ORIGINAL RESEARCH PAPER

Volume-7 | Issue-4 | April-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

SALIVARY LDH - A SUBSTITUTE FOR SERUM LDH AS A BIOMARKER IN POTENTIALLY MALIGNANT DISORDERS AND ORAL MALIGNANCY: A BIOCHEMICAL STUDY.

Oncology

Ramlal Gantala* Professor & HOD, Department of Oral Medicine & Radiology, SVS Institute of Dental Sciences, Mahabubnagar.*Corresponding Author

Srikar Muppirala Post graduate trainee, Department of Oral Medicine & Radiology, SVS Institute of Dental Sciences, Mahabubnagar.

Tejaswi Katne Post graduate trainee, Department of Oral Medicine & Radiology, SVS Institute of Dental Sciences, Mahabubnagar.

Rama Raju Devaraju Professor, Department of Oral Medicine & Radiology, SVS Institute of Dental Sciences, Mahabubnagar.

Rashmitha Arutla Assitant professor, Department of Oral Medicine & Radiology, SVS Institute of Dental Sciences, Mahabubnagar.

Leela Rani K Post graduate trainee, Department of Oral Medicine & Radiology, Govt. Dental College & Hospital, Afzalgunj, Hyderabad.

ABSTRACT

Tumor markers are the substances which quantitatively change in serum during tumor development. They have now been introduced in the diagnosis of potentially malignant disorders (PMDs) & malignant lesions. One such is Lactate Dehydrogenase (LDH) which is found in cells of almost all body tissues. LDH activity in serum increases as a marker of cellular necrosis. Serum LDH levels have been used as a biochemical marker in diagnosis of various body cancers. Profile of salivary LDH is similar to that found in oral epithelium indicating major source of salivary LDH is probably the oral epithelium shedding cells.

Aims and objectives: The current study was aimed to measure and compare LDH levels in serum and saliva in patients of oral sub mucous fibrosis (OSMF), oral leukoplakia (OL) and oral squamous cell carcinoma (OSCC).

Materials and methods: Clinically diagnosed and histopathologically confirmed 10 cases each of OL, OSMF and OSCC were selected and compared with control. After taking an informed consent, serum and unstimulated whole saliva was collected and processed for LDH measurement using LDH ERBA KIT.

Results: Increased LDH activity is seen in both serum as well as saliva in patients with OL, OSMF and OSCC in comparison to control.

Conclusion: salivary LDH estimation can prove to be a valuable substitute to serum LDH as a biochemical marker as it is a simple, non-invasive procedure and easily accepted by the patients.

KEYWORDS

Oral leukoplakia, Oral sub mucous fibrosis, Oral squamous cell carcinoma, lactate dehydrogenase.

INTRODUCTION

Cancer is one of the leading causes of adult deaths worldwide. Oral cancer is a serious problem in many countries. It accounts for significant mortality and is also responsible for extensive disfigurement, loss of function, behavioural changes, financial and sociologic hardship.1 Development of oral cancer is a multistep process, arising from pre-existing potentially malignant disorders (PMDs).[2]

Role of tumor markers in management of head and neck cancer has received increasing attention. Tumor markers in serum, tissue and other body fluids during neoplastic process are of clinical value in the management of patients with various body cancers. Among all the body fluids, blood has been the media of choice for the study of the biochemical markers by the medical community but it do have some inherent disadvantages.[3]

Collecting blood for investigation is an invasive procedure and has a potential risk of disease transmission through needle stick injuries. Despite the absence of charisma, however, a growing number of researchers are finding that saliva provides an easily available, non-invasive diagnostic medium for rapidly widening range of disease and clinical situations.[4]

Lactate dehydrogenase (LDH) activity is mainly due to genomic changes during malignant transformation. Increased LDH levels are due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoprotein. Value of LDH elevates in oral squamous cell carcinoma. (OSCC) and PMDs; this finding can be used for benefit of the patient in predicting prognosis.1 Consequently, LDH concentration in saliva as an expression of cellular necrosis can be considered to be a specific indicator for lesions affecting the integrity of the oral mucosa. Hence, the present study was done in an attempt "To Measure and Compare Serum and Salivary LDH Levels in Patients with Malignancy and PMDs"

MATERIALS AND METHODS:

Source of data:

The patients with oral malignancy and PMDs visiting the Department of Oral Medicine and Radiology, SVS Institute of Dental Sciences, Mahabubnagar were included for the study.

40 subjects were included in the study which were divided in to four groups

Group I: 10 Normal healthy subjects.
Group II: 10 Patients with oral sub mucous fibrosis (OSMF)
Group III: 10 Patients with oral leukoplakia (OL)
Group IV: 10 Patients with OSCC

Inclusion criteria

Patients who are clinically diagnosed and histopathologically confirmed as OSCC and oral PMDs.

Exclusion Criteria

- Patients treated for cancers (surgery, chemotherapy, radiotherapy)
- Systemic diseases known to increase serum LDH levels such as myocardial infarction, liver diseases, renal disease, and muscle dystrophy.
- Immuno compromised patients.
- Individuals with other mucosal lesions

Sample Collection

After obtaining informed consent from the patient, 5ml of blood was drawn from the peripheral veins (Brachial or Antecubital Vein) under aseptic conditions. Collected blood sample was kept in test tubes at room, 5 ml of unstimulated whole saliva was aseptically collected by the spit method in a wide mouthed container. Care was taken to see that the volunteers did not Consume food or chew gum at least one hour

before and smoke three hours before the saliva collection procedure. Following, the collected sample was centrifuged at 2500 rpm for 15 minutes and the samples will be diluted in 1:1 ratio with saline then it was assayed using the standard kit and measured spectrophotometrically at 340 nm. Since saliva supernatant was used which can be treated like serum the same kit was used to process both the samples.

Aims and Objectives

1. To measure and compare serum and salivary LDH levels in patients with PMDs and OSCC.
2. To correlate LDH levels among healthy individuals, PMDs and OSCC.
3. To evaluate whether salivary analysis of LDH can substitute serum LDH analysis.
4. To evaluate if these levels can be used as biomarker in the progression in to PMDs.

STATISTICALANALYSIS:

The data collected is statistically analysed by ANOVA and student 't' test. The data was analysed by using statistical package for social sciences software (SPSS software, version 22.0). All the 'p' values <0.05 were considered as statistically significant.

RESULTS AND DISCUSSION:

Of the 40 subjects included in the present study 28 were male and 12 were female, with a mean age of 45.85 years ranging from 25-75. Mean ages of the groups I, II, III & IV are 33.8, 48.9, 50.7 & 50.0 respectively.

Mean serum LDH levels in controls (group I) are 221.6±36.5 IU. In group II, III & IV the serum LDH are 540.6±117.4, 406.3±78.06 and 1076±186.5 respectively.

Mean salivary LDH levels in the groups I, II, III & IV are 200.3±13.74, 490.5±76.18, 354.0±56.43 and 1023±161.4.

Table 1 shows mean serum and salivary LDH levels in the study groups. Comparison of serum and salivary LDH values in different groups are depicted in table 2.

Table 1: Mean serum and salivary LDH levels in various groups.

	Group I	Group II	Group III	Group IV
Mean[serum]	221.6	540.6	406.3	1079
Std. Deviation	36.50	117.4	78.06	186.5
Mean[saliva]	200.3	490.5	354.0	1023
Std. Deviation	13.74	76.18	56.43	161.4

Table 2: Comparison of mean LDH values in various groups.

	Conrol		OSMF		Leukoplakia		OSCC	
Sample	Serum	Saliva	Serum	Saliva	Serum	Saliva	Serum	Saliva
Minimum	169.9	180.0	388.2	400.9	287.2	254.9	826.1	875.7
25% Percentile	189.9	185.6	440.4	422.7	313.7	300.2	895.2	891.9
Median	222.3	201.8	507.7	474.7	420.6	375.9	1069	961.6
75% Percentile	246.5	212.6	656.7	567.2	469.4	398.1	1235	1186
Maximum	280.7	220.1	702.1	585.8	511.2	419.8	1381	1316

In both serum and salivary samples, one-way ANOVA showed statistical significance (<0.0001) in all the 4 groups. [Tables 3, 4]

Table 3: one way ANOVA serum.

Groups	N	Range	Mean	Sd	P value
Control	10	169.9-280.7	221.6	36.5	<0.0001
OSMF	10	388.2-702.1	540.6	117.4	
Leukoplakia	10	287.2-511.2	406.3	78.06	
OSCC	10	826.1-1381.0	1079.0	186.5	

Table 4: one way ANOVA saliva.

Groups	N	Range	Mean	Sd	P value
Control	10	180.0-220.1	200.3	13.74	<0.0001
OSMF	10	400.9-585.8	490.5	76.18	
Leukoplakia	10	254.9-419.8	354.0	56.43	
OSCC	10	875.7-1316.0	1023.0	161.4	

Bonferroni's post-hoc test showed statistical significance in intra group multiple comparisons of saliva as well as serum samples.

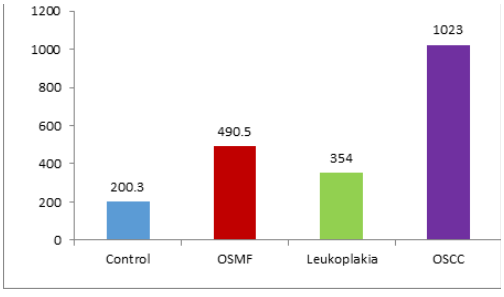
Karl Pearson's correlation coefficient(r) for groups I, II, III & IV are 0.783, 0.961, 0.951 and 0.817 respectively, with overall 'r' value of 0.984, $p \leq 0.004$

DISCUSSION

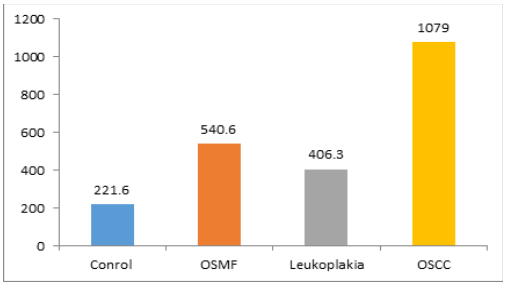
The use of biomarkers as a diagnostic tool during dental examinations could be helpful in early diagnosis of oral cancer. In recent years, total LDH and LDH isoenzyme activity has been used for screening oral potentially malignant disorders like OSMF, leukoplakia, lichen planus.[5]

Lactate dehydrogenase (LDH) is an intracellular enzyme that catalyzes 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 nonspecifically increases in many pathological conditions such as myocardial infarction, megaloblastic anemias, liver and renal diseases. LDH concentration in saliva could be a specific indicator for oral lesions that affect the integrity of the oral mucosa.[6]

In the present study the mean values of serum & salivary LDH values appears to be elevated in the groups II, III & IV compared to the controls. [Graphs 1,2].



Graph 1: mean serum LDH values



Graph 2: mean saliva LDH values

A study piloted by Langavad et al (1970)7 involving 29 OL, 25 OSMF, 10 OSCC and 32 healthy subjects in South Indian population to evaluate LDH isoenzyme patterns which proved that the mean isoenzyme ratio in OSMF did not differ statistically from the average ratio in OSCC, but significantly differed from that of control ($p < 0.05$) as well as from that of OL ($p < 0.01$) which were in concordance with the current study.

Schneider et al (1980) 8 conducted a study on 30 subjects to evaluate prognostic significance of serum LDH levels for malignant lymphoma who were treated, has shown a highly significant difference between survival patterns of patients and serum LDH levels. Patients with serum LDH levels greater than 500 IU experienced considerably shorter survival times, when compared to those patients whose LDH levels were 500 IU or less ($p = 0.003$). Though the disease entity taken were different significant elevation in LDH levels in cancer subjects were noticed in this study as well as in the present study

In a study done by Shipitzer et al (2007) 9 on 25 OSCC and 25 healthy subjects to evaluate biochemical parameters in the saliva and stated that there was 88% increase in the levels of salivary LDH in OSCC subjects when compared to control which was highly significant ($p = 0.002$) and is in accordance with the results of the present study.

In a study conducted by Shetty et al (2012) 10 75 subjects were included of which 25 healthy controls, 25 OL, 25 OSCC comparing salivary LDH levels and suggested that there is a significant increase in

salivary LDH levels in OSCC and OL than controls. Results of the present study reproduces the outcome obtained by Shetty et al.

Joshi et al (2012) 11 piloted a study to compare serum and salivary LDH levels in 7 subjects each of OL and OSCC who are clinically diagnosed with control and stated that the LDH activity increased in serum as well as saliva in patients with OL and OSCC when compared with control. Similar inference with higher sample size was noticed in the present study.

Kadiyala SK (2015) 2 conducted a study comparing salivary LDH levels on total of 60 subjects of which 20 were normal subjects, 20 OSMF, 20 OSCC and stated that the mean salivary LDH level in healthy controls were compared to OSMF and OSCC groups (126.7 ± 58.2 IU/L, 612.2 ± 328.9 IU/L and 515.7 ± 257.8 IU/L respectively) and proved to have a very high significant difference which is in accordance with the present study.

In a comparative study done by Rathore A et al (2015) 12, A total of 120 subjects of which 30 OSCC, 30 OL, 30 OSMF and 30 normal subjects were included to assess serum LDH levels and compare the same and observed that serum LDH activity was significantly ($P < 0.05$) increased in patients with OSCC, OL and OSMF. Even in the present study the serum and salivary levels are elevated.

Patel S et al (2015) 13 conducted a study on 75 subjects of which 25 healthy subjects, 25 with OL, 25 with OSCC to compare salivary LDH levels and revealed that salivary LDH levels showed tremendous increase from healthy control group to OL group to further increase in OSCC group. Bonferroni's post-hoc test results of the present study too showed the similar results backing the literature in terms of statistical significance.

Conclusion

Many researchers and investigators have been constantly probing to arrive at promising ways for early detection of cancer, so as to decrease the number of morbidity and mortality related to oral cancer. Lactate dehydrogenase is one of the biomarker which is elevated in both potentially malignant disorders and oral carcinoma. As serum is an invasive procedure which is not readily accepted by the patient. Henceforth, the present study stated that saliva can be a substitute to serum as it is simple, non- invasive and easily accepted by the patient.

REFERENCES:

1. Wood NK, Goaz PW. Differential Diagnosis of Oral and Maxillofacial Lesions. 5th ed. St. Louis, Missouri: Elsevier; 2006. p. 587
2. Kadiyala SK. A study of Lactate Dehydrogenase (LDH) levels in Oral Cancer and Oral Sub Mucosal Fibrosis Patients amoung the normal individuals. J Pharma Sci & Res Vol 7(7), 2015,455-57
3. Denny YP, CM Ho. The oral fluid MEMS/NEMS Chips (OFMNC): diagnostic and translational application. Adv Dent Res 2005;18:3-5.
4. Bigler LR, Streckfus CF, Dubinsky WP, et al. Salivary biomarkers for the detection of malignant tumors that are remote from the oral cavity. Clin Lab Med 2009;29:71-85.
5. Mohan N, Krithika S , Mathew S A study of salivary and serum lactate dehydrogenase levels in tobacco users and potentially malignant JMSCR 2017;5(2): 17638-43
6. Bhambal AM, Ingle N, Bhambal A. Salivary Lactate Dehydrogenase Enzyme Activity in Oral Submucous Fibrosis: A Biochemical and Clinicopathological Study. J Dent Oral Health. 2016; 2 (4) 40-6
7. Langavad E, Zachaiah J, Pindborg JJ. Lactate dehydrogenase isoenzyme patterns in leukoplakia, submucous fibrosis and carcinoma of the oral mucosa in South Indians. Acta Pathol Microbial Scand 1970;78:509-15
8. Schneider RJ, Seibert K, Passe S, Little C, Gee T, Lee BJ, et al. Prognostic significance of serum lactate dehydrogenase in malignant lymphoma. Cancer 1980;46:139-43
9. Shipitzer T, Bahar G, Feinmesser R, Nagler RM. A comprehensive salivary analysis for oral cancer diagnosis. J Cancer Res Clin Oncol 2007;133:613-17
10. Shetty SR, Chadha R, Babu S, Kumari S, Bhat S, Achalli S. Salivary lactate dehydrogenase levels in oral leukoplakia and oral squamous cell carcinoma: a bio chemical and clinicopathological study.Shetty SR et al J Cancer Res Ther 2012
11. Joshi PS, Chougule M, Dudankar M, Golgire S.Comparison between salivary and serum lactate dehydrogenase levels in patients with oral leukoplakia and oral squamous cell carcinoma- a pilot study. Int J Oral Maxillofac Path; 2012 :3(4): 07-12
12. Rathore A, Nagarajappa AK, sreedevi. Evaluation of serum lactate dehydrogenase in oral squamous cell carcinoma, oral leukoplakia and oral sub mucous fibrosis. J Indian Acad Oral Med Radiol 2015; 27: 29-34
13. Patel S, Metgud R . Estimation of salivarylactate dehydrogenase in oral leukoplakia and oral squamous cell carcinoma: A biochemical study. J Can Res Ther 2015;11:119-23