ORIGINAL ARTICLE
CYTOGENETIC PROFILE OF ACUTE LYMPHOBLASTIC LEUKAEMIA PATIENTS AND ITS ASSOCIATION WITH INDUCTION REMISSION STATUS

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Background: Acute lymphoblastic leukaemia is characterized by the presence of more than or equal to 20% lymphoblast (early lymphoid precursors) in peripheral blood and/or in bone marrow. Lymphoblast can infiltrate different organs and clinically patients can present with fatigue, pallor, fever, bone pain, bleeding or bruises and lymphadenopathy. ALL is the most common type of malignancy in children. To determine the cytogenetic abnormalities in patients of Acute Lymphoblastic Leukaemia as a predictor of response to induction chemotherapy. It was a descriptive cross-sectional study. **Methods:** This study was conducted at the Armed Forces Institute of Pathology, Rawalpindi over a period of six months from June to November 2019. Bone marrow and peripheral blood samples of newly diagnosed 80 patients of all the age groups and either gender, who received one month treatment for ALL,were analyzed for cytogenetic study. Patients who were previously diagnosed with ALL, who presented with relapse and those who required induction treatment outside the trial hospital were excluded. UK ALL 2011 treatment protocol was adopted for patients up to 25 years old and for patients above 25years old UK ALL 2014 treatment protocol as induction chemotherapy was adopted. Evaluation for remission was carried out at the termination of initial induction chemotherapy on day 29 of treatment. **Results:** A total of 80 patients were enrolled in the study, comprising 36 (45%) females & 44 (55%) males. The median age of paediatric patients was 5years (<19 years) who were 56/80 (70%) in number whereas the median age of adults was 27 years (>19 years) who constituted 24/80 (30%) of the participants. Cytogenetic of 51 (63.75%) patients revealed hyperdiploidy (chromosome number 51–66) whereas 29(36.25%) of the participants had miscellaneous mutations [(Hypodiploidy, t (9; 22), t (1; 19) and t (12; 21)]. On immunophenotyping 51/80 (63.7%) of the leukemias were of B cell origin and 29 (36.25%) of T-cell origin. **Conclusion:** Patients with hyperdiploidy, t (12;21) ETV6/RUNX1 and t(1;19)TCF3/PBX1 had better prognosis and higher remission rate compared to those with the other mutations like t(9;22)Ph$^+$ and hypodiploid which were associated with poor prognosis. Association of gender with remission was not statistically significant.

Keywords: Cytogenetics; Leukaemia; Remission

INTRODUCTION

Acute lymphoblastic leukaemia is characterized by the presence of more than or equal to 20% lymphoblast (early lymphoid precursors) in peripheral blood and/or in bone marrow. Lymphoblast can infiltrate different organs and clinically patients can present with fatigue, pallor, fever, bone pain, bleeding or bruises and lymphadenopathy. ALL is the most common type of malignancy in children. Fatality rate is high if left untreated with a cure rate of only 40% despite great advancements in the domain.[1,2] It accounts for 20% and 80% of all the cancers in adult and paediatric age groups, with an estimated prevalence of 3.5 and 2.2 per 100000 male and female population respectively.[3,4]

It is associated with various congenital, immunological, and environmental factors such as down syndrome, Li–Fraumeni syndrome, Neurofibromatosis, radiation or chemotherapy, and unusual immune response to infection.[5,6] Multiple genetic alterations result in excessive production of immature lymphocytes subsequently hampering the genesis of normal new blood components (red and white blood cells, platelets). Bone marrow examination constitutes the mainstay of diagnostic strategies. Some of the known prognostic markers of

acute lymphoblastic leukaemia include age, sex, molecular studies, immunophenotype, karyotype, white blood cell numbers and bone marrow examination[7,8]

Cytogenetic studies play a pivotal role in establishing the diagnosis, determining the appropriate treatment and help in predicting the prognosis of disease. The most common chromosomal abnormalities illustrated on cytogenetic evaluation are t (12; 21) (p13; q22) ETV6-RUNX1 with an estimation of 25% in children and 3% in adults. Prevalence of t (1; 19) (q23; p13) TCF3-PBX1, t (4;11) (q21;q23) MLL gene rearrangement and hyperdiploidy is 30–40% in children and 2–10% in adults. Prevalence of t (9; 22) (q34; q11) BCR-ABL rearrangement is 2–5% in paediatric patients compared with 20–25% in adults. The t (9; 22) and 11q23 are associated with the worst prognosis whereas t (12;21) and hyperdiploidy are associated with favourable outcomes.[9,10] Depending on prognostic features patients who respond well to treatment are considered as standard risk and those who do not achieve complete remission considered as high risk.[9]

The rationale of the study is to evaluate the cytogenetic abnormalities in patients of acute lymphoblastic leukaemia as a predictor of response to induction chemotherapy.

MATERIAL AND METHODS

This cross sectional study was conducted at Armed forces institute of Pathology, Rawalpindi after approval of ethical review board (AFIP/Trg/IRB/2018/103) over a period of six months from June to November 2019.The minimum sample size calculated for the study was 48 where the prevalence of Acute Lymphoblastic Leukemia in Asia was 3.2% with a 95% confidence level and 5% margin of error as reported by Khazaei *et al.*[11] With a purposive sampling a total of 80 participants were enrolled in the study.

Bone marrow and peripheral blood samples of 80 newly diagnosed patients of all the age groups and either gender who received one month treatment for ALL, was analyzed for cytogenetic after physical examination, complete blood counts, bone marrow morphology and immunophenotyping. Patients who were previously diagnosed with ALL, who presented with relapse and those who required induction treatment outside the trial hospital were excluded. Clinical features recorded were fever, bone pain, bleeding symptoms (petechiae, epistaxis, bruising, gum bleeding, blood in stool), hepatomegaly, splenomegaly, hyper leukocytosis (>30,000 in B-ALL and >50,000 in T-ALL) and CNS Status (I: <5 WBC in CSF without blasts, II: <5 WBC in CSF with

blasts, III: CSF >5 WBC in CSF with blasts). UK ALL 2011 treatment protocol was adopted for patients up to 25years old and for patients above 25years old UK ALL 2014 treatment protocol as induction chemotherapy was adopted. Evaluation for remission was carried out at the termination of initial induction chemotherapy on day 29 of treatment.

Patient's detailed history and examination was taken. Complete blood counts were performed on Sysmex automated haematology analyzer XN-3000. Bone marrow biopsy was performed. Peripheral blood and bone marrow smears were stained with Leishman and Geimsa stain. Diagnosis of Acute leukaemia was made on Blast morphology and blast percentage. ALL blasts were negative on Sudan black stain (cytochemical stain). Immunophenotyping was performed in every patient at diagnosis. Two colour flow cytometry was performed on FACS Flow Cytometer using monoclonal antibodies.

Cytogenetic analysis was done by Metaphase chromosome banding using conventional Geimsa banding technique. Bone marrow samples (5ml) were collected in sodium heparin tube and were processed immediately. To culture bone marrow samples Rose well Park Memorial Institute (RPMI) 1640 basal medium, containing 10% foetal calf serum for 24 h at 37 °C was employed later to achieve cell mitosis. Metaphase stage arrest treatment was instituted with 0.1 µg/ml of colcemid. Hypotonic potassium chloride solution and Carnoy's were used for harvest action and fixation. Giemsa trypsin banding technique used for analysis and karyotypes defined as per International System for Human Cytogenetic Nomenclature. Correlation with individual cytogenetic abnormalities was not feasible because of the low absolute number of patients with a specific abnormality.

Cytogenetic analysis grouped as hyperdiploidy (defined as chromosome number 51–66) and miscellaneous mutations [(Hypodiploidy(<46chromosomes), t (9;22), t (1;19) and t (12;21)]. Remission was defined as <5% blast cells on morphology in the presence of overall haematological recovery (neutrophils >1.0x10/L, platelets >100x10/L) and absence of extramedullary disease upon termination of induction therapy.[12]

UK ALL 2011 treatment protocol was adopted as induction chemotherapy for 1year to 25-year-old patients with vincristine, dexamethasone and asparaginase for standard risk as Regimen A. Regimen B was given in high risk which includes dexamethasone, vincristine, daunorubicin and asparaginase. UK ALL 2014 treatment protocol was adopted for 25–60-year-old patients which included vincristine, dexamethasone and daunorubicin.

Philadelphia negative patients were given asparaginase while Philadelphia positive were treated with imatinib. Evaluation for remission was carried out at the termination of initial induction chemotherapy on day 29 of treatment. Data was entered and analyzed by data management software IBM SPSS (version 23.0). The mean and standard deviation was reported for continuous variable whereas descriptive statistics for the categorical variable were presented as frequency and percentage with the Chi-Square test. A p-value of ≤0.05 was statistically significant.

RESULTS

A total of 80 patients enrolled in the study out of which 36 (45%) were females whereas 44 (55%) were males. The median age of paediatric patients was 5years (<19 years) who were 56 (70%) in number whereas the median age of adults was 27years (>19 years) who constituted 24 (30%) participants. Cytogenetic of 51 (63.75%) patients were representative of hyperdiploidy whereas 29 (36.25%) participants had miscellaneous mutations. Among miscellaneous mutations t (12; 21) was found in 12 (15%), t (1; 19) in 10 (12.5%), t (9; 22) in 5 (6.25%) and hypodiploidy in 2 (2.5%) patients.

Fifty-seven (71.2%) of the leukemias were of B cell origin and 23 (28.7%) T-cell origin at immunophenotyping. Thirty-two (40%) had hyper leukocytosis. 54 (67.5%) were of standard risk whereas 26 (32.5%) belonged to the high-risk group as per NCI Classification.

Fever and Bone pain were the most common clinical signs observed among the patients while pallor was the most common symptom. CNS presentation was less common.

Table-1: Correlation of cytogenetic with remission status

	Hyper diploidy	Miscellaneous	p-value
Remission	46 (74.2%)	16 (24.2%)	<0.001*
Remission Failure	5 (27.8%)	13 (72.2%)	

*Significant p-value

Table-2: Correlation of gender with remission status

	Male	Female	p-value
Remission	34 (77.3%)	28 (77.8%)	.95
Remission Failure	10 (22.7%)	8 (22.2%)	

*Insignificant p-value

Table-3: Clinical Presentation of Patients

Clinical Feature	N (%)
Fever	45 (56.25)
Bone Pain	37 (46.25)
Bleeding	19 (23.75)
Abdominal Pain	26 (32.5)
Pallor	60 (75)
Anorexia	14 (17.5)
Hepatomegaly	39 (48.75)
Splenomegaly	39 (48.75)
WBC count (x10⁹/L)	32.19±49.32
Haemoglobin (g/dl)	7.65± 2.62
Platelets (x10⁹/L)	68.29± 91.60
CNS	
I	75 (93.75)
II	3 (3.75)
III	2 (2.5)

DISCUSSION

Acute Lymphoblastic Leukaemia is a heterogeneous disorder with respect to its clinical, haematological, and genetic factors which has been well established over the years. In ALL, treatment and outcome of the disease highly depends on risk stratification, which relies on age at diagnosis, leucocyte count, immunophenotyping and cytogenetic abnormalities. These factors help in the decision of targeted therapies and in treatment plan of allogeneic stem cell transplant.[13,16]

Gender based study of our population revealed 44 (55%) males and 36 (45%) females with a male to female ratio of 1.2:1 (no significant correlation with gender) while Saima Naeem has reported that majority of the patients in her study were males (80%) with M:F 7:1. Fever was the most common presenting feature at diagnosis in both studies. On immunophenotyping both the studies found similar percentage of B-ALL (72%) and T-ALL (28%) respectively.[15]

In our study the median age for paediatric population was 5years whereas for adults was 27years. Age and WBC count at diagnosis are very important factors in risk stratification.[13] hyper leukocytosis was found in 40% of our cohort. Reddy P et al reported 42% with hyper leukocytosis and the median age of paediatric group in his study was 8years whereas 26.5years in adult group.[14]

To understand the genetic basis of ALL, cytogenetic has proved to be a very powerful tool over the years. Correlation of well-established cytogenetic abnormalities with age is strongly related to the disease outcome. In our cohort of patients hyperdiploidy (51%) was the most common chromosomal abnormality observed. Yu Wang et al in his study said that t (12; 21) ETV6/RUNX1 is associated with good prognosis and mostly present in children.[17] Hyperdiploidy (51%), t (12; 21)15%, t (1; 19)12.5% were mostly seen in children with favourable outcomes in our study. Other chromosomal abnormalities like hypodiploidy (2.5%) were also found in our patients. Patients with

hypodiploidy did not achieve complete remission at the end of induction therapy. Similar findings were reported by Clarie S and Christine J in their study.[16]

Risk of relapse and overall survival is predicted by response to induction therapy.[16] Patients with t(9;22)Ph$^+$ chromosome are considered as high risk cytogenetic group and are associated with disease relapse.[14,18] In our patients t(9;22) Ph$^+$ was seen in adult (6.25%) age group only out of which 1.25% did not respond well to treatment. Reddy P *et al* reported the similar findings in Ph$^+$ patients.[14]

Results of the study have shown that cytogenetic have a very significant role in diagnosis and prognosis of the disease. Cytogenetic also monitors the minimal residual disease (MRD) which is required to check the response to therapy. Extensive research work in ALL genetics and follow up for a longer time after treatment is required for patient's stratification and to determine the overall survival of ALL patients.

CONCLUSION

Majority of the population is presented with hyperdiloidy. Hyperdiploidy, t (12; 21) ETV6/RUNX1, t (1; 19) TCF3/PBX1 are associated with favourable outcomes compared to t (9; 22) Ph$^+$ and hypodiploidy which signifies the worst prognosis. Association of gender with remission was not statistically significant.

AUTHORS' CONTRIBUTION

HN: Literature review, data collection, result analysis, write-up. HSM: Literature review, proof reading. RM: Conceptualization of study design, write-up, proof reading. AM: Literature review. UB: Result analysis, proof reading. SJ: Result analysis.

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