ORIGINAL ARTICLE

CLINICO-HAEMATOLOGIC PARAMETERS AND ASSESSMENT OF POST-INDUCTION STATUS IN ACUTE LYMPHOBLASTIC LEUKAEMIA

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Background: Acute Leukaemia is a malignant disorder characterized by an abnormal proliferation of immature cells, called blasts. Classically, acute leukaemia is classified into acute myeloid leukaemia and acute lymphoblastic leukaemia depending on the lineage of the immature cells. Objective of the study was to evaluate the clinical presentations, analyze the haematologic parameters at time of diagnosis and assess the post-induction status in newly diagnosed ALL patients. This cross-sectional study was conducted in the Department of Haematology, Armed Forces Institute of Pathology, Rawalpindi from June to November 2019. **Methods:** A total of 55 newly diagnosed ALL patients were recruited including children, adults and elderly. Detailed medical history and physical findings were noted. Haematologic parameters were documented. Each patient was treated as per standard protocol and remission induction status was determined on day 29 of treatment. **Results:** The median age of the study cohort of 55 newly diagnosed ALL patients was 8.5 years. Males were 37 (67.3%) and females were 18 (32.7%) with a male to female ratio of 2:1. Paediatric group included 31 (56.4%) patients. Nine (16.4%) patients were in the adult group and 15 (27.3%) in the elderly age group. The time from onset of symptoms to diagnosis of acute lymphoblastic leukaemia was 98.87±79.21 days. Fever was the most common symptom but body aches were common among paediatric group while pallor was the most common sign. Mean WBC was 29.1±27.9 $x10^9$/l, Hb was 8.1±2.9 g/dl and platelet count was 60±41.8 $x10^9$/l B-acute lymphoblastic leukaemia was more common than T-acute lymphoblastic leukaemia. A total of 52 patients were assessed on day 29 to evaluate for post-induction remission status. The remission rate of our cohort of patients was 82.7%. **Conclusion:** Most of the patients were in paediatric age group and remission rate was better in this age group compared to elderly population. B-ALL was associated with good response to induction chemotherapy while patients with BCR-ABL1 gene rearrangement did not respond well to treatment. Identification of prognostic features at diagnosis will further help our clinicians to predict outcomes of the disease.

Keywords: Acute Lymphoblastic Leukaemia; Clinical features; Haematologic parameters; Post-induction remission status

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INTRODUCTION

Acute Leukaemia is a malignant disorder characterized by an abnormal proliferation of immature cells, called blasts.[1] Classically, acute leukaemia are classified into acute myeloid leukaemia and acute lymphoblastic leukaemia depending on the lineage of the immature cells.[2] Acute lymphoblastic leukaemia, also called acute lymphocytic leukaemia (ALL), can, thus, be defined as a clonal disorder identified by the presence of 20% or more blasts of lymphoid origin in the blood and/ or bone marrow replacing normal haematopoietic component.[3] The majority of leukaemia are of B cell origin while a quarter are T-cell ALL.[4]

ALL has a worldwide incidence of 1-4.75 cases per 100,000 annually.[5] Though seen in both adults and children, the disease is more common in children with a second peak seen in adults above the age of 40 years.[6] A slight male preponderance has been observed.[7] Clinically, the disease has an acute onset.[8] Symptoms are characteristically due to anaemia, thrombocytopenia and neutropenia which are a result of the replacement of normal haematopoietic elements by lymphoblasts.[9,10] Weakness, fatigue, fever, bruising are common symptoms.[11] Some patients may display bone tenderness while others may give a history of constitutional B symptoms.[12] On examination, patients may be pale and may have

lymphadenopathy, hepatomegaly and/or splenomegaly.[13]

Traditionally, risk stratification was done based on age, gender and the white cell count. In recent years, immunophenotypic, cytogenetic and molecular markers have been identified that determine the prognosis of disease.[18] Today, based on clinical parameters, morphologic findings, immunophenotypic, cytogenetics and molecular features, these patients are risk stratified and treatment decisions are taken accordingly.[19] Better understanding of underlying disease pathophysiology, identification of prognostic markers, years of clinical experience and advancements in therapeutics have remarkably improved the outcome of patients with acute lymphoblastic leukaemia.

In developing countries like Pakistan, resources are limited and advanced diagnostic facilities are only available at few centers and even where available, are not affordable. In such settings, clinico-haematologic parameters are important for risk stratification. We conducted this study with the aim to get an insight into the clinical presentations and laboratory parameters of our acute lymphoblastic leukaemia patients. This will not only enhance our knowledge of this disease but will also help us to comprehend the underlying pathophysiologic mechanisms. This study was planned to analyze the post-induction remission status. This will help our clinicians to identify high risk features and predict treatment response which will further facilitate in management decisions aimed at improving patient outcome.

MATERIAL AND METHODS

In this cross-sectional study, 55 patients presenting to Haematology Department, Armed Forces Institute of Pathology, Rawalpindi, Pakistan from CMH Paediatric Oncology, CMH Oncology and Armed Forces Bone Marrow Transplant Centre were investigated from June to November 2019. These were patients, of all ages and both genders, newly diagnosed as having acute lymphoblastic leukaemia. Patients who had received any treatment including steroids, radiotherapy or chemotherapy were excluded from the study. Written informed consent was taken from all the patients. The study was conducted after approval from the Institutional Ethical Review Board in accordance with the principles of the Declaration of Helsinki.

Detailed history was taken. Demographic details including age, gender and ethnicity were noted. Patients were divided into three groups based on their age. Patients till age 12 years were in the paediatric group, age >12 years to 60 years in the adult group and those >60 years in the elderly age group. Clinical history was taken and medical records were reviewed to assess the presenting signs and symptoms and their duration. Physical examination was performed and pallor, bruising, petechiae, lymphadenopathy, hepatomegaly and/or splenomegaly.

Complete blood counts were performed on Sysmex automated haematology analyzer XN-3000. Peripheral films were prepared and stained with Leishman's stain. Each peripheral film was examined, differential leukocyte count performed, blast count and morphology was documented. RBC morphology and platelet counts were documented. Blasts are small to medium sized with a high nucleus to cytoplasmic ratio and relatively condensed chromatin. Patients with mature B or FAB classification of L3 were not included in this study. On cytochemical staining, lymphoblasts are characteristically Sudan black negative. Supravital staining using New Methylene Blue was done for reticulocyte count.

Immunophenotyping was performed in all patients on FACS Flow cytometer (Beton-Dikinson Biosienes USA). Molecular study was done on RT-PCR of acute lymphoid gene panel including BCR-ABL1, E2A-PBX1 and TEL-AML1.Biochemical profile including liver function tests, renal function tests and LDH levels was done. Results of radiological investigations including ultrasonography and computed tomography scans were recorded. Pregnancy test was performed for married females of child bearing age before initiation of treatment.

Bone marrow examination was performed from posterior superior iliac spine under sterile conditions. Children were given ketamine while in adults the procedure was performed under local anaesthesia (plain lignocaine). Bone marrow aspiration was performed, slides prepared and stained with Leishman stain. Blast count and morphology as well as other haematopoietic components were evaluated. Bone marrow trephine biopsy was done and after processing as per standard protocol, H&E slides were examined. Degree and pattern of bone marrow infiltration and fibrosis was determined. Lumbar puncture was done and cerebrospinal fluid (CSF) was examined for the presence of blasts and lymphocyte count.

Each patient was risk stratified. Patients having white cell count $<50x10^9$/l and between age of 1-9 years were standard risk. High risk patients were defined by a white cell count of $>50x10^9$/l and age >10 yrs. or older.

Treatment protocol for children one year old and adults up to 25 years of age was UK ALL 2011. Standard risk patients received Regimen A induction

with dexamethasone, vincristine and asparaginase. High risk patients received Regimen B induction with dexamethasone, vincristine, daunorubicin and asparaginase. Adults above 25 years were given UK ALL 2014 protocol comprising of daunorubicin, dexamethasone and vincristine. Asparaginase was included in Philadelphia negative patients while Imatinib was included for Philadelphia positive patients. Elderly >60 years were assessed for performance status and co-morbidities. The UK ALL 2014 protocol was modified according to status of each patient to avoid drug toxicities. Complete blood counts, peripheral film examination and bone marrow morphology was performed on day 29 to assess the remission status. Complete remission was defined as normalization of counts defined as an absolute neutrophil count >1.0x10^9/l and platelet count >100x10^9/l, presence of <5% blasts in the bone marrow and absence of blasts on peripheral blood smear. Statistical analysis was done using SPSS 20. Results for qualitative variables were calculated using mean while percentage and frequencies were computed for categorical variables.

RESULTS

A total of 55 newly diagnosed acute lymphoblastic leukaemia patients were recruited over a period of six months. The median age of the study cohort was 8.5 years. Of these males were 37 (67.3%) and females were 18 (32.7%) with a male to female ratio of 2:1. The median age of the study cohort was 8.5 years. The patients were categorized into 3 groups based on their age. Paediatric group included 31 (56.4%) patients. Nine (16.4%) patients were in the adult group and 15 (27.3%) in the elderly age group. Each age group stratified according to gender is shown in Figure 1.

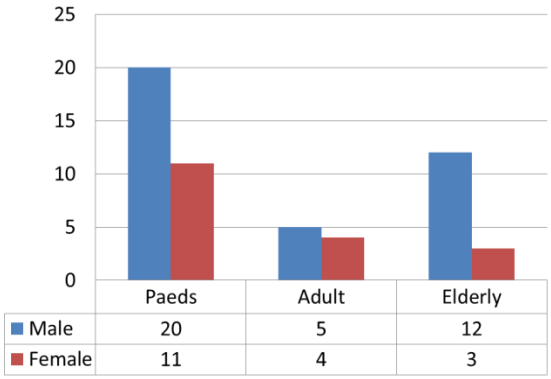

Figure-1: Each age group stratified according to gender

Among our study population of 55 newly diagnosed ALL patients; 26 (47.3%) were Punjabi, 21 (38.2%) were Pashtoon, 3 (5.5%) were Sindhi, 3 (5.5%) were Kashmiri, 1 (1.8%) was Balochi while 1 (1.8%) patient was from Gilgit-Baltistan. The time from onset of symptoms to diagnosis of acute lymphoblastic leukaemia was 98.87±79.21 days. The most common clinically reported symptom at time of presentation was fever and infections. Physical examination revealed pallor in majority of the patients while lymphadenopathy was observed in more than half the patients. Splenomegaly was also a prominent finding as shown in Figure-2.

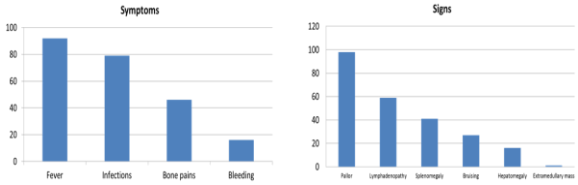

Figure-2: Symptoms and Signs of ALL cohort

Laboratory parameters of our cohort of patients were assessed (Table-1). Average WBC was 29.1±27.9, average haemoglobin was 8.1±2.9, average platelet count was 60±41.8, peripheral blood blasts were 70±28 and bone marrow blasts were 80±20. Bone marrow fibrosis was found in 58.2% and CNS involvement in 7%.

Table-1: Lab Parameters of study cohort

Parameters	
Mean WBC (x 10^9/L)	29.1 ± 27.9
Mean Hemoglobin (g/dl)	8.1 ± 2.9
Mean Platelet (x 10^9/L)	60 ± 41.8
PB Blasts (%)	70 ± 28
BM Blasts (%)	80 ± 20
BM fibrosis > grade I (%)	58.2
CNS involvement (%)	7.3
Mean LDH levels (U/l)	760 ± 510

One patient in the paediatric age group died on the second day of initiating induction therapy while one child died in the second week of treatment. One of the patients in the elderly group died on the day of initiating treatment. Febrile neutropenia was the most common treatment-related complication observed. A total of 52 patients (Table-2) were assessed on day 29 to evaluate for post-induction remission status. The remission rate of our cohort of patients was 43 (82.7%). Nine (17.3%) patients did not achieve post-induction remission. The majority of these patients who did not achieve remission were in the elderly age group and mostly male patients. On

Immunophenotyping (>20% blasts for B-ALL were positive for tdt, CD10, CD19, CD22, cCD79a and for T-ALL cCD3, CD5 and CD7 was positive) 90% patients were positive for B-ALL and 9.6% for T-ALL. Molecular studies by real-time PCR showed BCR-ABL1 rearrangement in 4(7.7%) patients, TEL-AML1 fusion gene in 6(11.5%) and E2A-PBX1 fusion gene in 5(9.6%).

Table-2: Comparison of Clinico Haemotological Parameters of Patients with/without Remission

Parameters	In Remission (n=43)		Not in Remission (n=9)	
	n	%	n	%
Age				
< 12	30	96.8	1	3.2
13-45	7	77.8	2	22.2
46-60	9	60.0	6	40.0
Gender				
Male	29	78.4	8	21.6
Female	17	94.4	1	5.6
WBC				
< 10x10^9/L	25	92.6	2	7.4
10-50 x 10^9/L	8	80.0	2	20.0
More than 50	8	61.5	5	38.5
Immunophenotyping				
B-ALL	40	85.1	7	14.9
T-ALL	2	40.0	3	60.0
Molecular Finding				
BCR-ABL1	1	25.0	3	75.0
TEL-AML1	6	100.0	0	0.0
E2A-PBX1	4	80.0	1	20.0
CNS Disease	1	25.0	3	75.0

DISCUSSION

Acute lymphoblastic leukaemia is the clonal proliferation of lymphoid precursor cells in the blood, bone marrow and extramedullary sites.[3] It is predominantly seen in children with a second peak in the elderly age group.[5] While much is known about clinical presentations and disease course, most of the data is from the Western population. Considering geographic and ethnic differences may affect disease biology, here we present data from our ALL patients, of Asian origin.

In our study, there was a male preponderance with a male to female ratio of 2:1 and most males 8 (21.6%) didn't respond well to induction treatment. Similar results have been reported in the Chinese population. Su-Yi Li *et al* reports a male to female ratio of 1.841.[20] In another study, a ratio of 1.85 has been reported in the Iranian population[21] with poor remission status. However, a female predominance has been reported in a study conducted at the University of Tokyo, Japan.[22]

The time from onset of symptoms to first diagnosis was 98.87±79.21 days. This could be attributable to the fact that Pakistan is a developing country where majority of people cannot afford good healthcare facilities nor do they have access to tertiary care centers for timely diagnosis and proper management. This could also account for the early deaths of the patients on just initiating treatment as they reported at a later stage of their disease. This study may help guide clinicians regarding the signs and symptoms as well as laboratory parameters of ALL patients ensuring timely referral and diagnosis. Prompt diagnosis and initiation of treatment may improve patient outcome.

Our study reports remission rate of 96.6% in our paediatric cohort. A similar study conducted at St. Jude Children's Research Hospital, Memphis, US on a much larger number of patients reports a post-induction remission rate of 97.6%.[23] However, in our study the CR rate was 77.8% in the adult sub-group and 60% in the elderly sub-group. A lower CR rate in the elderly age group is most likely due to the presence of co-morbidities and modified/reduced chemotherapy regimens.

Complete blood counts play a pivotal role in which, raised TLC, low haemoglobin and low platelet counts are prominent features.[15,16] In our study 27(52%) patients had low TLC ($<10x10^9$/L) and 13(25%) showed high TLC ($>50X10^9$ /L). High TLC is not a good prognostic marker for ALL, 5(38.4%) patients with raised TLC couldn't achieve complete remission.

On Immunophenotyping 47(90.3%) patients were diagnosed with B-ALL and 5(9.6%) were diagnosed with T-ALL out of which B-ALL responded well to induction treatment. Daniel William et al[24] observed similar findings on immunophenotyping in Brazilian patients. Most of them were diagnosed as B-ALL 89.5% and 10.5% were diagnosed as T-ALL. In similar Brazilian study CNS involvement was seen in 6.6% patients which is a poor prognostic marker of ALL while in our study 7.7% individuals had CNS disease.

Identification of molecular and cytogenetic features may be beneficial in modifying treatment regimens to improve the post-induction remission status. Post-induction remission status leads to deciding the next step of management and aids in predicting patient outcome. In our study most of the patients with BCR-ABL1 rearrangement didn't receive complete remission while patients with TEL-AML1 fusion gene and E2A-PBX1 fusion gene showed good response to induction therapy.

Limitation of our study is the small sample size. Further studies with a larger sample size may prove to be useful in establishing a database of our patients. Moreover, incorporation of cytogenetic studies in future will further strengthen the findings. However, we have elaborated the presenting clinical

features and the baseline haematologic laboratory parameters which are a good indication towards diagnosis and may help clinicians in instituting appropriate treatment.

AUTHORS' CONTRIBUTION

HN: Literature review, data collection, write up, result analysis, discussion. HSM: Literature review, proof reading. RM: Literature review, result analysis, proof reading. AM: Literature review. SZ: Data collection, result analysis. UN: Result analysis

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