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SALIVARY AMYLASE AND TOTAL PROTEIN LEVELS IN LEUKEMIAS

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A B S T R A C T

Background: Oral manifestations are produced during different stages of leukemia and often reflects underlying systemic disesae, can be used as diagnostic indicator.This study was done to evaluate various oral complications produced by leukemia patients and to estimate the salivary amylase and salivary total protein levels in these patients to support the diagnostic value of saliva.

Material and Methods: A total of thirty patients of leukemia after confirmed diagnosis, who were not on chemotherapy drugs were included in the study group. 30 age and sex matched healthy individuals were enrolled as controls. Unstimulated saliva was analyzed for salivary amylase and salivary total proteins by autoanalyzer the same day.

Result: Patients in leukaemia group showed a significant increase in mean serum amylase levels when compare to controls (p=0.02).Although the mean salivary protein levels were higher in leukemia group but this difference was not significant statistically (p= 0.355).

Conclusion: As leukemia patients showed higher salivary amylase and salivary total protein levels than control group and there is associated oral complications in leukemia group. This indicates decline in oral health with associated changes in saliva composition in leukemia patients. Hence supports the diagnostic value of saliva in coexisting systemic diseases such as leukemias.

INTRODUCTION

Leukemias are malignant neoplasm of hematopoietic stem cells, characterized by diffuse replacement of the marrow by neoplastic cells. It is primarily a disease of the adults between the ages of 25 to 50 years with a peak incidence in fourth and fifth decades of life.They include: Lymphoid neoplasm – a diverse group of tumors of B-cell, T-cell and NK-cell origin, myeloid neoplasm, histiocytes: uncommon proliferative lesions of macrophages and dendritic cells.[1]Leukemias are also classified on the basis of clinical characteristics as acute and chronic leukemia.[2] Therefore categorized into acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), chronic myelogenous leukemia (CML), and chronic lymphocytic leukemia (CLL). [3]

The etiology of leukemia is not well known and is considered as multifactorial. Risk factors associated with leukemia are smoking, radiation hazard, viral infections, chemical compounds like benzene, chromosomal abnormalities and/or family history of leukemia.[4]Clinical features include fatigue, weakness, anorexia, weight loss, fever with or without identifiable infections, signs of abnormal hemostasis (bleeding, easy bruising), lymphadenopathy, nonspecific cough, headache, hepatomegaly, spleenomegaly.[1]Initial diagnosis of leukemia is mainly made on the basis of clinical presentation of the patient, followed by confirmed diagnosis based on identification of abnormal hematopoietic cells in blood and bone marrow aspirates. Further characterization is done by cytochemical staining, immunophenotyping and cytogenetic analysis of chromosomal abnormalities.[1]

As leukemia involves the whole body during different stages of disease progression resulting in a variety of oral manifestations, which are often mild and masked by the major manifestation of systemic disease.[5]Among the different type of oral manifestation produced by leukemia, most frequent is paleness of oral mucosa/local abnormal color of the gum, gingival petechiae, ecchymosis, bleeding associating painless gingival hyperplasia, hemorrhages, ulcerative necrotic lesions and buccal infections.[6]

Saliva being diagnostic fluid offers advantages over blood/serum for its non-invasive nature, easy storage and transportation, cost-effectiveness and safe handling. These characterstics of saliva make it possible to monitor several biomarkers and also its use in many circumstances in which blood and urine sampling is not available. Saliva seems better for diagnostic purposes because of its linkage with traditional biochemical parameters which appears in different forms in the circulation.[7]Hence this study was done to evaluate various oral complications produced by leukemia patients during their course of disease and to estimate salivary amylase and salivary total protein levels in these patients to support the diagnostic value of saliva.

MATERIALS AND METHODS

The present study was conducted in the Department of Biochemistry, in collaboration with the Department of

Medicine (Clinical Hematology unit); Pt. B.D. Sharma, University of Health Sciences, Rohtak. Thirty patients of leukemia after confirmed diagnosis, who were not on chemotherapy drugs were included in the study group. The diagnosis was made by history, clinical examination, total and differential leukocyte count, bone marrow examination and cytogenetic studies. 30 age and sex matched healthy volunteers were taken as controls.

Unstimulated saliva was obtained when subject seated quitely with his or her head flexed forward allowing the saliva to passively drip from the mouth to a collection container [8] and then transferred to sterile collecting tube. Samples were centrifuged and pellet was discarded. Supernatant was analyzed for salivary amylase and salivary total protein by autoanalyzerthe same day. IBM SPSS ver. 20 was used for various statistical analysis. Data was expressed in mean ± SD. P≤0.05 was considered significant.

RESULT

In the present study thirty patients with confirmed diagnosis of leukemia were included from the department of Radiotherapy. Out of 30 cases, 11 (36.6%) had AML, 6 (20%) had ALL, 9 (30%) had CML and 4 (13.3%) had CLL. The age of study group varied from 17 to 68 years, with a mean age of 43.8 years. Out of 30 cases 22 were males and 8 were females. The AML group had 7 males and 4 females. The ALL group consisted of 4 males and 2 females, the CML group consisted of 7 males and 2 females and the CLL group had 4 males.

Table I Distribution of cases according to type of leukemias

Type of leukemia	No. of cases(out of 30)	Percentage (%)
AML	11	36.6%
ALL	6	20%
CML	9	30%
CLL	4	13.3%

Table II Distribution of cases according to Gender

Type of leukemia	Males	Females
AML	7	4
ALL	4	2
CML	7	2
CLL	4	Nil

Table III Comparison of salivary amylase and salivary total protein in controls and cases

Parameter	Control	Cases	Significance
Salivary amylase	28.45±11.60U/L	46.3±21.69U/L	P<0.05 Significant
Salivary total protein	0.38±0.10g/dL	0.41±0.10g/dL	p>0.05 Non-significant

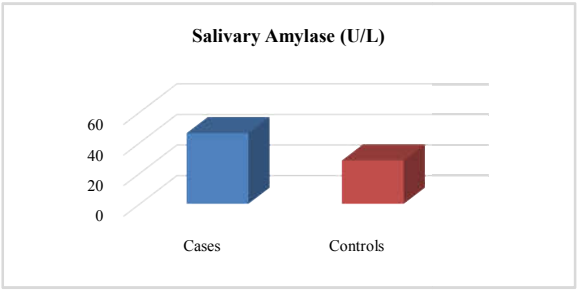

Figure I Salivary Amylase in control and cases

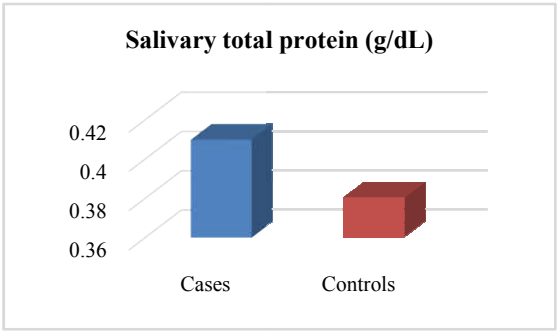

Figure II Salivary total proteins in control and cases

Table IV Comparison of salivary amylase and salivary total protein in acute and chronic leukemia

Parameter	Acute leukemia (n=17)	Chronic leukemia (n=13)	Significance
Salivary amylase	37.62±20.92U/L	59.75±13.53U/L	P<0.05 Significant
Salivary total protein	0.42±0.11g/dL	0.40±0.10g/dL	p>0.05 Non-significant

Salivary amylase in leukaemia patients ranged from 15-76U/L while in control group serum amylase value rangedfrom 14-58U/L. Mean serum amylase levels in leukemia patients were 46.3±21.69U/L and higher than the control group mean serum amylase levels being 28.45±11.60U/L and this difference was statistically significant (p=0.02). Mean serum amylase levels were 59.75±13.53U/L in chronic leukaemia patients and were significantly higher (p=0.016) than acute leukaemia patients salivary amylase levels being 37.62±20.92U/L.

The mean salivary total protein levels were 0.38±0.10g/dL in control group, while in leukaemia patient value was 0.41±0.10g/dL. This difference was not significant statistically (p= 0.355). In chronic leukaemia patients mean serum protein levels were 0.40±0.10g/dL and in acute leukamia value was 0.42±0.11g/dL. This difference was not significant statistically.(p=0.754)

Table V Oral manifestations in different leukemias

Oral manifestaion	AML (n=11)	ALL (n=6)	CML (n=9)	CLL (n=4)
Paleness of gum	9	5	7	2
Gingival hyperplasia	5	Nil	1	1
Gingival petechiae	3	2	Nil	Nil
Gingivitis	4	Nil	3	3
Enlarged submandibular lymphnodes	6	3	5	3
Ecchymosis	4	Nil	2	Nil
Ulcerative necrotic lesion	3	5	1	Nil

Out of 30 cases, 23 (76.6%) patients had discolouration (pallor) of the gum (9 AML, 5 ALL, 7 CML and 2 CLL patients); 7(23.3%) patatients had gingival hyperplasia (5 AML, 1 CML and 1 ALL); 10 patients had gingivitis (4 AML, 3 CML and 3 CLL); 17 patient had enlarged submandibular lymph nodes (6 AML, 3 ALL, 5 CML and 3 CLL); 5 (16.7%) cases had gingival petechiae (3 AML and 2 ALL patients); and 6 (20%) patient had ecchymosis (4 AML, 2 CML); 9(30%) patients had ulcerative necrotic lesion (3 AML, 5 ALL and 1 CML) and none patient showed gum hemorrhages.

DISCUSSION

Leukemia during its different stages of progression involves the whole body system and produces systemic manifestations.

Oral manifestations often reflects the underlying systemic disease and therefore saliva has been used recently as an diagnostic indicator of oral health[9-11]in leukemia and many other systemic diseases where function of salivary gland and composition of saliva is affected. Salivary components are now used as surrogates for systemic biomarkers in blood.[12]
Our study reported mean salivary total protein levels 0.38±0.10g/dL in control group , while the mean values in leukaemia patient was 0.41±0.10g/dL. Although salivary proteins levels are higher in leukemic patients but this difference was not significant statistically (p= 0.355) (Table III, Figure II).

In the oral cavity saliva serves as the first barrier defending against the microbial invasion by mechanical, nonimmunologic (non-specific) and immunologic (specific) means.[13]Salivary proteins (e.g. lysozyme, peroxidase, myeloperoxidase,lactoferrin) along with salivary components (thiocyanate, chlorine, hydrogen peroxide) are mainly responsible for innate immunity via there immune activator and/or immune modulator properties.[13,14] Their levels undergoes considerable fluctuations in various physiological and pathological conditionss such as physical exercise, age, tobacco smoke, diabetes , hyper-IgE syndrome and caries etc. [15]

The effect of these salivary proteins are cumulative and /or synergistic providing molecular defense in the oral cavity. Local concentration of these proteins near the mucosal surfaces (mucosal transudate), periodontal sulcus (gingival circular fluid) and oral ulcers (transudate) is very high and also reinforced by immune and/or inflammatory reactions of oral mucosa. Binding of salivary proteins onto mucosal surfaces improve their resistance against proteolytic degradation via microbial proteases.[14] They interfere bacteria metabolism, its adhesion and cause the bacterial aggregation, so that they can be swallowed.[13] The salivary levels of total protein increase through β- sympathetic activity in the salivary glands, since saliva scretion is mainly evoked by the action of adrenergic mediators.[16]Salivary glands may respond to periodontitis by enhanced synthesis of some acinar proteins and thereby an increase in the protective potential of saliva [17-19]

The mean serum amylase levels in our study in leukemia patients were 46.3±21.69U/L and higher than the control group mean serum lamylase levels being 28.45±11.60U/L and this difference was statistically significant (p=0.02) (Table:III, Figure:I).

Amylase is a highly abundant protein in saliva, having endoglycosidase activity. It has role in acquired pellicle formation on tooth surfaces. In salivary fluid amylase is one of the most important digestive enzyme and thus α- amylase is prone to alterations in response to the cell damage caused by chronic periodontitis. α- amylases has higher concentration in saliva and are main constituents of lycoproteinaceous thin film formed on teeth. Formation of a preliminary film is later followed by the dental plaque formation which contributes to various oral diseases, dental caries and periodontitis.[20,21] The salivary α- amylase can bind specifically to the most common oral bacteria, streptococcus species. [22]

Amylase inhibits bacterial growth by binding to lipopolysaccharide of bacteria which is structural part of bacteria. It also inhibits bacterial toxin mediated tissue destructive inflammatory reactions.[23] Thus, it was demonstrated that α-amylases from human saliva, porcine pancreas and rice show a significant cell growth inhibitory activity against Porphyromonas gingivalis species, and interfere with the adherence and biofilm formation of Aggregatibacter actinomycemcomitans, indicating that α-amylase could be effective in preventing periodontal diseases.[24,25]

Unstimulated saliva was used because there is a prevalent resting condition in the oral cavity for most of the 24-hour period and, as the salivary secretion is a reflex response which can be influenced by several stimuli such as the accumulation of plaque-derived substances or inflammatory products.[26] Other studies had reported a positive relationship between basal values of amylase, proteins and mucin with CAL (clinical attachment loss) and PPD (probing pocket depth).[27] This is mainly explained by the fact that inflammatory products may trigger salivary secretion via neural pathways.[26]

Levels of amylase, mucin and proteins in saliva from adults with or without chronic periodontal disease were evaluated in a previous study. They reported higher concentration of mucin, amylase and proteins were found in saliva from patients compared with healthy subjects. [17] Ashok L *et al* also reported raised salivary amylase and salivary proteins levels in leukemic patient when compared with healthy controls.[28]

Out of 30 cases, 23 (76.6%) patients had discolouration (pallor) of the gum (9 AML, 5 ALL, 7 CML and 2 CLL patients); 7(23.3%) patatients had gingival hyperplasia (5 AML, 1 CML and 1 ALL); 10 patients had gingivitis (4 AML, 3 CML and 3 CLL); 17 patient had enlarged submandibular lymph nodes (6 AML, 3 ALL, 5 CML and 3 CLL); 5 (16.7%) cases had gingival petechiae (3 AML and 2 ALL patients); and 6 (20%) patient had ecchymosis (4 AML, 2 CML); 9(30%) patients had ulcerative necrotic lesion (3 AML, 5 ALL and 1 CML) and none patient showed gum hemorrhages(Table: V).

Acute leukemia patients often present to dentists with different type of oral manifestations at various stages of disease. Gingival enlargement is the most commonly seen oral manifestation in leukemia patients. Other manifestations include paleness of oral mucosa/local abnormal color of the gum, gingival petechiae, ecchymosis, hemorrhages, ulcerative necrotic lesions and buccal infections.[6]In chronic leukemia oral manifestations are non-specific and less common.In these patients oral lesion occurs because of infiltration in local tissues by leukemic cells, anemia, thrombocytopenia and immunodeficiency state. Dreizen *et al* also reported gingival hyperplasia in acute leukemia patients both ALL and AML, which disappears totally or partially in most of cases after chemotherapy administration.[29-31]

CONCLUSION

As leukemia patients showed higher salivary mylase and salivary total protein levels than control group and there is associated oral complications in leukemia group. This indicates decline in oral health with associated changes in saliva composition in leukemia patients. Hence supports the diagnostic value of saliva in coexisting systemic diseases such as leukemias.

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