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COMPARATIVE ANALYSIS ON ALKALINE PHOSPHATASE, LACTATE DEHYDROGENASE, AND IMMUNOGLOBULINS INSALIVA OF PATIENTS SUFFERING FROM ORAL MALIGNANCY LIKE ORAL SQUAMOUS CELL CARCINOMA

Biochemistry

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ABSTRACT

Biochemical changes have been occurring in biological fluids and tissues of different types of malignancies. Most molecules found in blood and urine is found in saliva, but their concentrations were estimated to be one tenth to one thousandth of that in the blood. The present study aims to measure the levels of Lactate dehydrogenase & Alkaline Phosphatase enzymes and Immunoglobulins in saliva of Oral Squamous Cell Carcinoma (OSCC) patients, and apparantly healthy individuals as control group. Unstimulated (resting) saliva was collected from 30 newly diagnosed, untreated OSCC patients, in addition to 30 healthy individuals. According to the results of this study, saliva enzymes showed a significant increase in Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP) levels of OSCC in comparison to control group. Patients with OSCC resulted a significantly increase in saliva immunoglobulins and increase in saliva IgA was also noticed in comparison to control group. Saliva Immunoglobulins IgG and IgM were also higher in OSCC patients In conclusion, in a local malignancy like OSCC; the changes are more prominent in saliva.

KEYWORDS

INTRODUCTION

Humans have always been challenged by some or other diseases, even a smallest disease will have an extensive impact if the cause is not known, i.e. because the basic aspect of treatment lies on etiopathology[1]. Today's scientific world has tried to develop a long time unknown relationship between neoplasia and carcinogens. Oral cancer is an important global concern accounting for an estimated 2, 75,000 cases and 1, 28,000 deaths annually [2]. Squamous cell carcinomas of head and neck are a disease associated with major morbidity and mortality [3]. Oral squamous cell carcinoma (OSCC) is the 6th most common human cancer that encompasses at least 90% of all oral malignancies [4]. Prevention and early diagnosis remain the best instrument in our armamentarium for reducing the death rate associated with oral cancer [5].

The early diagnosis and treatment of cancer are based on the concept that oral cancer develops overlong period of time, going through intermediate stages of different biological significance. Treatment at this early or pre-invasive stages offer the best prognosis and even the chances of cure [6]. The starting point of cancer is the mucosal epithelium which when subjected to exogenous sand endogenous factors (carcinogens) produce changes that are reactive and reversible but with progressive loss of normal control mechanism they lead to precancerous state [7]. Recently, the role of tumor markers in management of head and neck cancer has received increasing attention. Tumor markers are biochemical substances elaborated by tumor cells either due to the cause or effect of malignant process [6]. These marker scan be normal endogenous products that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells [8]. Continuing search for ideal tumor markers in serum, tissue and other body fluids during neoplastic process is of clinical value in the management of patients with various malignancies.

Unfortunately none of the tumor markers reported to date is ideal [6]. Saliva is a complex and dynamic biologic fluid, which over the years has been recognized for the numerous functions it performs in the oral cavity. Modern technology, however, has unveiled a plethora of compounds never before detected in saliva (e.g. drugs, pollutants, hormones; but also biomarkers of bacterial, viral, and systemic diseases). Other advantages of saliva over serum are that it is easy to collect, store and transport, easy to handle for diagnostic procedure, it does not clot, so lessens the manipulation required [8, 9].

The enzyme lactate dehydrogenase (LDH) is a ubiquitous enzyme that was discovered in the early period of enzymology. This enzyme catalyzes the reaction of lactate production via pyruvate reduction during anaerobic glycolysis. LDH concentration in saliva, as an expression of cellular necrosis, could be a specific indicator for oral lesions that affect the integrity of the oral mucosa [11].

LDH have been of considerable interest to the biochemical oncologist and serum LDH and its isoenzyme levels have been studied extensively in various body cancers and increased levels have been observed. Serum LDH isoenzyme determination in carcinomas has been found to be useful in diagnosis as well as an important prognostic parameter [12].

Studies on analysis of salivary LDH either total or isoenzymes OSCC patients have not been carried out extensively. So the present study was designed to evaluate and inter compare the salivary lactate dehydrogenase enzyme levels in normal individuals, patients with oral squamous cell carcinoma and to inter compare the salivary lactate dehydrogenase enzyme levels in different histopathological grades of oral squamous cell carcinoma.

Salivary alkaline phosphatase in oral potentially malignant disorders- In 2016 a study was conducted where they observed that the S-ALP enzyme was significantly greater in diabetes mellitus, smokers and subjects with potentially malignant disorders without any periodontitis compared to systemically healthy individuals [23]. Based on the findings of their study, they emphasized that the screening of premalignancies and malignant lesions can be made by measuring salivary AP levels [23]. In one more study conducted 2014, salivary AP and lactate dehydrogenase resulted to be equally sensitive markers for the early detection of oral carcinoma [24]. Comprehensive salivary analysis revealed an overall altered salivary composition in OSCC, indicating a compromised oral environment in these patients and suggesting salivary analysis as a new diagnostic tool for oral cancer [11]. Studies have even tried to determine the utility of salivary IgA as a biomarker in oral precancer and cancer. Researchers have found increased levels of serum IgA in patients with OSCC as compared to healthy controls.[11] It has also been reported that the levels of serum IgG increase with the progression of the disease [11]. Since IgA is associated with local immune response and saliva is in direct constant contact with oral lesions, salivary IgA is proposed to accurately reflect the changes caused by OPMD and OSCC in the oral cavity. The increase in salivary IgA in OSCC is proposed to be due to increased local infection, increased antigenic inflammatory stimulus, increased local synthesis, and local host reaction to the disease[19].

MATERIAL AND METHOD

Study population

Patients were selected from those attending the OPD of the department of Biochemistry, Oral Pathology and Oral Medicine of the institution and associated medical colleges. A routine general physical examination to assess the vital signs was carried out. Study subjects were assigned to one of the three groups:

Group I Comprised of 30 healthy individuals of comparable age, Group II comprised of 30 otherwise healthy and consenting patients with oral squamous cell carcinoma patients.

Collection of data

None of the study group was on a therapeutic regimen or suffering from any systemic conditions that could have affected the salivary LDH level in the body. Patients undergoing cancer therapy and suffering from oral and systemic conditions known to alter lactate dehydrogenase enzyme levels were excluded from the study. Participants were informed in detail about the planned study and written informed consents were obtained. An ethical clearance certificate from the Institution's ethical committee was obtained prior to the study. Patients were asked to sit comfortably with head in upright position; they were asked to rinse the oral cavity using 30 ml of normal water and then were asked to accumulate the saliva in their oral cavities (unstimulated whole saliva) for 5 min. The patients were asked to spit the accumulated saliva in sterile, disposable container, until a minimum desired quantity of 2 ml was obtained. Biopsy specimens obtained from primary OSCC patients were processed and stained with hematoxylin andeosin. Sections of OSCC were histopathologically graded for differentiation of carcinoma. These containers were then kept in an ice box and were brought to the biochemistry laboratory, within 2 hours and biochemical estimation of LDH with the help of Semiautomatic Analyzer by using Biovision Lactate Dehydrogenase Activity Colorimetric Assay Kit.

Saliva samples were collected from OSCC patients preoperatively.

The collected saliva was centrifuged at2500 rpm for 10 min within one hour from collection to eliminate debris and cellular matter. All samples were kept at (-20° Ċ) in polyethylene tubes until analyses [15]. The saliva supernatant was then analyzed.

Calculation:

ΔOD / mn. x 8095 = U / L

The concentration of standard = 8095 U/L (Unit per liter).

Enzyme activity is expressed in international units (IU), that is equivalent to the amount of enzyme that catalyzes the conversion of 1 μ mole of substrate per minute [16].

Alkaline Phosphatase Principle

Colorimetric determination of alkaline phosphatase activity was made according to following reaction:

$$Phenylphosphate \xleftarrow[pH10]{ALP} \rightarrow phenol + phosphate$$

The librated phenol was measured in the presence of 4-aminoantipyrine and potassium ferricyanide [16].

Radial Immunodiffusion assay (RID):

RID was used for measuring the concentration of various soluble antigens in biological fluids as follow:

1. RID plates (supplied in foil pouches). These contain mono specific antibody to IgG, IgA, and IgM in agarose gel. Up to fourteen samples can be run per plate.

Preservatives: 0.1% sodium azide, 0.1% Є-amino-n-caproic acid (EACA), 0.01%thiomersal. (Sodium ethylmercurithiosalisalate), 0.01% benzamidine .

RESULTS

Biochemical Analysis of LDH and ALP in OSCC:

Concentrations of LDH and ALP enzymes in patients with OSCC showed the following changes:

A significant increase in saliva LDH levels of OSCC patient in comparison to that of control group.

The results also showed a highly significant increase in serum ALP levels of OSCC patients in comparison with that of control group while the increase in salivary ALP levels of OSCC patients was not significant in comparison with control group as shown in Table 1 below

Table 1: Saliva enzymes concentration in OSCC patients in comparison to the control group

Enzymes	OSCC patients	Control	P value
LDH U/L	1440.21±335.75	382.1±186.53	0.000
ALP U/100ml.	220.17±36.91	89.53±22.91	0.000

P<0.05=statistically significant. S=Significant, HS=Highly significant, NS=Not significant,

LDH= Lactate dehydrogenase, SD=Standard deviation

Biochemical Analysis of immunoglobulins in OSCC patients:

Table 2: The mean concentration of Immunoglobulins in saliva of OSCC patients and control group

Immunoglobulins	OSCC patients	Control	P value
IGA	5.94±0.82	3.06±1.47	0.000
IGG	19.45±2.54	10.33±2.78	0.002
IGM	4.12±1.23	35.9±0.42	0.000

P<0.05=statistically significant. S=Significant, HS= Highly significant, NS=Not significant,

LDH=Lactate dehydrogenase, SD=Standard deviation

DISCUSSION

The results showed that there was a significant increase in serum LDH activity of OSCC patients as compared to control group. These findings indicate that gradual changes in the percentage distribution of LDH isoenzymes may represent a useful parameter of disease activity in patients with OSCC [24]. Salivary LDH activity was significantly higher than that of normal saliva. Other fluids were used for measuring LDH activity in different pathological conditions.

The present study indicates that an abnormal elevation in salivary LDH activity represents a release from pathologically altered cells rather than an increased biosynthesis [24].

In the present study, a non significant increase in salivary ALP level in OSCC patients was observed when compared with control group.

Comparing salivary ALP concentration in OSCC patient and periodontitis patients, the results show not significant differences.

The elevation of salivary ALP may be due to the secretion of saliva.

The increase in salivary IgG and IgM levels in saliva of OSCC patients could be tentatively attributed to their leakage from interstitial fluids through the torn and damaged oral mucosa. In control group, the intact epithelium prevented the leakage of immunoglobulins [25].

Another source of these immunoglobulins may be the locally accumulated plasma cells beneath the tumor [26].

The predominant immunoglobulin in saliva of healthy is secretory IgA, which is secreted by salivary glands through the localized plasma cells. The reduction in secretory IgA in head and neck cancer patients may be due to general depression in cell mediated and humoral immune response [27]. Salivary IgA will be decreased even in comparison with normal control, due to the depressed secretory ability of the salivary glands due to the immune defect in patients with malignancy [28]. The only secretory immunoglobulin is reduced because it is consumed by the defense mechanism.

The present study indicates that abnormal elevation in saliva biochemical findings in OSCC patients represented a released from pathologically altered cells rather than an increased biosynthesis caused by the leakage from interstitial fluids through the torn and damaged oral mucosa and gingival sulci.

CONCLUSION

This study indicates the abnormal elevation in saliva biochemical findings in OSCC patients. Considering the rarity of studies done on total salivary LDH in OSCC, we can definitely say that our study is one of the pioneer studies in this field. Salivary LDH estimations may serve as a feasible, simple and convenient approach for investigating oral precancer and cancer. Further interventional studies with large sample size are needed to evaluate whether LDH estimations may serve for screening of oral precancer and as predictors of response to therapy and prognosis.

REFERENCES:

1. Brew P. 2003. ABC of Clinical Haematology, 2nd edition, by BMJ Books, BMA House, Tavistock Square, London WC1H 9JR.
2. Ribeeiro KC, Kowalski LP, Latoree MR. Impact of comorbidity,symptoms and patients'characterstics on the prognosis of oral carcinoma.Arch Otolaryngol HeaD

Neck Surg. 2000; 126: 1079-1085.
3. Kantola S, Parikka M, Jokinen K.Prgnostic factors in tongue cancer-relative importance of demographic,clinical and histopathological factors. Br J Cancer. 2000; 83:614-619.
4. Sahu, R, Zeliq, U, Hulehel M, Brosh N, Talyshinsky N, Ben-Harosh M, Mordechai, SM (2009). Continuous monitoring of WBC in an adult leukemia patients using advanced FTIR- Spectroscopy,Leukemia Research, 30(6):687-693.
5. Zamkoff, KW, Kirshner, JJ. Marked hypophosphatemia associated with acute myelomonocytic leukemia. Arch. Intern Med.1980; pp.140, 1523.
6. Pfluger KH, Gramse M, Grop PC, Havemann K. Ectopic ACTH Production with autoantibody formation in a patient with acute myleloblastic leukemia. N Engl J Med.1981; pp.305, 1632.
7. Bratt G, Bromback M, Paul C. Factors and inhibitors of blood coagulation and fibrinolysis in acute nonlymphoblastic leukemia. Scand J Haematol, 1985; pp.34
8. Pui CH, Doge RK, Dahl GV. Serum lactate dehydrogenase level has prognostic value in childhood acute lymphoblastic leukemia. Blood. 1985; 66(4):778-82.
9. Welch JC, Lilleyman JS (1995). Immunoglobulin concentration in untreated lymphoblastic leukemia. Pediatr Haematol Oncol, pp.12, 545.
10. Bashar HA, Hassan IY, Raja KK, Ferial AH and Khalid BM. Gingival fine needle aspiration cytology in acute leukemia. J of Oral Pathology and Medicine.2008; 31(1):55-58.
11. Soames JV, Southam, 2003. Tumers melanocytic naevi and malignant melanoma. In: Oral Pathology.3th ed., Oxford University Press. Oxford, J. Oral epithelial. pp.159-179.
12. Das BR, Nagpl JK. Understanding the biology of oral cancer. Med Sci Monit.2002; 8(11):258-267.
13. Manoharan S. Kolanjiappan K. Suresh K. Panjamurthy, K. 2005. Lipid peroxidation and antioxidants status in patients with oral squamous cell carcinoma. Indian J. of Med. Res., 122:529-534.
14. Gene Mayer, 2008. Microbiology and Immunology. 7th ed. Chapter 3. University of south Carolina, school of medicine.
15. Abdulla BH. 2002. Saliva cytological fundings and measurement of some enzymes and essential elements levels in serum and saliva of adult acute leukemic patients. Thesis, College of Dentistry, University of Baghdad.
16. Wolf PL. Clinical significance of an increased or decreased serum alkaline phosphatase level. Arch Pathol Lab Med.1990; 102:497–501.
17. Mancini G, Carbonava AO, Heremans JF. (1965). Immunochemical quantitaion of antigen by single radical immunodiffusion. Immunochemistry 2: 235-254.
18. Flanagan NG, Ridwny JC, Platt CC, Rowlands AJ asnd whitson A. 1989. Lactate dehydrogenase estimation in hematological malignancies. Clin. Lab. Haematol, 11(1):17-26.
19. Patel PS Adhurya SG, Balar DB. Serum lactate dehydrogenase and its isoenzymes in leukemia patients. Neoplasma,1994; 41(1):55-59.
20. Wa, J, Fantasia JE, Kaplan R (2002).Oral manifestations of acute leukemia: a case report and review of the classification of leukemia. J Periodontal. 73(10):1228-1232.
21. Abdulla BH 2002. Saliva cytological fundings and measurement of some enzymes and essential elements levels in serum and saliva of adult acute leukemic patients. PhD. thesis, College of Dentistry, University of Baghdad.
22. Al Rawi NH. 2001. Measurment of trace elements, enzymes and proteins in saliva, serum and tissue extract in patients with oral cancer. PhD. thesis. college of Dentistry, University of Baghdad.
23. Nakamura M, Slots J.Salivary enzymes origin and relationship to periodontal disease. J. of periodontal research.1983; 18:559-569.
24. Gorogh T, Wers JJE, Lipperk B. Lactate dehydrogenase isoenzyme in OSCC of the oral cavity. Oral Path. and Med.2006; 19(2):56-59.
25. Kashmoola MA .2000. Quantitative analysis of trace elements, nucleosides and immunoglobulins in saliva of patients with oral cancer. PhD. thesis. College of Dentistry, University of Baghdad.
26. Loning T, Burkhardt A. Plasma cell immunoglobulin synthesis in oral precancer and cancer. Pathol Anat. 1979; 348:109-20.
27. Edgar WM, amp O, Mullane DM. 1996. Saliva and Oral Health. 2nd ed.,British Dental Association, London. pp.24-29.
28. Wanebo HJ, Jun MY, Strong EW, Oettgen H. T- cell deficiency in patients with squamous cell cancer of the head and neck. Am. J. Surg.1975; 130:445-51.