www.sphinxsai.com

International Journal of ChemTech Research
CODEN (USA): IJCRGG ISSN : 0974-4290
Vol.6, No.5, pp 3014-3018, Aug-Sept 2014

Expression of Salivary Biomarkers – Alkaline Phosphatase & Lactate dehydrogenase in Oral Leukoplakia

M. Dhivyalakshmi and T. N. Uma Maheswari*

Saveetha Dental College, Saveetha University, 162, PH Road, Velappanchavadi, Chennai – 600077, India.

***Corres.author: umasamsi@gmail.com**
Contact Number: 9840958339

Abstract:
Aim: To find out the significance of Lactate dehydrogenase, Alkalinephosphatase in salivary samples of oral leukoplakia, oral squamous cell carcinoma cases and control groups and to ensure whether estimation of these markers in leukoplakia is valuable in assessing the malignant risk potential
Methods: 5 ml of unstimulated saliva was collected from the patients by spit method in a calibrated test tube. It was then immediately centrifuged at 2500 rpm for 15 minutes. The resulting supernatant was then separated into 1 ml aliquots and subjected for further biochemical assay analysis using standard kit method. The samples will then be diluted in 1:1 ratio with saline and assayed using standard kit and measured using auto analyzer.
Results: Both Alkaline phosphatase and Lactate dehydrogenase have been found to be statistically significant with a p value of 0.001 for Alkaline phosphatase and <0.001 for Lactate dehydrogenase.
Conclusion: Both Alkaline phosphatase and Lactate dehydrogenase are sensitive markers for the detection of leukoplakia hence helpful in early detection of oral carcinoma. Statistical analysis also proves that Lactate dehydrogenase could be more reliable marker in detection of oral carcinoma in comparison with Alkaline phosphatase.
Keywords: Leukoplakia, Oral Squamous Cell Carcinoma, Saliva, Lactate dehydrogenase, Alkaline phosphatase.

Introduction:

Cancer is the second most common disease in India responsible for maximum mortality with about 0.3 millions deaths per year (1). In India, the age standardized incidence rate of oral cancer is 12.6/100,000 population and a sharp increase in the incidence rate of this cancer has been reported in recent years (2). The main reason is attributed to the usage of tobacco yet 1.93% is not related to tobacco (3) and genetics constitute 5-10%. Potentially malignant lesions of oral cavity are relatively common occurring in about 2.5% of the population (4), with a malignant transformation rate in various studies & locations that range from 0.6 to 20% (3). Oral leukoplakia is the most commonly occurring precancerous lesion of the oral cavity representing 85% of such lesions (5).

Potentially malignant lesions areusually asymptomatic and they are diagnosed by the dentist during routine dental examination.Once the patient is diagnosed as having oral leukoplakia, the treatment is first directed towards elimination of risk factors like smoking & alcohol. If the lesion does not heal in 2 to 3 weeks after elimination of risk factor, then conservative treatment like Vitamin therapy, Antioxidants or surgical treatment like conventional surgery, cryosurgery, electrocautery, CO_2 laser should be considered (3).

Though biopsy is mandatory before making any treatment plans (3), biopsy is an invasive procedure and all patients may not be willing for biopsy as the lesion is usually asymptomatic. There are various non-invasive techniques in detecting oral pre-malignancies, which include ViziLite, Microlux DL system, Orascopic DK system & VELscope system & cytopathology by oral CDx Brush test system (6).As most of the homogenous leukoplakia is asymptomatic thepatients may not be willing either for an invasive procedure like biopsy or for non-invasive and expensive procedures like ViziLite or VELscope.So switching on to a non-invasive and relatively inexpensive procedure like Sialodiagnosis becomes essential.

There are several body fluids which can be used for diagnostic purposes like saliva, urine, cerebrospinal fluid etc., but Sialodiagnosis has its own advantages as it is non - invasive and the technique is easier to perform. It has also been shown that salivary levels of biomarkers are found to be equally sensitive as serum levels (7).

Use of saliva in evaluating the biomarkers for early diagnosis of cancer risk potential may be more appropriate in oral cancer, as saliva reflects most of the oral diseases & effects of oral mucosa in cancer can be better reflected in saliva as it bathes the entire oral cavity (8)

Cellular Alkaline phosphatase (ALP) is increasingly recognized as an important marker of induction of tumor cell differentiation (9). The development of cancer is associated with a high glycolytic activity with a shift from aerobic to anerobic glycolysis. With the increase in the glycolytic activity the concomitant increase in lactate dehydrogenase (LDH) enzyme may be reflected in certain tissues (10).

Materials & Methods:

The study involved 42 subjects with age range of 30-70 years reporting to the Department of Oral Medicine and Radiology. Fourteen healthy individuals without the habit of tobacco usage formed the first group. Fourteen clinically diagnosed cases of leukoplakia formed the second group. Fourteen clinically and histopathologically diagnosed cases of Oral Squamous Cell Carcinoma comprised the third group. Patients with history of diabetes, hypertension, cardiac, liver, renal diseases, muscle dystrophy, periodontitis were excluded from the study. Ethical clearance was obtained from the institution .5 ml of unstimulated saliva was collected from each of the patients by the spit method in a calibrated test tube after getting a prior informed consent from them. Care was taken to see that the volunteers did not consume food or chew gum at least one hour before the collection procedure. Following collection, saliva was immediately centrifuged at 2500 rpm for 15 minutesto remove squamous cells and cell debris. The resulting supernatant was separated into 1 ml aliquots and subjected for further biochemical assay analysis using standard kit method.The samples will be diluted in 1:1 ratio with saline & assayed using the standard kit & measured using auto analyzer & the LDH & ALP concentrations were expressed in terms of IU/L.

Results:

The mean values for ALP were found to be about 11.88 IU/L, 23.04 IU/L and 35.43 IU/L respectively for control, oral leukoplakia and oral squamous cell carcinoma cases respectively (Figure 1).The mean values for LDH were found to be about 79.70 IU/L, 102.54 IU/L and 268.57 IU/L for control, oral leukoplakia and oral squamous cell carcinoma respectively (Figure 2) A one way ANOVA was performed to compare the data among the three groups and it was found that the data differed significantly between the three groups with a p value of 0.001 for alkaline phosphatase and <0.001 for lactate dehydrogenase.

(Figure 1):

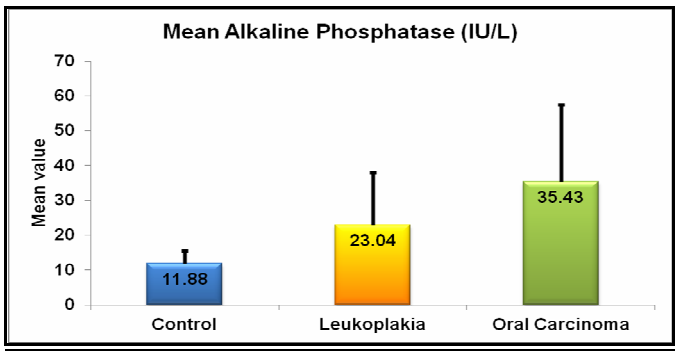
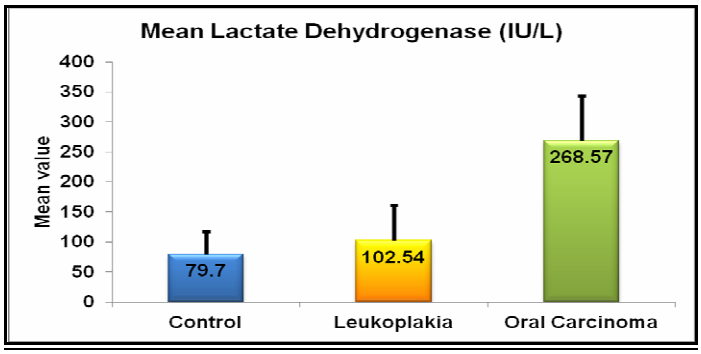


Figure 2:



Sensitivity Specificity Analysis:

Alkaline Phosphatase and Leukoplakia :(Table 1):

Parameter	Estimate	95% CI (Lower – Upper)
Sensitivity	64.29%	45.83, 79.29
Specificity	92.86%	68.53, 98.73
Positive Predictive Value	94.74%	75.36, 99.06
Negative Predictive Value	56.52%	36.81, 74.37
Diagnostic Accuracy	73.81%	58.93, 84.70

Lactate Dehydrogenase &Leukoplakia :(Table 2):

Parameter	Estimate	95% CI (Lower – Upper)
Sensitivity	64.29%	45.83, 79.29
Specificity	85.71%	60.06, 95.99
Positive Predictive Value	90.00%	69.90, 97.21
Negative Predictive Value	54.55%	34.66, 73.08
Diagnostic Accuracy	71.43%	56.43, 82.83

Alkaline Phosphatase & Oral Carcinoma: (Table 3)

Parameter	Estimate	95% CI (Lower – Upper)
Sensitivity	57.14%	32.59, 78.62
Specificity	92.86%	77.35, 98.02
Positive Predictive Value	80.00%	49.02, 94.33
Negative Predictive Value	81.25%	64.69, 91.11
Diagnostic Accuracy	80.95%	66.70, 90.02

Lactate Dehydrogenase & Oral Carcinoma (Table 4):

Parameter	Estimate	95% CI (Lower – Upper)
Sensitivity	100.00%	78.47, 100.00
Specificity	82.14%	64.41, 92.12
Positive Predictive Value	73.68%	51.21, 88.19
Negative Predictive Value	100.00%	85.69, 100.00
Diagnostic Accuracy	88.10%	75.00, 94.81

Discussion:

Literature review reveals studies in salivary biomarkers for leukoplakia started in 1970's, yet maximum number of studies is being conducted in the last 13 years. Salivary biomarkers have been studied in 208 leukoplakia patients, 283 OSCC patients & 300 controls. Maximum number of leukoplakia patients studied in a clinical trial is 32 (11) by Jie Wei et al in the year 2011 for the markers Gamma amino butyric acid,

phenylalanine, valine, n-eicosanide, lactic acid. In 9 trials, marker in leukoplakia is compared with that of OSCC and in 3 trials with control only (12, 13, 14). Many numbers of studies were conducted in Lactate dehydrogenase and studies have proven it can be used as a reliable marker for the early detection of OSCC (15, 16, 17, 18).

Lactate Dehydrogenase has been studied in 4 clinical trials (15, 16, 17, 18) and Alkaline phosphatase in 1 trial (16).A study similar to our clinical trial done in the year 1992, were Alkaline phosphatase, Acid phosphatase and Lactate dehydrogenase were studied in control, leukoplakia (n=7) and oral carcinoma cases (n=100) and concluded that all 3 markers will increase 1.5-6 times than control in carcinoma cases (16). The results of the current research have not only proved that both are significant, it has also proved that Lactate dehydrogenase seems to be more significant marker than Alkaline phosphatase in detection of oral carcinoma. In another comparative study in which Alkaline phosphatase and Lactate dehydrogenase estimation was done in both serum and saliva in acute leukemia (n=70) and Oral Squamous Cell Carcinoma patients (n=20), 20 control and 12 periodontitis patients. The results of the study proved that both serum and saliva are reliable diagnostic tools while saliva seems to be more significant in oral malignancies like OSCC. Thus this study proves the significance of saliva as a diagnostic tool especially in oral lesions such as oral potentially malignant lesions and oral malignancies (19).

The progression of oral leukoplakia to carcinoma though unpredictable it has been reported that non homogenous leukoplakia has a greater risk of carcinomatous transformation (20-25%) than homogenous leukoplakia (0.6-5%) (20). It is true as in this current research, cases of speckled leukoplakia have been found to be with higher values. But there was a case of homogenous leukoplakia in which the LDH value (224.30 IU/L) was found to be even higher. This could be attributed to other reasons such as Loss of Heterozygosity at either 3p or at 9p in keratinocytes of oral leukoplakia which is associated with carcinomatous transformation of the lesion (21).It is certain that leukoplakias with malignant potential and those without malignant potential cannot be distinguished clinically (22). Thus this study proves that we should not rely only upon clinical diagnosis and a co-relation between clinical diagnosis and investigations has to be made to direct the patients toward proper treatment.

In conclusion, Salivary Alkaline phosphatase and Lactate dehydrogenase are equally sensitive markers for the early detection of oral carcinoma.Statistical analysis also proves that Lactate dehydrogenase could be more reliable marker than Alkaline phosphatase in the detection of oral carcinoma. Salivary diagnosis should be performed in all the dental institutions to assess the malignant risk potential of potentially malignant disorders and thus quality of life of patients can be improved.

Acknowledgement:

ICMR funds are greatly acknowledged.

Conflicts of Interest:

"The author(s) declare(s) that there is no conflict of interests regarding the publication of this paper."

References:

1. Imran Ali, Waseem A.Wani, Kishwar Saleem.,Cancer Scenario in India with Future Perspectives,J Cancer Therapy.,2011,8, 56-70.
2. Misra C, Majumder M, Bajaj S, Ghosh S, Roy B, Roychoudhury S., Polymorphism at p53, p73, and MDM2 loci modulate the risk of tobacco associated leukoplakia and oral cancer, Mol Carcinog., 2009,48,790-800.
3. Prasanna Kumar Rao J.,Potentially malignant lesion – oral leukoplakia, Global Advanced Research Journal of Medicine and Medical Sciences., 2012; 1(11):286-291.
4. McCullough MJ, Prasad G, Farah CS.,Oral mucosal malignancy and potentially malignant lesions: an update on the epidemiology, risk factors, diagnosis and management,Aust Dent J., 2010 Jun, 55 Suppl 1:61-65.doi:10.1111/j.1834-7819.2010.01200.x.
5. Neville BW, Damm DD, Allen CM, Bouquot JE.,Epithelial Pathology In: Saunders 3rd ed. Philadelphia, Oral and maxillofacial pathology, 2009, 388.

6. Masthan KMK,AravindhaBabu N, Kailash Chandra Dash, Elumalai M.,Advanced diagnostic aids in oral cancer, Asian Pacific J Cancer Prevention 2012,13,3573-3576.
7. Rajkumar K, Ramesh kumar A, Ramyamalini V et al.,Estimation of serological and salivary biomarkers in patients with oral squamous cell carcinoma, premalignant lesions & conditions, SRM University Journal of Dental Sciences., 2010,1(1),14-19.
8. Greabu M, Battino M, MohoraM et al., Saliva – a diagnostic window to the body, both in health and in disease, J Med Life., 2009 Apr-Jun,2(2),124-32.
9. Xiao B, Guo J, Liu D, Zhang S., Aloe – emodin induces in vitro G2/M arrest & alkaline phosphatase activation in human oral cancer KB cells, OraOncol., 2007,43(9),905-10.
10. Zodag HA, Klein F., Clinical applications of lactate dehydrogenase isoenzymes:Alteration in malignancy, Ann N Y Acad Sci.,1968,151,578-80.
11. Wei J, Xie G, Zhou Z.,Salivary metabolite signatures of oral cancer and oral leukoplakia,Int J Cancer., 2011,129(9),2207-17.
12. Airoldi M, Negri L, Pedani F, Gerbino G, Camoletto D, Piantino P.,Serum and salivary CEA in benign and malignant lesions of the oral cavity, Boll SocItalBiolSper., 1984,60(4),865-70.
13. Brailo V, Vucicevic-Boras V, Cekic-Arambasin A, Alajbeg IZ, Milenovic A, Lukac J.,The significance of salivary interleukin – 6 and tumor necrosis factor alpha in patients with oral leukoplakia, Oral Oncol., 2001,43(1),37-41.
14. Hiroshi Kobayashi,Kumagai K, Gotoh A.,Upregulation of epidermal growth factor receptor 4 in oral leukoplakia,International Journal Of Oral Science.,2013,5,15-20.
15. Langavad E, Zachariah J, Pindborg JJ.,Lactate dehydrogenase isoenzyme patterns in leukoplakia, submucous fibrosis & carcinoma of the oral mucosa in South Indians,ActaPathol Microbial Scand., 1970,78,509-15.
16. Bassalyk LS, Guskova NK, Pashintseva LP, Liubimova NV.,Enzyme and isoenzyme activity in patients with malignant tumors of the oral mucosa,VoprOnkol., 1992,38(3),291-9.
17. Shetty SR, Chadha R, Babu S, Kumari S, Bhat S, Achalli S.,Salivary LDH levels in oral leukoplakia and oral squamous cell carcinoma: A biochemical and clinicopathological study, J Can Res Ther., 2012,8(6),123-125.
18. PriyaShirish Joshi, MadhuriChougule, Mahesh Dudanakar, SomeshwarGolgire.,Comparison between salivary and serum lactate dehydrogenase levels in patients with Oral leukoplakia and oral squamous cell carcinoma – a pilot study, International Journal of Oral and Maxillofacial Pathology., 2012,3(4),07-12.
19. Khalida S. Merza, Sanad B. Alaaraji, Bashar H. Abdullah.,Comparative study on lactate dehydro genase, alkaline phosphatase and immunoglobulins in serum and saliva of acute leukemia and oral squamous cell carcinoma patients, Iraqi Journal of Science., 2010,51(2),262-270.
20. Feller L, Lemmer J.,Oral leukoplakia as it relates to HPV Infection: A Review,International Journal of Dentistry., 2012,doi:10.1155/2012/540561.
21. Lee JJ, Hong WK, Hittelman WN et al.,Predicting cancer development in oral leukoplakia: ten years of translational research, Clinical Cancer Research., 2000,6(5),1702-1710.
22. Feller L, Lemmer J.,Field Cancerization and oral leukoplakia, In: Dakubo G D, ed. Field Cancerization: Basic Science and Clinical Applications, Ontario, Canada: Nova Science, 2011, 95-111.

*****