RESEARCH ARTICLE

Chromosomal Abnormalities in Pakistani Children with Acute Lymphoblastic Leukemia

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Abstract

__Background__: Cytogenetic abnormalities have important implications in diagnosis and prognosis of acute leukemia and are now considered an important part of the diagnostic workup at presentation. Karyotype, if known at the time of diagnosis, guides physicians to plan appropriate management strategies for their patients. __Aim and Objectives__: To determine the cytogenetic profile of acute lymphoblastic leukemia (ALL) in Pakistani children 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 2011 was performed. 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 153 patients were diagnosed as ALL during the study period, of which 127 samples successfully yielded metaphase chromosomes. The male to female ratio was 1.8:1. A normal karyotype was present in 51.2% (n=65) of the cases whereas 48.8% (n=62) had an abnormal karyotype. Most of the abnormal cases showed hyperdiploidy(13.4%) followed by t(9;22)(q34;q11.2) (7.08%). __Conclusions__: This study revealed a relative lack of good prognostic cytogenetic aberrations in Pakistani children with ALL.

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

Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by accumulation of lymphoblasts. It accounts for 75-80% of childhood leukemias and various subtypes of the disease can be defined based on cell morphology, immunophenotype, karyotype and gene expression characteristics. Over the past several years, diagnosis and treatment of ALL in children has improved significantly and approximately 80% of children with ALL now survive into adulthood (Vrooman and Silverman, 2009; Aburn and Gott, 2011; Tharnprisan et al., 2013).

Cytogenetic analysis in hematological malignancies like many other diseases, plays a significant role in understanding the pathophysiology as well as clinical behavior of the condition (Mazloumi et al., 2012; Gil et al., 2013). In fact, for acute lymphoblastic leukemia, like other malignant conditions, karyotype is one of the prognostic indicators (Borowitz et al., 2008; Iacobucci et al., 2012). Other important prognostic indicators in ALL include age (good prognosis in 1-9 years) (Hilden et al. 2006; Tharnprisan et al., 2013), gender (better prognosis in girls) (Pieters and Carroll, 2008), white blood cell count (Landau and Lamanna, 2006) (good prognosis if <50x 10^9/L at presentation), immunophenotype and minimal residual disease (MRD) detection (Basso et al., 2009)

(high relapse risk with MRD of 1% or more at the end of remission induction therapy and those with MRD of 0.1% or more during continuation therapy). Numerous cytogenetic abnormalities have been found associated frequently with distinct immunologic phenotypes of ALL and characteristic outcomes (Pui et al., 2008; Harrison, 2009; Vrooman and Silverman, 2009). Both structural and numerical chromosomal abnormalities are detected recurrently in approximately 80 percent of ALL (Harrison et al., 2005; Moorman et al., 2010). There are considerable differences in types of cytogenetic abnormalities detected in different age groups. For instance, t(9;22) is detected more commonly in adults (Harrison et al., 2005; Moorman et al., 2010) as compared to children. Whereas, t(4;11), t(12;21) and hyperdiploidies are more common in children (Harrison et al., 2005; Hilden et al., 2006; Moorman et al., 2010).

These cytogenetic abnormalities also differ in overall prognosis of the disease including response to chemotherapy and subsequent chances of relapse. For example, certain translocations, such as t(4;11) and t(9;22), are associated with resistant disease and may require intensive chemotherapy (Aricó et al., 2010). In comparison, the t(12;21) (Forestier et al., 2008; Pieters and Carroll, 2008), t(1;19), and hyperdiploidy (47 to 57 chromosomes) are associated with encouraging outcomes

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(Harrison et al., 2005; Pieters and Carroll, 2008).

Cytogenetic data of Pakistani children with ALL is unavailable. Therefore, this study aimed in determining the cytogenetic profile of Pakistani children with ALL in order to provide an insight into the prognosis and hence proper management of these patients.

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 2011 were included in the analysis. All cases of acute myeloid leukemia and undifferentiated leukemia were excluded.

Diagnosis

In all cases, the diagnosis was confirmed by morphology and appropriate cytochemical staining. Immunophenotyping by either immunohistochemistry or by flow cytometry was performed where possible by the use of standard methodologies.

Cytogenetic analysis

Metaphase chromosome banding was performed using conventional Giemsa banding (G banding) technique. 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.

Ethical issues

An ethical exemption to conduct this analysis was granted by the institutional ethical review committee. Written and informed consent was taken from parent/ guardian of all children 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 153 children younger than 15 years were diagnosed with ALL during the study period. There were more male than female (M:F 1.8:1). Cytogenetic analysis couldn't be performed in n=26 (16.9%) cases either due to inadequacy of the sample or no metaphases were yielded on bone marrow culture. Out of total (n=127) successfully

Table 1. Age, Sex and Cytogenetic Features of the Study Population

Total number of patients	153
Age (Mean±S.D)	7.05±4.4
Sex ratio, M:F	1.8:1
Number of cases cancelled	26 (16.9%)
Normal karyotype*	65 (51.2%)
Abnormal karyotype*	62 (48.8%)
Aneuploidies*	24 (18.9%)
Translocations*	18 (14.2%)
Additions*	10 (7.87%)
Deletions*	6 (4.72%)
Derivatives*	4 (3.1%)

*Numbers and percentages presented are out of total successful 127 cases

completed samples, 51.2% (n=65) had a normal karyotype whereas, in 48.8% (n=62) cases, various cytogenetic abnormalities were detected. Both the numerical and structural chromosomal abnormalities were detected; aneuploidies and translocations being the commonest. Table 1 shows the age, sex and cytogenetic features of 153 patients with ALL.

Aneuploidies

Hyperdiploidy (47-57 chromosomes) was the commonest chromosomal abnormality identified in this study (n=17, 13.4%). Near-triploidy (58-80 chromosomes) and near-tetraploidy (81-103 chromosome) was detected in five (3.9%) and one patients (0.8%) respectively. There was a single case with 28 chromosomes (hyperhaploidy, 24-34 chromosomes).

Translocations

Nine (7.1%) patients were identified to harbor t(9;22)(q34;q11.2) whereas, two (1.6%) patients had t(1;19)(q25;p13.3). Seven other translocations which were present in seven different patients included t(1;7) (p34.3;q36), t(2;6)(q21.3;q27), t(2;9)(q33;p13), t(5;9) (q13;p24), t(2;14)(p11.2;q32), t(9;14)(p24;q11.2) and t(9;17)(p12;q11.2).

Other abnormalities

Other chromosomal abnormalities like duplications, additions, deletions and derivatives were identified in different patients (Table 1).

Discussion

This study underscored several important facts regarding ALL in Pakistani children. Literature search revealed that, it is the largest study detailing cytogenetic profile of Pakistani children with ALL. The mean age of study population was 7±4.4 which is comparable to other reported literature (Yasmeen and Ashraf, 2009). Although ALL is more common in boys than girls; male to female ratio was found to be 1.8:1 in this study. Yasmeen and Ashraf (2009) reported male to female ratio of 1.7:1 in Pakistani children. These figures indicate that incidence of ALL is almost double in Pakistani boys than in girls. On the other hand, this figure may unfortunately reflect male predominance in our society; males are given

more importance than females and hence, females are kept deprived of medical facilities mainly due to cost constraints.

Overall, both numerical and structural cytogenetic abnormalities were detected in 48.8% (n=62) of patients. In our study, hyperdiploidy (47-57 chromosomes) was detected in 13.4% of patients (n=17) whereas usual prevalence of this abnormality as reported in literature is around 25% (Pui et al., 2008). Surprisingly, t(12;21)(p13;q22) which is the commonest translocation in children with ALL and carries good prognosis was not found in Pakistani population. Prevalence of t(9;22)(q34;q11.2) ranges from 3-5% in pediatric ALL (Schultz et al., 2009), however, it emerged as the commonest translocation in Pakistani population (7.08%).

Besides being the largest cytogenetic study in Pakistani children with ALL, another strength of our study is use of conventional cytogenetic method for karyotype determination. One advantage of this method is that, it provides status of all chromosomes and hence, it identifies all the changes present in karyotype. However, due to its inherent low sensitivity as compared to more sophisticated methods like fluorescence in situ hybridization (FISH) and polymerase chain reaction (PCR), there is always a chance of missing some abnormalities. FISH and PCR target only specific lesion in question and therefore, information about other possible findings is not provided.

We could not compare our findings with immunophenotype (B or T lineage) of ALL however, specific cytogenetic abnormality when present, independently provides strong predictions as far as the prognosis of the disease is concerned. The relevance of gene set analysis also remains unclear (Soheila et al., 2013).

In conclusion, this study shows relative lack of good prognostic cytogenetic abnormalities like t(12;21)(p13;q22) and hyperdiploidy (47-57 chromosomes) in Pakistani children with ALL. Prevalence of poor prognostic cytogenetic aberrations like t(9;22)(q34;q11.2) is comparable to available international literature.

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