COMPARATIVE STUDY ON LACTATE DEHYDROGENASE, ALKALINE PHOSPHATASE AND IMMUNOGLOBULINS IN SERUM AND SALIVA OF ACUTE LEUKEMIA AND ORAL SQUAMOUS CELL CARCINOMA PATIENTS

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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 are 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 phpsphatase enzymes and Immunoglobulins in serum and saliva of Acute Leukemia (AL) and Oral Squamous Cell Carcinoma (OSCC) patients, and apparantly healthy individuals as control group.

Unstimulated (resting) saliva and serum were collected from 70 newly diagnosed, untreated AL patients and 20 OSCC patients, in addition to 20 healthy individuals and 12 adults with periodontitis.

According to the results of this study, serum and saliva enzymes showed a significant increase in Lactate Dehydrogenase (LDH) and Alkaline Phosphatase (ALP) levels of AL and OSCC in comparison to control group. Serum and saliva IgG showed non significant increase, whereas IgA level was reduced and IgM showed significant increase in AL patients in comparison to the control group.

Results on OSCC patients showed a significantly increase in serum and saliva immunoglobulins but saliva IgA was reduced in comparison to control group.

The levels of serum LDH and ALP in AL patients were higher than that in OSCC patients, whereas the levels of saliva LDH and ALP in AL were lower than that of OSCC patients. The serum IgG and IgM levels were higher in AL patients than that of OSCC patients, whereas serum IgA was lower in AL patients. Saliva immunoglobulins were higher in OSCC patients than that of AL patients.

In conclusion, a disseminates malignancy, like AL, causes changes in the levels of Lactate dehydrogenase and Alkaline phosphatase in blood as well as in saliva. However, the changes in blood are more striking than that is saliva while in a local malignancy like OSCC, the changes are more prominent in saliva than that in blood.

دراسة مقارنة لأنزيمي اللاكتيت ديهايدروجيني والفوسفاتيز القاعدي والبروتينات المناعية في مصل ولعاب مرض ابيضاض الدم الحاد وسرطان الفم الحرشفي

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الخلاصـــة

تهدف هذه الدراسة الى قياس التغييرات في مستوى انزيمي اللاكتيت ديهايدروجنيز والفوسـفاتيز القاعـدي والغلوبيولينات المناعية في مصل ولعاب مرضى ابيضاض الدم الحاد وسرطان الفم الحرشفي وتقارن ذلك بالاشخاص غير المصابين.

تم اخذ نماذج مصل الدم واللعاب من ٧٠ مصاباً بأبيضاض الدم الحاد و٢٠ مصاباً بسرطان الفم الحرشفي اضافة الى ٢٠ شخصا غير مصاباً و١٢ اخرين مصابين بالتهاب ماحول الاسنان.

أظهر التحليل الانزيمي في مصل ولعاب مرضى ابيضاض الدم الحاد زيادة معنوية فـي نشـاط انزيمـي اللاكتيت ديهايدروجنيز والفوسفاتيز القاعدي في مصل و لعاب مرضى ابيضاض الدم الحاد وسرطان الفم الحرشفي مقارنة بالاشخاص غير المصابين.

كما أظهرت نتائج الاختبارات المناعية في مصل ولعاب مرضى ابيضاض الدم الحاد زيادة غير معنوية في مستوى الغلوبيولين المناعي صنف IgG وانخفاظاً في مستوى الغلوبيولين المناعي صـنف IgA وزيـادة معنوية في مستوى الغلوبيولين المناعي صنف IgM مقارنة بالاشخاص غير المصابين. اما في حالة مرضى سرطان الفم الحرشفي فقد لوحظ زيادة معنوية في مستوى الغلوبيولينات المناعية ماعدا الغلوبيولين المناعي صنف IgA، اذ انه اظهر انخفاظا في لعاب مرضى سرطان الفم الحرشـفي مقارنـة بالأشـخاص غيـر المصابين.

وعند مقارنة النتائج تم التوصل الى ان مستوى انزيمي اللاكتيت ديهايدروجنيز والفوسفاتيز القاعـدي فـي مصل مرضى ابيضاض الدم الحاد هو أعلى من مستواه في مصل مرضى سرطان الفم الحرشفي بيـد ان مستوى هذان الانزيمان في لعاب مرضى ابيضاض الدم الحاد هو اقل من مستواهما فـي لعـاب مرضـى سرطان الفم الحرشفي .

تبين ان مستوى الغلوبيولينات المناعية صنف IgG وIgM في مصل مرضى ابيضاض الدم الحاد هو أعلى من مستواه في مصل مرضى سرطان الفم الحرشفي بينما كان مستوى الغلوبيولين المناعي صنف IgA في مصل مرضى ابيضاض الدم الحاد هو اقل.

واتضح ان مستوى الغلوبيولينات المناعية في لعاب مرضى سرطان الفم الحرشفي هو أعلى من مستواه في لعاب مرضى ابيضاض الدم الحاد.

نستنتج من هذه الدراسة بأن السرطان المنتشر مثل ابيضاض الدم يسبب تغييرات في مستويات الانزيمـات والبروتينات الكلية والمناعية المدروسة بالدم واللعاب ولكن هذه التغييرات تكون أشد في الدم مما هي فـي اللعاب، بينما في السرطان الموضعي مثل سرطان الفم الحرشفي تبرز هذه التغيرات في اللعاب أكثر مما في الدم.

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Introduction

Prior to treatment, a mild increase in serum uric acid and lactic dehydrogenase levels are frequent. Both levels are higher in

myelomonocytic and monocytic variants than other acute myeloid leukemia (AML) phenotypes [1]. Abnormalities of sodium, potassium, calcium, or hydrogen ion concentration are infrequent and usually mild[2]. Severe hyponatremia associated with inappropriate antidiuretic hormone secretion has occurred at presentation. Hypokalemia is a more frequent finding at presentation and is related to kaliuresis, although the reason for the proximal renal tubular dysfunction is unclear [3].

Spectral changes that occurred in white blood cell (WBC) of an adult acute myeloid leukemia patients and their possible utilization for monitoring biochemistry of WBC were investigated. The phosphate absorbance from nucleic acids and the lipid-protein ratio in WBC decreased immediately after treatment and then increased to levels of the control group. Similar observations were recorded in child patients with acute lymphoblastic leukemia who were used as test cases [4].

Hypophosphatemia, as a result of phosphate uptake by leukemic cells, can occur [5]. Ectopic adrenocorticotropic hormone secretion [6], circulating immune complexes and abnormal concentrations of coagulation factors or their inhibitors [7] may be present.

The level of serum lactate dehydrogenase (LDH) is increased in most patients with ALL and is well correlated with the size of leukemic infiltrate [8]. Increased levels of serum uric acid are common in patients with a large leukemic cell burden. This finding reflects an increased rate of purine catabolism. Patients with massive renal involvement can have increased levels of creatinine, urea nitrogen, uric acid, and phosphorus. Serum immunoglobulin levels (mostly IgA and IgM classes) are mostly decreased in approximately one third of that in children with ALL [9].

Oral findings in AL are common and could be the presenting feature of the disease, namely, gingival enlargement, ulceration, bleeding, and infection. Gingival enlargement in AL is either due to leukemic infiltration, or due to reactive hyperplasia. Leukemic gingival enlargement is mostly seen in patients with acute myeloid leukemia, particularly M4 and M5 subtypes, however, it can also seen in all types of leukemia [10].

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].

Total cholesterol and esterified cholesterol were markedly increased whereas phospholipids and free fatty acids were significantly decreased in tumor tissues as compared to normal tissues.

The level of thiobarbituric acid reactive substance (TBARS) was significantly increased in plasma, erythrocytes and erythrocyte membranes of oral cancer patients as compared to healthy subjects, and were gradually increased from stage II to stage IV of oral cancer patients. The levels of vitamin E and reduced glutathione were significantly decreased in oral cancer patients as compared to healthy subjects, and were gradually decreased from stage II to stage IV of oral cancer patients. The activities of The antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase were also significantly decrease in oral cancer patients as compared to healthy subjects and gradually decreased from stage II to stage IV of oral cancer patients [12].

Superoxide dismutase, catalase and glutathione peroxidase serve as the back bone of cellular antioxidant defense mechanism. Lowered activities of these enzymes have been reported in various pathological conditions including oral carcinogenesis [13].

Immunoglobulin is a group of Y- shaped protein molecules synthesized in blood plasma by B-lymphocytes and functioning as an antibody in an immune response. Among the most classes are immunoglobulin G (IgG), an antibody is produced towards the end of a primary immune response and the main antibody is in a secondary response; immunoglobulin A (IgA), secreted locally in the gut and bodily fluids, such as saliva, tears, and milk; and immunoglobulin M (IgM), the first class of immunoglobulin appearing in a primary immune response [14].

Materials & Methods

Saliva and blood samples were taken from the same AL patient, before receiving cytotoxic chemotherapy. Saliva and blood samples were collected from OSCC patients preoperatively. Blood samples were centrifuged at 2000 rpm for 10 min. The collected saliva was centrifuged at 2500 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]. Unhemolysed serum and saliva supernatant

were analyzed. The control groups consisted of 20 non-hospitalized adults with no history of systemic disease. Saliva samples were taken from 12 cases selected as periodontitis from patients enrolled for treatment at the department of Periodontal, College of Dentistry.

Enzymes Assay

Lactate Dehydrogenase

Kinetic determination of the lactate dehydrogenase activity optimized the test according to the recommendation of SFBC (societe francaise de biologic clinique)

$$Pyruvate + NADH + H^+ \xleftrightarrow{LDH} Lactate + NAD^+$$

The activity of LDH is shown by the variation of optical density at 340 nm which is proportional to the quantity of NADH oxidized.

Reconstitute one vial of reagent 2 with 10 milliliters of buffer / reagent 1. This working reagent is stable 15 days at 2-8 Ċ or 24 hours at 20-25 Ċ. Pipette into cuvette, 1 ml. working reagent, equilibrate at 37 Ċ. 20 µl. sample was added, mix and wait, for 1 min. measure the extinction decrease per min.

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 µmole of substrate per minute [16].

Alkaline Phosphatase Principle

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

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

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 monospecific antibody to IgG, IgA, 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 ethylmercurithiosalisal-ate), 0.01% benzamidine .
2. Calibrators: These are supplied in stabilized liquid form as a set of three containing high, medium and low concentrations of immunoglobulin.
   Preservatives: 0.1% sodium azide, 0.1% EACA, 0.01% benzamidine.
3. 7% Bovine serum albumin (BSA) solution. This is supplied in stabilized liquid form and is included for use as a diluents. Preservatives: 0.1% sodium azide, 0.1% EACA, 0.01% benzamidine.
4. Control serum. This is supplied in stabilized liquid form and is included for use as marked on the vial label.
   Preservatives: 0.1% sodium azide, 0.1% EACA, 0,01% benzamidine;

Specimen Collection and Preparation

Blood samples should be collected by vencapuneture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum was stored at 2–8Ċ for up to 48 hours prior to assay or for prolonged storage aliquoted and kept at – 20Ċ.

Results

Biological analysis of LDH and ALP in AL

Serum and saliva LDH and ALP concentrations were found to be higher in AL patients than in control groups. (Table 1)

Table 1: Concentration serum and saliva enzymes in AL patients in comparison to the control group.

Enzymes LDH (U/L) ALP (U/100ml.)	AL patients χ ± SD N= 70	Control χ ± SD N= 20	P.V.	C.S.
LDH serum	634.2 ± 406.18	229.5 ± 57.4	P< 0.001	HS
LDH saliva	319.2 ± 126.2	211.6 ± 97.7	P< 0.05	S
ALP serum	13.55 ± 5.50	6.6 ± 2.71	P< 0.05	S
ALP saliva	1.59 ± 0.74	1.03 ± 1.74	P< 0.05	S

(Table 2), ALP enzyme concentration was significantly higher in AL patients then in periodontitis group. Although the concentration of LDH enzyme in saliva of AL patients was higher than that of periodontitis, however the differences were not significant.

Table 2: The saliva enzymes concentration in AL patients in relation to patients with periodontitis.

Enzymes in saliva	AL patients χ ± SD N= 70	Periodontitis group χ± SD N= 12	P.V.	C.S.
LDH U/L	319.2 ± 126.2	270.9 ± 74.1	P> 0.05	NS
ALP U/100ml.	1.59 ±0.74	1.05 ± 0.36	P< 0.05	S

Serum and saliva enzymes concentrations (LDH and ALP) were higher in ALL than AML patients (Table 3). Serum LDH was non significantly higher in ALL patients than in AML, while saliva LDH in ALL patients was significantly higher than that of AML patients. Serum ALP in ALL patients was significantly higher than that of AML patients while salivary ALP in ALL patients was non significantly higher than that of AML.

Table 3: Comparison between enzyme levels in serum and saliva of AML and ALL patients

Enzymes LDH U/L ALP U/100ml.	ALL $\chi \pm SD$ N= 27	AML $\chi \pm SD$ N=38	P.V.	C.S.
LDH serum	676.4 ± 423.2	608.2 ± 424.1	P > 0.05	N.S.
LDH saliva	351.8 ± 153.2	288.5±107.3	P < 0.05	S
ALP serum	15.46 ± 7.22	11.7±3.73	P < 0.05	S
ALP saliva	1.78 ± 0.805	1.48±0.803	P > 0.05	N.S.

Biological analysis of immunoglobulins in AL

Table (4) represents the concentration of serum and saliva immunoglobulins levels in AL patients and in control group.

Table 4: The Mean concentration of Immunoglobulins in serum and saliva of AL patients and control group .

Immunoglobulins	AL $\chi \pm SD$ N=70 mg/dl	Control $\chi \pm SD$ N= 20 Mg/dl	P.V.	C.S.
Serum IgG	1951.1± 464.9	1645.1± 540.4	P> 0.05	NS
Saliva IgG	10.92 ± 3.03	7.82 ± 4.97	P >0.05	NS
Serum IgA	354.2 ± 85.96	359.5 ± 113.23	P> 0.05	NS
Saliva IgA	14.56 ± 3.79	48.1 ± 5.67	P< 0.001	HS
Serum IgM	259.9 ± 79.02	213.3 ± 82.3	P< 0.05	S
Saliva IgM	8.08 ± 1.83	3.83 ± 1.98	P< 0.001	HS

A comparison between ALL and AML patients in the concentration of immunoglobulin in serum and saliva are presented in Table (5).

Table 5: Comparison between Immunoglobulin concentration in serum and saliva of AML and ALL patients

immunoglobulin mg/dl	ALL $\chi \pm SD$ N=27	AML $\chi \pm SD$ N= 38	P.V.	C.S.
IgG serum	1925.9 ± 475.6	1972.6 ± 515.7	P> 0.05	NS
IgG saliva	9.185 ± 2.06	11.76 ± 2.56	P< 0.001	HS
IgA serum	351.66 ± 101.9	354.8 ± 87.8	P> 0.05	NS
IgA saliva	12.51 ± 3.09	16.16 ± 3.77	P< 0.001	HS
IgM serum	263.6 ± 75.6	252.2 ± 91.29	P> 0.05	NS
IgM saliva	7.87 ± 1.79	8.16 ± 2.05	P> 0.05	NS

When comparing the mean of saliva immunoglobulins of AL patients with that of periodontitis group, as showed in Table (6), saliva IgG concentration was significantly higher in patients with periodontitis than in AL patients while saliva IgM concentration was significantly higher in periodontitis patients. Saliva IgA concentration was non significantly higher in AL patients than that of patients with periodontitis.

Table 6: Comparison between saliva immunoglobulins in AL patients and patients with periodontitis.

Immunoglobulins in saliva mg/dl	AL $\chi \pm SD$ N=70	Periodontitis $\chi \pm SD$ N=12	P.V.	C.S.
IgG	10.92±3.02	19.67±6.33	P<0.001	HS
IgA	14.86±3.79	14.30±4.18	P>0.05	NS
IgM	8.08±1.83	11.61±3.44	P<0.05	S

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 serum and 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 non significant in comparison with control group as shown in Table (7).

Table 7: Serum and saliva enzymes concentration in OSCC patients in comparison to the control group.

Enzymes LDH U/L ALP U/100ml.	OSCC patients $\chi \pm SD$ N= 20	Control $\chi \pm SD$ N= 20	P.V.	C.S.
LDH serum	311.2 ± 86.1	229.5 ± 57.4	P< 0.05	S
LDH saliva	325.4 ± 156.5	211.6 ± 97.7	P< 0.05	S
ALP serum	11.06 ± 3.60	6.67 ± 2.71	P< 0.001	HS
ALP saliva	2.24 ± 2.94	1.03 ± 1.74	P> 0.05	NS

Biochemical Analysis of immunoglobulins in OSCC patients:

Table (8) represents mean serum and saliva immunoglobulins concentration in OSCC patients and in control group.

Table 8: The mean concentration of Immunoglobulins in serum and saliva of OSCC patients and control group.

Immunoglobulins	OSCC patient $\chi \pm SD$ N=20 mg/dl	Control $\chi \pm SD$ N= 20 mg/dl	P.V.	C.S.
Serum IgG	1814.6 ± 445.2	1645.1 ± 450.4	P> 0.05	NS
Saliva IgG	20.7 ± 5.07	7.82 ± 4.97	P< 0.001	HS
Serum IgA	431.4 ± 169.9	359.5 ± 113.23	P> 0.05	NS
Saliva IgA	40.09 ± 15.66	48.1 ± 5.67	P< 0.05	S
Serum IgM	256.1 ± 172.3	213.3 ± 82.3	P> 0.05	NS
Saliva IgM	14.52 ± 3.21	3.83 ± 1.98	P< 0.001	HS

When comparing the mean of saliva immunoglobulins in OSCC patients with that of

periodontitis group, as showed in Table (9), all the results showed increase in the biochemical parameters concentration in saliva of OSCC patient.

Table 9: Saliva immunoglobulins in OSCC patients in relation to patients with periodontitis.

Immunog-lobulins	OSCC patients $\chi \pm SD$ N=20	Periodontitis patients $\chi \pm SD$ N=12	P.V.	C.S.
IgG mg/dl	20.7 ±5.09	19.67 ± 6.33	P > 0.05	NS
IgA mg/dl	40.09 ± 15.66	14.305 ± 4.18	P < 0.001	HS
IgM mg/dl	14.52 ± 3.21	11.61 ± 3.44	P < 0.001	HS

Comparison between AL and OSCC

As seen from table (10) the serum concentrations of LDH & ALP enzymes were significantly higher in AL patients than these with OSCC. While the reverse is true in saliva samples. The serum concentrations of IgG and IgM were non significantly higher in AL patients than these with OSCC. While saliva concentration of IgG and IgM were significantly higher in OSCC patients than these with AL. The serum and saliva concentrations of IgA were significantly higher in OSCC patients than these of AL.

Table 10: Comparison between AL and OSCC patients in relation to serum and saliva enzymes and immunoglobulins.

Biochemical	AL patients $\chi \pm SD$ N=70	OSCC patients $\chi \pm SD$ N=20	P.V.	C.S.
LDH serum U/L	634.2 ± 406.18	311.2 ± 86.1	P< 0.001	HS
LDH saliva U/L	319.2 ± 126.2	325.4 ± 156.5	P> 0.05	NS
ALP serum U/100ml	13.55 ± 5.50	11.06 ± 3.60	P< 0.05	S
ALP saliva U/100ml	1.59 ± 0.74	2.24 ± 2.94	P> 0.05	NS
IgG serum mg/dl	1951.1 ± 464.9	1814.6 ± 445.2	P> 0.05	NS
IgG saliva mg/dl	10.92 ± 3.02	20.7 ± 5.09	P< 0.001	HS
IgA serum mg/dl	354.2 ± 85.96	431.4 ± 169.9	P< 0.05	S
IgA saliva	14.56 ± 3.79	40.09 ± 15.66	P< 0.001	HS
IgM serum mg/dl	259.9 ± 79.01	256.1 ± 172.3	P> 0.05	NS
IgM saliva mg/dl	8.08 ± 1.83	14.52 ± 3.21	P< 0.001	HS

Discussion

Malignant cells have a distinctive type of metabolism in which the glycolytic sequence and the tricarboxylic acid cycle are poorly integrated, hence the cells tend to utilize five to ten times as much as glucose as do normal cells, converting most of it into lactate. Escape of LDH, due to damage of cells in any of these tissues, will tend to produce elevated serum levels [17].

The elevated level of serum LDH in AL is possibly due to this distinct type of metabolism of malignant leukemic cells which may reflect basic difference in cell proliferation and turnover [18].

In the present study, both ALL and AML patients have higher serum LDH levels than the control group. Elevation of serum LDH in AL patients could be due to the tumor burden activity which reflects the function of leukemic cell number and turnover. This was proved by the findings that LDH was significantly increased with the increase of WBC and absolute blast cells, which means an increase in tumor burden [19].

Alteration of cellular enzyme level in blood is a reflection of the presence of some abnormality in the disease tissue or organ. This abnormality may be due to an altered amount of the enzyme forming tissue, an altered rate of synthesis of these enzymes within the tissue of origin, or an alteration in the permeability of the cell member brought about by the pathological condition.

In this study, a significant increase of LDH levels in whole unstimulated centrifuged saliva compared with that of control group and the saliva LDH levels of normal subject were lower but comparable to the normal serum level.

Oral signs and symptoms may indicate a serious underlying systemic disease [18].According to these issues, it is convincing to state that the LDH activity in the whole centrifuged saliva is a net result of enormous LDH activity in gingival fluid being diluted with the pure saliva secretions with added activity of the epithelial cells, leukocytes and bacteria to reach a value which is comparable to serum level [20].

Although patients with periodontitis group had higher saliva LDH level than the normal control, the level in AL group remains significantly higher.

Saliva in AL patients, as it is a fluid bathing a tissue which could be infiltrated by Leukemic cells and/or the fluid itself (saliva), is containing leukemia cells migrating through the gingival sulcus. In contrast to this, in normal person, the saliva is a fluid bathing a normal gingival tissue and containing neutrophils, mainly, increased in periodontitis and/or gingivitis. From this supposition, one could assume that the gingival tissue infiltration in Leukemic patients with the increased influx of leukemic cells are the key

factors that influence LDH activity in saliva especially in AML group [21].

In the present study, a significant increase was noticed in the level of ALP in the serum of AL patients compared with that of control group. The patients with ALL had higher total serum level than the AML group with significant differences. Liver infiltration seen in leukemic patients can exhibit marked elevations of ALP and these correlate with the extent of liver involvement [22].

Hepatomegaly, which is due to leukemic infiltration of the liver is mostly seen in ALL rather than AML patients. In AML group, the monocytic leukemias are the most common to be involved with extramedullary infiltration including the liver. These points could explain the increased ALP activity in these variants of AL.

Alkaline phosphatase enzyme was measured in whole supernatant saliva of AL patients, patients with periodontitis and of healthy individuals. In patients with periodontitis, the levels increased significantly and saliva showed lower ALP activity than serum. The origin of this enzyme include neutrophils, bacteria, oral epithelial cells and the gingival crevicular fluid [23].A significant increase of saliva ALP levels in both ALL and AML patients, was noticed when compared with that of the normal subjects and the periodontitis groups.There was a non significant increase in saliva ALP of ALL patients when compared with that of AML patients. The level of ALP enzyme in saliva, is in harmony with that in serum, being higher in ALL group, this is confirmed by significant association seen in the current study between the level in serum and that in saliva.

This study demonstrates clearly that the mean concentration of IgG was non significantly higher in AL patients compared with control group, while the concentration of IgA was non significantly decreased. The concentration of IgM in serum of AL patients was found to be elevated significantly in comparison to control group. In comparing AML and ALL, the results show that non significant differences existed between AML and ALL in serum IgG and IgA concentration, although the concentrations in AML was higher than ALL. In case of IgM concentration, the difference was non significantly increased in ALL. The mechanism underlying long- term antibodies are not understood because the half life of human serum immunoglobulin is 3 to 4 weeks. A sustained level of antigen- specific antibodies in serum would require a constant production of antibodies. Concerning the immunoglobulin level in AL, the studies shows controversial findings.

Concerning the analysis of immunoglobulin in saliva, to the best of our knowledge, no previous study was trailed regarding the analysis of immunoglobulin in saliva of leukemic patients.

The reproducible elevation of salivary IgG and IgM of AL patients observed in this study may be a result of the natural antibody response to antigens of AL. The elevation was non significant in IgG concentration and highly significant in IgM. However, the interesting finding was the decrease in salivary IgA levels of AL patients. This could be explained by the fact that IgA is the major immunoglobulin present in saliva. It is the secretary defense against microorganism which could be consumed by this defense mechanism against microbial Ag present in the oral cavity.

Patients with periodontitis group had higher saliva immunoglobulins level than the normal group and higher than AL patients. This denotes that local production of antibody is present in oral mucosa in normal individual.

The results showed that there was a significant increase in serum LDH activity of OSCC patients as compared to control group. The elevation of LDH was also demonstrated in a variety of cancers including liver, acute leukemia, lymphoma, ovarian, breast, colon, stomach and lung cancers. 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 serum and salivary LDH activity represents a release from pathologically altered cells rather than an increased biosynthesis [24].

Serum ALP activities were measured for OSCC patients which showed a highly significant increase in comparison with ALP activity of normal control. This increase in serum ALP activity is potentially a useful indicator for early detection of malignancies.

The elevation level of ALP in OSCC may be due to the tissue or bone distruction.

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 non significant differences.
The elevation of salivary ALP may be due to the secretion of saliva fluid bathing tumor in addition to that from serum.
In the present study, non significant increase in serum immunoglobulin (IgG, IgA and IgM) levels observed in OSCC patients when compared with that of the control group and high significant elevation of salivary IgG and IgM of OSCC patients is observed when compared with its levels of controls. However, the interesting finding is the decrease in salivary IgA levels of OSCC patients.
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.
In comparing the biochemical parameters (LDH and ALP) between AL and OSCC patients in serum and saliva, this study shows that there was a highly significant increase in serum LDH in AL patients in comparison with OSCC patients. In contrast, salivary LDH was non significantly increased in OSCC patients in comparison with AL patients .The reason of this variation may be due to that the tumor burden in the oral cavity is larger than that of leukemic cell in oral tissue and saliva, while the tumor burden is larger in blood in AL than in OSCC.
Also the results showed a significant increase in serum ALP of AL patients in comparison with OSCC patients but it was non significant in saliva ALP of OSCC patients, in comparison with AL patients for the same previous reason.
The present study shows a non significant increase in serum IgG and IgM of AL patients in comparison with OSCC patients but the level of IgA was significantly increased in serum of OSCC patients in comparison with AL patients. On the other hand, the level of saliva IgG, IgA and IgM was highly significantly increased in OSSC patients in comparison with AL patients. In OSCC the main tumor burden is the malignant tissue in oral cavity, which bathed by saliva, while in AL, the main tumor burden is in blood, boon marrow, which affect the oral cavity either directly by tissue infiltration or indirectly by tissue destruction due to defective immune response, with the changes in the biochemical parameters. 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.

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