International Journal of Dentistry Research

Research Article

IJDR 2017; 2(2): 31-35

www.dentistryscience.com

Estimation of serum and salivary lactate dehydrogenase levels among healthy individuals and oral cancer patients- A clinical and biochemical study

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Abstract

Background: Cancer is one of the most common causes of morbidity and mortality today, with more than 10 million new cases and more than 6 million deaths each year worldwide. Hence an accurate and sensitive method for detecting early oral cancerous lesions as well as predicting regional recurrence and/or spreading metastases is of paramount importance. The objective of the present study was to estimate the serum and salivary lactate dehydrogenase levels among healthy individuals and in patients with oral cancer. Also the present study aimed to suggest saliva as a better diagnostic tool than serum, as it can be easily collected without the need for breaking the skin barrier, thereby greatly reducing the risk of contamination among the patient and the personnel. **Materials and Methods:** In this study lactate dehydrogenase levels were estimated in 30 healthy individuals and 30 oral cancer patients using Spectrophotometry. **Results:** The mean values for serum and salivary lactate dehydrogenase levels were higher in oral cancer patients in comparison with healthy individuals. The mean serum and salivary values among the study group showed marked increase in salivary LDH levels than the serum LDH values. Comparison of serum and salivary LDH levels between control group and study group was highly significant ($p<0.001$). Correlation between serum and salivary LDH values among the control group and study group yielded highly significant values ($p<0.001$) for salivary LDH. **Conclusion:** With the application of a standardized method for saliva collection, storage and handling, the LDH levels in whole saliva could be useful as a biochemical marker in the detection of oral malignancy.

Keywords: Antioxidants, Saliva, Serum, Lactate Dehydrogenase, Oral potentially malignant disorders, Oral cancer.

INTRODUCTION

There is an increasing attention towards the use of biomarkers in the diagnosis of potentially malignant disorders and cancerous patients in the recent years [1]. Biomarkers are being used increasingly in bodily fluids for early detection of cancer, prognostic patient stratification of potentially malignant as well as malignant disorders and in patient surveillance after primary treatment.

All along the history of clinical investigations Biomarker estimation mainly focused on blood or body tissues as the medium. But in the recent years, due to the several advantages it holds, saliva has immerged as a potential test medium. Saliva as a potential source of biomarker holds benefits like ease and comfort in obtaining the sample with reduced risk of contamination among patients and/ or personnel [1].

LDH (Lactate dehydrogenase) is an 'ubiquitous' enzyme which was discovered in the early period of enzymology [2]. When the cells of human body become extracellular upon cell death, LDH becomes detectable in the cell cytoplasm. Hence the extracellular presence of LDH is usually related to cell necrosis and tissue breakdown. Therefore its serum activity non-specifically increases in various pathological conditions [3].

During the aerobic glycolysis LDH is formed within the cell. Glucose which is used primarily for the production of pyruvate enters the mitochondrial matrix. There it is oxidized into acetyl CoA by the action

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of pyruvate dehydrogenase which enters the citric acid cycle. In an anaerobic medium, pyruvate thus formed is reduced to Lactate by the catalystic enzyme LDH, using Nicotinamide Adenine Dinucleotide (NAD) as a coenzyme [3].

The literatures existing about the levels of LDH activity in saliva are scanty and show variable results depending on the diversity of sampling, the handling and the methods of analysis used. The information obtained from this study could be useful in determining LDH as a salivary biomarker in the early detection and diagnosis of oral cancer.

MATERIALS AND METHODS

A randomised case control study was performed on 60 subjects who had reported to department of Oral Medicine and Radiology. Ethical clearance was obtained prior to conducting the study. Informed consent was obtained from these subjects after informing the nature and purpose of the study. The subjects included in the study were between 20 years to 70 years of age. A detailed case history was recorded and thorough oral examination was performed. The total sample size was 60 subjects. The control group (Group C) consisted of 30 healthy subjects without any oral mucosal lesions and with no history of substance abuse. The study group (Group S) consisted of 30 subjects with histo-pathologically confirmed oral cancer. Subjects with history of any systemic diseases, other known malignancies, pregnant women and subjects on medications or with any other oral mucosal lesions were excluded from the study.

The patients were asked not to consume any food for 2 hours prior to the collection of saliva. Following a thorough mouth rinse with distilled water, saliva was allowed to accumulate in the mouth for 5 minutes under resting conditions. Spit method was used to collect accumulated saliva. 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 subjects (30 healthy subjects and 30 subjects with oral cancer) was collected. After collection, saliva was centrifuged at 800 rpm for 10 minutes at 48 °C. The supernatant collected was subjected to biochemical analysis.

Venous blood which was collected from the ante-cubital vein and serum was extracted. It was placed in plastic vials containing 3% citric acid and stored at -20 °C and analysis was carried out within 24 hours.

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

The activity of serum and salivary LDH was measured in each group and compared. Statistical data analysis was carried out using SPSS version 18. ANOVA test for comparison between the groups, Chi- Square Test for the association of the age, gender with different parameters. Correlations between the groups were done using Pearson's correlation.

RESULTS

A randomised case-control study on 30 healthy subjects and 30 subjects with oral cancer was conducted.

Group C consisted of 30 healthy subjects without any oral lesions, no tobacco related habits and Group S consisted of 30 subjects with oral cancer.

Demographic data analysis of the study groups:

Demographic data analysis of group C:

In this group the age of the subjects ranged from 20 to 70 years. Majority (40 %) of these cases were within 20-30 years, followed by <20 years (20%), 30-40 years (13.3%), 40-50 years (13.3%), 50-60 years (6.7%) and 60-70 years (6.7%) [Table 1]. The mean age in this group was 34.9 years [Table 2]. Males comprised 53.3% of this group (16/30), while females formed the remaining 46.7% of the group (14/30) [Table 3].

Table 1: Age Distribution

Age- groups		Oral cancer	Control
=20	Count	0	6
	%	.0%	20.0%
20 -30	Count	0	12
	%	.0%	40.0%
30 -40	Count	2	4
	%	6.7%	13.3%
40 -50	Count	6	4
	%	20.0%	13.3%
50 – 60	Count	12	2
	%	40.0%	6.7%
60 – 70	Count	7	2
	%	23.3%	6.7%
=70	Count	3	0
	%	10.0%	.0%
Total	Count	30	30
	%	100.0%	100.0%

Table 2: Age distribution in each group

	N	Mean	Std. Deviation	Minimum	Maximum
Oral Cancer	30	57.2667	9.54096	37.00	76.00
Control	30	34.9000	14.87500	20.00	70.00

Table 3: Gender distribution in each group

Gender		Oral Cancer	Control	Total
Male	Count	20	16	36
	%	66.7%	53.3%	60%
Female	Count	10	14	24
	%	33.3%	46.7%	40%
Total	Count	30	30	60
	%	100.0%	100.0%	100.0%

Demographic data analysis of group S:

In this group the age of the subjects ranged from 20 to 70 years. Majority (40%) of these cases were within 50-60 years, followed by 60-70 years (23.3%), 40-50 years(20%), >70 years (10%), 30-40 (6.7%) [Table 1]. The mean age in the group was 57.27 [Table 2]. Males comprised 66.7% of this group (20/30), while females formed the remaining 33.3% of the group (10/30) [Table 3].

Analysis of mean serum LDH and mean salivary LDH values between control and cancer groups:

Mean serum LDH levels:

The mean serum LDH level in Group C was 390.8667+ 71.0953 IU/L whereas the mean serum LDH level of Group S was 540.5033+ 88.8147 IU/L respectively [Table 4].

Mean salivary LDH levels:

The mean salivary LDH levels in Group S was estimated as 906.4183+239.46458 IU/L and seen to be higher than that of Group C which was calculated to be 201.3700+89.1439 IU/L [Table 5].

Table 4: Mean serum LDH levels

		N	Mean	Std. Deviation	Minimum	Maximum
Serum LDH	Control	30	390.8667	71.0953	88.80	543.20
	Oral Cancer	30	540.5033	88.8147	345.90	691.40
ANOVA						
			F		Sig.	
Saliva LDH	Between Groups		97.258		0.000	

Table 5: Mean salivary LDH levels

		N	Mean	Std. Deviation	Minimum	Maximum
Salivary LDH	Control	30	201.3700	89.1439	69.40	378.50
	Oral Cancer	30	906.4183	239.4646	376.10	1560.00
ANOVA						
			F		Sig.	
Serum LDH	Between Groups		20.524		0.000	

Duration of areca use in oral cancer:

Serum LDH levels: Serum LDH levels in areca users of 1-10 year duration was 511.0000+80.3273 IU/L, while those who chewed areca for 10-20 years, 20-30 years and 30 years was 546.8667+83.2856 IU/L, 518.3000+106.7784 IU/L and 594.1200+58.2612 IU/L respectively [Table 6].

Salivary LDH levels: Salivary LDH levels in areca users of 1-10 year duration was 941.450000+418.2537 IU/L, while those who chewed areca for 10-20 years, 20-30 years and more than 30 years was 839.6767+87.9725 IU/L, 878.7821+242.1149 IU/L and 900.3180+231.0400 IU/L respectively [Table 6].

Table 6: Serum and salivary LDH levels and duration of areca use in oral cancer

Duration of areca usage (In Years)	Serum LDH U/L (85-300 IU/L)	Salivary LDH U/L (360-430 IU/L)
1-10	511.0000±80.3273	941.4500±418.2537
10-20	546.8667±83.2856	839.6767±87.9725
20-30	518.3000±106.7784	878.7821±242.1149
>30	594.1200±58.2612	900.3180±231.0400

Frequency of areca use in oral cancer:

Serum LDH levels: Serum LDH levels in areca users with frequency less than 10 times/day was 550.1652+94.0360 IU/L, while those who chewed areca for more than 10 times/day was 469.0500+55.0237 IU/L [Table 7].

Salivary LDH levels: Salivary LDH levels in areca users with frequency less than 10 times/day was 866.7409+229.6702 IU/L, while those who chewed areca for more than 10 times/day was 947.6150+92.7411 IU/L [Table 7].

Table 7: Serum and salivary LDH levels and frequency of areca use in oral cancer

Frequency of areca usage (Per Day)	Serum LDH U/L (85-300 IU/L)	Salivary LDH U/L (360-430 IU/L)
< 10 times	550.1652+94.03596	866.7408+229.6702
> 10 times	469.0500+55.0237	947.6150+92.7411

Analysis of statistical significance:

Serum LDH levels: Comparision of the serum LDH levels of Group C with Group S showed statistically highly significant result (p< 0.001). [Table 8].

Salivary LDH levels: When the salivary LDH levels of group C was compared to group S the difference was highly significant (p<0.001) [Table 9].

Table 8: Correlation of serum LDH levels between control and oral cancer group

	Groups	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Z	Asymp. Sig. (2-tailed)
SERUM LDH IU/L (85-300 IU/L)	Control	30	18.1	543	78.000	-5.500	<0.001
	Oral Cancer	30	42.9	1287			

Table 9: Correlation of salivary LDH levels between control and oral cancer group

	Group	N	Mean Rank	Sum of Ranks	Mann-Whitney U	Z	Asymp. Sig. (2-tailed)
SALIVARY LDH U/L (360-430 IU/L)	Control	30	15.53	466	1.000	-6.638	<0.001
	Oral Cancer	30	45.47	1364			

Correlations between group C and group S was done using Mann Whitney U test. The serum LDH levels on comparison between the groups was statistically significant (p<0.001) [Table 8]. The values of salivary LDH levels between the groups was highly significant (p<0.001) [Table 9].

Pearson's correlation was calculated for correlation between the groups and positive correlation (r=0.407) but a moderate correlation. This indicated that, as the serum LDH levels increase salivary LDH levels also increases. This correlation suggests that saliva can be considered as better diagnostic medium than serum.

DISCUSSION

In the recent years various hypotheses have been, out of which three mechanisms have been heighted as responsible for the increase in the level of serum LDH [4]. The reasons could be due to induction process initiated by the tumor, tissue necrosis and cellular degeneration involving normal tissues and resultant muscle degeneration caused by protein deficit.

Oral squamous cell carcinoma (OSCC) is the sixth most common human cancer that that affects at least 90% of all oral malignancies. It is one of the major causes of morbidity and mortality in the world [5,6].

Salivary analysis for biomarkers has been promoted as a noninvasive alternative to serum analysis in diagnosis, management and prognosis of oral cancer that may also help in future prediction of oral cancer [7].

This enzyme is mostly found in all body tissues but mainly concentrated in liver, heart, kidneys, red blood cells, lungs, brains and muscles. The salivary LDH isoenzymes profiles are similar to that of salivary LDH isoenzymes, present in the oral epithelium, which indicates that the major source of LDH is the oral epithelium probably, which is derived from the surface exfoliated cells. Presence of LDH a known cellular necrosis marker activity in saliva and the increase of this could be suggestive of a specific indication of oral mucosa breakdown. Hence it was suggested that the main source of LDH in whole saliva was not from the salivary glands but the oral epithelium. Furthermore, various pathologic factors may affect the oral mucosa, leading to various epithelial alterations and even to the development of oral squamous cell carcinoma which in turn causes release of LDH into the saliva [8].

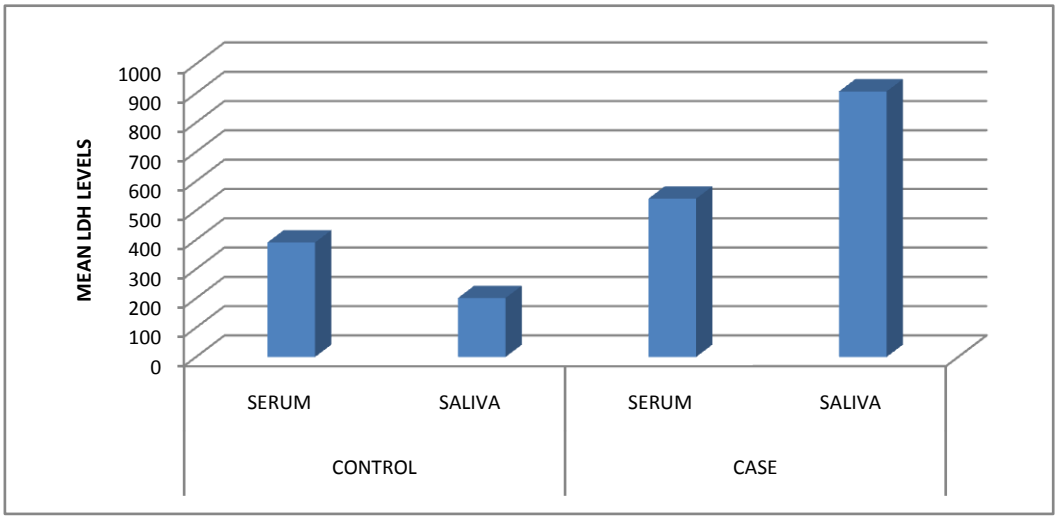
Even though saliva collection is far easier, non-invasive and economic than blood collection, only very few studies have been performed in saliva [8]. Therefore our study is of great interest to study and characterize salivary LDH as a potential diagnostic tool. Also saliva as test medium holds numerous advantages as it can be collected easily, may be collected by any members of the dental team without any special training, with no need for breaking the skin barriers and thereby greatly reducing the risk of contamination among patient as well as personnel [9]. The salivary LDH levels have been rarely studied in oral cancer patients. In the present study both serum and salivary LDH have been estimated, compared as well as correlated so as to determine the better estimate.

In 1955, Wroblewski and La Due successfully measured the normal values of serum LDH using a spectrophotometer. They gave the normal range as 260 to 850 units per ml (85-300 IU/L) [10]. Using the same method Elliot and Wilkinson (1961) estimated the normal range to be 150-500 units per ml (72 to 240 IU/L) [11]. King J in 1959 using the colorimetric method determined the normal range of serum LDH to be from 70-240 IU/L [12]. In the present study we have measured the LDH values among the controls using spectrophotometer and it was found to be in the range of 390.8667+ 71.0953 IU/L for serum and 201.3700 + 89.1439 IU/L for saliva [Table 4, 5].

Very few studies are present in literature comparing, correlating serum and salivary LDH values among healthy individuals and oral cancer patients. The study results showed an increase in serum LDH levels as well as salivary LDH levels which was estimated to be 540.50+ 88.81 IU/L and 906.4183+239.4646 IU/L respectively among the cancer patients when compared to the control group in both serum and saliva which was estimated to be 390.8667+ 71.0953 IU/L and 201.3700 + 89.1439 IU/L respectively. Also there was a marked increase in salivary LDH level more than the serum LDH levels in the oral cancer group [Table 10, Graph I].

Table 10: Correlation of salivary and serum LDH levels in each group

Group		Mean	Std. Deviation	N	Pearsons correlation (r)	p value
Control	SERUM LDH U/L (85-300 IU/L)	390.8667	71.0953	30	0.076	0.692
	SALIVARY LDH U/L (360-430 IU/L)	201.3700	89.1439	30		
Oral Cancer	SERUM LDH U/L (85-300 IU/L)	540.5033	88.8147	30	-0.233	0.214
	SALIVARY LDH U/L (360-430 IU/L)	906.4183	239.4645	30		



Graph I: Comparision of mean values of serum and salivary LDH levels in each control and oral cancer (study) group

In the present study, the correlation of serum LDH and salivary LDH levels values between the control and oral cancer group showed highly significant values ($p< 0.001$) [Table 8, 9].

One of the study by Shpitzer T *et al* on 19 tongue cancer patients, LDH level was measured along with other biomarkers by kinetic spectrophotometry wherein the activity of LDH was seen to be considerably increased [9]. In the present study also the total LDH activity in saliva which was determined by spectrophotometry which was seen to increase among oral cancer patients when compared to controls.

The various components of the tobacco products cause direct contact and continuous irritation to the oral tissues and thus initiating the disease process. The period between initiation of chewing habit and the development of clinical symptoms of cancer varies tremendously depending on the type, frequency and duration of the tobacco chewing habit [Table 6, 7]. Our study presented here shows an increase in mean salivary LDH levels with increase in frequency of the habit more so when areca is used more than 10 times per day [Table 7]. The serum LDH level does not show an increase in level with increase in duration of the habit but the salivary LDH levels are increased markedly for patients who gave history of areca use of more than 30 years [Table 6]. The increased LDH concentration in saliva may be considered as a specific indicator for oral lesions that disrupt the integrity of the oral mucosa, hence could be considered as an expression of cellular necrosis [13]. In our study there is a significant increase in the salivary LDH values between healthy individuals and oral cancer patients hence it can be considered as a biomarker for early detection and management of oral cancer patients.

CONCLUSION

The current study was aimed to evaluate salivary LDH as a biomarker in the early pathogenesis of oral cancer. Recent studies have revealed that changes in salivary LDH could precede dysplastic changes in oral epithelium. The encouraging results of the present study indicates that salivary estimation of lactate dehydrogenase levels can be used as biomarker in the early diagnosis of oral cancer; and that saliva can be used as an effective diagnostic tool and as an adjunct to serum analysis.

Conflict of interest: None.

Financial support and sponsorship: Nil.

REFERENCES

1. Rai B, Jain R, Anand SC, Simmi K. Salivary lactate dehydrogenase isoenzymes in oral lichenplanus. Adv. in Med. Dent. Sci., 2008;2(1):1-3.
2. Narang APS, Greval RS, Chopra H, Kalra CS. The role of two enzymes (LDH and PHI) and A tumour marker (CEA) in the prognostic evaluation of head and neck malignancy. Indian Journal Of Otolaryngology and Head and Neck Surgery 2001;53(1):76-80.
3. Vıctor Alonso De La Pen, Diz Dios P, Sierra RT. Relationship between lactate dehydrogenase activity in saliva and oral health status. Archives of Oral Biology 2007;52;911-15.
4. Henry RJ, Chiamori N, Golub OJ, Berkman S. Revised spectrophotometric methods for the determination of glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, and lactic acid dehydrogenase. Am J Clin Pathol 1960;34:381-98.
5. Jefferies S, Foulkes WD. Genetic mechanisms in squamous cell carcinoma of the head and neck. Oral Oncol. 2001;37:115-26.
6. Massano J, Regateiro FS, Januário G, Ferreira A.Oral squamous cell carcinoma: Review of prognostic and predictive factors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:67-76.
7. Nagler RM. Saliva as a tool for oral cancer diagnosis and prognosis. Oral Oncol 2009;45:1006-10.
8. Nagler RM, Lischinsky S, Diamond E, Klein I, and Reznick AZ. New insights into salivary lactate dehydrogenase of human subjects; J Lab Clin Med. May 2001. 137;5: 363-369.
9. Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, Gavish M, Nagler RM. Salivary analysis of oral cancer biomarkers. British Journal of Cancer. 2009; 101: 1194 – 1198.
10. Wroblewski F, La Due JS. Lactic dehydrogenase activity in blood. Proc Soc Exper Boil and Med 1955; 90: 210-213.
11. Varley H, Bell M, Gowenlock AH. Practical clinical biochemistry 4th Ed: Arnold – Heinmann Publishers (Ind.) Pvt. Ltd.; New Delhi; 1975, 278-283.
12. King J. Routine method for estimation of lactic dehydrogenase activity. J M Lab. Tech. 1966; 16: 265-272.
13. Joshi PS and Golgire S. A study of salivary lactate dehydrogenase isoenzyme levels in patients with oral leukoplakia and squamous cell carcinoma by gel electrophoresis method. J Oral Maxillofac Pathol. 2014;18(1):S39-44.