ASSOCIATION OF HYPERDIPLOIDY WITH REMISSION STATUS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA AFTER INDUCTION THERAPY

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ABSTRACT

Objective: To determine the frequency of hyperdiploidy in childhood acute lymphoblastic leukemia (ALL) and its association with remission status after induction therapy.

Study Design: Observational study.

Place and Duration of Study: Department of Haematology, Armed Forces Institute of Pathology (AFIP) and Pediatric Oncology Department Combined Military Hospital (CMH) Rawalpindi, from Apr 2017 to Apr 2018.

Patients and Methods: All diagnosed cases of ALL between 1-12 years of age were selected by convenient non-probability sampling. All other cases of leukemias including patients of acute undifferentiated leukaemia, acute myeloid leukaemia and patients of ALL who did not yield successful cytogenetic culture or those who died during the induction therapy were excluded from the study. Diagnosis of ALL was done on the basis of morphology, cytochemistry, immunophenotyping and cytogenetics. Cytogenetic analysis was done by using conventional Giemsa banding. Karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN). On the basis of cytogenetics two groups were made one with hyperdiploidy and another without hyperdiploidy. Hyperdiploidy was defined as chromosomes >46. All patients of childhood ALL were treated with 'UK ALL 2011' protocol and their remission status was assessed after 1 month of induction therapy by bone marrow examination. Remission status of 2 groups of ALL with and without hyperdiploidy were compared by using chi square test

Results: Out of total 80 patients of ALL, 62 (77.5%) yielding successful cytogenetic culture were included in the study. Mean age at diagnosis was 5.6 ± 2.9 years and male to female ratio was 2.4:1. Analytical immunocytometry revealed 58 (93.5%) as B-ALL while 4 (6.55%) were T-ALL. Hyperdiploidy was detected in 19 (30.6%) and t (9:22) (q34:31) in 1 (1.6%). In all other patients no cytogenetic abnormality was detected. Out of 62 patients, Overall complete haematological remission (CHR) was achieved in 47 (75.8%). Out of 19, patients with hyperdiploidy 18 (94.7%) achieved CHR, as compared to other group without hyperdiploidy in which 29 (67.4%) achieved CHR. The difference was statistically significant (*p*-value 0.021).

Conclusion: Remission rate was 94.7% in patients of childhood ALL with hyperdiploidy. Patients with hyperdiploidy achieve higher CHR rate as compared to patients without hyperdiploidy.

Keywords: Acute Lymphoblastic Leukemia, Complete Haematological Remission (CHR), Hyperdiploidy.

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INTRODUCTION

Most common type of malignancy in children is acute lymphoblastic leukemia (ALL). It constitutes around 75% to 80% of pediatric leukemias. Increase in the early lymphoid precursors in bone marrow is the typical feature of childhood ALL[1,2]. Fever, bruises and bone pains are the frequent presentations of childhood ALL[3]. Diagnosis of childhood ALL is based on morphology, immunophenotyping, karyotyping and gene expression[1]. Cytogenetic studies have a major role in establishing diagnosis and determining the optimal therapy[2]. Some of the cytogenetic abnormalities have established prognostic impact. Cytogenetic abnormalities with favourable prognosis are hyperdiploidy, t (12:21) (p13;q22) ETV6-RUNX1 and those with poor prognosis are 11q23 (MLL gene rearrangement) and Ph+ALL. Other prognostic indicators are age, WBC count, flow cytometry and induction

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response[4,5]. Hyperdiploidy is defined as having more than diploid number of chromosomes i.e >46 chromosomes. Hyper-diploidy in general has good prognosis[6-9]. Hyperdiploidy is self-sufficient indicator of good prognosis[9,10]. Present management of ALL of childhood cures about 80% of patients. Main cause of relapse is treatment failure or may be due to inadequate treatment. Some of the patients do not achieve complete haematological remission (CHR) after induction therapy and are considered as high risk having poor prognosis. Such patients are candidates for allogenic stem cell transplantation[11,12]. Remission induction is a multiagent therapy which attains complete remission in about >95% of cases. Three to four drugs (prednisolone, vincristine, asparaginase with or without daunorubicin) are used for the treatment of childhood ALL depending upon the risk groups of patients[13]. Complete haematological remission is defined as absolute neutrophil count (ANC) 1.0 x 10^9/L, platelet count 100,000/ul and bone marrow blast <05% after induction therapy[14].

Aim of the study was to evaluate frequency of hyperdiploidy in childhood ALL and its association with remission status after induction therapy in Pakistani pediatric patients.

PATIENTS AND METHODS

This observational study was conducted at Armed Forces Institute of Pathology (AFIP) Rawalpindi and Pediatric Oncology Department Combined Military Hospital (CMH) Rawalpindi from April 2017 to April 2018.

All newly diagnosed cases of ALL between 1-12 years of age were selected by convenient non-probability sampling. Sample size was calculated by using WHO calculator. All other cases of leukemias including patients of acute undifferentiated leukaemia, acute myeloid leukaemia and patients of ALL who did not yield successful cytogenetic culture or those who died during the induction therapy were excluded from the study.

Study was started after the approval of the institutional review board and consent was taken from the patients. Peripheral blood and bone marrow samples were collected. Bone marrow samples were sent for immunophenotyping, cytogenetic studies and molecular analysis. patient was diagnosed as a case of ALL, if the patient had >20% blasts, Sudan black negativity of the blast cells and characteristic immunophenotype (for B-ALL >20% of Blast cells express tdt, CD10, CD 19, CD 22, cCD79a and for T-ALL, cCD 3, CD 5, CD 7). Cytogenetic studies were also carried out. On the basis of cytogenetics two groups were made one with hyperdiploidy and other group without hyperdiploidy.

Chromosome analysis was done by metaphase chromosome banding using conventional Giemsa banding technique. Bone marrow samples (5ml) were collected in sodium heparin tubes and were processed immediately, cultured by standard methods and harvested (by mitotic inhibitor and addition of hypotonic solution). Fixation was done by methanol and glacial acetic acid in a ratio of 3:1, after fixation was treated with trypsin and stained with Giemsa. Slides were examined under light microscopy and minimum of 20 metaphases were analyzed and interpreted according to the international system of cytogenetic nomenclature (ISCN).

Risk stratification of patients was done according to National Cancer Institute's criteria. Standard risk was defined as age <10 years and WBC count of <50,000 x 10^9/L and high risk was defined as age ≥10 years of age and WBC count of ≥50,000 x 10^9/L. Patients having high risk cytogenetics i.e. (t(9:22 and t(11q23; variable)) and patients of T-ALL were also included in high risk category.

Patients of ALL included in the study were treated with 'UK ALL 2011' protocol. Standard risk group received regimen-A chemotherapy and high risk group received regimen-B chemotherapy. Regimen-A included three drugs (dexamethasone, vincristine and asparaginase) and Regimen-B included four drugs (dexa-methasone, vincristine, asparaginase and daunorubicin)

Remission status was assessed after 1 month of induction chemotherapy by blood complete counts and bone marrow examination. Complete haematological remission (CHR) was defined as absolute neutrophil count (ANC) of 1.0 x 10^9/L, Platelet count of ≥100,000/ul, bone marrow aspirate with <05% blasts and no extramedullary disease after 1 month of induction therapy.

Data were analyzed by using SPSS 24. Quantitative variables were represented by mean ± SD and qualitative variables were measured as frequencies and percentages. Comparison of remission status between the 2 groups (with or without hyperdiploidy) was done by using chi square/Fisher's exact test. A *p*-value less than or equal to 0.05 considered as a significant *p*-value.

RESULTS

Out of total 80 patients of ALL, 62 patients yielded successful cytogenetic culture and were included in the study. Of them, 54 (87%) patients were between 1-9 years of age and 8 (13%) patients were ≥10 years of age. Mean age at diagnosis was 5.6 ± 2.9 years. There were 44 (71%) boys and 18 (29%) girls with a male to female ratio of 2.4:1 (M: F 2.4:1). Nineteen patients (30.64%) had WBC ≥50 x 10^9/L while 43 (69.35%) had WBC <50 x 10^9/L. On immuno-phenotype 58 (93.5%) were B-ALL and 4 (6.55%) were T-ALL. Hyperdiploidy was detected in 19 (30.6%) and t (9:22) (q34:31) in 1 (1.6%). In all other patients, no cytogenetic abnormality was detected (table-I). After treatment, overall complete haematological remission was achieved in 47 (75.8%). Out of 19, 18 (94.7%) patients with hyperdiploidy achieved complete haematological response (CHR), as compared to other group without hyperdiploidy 29 (67.4%) achieved CHR, which was stastically significant (*p*-value 0.02) (table-II).

Table-I. Characteristics of the patients of acute lymphoblastic leukaemia included in the study (N=62).

Patients characteristics		Hyperdiploidy n=19 (30.6%)	Without hyperdiploidy n=43 (69.4%)
Gender distribution	Male	16 (84.2%)	28 (65.1%)
	Female	03 (15.8%)	15 (34.9%)
Age	<10 years	17 (89.5%)	37 (86.0%)
	≥10 years	02 (10.5%)	06 (14.0%)
Risk groups	Standard	16 (84.2%)	26 (60.5%)
	High	03 (15.8%)	17 (39.5%)

Table-II: Remission status between 2 groups with and without hyperdiploidy.

	Hyperdiploidy	Other group without hyperdiploidy	*p*-value
Remission	18 (94.7%)	29 (67.4%)	0.02
Not in remission	01 (5.26%)	14 (32.6%)	

DISCUSSION

The study has validated various facts regarding ALL in children. Mean age was 5.6 ± 2.9 years which was comparable to the other published studies from Pakistan[1,15,16]. Study conducted in India by Kulkarni *et al* reported mean age of 5.7 years SD ± 0.23 which is comparable to our study[17]. Male to female ratio was 2.4:1 in our study which is higher side than the figures reported literature from Pakistan. Male to female ratio reported in earlier studies by Shariq *et al*, Yasmeen *et al* and Zulfiqar *et al* were 1.8, 1.7 and 2.1 respectively[1,15,18]. Kulkarni *et al* reported a male to female ratio of 3.2:1 which is higher than the one reported in our study[17]. Our study revealed hyperdiploidy in 19 (30.6%) of 62 patients, which is comparable with the data reported in international literature[18,19]. Normal karyotype was seen in 42 (67.7%) patients which was higher than the published studies in Pakistan[1,20]. Our study revealed 93.5% B-ALL and 6.4% T-ALL which is comparable with the literature available in Pakistan but was not

comparable with the study conducted by Shrappe *et al*[11,19].

In this study, complete haematologic remission was achieved in 47 (75.8%) out of 62 patients. CHR in patients with hyperdiploidy was 18 (94.7%) and in patients without hyperdiploidy 29 (67.44%). Overall complete remission rate and remission rate in patients with hyperdiploidy was comparable with the international data, Shrappe *et al* have reported overall complete remission rate of 75% and Dastugue *et al* have reported CHR of 99.6% in ALL patients with hyperdiploidy[11,18].

Our study showed stastically significant difference between the proportion of patients with hyperdiploidy achieving CHR and proportion of patients without hyperdiploidy achieving CHR (p=0.021). Achievement of complete haematological remission after induction chemotherapy is a good prognostic sign. Cytogenetic studies should be performed in all cases of ALL for hyperdiploidy because of its association with good prognosis.

The limitations of the study are that this is a single center study with small sample size; multicentered studies are required to ascertain the overall survival of patients with different cytogenetic abnormalities. Long term follow is required to assess the overall survival of the patients

CONCLUSION

Remission rate was 94.7% in patients of childhood ALL with hyperdiploidy. Patients of ALL with hyperdiploidy had a higher CHR rates as compared to patients of ALL without hyperdiploidy

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any authors.

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