

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

Janan GH^{1*},
Zainab AJ¹ and Lamia MALN²

1 Department of Paediatrics, Basrah University, Iraq

2 Department of Biochemistry, Basrah University, Iraq

Abstract

Background: Saliva has found to be use 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

*Corresponding author: Janan GH

✉ jenan_ah03@yahoo.com

Department of Paediatrics, Basrah University, Iraq.

Tel: +964(0)7801000820

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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 OTP [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 ALLP [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 (cyIgM, cyCD3) fluorescence, Cytogenetic analysis of leukemic 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 ce-6, and tumor necrosis factor released from the leukemic cells [10] Anemia, fatigue and lethargy are common manifestations of anemia in patients with ALLP [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 ALLP [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 leukaemia is diagnosed [19]. Saliva has found to be use 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 (α -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 [22]. The concentration of amylase 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 ALLP [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 P9P/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 a 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 clearly 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 leukaemia 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 $<50,000/\mu\text{L}$ are used to define standard risk. Children who are >10 years of age or who have an initial leukocyte count of $>50,000/\mu\text{L}$ 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-well microtiter plate with controls provided.

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

Assay principle

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

Protein + Cu^{2+} + 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 μ L 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 \pm 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
	No	%	No	%	
≤ 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
	No.	%	No.	%	
Male	12	40.0	32	53.3	>0.05
Female	18	60.0	28	46.7	
Total	30	100	60	100	

This Table shows the percentage of children their ages ≤ 4 years represent (33.3%) for ALL cases and (30.0%) for control, for those children of their ages range from (>4-9) years represent (46.7%) for ALL cases and (43.3%) for control and for those of their ages range from (>9-14) years represent (20.0%) for ALL cases and (26.7%) for control, $p > 0.05$.

Male patients with ALL and control group were (40.0%, 53.3%) respectively. While female was (60.0%, 46.7%) respectively as shown in **Table 2**. There was no significant difference regarding sex of patients with ALL and control group ($p > 0.05$).

Salivary amylase and total protein levels were measured for patients with ALL before starting chemotherapy and control group the results are presented in **Table 3**.

Analysis of the data revealed that at the time of diagnosis the mean value of the salivary amylase (817.05 ± 328.10) was statistically significantly higher in cases compared with that in control group (218.54 ± 268.89), ($p < 0.0001$). The mean value of salivary total protein (10.20 ± 2.3) of cases was also significantly higher as compared with the control group (7.30 ± 0.8) ($p < 0.0001$).

Salivary amylase and total protein levels were measured for patients with ALL before starting chemotherapy and control group and compared between both of group according to the sex the results are presented in **Tables 3 and 4**.

This Table shows that salivary amylase level is significantly higher among males and females with ALL compared to males and females in the control group ($p < 0.0001$).

Table 5 shows that out of 30 patients with newly diagnosed ALL; 17(56.7%) of them with standard risk and the remaining 13(43.3%) with high risk.

Salivary amylase and total protein levels were measured for

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 \pm SD*		p-value
		Before Chemotherapy	During Chemotherapy	
≤ 4 years (10)	Amylase	885.83 \pm 229.27	379.9 \pm 86.62	0.0001
	Total protein	9.21 \pm 0.94	9.14 \pm 1.28	>0.05
>4-9 years (14)	Amylase	697.15 \pm 259.33	282.99 \pm 115.54	0.0001
	Total protein	10.27 \pm 1.72	15.56 \pm 20.90	>0.05
> 9-14 years (6)	Amylase	982.18 \pm 521.97	434.21 \pm 307.68	<0.001
	Total protein	11.70 \pm 3.169	11.70 \pm 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 \pm SD*		p-value
		before chemotherapy	during chemotherapy	
Male (12)	Amylase	863.14 \pm 336.69	378.94 \pm 172.17	0.0001
	Total protein	10.35 \pm 1.75	10.23 \pm 1.89	>0.05
Female (18)	Amylase	786.32 \pm 328.31	323.31 \pm 168.01	0.0001
	Total protein	10.11 \pm 2.25	14.26 \pm 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 \pm SD*	p-value
Amylase before chemotherapy U/mL	Male	12	863.14 \pm 336.69	0.54
	Female	18	786.32 \pm 328.31	
Amylase during chemotherapy U/mL	Male	12	378.94 \pm 172.17	0.39
	Female	18	323.31 \pm 168.01	
Total protein before chemotherapy g/dL	Male	12	10.35 \pm 1.75	0.74
	Female	18	10.11 \pm 2.25	
Total protein during chemotherapy g/dL	Male	12	10.23 \pm 1.89	0.41
	Female	18	14.261 \pm 1.85	

patients with ALL before starting chemotherapy and compared between the two main risk groups the results are presented in **Table 6**.

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

Also, the differences were statistically not significant between the mean value of salivary total protein (9.82 \pm 1.70) of standard risk group when compared with high risk group of cases (10.70 \pm 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-Asparaginase 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.

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