Serum and salivary lactate dehydrogenase levels as biomarkers of tissue damage among cigarette smokers. A biochemical study

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SUMMARY

Objective. To estimate the serum and salivary lactate dehydrogenase levels in cigarette smokers and non-smokers.

Materials and Methods. In this study lactate dehydrogenase levels were estimated in 30 healthy individuals with no tobacco related habits and in 30 patients with history of smoking cigarettes for a minimum of 2 years using Spectrophotometry.

Results. The mean values for serum and salivary lactate dehydrogenase levels were higher in cigarette smokers when compared to non-smokers. Serum lactate dehydrogenase levels on comparison between the groups was statistically significant (p=0.04). The values of salivary lactate dehydrogenase levels between the groups was highly significant (p<0.001).

Conclusion. Cigarette smoking leads to an increase in serum as well as salivary Lactate dehydrogenase levels as indicator of tissue damage in the oral cavity. The present study indicates saliva as a better test medium than serum in determination of lactate dehydrogenase levels.

Key words: antioxidants, saliva, serum, cigarette smoking, lactate dehydrogenase.

INTRODUCTION

The most common cause of morbidity and mortality annually today is tobacco use, which causes more than 10 million new deaths and worldwide more than 6 million pre-mature deaths due to cancer (1). Of the total malignancies, the cancers of oral cavity are found to be 2% in Western Europe and North America, but in India it is up to 50% (2). Among high income countries 20% of all deaths are caused due to cancer whereas in low-income countries it is 10%. The cancer epidemic is low in high income countries, and high in low-income and middle-income countries due to high or increased levels of cancer risk factors (3). The percentage of cancer deaths occurring due to tobacco related substance abuse, alcohol consumption, unhealthy diet practices, sedentary lifestyles and infections stand to about 43% (4, 5). Cancer of the oral cavity, pharynx, larynx, oesophagus, stomach, pancreas, liver, kidney, ureter, urinary bladder, uterine cervix and bone marrow have been linked to tobacco use. Synergistic actions of tobacco use and alcohol consumption are causative to cancer of the oral cavity, pharynx, larynx and oesophagus (5).

In the world one-third of adults (1.3 billion people) are known to be smokers. Over 4000 bioactive chemical compounds have been isolated from cigarette smoke, of which more than 300 carcinogens have been identified in smoke or in its water-soluble components that leach into saliva (6). Oral cancer has been documented as one of the most common cause of malignancy in the head and neck region, with a global incidence of over 369,200 new cases reported in 2012. These cancers are responsible for nearly 145,328 deaths worldwide every year. The majority incidences of these cancers are usually reported from South and Southeast Asia and few countries of southern Europe (7).

A nineteen-fold increase of prevalence of oral SCC is seen when tobacco consumption is associated with alcohol, one hundred and twenty three-fold increase with chewing tobacco habits. The prevalence
of SCC among cigarette smokers is 4–7 times higher than among the non-smokers (7). Saliva bathes the mucosa of oral cavity up to the larynx and thus saliva intervenes and interacts with the cigarette smoke before it affects the mucosa (8).

Lactate Dehydrogenase (LDH) an ubiquitous enzyme as it is detectable in cytoplasm of almost every cell of the human body (9). Within the cell during the aerobic glycolysis, glucose is used for the production of pyruvate. Pyruvate enters the mitochondrial matrix, where it is oxidized by the action of pyruvate dehydrogenase, being transformed into acetyl CoA which enters citric acid cycle. In an anaerobic medium, pyruvate is reduced to Lactate, catalyzed by LDH, which uses Nicotinamide Adenine Dinucleotide (NAD) as a coenzyme (10).

LDH is an enzyme found in cytoplasm of almost every human cell, which becomes extracellular following cell necrosis, cell death and tissue breakdown (10). Increase in the activity of serum LDH levels among cigarette smokers has been attributed to smoking induced skeletal muscle damage which leak cellular contents along with LDH into the serum (11).

The existing literatures about the levels of LDH activity in saliva is scarce and shows variable results depending on the diversity of sampling, the handling and the methods of analysis used. The information obtained from the study could be useful in determining salivary LDH enzyme levels in smokers and non-smokers, to assess the level of tissue damage and risk of smoked tobacco in oral carcinogenesis.

The present study was aimed to estimate and compare serum and salivary LDH levels in cigarette smokers and in non-smokers, so as to establish salivary lactate dehydrogenase as a biomarker of tissue damage among cigarette smokers. The advantage is that salivary Lactate dehydrogenase is a better test medium since the collection is easy and non-invasive.

**MATERIALS AND METHODS**

Informed consent was obtained from the patients included in the study. Subjects between 20 years to 60 years of age, with no history of any tobacco related habits, systemic diseases, and who are not on any medications were included in the study as control group. The study group consisted of patients who
Subjects with any other tobacco related habits, systemic diseases, on any medications, any clinically detectable oral mucosal lesions and subjects with history of alcohol consumption were not included in the study.

The patient was asked not to consume any food 2 hours prior to the collection of saliva. Following a thorough mouth rinse using distilled water, saliva was allowed to accumulate in his or her mouth for 5 minutes. Accumulated saliva was collected by spit method (12). 2 ml of collected saliva was stored at a temperature of -200°C in plastic vials and analysis was carried out within 24 hours. Unstimulated saliva of 60 volunteers (30 smokers and 30 non-smokers) was collected.

Serum was extracted from venous blood which was collected from the ante-cubital vein. It was placed in plastic vials containing 3% citric acid and stored at -200°C and analysis was carried out within 24 hours.

Serum & saliva samples were then centrifuged and then analysed using standard kit (AGAPPE diagnostics). The samples were then subjected to Spectrophotometry method for estimation of serum & salivary lactate dehydrogenase levels.

Blood was collected from antecubital vein in plastic vials containing 3% citric acid. Centrifuged at 2500 rpm for 10 minutes, serum was separated and stored at -20°C. The analysis was carried out within 24 hours using GBC Avanta atom absorption spectrophotometer.

The LDH Working Reagent Composition was as follows:

1. LDH-P (S.L) R1: Tris buffer (pH 7.4) 80 mmol/L, Pyruvate 1.6 mmol/L, Sodium chloride 200 mmol/L.

2. LDH-P (S.L) R2: NADH 240 mmol/L.

1000 μL of the Working Reagent and 10 μL of the sample (serum/Saliva) was added. It was mixed and incubated at 37°C for 1 minute. The change was measured in the absorbance at 340 nm.

The activity of salivary LDH was measured in each group and compared. Statistical analysis was carried out using SPSS version 18. Statistical comparisons were done by Mann-Whitney U and Wilcoxon W test.

RESULTS

A case-control study on 30 cigarette smokers and 30 healthy individuals without any oral mucosal lesions was conducted.

Group A consisted of 30 healthy subjects without any oral lesions, no tobacco related habits. Group B consisted of cigarette smokers with a minimum of 2 years of smoking duration. All the subjects in the study were males.

In group A the age of the subjects ranged from 20 to 70 years. Majority (40.0%) of these cases were within 20-30 years, the mean age in this group being 34.9 years. In group B the age of the subjects ranged from 20 to 70 years. Majority (21.9%) of these cases were within 20-30 years. The mean age in this group was 43.31 years.
The mean values for serum and salivary LDH levels were higher among cigarette smokers than in non-smokers, which was calculated to be 532.6±77.1 IU/L and 422.2±94.9 IU/L respectively.

The correlations between non-smokers and smokers was done using Mann Whitney U test. The serum LDH levels on comparison between the groups was statistically significant (p=0.04) (Table, Figure 1). The values of salivary LDH levels between the groups was highly significant (p<0.001) (Table, Figure 2). The relation between serum and salivary LDLH levels among both the groups is depicted in Figure 3. The inter-durational comparison between serum and salivary LDH levels was carried out using Kruskal wallis test which was found to be not significant (Figures 4, 5). Correlation between frequency of smoking (grouped into <10 years and >10 years) and LDH levels among the two groups was also found to be not significant (Figures 6, 7). This correlation suggests that saliva can be considered as better diagnostic medium than serum.

**DISCUSSION**

The classical research of Warburg and associates in 1930 paved way for studies of enzymes in cancer. He reported that cancer tissue exhibited a greater degree of aerobic glycolysis than normal tissue (13).

Many investigators have worked on cancer enzymology especially, on the role of lactate dehydrogenase in various diseases. The variation in the levels of LDH in various systemic diseases has been well documented in tissue samples and serum.

LDH activity and isoenzyme patterns have been studied extensively in the various tissues mentioned and in the plasma; only a few evaluations have been performed in saliva and none in the oral epithelium (14). This, despite the fact that saliva collection is far

### Table. Comparision of serum and salivary LDH levels in group A and group B

<table>
<thead>
<tr>
<th>habit</th>
<th>N</th>
<th>Mean Rank</th>
<th>Sum of Ranks</th>
<th>Mann-Whitney U test</th>
<th>Wilcoxon W test</th>
<th>Z</th>
<th>Asymp. Sig. (2-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SERUM LDH U/L (350-550)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>30</td>
<td>26.65</td>
<td>799.50</td>
<td>334.500</td>
<td>799.500</td>
<td>-2.050</td>
<td>0.040</td>
</tr>
<tr>
<td>Group B</td>
<td>30</td>
<td>36.05</td>
<td>1153.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>SALIVARY LDH U/L (350-750)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group A</td>
<td>30</td>
<td>15.57</td>
<td>467.00</td>
<td>2.000</td>
<td>467.000</td>
<td>-6.733</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group B</td>
<td>30</td>
<td>46.44</td>
<td>1486.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td></td>
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</tbody>
</table>
easier, non-invasive, and cheaper than blood collection. It has been suggested that the main source of LDH in whole saliva was the oral epithelium, and not the salivary glands. LDH activity in saliva indicates oral mucosal breakdown, as LDH is a specific marker for cellular necrosis. Furthermore, various pathologic factors may affect the oral mucosa, leading to various epithelial alterations and may even lead to the development of oral squamous cell carcinoma. Therefore it was of great interest to study and characterize salivary LDH with its potential as a non-invasive diagnostic tool (14).

Saliva as test medium holds several advantages, as it can be collected easily, can be collected by all members of the dental team without the need for breaking the skin barriers, thereby greatly reducing the risk of contamination among patient and personnel (15).

Wroblewski and LaDue in have successfully measured the normal values of serum LDH using a spectrophotometer. They gave the normal range as 200 to 650 units per ml (85-300 IU/L) (16). Using the same method Elliot and Wilkinson have estimated the normal range to be 150-500 units per ml (72 to 240 IU/L) (17). The colorimetric method was developed by King J who had determined the normal range to be from 70-240 IU/L (18). In our study we have measured the LDH values using a spectrophotometer and among the controls the values were found to be in the range of 85-300 IU/L for serum and 360-430 IU/L for saliva.

Since LDH is detectable in cytoplasm of almost every cell of the human body its extracellular presence may indicate cell necrosis and tissue breakdown secondary to a pathological process (10). The "field cancerization" concept is the currently accepted explanation for the carcinogenic effect of cigarette smoke on oral mucosa. It states that when the oral epithelial cells are under constant, direct attack of cigarette smoke reagents, the effects gradually accumulate and result in a step-wise malignant transformation. Further evidence for the suggested role of free radicals in the pathogenesis of evolving oral SCC in the human oral cavity during areca quid chewing, may be due to the fact that the activity causes oxidative DNA damage to the surrounding tissues. Nishioka et al. in his study has found that saliva inhibited the mutagenicity of well-known oral cancer inducers such as cigarette smoke, 4-nitroquinoline 1-oxide and benzopyrene (19).

Leyva Huerta et al. conducted study to evaluate enzymatic activity of LDH in 15 male patients which consisted of smokers with chronic periodontitis, non-smokers with chronic periodontitis, and healthy subjects were in the LDH values were higher in smokers. They attributed the presence of intra-cellular enzymes like LDH in the gingival crevicicular fluid and saliva to cell death and tissue damage (20).

Nagler et al. carried out a study on 16 healthy subjects with a median age of 32 years. LDH activity and isoenzyme pattern was analysed in Saliva, plasma and oral epithelium following exposure of saliva and plasma to cigarette smoke. The LDH isoenzyme profiles at zero time, after 1 hour of exposure to CS, and after 3 hours of CS exposure were recorded. The whole salivary LDH isoenzyme profiles completely changed after 3 hours of CS exposure, whereas exposure of plasma to CS for 3 hours did not significantly affect the LDH isoenzyme profile (14). Though serum levels of LDH did not alter significantly in our study as reported by Nagler RM, the salivary LDH levels significantly increased in contradiction to the above study.

In a study carried out by De La Pen et al. on 175 volunteers who were over 18 years of age, oral and perioral examination was performed on the volunteers to determine oral health status. LDH activity was determined from stimulated whole saliva. The increase in LDH activity was associated with presence of calculus and pockets greater than 5 mms. The study concluded that LDH activity in whole saliva could be useful as a biochemical marker of periodontal disease status. (10) Studies have shown that cigarette smoke causes local tissue damage in the oral cavity, leading to increased LDH levels secondary to oral mucosal breakdown. This may be the reason for increased salivary LDH levels in our study (20).

Recent study conducted by Rai et al. among 4 groups which included smokers, non-smokers with healthy periodontium; and smokers, non-smokers with periodontal disease. They observed that a significant rise in salivary LDH was observed in smokers when compared to non-smokers (21). Similar results were observed in our study.

Rai et al. carried out a study in oral lichen planus (OLP) patients and healthy controls concluded that screening of OLP by measuring salivary LDH may be a feasible, simple and convenient approach (22).

There have been numerous studies on antioxidant activities in serum and saliva of Oral Cancer patients (15, 23-25). But there was no study comparing, correlating serum and salivary LDH values among smokers and non-smokers to date.

In a study by Shpitzer et al. on 19 tongue cancer patients, measuring the levels of 8 salivary markers including LDH determined by kinetic spectrophotometry, the activity of LDH had considerably increased
In the present study also the total LDH activity in saliva was seen to increase in cigarette smokers when compared to controls, which were determined by spectrophotometry.

Langvad et al. studied the LDH isoenzyme pattern of biopsies from Indian population with oral leukoplakia, submucous fibrosis and carcinoma of the oral mucosa as well as that of oral mucous membrane biopsies from clinically normal Indians. The mean isoenzyme ratio of Indian control biopsy was significantly above that reported for Danish control biopsies. They concluded that the high isoenzyme ratio of the Indian control material represented an early precancerous condition, probably preceding the histopathological manifestations of precancerous lesions (25). In this study the total LDH activity in serum was seen to increase in cigarette smokers, even better increase was noticed in the salivary LDH levels among smokers when compared to total LDH activity among the controls.

CONCLUSION

The current study was aimed to evaluate salivary LDH as a biomarker in the early pathogenesis stage of oral pre-cancer. Recent studies have revealed that changes in salivary LDH could precede dysplastic changes in oral epithelium. The outcome of our study reveals a possible relation between cigarette smoking and elevated salivary LDH levels which may be secondary to local tissue damage in oral cavity.

CONFLICT OF INTERESTS

None.

REFERENCES