RESEARCH ARTICLE

Frequency of Chromosomal Abnormalities in Pakistani Adults with Acute Lymphoblastic Leukemia

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Abstract

Background: The difference in prognosis of adult and childhood acute lymphoblastic leukemia (ALL) can be attributed largely to variation in cytogenetic abnormalities with age groups. Cytogenetic analysis in acute leukemia is now routinely used to assist patient management, particularly in terms of diagnosis, disease monitoring, prognosis and risk stratification. Knowing about cytogenetic profile at the time of diagnosis is important in order to take critical decisions in management of the patients. Aim and Objectives: To determine the frequency of cytogenetic abnormalities in Pakistani adult patients with ALL in order to have insights regarding behavior of the disease. Materials and Methods: A retrospective analysis of all the cases of ALL (≥15years old) diagnosed at Aga Khan University from January 2006 to June 2014 was performed. Phenotype (B/T lineage) was confirmed in all cases by flow cytometry. Cytogenetic analysis was made for all cases using the trypsin-Giemsa banding technique. Karyotypes were interpreted using the International System for Human Cytogenetic Nomenclature (ISCN) criteria. Results: A total of 166 patients were diagnosed as ALL during the study period, of which 151 samples successfully yielded metaphase chromosomes. The male to female ratio was 3.4:1. The majority (n=120, 72.3%) had a B-cell phenotype. A normal karyotype was present in 51% (n=77) of the cases whereas 49% (n=74) had an abnormal karyotype. Of the abnormal cases, 10% showed Philadelphia chromosome; t(9;22)(q34;q11.2). Other poor prognostic cytogenetic subgroups were t(4;11)(q21;q23), hypodiploidy (35-45 chromosomes) and complex karyotype. Hyperdiploidy (47-57 chromosomes) occurred in 6.6%; all of whom were younger than 30 years. Conclusions: This study showed a relatively low prevalence of Philadelphia chromosome in Pakistani adults with ALL with an increase in frequency with age (p=0.003). The cumulative prevalence of Philadelphia-negative poor cytogenetic aberrations in different age groups was not significant (p=0.6).

Keywords: ALL - cytogenetics - G-banding - metaphase - adult - Pakistan

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Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by clonal expansion and accumulation of hematopoietic blasts of lymphoid lineage. Incidence of ALL declines with age nonetheless, its prognosis in adults has always been inferior to children (Faderl et al., 2010; Jinnai et al., 2010). Several age dependent biological and host factors have been described (Jiang et al., 2013; Wang et al., 2014) including, differences in sensitivities of leukemic cells to corticosteroids, metabolism of chemotherapy (Kaspers et al., 1995), depleted marrow reserve and increased extramedullary toxicity (Mishra et al., 2006; Ribera and Oriol, 2009). However, the most important differences lie in cytogenetic and molecular characteristics. Distinct cytogenetic abnormalities have been found associated frequently with immunologic phenotypes of ALL and characteristic outcomes (Moorman et al., 2010a). Amongst these recurring cytogenetic abnormalities, t(9;22) (q34;q11.2) or Philadelphia chromosome accounts for about 25% of ALL and is associated with resistant disease even after intensive chemotherapy (Lee et al., 2011). Non-Philadelphia high risk cytogenetic subgroups include t(v;11q23); hypodiploidy (35-45 chromosomes) and complex karyotypes (Gómez-Seguí I et al., 2012). Hyperdiploidy (47-57 chromosomes) and t(12;21)(p13;q22) carry rather better prognosis with good response to treatment and lower risk of relapse (Kaspers et al., 1995). Several studies have demonstrated increase in the frequency of poor cytogenetic risk groups and decrease in the frequency of good cytogenetic risk groups with increasing age (Moorman et al., 2010a; Moorman et al., 2010b).

The 2008 World Health Organization classification is based on specific selection of those cytogenetic lesions which are associated with distinctive clinical or phenotypic properties, have important prognostic implications, demonstrate evidence that they are biologically distinct

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and are generally mutually exclusive with other entities (Vardiman et al., 2009).

Cytogenetic analysis is now routinely used in pediatric setting to assist patient management, particularly in terms of diagnosis, disease monitoring, prognosis, and risk stratification. If available at the time of diagnosis, cytogenetic analysis in adult patients with ALL can provide clear answers to physicians and hence can aid in taking critical decisions for appropriate management (Pullarkat et al., 2008). The cytogenetic data of Pakistani adults with ALL is scarce. Literature search retrieved a single cytogenetic study in patients with ALL from our country comprising of 69 subjects only (Khalid et al., 2007). The current study aimed in determining the frequency of chromosomal abnormalities in Pakistani adult patients with ALL in order to have an insight about the behavior of this condition.

Materials and Methods

Study area and subjects

This was a retrospective analysis performed at Aga Khan University Hospital in the department of hematology. All patients diagnosed as ALL who were ≥15 years of age from January 2006 to June 2014 were included in the analysis. All cases of acute myeloid leukemia, undifferentiated leukemia and Burkitt lymphoma/leukemia (ALL L3 according to French-American-British (FAB) classification) were excluded.

Diagnosis

In all cases, the diagnosis was confirmed by morphology, appropriate cytochemical staining and immunophenotyping by flow cytometry. Flow cytometry was performed on FACScan (Becton Dickinson, Miami Lakes, FL, USA) before September 2007 and on FC500 MCL Flow Cytometer (Beckman Coulter, Miami, FL, USA) afterward. Monoclonal antibodies against following antigens were used: T-lineage-associated antigens CD3, CD5, CD7; B-lineage antigens CD19, CD20, CD79a, CD22; and antigens TdT and CD10.

Cytogenetic analysis

Analysis was performed on pretreatment bone marrow samples by the use of conventional G-banding techniques. Bone marrow samples were cultured using standard culture techniques followed by harvesting (incubation, centrifugation and addition of hypotonic solution). After addition of fixative (3:1 methanol to glacial acetic acid) and trypsin treatment, Giemsa staining was performed. Slides were examined under microscope and at least 20 mitosis were analyzed whenever possible.

Data handling

Chromosomal abnormalities were identified and described according to the International System for Human Cytogenetic Nomenclature (ISCN 2005, 2009). Age, gender and types of cytogenetic abnormalities were included for analysis and results were expressed as frequencies. Categorical variables were compared by the use of the Chi-square test or Fisher Exact test. Significance of mean age between two groups was calculated by Independent-Samples T-Test.

Ethical issues

An ethical exemption to conduct this analysis was granted by the institutional ethical review board (2888-Med-ERC-14). Written and informed consent was taken from all patients as per institutional policy before collecting bone marrow samples. Relevant counseling regarding prognostic impact of the detected abnormality was provided to all who followed up in outpatient department or in the wards.

Results

A total of 166 adults aged 15 years or older were diagnosed with ALL during the study period. Overall there were more male than female subjects (M:F 3.4:1), and this ratio did not vary substantially with age or B/T phenotype (Table 1). The majority of patients had B-lineage ALL (n=120, 72.3%), whereas 45 (27.1%) had T-ALL and 1 (0.6%) patient was described as biphenotypic (B/T) ALL. The patients with T-ALL were younger with a median age of 22.2±6.4 (Range 15-41 years) versus 28.6±13.8 (Range 16-77 years) in B-ALL (p=0.003). All 6 patients older than 60 years had B-cell phenotype.

Cytogenetic analysis: A total of 151 (91%) patients had a successful cytogenetic result. No metaphase chromosome was yielded in 15 (9%). Cytogenetics revealed a normal karyotype in 77 (51%) patients (Table 2). Fifty seven of 120 (47.5%) with B-ALL and 20 of 45 (44.4%) patients with T-ALL had normal karyotype. The single case with combined B and T phenotype had unsuccessful cytogenetic result.

Translocations

The most prevalent specific chromosomal abnormality was the Philadelphia chromosome, t(9;22)(q34;q11.2), which was present in 15 (10%) of 151 patients. Its prevalence in different age groups was: 15 to 19 years, 1 (1.9%) of 53; 20 to 29 years, 5 (9%) of 55; 30 to 39 years 4 (23.5%) of 17; 40 to 49 years, 2 (16.7%) of 12; 50 to 59 years 2 (25%) of 8; 60 to 69 years, 0 of 5; 70 to 79 years, 1 of 1. Translocation (4;11)(q21;q23) occurred in 3 patients. All patients with t(9;22)(q34;q11.2) and t(4;11)(q21;q23) had B-cell phenotype. Other established translocations such as, t(12;21)(p13;q22), t(5;14)(q31;q32) and t(1;19)(q23;p13.3) were not detected in any patient.

Aneuploidies

Overall aneuploidies were detected in 17 (11.2%)

Table 1. Demographic Features of 166 Adults with ALL

	Age in years			Total
	15-29	30-59	≥60	
No. of Patients	121	39	6	166
Sex ratio M:F	3.5:1	3.3:1	2:1	3.4:1
Immunophenotype n(%)				
B-ALL	82 (67.8)	32 (82)	6 (100)	120 (72.3)
T-ALL	38 (31.4)	7 (18)	0	45 (27.1)
Biphenotypic	1 (0.8)	0	0	1 (0.6)

Table 2. Cytogenetic Features of 151 Adults with ALL

		Age in years			
		15-29	30-59	≥60	Total
Cytogenetic Investigations n (%)	Successful	108/121 (89)	37/39 (95)	6/6 (100)	151/166 (91)
	Normal	55 (51)	20 (54)	2 (33.3)	77 (51)
	Abnormal	53 (49)	17 (46)	4 (66.6)	74 (49)
Chromosomal abnormality n (%)	t(9;22)(q34;q11.2)	6 (5.5)	8 (21.6)	1 (16.6)	15 (10)
	t(4;11)(q21;q23)	1 (0.9)	2 (5.4)	0	3 (2)
	Hyperdiploidy (47-57 chromosomes)	10 (9.2)	0	0	10 (6.6)
	Hypodiploidy (35-45 chromosomes)	1 (0.9)	0	0	1 (0.6)
	Near-triploidy (58-80 chromosomes)	2 (1.8)	0	0	2 (1.3)
	Near-tetraploidy (81-103 chromosomes)	4 (3.7)	0	0	4 (2.6)
	del(6)(q23q27)	3 (2.7)	0	0	3 (2)
	del(12)(p13)	2 (1.8)	0	1 (16.6)	3 (2)
	del(9)(p13)	1 (0.9)	1 (2.7)	0	2 (1.3)
	Complex*	6 (5.5)	3 (8.1)	2 (33.3)	11 (7.2)
	Others	17 (15.7)	3 (8.1)	0	20 (13.2)

Numbers and percentages presented are out of total cases with successful cytogenetic results in each age group (108 in 15-29 years, 37 in 30-59 years and 6 in ≥60 years); *Three or more clonal chromosomal abnormalities in the absence of aforementioned established chromosomal abnormalities

patients (Table 2). All patients with aneuploidies were younger than 30 years without significant difference in between 15-19 year (n=8, 47%) and 20-29 year (n=9, 53%) age groups (p=1.0). The sub categories included: hyperdiploidy (47-57 chromosomes) in 10 (9 B-cell, 1 T-cell), near-triploidy (58-80 chromosomes) in 2 (Both B-cell), near-tetraploidy (81-103 chromosomes) in 4 (3 B-cell, 1 T-cell) and hypodiploidy (35-45 chromosomes) in 1 (T-cell).

Other abnormalities

Other chromosomal abnormalities comprised of del(6)(q23q27) in 3, del (12)(p13) in 3, del(9)(p13) in 2 and complex karyotype in 11 (Table 2). Complex karyotype was defined as presence of three or more clonal chromosomal abnormalities in the absence of aforementioned established chromosomal abnormalities. Twenty patients had miscellaneous chromosomal abnormalities including deletions, additions, inversions, other translocations and marker chromosomes.

Discussion

To the best of our knowledge, we have reported the largest cytogenetic data in Pakistani adults with ALL. Several important facts were noticed. The mean age of study population was 27±12.5 with male:female ratio of 3.4:1. The bimodal age distribution of ALL was not observed in this study as its prevalence continued to decline with an increase in age. The gender distribution in different age groups also did not vary considerably (Table 1). Of successful cytogenetic results, recurrent chromosomal abnormalities were observed in 35 (23%) patients. The normal and miscellaneous cytogenetic groups collectively comprised of 116 (77%) cases.

The prevalence of Philadelphia chromosome, t(9;22)(q34;q11.2) was 10% in this study whereas, its prevalence varying from 15-30% has been described in several other studies (Pullarkat et al., 2008; Moorman et al., 2010a). In this study its prevalence increased with age till the fourth decade (p=0.003). Similar findings have been described in other studies (Moorman et al., 2010a). Thus, although there was a significant difference in the incidence of t(9;22)(q34;q11.2) between ages 15 and 30 years (5.5%) and those older than 30 years of age (21%; p=0.01), there was no difference between those ages 30 to 60 years (21.6%) and older than 60 years of age (16.6%; p=1.0).

Although the incidence of t(12;2 1)(p13;q22) in ALL declines with age, none of the patient in this study had this abnormality. The lower frequency of this abnormality in Pakistani children has also been documented in literature (Shaikh et al., 2014). Hyperdiploidy (47-57 chromosomes) which carry good prognosis was observed in 9 patients with B-cell phenotype. Its occurrence in younger age group (15-29 years) in this study is in accordance with the literature (Faderl et al., 2010; Chilton et al., 2013). The difference between 15-19 year and 20-29 year age group was not significant (p=1.0). Three patients with T-cell phenotype individually had hyperdiploidy, hypodiploidy and near-tetraploidy. The rare occurrence of these anomalies in T-ALL has been described in the literature (Healey et al., 2009; Garcia et al., 2011).

The collective prevalence of Philadelphia-negative patients with high-risk cytogenetics, defined as t(4;11)(q21;q23), hypodiploidy, and complex karyotype was not significant among different age groups (p=0.6). Twenty patients had miscellaneous chromosomal abnormalities but were too infrequent to be analyzed separately.

Besides being the largest cytogenetic data in Pakistani adults will ALL, other strengths of this study include the confirmed lineage specific (B/T phenotype) diagnosis of all cases by flow cytometry and use of conventional cytogenetic method for karyotype determination. Conventional cytogenetic provides status of all chromosomes and hence, it identifies all the changes present in karyotype. Nonetheless, a fraction of cases are liable to omission due to its inherent low sensitivity. More sophisticated methods like fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR) have higher yields but target only specific lesion in question and therefore, information about other possible findings is not provided.

In conclusion, this study showed recurrent cytogenetic abnormalities in 23% Pakistani adults with ALL. Despite relatively low prevalence (10%) of t(9;22)(q34;q11.2), it emerged as the most common specific chromosomal

abnormality with an increase in frequency with age. The cumulative prevalence of non-Philadelphia poor cytogenetic abnormalities however, did not vary significantly with age.

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