ESTIMATION OF SERUM LACTATE DEHYDROGENASE LEVELS IN ORAL SUBMUCOSAL FIBROSIS PATIENTS, AND TOBACCO CHEWERS WITHOUT ANY POTENTIALLY MALIGNANT DISORDERS

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ABSTRACT: Oral cancer is the commonest cause of death in Indian population. It may arise from potentially malignant disorders. Early diagnosis and appropriate treatment will prevent progression of disease condition from premalignant level to malignant level. A simple biochemical parameter that helps to diagnose the disease in early stage is lactate dehydrogenase enzyme. LDH is present in almost all the cells of our body. According to Warburg hypothesis in case of hyperplasia the LDH level will be increased due to increased level of anaerobic glycolysis.

KEYWORDS: Serum Lactate Dehydrogenase, Potentially Malignant Disorders, OSMF

I. INTRODUCTION
Oral cancer is the commonest cause of death in Indian population. An estimated five-year survival has remained approximately 50% despite the advanced treatment modalities available. A detrimental factor is the lack of early diagnosis.¹ The main reason is attributed for the usage of tobacco, in some cases it arises from a potentially malignant disorder.² Potentially malignant disorder of oral cavity are relatively common occurring in about 2.5% of the population, with a malignant transformation rate in various studies and locations that range from 0.6 to 20%.³ Of all potentially malignant disorders OSMF is of greater concern because of its disabling nature and relatively greater chances of malignant transformation.

Oral Submucosal Fibrosis is an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Occasionally it is preceded by and/or associated with vesicle formation and is always associated with juxta-epithelial inflammatory reaction followed by progressive hyalinization of lamina propria. The later subeothelial and submucosal myofibrosis leads to stiffness of the oral mucosa and deeper tissues with progressive limitation in the opening of the mouth and protrusion of the tongue, thus causing difficulty in eating, swallowing and phonation.⁴ Early diagnosis and appropriate treatment will prevent progression of disease condition from premalignant level to malignant level. A simple biochemical parameter that helps to diagnose the disease in early stage is lactate dehydrogenase enzyme.

Lactate dehydrogenase (LDH) is a cytoplasmic enzyme present essentially in all body tissues. Catalyses the reaction of lactate production via pyruvate reduction during anaerobic glycolysis. Its extracellular presence is always related to cell necrosis and tissue breakdown. Serum LDH non-specifically increases in many pathological conditions such as myocardial infarction, megaloblastic anaemia, liver and renal diseases.⁵ studies have also suggested increased level of LDH in OSCC and several potentially malignant disorders due to increase in mitotic index and increased lactic acid production by breakdown of glycoproteins.⁶ Therefore Purpose of this study is to estimate the LDH levels in serum of subjects with OSMF and tobacco chewers without any lesions and compare them with normal individual.

II. MATERIALS AND METHOD

A. Source of data:
Patients were selected from those attending the Out Patient Department (OPD) of Maitri college of dentistry and research centre and divided into three groups as follows:
Study Groups:

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Group I: Normal individuals: 20 individuals
Group II: tobacco chewers without any lesions: 20 individuals
Group III: Patients with OSMF: 20 individuals

Total Sample Size: 60 individuals

B. Inclusion criteria:
- Patient’s willingness to participate.
- Subjects in the age group of 20-60 years irrespective of sex.
- Patients of OSMF.
- Individuals having habit of tobacco chewing without any lesions

C. Exclusion criteria:
- Systemic diseases known to increase serum LDH levels such as MI, liver diseases, renal disease, and muscle dystrophy
- Other oral conditions known to increase salivary LDH levels like periodontitis
- Patients with history of consumption of aspirin, narcotics or alcohol, and recent anesthesia.

D. Collection of blood sample
Collection of serum sample was done by the method recommended by National Institute of Health. Five millilitres of blood was drawn from the peripheral veins under aseptic conditions. Collected blood sample was kept in test tubes at room temperature for 30-60 minutes to allow sedimentation of cellular fraction of blood. Later, the sedimented blood sample was centrifuged at 3000 rpm for 10-15 minutes. Supernatant serum was separated out with the help of a micro-pipette. The quantification of LDH procedure was carried out on the same day that the blood was drawn.

E. Biochemical analysis
Biochemical analysis was carried out with a semi-automated machine and infinite liquid LDH reagent (UV kinetic) kit was used manufactured by Reckon Diagnostic Pvt. Ltd., Vadodara, India. contained 1 LDH (Coenzyme) and 2 LDH (Buffered substrate). One tablet of 1 LDH with 1.1 ml of 2 LDH were mixed gently to dissolve their contents. They were used after 5 minutes. It works on the principle that LDH catalyses the oxidation of lactate to pyruvate accompanied by the simultaneous reduction of NAD to NADH. LDH activity in serum is proportional to the increase in absorbance due to the reduction of NAD.
Now 1 ml of working reagent was mixed with 0.05 ml of the serum sample. Reading of first absorbance of the test was noted exactly at 1 minute and thereafter at 30, 60 and 90 seconds at 340 nm of light. Determination of mean change in absorbance per minute and calculation of test results in the semi-automated biochemistry analyser was done and the results were obtained.

III. RESULTS
The comparisons of mean LDH levels between groups were done using ANOVA followed by Tukey HSD for multiple comparisons.

A. Sex wise comparison of LDH levels between study population and control
In the control group, the mean value of LDH was 255.07± 9.34 for males, 296.6 IU/L for females and total of 259.23±15.81 IU/L. In the tobacco chewers mean value of LDH was 273.00± 15.78 IU/L for males and total of 273.00±15.78 IU/L ± 33.34. In the patients with OSMF mean value of LDH was 323.42±30.28 IU/L for males 316.8 IU/L for females and total of 322.76±28.63 IU/L [Table 1 and Graph 1].

B. Comparative analysis of IDH level in various groups
Mean LDH level in control group, OSCC group, tobacco chewer group and OSMF group subjects was 259.23 IU/L, 273.00 IU/L, and 322.76 IU/L respectively. [Tables 2 and 3, and Graph 2]. ANOVA results showed a significant F ratio (F = 25.409; P < 0.001) and individual group comparison using Tukey HSD showed a significantly very high mean LDH levels with OSMF subjects and very low levels in controls. Mean LDH levels of OSMF subjects were significantly higher than control and tobacco chewer group subjects. Mean levels of LDH of tobacco chewer was higher than control group subjects.
Oral submucous fibrosis is a chronic insidious and a disabling condition. The popularity and commercial availability of tobacco and betel nut products etc. has been associated with a sharp increase in the frequency of OSMF. Tumor markers present in serum during neoplastic transformation have a significant value in the early diagnosis of the disease. One of the main enzyme involved in anaerobic glycolysis is the lactate dehydrogenase Which is overexpressed by cancer cells and is linked with the tumors in hypoxia.

Lactate dehydrogenases (LDHs) belong to a wide group of 2-ketoacid:NADP-dependent dehydrogenases that catalysse the reversible conversion of 2-hydroxyacids to the corresponding 2-ketoacids. Lactate dehydrogenase catalyses the oxidation of L-lactate to pyruvate with Nicotinamide-Adenine Dinucleotide (NAD+) as the hydrogen acceptor.

### Table 1. Sex wise comparison of LDH levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Male (N)</th>
<th>Female (N)</th>
<th>Total (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>255.07±9.34 (n=18)</td>
<td>296.6 (n=2)</td>
<td>259.23±15.81 (n=20)</td>
</tr>
<tr>
<td>Tobacco (2)</td>
<td>273.00±15.78 (n=20)</td>
<td>-----</td>
<td>273.00±15.78 (n=20)</td>
</tr>
<tr>
<td>OSMF (3)</td>
<td>323.42±30.28 (n=18)</td>
<td>316.8 (n=2)</td>
<td>322.76±28.63 (n=20)</td>
</tr>
</tbody>
</table>

Significance:
- T 3/1 = 6.4 ; p = 0.001*
- T 2/3 =4.6 ; p = 0.001*
- T 2/1 = 2.9; p = 0.009*

### Table 2. Comparative analysis of LDH

<table>
<thead>
<tr>
<th>(I) group</th>
<th>(J) group</th>
<th>Mean Difference (I-J)</th>
<th>Std. Error</th>
<th>Sig.</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>osmf</td>
<td>tobacco</td>
<td>49.760*</td>
<td>9.377</td>
<td>.001*</td>
<td>26.51 - 73.01</td>
</tr>
<tr>
<td>osmf</td>
<td>control</td>
<td>63.530*</td>
<td>9.377</td>
<td>.001*</td>
<td>40.28 - 86.78</td>
</tr>
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</table>

### Table 3. Multiple Comparisons (Tukey HSD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sample (N)</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
<th>F value</th>
<th>p value</th>
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<tbody>
<tr>
<td>osmf</td>
<td>20</td>
<td>322.76</td>
<td>28.630</td>
<td>9.054</td>
<td>289</td>
<td>397</td>
<td>25.409</td>
<td>0.001*</td>
</tr>
<tr>
<td>tobacco</td>
<td>20</td>
<td>273.00</td>
<td>15.783</td>
<td>4.991</td>
<td>241</td>
<td>289</td>
<td>241</td>
<td>297</td>
</tr>
<tr>
<td>control</td>
<td>20</td>
<td>259.23</td>
<td>15.811</td>
<td>5.000</td>
<td>244</td>
<td>297</td>
<td>241</td>
<td>297</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>285.00</td>
<td>34.345</td>
<td>6.271</td>
<td>241</td>
<td>397</td>
<td>241</td>
<td>297</td>
</tr>
</tbody>
</table>

Graph 1. Sex wise comparison of LDH levels

Graph 2. Comparison of mean LDH value in various groups

**IV. DISCUSSION**

Oral submucous fibrosis is a chronic insidious and a disabling condition. The popularity and commercial availability of tobacco and betel nut products etc. has been associated with a sharp increase in the frequency of OSMF. Tumor markers present in serum during neoplastic transformation have a significant value in the early diagnosis of the disease. One of the main enzyme involved in anaerobic glycolysis is the lactate dehydrogenase Which is overexpressed by cancer cells and is linked with the tumors in hypoxia.

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The enzyme is composed of four peptide chains of two types: M (Muscle) and H (Heart), each under separate genetic control. Lactate dehydrogenase, a cytoplasmic enzyme is present essentially in all major organ systems. The extracellular appearance of LDH is used to detect cell damage or cell death.\textsuperscript{10} Results of our study suggested that males were more commonly affected from OSMF than females. Similar observations were made in studies conducted by Rajendran R (1994).\textsuperscript{11} Results clearly suggest that mean levels of LDH were significantly higher in OSMF group subjects compared to control subjects. Similar observations were made in studies conducted by Joshi PS \textit{et al} (2012), and Kamath VV \textit{et al} (2013).\textsuperscript{6,12} Kamath \textit{et al.} (2013) in their study found that tissue breakdown releases LDH and therefore, LDH can be measured as a surrogate for tissue breakdown, e.g., haemolysis. Elevated levels were seen in OSMF patients indicating evidence of tissue breakdown.\textsuperscript{12} The serum LDH level we found in this study among control group was in accordance with the value found by Drent \textit{et al.}1996.\textsuperscript{13} As per our knowledge no literature is available for comparison of serum LDH levels of tobacco chewers with control group, but according to this study the mean LDH level of individual with habit was greater than the control group.(although it was statistically not significant) The LDH activity in OSMF cases can be related to muscle fatigue.\textsuperscript{14} Another reason may be hypoxia, which triggers glycolytic pathways. Increased hypoxia plays a role in malignant transformation and progression of OSMF.\textsuperscript{15}

\section*{V. CONCLUSION}

Based on the results of this study it can be concluded that the activities of LDH enzyme was significantly increased in OSMF patients. The clinical diagnosis supplemented with LDH levels can gain diagnostic importance in near future. The LDH levels can also be used as valuable aid in monitoring treatment outcomes in OSMF patients.

\section*{REFERENCES}

5. Glick JH. Serum lactate dehydrogenase isoenzyme and total lactate dehydrogenase values in health and disease, and clinical evaluation of these test by means of discriminant analysis. Am J Clin Pathol 1969;