



RESEARCH ARTICLE

COMPARISON AND CORRELATION OF LACTATE DEHYDROGENASE LEVELS OF SALIVA AND SERUM IN HEALTHY SUBJECTS, PATIENTS WITH GINGIVITIS AND CHRONIC PERIODONTITIS

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ABSTRACT

**Background:** Periodontal disease is a chronic tissue-destructive inflammation, which destroys tooth-supporting structures and ultimately leads to tooth loss. Early diagnosis and treatment of periodontal diseases can reduce the risk of tooth loss in general population. Several biomarkers like Lactate dehydrogenase (LDH) have been proposed for early detection of periodontal disease from saliva, serum and gingival cervical fluid.

**Aims:** To assess and correlate the LDH levels in saliva and serum of healthy subjects, subjects with chronic gingivitis and periodontitis and to compare the saliva and serum LDH levels in all three groups.

**Materials and methods:** Total 33 subjects aged 20– 45 years were considered for the study. The subjects were divided into three groups based on periodontal parameters as healthy subjects, chronic generalized gingivitis and chronic generalized periodontitis. Two ml of un-stimulated whole saliva and two ml of blood was collected from all the subjects and the level of LDH in both saliva and serum were measured and compared using commercially available kit.

**Statistical analysis:** Statistical analysis was carried out using student t test and ANOVA with the help of SPSS software and P value <0.05 was considered as significant.

**Results:** The clinical parameters like gingival index, plaque index, probing pocket depth and clinical attachment level were statistically significant between healthy subjects, patients with generalized gingivitis, and those with chronic periodontitis. Serum and Salivary LDH levels were found to be within normal limits in healthy subjects where as it was significantly increased in patients with gingivitis and chronic periodontitis. The activity of LDH increased linearly as the disease progressed.

**Conclusion:** LDH levels in saliva as well as serum increased with periodontal disease progression. There was no significant difference observed between saliva and serum LDH levels. Estimation of salivary LDH can be used as risk predictor in patients with periodontal disease.

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INTRODUCTION

Periodontal disease is chronic tissue-destructive inflammation which degrades the attachment apparatus of the teeth causing

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mobility leading to tooth loss, thereby affecting the quality of individual's life. The dental plaque consisting of an array of microorganisms, firmly attached to the underlying oral structures, not easily detected by the naked eye is the prime cause of periodontal disease. (Nepale et al., 2014) Diagnosis of periodontal disease relies primarily on clinical examination of gingiva, Bleeding on probing (BOP), Probing pocket depth (PPD) and radiographic parameters (alveolar bone loss).

(Croxon, 1984) Radiographs like conventional, digital periapical radiographs and panoramic can be used for detection of periodontal diseases. (Ashwinirani *et al.*, 2015) Early diagnosis and treatment of periodontal diseases can reduce the risk of tooth loss in general population. Several biomarkers from tissue and fluids are being researched for early detection of periodontal disease. Many biomarkers in saliva, serum and gingival cervicular fluid (GCF) have been proposed as a diagnostic test for periodontal diseases e.g. aspartate and alanine aminotransferase (AST, ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline and acidic phosphatase (ALP, ACP), gamaglutamiltransferase (GGT). (Numabe *et al.*, 2004; Kaufman and Lamster, 2000; Ozmeric, 2004) Lactate dehydrogenase (LDH) is a ubiquitous enzyme which plays a significant role in the clinical diagnosis of pathologic processes. The extracellular appearance of LDH is used to detect cell damage; raised level of LDH is an indicator of disease progression and cell death. In this background the present study was conducted to assess and correlate LDH levels in healthy subjects, patients with chronic generalized gingivitis and chronic periodontitis and to compare the LDH levels in saliva and serum in all the three groups.

## MATERIALS AND METHODS

The study was conducted in the Department of Periodontology in collaboration with Department of Biochemistry, Krishna Institute of Medical Science, Karad, India. The research protocol was initially submitted to the institutional ethical committee and ethical clearance was obtained before commencing the study. The study was conducted during the period from July 2014 to March 2015. An informed consent was obtained from all patients before enrolling them in the study. Subjects willing to participate in the study with at least 20 or more natural teeth were included in the study. Subjects with any known systemic diseases, subjects who had used antibiotics during the previous six months, Pregnant and lactating women, subjects who use tobacco in smoked or smokeless form were excluded from the study. After purposive sampling technique 33 subjects aged between 20 – 45 (Mean age  $31.66 \pm 8.55$  years) were considered for the study.

### Clinical Examination

The following clinical periodontal parameters like plaque index (PI), gingival index (GI), clinical attachment loss (CAL) and probing pocket depth (PPD) were recorded in a structured proforma. The GI was used to diagnose patients with gingivitis as per by Loe and Sillness. (Loe, 1967) The UNC-15 periodontal probe (Hu-Friedy, Chicago IL.W) was inserted parallel to the vertical axis of the tooth and walked circumferentially around each surface of the tooth with constant probing force for PPD and CAL measurements. The CAL was measured from cementoenamel junction to the base of gingival sulcus and PPD was measured from gingival margin to the base of gingival sulcus. The participants were classified as having chronic periodontitis based on 1999 consensus classification of periodontal diseases. (Todorovic *et al.*, 2006) All the parameters were recorded by a single researcher and the values were validated and confirmed by a senior periodontist to minimize the observer bias. The patients were divided into three groups as per clinical examination using the periodontal parameters.

**Group 1 (n=11):** Healthy subjects with absence of gingival inflammation or bleeding on probing.

**Group 2 (n=11):** Subjects suffering from chronic generalized gingivitis without clinical attachment loss.

**Group 3 (n=11):** Subjects suffering from chronic generalized periodontitis

### Saliva and blood sample collection

Two ml of un-stimulated whole saliva sample was collected in the morning from 10 AM to 11 AM, two hours after the last meal to standardize the collections according to the circadian rhythm. The subjects were requested to rinse their mouth thoroughly with distilled water before the sample collection. Five minutes after the oral rinse, the subjects were requested to swallow any residual saliva in their mouth. They were asked to refrain from talking and asked not to cough up mucus as saliva is collected in their oral cavity. The saliva collection was done using spitting method, where patient is allowed to accumulate saliva for 60 seconds in mouth and then spit in collection vessel until the required quantity was collected. Two ml of blood sample was collected from cubital fossa in a vacutainer by routine method. The saliva and blood samples were transported to the laboratory in a standard gel coolant pack to maintain the temperature between  $2^{\circ}\text{C}$ -  $4^{\circ}\text{C}$ .

### Biochemical Analysis

LDH levels in saliva and serum were determined using commercially available kits (Infinite Lactate Dehydrogenase; Accurex Biomedical Pvt. Ltd., Thane, India) with the Clinical Chemistry Analyzer (EM 360, ERBA Diagnostics, Mannheim, Germany).

### Statistical Analysis

Statistical analysis was carried out using Student *t* test for the comparison of the salivary and serum LDH levels in the same group. One-Way ANOVA test was used to compare the salivary and serum LDH levels between the three groups. The tests were carried out using the SPSS software version 22 (SPSS Inc., Chicago, Illinois) and P value  $\leq 0.05$  was considered significant.

## RESULTS

The 33 subjects enrolled in the study were categorized in to different groups comprising of 11 in each group. The socio demographic variations are explained in (Table 1). On intergroup comparison, the differences in clinical parameters like GI, PI, PPD and CAL were statistically significant between healthy subjects, patients with generalized gingivitis, and those with chronic periodontitis ( $p < 0.0001$ ) (Table 2). LDH levels in Serum and Saliva were found to be within normal limits in healthy subjects (Serum LDH level:  $291.90 \pm 209.87$ ; Salivary LDH level:  $289.08 \pm 338.69$ ) where as it was increased in patients with gingivitis (Serum LDH level:  $358.09 \pm 119.97$ ; Salivary LDH level:  $370.20 \pm 141.50$ ), the difference was not statistically significant for both serum and saliva ( $p$  value =  $> 0.05$ ). Serum and salivary LDH levels were found to be significantly increased in patients with chronic periodontitis (Serum LDH level:  $592.36 \pm 328.50$ ; Salivary LDH level:  $869.49 \pm 597.05$ ). Difference between gingivitis group and periodontitis group was statistically significant (Serum 'p' value =  $0.0139$ ; Saliva 'p' value =  $0.0041$ ).

**Table 1. Socio demographic data**

	Group 1	Group 2	Group 3
Study Sample (N)	11	11	11
Age (mean)	22.09±1.37	34.45±7.313	38.45±4.29
Gender			
Males	4(36.36%)	7(63.63%)	3(27.27%)
Females	7(63.63%)	4(36.36%)	8(72.72%)

**Table 2. Clinical periodontal parameter**

	Probing Pocket Depth	Clinical Attachment Loss	Plaque Index	Gingival Index
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Group 1	0.14 ± 0.18	0.06 ± 0.08	0.80 ± 0.11	0 ± 0.00
Group 2	0.67 ± 0.37	0.12 ± 0.08	2.28 ± 0.15	2.51 ± 0.25
Group 3	2.60 ± 0.29	3.31 ± 0.19	2.48 ± 0.21	3.18 ± 0.25
P value	<0.0001	<0.0001	<0.0001	0.0001
F Value	214.33	2223.0	332.94	732.26

**Table 3. Comparisons of serum and salivary ldh levels**

Groups	Saliva LDH level		Serum LDH Level		P Value	t Value
	Mean	SD	Mean	SD		
Group 1	289.08	338.69	291.90	209.87	0.98	0.02
Group 2	370.20	141.50	358.09	119.97	0.83	0.21
Group 3	869.49	597.05	592.36	328.50	0.19	1.35

The activity of LDH increased linearly as the disease progressed. (Table 3) Comparison of LDH levels between serum and saliva in all three groups showed no statistically significant differences. (Table 3)

## DISCUSSION

The LDH in the whole saliva within the oral cavity may originate from various sources, because whole saliva is a combination of secretions from both major and minor salivary glands, fluids diffused through the oral epithelium, gingiva, cellular and other debris. It is logical to assume that pathological alternations of oral epithelium like dysplasia or cancer may result in alternation of LDH levels in saliva. Therefore, salivary LDH may be evaluated for possible oral mucosal pathologies in a manner similar to that used for evaluating tissue pathologies in heart, muscle, or liver by LDH detection in plasma. Serum LDH has a widespread distribution in the body.

It is released into the peripheral blood after cell death caused by ischemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisonings. However, collection of GCF in a routine clinical setting has inherent problems. The use of saliva as a source to measure these enzyme biomarkers offers several advantages over GCF as it is faster and more convenient for both the patient and the practitioner. Periodontal diseases are treated using nonsurgical treatment methods and surgical treatment methods. The surgical periodontal treatment is usually rendered as a definitive treatment to patients after the preliminary nonsurgical periodontal treatment. The American Academy of Periodontology treatment guidelines considers the nonsurgical periodontal treatment as the least invasive and most cost-effective approach to periodontal health. (Azodo and Ojehanon, 2016) Salivary LDH is most useful enzyme for the screening of periodontitis. Studies showed increased LDH activity in the saliva of subjects with increased probing depth than in individuals with healthy periodontium.

(Nagler *et al.*, 2001) Previous studies showed salivary LDH has also been used as a screening test to detect the presence of periodontitis in pregnant women. (Kugahara *et al.*, 2008) Study done by Todorovic *et al* shows that the activity of CK, LDH, AST, ALT, GGT, ALP and ACP in saliva from patients with periodontal disease before and after periodontal therapy showed significantly decreased salivary enzymes after periodontal therapy. (Loe, 1967) In our study level of LDH was less in healthy subjects when compared with subjects with gingivitis and periodontitis, this shows direct relation between LDH and periodontitis. The results of our study were consistent with previous study, where they found significant positive correlation between CAL and LDH levels among smokers. (Kugahara *et al.*, 2008) In the present study, the salivary LDH levels were higher in patients with periodontitis than in healthy subjects or in patients with gingivitis, which is in consistent with the results of previous study. (Nomura *et al.*, 2006) Increased serum and salivary LDH activities may be due to cellular necrosis seen in periodontal diseases. The serum LDH levels were also higher in patients with chronic periodontitis than in patients with chronic generalized gingivitis and healthy subjects. There were no significant intra-group differences in salivary and serum LDH levels, which suggests that salivary LDH level, can be used as a substitute for serum LDH level as a biomarker for periodontal diseases since collection of saliva is easier and non invasive.

## Conclusion

Based on the results of this study it was concluded that LDH levels in saliva as well as serum increased with disease progression (healthy subjects < patients with gingivitis < patients with chronic periodontitis). Correlating the saliva and serum LDH levels there was no significant differences. Therefore, salivary LDH levels can be considered as feasible and useful biochemical marker of the functional condition of periodontal tissues. It can offer new possibilities for accurate and efficient diagnosis and disease treatment. Future studies with larger sample size should be carried out to validate these findings.

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