Section A-Research paper

Estimation of salivary lactate dehydrogenase in oral submucous fibrosis, oral lichen planus and lichenoid reaction - A Case Control Study

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ABSTRACT:

AIM:

To determine the level of salivary lactate dehydrogenase(LDH) in oral submucous fibrosis (OSMF), oral lichen planus (OLP) and lichenoid reaction (LRs).

METHODS:

Clinically diagnosed 90 cases of OSMF, OLP and LRs were considered and 30 healthy individuals were considered as control group to compare the salivary LDH levels. Unstimulated saliva (2ml) was collected in aseptic conditions and was processed for estimation of salivary LDH.

RESULT:

The results showed a positive relation of increased levels of salivary LDH between the OSMF and OLP & LRs participants and healthy individuals with statistical significance. Thus, justifying the belief that levels of salivary LDH can be utilized in early detection of OPMDs and making salivary LDH level estimation a potential noninvasive alternative to other invasive procedures for diagnosis.

KEYMESSAGE:

The levels of salivary LDH can be utilised in early detection of OPMDs and making salivary LDH level estimation a potential noninvasive alternative to other invasive procedures for diagnosis.

Key words: Salivary lactate dehydrogenase, Oral Submucous fibrosis, Oral Lichen Planus, Oral Lichenoid reaction

INTRODUCTION

Oral cancer has been accounted as a concerning problem in many parts of the globe with highest incidence seen in Southeast Asia.[1] Oral cancer is always preceded by various clinically visible changes in the oral mucosa.These changes occurring in the oral cavity can be either white or red lesions having a variable potential for malignant transformation. WHO has termed these changes occurring in the oral cavity as "oral potentially malignant disorders".[2,3]

In recent times, the frequency of OPMD has increased worldwide which are most commonly seen in Asian population.[4] The OPMDs vary in their malignant transformation with OSMF reported to have a malignant transformation of about 4.5% to 7.6% and OLP and LRs is 1-2% and 2.4% respectively.[5,6,7] As the percentage seems to be relatively high, hence early diagnosis of such entities is highly essential for the disease outcome and prognosis. It has been seen that the prognosis rate drops to 50 % or even higher when diagnosed at Stage III.[8,9] Biopsy is generally considered as a gold standard, however the procedure involves various limitations. Due to the disadvantages and challenges, studies have been carried out to establish alternative methods and techniques which can be used for early detection of premalignant lesions and conditions. One such alternative and promising technique is the use of tumour biomarkers.

Lactate dehydrogenase (LDH) is one of the biomarkers which is being used in the early detection of premalignant lesions and conditions.[10] Estimation of these tumor biomarkers can be done be by using various body fluids, among which saliva can be used as a essential tool in early diagnosis of such premalignant disorders. However, it is seen that routine use of such techniques is still lacking.[9]

Hence, the present study was designed to estimate the levels of salivary LDH in participants having OSMF, OLP and LRs.

MATERIALS AND METHODS

STUDY GROUPS:

The study was approved by the Institutional Ethics Committee (Ref.No.DYPDCH/EC/648/43/2021). A total of 120 participants using convenience sampling technique were considered and segregated as: Group A : control, Group B: Habit without lesion, Group C : OSMF, Group D: OLP and LRs. Inclusion criteria included: participants from age from 10-90 years age group and clinically diagnosed cases of OSMF, OLP and LRs. Participants having any systemic diseases, undergone any surgical procedure and having any other mucosal lessons were excluded from the study.

SALIVA COLLECTION:

A written informed consent was obtained from each patient before the collection of saliva. About 2ml of unstimulated saliva was collected from different study groups during 9-11AM. Patient were asked not to eat and drink 60mins prior to saliva collection. The saliva was collected through the spit technique and the patient was explained about the technique. The samples collected from each group was then sent to laboratory (CCL) for further analysis of enzyme level

STATISTICAL ANALYSIS:

The data was entered and analyzed using the Statistical Package for Social Sciences (SPSS) for Windows, Version 28.0. (Armonk, NY: IBM Corp) and a p-value $\leq$ of 0.05 was considered statistically significant. Kolmogorov–Smirnov and Shapiro Wilk tests were performed to determine the normality of the data for salivary LDH levels for four groups. Mann Whitney U test was used to compare the distributions over groups. Kruskal Wallis test was used to compare the distribution of variables over groups. Dunn’s test was used as post hoc analysis.

RESULTS:

Different age groups were considered for the study, with a mean age of 36 ± 12.42 years. The salivary LDH levels (IU/L) of different age range were compared and no statistical significant difference were found.(Table/fig 1)

However, the age range of 45 to 59 years showed the highest mean salivary LDH level of 1026.58 ± 536.62 IU/L. Highest level of salivary LDH was noted in the age range of 47. Both male and female participants were included in the study. Out of 120 participants, 95 (79.1%) were male and 25 (20.9)% were female.(Table/fig 2) Among the participants, the male participants(604.51±461.05IU/L) showed higher salivary LDH level than female participant (564.24 ± 401.26 IU/L). However, there was no statistical significance noted.

To assess the effect of duration and frequency on the salivary level of LDH, the samples were categorised into three group with duration of 1-10 years, 11-20 years, and 21-30 years and participants who consumed tobacco more than five times per day, participants who consumed tobacco less than five times per day. The highest value was noted in participants with habit duration of 21-30 years having a value of 1160 ± 389.8 IU/L. There was statistical significance noted having a p-value of 0.025. (Table/fig 3) Among the two groups, the level of LDH was increased with increased frequency of more than five times a day having a p-value of <0.001 which was highly significant. (Table/fig 4)

On analysing the effect of type of tobacco habit on salivary LDH level, participants on chewable tobacco showed higher level of salivary LDH 660.14 ± 494.47 IU/L compared to the smokers 540.96 ± 398.78 IU/L with a p-value of < 0.001. The OLP participants with habit history showed higher LDH level of 1035.16 ± 472.25 IU/L having a p-value of <0.001. (Table/fig 5)

Pairwise comparison of salivary LDH among different groups was done. There was highly statistical significance and LDH levels was noted in OLP (966.53 ± 477.49 IU/L and 0.01 ^{KW}*), OSMF (646.46 ± 483.89 IU/L and 0.01 ^{KW}*) when compared to that of control group. Apart from

the control even statistical significance was noted between OLP and OSMF (0.05 ^{KW}*). (Table/fig 5)

DISCUSSION

The second most common cause of death worldwide after heart diseases in developed countries is cancer, with third leading cause of mortality following heart diseases.[11,12] Regardless of the accessibility of the oral cavity to direct examination, the frequency of early detection of these malignancies are still lacking until late stage.[9,13] Every hour the disease kills one person more than other malignancies. Early detection using biomarkers followed by appropriate treatment, can increase cure rates to 80 or 90%, and can greatly improve the quality of life by minimising extensive, debilitating treatments.[9,13]

The present study was designed to evaluate the levels of salivary LDH in OPMDs using easy and simplified technique and compare these levels with levels in healthy individuals and analyse if it can be considered as a potential biomarker.

The mean value of salivary LDH in different groups were seen gradually increasing in the following order: control<habit without lesion<OSMF<OLP and LRs. This was an interesting finding since the mean salivary LDH levels were significantly more in OLP and LRs group as compared to OSMF group**.** There has always been difference of opinion regarding the malignant potential of OLP and lichenoid reactions but with this finding it can be presumed that the malignant potential of OLP might be more than OSMF if salivary LDH is considered as a biomarker for malignancy process. The current finding may serve as the basis for future detailed studies on the salivary LDH level comparison between OLP and oral cancer to further support this presumption.

Literature review revealed that there are few studies which have attempted in identifying relationship between the level of salivary LDH and oral sub mucous fibrosis. In 2015, Panda A et al [4] analysed salivary LDH levels in OSMF patients and compared it with healthy individuals and found statistically significant difference. Similar results were seen in the present study having a significant LDH value. Other studies which were in consistent with this finding are studies

conducted by Sivaramakrishnan *et al.*[15] in 2015 to evaluation LDH enzyme activity in saliva of OSMF patients showed that salivary LDH levels were greater in OSMF patients and was statistically significant, study by Kallalli B N et al [5] and Mantri T et al [14] also showed positive correlation.

As tobacco-related cancers represent the most preventable form of cancer in our society, which highlights its potential for early detection. The present study has included participants with tobacco habit but without any oral lesion (Group B) to emphasise the importance of estimation of salivary LDH in this group of population. The salivary LDH level was higher in group B, however it was not statistically significant.

In the present study, we had also included participants having OLP and OLRs and found significantly higher mean level of salivary LDH when compared with healthy individuals. This was in accordance with the studies conducted by Gholizadeh N et al.[7] While inferencing the results, the participants of middle age group had showed increased habit of tobacco usage which was reflected in the level of salivary LDH. The possible explanation for higher mean salivary LDH level in 45-59 years group may be attributed to increased duration and frequency of tobacco usage which was observed in this age group. This can also be found in studies by Mishra S et al[9] and Awasthi N[16] where individuals in middle-aged group had higher predominance of tobacco associated lesions and thus suggested that tobacco usage can potentially change the levels of LDH in saliva.

In the present study, the duration and frequency of tobacco usage among different participants were studied. The level of salivary LDH was highest in duration of 21-30 years and tobacco frequency of more than 5 times a day. Similar findings were also observed in study conducted by Javaraiah R K et al[17], where the mean salivary LDH levels were found to increase with increase in frequency and duration of habit.

The present study included participants having tobacco in both smoke and smokeless forms. The results showed increase in level of salivary LDH in these groups when compared to that of healthy individuals. Salivary LDH level of participants having tobacco chewing habit showed higher level

of salivary LDH compared to the smokers having a significant statistical value of < 0.001. According to the Global Adult Tobacco Survey India (GAT-2) 2016-17, the percentage distribution of tobacco usage between adults is more in smokeless tobacco users compared to the smokers.

The study had showed a positive correlation of salivary LDH and OPMDs. However, this study has limitations also as the histopathological examination was not done for the participant with oral lesions. Apart from the shortcomings there were few strength also as this study has included participants with habit history but without lesion. This will not only help in preventing the occurrence of the oral lesions but will also help in proper counselling for tobacco cessation among young population.

CONCLUSION:

The present study showed a positive relation of increased levels of salivary LDH between the OSMF and OLP & LRs participants and healthy individuals thus, justifying the belief that levels of salivary LDH can be utilised in early detection of OPMDs and making salivary LDH level estimation a potential noninvasive alternative to other invasive procedures for diagnosis.

STRENGTH & LIMITATION:

- A systemic review (PROSPERO registration id CRD42022366117) was done and the findings shortcomings of previous studies were covered in the present study.
- Apart from the clinically diagnosed cases of oral lesions, participants with habit history but without lesion were also included. This will not only help in preventing the occurrence of the oral lesions but will also help in proper counselling for tobacco cessation.
- A comparison was done between both types of tobacco i.e smokeless and smoke
- Histopathological examination was not conducted.

FUTURE SCOPE:

As there was positive co relation between the salivary LDH level with the oral lesions, hence in future more studies of larger sample size with stringent inclusion and exclusion criteria should be conducted

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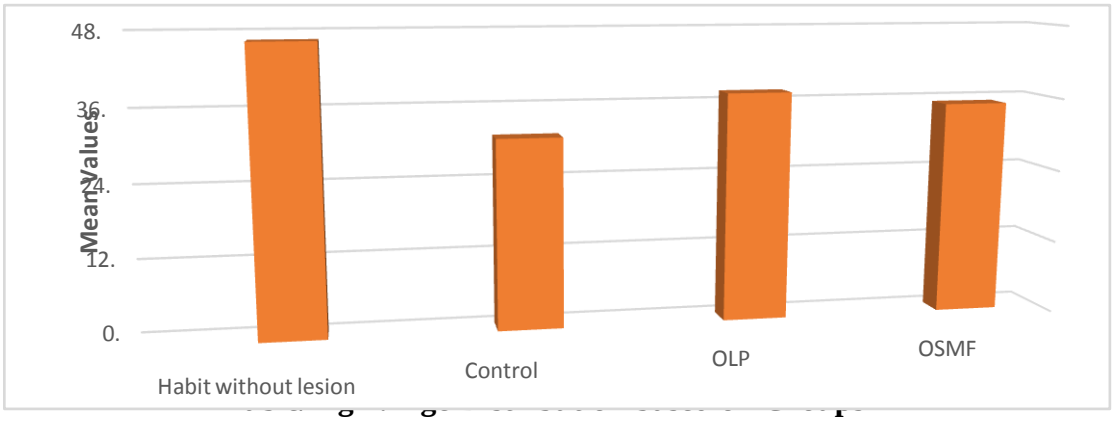

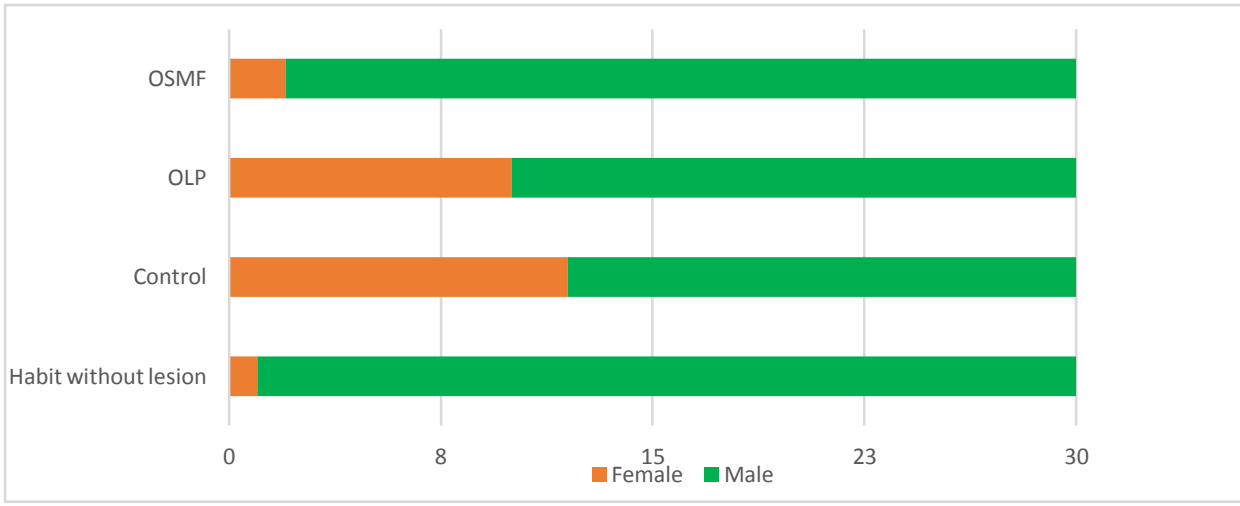

Table/Fig 2: Gender Distribution based on groups

Variables	Sub category	Salivary LDH levels (IU/L)	p-value
Duration (In Years)	1-10	457.66 ± 329.39	**0.025^{KW*}**
	11-20	1088.25 ± 667.78	
	21-30	1160 ± 389.81	
	None	660.14 ± 494.47	

*(Abbreviation: KW – Kruskal Wallis test, MW-Mann Whitney test, *- indicates statistical significance)*

Table/fig 3: Salivary LDH level based on duration

Variables	Sub category	Salivary LDH levels (IU/L)	p-value
Frequency	>5	854.3 ± 475.76	**<0.001^{MW}***
	<5	485.4 ± 283.02	
	None	660.14 ± 494.47	

*(Abbreviation: KW – Kruskal Wallis test, MW-Mann Whitney test, *- indicates statistical significance)*

Table/fig 4: Comparison of variables with Salivary LDH levels (IU/L)

Variables	Type	Salivary LDH levels (IU/L)	p-value
Type of Habit	Smoke	540.96 ± 398.78	**<0.001^{MW}***
	Smokeless	660.14 ± 494.47	

*(Abbreviation: KW – Kruskal Wallis test, MW-Mann Whitney test, *- indicates statistical significance)*

Table/fig 5: Comparison of OLP with Salivary LDH levels levels (IU/L)

Salivary LDH levels	Control	Habit without lesion	OSMF	OLP
Control	-	0.88	0.01^{KW}*	0.01^{KW}*
Habit without lesion	0.88	-	0.10	0.01^{KW}*
OSMF	0.01^{KW}*	0.10	-	0.05^{KW}*
OLP	0.01^{KW}*	0.01^{KW}*	0.05^{KW}*	-

*(Abbreviation: KW – Kruskal Wallis test, MW-Mann Whitney test, *- indicates statistical significance)*

Table/fig 6 : Pairwise comparison of salivary LDH levels in the study groups