Evaluation of Salivary Amylase and Total Protein in Children with Acute Lymphoblastic Leukemia in Basrah Pediatric Oncology Center

Abstract

Background: Saliva has found to be used as a diagnostic aid in an increasing number of clinical situations and in systemic disease that can affect salivary gland function and composition.

Objective: To assess salivary amylase and total protein content in un-stimulated whole saliva in children with Acute Lymphoblastic Leukemia (ALL) at time of diagnosis and during induction phase of chemotherapy.

Patients and methods: Thirty newly diagnosed children with acute lymphoblastic leukemia aged (1–14) years were recruited. Sixty healthy children matched for age and sex were regarded as control. Amylase and total protein were estimated in un-stimulated saliva from all subjects under study.

Results: In children with ALL, the mean value of salivary amylase (817.05 ± 328.10 U/L) and total protein (10.20 ± 2.03 g/dl) were significantly higher before induction of chemotherapy than the controls (188.04 ± 124.7 U/L and 7.30 ± 0.82 g/dl, respectively) (p<0.0001). While during induction phase of chemotherapy only salivary amylase decreased significantly (p<0.0001). The mean value of the salivary amylase and total protein were significantly elevated at the time of diagnosis in patients of both sexes with ALL than in controls (p<0.0001). However, within the ALL group, there were no significant differences in mean value of the salivary amylase and total protein between males and females at the time of diagnosis and during induction phase of the chemotherapy (p>0.05).

Conclusion: Salivary amylase level significantly increases at time of diagnosis of acute lymphoblastic leukemia (ALL) and decreases during the induction phase of treatment with chemotherapy. So it can be regarded as diagnostic and prognostic indicator for acute lymphoblastic leukemia.

Keywords: Salivary amylase; Malignancies; Gastrointestinal tract; Oral mucosa

Introduction

OT Cancer is predominantly a disease of ageing and is very rare in childhood, in western populations; only around 0.5 percent of all cancers occur in children aged less than 15 years OT [1]. Hematopoietic neoplasm constitute more than 40% of malignancies in children and represent a wide range of disorder that include acute and chronic leukemias, lymphomas, and histiocytic malignancies [2]. The leukemias are the most common malignant neoplasms in childhood, accounting for about 41% of all malignancies that occur in children <15 yr of age [3]. The incidence of Acute Lymphoblastic Leukemia varies significantly throughout the world, with the rates ranging from nine to 47 per million for male children, and from seven to 43 per million for females. P-Acute lymphoblastic leukemia has a striking peak incidence between 2-6 years of age and occurs slightly more frequent in boys than girls [4]. Toxic exposure in utero to maternal Epstein-Barr virus (EBV) reactivation or other viral entities such
as cytomegalovirus and herpes simplex virus has been proposed as a causative factor in some infants and children with ALL [5]. Monozygotic twins carry a small but definite increased risk of concordant ALL [6]. The precise pathogenetic events leading to the development of ALL are unknown. Only, a minority (5%) of cases are associated with inherited, predisposing genetic syndromes [7]. There have been several attempts to classify ALL cells morphologically using criteria such as cell size, nuclear to cytoplasmic ratio, nuclear shape, number and prominence of nucleoli, nature and intensity of cytoplasmic staining, presence of cytoplasmic granules, prominence of cytoplasmic vacuoles and the character of the nuclear chromatin [2]. Based on the lymphoblast morphology on Romanovsky stained, the French-American-British (FAB) Cooperative group has recognized three distinct ALL subsets-L1, L2, L3 [8].

The ability to detect intracellular antigen by FCM has considerably improved the diagnostic accuracy of immunophenotyping [4]. Surface antigens are considered positive if 20 percent or more of the leukemic cell express antigen with more than 98 percent fluorescence intensity, as compared with negative control cells [9]. Positivity for terminal deoxynucleotide transferase (TdT) and cytoplasmic antigen (cy) is defined as more than 10 percent of cells exhibiting nuclear (TdT) or intracytoplasmic (cy) fluorescence, Cyto genetic analysis of leukemia blasts provides important information in up to 90 percent of ALL that is clinically relevant for diagnosis and prognosis of ALL [9]. The clinical presentation of ALL varies. Symptoms may appear insidiously or acutely. The presenting features generally reflect the degree of marrow failure and the extent of extramedullary spread patient can present with fever which could be induced by infection or by pyrogenic cytokines (e.g., interleukin-1, interleukin-6, and tumor necrosis factor released from the leukemic cells) [10]. Anemia fatigue and lethargy are common manifestations of anemia in patients with ALL [7].

Arthralgia and bone pain, more than a fourth of patients, especially young children, may have a limp, bone pain, arthralgia, or an unwillingness to walk because of leukemic infiltration of the periosteum, bone, or joint or because of expansion of the marrow cavity by leukemic cells [6].

Bleeding manifestations: by gum bleeding, petechial bleeding, sometimes retinal bleeding, and easy bruising due to thrombocytopenia, hematomas are less frequent [11]. Most patients have some extramedullary disease at diagnosis, and extramedullary relapse is a known complication of the disease. The most commonly affected extramedullary sites of disease include the central nervous system, testes, lymph nodes, liver, spleen, and kidney. Of these sites, the CNS and the testes have the greatest clinical significance [12]. CNS involvement at diagnosis is relatively uncommon; the incidence of less than 5%, usually is not associated with symptoms [13]. Overt testicular involvement at the time of diagnosis occurs in approximately 2% of males, most commonly in T-cell ALL [14,15]. Asymptomatic lymphadenopathy and hepatosplenomegaly occur among more than one half of patients [13]. The gastrointestinal tract (GIT) is frequently involved in ALL. The most common manifestation is bleeding [16].

The oral manifestations include pallor, gingival enlargement, petechiae and ecchymoses [17]. Other oral complications include infections of the mucosa salivary gland dysfunction, taste dysfunction, and pain [18]. Leukemic infiltrates in the GIT are usually clinically silent until terminal stages when necrotizing enteropathy might occur. The most common site for this is the cecum, giving rise to a syndrome known as typhlitis [16]. Enlarged salivary glands and priapism. In some patients, infiltration of the tonsils, adenoids, appendix or mesenteric lymph nodes leads to a surgical intervention before leukemia is diagnosed [19]. Saliva has found to be useful as a diagnostic aid in an increasing number of clinical situations and in systemic disease that can affect salivary gland function and composition [17]. Biomarkers detected in saliva can be valuable in a wide range of clinical pathology, forensic medicine and sport medicine [20]. Amylase is one of the most important salivary digestive enzymes. It consists of two families of isoenzymes, of which one set is glycosylated and the other contains no carbohydrate [21]. It has been suggested that amylase accounts for 40 to 50% of the total salivary gland-produced protein, most of the enzyme being synthesized in the parotid gland [21]. It increases with the salivary flow rate, and it is generally considered to be a reliable marker of serous cell function [23]. Patients with leukemia had higher amylase activity and elevated concentration of salivary total protein at the time of diagnosis [17]. Therapy for ALL typically consists of a brief remission-induction phase followed by intensification (or consolidation) therapy to eliminate residual disease, and then prolonged continuation treatment to maintain remission. All patients also require treatment directed to the CNS early in the clinical course to prevent relapse due to leukemic cells sequestered in this site [24]. Remission induction therapy aims at eradicating more than 99% of the initial leukemic cell burden and restoring normal hematopoiesis. This treatment phase typically lasts 4 to 6 weeks A two-drug remission induction regimen of weekly vincristine and daily prednisone results in remission in 80% to 90% of children with ALL [12,25]. Once remission has been achieved, systemic treatment in conjunction with central nervous system (CNS) sanctuary therapy follows [26]. All or its treatment can lead to thrombocytopenia [27]. Platelet transfusions should be given therapeutically for overt bleeding and may be indicated when platelet counts are less than 10 × 10^9/liter [12]. Transfusion of packed red cells is indicated in patients with anemia and marrow suppression but should be delayed until the leukocyte count is reduced in patients with extreme hyperleukocytosis [27]. The major salivary glands are the parotid glands, submandibular glands and sublingual glands. Minor salivary glands are situated on the tongue, palate, and buccal and labial mucosa, they are small mucosal glands with primarily mucous secretion [28]. Salivary fluid is an exocrine secretion consisting of approximately 99% water, containing a variety of electrolytes (sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate) and proteins, represented by enzymes, immunoglobulins and other antimicrobial factors, mucosal glycoproteins, traces of albumin and some polypeptides and oligopeptides of importance to oral health [29]. There are also glucose and nitrogenous products, such as urea and ammonia [30]. Many functions have been ascribed to saliva, including its role as a lubricant that coats the mucosa and helps protect the...
oral tissues against mechanical, thermal and chemical irritants [31]. Saliva is also involved in initial enzymatic digestion through one of its major constituents, amylase [32].

Other functions of human saliva include initiation of digestion through α-amylase buffering capacity acting as an ion reservoir that facilitates the remineralization of teeth antimicrobial activity involving secretory immunoglobulin A, lysozyme, lactoferrin and myeloperoxidase agglutination, resulting in the clearance of bacterial cells; pellicle formation; providing a solvent and acting as a medium where tastants derived from foods are presented to taste buds; and acting as a medium for moistening dry foods to aid swallowing [28,31]. Aggressive treatment of malignant disease may produce unavoidable toxicities to normal cells. The mucosal lining of the gastrointestinal tract, including the oral mucosa, is a prime target for treatment-related toxicity by virtue of its rapid rate of cell turnover [33]. A variety of complications involving the oral cavity may be seen in patients with cancer, either as a result of the disease itself or of treatment with chemotherapeutic agents, during chemotherapy most complications are the result of myelosuppression, immunosuppression and/or direct cytotoxic effect on oral tissue. Major clinical problem encountered in the oral cavity include ulcers, local or systemic infection and hemorrhage [34]. This risk results from multiple factors, including high rates of cellular turnover for the lining mucosa, a diverse and complex microflora, and trauma to oral tissues during normal oral function [35]. Chemotherapy may also adversely affect the salivary gland resulting in an alteration of the quality and quantity of saliva [34]. However, it has not been possible to draw consistent conclusions about the effects of cancer chemotherapy on salivary gland function [18]. Ulcerative oral mucositis occurs in approximately 40% of patients receiving chemotherapy, intensive chemotherapy can cause ulcerative mucositis that initially emerges approximately 2 weeks after initiation of high-dose chemotherapy [36,37]. Normal salivary gland function promotes mucosal health. Oral mucositis can be complicated by infection in the immunocompromised patient. Also, oral organisms can disseminate systemically in the setting of ulcerative oral mucositis and profound, prolonged neutropenia [33,38]. Oral evaluation and management of patients scheduled to undergo myeloablative chemotherapy should occur as early as possible before initiation of therapy. To maximize outcomes, the oncology team should carefully advise the dentist as to the patient’s medical status and oncology treatment plan. In turn, the dental team should delineate and communicate a plan of care for oral disease management before, during, and after cancer therapy [37]. This case control study was carried out to estimate salivary amylase and total protein content in un-stimulated whole saliva in acute lymphoblastic leukemia at time of diagnosis and during induction phase of chemotherapy.

Subjects and Methods

A case control study has been carried out on children and adolescents with newly diagnosed acute lymphoblastic leukemia over ten months (from the first of January till the end of October 2011). The study was carried out in Basrah governorate and the data were collected from Basrah oncology pediatric center. A total of 30 newly diagnosed patients with ALL according to comprehensive diagnostic work up, their ages range from (1-14) years, 12 were males and 18 were female. The control group consisted of 60 healthy children 32 males and 28 females, age and sex matched between ALL cases and control group was done. Exclusion criteria were patients with psychiatric, endocrine, cardiovascular, or other chronic disease, or those patients receive medications as psychoactive drugs, beta-blockers and glucocorticoids [39].

Data collection

A special questionnaire was designed for the purpose of the study. The Following information was taken: name, date of birth, sex, age at diagnosis and residence. ALL cases were classified according to risk group into standard and high-risk groups depending on the characters of ALL classification according to age of the patient at the time of diagnosis and the initial leukocyte count. Age between 1–10 years and a leukocyte count of <50,000/μL are used to define standard risk. Children who are >10 years of age or who have an initial leukocyte count of >50,000/μL are considered to be at higher risk [40].

Methods

Two ml of un-stimulated saliva sample of all the study subjects were collected in the morning from the floor of mouth by a sterile collecting tubes before and during induction phase of chemotherapy and kept immediately in refrigerator for further analysis.

Salivary amylase enzyme: Estimate by using colorimetric α-(AMYLASE ASSAY KIT).

Test principle: This method utilizes a chromogenic substrate, Ethyliden-p-nitrophenol linked with maltotriose [40]. The enzymatic action of α-amylase on this substrate yields Ethyliden-p-nitrophenol, which can be spectrophotometrically measured at 405 nm. The amount of α-amylase activity present in the sample is directly proportional to the increase in absorbance at 405 nm. For ease of use, the reaction is read in a 96-wellmicrotiter plate with controls provided.

Salivary total protein: Was estimated by using colorimetric method (Biruet reagent) [41,42].

Assay principle

In alkaline medium the copper reacts with the peptide bonds of proteins to form the characteristic pink to purple Biruet complex. Sodium potassium tartarate prevent copper hydroxide precipitation, and potassium iodide prevents the auto-reduction of copper.

Protein +CuP2+P Alkaline PH Cu–Protein complex (18)

The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in the absorbance at 546 nm.

Procedure

One ml of biuret reagent was pipetted into 3 test tubes marked blank, standard and test, 20 μL of saliva was added to the test
tube marked test, 20 μL of total protein standard was added to the test tube marked standard. Then solutions were mixed well and incubated at 37 PoPC for 10 minutes. The absorbance of the test and standard was read against reagent black at 546 nm with 30 minutes.

Calculation of total salivary protein was done by using the following equation:

Absorbance of test-
• Total protein concentration (gm/dl)=X 6
Absorbance of Std-
• Expected values: Children (<1 years) 4.8-7.6 g/dl
• (>1 years): 6.0-8.0 g/dl
• Adult: 6.6-8.7 g/dl

Statistical analysis
Statistical analysis was done using SPSS program (15), data were expressed by mean ± Standard Deviation. Comparisons of proportions was performed by crosstab using Chi-Square test. The t-test used for quantitative comparison of biochemical variables before and during induction of chemotherapy. Lastly by using paired t test for comparison of biochemical variables for the same cases. For all tests p-value of <0.05 was considered as statistically significant.

Results
Table 1 shows characteristics of cases with acute lymphoblastic leukemia and control group according to age groups. A total of 30 children with newly diagnosed ALL and 60 children of control group were included in this study, their ages range from (1-14) year (Table 1).

Table 1 Distribution of cases and control group according to age groups.

| Age/year | ALL cases | %  | Control | %  | p-value |
|----------|-----------|----|---------|----|---------|
| ≤ 4      | 10        | 33.3 | 18      | 30.0 | >0.05   |
| >4-9     | 14        | 46.7 | 26      | 43.3 |
| >9-14    | 6         | 20.0 | 16      | 26.7 |
| Total    | 30        | 100  | 60      | 100  |

Table 2 Characteristics of cases with ALL and control according to sex.

| Sex      | ALL cases | %  | Control | %  | p-value |
|----------|-----------|----|---------|----|---------|
| Male     | 12        | 40.0 | 32      | 53.3 | >0.05   |
| Female   | 18        | 60.0 | 28      | 46.7 |
| Total    | 30        | 100  | 60      | 100  |
**Table 3** Salivary amylase and total protein levels at time of diagnosis.

| Variable                                           | Group          | N   | Mean ± SD          | p-value |
|----------------------------------------------------|----------------|-----|--------------------|---------|
| Salivary amylase level before chemotherapy U/mL    | ALL Cases      | 30  | 817.05 ± 328.10    | <0.0001 |
|                                                    | Control        | 60  | 188.04 ± 124.7     |         |
| Total salivary protein level before chemotherapy g/dL | ALL Cases      | 30  | 10.20 ± 2.03       | <0.0001 |
|                                                    | Control        | 60  | 7.30 ± 0.82        |         |

**Table 4** Salivary amylase and total protein levels in both sex of ALL cases and control group.

| Sex       | Group                        | Amylase level before chemotherapy U/mL Mean ± SD | Total protein level before chemotherapy g/dL Mean ± SD | p-value |
|-----------|------------------------------|-----------------------------------------------|-------------------------------------------------------|---------|
| Male      | ALL cases (12)               | 863.14 ± 336.69                               | 10.35 ± 1.75                                          | <0.0001 |
|           | Control (32)                 | 185.65 ± 132.66                               | 7.26 ± 0.81                                           |         |
| Female    | ALL cases (18)               | 786.32 ± 328.31                               | 10.11 ± 2.25                                          | <0.0001 |
|           | Control (28)                 | 190.76 ± 117.25                               | 7.36 ± 0.84                                           |         |

**Table 5** Classification of ALL according to risk groups.

| Risk level  | Frequency | %    |
|-------------|-----------|------|
| Standard risk| 17        | 56.7%|
| High risk   | 13        | 43.3%|
| Total       | 30        | 100% |

**Table 6** Salivary amylase and total protein levels between the two main risk groups before starting the chemotherapy.

| Variable                                           | Risk Group       | N   | Mean ± SD          | p-value |
|----------------------------------------------------|------------------|-----|--------------------|---------|
| Amylase level before chemotherapy U/mL             | Standard risk    | 17  | 748.80 ± 253.08    | >0.05   |
|                                                    | High risk        | 13  | 906.30 ± 399.41    |         |
| Total protein level before chemotherapy g/dL       | Standard risk    | 17  | 9.82 ± 1.70        | >0.05   |
|                                                    | High risk        | 13  | 10.70 ± 2.38       |         |

**Table 7** Salivary amylase and total protein levels before and during induction phase of chemotherapy.

| Variable                                           | No   | Mean ± SD          | p-value |
|----------------------------------------------------|------|--------------------|---------|
| Amylase level before chemotherapy U/mL             | 30   | 817.05 ± 328.10    | 0.0001  |
| Amylase level during chemotherapy U/mL             | 30   | 345.56 ± 168.99    |         |
| Total protein level before chemotherapy g/dL       | 30   | 10.20 ± 2.03       | 0.362   |
| Total protein level during chemotherapy g/dL       | 30   | 12.65 ± 14.37      |         |
Table 8 Salivary amylase and total protein levels of ALL cases before and during induction phase of chemotherapy according to age groups.

| Age/No.  | Variable       | Mean ± SD  | p-value |
|----------|----------------|------------|---------|
|          | Before Chemotherapy | During Chemotherapy |         |
| ≤ 4 years (10) | Amylase  | 885.83 ± 229.27 | 379.9 ± 86.62 | 0.0001 |
|          | Total protein  | 9.21 ± 0.94  | 9.14 ± 1.28 | >0.05 |
| >4-9 years (14) | Amylase  | 697.15 ± 259.33 | 282.99 ± 115.54 | 0.0001 |
|          | Total protein  | 10.27 ± 1.72  | 15.56 ± 20.90 | >0.05 |
| > 9-14 years (6) | Amylase  | 982.18 ± 521.97 | 434.21 ± 307.68 | <0.001 |
|          | Total protein  | 11.70 ± 3.169 | 11.70 ± 3.15 | >0.05 |

Table 9 Salivary amylase and total protein levels of ALL cases before and during induction phase of chemotherapy according to sex.

| Sex /No. | Variable      | Mean ± SD  | p-value |
|----------|---------------|------------|---------|
|          | before chemotherapy | during chemotherapy |         |
| Male (12) | Amylase  | 863.14 ± 336.69 | 378.94 ± 172.17 | 0.0001 |
|          | Total protein  | 10.35 ± 1.75  | 10.23 ± 1.89 | >0.05 |
| Female (18) | Amylase  | 786.32 ± 328.31 | 323.31 ± 168.01 | 0.0001 |
|          | Total protein  | 10.11 ± 2.5  | 14.26 ± 18.53 | >0.05 |

Table 10 Salivary amylase level and total protein between the males and females of ALL cases before and during induction phase of chemotherapy.

| Variable                  | Sex   | N   | Mean ± SD  | p-value |
|---------------------------|-------|-----|------------|---------|
| Amylase before chemotherapy U/mL | Male   | 12  | 863.14 ± 336.69 | 0.54    |
|                           | Female | 18  | 786.32 ± 328.31 |         |
| Amylase during chemotherapy U/mL | Male   | 12  | 378.94 ± 172.17 | 0.39    |
|                           | Female | 18  | 323.31 ± 168.01 |         |
| Total protein before chemotherapy g/dL | Male   | 12  | 10.35 ± 1.75  | 0.74    |
|                           | Female | 18  | 10.11 ± 2.25  |         |
| Total protein during chemotherapy g/dL | Male   | 12  | 10.23 ± 1.89  | 0.41    |
|                           | Female | 18  | 14.26 ± 1.85  |         |

Patients with ALL before starting chemotherapy and compared between the two major risk groups the results are presented in Table 6.

The differences between the mean value of salivary amylase (748.80 ± 253.08) before starting chemotherapy were statistically not significant in standard risk group when compared with high risk group of cases (906.30 ± 39941) (p>0.05).

Also, the differences were statistically not significant between the mean value of salivary total protein (9.82 ± 1.70) of standard risk group when compared with high risk group of cases (10.70 ± 2.38) (p>0.05). Salivary amylase and total protein levels were measured for patients with ALL before and during induction chemotherapy. The mean values were compared. As shown in Table 7.

Analysis of the data by using of paired t test when compared between salivary amylase level of cases before and during induction of chemotherapy revealed the differences statistically significant (p<0.0001). While the differences in salivary total protein level before starting chemotherapy and during induction of chemotherapy was statistically not significant (p>0.05).

Salivary amylase and total protein levels of ALL cases were measured before and during induction phase of chemotherapy and the mean differences according to age groups are presented in Table 8. The mean values of salivary amylase levels in cases before starting chemotherapy were significantly higher than that for cases during induction phase of chemotherapy for all age groups, while the mean values of salivary total protein levels in ALL cases before starting chemotherapy were statistically not significant than that during induction phase of chemotherapy (p>0.05).

The mean differences of Salivary amylase and total protein levels of ALL cases before and during induction phase of chemotherapy according to sex were measured and the results presented in Tables 9 and 10. The mean differences of salivary amylase levels for males and females cases of ALL before starting chemotherapy was statistically significant than that for males cases during induction phase of chemotherapy with (P < 0.0001) for both, and the mean differences of salivary total protein level in males and females cases before starting chemotherapy was statistically not significant than that for cases during induction phase of chemotherapy with (p>0.05) (Table 10).

The mean difference of salivary amylase and total protein levels for males and females’ cases of ALL before starting chemotherapy was statistically not significant, (p>0.05) and the mean difference
of salivary amylase and total protein levels also not significant during induction phase of chemotherapy, (p>0.05).

**Discussion**

Leukemia is a fatal disease in which there is an abnormal proliferation of precursors of white components of the blood. It is not a single disease but is a group of disorders involving blood producing organs [17]. Salivary gland dysfunction may occur as a result of cancer or it is therapy. Affected patients may develop significant oral, dental, and upper gastrointestinal sequel [18].

During recent years it has become apparent that saliva is critical for maintenance and function of all tissue in the mouth. Therefore, any situation that disturb saliva production will probably have broad negative sequel in the mouth and may result in systemic complications [17,33]. Oral problems may make difficult for a child to receive all of his or her cancer treatment. For many leukemia patients the oral complications are very painful and have potentially lethal consequences. Sometimes the leukemia treatment must be stopped completely [42].

The present study conclude that the mean values for amylase level in the ALL cases before starting chemotherapy was significantly different from the mean values seen in control subjects, this result is similar to that reported by Rahemtulla et al. [33], where they study a newly diagnosed ALL patients, analysis of their data revealed that ALL patients had significantly higher mean values for salivary amylase activity than the control group before starting the chemotherapy, this result is also in agreement with another study reported by Ashok LP [17]. These results suggest that notable changes occur in saliva as a result of the disease itself.

In this study the mean values for amylase level before chemotherapy was statistically significantly different from the mean values for amylase level in ALL cases during the induction phase of chemotherapy (p<0.0001), result was similar to that reported by Rahemtulla et al. [31] and Ashok [17]. The reduction in salivary amylase during the induction phase may be due to the effect of chemotherapy especially the L-Asparginase which interferes with salivary amylase in saliva and gastroenteritis [3].

Current study revealed that the mean values of total protein for patients with ALL are significantly higher than the mean values of total protein for control group (p<0.0001). These findings are consistent with those of Rahemtulla et al. [34]. Also, our result similar to that reported by Ashok [17].

This result could have several different explanations for example, the whole saliva is a complex mixture of glandular secretions, oral microorganisms, epithelial cells, erythrocytes and polymorph nuclear leukocytes and some food debris. Poor oral hygiene due to malaise due to the disease results in increased dental plaque all these causes can contribute to an increase in total protein concentration in saliva.

In present study during the induction phase of chemotherapy the concentration of salivary total protein statistically was not significantly different from that of the ALL cases before starting the treatment with (p=0.362) this result similar to that reported by Rahemtulla et al. [34] and Ashok [17].

Hemorrhages and oral ulceration and gingivitis are common findings in leukemia patients after starting the chemotherapy and because blood is mixed with saliva, all these causes contribute to an increase in total protein concentration after starting the chemotherapy.

There is no significant difference in mean values of amylase and total protein when compared between the standard and high risk groups of ALL cases and also there is no significant difference in mean values of amylase and total protein between males and females of ALL cases, this mean that there is no relation between the changes in amylase and total protein levels in the saliva and the sex and the risk groups of the patient.

**Conclusion**

From this study it can be concluded that Salivary amylase level significantly increases at time of diagnosis of acute lymphoblastic leukemia and significantly decreases during the induction phase of treatment with chemotherapy and it can be regarded as diagnostic and prognostic indicator for ALL. Further studies with larger sample size are required for a better understanding the effect of acute lymphoblastic leukemia and chemotherapy on saliva component.

**References**

1. Stiller CA (2004) Aetiology and epidemiology of acute lymphoblastic leukemia. Pediatric Oncology, 3rd edition. Arnold Co 3: 24.
2. Margolin JF, Steuber CP, Poplack DG (2006) Acute lymphoblastic leukemia. 5th edition. Philadelphia, Lippincott William and Wilkins 538: 579.
3. Tubergin DG, Archie B (2007) The leukemias. 18th edition, Philadelphia. WB Saunders Co pp: 2116-2143.
4. Smith OP, Hann I (2004) Pathology of leukemia. 3rd edition. London. Arnold Co 83: 100.
5. Moore JO (2007) Epidemiology, risk factors, and classification acute lymphoblastic leukemia. Clinical Malignant Hematology pp: 103-108.
6. Maia AT, Van Der Velden VH, Harrison CJ (2003) Prenatal origin of hyperdiploid acute lymphoblastic leukemia in identical twins. Leukemia pp: 2117-2202.
7. Puig CH (2006) Acute lymphoblastic leukemia Williams hematology. 7th edition, McGrow-Hill Companies pp: 1321-1335.
8. Kantarjian HM, Fadri S (2004) Acute lymphoid leukemia in adults. Abeloff’s clinical oncology, 3rd edition, Philadelphia. Churchill Livingstone pp: 2793-2817.
9. Schorpppe M, Pieters R (2004) Acute lymphoblastic leukemia. Pediatric oncology, 3rd edition, London Arnold Co pp: 230-253.
10. Campana D, Puig CH (2004) Childhood leukemia. Abeloff’s Clinical Oncology, 3rd edition, Philadelphia. Churchill Livingstone pp: 2731-2784.
11. Reinhold M, Vishwas S (2007) Acute Lymphoblastic Leukemias. Modren hematology biology and clinical management. 2nd edition Humana Press Inc, USA pp: 173-192.
12 Stacey B, Steeuber CP, Poplack DG (2008) Clinical manifestation of acute lymphoblastic leukemia. Hematology basic principles and practice. 5th edition, Churchill Livingstone pp: 1070-1076.

13 Stuber CP, Poplack DG (2003) Acute lymphoblastic leukemia. Rudolph Paediatrics, 2nd edition, McGraw-Hill: pp: 1595-1600.

14 Hijiya N, Liu W, Sandlund JT (2005) Overt testicular disease at diagnosis of childhood acute lymphoblastic leukemia: Lack of therapeutic role of local irradiation. Leukemia 19: 403-1399.

15 Sirvent N, Suciu S, Bertrand Y (2007) Overt testicular disease (OTD) at diagnosis is not associated with a poor prognosis in childhood acute lymphoblastic leukemia: Results of the EORTC CLG Study 58881. Pediatric Blood Cancer 49: 328-344.

16 Steven A, Mark A, Banu A, Arlene R (2005) Leukemias: Manual of pediatric and oncology pp: 415-450.

17 Ashok L, Sujatha GP, Hema G (2010) Estimation of salivary amylase and total proteins in leukemia patients and its correlation with clinical feature and radiographic finding. Indian J Dent Res 21: 486-490.

18 Jensen SB, Pedersen AM, Vissink A (2010) A systematic review of salivary gland hypofunction and xerostomia induced by cancer therapies: Prevalence, severity and impact on quality of life support. Care Cancer 18: 60-103.

19 Pui CH, Cherng D (2005) Childhood acute lymphoblastic leukemia. Postgraduate Hematology 5th edition. Blackwell Publishing Ltd pp: 542-557.

20 Kagami H, Hiramatsu Y, Hishida S (2000) Salivary growth factors in health and disease. Adv Dent Res 14: 99-102.

21 Mäkinen KK (1989) Salivary enzyme. Boca Raton, Florida, USA pp: 93-120.

22 Noble RE (2000) Salivary alpha-amylase and lysozyme levels: A non-invasive technique for measuring parotid vs. submandibular sublingual gland activity. J Oral Sci 42: 66-83.

23 Almstahl A, Wikstrom M, Groenink J (2001) Lactoferrin, amylase and mucin MUC5B and their relation to the oral microflora in hyposalivation of different origins. Oral Microbiol Immunol 16: 337-345.

24 Pui CH, Evans WE (2006) Treatment of acute lymphoblastic leukemia. N Engl J Med 354: 166.

25 Bostrom BC, Sensel MR, Sather HN (2003) Dexamethasone vs. Prednisone and daily oral vs. weekly intravenous mercaptopurine for patients with standard-risk acute lymphoblastic leukemia: A report from the Children's Cancer Group. Blood 101: 3809.

26 Pui CH, Crist WM (2000) Acute lymphoplastic leukemia. Childhood Leukemias pp: 288-313.

27 Lowe EJ, Pui C-H, Hancock ML (2005) Early complications in children with acute lymphoblastic leukemia presenting with hyperleukocytosis 45: 10.

28 Sariri R, Damirch A (2010) Alternations in salivary amylase due to exercise intensity. Pharmacology Online 3: 263-269.

29 Almeida PD, Gregio AM, Machado MA, Lima AA, Azevedo LR (2008) Saliva composition and functions: A comprehensive review. J Contemp Dent Pract 3: 72-80.

30 Humphrey SP, Williamson RT (2001) A review of saliva: Normal composition, flow and function. J Prosthodont 85: 162-169.

31 Featherstone JD (2003) The caries balance: Contributing factors and early detection. J Can Dent Assoc 31: 33-129.

32 Humphrey SP, Williamson RT (2001) A review of saliva: Normal composition, flow and function. J Prosthodont 85: 162-169.

33 Lalla RV, Brennan MT, Schubert MM (2011) Oral complications of cancer therapy. Pharmacology and Therapeutics for Dentistry pp: 98-782.

34 Rahmatulla BM, Techanitwisad I, Rahematulal F, Tonya O (1992) Analysis of salivary components in leukemia patients receiving chemotherapy. Cancer 73: 35-46.

35 Keefe DM, Schubert MM, Elting LS (2007) Updated clinical practice guidelines for the prevention and treatment of mucositis. Cancer 109: 780-820.

36 Jellema AP, Slotman BJ, Doornaert P (2007) Impact of radiation-induced xerostomia on quality of life after primary radiotherapy among patients with head and neck cancer. Int J Radiat Oncol Biol Phys 69: 60-75.

37 Sonis ST (1998) Mucositis as a biological process: A new hypothesis for the development of chemotherapy-induced stomatotoxicity. Oral Oncol 34: 39-43.

38 Schubert MM, Peterson DE (2009) Oral complications of hematopoietic cell transplantation. Thomas hematopoietic cell transplantation: Stem cell transplantation. 4th edition, Wiley-Blackwell pp: 1589-1607.

39 Nicolas R, Hutta M, Enrique F, Clemens K (2006) The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. Psychophysiology 43: 645-652.

40 Wallenfels K, Foldi P, Niermann H, Bender H, Linder D (1978) The enzymic synthesis, by trans-glucosylation of a homologous series of glycosidically substituted malto-oligosaccharides and their use asamylase substrates. Carbohydrate Research 61: 359-368.

41 Foo YA, Brosalki SB (1986) Analytical reviews in clinical biochemistry: Calcium measurement. Biochem 23: 624-637.

42 Runge ME, Edwards D (2000) Orthodontic treatment for an adolescent with a history of acute lymphoblastic leukemia. Pediatric Dentistry 22: 494-498.