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SALIVARY LACTATE DEHYDROGENASE LEVELS IN ACTIVE CIGARETTE SMOKERS AND PASSIVE SMOKERS AS BIOMARKER FOR ORAL CANCER.

Dental Science

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ABSTRACT

Purpose : To assess the salivary lactate dehydrogenase levels in active , passive smokers and healthy controls as biomarker for oral cancer.

Materials and Methods : The study included subjects residing in Madurai city. A total 75 Subjects were included in the study (25 active smokers , 25 passive smokers and 25 healthy controls) , informed consent were obtained from all the subjects prior to the start of the study. The source of data was primary in nature which included structured interview to collect demographic details and details regarding smoking and exposure to smoke , clinical examination and salivary analysis for assessing the LDH was done.

Results: The age of the study participants ranged from 18 to 60 years . The mean LDH value for active smokers , passive smokers and healthy controls were 433U/L , 356.5U/L and 230.6 U/L respectively. There was a positive correlation between the duration , type and frequency of smoking , consumption of cigarette / beedi and LDH level among active smokers.

Conclusion : The present study reveals a association between cigarette smoking (both active and passive) and elevated salivary LDH levels. Estimation of salivary LDH level can as a serve reliable marker to monitor the tobacco smokers.

KEYWORDS

salivary lactate dehydrogenase, passive smokers, biomarker, oral cancer.

INTRODUCTION

"A cigarette is the only consumer product which when used as directed kills its consumer" states Dr. Gro Harlem Brundtland.[6]

Oral cancer represents only about 3 % in US population but in India it accounts for 30% of all cancers . In India it accounts for 86% of worlds oral cancer cases (National Institute of Public Health February 2011), thus oral cancer is a common human malignancy with an increasing incidence and with high mortality rate of 50% which has not changed significantly in more than 50 years. Its survival rates increase significantly when it is detected and treated early. In India oral cancer ranks first among all cancer cases in males and is the third most common among females in many regions. Cancer is one of the leading causes of adult deaths worldwide. Oral cancer is a serious problem in many countries. The development of oral cancer is a multistep process, arising from pre-existing potentially malignant disorders.[9]

Recently, the role of tumor markers in management of head and neck cancer has received increasing attention. 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 does have some inherent disadvantages. 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. Lactate dehydrogenase 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 and potentially malignant disorders; this finding can be used for benefit of the patient in predicting prognosis. LDH level is increased in both serum and saliva during the malignant changes of the tissue. But LDH is predominantly found in saliva than serum.[4] 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.[4] LDH has also been shown to be elevated among smokers leads to tissue necrosis and higher risk to oral cancer. The Cigarettes the smoke consists of many carcinogenic chemicals that include nicotine, tar and gaseous compounds including carbon monoxide (CO). Chronic smoking causes deposition of carbon monoxide resulting in tissue hypoxia. Increased carboxyheamoglobin and decreased oxyhemoglobin has resulted in respiratory acidosis and electrolyte imbalance leading to increase in LDH level in serum and saliva.[7]

The hypothesis of the present study is there any association between the LDH level among active, passive smokers. The present study is aimed to evaluate the salivary LDH in active smokers, passive smokers and among the control individuals to correlate the LDH levels as a biomarker for oral cancer using the relatively non -invasive saliva as the diagnostic tool.

Literature has shown that smoking, not only affect the person who is smoking but also the smoke inhalers (passive smoking). As per the literature research there is no study done with respect to LDH level among passive smokers.

MATERIALS AND METHODS

A Cross sectional study was undertaken to assess and compare the lactate dehydrogenase value among active smokers, passive smokers and healthy controls among the people residing in Madurai city. The total study subjects were 75, each group consist of 25 subjects. The sample size was calculated from the results of pilot study. The duration of the study was 1 month which was conducted in May 2018. The subjects were included after obtaining the informed consent. The study subjects were randomly selected among the residents of Madurai city, household visit was conducted, after obtaining the informed consent, information was collected regarding smoking habits among family members. The subjects aged 18 – 60 years were included in the study and the active smokers who smokes at home for atleast 5 years has been

included. They were clinically assessed for oral mucosal lesions only those who did not have any oral mucosal lesion were included. From these, household who had a family member with the habit of smoking, active smoker and in the same household the family member having maximum exposure was selected in the passive smoker group were selected. Controls were selected from household where none of the family members smoked and from where smoking was prohibited. In the present study the subjects who has systemic disease, the subject who has the habit of chewing smokeless tobacco and alcohol and those who were on medications were excluded. The ethical clearance was obtained from the Institutional Review Board Best Dental Science College, Madurai (BDSC2542018).Demographic details were collected from each subject through structured interview in the local language (Tamil) and collected data was recorded by the principal investigator. The subjects were assured about the confidentiality of the information. The details regarding Age, Gender, Address, Education, Occupation, Oral habits, Other habits, Type of smoke tobacco, Duration of smoking, Frequency of smoking, No of cigarrate / beedi per day were recorded. The saliva was collected from each subject using a sterile container and transported to the laboratory for assessing the level of LDH among active smokers, passive smokers and healthy controls. 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 collected by drooling method, 1ml of collected saliva was stored in plastic vials and transported to laboratory through frozen bag and analysis was carried out within 24 hours using LDH kit.[9]

RESULT

The total study subjects were 75, each group consist of 25 subjects. The age of the study participants ranged from 18 to 60 years. The mean age group among active smokers, was 41.5 years, for passive smokers was 37.0 and for healthy controls was 36.4. All the active smokers were males. In passive smokers 6 were males and 19 were females whereas in healthy controls 14 were males and 11 were females. The mean LDH value for active smokers, passive smokers and healthy controls were 433 U/L, 356.5 U/L and 230.6 U/L respectively. A statistically significant difference (0.007) was observed between the mean LDH of active smokers and healthy controls. The higher level of LDH was observed among 48 % (12) of active smokers, 36% (9) of passive smokers and 16% (4) of healthy controls (Table 1). Majority of the smokers were using cigarette (80%) and only 20% used beedi for smoking. The subjects who smoke full cigarette were 68% and 32% use half cigarette. The 'p' value was 0.013 Significant. Pearson's correlation demonstrated a positive correlation between the duration of smoking and LDH level among active smokers, hence the increase in duration increases the LDH level (graph 1). The subjects who smoke up to 5 cigarettes / beedi per day had the mean value of LDH as 230 U/L and the subjects who smoke above 5 cigarettes / beedi per day had the mean value of LDH as 653 U/L. The 'p' value was <0.001 Significant. There was a positive correlation between the frequency of smoking and LDH level among active smokers, The mean value of LDH for those who smokes cigarette was 331 U/L and the mean value for those who smoke beedi was 840 U/L . The mean value of LDH was higher among the subjects who smoke full cigarette (572 U/L) than the subjects who smoke half cigarette (138) .The mean value of LDH among Active smokers whose duration of smoking up to 10 years was 221 U/L and Above 10 years was 552 U/L.(Table 2 and Graph 2).

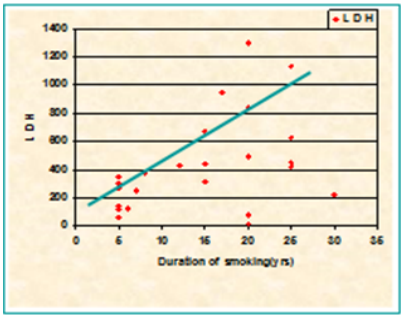
Table 1: LDH level of the study participants based on mean scores

Group	LDH Score					
	Normal (Up to 414)		Abnormal (>414)		Mean	SD
	No.	%	No.	%		
Active Smokers (n=25)	13	52.0*	12	48.0	433.0*	330.8
Passive Smokes (n=25)	16	64.0	9	36.0	356.5	397.0
Healthy Controls (n=25)	21	84.0*	4	16.0	230.6*	139.1

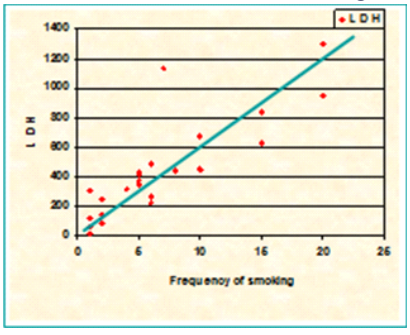
***Statistically significant**

Table 2: Mean scores of LDH level based on type of smoking, Consumption of cigarette/ beedi, duration of smoking and frequency of smoking.

Type of smoking.	Cigarette	No of subjects (%)	Mean	SD	'p' Value
		20 (80)	331	251	**< 0.001 Significant**
	Beedi	5 (20)	840	313.4	
Consumption of cigarette/beedi.	Full	17 (68)	572	309.9	**0.005 significant**
	Half	8 (32)	138	95	
Duration of smoking	Up to 10 years	9 (36)	221	112.5	**0.013 Significant**
	Above 10 years	16 (64)	552	355.5	
Frequency of smoking(per day).	Up to 5 cigarette/ beedi	12 (48)	230	144.1	**< 0.001 Significant**
	> 5 cigarette/ beedi	13 (52)	653	338.4	



Correlation coefficient 'r' = 0.535, Positive correlation
Graph 1: Correlation between duration of smoking and LDH level



Correlation coefficient 'r' = 0.839, Positive correlation
Graph 2: Correlation between frequency of smoking and LDH among active smokers

DISCUSSION

LDH activity and isoenzyme patterns have been studied extensively in the various tissues as mentioned along with the plasma; LDH level in saliva has been noted but only a few evaluations have been performed in saliva, despite the fact that saliva collection is far easier, non – invasive and cheaper than blood collection. Saliva as a test medium holds several advantages and it can be collected easily, 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.[9] Clinical significance of salivary biomarkers in various malignancies is studied by several investigators.[7] Among these biomarkers LDH is a specific marker for mucosal necrosis. Its extracellular presence is always related to cell necrosis and tissue breakdown.[7]

In the present research we have evaluated the level of salivary LDH among active smokers, passive smokers and healthy controls. The LDH level was analysed in the saliva of the subjects since it is non-invasive method of collecting the samples. The present study have included smokers because many studies proved that smoking leads to tissue necrosis and also they are more prone to oral cancer.

The present research was the first Indian study to compare the level of LDH among active smokers, passive smokers and healthy control. In the current research we have analysed LDH value using automated analyzer, spectrophotometer or photometer with cell holder thermostatable at 25, 30 or 37ºC and able to read at 340 nm. The reagents used for analysis were Reagent A: Tris 100 mmol/L, pyruvate 2.75 mmol/L, sodium chloride 222 mmol/L, pH 7.2 and Reagent B: NADH 1.55 mmol/L, sodium azide 9.5 g/L.(Biosysytems – Reagents and Equipments).The reference value for LDH according to the method we used was 207 U/L -414 U/L.[10]In the present study drooling

method was used to collect the unstimulated saliva incontrast to the spit method employed in other studies,[9,7,10,5.] As the latter method dilutes the saliva due to activation of masticatory muscles which makes detection of the biomarker difficult. LDH is also influenced by other systemic conditions including malignancies, myocardial infarction, liver disease, megaloblastic anemia, renal disease therefore the present research have excluded all of the above mentioned conditions to avoid the false result.[5]

All the active smokers were males, in passive smokers 6 were males and 19 were females whereas in healthy controls 14 were males and 11 were females. The reason for the difference in the gender distribution may be due to high prevalence of smoking among males and smoking being socially unaccepted among females. The mean value of LDH for those who smokes cigarette was 331 U/L and the mean value for those smokes beedi was 840 U/L. Suggestive of beedi causing more harm to the oral tissues.

The literature review reveals only a small number of articles about the LDH levels among smokers and thus the comparison is limited and this being the first research to compare the passive smokers with active smokers. The mean LDH value for active smokers, passive smokers and healthy controls were 433 U/L, 356.5 U/L and 230.6 U/L respectively. A statistically significant difference was observed between the active smokers and healthy controls. The higher level of LDH was observed among 48 % (12) of active smokers, 36% (9) of passive smokers and 16% (4) of healthy controls. A positive correlation was demonstrated between the duration and frequency of smoking and salivary LDH level suggestive of dose response. This higher level was due to the local tissue damage in oral cavity due to cigarette smoke. The Cigarettes the smoke consists of many carcinogenic chemicals that include nicotine, tar and gaseous compounds including carbon monoxide (CO). Chronic smoking causes deposition of carbon monoxide resulting in tissue hypoxia. Increased carboxyheamoglobin and decreased oxyhemoglobin has resulted in respiratory acidosis and electrolyte imbalance leading to increase in LDH level in serum and saliva.[7] This result is in line with the studies conducted by Rai et al[1] and Kumuda Rao et al[9] where the smokers showed high level of LDH than non-smokers and N. Mohan et al[7] found LDH activity increased in serum as well as saliva in tobacco users and potentially malignant disorders in comparison to normal control and he also emphasized that salivary LDH estimation can prove to be a valuable substitute to serum LDH as a biochemical marker.

Literature review also reveals few articles wherein the LDH level was higher among the subjects with precancerous and cancerous lesions: The results of the studies reveals increase in LDH level among oral lichenplanus[5],oral leukoplakia[10,3], oral precancerous, cancerous patients[4] and oral squamous cell carcinoma patients and LDH was the sensitive marker for early detection of all of these conditions. Kavyashree lokesh et al suggested that LDH as a potential biomarker by correlating LDH level with the histopathological grading of the tumours.[5]

Although this study unveals the adverse effect of passive smoking, the generalisability was limited due to short duration and small sample size. Irrespective of the above shortcomings the study has opened new vistas in understanding passive smoking as a risk for malignancy and LDH as a reliable marker. Further longitudinal prospective studies will help in understanding and strengthening the relationship between the LDH and oral cancer.

CONCLUSION

This study shows a statistically significant difference in salivary LDH level among active, passive smokers and healthy controls. The study is an evidence that you don't need to smoke just being a passive smoker is enough to cause tissue damage. The outcome of the present study reveals a possible relation between cigarette smoke inhalation and elevated salivary LDH levels which may be secondary to local tissue damage in oral cavity which occur in conditions like oral cancer. Estimation of salivary LDH level could be a reliable marker for oral cancer.

CONFLICT OF INTEREST - NO CONFLICT OF INTEREST.

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