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"SERUM LACTATE DEHYDROGENASE: A CROSS LINK BETWEEN CHRONIC PERIODONTITIS AND TOBACCO"

Dental Science

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ABSTRACT

Background and objectives: Lactate dehydrogenase [LDH] is an intracellular enzyme released by inflammatory cells during tissue destruction. Present study was conducted to estimate and compare serum LDH levels among healthy patients, Chronic Periodontitis (CP) patients, smokers with chronic periodontitis and smokeless tobacco users with chronic periodontitis.
Materials and methods: 120 subjects were selected and divided into 4 groups of 30 each. Serum samples collected were analyzed for LDH and Statistical analysis was obtained.
Results: Statistically significant increase in serum LDH levels was found in tobacco users compared to CP and healthy patients. Smokeless tobacco users showed significantly higher LDH levels compared to smokers. Clinical parameters were positively correlated with LDH levels.
Conclusions: LDH is a marker of tissue integrity, its raised levels is attributed to cell death and tissue breakdown in periodontitis.

KEYWORDS:

Lactate dehydrogenase,Periodontitis,Serum,Tobacco

INTRODUCTION

Periodontitis, the second most common oral disease next to dental caries is a chronic inflammatory disorder that leads to disruption of epithelial and connective tissue elements through a complex interaction between the perio pathogens and the host defense system.[1] Host microbial interactions lead the stromal, epithelial, inflammatory or bacterial cells to release several enzyme families and inflammation markers. The analysis of these enzymes and inflammation markers in serum of periodontitis patients can provide an insight into the pathogenesis and thus aids in making a prompt diagnosis of the periodontal disease.[2]

Biochemical markers can detect inflammatory changes in short period of time. Estimation of enzymes such as lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) in gingival crevicular fluid, saliva, and serum to assess periodontal disease has been proposed.[3] Lactate dehydrogenase (LDH) is a ubiquitous cytoplasmic enzyme, that gets released into the extracellular environment upon cellular lysis and cell death. Within the cell, glucose is used principally for the production of pyruvate in the glycolysis pathway. In an anaerobic medium, pyruvate is reduced to lactate in a reversible reaction catalyzed by lactate dehydrogenase (LDH), which uses nicotinamide adenine dinucleotide as a coenzyme. It shows wide application in medicine as a diagnostic aid to assess cell destruction and damage.4,5,6 Its serum activity is shown to increase non-specifically in many pathological conditions such as myocardial infarction, liver diseases, megaloblastic anaemia's, renal diseases, malignant diseases, progressive muscular dystrophy and pulmonary embolism.

The prevalence of periodontitis is contributed by various lifestyle related factors including smoking, alcohol consumption, and obesity.[7] A large percentage of general population is known to be habitual users of tobacco products. A prevalence study reported that 75 percent of patients referred to periodontists compared to 54 percent of patients in general practices, were either current or past smokers.8 Cigarette smoke (CS) is considered a risk factor in various systemic diseases like cardiovascular diseases, lung cancer, respiratory and other chronic inflammatory diseases.[9] Free radicals and other reactive oxygen and nitrogen species produced from the tar and gas phases of cigarette smoke leads to various smoking-related diseases.[10] Cigarette smoke releases oxygen free radicals and volatile aldehydes which acts on polyunsaturated fatty acid rich cell membranes leading to increased permeability and altered fluidity of the membrane, and thereby causing cellular leakage.[11] The high frequency of lesions occurring in smokers, ranging from periodontal diseases to dental caries and oral carcinoma is due to local noxious effect of tobacco smoke.[12]

Among cigarette smokers, there is widespread periodontal destruction, but in smokeless tobacco users the oral effects are localized to the site of placement. The primary periodontal alteration in smokeless tobacco users is localized gingival recession which occurs in 25-30 percent of these users, and white mucosal lesions occuring in 50-60 percent of users.[13,14] Bagchi et al. demonstrated that smokeless tobacco extracts caused oxidative damage leading to cell apoptosis.[15] Furthermore, smokeless tobacco extract produces oxidative tissue damage and apoptosis and is more toxic than nicotine in terms of their respective oxidative stress actions.[16] Risk factors for tobacco include increased oxidative stress and long-term inflammation.[17]

Thus our aim is to examine the feasibility and reliability of these parameters to adopt as a routine test in the diagnosis of Periodontitis. The ability to diagnose the progression of periodontitis and to identify patients at risk of progression using a non-invasive method would provide useful information in clinical practice. Serum is readily accessible and has many advantages over other bio fluids. The present study was designed to compare the levels of LDH in serum among healthy patients, patients with Chronic Periodontitis, smokers with chronic periodontitis and smokeless tobacco users with chronic periodontitis and to explore the possibility of LDH as a biochemical marker and reliable diagnostic aid of Periodontal diseases.

MATERIALS AND METHODS

A total of 120 subjects, aged 18-60 years, were randomly selected from

the Out-patient department, Department of Periodontics PMNM Dental College, Bagalkot. The protocol for this cross-sectional study was approved by the institutional ethical committee. Prior to enrolment in the study, a written consent was obtained from the candidates who fulfilled the inclusion criteria.

The selection of patients was made according to the criteria approved by the 1999 International Workshop for the classification of periodontal diseases and conditions.18 Further, Patients were divided into four groups as clinically healthy periodontium (Group I), chronic periodontitis (Group II), smokers with CP (Group III) & gutkha chewers with CP (Group IV). All the study participants with no history of any acute/chronic systemic disorders were included. Subjects belonging to the group III were enrolled if they had smoked ≥100 cigarettes in their lifetime and currently smoked. Gutkha chewers with chronic periodontitis (Group IV) were enrolled if they regularly chewed smokeless tobacco at least one sachet daily for atleast 12 months.19 Pregnant women, lactating mother, individuals with trauma or who underwent recent tooth extraction or who had received any periodontal / antimicrobial and anti-inflammatory therapy or vitamin supplements in last three months before sampling were excluded from the study. One calibrated examiner obtained all the measurements so as to reduce intra-examiner variability for Gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL). Both PPD and CAL were recorded using the Williams graduated periodontal probe at four sites around all present teeth, excluding the third molars.

BIOCHEMICAL ANALYSIS

2ml of venous blood was drawn with aseptic precautions from median cubital vein into a serum separating tube. Serum obtained was immediately processed (centrifuged at 2500 rpm for 15 min) and transferred into eppendorf tubes which were then stored in the refrigerator. These were then transferred for analysis of LDH on the same day by StatFax 3300semi automated dry chemistry analyzer. The samples were analyzed using optimized kinetic method of Deutsche Gesselschaft fur Klinische Chemie (DGKC)

$$\text{Pyruvate} + \text{NADH} + \text{H+} \xleftrightarrow{\text{LDH}} \text{Lactate} + \text{NAD+}$$

The rate of absorbance changing at l=340nm is directly proportional to LDH activity.

STATISTICAL ANALYSIS

Statistical analysis of the obtained data was done by applying one way ANOVA and Post hoc analysis tests using computer software IBM Statistical Package for Social Sciences Version 20. It showed significant P value (P< 0.001).

RESULTS

Mean age of the study participants was 44.27 ± 10.47 years. The mean levels of serum LDH of 291.30 ± 30.55U/L, 414.73 ± 104.61 U/L, 451.17 ± 79.90 U/L and 494.55 ± 85.15 U/L was observed among periodontally healthy, chronic periodontitis, smokeless tobacco users with chronic periodontitis and smokers with chronic periodontitis patients, respectively.(Table 1)

TABLE 1: COMPARISON OF MEAN LACTATE DEHYDROGENASE AMONG THE FOUR GROUPS USING ANOVA TEST

Groups	N	Mean	Standard deviation	F value	P value
Healthy	30	291.30	30.55	35.93	<0.001*
Chronic periodontitis	30	414.73	104.61		
Smokers with Chronic Periodontitis	30	451.17	79.90		
Smokeless tobacco users with Chronic Periodontitis	30	494.55	85.15		
Total	120	412.94	109.52		

P < 0.05 – Significant, P < 0.001 – Highly significant

All the periodontal parameters i.e. GI, PPD and CAL showed positive correlation with all the groups. (Table 2,3,4) According to the increase in the GI, PPD and CAL, there was significant increase in the LDH level among all the groups. Comparison of mean serum LDH levels in different groups by ANOVA test showed statistically significant difference $P \leq 0.001$.

TABLE 2: CORRELATION BETWEEN THE GINGIVAL INDEX AND LACTATE DEHYDROGENASE LEVELS AMONG THE FOUR GROUPS USING ANOVA TEST

Groups	N	Mean	Standard deviation	F value	P value
Healthy	30	0.607	0.1964	513.042	<0.001*
Chronic periodontitis	30	2.097	0.2470		
Smokers with Chronic Periodontitis	30	2.207	0.2083		
Smokeless tobacco users with Chronic Periodontitis	30	2.360	0.1102		
Total	120	1.818	0.7345		

TABLE 3: CORRELATION BETWEEN THE POCKET PROBING DEPTH AND LACTATE DEHYDROGENASE LEVELS AMONG THE FOUR GROUPS USING ANOVA TEST

Groups	N	Mean	Standard deviation	F value	P value
Healthy	30	0.607	0.1964	513.042	<0.001*
Chronic periodontitis	30	2.097	0.2470		
Smokers with Chronic Periodontitis	30	2.207	0.2083		
Smokeless tobacco users with Chronic Periodontitis	30	2.360	0.1102		
Total	120	1.818	0.7345		

P < 0.05 – Significant, P < 0.001 – Highly significant

TABLE 4: CORRELATION BETWEEN THE CLINICAL ATTACHMENT LEVEL AND LACTATE DEHYDROGENASE LEVELS AMONG THE FOUR GROUPS USING ANOVA TEST

Groups	N	Mean	Standard deviation	F value	P value
Healthy	30	0.607	0.1964	513.042	<0.001*
Chronic periodontitis	30	2.097	0.2470		
Smokers with Chronic Periodontitis	30	2.207	0.2083		
Smokeless tobacco users with Chronic Periodontitis	30	2.360	0.1102		
Total	120	1.818	0.7345		

P < 0.05 – Significant, P < 0.001 – Highly significant

DISCUSSION

Periodontitis is a chronic inflammatory condition characterized by persistent inflammation, annihilation of connective tissue matrix and ruining of alveolar bone which is caused by bacterial infection of gingival tissues.[20] Periodontitis begins with a microbial infection, followed by a host-mediated destruction of soft tissue, clinically significant connective tissue and bone destruction caused by hyper activated or primed leukocytes and the generation of cytokines eicosanoids, and matrix metalloproteinases. Breakdown products are released into periodontal tissues and migrate towards the gingival sulcus as a consequence of resorption.[21] Periodontal disease reveals any one or more of their three phase of damage i.e inflammatory phase, connective tissue degradation phase or bone turnover phase wherein each of these is supposed to reflect in oral cavity.[22]

Numerous intracellular enzymes in the serum have been proposed as diagnostic tests for periodontal diseases, such as LDH, AST, ALT etc. These enzymes are mostly present in cells of soft tissues and are included in the metabolic process of cells. The metabolic change in the inflamed soft tissues is reflected by their increased release from the damaged cells of soft tissues.[23] LDH is a cytosolic enzyme, which is essentially present in all tissues involved in glycolysis. The enzyme leaks into extracellular fluids and then into body fluids with any destructive process of these tissues. Hence an elevated concentration of this enzyme released into the blood stream from the damaged tissues becomes a definitive diagnostic and prognostic criterion for various diseases and disorders and a study conducted of its isoenzyme has shown its importance in the location of tissue damage.[24] These enzyme release in high amounts in to blood because of the metabolic changes in inflamed gingiva.[21]

Malignant cells have a distinctive type of metabolism in which the glycolytic sequence and the tricarboxylic acid cycle are poorly

integrated, hence the cells tend to utilize five to ten times as much as glucose as do normal cells, converting most of it into lactate. Escape of LDH, due to damage of cells in any of these tissues, will tend to produce elevated serum levels.[25] Alteration of cellular enzyme level in blood is a reflection of the presence of some abnormality in the disease tissue or organ. This abnormality may be due to an altered amount of the enzyme forming tissue, an altered rate of synthesis of these enzymes within the tissue of origin, or an alteration in the permeability of the cell member brought about by the pathological condition.[26]

This explains the findings in the present study that the values of serum LDH activity in individuals with periodontal disease were found to be significantly higher than those obtained in patients with a healthy periodontium. This is in concurrent with findings of Smith et al who reported in a study that LDH levels were more in patients with increased probing depth compared to healthy probing depth.[27]

Cigarette smoke is a complex, oxidizing milieu possessing an array of free radicals and reactive oxygen species. The sustained release of reactive free radicals from the tar and gas phases of smoke imposes an oxidant stress, promotes lipid peroxidation and consequently disturbs the antioxidant defense systems in blood and tissues of smokers.[28] Cell membranes being primarily composed of lipids especially polyunsaturated fatty acids are particularly susceptible to attack by these free radicals from cigarette smoke, leading to increased permeability and altered fluidity of the membrane, and thereby causing cellular leakage. In the present study, cigarette smoke exposure resulted in a significant elevation of LDH in serum, which may be due to the increased leakage of this enzyme from the necrotic tissues into circulation.

Tobacco consists of innumerable chemical adjuncts and preservatives with carcinogenic potential responsible for multitude of oral and systemic maladies.[29] Toxicity of smokeless tobacco is attributed not only to its nicotine content but also to various other biologically active substances such as aldehydes and reactive oxygen species (ROS).[29] In the present study, an increase in LDH activity in smokeless tobacco users could be due to the cytotoxic effect of tobacco on blood cells leading to ROS – mediated cellular damage and LDH release.

CONCLUSION

The present study concludes that if LDH is adequately exploited can be used to measure and detect the rate of progression of an inflammatory condition. A development of a chairside test will be helpful in such cases. However, the LDH level may not be able to pinpoint the exact location of damage. It could also be used to detect the progression after treatment.[23]

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