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Assessment of Enzymatic and non-enzymatic markers in Oral cancer

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Abstract:

Oral cancer is a malignant neoplasia that develops on the lip or in the oral cavity, commonly referred to as squamous cell carcinoma (OSCC) as 90% of malignancies in the dental area have squamous cell origins [1]. Although there has been a rise in oral cancer diagnoses among those under 40, the disease's incidence rises with age, reaching its peak in those over 60. The present study aimed to estimate the levels of Sialic acid, LDH and ALP in the given serum sample and to determine the role played by Sialic acid, LDH and ALP in oral cancer. The study group consists of 30 control groups with no complications and 30 oral cancer patients. 5ml venous blood sample was taken, centrifuged and serum was collected . Serum was analyzed for LDH, ALP and Sialic acid. The mean age of the study population, the case group was 50.25±10.58 and the control group was 26.2±1.28. Males were 80% and Females were 20 % in the case group and in the control groups, males and females were 50% each. Most of the patients had the habits of smoking, alcohol consumption and tobacco chewing. Significantly higher levels of LDH, ALP and Sialic acid was observed in the serum of Oral cancer patients. ALP, LDH and sialic acid may be considered sensitive markers for the diagnosis of dysplasia with precancerous and malignant lesions. As a result, it can aid with the early diagnosis of oral carcinoma.

Keywords: OSCC, ALP, LDH, Sialic acid

Introduction:

Cancer is a disease characterized by abnormal metabolism and signaling, which allows transformed cells to divide uncontrollably and survive. Oral cancer is a malignant neoplasia that develops on the lip or in the oral cavity, commonly referred to as squamous cell carcinoma

(OSCC) as 90% of malignancies in the dental area have squamous cell origins (1). Although there has been a rise in oral cancer diagnoses among those under 40, the disease's incidence rises with age, reaching its peak in those over 60. Established symptoms of oral cancer include a non-healing ulcer, visible red or white patches, mouth swelling, and tongue soreness (2). The principal etiologic factors for the development of oral cancer are tobacco use, particularly smokeless tobacco and heavy alcohol consumption. In addition, a variety of suspected risk factors such as poor oral hygiene, viral infection, occupational exposure, and malnutrition have been identified as possible causes of oral cancer (3). High levels of glycolytic activity, specifically a transition from aerobic to anaerobic glycolysis, have been associated with the development of oral cancer. LDH is a biomarker for cancer detection found in almost all cell types (4). It has been shown that increased LDH levels are present in oral cancer as well as several other potentially malignant conditions (5). Neoplastic transformation of the cell results in changes to the structural components of glycoproteins and the cell membrane. Increasing sialic acid concentrations on the cell surface is one of these. As a result, neoplasms frequently exhibit an increase in the quantity of sialic acid on the surface of the tumour cell, and some of these cells release or shed sialic acid, raising the concentration in the blood (6). Alkaline phosphatases (ALP) are a class of enzymes that catalyze the hydrolysis of phosphate esters in an alkaline environment. In healthy individuals, ALP originates mainly from the liver and bones, with a small quantity from the intestines, placenta, kidneys, and leukocytes. Serum ALP levels increase in patients with primary and metastatic liver and bone tumours, including colorectal cancer hepatic metastasis and breast cancer bone and liver involvement. An increased serum ALP in cancer patients may be a sign of metastatic diseases. Furthermore, the effect of ALP in OSCC is rarely studied (7).

The present study aimed to estimate the levels of Sialic acid, LDH and ALP in the given serum sample and to determine the role played by Sialic acid, LDH and ALP in oral cancer.

Materials and Methods:

Ethical statements:

This study was approved by the Ethics committee of KS Hegde Medical Academy, Nitte (Deemed to be University). 60 patients reporting to the Department of Oral Medicine and Radiology A.B.Shetty Memorial Institute of Dental Sciences, Deralakatte, Mangalore were recruited for the study after taking informed consent.

Patient population: The study group consists of 30 control groups with no complications and 30 oral cancer patients. 5ml venous blood sample was taken, centrifuged and serum was collected and stored at 4°C for subsequent analysis.

Control group:
The study group consists of 30 control groups with no complications.

Estimation of sialic acid by DPA method
0.2ml of serum and 0.2ml sialic acid is pipetted into a clean test tubes. To each test tube added 4.8ml of 5% TCA and placed in boiling water bath. After 15minutes, filter the cooled sample

through Whatmann No.1 filter paper. Then 2ml of the filtrate pipetted into each tubes. Reagent blank containing 2ml of 5% TCA was prepared. 4ml of acid mixture pipetted into tubes marked blank. 4ml of diphenylamine reagent was pipetted into the test tubes marked test and standard. All the test tubes mixed gently on the vortex mixture and placed it in a boiling water bath for 30minutes.The absorbance was measured at 530nm in a colorimetric or spectrophotometer setup.
Calculations: AbsT - AbsT-B/AbS - AbsS-B × 20= mg sialic acid/100ml.

Where,

AbsT= absorbance at 530nm for test., AbsT-B = absorbance at 530nm for test blank., Abs= absorbance at 530nm for standard., AbsS-B = absorbance at 530nm for standard blank.

Estimation of serum LDH:
Working reagent consists of Tris buffer - 80mmol/L, Pyruvate - 1.6mmol/L, sodium chloride - 200mmol/L- (Reagent 1), NADH - 240micro mol/L (Reagent 2). 4 volumes of reagent 1 (RI) mixed with I volume of reagent 2 (R2).
1ml of working reagent is taken in a test tube. To this 0.02ml of serum sample is added. Mixed and incubated at 37℃ for 1 minute. The change in absorbance per minute was measured (delta OD/min) for three minutes.

Calculation:

LDH-activity: delta Absorbance /Min-8109

Estimation of Serum ALP:
Working Reagent: Dissolve 1 substrate vial in 5ml (7×5ml pack) of buffer reagent this working reagent is stable for atleast 45 days when stored at 2-8°C. 0.02 ml of serum sample was taken in a test tube. To this added 1ml of working reagent. Incubated at 37°C for 1minute. The change in absorbance per minute at 405nm for 3 minutes was measured.
C: ALP activity in IU/L= delta Absorbence-2754>tf

Statistical analysis:
Statistical analysis was carried out using). Results were expressed as mean ± Standard Deviation (SD). All data collected from experiments were performed in three replicates and analyzed using students t-test. A value of P *P < 0.05, **P < 0.01, ***P < 0.001, and **** P < 0.0001 are considered statistically significant.

Results:

Demographic and Clinical characteristics:

The present study was carried out to estimate the levels of Sialic acid, LDH and ALP in the given serum sample and to determine the role played by Sialic acid, LDH and ALP in oral cancer. The study group consists of 30 control groups with no complications and 30 oral cancer patients. 5ml venous blood sample was taken, centrifuged and serum was collected and stored

at 4°C for subsequent analysis. The mean age of the study population, the case group was 50.25±10.58 and the control group was 26.2±1.28. Males were 80% and females were 20% in the case group and in the control groups, males and females were 50% each. Most of the patients had habits of smoking, alcohol consumption and tobacco chewing **(Table 1).**

Table 1:Baseline characteristics of study subjects (value are expressed as mean +/-SD)

Case		Control	
Male	8 (80%)	Male	5 (50%)
Female	2 (20%)	Female	5 (50%)
Mean Age	50.25±10.58	Mean age	26.2±1.28

Table 2: Clinical characteristics of the study subjects (value are at mean ±SD)

Parameter	Control	Oral cancer patient	P-value
Serum Lactate dehydrogenase IU/L	6.47±1.07	9.918±1.36	<0.0001
Serum Alkaline phosphatase IU/L	163.2±57.92	415.1±66.74	<0.0001
Serum Sialic acid (mg/dl)	73.12±9.71	124.6±27.81	<0.0001

Significantly higher levels of LDH, ALP and Sialic acid was observed **(Table 2)**

Discussion

Oral cancer remains a lethal disease around the world, affecting over 52,000 individuals and accounting for 28.6% of all deaths in Taiwan in 2019. Oral cancer primarily affects the mucosal surfaces of the oral cavity, pharynx (throat), larynx, paranasal sinuses, nasal cavity, and salivary glands. Squamous cells are responsible for most oral malignancies (more than 90%). Males are much more likely to develop oral cancer than females (8).

Biomarkers are crucial components of precision medicine because they serve as indicators and guides for disease prevention, early detection, and personalized therapy. Biomarkers are frequently obtained from body fluids or solid tissues, and the measured quantities either represent the quantifiable risk of diseases or the outcome of therapies.

Almost all bodily tissues contain the enzyme LDH. Increased serum LDH activity is thought to be a hallmark of cellular necrosis, and serum LDH levels have been utilised as a biochemical diagnostic in the detection of cancers such as oral, laryngeal, and breast cancer. LDH activity is mostly a result of chromosomal alterations during malignant transformation. Increased LDH levels are caused by an increased mitotic index as well as increased lactic acid synthesis by tumour cells as a result of glycoprotein breakdown (9). In the present study serum LDH levels were statistically elevated in oral cancer patients as compared to controls. In the case of Wilms' tumour, LDH is employed as a marker for both diagnostic and treatment response monitoring. Most tumour tissues, including precancerous lesions, show increased total LDH activity (10).

ALP is an enzyme found in a variety of tissues but is especially abundant in the liver, kidneys, placenta, and bones. It is expressed on the surface of osteoblasts in bone and plays a role in mineralization by removing phosphate groups from a variety of molecules, including nucleotides, proteins, and alkaloids. ALP plasma levels are frequently elevated in the presence of tumour bone lesions. The relevance of ALP as a marker of skeletal involvement has been examined in various solid malignancies, including prostate cancer (11). The normal level of alkaline phosphatase in adults ranges from 80-290 IU/L at 370 and in children 245-770 IU/L at 37°C.In the present study serum ALP levels were statistically higher in oral cancer patients than in controls. Certain cancers have been linked to abnormal ALP expression and activity. Patients with bone metastases may have high serum ALP levels in some circumstances, such as prostate cancer, liver metastasis, pancreatic cancer, lung cancer, non-small cell lung cancer, and testicular cancer. Low ALP activity has been associated with breast and colorectal cancers (12).

Many research has investigated the role of sialic acid in gynecological cancers, with the majority focusing on ovarian cancer (OC) and, to a lesser extent, endometrial and cervical cancer. Sialic acid expression changes may help with diagnosis or indicate tumour progression. 33 In OC, abnormal sialylation has been observed in early-stage tumours, highlighting its potential as a diagnostic biomarker (13). Serum sialic acid levels were significantly higher in oral cancer patients as compared to controls. There is no significant difference in the serum sialic acid level in gender. Age and sex do not influence sialic acid levels in serum.

The elevated level of serum lactate dehydrogenase, alkaline phosphatase and sialic acid indicates the malignancy such as oral cancer. Hence elevated level of these enzyme and glycoconjugate activities is considered as a marker in oral cancer.

Conclusion:

From this study, we found that serum LDH, ALP and sialic acid elevated in serum of oral cancer patients. Cells produce LDH an isoenzyme that catalyzes the conversion of lactate to pyruvate. This is an important step in energy production. In the case of cancer rapid cell division occurs and forms a mass of cell. As the cell number increases LDH level is also elevated. Serum ALP levels also elevated in oral cancer patients. Sialic acid is glycoconjugate which helps in malignancy transformation. An increase in the levels of sialic acid in oral cancer indicates its importance as a tumour marker. Hence elevated levels of serum LDH, ALP and

serum sialic acid can used as a marker in oral cancer. In conclusion, ALP, LDH and sialic acid may be considered sensitive markers for the diagnosis of dysplasia with precancerous and malignant lesions. As a result, it can aid with the early diagnosis of oral carcinoma.

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Conflict of Interest:
The author declares no conflict of Interest.

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