group, and both males and females.**

Groups	LDH (U/L) in saliva		LDH (U/L) in serum	
	Mean	±SD	Mean	±SD
<b>CP</b>	242	±23.514	250	±41.631
<b>Control group</b>	123	±22.432	134	±50.710
<b>Male with CP</b>	245	±13.870	247	±33.098
<b>Female with CP</b>	236	±43.953	240	±112.809

LDH=lactate dehydrogenase enzyme

**Table (4): comparison between the level of LDH(U/L) in saliva and serum in both CP and control group and in male and female with CP.**

Enzyme	Independent t-test	P-value	Sig.
In CP	24.25	0.06	NS
In control group	17.8	0.076	NS
Between Gender	35.14	0.04	S

P=probability  
 Sig.=significant  
 NS=non-significant

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