IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 3 Ver.7 March. (2018), PP 20-26
www.iosrjournals.org

Level of Serum Lactate Dehydrogenase Enzyme in Oral Lichen Planus-A Biochemical Study

Arya S Nalin[1], Rajeev R[2], Giju Baby George[3], Sheeba Padiath[4]
[1] *Assistant professor, Department of Oral Medicine and Radiology, Government Dental College/ Trivandrum,Kerala,India*
[2] *Assistant professor, Department of Oral Pathology, Government Dental College/ Trivandrum,Kerala,India*
[3]*Principal, Anoor Dental College, Moovatupuzha,Kerala ,India*
[4]*Professor, Department of Oral Medicine and Radiology, Mar Baselios Dental College/ Thankalam,Kerala,India*
Corresponding author: Dr R.Rajeev

Abstract: ***Aims and Objectives:*** *Evaluation of alteration in serum LDH in patients with erosive lichen planus and to compare it with that of controls.* ***Materials and Methods:*** *Blood samples were collected using standardized methods in subjects divided equally into two groups [control group and oral lichen planus group (n=20)].Biochemical assays for LDH was done. The serum levels of LDH in patients with erosive lichen planus was compared with that of the controls.****Results:*** *This study showed no significant difference in serum levels of LDH among Lichen planus and control group. (MD = 37.000, p-value = 0.427).* ***Conclusion:*** *Simce the histochemical study of oral mucosal tissue has demonstrated a marked difference between lactate dehydrogenase activity in oral lichen planus lesions as compared to normal and chronically inflamed oral mucosa; definite biochemical alterations may occur in serum also concurrently with the development of oral erosive lichen planus. But in order to establish this factor, multi centric trails including the follow-up of patients are necessary to understand the true value of serum LDH level as a prognostic parameter in oral lichen planus*
.

Keywords: *Lactate dehydrogenase, Oral lichen planus, Oral potentially malignant disorders*

Date of Submission: 28-02-2018 Date of acceptance: 17-03-2018

I. Introduction

Oral lichen planus (OLP) is considered as a chronic inflammatory disease of unknown etiology [1]. Even though the exact cause is unknown, activated cytotoxic T-cells are found to be located close to damaged basal keratinocytes, which may suggest that they are responsible for the damage and supports the theory of a cell-mediated immune response actively participating in the local pathogenic mechanisms in OLP [2]. In the oral cavity, the disease assumes somewhat different clinical appearance than on the skin, and has six classical clinical presentations as described in the literature [3]. The most common entity is the reticular form which are usually asymptomatic, bilaterally/symmetrically anywhere in the oral cavity but most common on buccal mucosa, tongue, lips, gingiva, floor of mouth, palate and may appear weeks or months before the appearance of cutaneous lesions. An erosive form of this disease presents as chronic multiple oral mucosal ulcers. Erosive lesions of lichen planus occur in the severe form of the disease when extensive degeneration of the basal layer of epithelium causes a separation of the epithelium from the underlying connective tissue [4].

Lactate dehydrogenase (LDH) is a ubiquitous enzyme that plays a significant role in the diagnosis of pathologic processes [5].This enzyme catalyzes the reaction of lactate production via pyruvate reduction during anaerobic glycolysis [6]. Lactate dehydrogenase is believed to vary according to the metabolic requirement of each tissue, and alteration in LDH levels has been observed during development, under changing biological conditions, and in response to pathological processes [7]. Lactate dehydrogenase activity in serum increases as a marker of cellular necrosis [8]. Increased LDH levels are due to increased mitotic index and more lactic acid production by tumour cells due to breakdown of glycoprotein [9]. It has been found that the serum LDH levels are increased in potentially malignant disorders and malignancy [6] .The histochemical study of oral mucosal tissue also demonstrated a marked difference between lactate dehydrogenase activity in oral lichen planus lesions as compared to normal and chronically inflamed oral mucosa [9]. In addition to a generalized increase in enzyme activity, there was a marked increase localized to the area of the basal layer. This histochemical confirmation of an altered metabolism in lichen planus lesions is of interest, particularly since an increase in lactate dehydrogenase activity is suggestive of an increase in glycolysis whereby glucose is metabolized anaerobically to lactic acid rather than aerobically to carbon dioxide.

With the above background, the aim of the study was to evaluate serum levels of LDH in patients with erosive lichen planus and to compare it with healthy controls.

II. Materials and methods

The study was conducted in a tertiary dental care centre of Kerala, South India which included10 patients with erosive form of lichen planus in the oral cavity and 10 healthy looking volunteers those were age- and sex-matched with the patients All of the OLP patients were diagnosed clinically, and the diagnoses were confirmed through histopathologic examination according to the modified WHO diagnostic criteria for OLP. Relevant history of each patient was recorded thoroughly, to rule out any systemic illness or any prior therapy. After thorough evaluation, 10 subjects for the control group were selected from age and sex matched subjects. These subjects were ruled out for the presence of any systemic illness by taking thorough history. Further, these subjects did not have any oral tissue abuse habits or any obvious oral lesions. Accordingly the subjects for the study were grouped as follows:
Group I (control), Group II (Erosive lichen planus) [Table 1]

Inclusion &Exclusion criteria for cases

	Inclusion Criteria	**Exclusion Criteria**
	• Patients willing to participate • Subjects in the age group of 20 to 50 years irrespective of sex • Clinically and histopathologically diagnosed cases of erosive lichen planus	• Patients not willing for participation in the study • Patients undergoing chemotherapy, radiotherapy, or any surgical procedure for oral cancer • Patients with a history of heart failure (myocardial infarction) within past 2 weeks • Patients taking procainamides and other drugs used to treat arrhythmia, pulmonary infarction, and stroke. • Patients suffering from hepatitis, hypothyroidism, anaemia (haemolytic or pernicious anaemia), lung disease, liver disease, kidney disease, pancreatitis, muscle trauma, and muscular dystrophy. • Patients with history of consumption of aspirin, narcotics or alcohol,

Method of data collection

1. All patients fulfilling the inclusion criteria were informed about the study and only those who agree were enrolled in the study.
2. An informed consent was obtained from each patient.
3. Incision biopsy was performed at clinically most representative area in erosive lichen planus cases.
4. Tissue were fixed in formalin
5. The specimen was then processed and cut by a rotary microtome to obtain paraffin sections of 5 microns thickness.
6. Each section was stained using Haematoxylin -Eosin and observed under microscope.
7. Only those cases concurring histopathologically with the features of erosive lichen planus were included in the study.
8. From both cases and controls, a volume of 5 ml blood collected from the antecubital vein by using standard aseptic precautions was transferred to a sterile test tube and allowed to clot at room temperature for 2 hrs. The serum was then separated out, and centrifuged at 2000 rpm for 10 min.
9. LDH was estimated by means of a standard kit method.

Biochemical analysis

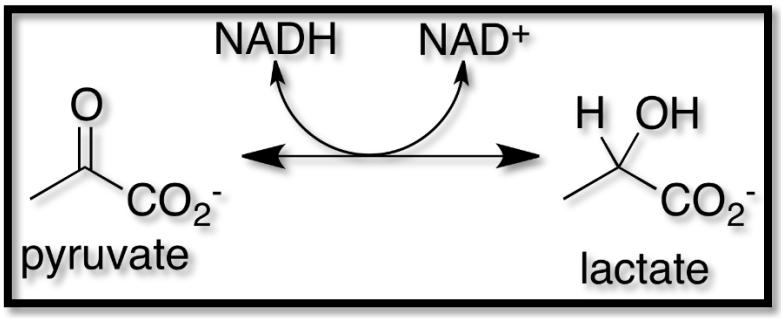
Biochemical analysis was carried out with a fully automated machine (Dimension RxL Max Integrated Chemistry System made by Siemens Industry Limited, Kolkata, India). Reagent kit used for the estimation of LDH was manufactured by Siemens known as Flex reagent cartridge LDI [LDI Flex reagent cartridge, Cat. No.DC35]. Flex r dimension LDI method is an *in vitro* diagnostic test for the quantitative measurement of LDH in human serum and plasma on the Dimension R clinical chemistry system. This method is standardized to the International Federation of Clinical Chemistry [IFCC] lactate dehydrogenase [LD] primary reference method procedure at 37 degree Celsius, adapted to the Dimension R clinical chemistry system.

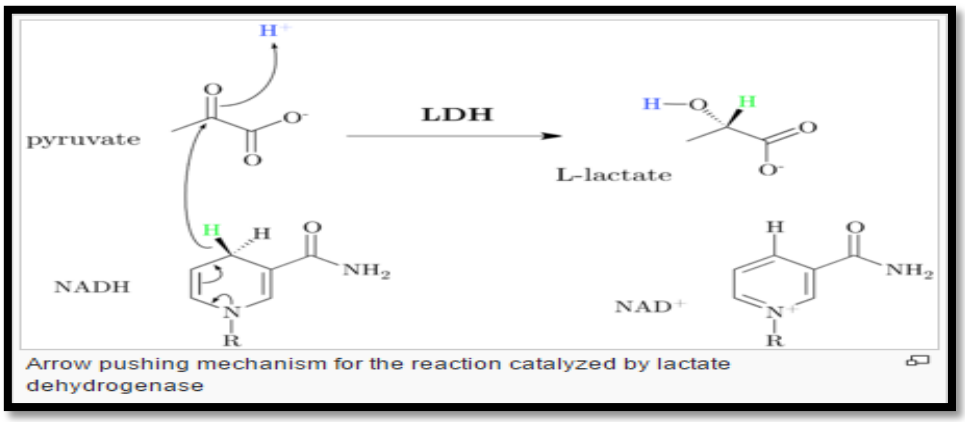
Principle

It works on the principle that LDH catalyses the reduction and conversion of the substrate pyruvate to lactate in the presence of NADH.

The reaction catalyzed by LDH is as follows;

Pyruvate + NADH + H+ → L-Lactate + NAD





Reagents:

Wells	Form	Ingredient	Concentration[b]
1-4 Reagent 1	Liquid	N-Methyl-D-glucamine	1091 mmol/l
		L(+)Lactate	168 mmol/l
		NaCl	513 mmol/l
5-6 Reagent 2	Liquid	NAD^+	16.5 mmol/l
		NAD Lithium salt	36.0 mmol/l
		Preservative	
		Stabiliser	

Reagent details of lactate dehydrogenase

- Wells were numbered consecutively from the wide end of the cartridge.
- Nominal value per well in a cartridge.
- Wells 5-6 contained preservative and stabilizer.
- All reagents were in liquid form and was ready to use.
- Sampling reagent delivery, mixing, processing and result printing were automatically performed by the dimension system.

Test conditions:

- Sample volume :8 microliter
- Reagent 1 volume :106 microliter
- Reagent 2 volume :50 microliter
- Reaction time :7.5 min
- Type of measurement :bio chromatic rate
- Calculated from test initiation to final result.

Statistical analysis

The data were analyzed with Student's independent *t*-test. All statistical analysis was performed with the program Statistical Package for Social Science (SPSS Statistics Base 17.0 software SPSS Inc. 233 South Wacker Drive, 11th floor, Chicago) and $P < 0.05$ was accepted as statistically significant.

III. Results

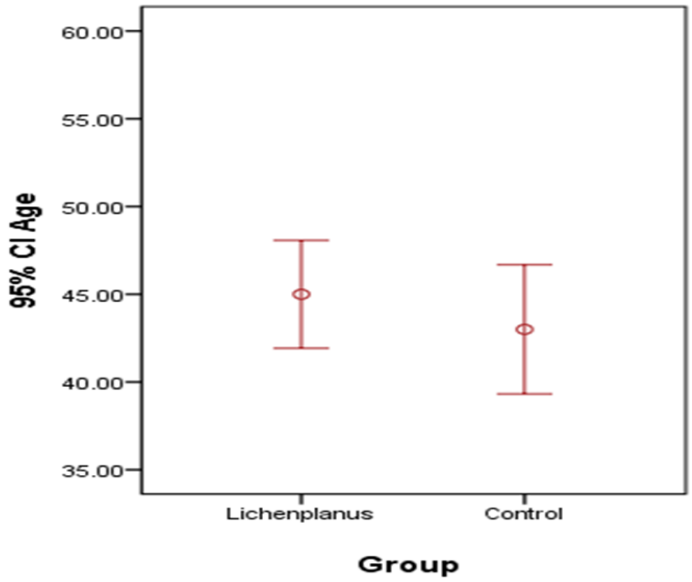
The mean age of cases and control were 45.000 and 43.000 respectively [Table1, Graph1]. The arithmetic mean of serum LDH level of each group was calculated. The standard deviation of each parameter was measured. The observed mean serum LDH level was 144.500 IU/L in the control group. In erosive lichen planus cases, the mean serum LDH level was 181.500 IU/L [Table 2, Graph 2]. There was no statistically significant difference in serum LDH level between two groups. (p=0.427)

Table 1: distribution of study groups

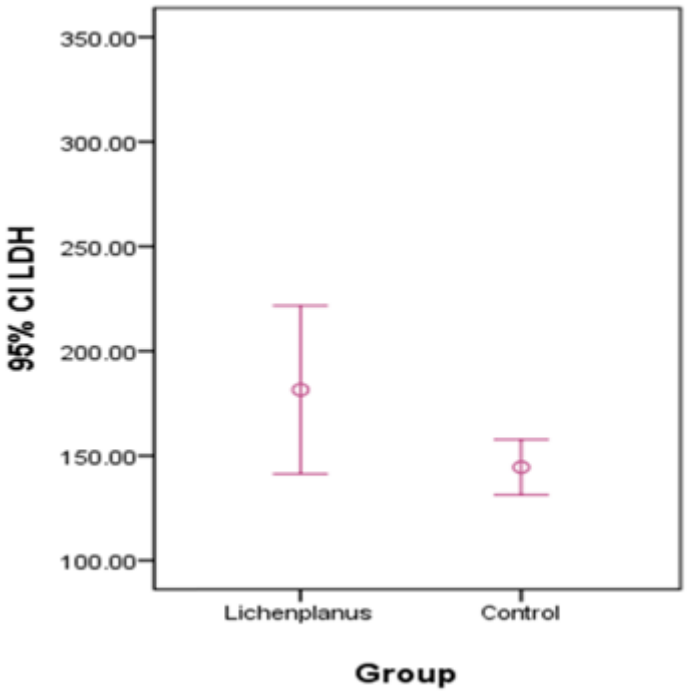
	N	Mean	Std. Deviation	Std. Error
Lichen planus	10	45.000	4.295	1.358
Control	10	43.000	5.142	1.626
Total	40	45.425	7.089	1.121

Table 2: mean serum LDH value in cases &control groups

	Group	N	Mean	Std. Deviation	Std. Error
LDH	Lichen planus	10	181.500	56.139	17.753
	Control	10	144.500	18.441	5.831
	Total	20	210.850	74.456	11.772



Graph 1: Error bar graph of age



Graph 2: Error bar graph of serum levels of LDH

IV. Discussion

Chronic inflammation is a pathological condition characterized by continued active inflammatory response and tissue destruction. Inflammatory process induces oxidative stress and reduces cellular antioxidant capacity. Lactate dehydrogenase is a cytoplasmic enzyme present essentially in all major organ system [10].The extracellular appearance of LDH is used to detect cell damage or cell death. Due to its extraordinarily widespread distribution in the body, serum LDH is abnormal in a lot of disorders. It is released into the peripheral blood after cell death caused by ischemia, extreme temperatures, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisoning [11].Various studies have shown that LDH is released during tissue injuries. Serum LDHs have been studied extensively in various cancers and increased levels have been observed [12]. As cells progress from normal through premalignant to the malignant condition, their chemical character may diverge from normal. Malignant cells have a distinctive type of metabolism. These cells alter the biochemical parameters, which are either increased or decreased [13]. Blood acts as a unique medium, which reflects various biochemical changes occurring in the body due to malignancy.
Carcinogenic changes have tremendous influence in increasing LDH activity. These carcinogenic changes may lead to decreased lactate to pyruvate conversion resulting in an anomaly in the regeneration of NAD+ that may interfere with glycolysis part of carbohydrate metabolism. Malignant tumor tissue or contiguous tissue damaged by tumor liberates enzymes into circulation that contributes toward abnormal increase in enzyme levels. Increased LDH levels are due to increased mitotic index and more lactic acid production by tumor cells due to breakdown of glycoproteins.Lactate dehydrogenase and its isoenzymes have been of considerable interest to the biochemical oncologist and serum LDH isoenzyme levels have been studied extensively in lung, breast, cervical and gastric cancers [14].The fundamental alterations in cancer cell are the change from aerobic respiration to increased aerobic glycolysis and anaerobic glycolysis, that is, fermentation [15]. According to Gerson and Silverman, protein synthesis and mitotic activity takes place at a greater rate in leukoplakic epithelium, than in nonkeratinized epithelium. Both protein synthesis and cell division are energy consuming process. The requirement for energy is thus greater in leukoplakic epithelium and this may account for an increase in glycolysis[16]. The progressively increasing serum LDH level is positively correlated with the degree of cellular atypia suggesting that serum LDH level can serve as a biochemical tool, in assessing the malignant potential of premalignant lesions. The significant increase in serum LDH level correlates with the increased cellular activity. Görögh *et al*. studied LDH isoenzymes in human epithelial cells from squamous cell carcinomas and healthy tissues of the oral cavity. They concluded that gradual changes in the percentage distribution of LDH isoenzymes may represent a useful parameter of disease activity in patients with squamous cell carcinoma[17]. Although the WHO has categorized OLP as a precancerous condition, the risk of malignant transformation of OLP remains a subject of debate in the literature. Some authors accept the possible malignant potential of OLP,

while others oppose this suggestion[18, 19]. It is uncertain,what mechanisms could cause malignant transformation of OLP. A cytokine-based microenvironment arising from chronic inflammation of OLP may induce genetic alterations of epithelial cells to progress to malignancy [20-25]. Expression of apoptosis and cell cycle regulating proteins such as p53 protein, p21 protein, p16 protein, bcl-2, and bax is also altered in the transformation process [26-30]. These molecular changes may be useful in further understanding malignant processes associated with OLP. Clinically, OLP lesions are known to be more chronic in nature than cutaneous lichen planus. The atrophic and erosive forms are believed to account for the vast majority of cases of malignant transformation of OLP to OSCC [31].The etiology behind OLP developing into OSCC also is poorly understood and many different hypotheses have been suggested. Some investigators propose that a lack of the expected keratinocyte apoptotic response to the cell-mediated attack may be etiologic in cancerous transformation [32].

The present study was thus carried out to evaluate the serum levels of LDH in patients with erosive lichen planus, so as to utilize this biochemical measurement as an adjunct to assess the clinical severity of the condition and to predict the malignant transformation potential well before they are clinically or histologically apparent and to monitor the progress of the disease at every step. None of our lichen planus cases were showing histological evidence of epithelial dysplasia. The lack of cytologic atypia in our cases is reflected in the serology of those patients with no evidence of a significant increase in LDH level. There may be a subset of lichen planus cases; even though very small in number where the molecular alterations are happening which ultimately ending up in a malignant change. Alteration in serum levels of LDH in OLP was suspected because of the increased rate of epithelial degeneration that occurs in OLP.Studies have proven that the alteration in serum LDH can be correlated with the amount of epithelial degeneration that is happening. But this study failed to rule out the relationship between OLP and serum LDH. This might be attributed to the fact that the severity of the epithelial destruction in our samples might be not up to the level of causing elevation in serum LDH [33, 34].

V. Conclusion

Serum LDH levels increase in oral premalignant lesions/conditions and OSCC. Serum LDH estimation can prove to be a valuable biochemical marker; as it is a simple procedure and may be easily accepted by the patient. Thus, the present study emphasize on further research to be conducted by including all variants of lichen planus cases with detailed description of its pathologic features and to evaluate the usefulness of serum LDH estimation to assess clinical severity of the condition and to predict the malignant transformation. Above all, studies including the follow‑up of patients are necessary; in order to understand the true value of serum LDH level as a prognostic parameter in oral lichen planus.

References

[1] Schlosser BJ. Lichen planus and lichenoid reactions of the oral mucosa. Dermatologic Therapy 2010; 23: 251–67

[2] Kilpi AM. Activation marker analysis of mononuclear cell infiltrates of oral lichen planus in situ.

[3] Canto AM, Müller H, Freitas RR, Santos PS. Oral lichen planus (OLP): Clinical and complementary diagnosis. A Bras Dermatol. 2010; 85:669–75.

[4] 4 -Greenberg M S. Ulcerative, vesicular, and bullous lesions. In: Greenberg M S, Glick M. Burket's oral medicine diagnosis and treatment. 1th ed. BC Deckerand J Dental Res 1987; 95:174-80. Inc; 2003

[5] Kallalli BN, Rawson K, Muzammil, Singh A, Awati MA,Shivhare P. Lactose dehydrogenase as a biomarker in oral cancer and oral submucous fibrosis. J Oral Pathol Med2016Oct;45(9):687-690.

[6] Pereira T, Shetty S, Pereira S. Estimation of serum lactate dehydrogenaselevel in patients with oral premalignant lesions/conditions and oral squamous cellcarcinoma:aclinicopathologicalstudy.

[7] Drent M, Cobben NA, Henderson RF, Wouters EF, vanDieijen-Visser M. Usefulness of lactate dehydrogenase andits isoenzymes as indicators of lung damage or inflammation.Eur Respir J 1996 Aug;9(8):1737-1742.

[8] Lokesh K, Kannabiran J, Rao MD. Salivary lactate dehydrogenase (LDH) – a novel technique in oral cancer detection and diagnosis. J Clin Diagn Res 2016 Feb;10(2):ZC34-ZC37

[9] Gerald Shklar,. Lactic Dehydrogenase activity in oral lichen planus lesionseThe Journal of Investigative Dermatology Vol. 45, No. 3

[10] Kallalli BN, Rawson K, Muzammil, Singh A, Awati MA,Shivhare P. Lactosedehydrogenase as a biomarker in oral cancer and oral submucous fibrosis. J Oral Pathol Med 2016Oct;45(9):687-690. 12.

[11] Nagler RM, Lischinsky S, Diamond E, Klien I, Reznick AZ.New insights into salivary lactate dehydrogenase of human subjects. J Lab Clin Med 2001 May;137(5):363-369.

[12] Patel S, Metgud R. Estimation of salivary lactate dehydrogenasein oral leukoplakia and oral squamous cell carcinoma:a biochemical study. J Can Res Ther 2015 Apr;11(1):119-123.

[13] .Dabelsteen E, Clausen H. Tumor‑associated carbohydrate antigens. J Oral Pathol 1987;16:196‑8.

[14] Bigler LR, Streckfus CF, Dubinsky WP. Salivary biomarkers for thedetection of malignant tumors that are remote from the oral cavity.Clin Lab Med 2009;29:71‑85.

[15] Vinzenz K, Schönthal E, Zekert F, Wunderer S. Diagnosis of head and neck carcinomas by means of immunological tumour markers (Beta‑2‑microglobulin, immunoglobulin E,ferritin, N‑acetyl‑neuraminic acid, phosphohexose‑isomerase).J Craniomaxillofac Surg 1987;15:270‑7.

[16] Gerson S, Silverman S Jr. Activity levels of some oxidoreductases of carbohydrate metabolism in leukoplakic and normal human buccal epithelium. J Oral Pathol 1973;2:77‑85

[17] Görögh T, Eickbohm JE, Ewers R, Lippert B. Lactate dehydrogenase isoenzymes in squamous cell carcinomas of the oral cavity. J Oral Pathol Med 1990;19:56‑9.

[18] I.R. Kramer, R.B. Lucas, J.J. Pindborg, et al.Definition of leukoplakia and related lesions: an aid to studies on oral precancerOral Surg Oral Med Oral Pathol, 46 (1978), pp. 518-539

[19] J. Sudbo, S.M. Lippman, J.J. Lee, et al.The influence of resection and aneuploidy on mortality in oral leukoplakia N Engl J Med, 350 (2004), pp. 1405-1413

[20] Das BR, Nagpal JK. Understanding the biology of oral cancer. Med Sci Monit. 2002;8:RA258–67.[PubMed]

[21] Vielba R, Bilbao J, Ispizua A, Zabalza I, Alfaro J, Rezola R, et al. p53 and cyclin D1 as prognostic factors in squamous cell carcinoma of the larynx. Laryngoscope. 2003;113:167–72. [PubMed]

[22] Nagpal JK, Mishra R, Das BR. Activation of Stat-3 as one of the early events in tobacco chewing-mediated oral carcinogenesis. Cancer. 2002;94:2393–400. [PubMed]

[23] Shpitzer T, Hamzany Y, Bahar G, Feinmesser R, Savulescu D, Borovoi I, et al. Salivary analysis of oral cancer biomarkers. Br J Cancer. 2009;101:1194–8. [PMC free article] [PubMed]

[24] Shimakage M, Horii K, Tempaku A, Kakudo K, Shirasaka T, Sasagawa T. Association of Epstein-Barr virus with oral cancers. Hum Pathol. 2002;33:608–14. [PubMed]

[25] Eichel HJ, Conger N, Chernick WS. Acid and alkaline ribonucleases of human parotid, submaxillary, and whole saliva. Arch Biochem Biophys. 1964;107:197–208. [PubMed]

[26] Hu Z, Zimmermann BG, Zhou H, Wang J, Henson BS, Yu W, et al. Exon-level expression profiling: A comprehensive transcriptome analysis of oral fluids. Clin Chem. 2008;54:824–32. [PMC free article][PubMed]

[27] Hasselmann D, Rappl G, Tilgen W, Reinhold U. Extracellular tyrosinase mRNA within apoptotic bodies is protected from degradation in human serum. Clin Chem. 2001;47:1488–9. [PubMed]

[28] . Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: Important and underappreciated mediators of cell-to-cell communication. Leukemia.2006;20:1487–95. [PubMed]

[29] García JM, García V, Peña C, Domínguez G, Silva J, Diaz R, et al. Extracellular plasma RNA from colon cancer patients is confined in a vesicle-like structure and is mRNA-enriched. RNA. 2008;14:1424–32.[PMC free article] [PubMed]

[30] Skog J, Würdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers.Nat Cell Biol. 2008;10:1470–6. [PMC free article] [PubMed]

[31] Eisen D. The clinical features, malignant potential,and systemic associations of oral lichen planus: a study of 723 patients. J Am Acad Dermatol. 2002;46:207-214.

[32] Ismail SB, Kumar SK, Zain RB. Oral lichen planus and lichenoid reactions: etiopathogenesis, diagnosis,management and malignant transformation. J Oral Sci. 2007;49:89-106

[33] L. Feller and J. Lemmer, "Field Cancerization and Oral Leukoplakia," In: G. D. Dakubo, Ed., Field Cancerization: Basic Science and Clinical Applications, Nova Science Publishers, Ontario, 2011, pp. 95-111.

[34] Wallimann T, Dolder M, Schlattner U, Eder M, Homemann T, O'Gorman E, Ruck A, Brdiczka D. Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. Biofactors. 1998; 8:229–234.

Rajeev R "Level Of Serum Lactate Dehydrogenase Enzyme In Oral Lichen Planus-A Biochemical Study" IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 3, 2018, pp 20-26