RESEARCH ARTICLE

CYTOGENETICS FINDINGS OF PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN WEST INDIAN REGION.

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Manuscript Info

Abstract

Acute lymphoblastic leukemia (ALL) is one of the most common malignancies in India. Detection of chromosomal abnormalities by conventional karyotyping and Fluorescent in situ hybridization (FISH) plays a very important role in classification and treatment of ALL patients. The aim of this study was to define the frequency and types of chromosomal aberrations in B-ALL patients of West India. In this study, we analyzed bone marrow samples of 35 (18 adults and 17 childhood) West Indian patients with B-ALL using the conventional G-banding and FISH technique. Total 24 patients (68.57%) showed chromosomal alterations, including numerical and/or structural abnormalities. Hyperdiploidy was most common (11.43%) numerical abnormality while the most common fusion oncogenes were BCR-ABL1/t(9;22) in adults (33.33%) and ETV-RUNX1/t(12;21) in children (23.53%). The incidence of 11q23 and t(1;19) were also high than those reported in the previous studies. There was a difference in the prevalence of abnormalities in the current study, maybe due to the geographical variation and/or small sample size.

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Introduction:

Acute lymphoblastic leukemia (ALL) is a clonal proliferation of lymphoid precursor cells in the bone marrow. ALL is the most common malignancy diagnosed in children, accounting for one-fourth of all childhood cancers. In India, the incidence of childhood cancer ranges from 38 to 124 per million children per year, where 60 to 85% of all leukemia reported are ALL (Arora et al., 2009).

ALL is a genetic disease because most patients harbor acquired genetic mutations that results in increased proliferation, prolonged survival and/or impaired differentiation of lymphoid progenitor cells. In majority of the patients with ALL the genetic alterations are present in the form of numerical and/or structural chromosomal aberrations (Mrozek et al., 2009). The chromosomal aberrations play a major role in the prognosis and treatment outcome of ALL (Sabir et al., 2012), hence detection of these abnormalities is necessary. The detection can be done by conventional cytogenetic method like karyotyping and molecular cytogenetic techniques like Fluorescent in-situ hybridization (FISH). These methods are helpful in assessing the classification, risk stratification and prediction of outcome of ALL patients (Goud et al., 2015).

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Conventional G-banding is commonly used to detect chromosomal aberrations (Chang et al., 2006). However, this method cannot be used for identification of certain translocations like t(12;21)/ETV-RUNX1, which requires FISH for detection (Spathas et al., 1999). Hence, chromosomal analysis with FISH is more useful in diagnosis of certain cryptic rearrangements (Nordgren et al., 2002). In this study interphase-FISH was used as a complementary method along with G-banding for some ALL patients to confirm cytogenetic findings and for the poor quality samples or slides with no metaphases. The combination of both the method can improve the detection of genetic abnormalities (Goud et al., 2015).

The distribution of subtypes of ALL varies among population, maybe due to racial, geographical and genetic variations (Perez-Vera et al., 2001). The prevalence of ALL is more in male to that of the female and the male to female ratio in most resource rich countries is around 1.2:1 (Arora et al., 2009). Also, there is significant difference between the childhood and adult ALL as indicated by the prevalence of various chromosomal aberrations and other factors (Sabir et al., 2012). Hence, Marwaha and Kulkarni (2011) have emphasized on accurate estimation of incidence and prevalence of ALL in India, which would help to estimate the disease burden, its impact on population and to develop effective treatment strategies (Siddaiahgari et al., 2015).

Many studies have been conducted on ALL, based on the cytogenetic findings of the patients with ALL (Safaei et al., 2013; Siddaiahgari et al., 2015), FISH analysis of childhood and adult ALL patients (Goud et al., 2015) and Epidemiology of childhood cancers in India (Arora et al., 2009). Also, studies based on significant fusion oncogenes in patients with ALL have been conducted (Sabir et al., 2012).

In the current study, we present the cytogenetic findings of 35 B-ALL patients and compare the distribution of numerical abnormalities and four most commonly occurring fusion oncogenes between children and adults. The aim of this study is to define the frequency and types of chromosomal translocations and fusion oncogenes in ALL patients using G-banding and FISH technique and compare our findings with the data reported in the previous studies.

Materials and methods:

Patients:
35 patients of both gender, aged between 5 months and 76 years, suspected of B-lineage ALL, were included in this study. The study was conducted between 2015 and 2017 and patients were analyzed by conventional cytogenetic and molecular analysis technique in our centre.

Cytogenetic analysis:
The bone marrow samples were cultured in 5ml of MarrowMax Media (Gibco-Invitrogen-USA) at 37°C for 12-14 hours. After incubation, the cells were treated with 200µl of colchicine (Sigma-Aldrich, Germany) for 1 hour to arrest the cells at metaphase stage followed by harvesting the cells with hypotonic solution (0.075 M KCL) at 37°C for 20 minutes. Then, the cells were fixed using 3-4ml of Carnoy’s fixative (methanol: glacial acetic acid 3:1) at 4°C for 2 hours. Fixed cell suspension was dropped over pre-chilled slides and slides were aged overnight at 64°C followed by staining using the standard G-banding technique (Seabright, 1971). At least 20 metaphases were analyzed using Zeiss Axio Imager Z2 microscope and image was captured using Ikaros karyotyping software (MetaSystems, Germany). Chromosome identification and karyotype designation were made according to International System of Human Cytogenetic Nomenclature (ISCN, 2009).

Fluorescent in-situ hybridization (FISH) analysis:
In this method, the cells were directly harvested with hypotonic solution (0.075 M KCL) at 37°C for 20 minutes. It was followed by fixation and slide preparation similar to cytogenetic protocol. Then, 10µl of dual colour LSI/CEP probes (Vysis) were applied on the selected areas of slide and the slides were hybridized overnight at 37°C. After hybridization, the cells were counterstained with DAPI (4, 6-diamidino 2-phenylindole) and at least 100 interphase nuclei were examined under Zeiss Axio Imager Z2 with appropriate dual filters. The images were captured using the Isis Software (MetaSystems, Germany).

Results:
In the present study, we analyzed 35 patients suspected of having B-lineage ALL comprising of 25 (71.42%) males and 10 (28.58%) females (M:F ratio= 2.5:1). Children accounted for 17 cases (49%) at mean age of 6.08 years (aged 05 months to 14 years) and adults comprised of 18 cases (51%) at mean age of 34.1 years (aged 15 to 76 years). Of
the 35 patients, 30 (85.71%) showed successful cultures. Total 24 patients (68.57%) showed chromosomal alterations while 11 (1.43%) displayed normal karyotype.

**Numerical chromosomal abnormalities:**
Hyperdiploidy was seen in 11.43% (4/35) patients, while high hyperdiploidy i.e. 51 to 67 chromosomes (Fig.1) was observed in 5.55% (1/18) adults and 5.88% (1/17) children. Hypodiploid karyotype (35-45 chromosomes) was seen in 11.11% (2/18) of adult ALL patients and no hypodiploidy was observed in children (Table 1).

**Structural chromosomal abnormalities:**
The figures 2A and 3A represents normal cells for BCR-ABL fusion and MLL rearrangement respectively. We found that 65.70% (23/35) of B-ALL patients harbored one or more of the fusion oncogenes, with the distribution of BCR-ABL1 (Fig.2B) in 33.33% (6/18) adults and 11.76% (2/17) children, ETV-RUNX1 (TEL-AML1) in 16.66% (3/18) adults and 23.53% (4/17) children, TCF-(E2A) PBX1 in 16.66% (3/18) adults and 11.76% (2/17) children, 11q23 (MLL) rearrangement (Fig.3B) in 22.22% (4/18) adults and 29.41% (5/17) children. Two patients showed 6q deletion (Fig.1). Other complex changes observed were deletion 1p (Fig.1), deletion 11.2, der(22), der(9;22), t(2;5), t(2;14), t(10;11) and t(12;13) in one patient each (Table 1).

**Discussion:-**
In the present study, we determine the frequency and types of chromosomal aberrations in 35 patients (17 children and 18 adults), suspected of having B-ALL and compare our findings with other relevant reports. We used conventional karyotyping and FISH techniques to detect the numerical abnormalities such as hypodiploidy and hyperdiploidy and the prevalence of four most common oncogenes i.e. BCR-ABL/t(9;22), ETV-RUNX1/t(12;21), TCF3-PBX1/t(1;19) and 11q23 rearrangement in the patients with B-ALL.

In this study, the bone marrow culture of 14.28% (5/35) cases yielded no metaphase or the quality of chromosome was too poor to be analyzed. This result is comparable to the findings in the earlier studies (Safaei et al., 2013). The incidence of normal karyotype in the previous studies is reported as 48.32% (Siddaiahgari et al., 2015) and 38.30% (Safaei et al., 2013) while it was 31.42% in our study maybe, due to the improvement in the culture techniques and introduction of interphase FISH technique, which led to higher detection of chromosomal abnormalities as in many other studies which used FISH (Goud et al., 2015).

In previous studies, hyperdiploidy (47 to >65 chromosomes) was seen in 32.80% (Safaei et al., 2013), 14.20% (Mazloumi et al., 2012) and 10.11% (Siddaiahgari et al., 2015) whereas, it was 11.43% (4/35) in our study. High hyperdiploidy (51 to 67 chromosomes) has higher incidence in children than in adults (25-30% vs. 2-10%) (Mrozek et al., 2009), while in our study it was 5.55% (1/18) in adults and only 5.88% (1/17) in children. The prevalence of hypodiploidy is equal among childhood and adult cases, at 5-6% (Secker-Walker et al., 1997), but it is high in Indian pediatric population i.e. 37.50% (Settin et al., 2007) and 10.34% (Ahmad et al., 2008). Present study showed hypodiploidy in 11.11% (2/18) of adults but no hypodiploidy was observed in children.

In our study, we found that 65.7% (23/35) of suspected ALL patients having one of the four fusion oncogenes. According to the previous studies, t(9;22)(q34;q11.2)/BCR-ABL1 generally found in 11-29% adults (Secker-Walker et al., 1997; Pullarkat et al., 2008), while it is relatively rare in children at 1-5% (Moorman et al., 2010). Similar results were observed in our study as BCR-ABL1 was detected in 33.3% (6/18) and 11.7% (2/17) children. In our study, t(12;21)(p13;q22)/ETV-RUNX1 (TEL-AML1) was observed in 23.53% (4/17) children, which is comparable to the earlier reported studies (25-30%) (Forestier et al., 2008). In the frequency of some of these fusion oncogenes, there were significant differences as compared to the previous reports. For example, ETV-RUNX1 is found in only 3% adults with B-ALL according to other studies (Sabir et al., 2012) but it was detected in 16.6% (3/18) of adults in our study indicating higher prevalence of this rearrangement in the West Indian population.

In addition, translocation (1;19)(q23;p13.3)/TCF3(E2A)-PBX1 was found in 16.66% (3/18) adults and 11.76% (2/17) children in our study, again much more common than the previously reported studies (Mancini et al., 2005). The 11q23 MLL rearrangement is majorly detected in infants less than 6 months old, the incidence in older children and adults is much lower i.e. 1.5% and 5-10% respectively (Marchesi et al., 2011), but in our study its occurrence was high at 25% (4/16) in older children (1 to 14 years) and 22.22% (4/18) in overall adults.

With respect to translocations such as t(2;5), t(2;14), t(10;11), t(12;13) were also observed in our study but no similar reports were found in the previous studies.
The study concludes that, there is difference in the prevalence of numerical abnormalities and the fusion genes in the studied population, which could be due to the geographical variations and/or other unknown factors. The larger sample size could throw better light in this respect.

### Table 1: Karyotypes and FISH results of patients suspected of B-ALL

| Sr. No. | Age | Sex  | Karyotype                                                                 | FISH probes          | FISH probes |
|---------|-----|------|----------------------------------------------------------------------------|----------------------|-------------|
| 1.      | 05  | Male | 46, XY                                                                    | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Positive |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Negative |
| 2.      | 17  | Female | 46, XY                                                                 | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Negative |
| 3.      | 08  | Male | 46, XY                                                                    | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Positive |
|         |     |      |                                                                           | 3. MLL               | 3. Positive |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Positive |
| 4.      | 20  | Male | No Karyotype                                                               | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Positive |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Negative |
| 5.      | 11  | Male | 55-61, XY, +del(1p), +4, del(6q), +8, +10, +11, +12, +14, +17, +18, +21, +21, +22 | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Positive |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Positive |
| 6.      | 52  | Male | 46, XY                                                                    | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Positive |
| 7.      | 01  | Male | 46, XY                                                                    | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Positive |
| 8.      | 24  | Male | 46, XY                                                                    | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Positive |
| 9.      | 18  | Male | 46, XY                                                                    | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Positive |
| 10.     | 11  | Female | 46, XX                                                                   | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Positive |
| 11.     | 17  | Male | 55, XY, t(9;22)(q34;q11.2), +15, +17, +21, der(22), del(q11.2), +5 mar | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Negative |
| 12.     | 03  | Female | 46, XX                                                                   | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Negative |
| 13.     | 47  | Male | 45, X-Y, t(9;22)(q34;q11.2) /46, XY, t(9;22)(q34;q11.2)                  | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Negative |
| 14.     | 33  | Male | 48, XY, +6, +8, t(9;22)(q34;q11.2) / 47, XY, +6, t(9;22)(q34;q11.2)       | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Negative |
| 15.     | 01  | Male | 46, XY                                                                    | 1. BCR-ABL           | 1. Positive |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
| 16.     | 47  | Male | 45, XY, t(2;14)(q23;q12), der(9;12)(q24;q12), -12 / 46,XY                | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
|         |     |      |                                                                           | 3. MLL               | 3. Negative |
|         |     |      |                                                                           | 4. TEL-AML           | 4. Positive |
| 17.     | 09  | Male | 46, XXY, t(4;11)(q12;q14), +del(6q), -17                                  | 1. BCR-ABL           | 1. Negative |
|         |     |      |                                                                           | 2. E2A               | 2. Negative |
| Case | Age | Gender | Karyotype | Cytogenetic Findings |
|------|-----|--------|-----------|---------------------|
| 18   | 55  | Female | 46, XX    |                     |
| 19   | 36  | Male   | 46, XY, t(4;11)(q21;q23) /46, XY | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Positive  
4. Negative |
| 20   | 03  | Male   | 46,XY     | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Positive  
4. Positive |
| 21   | 06  | Female | Multiple Anomalies with marker chromosome | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Positive  
2. Positive  
3. Negative  
4. Negative |
| 22   | 28  | Male   | 46, XY    | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Positive  
2. Positive  
3. Negative  
4. Negative |
| 23   | 03  | Male   | No Karyotype | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Negative  
4. Positive |
| 24   | 06  | Male   | 46, XY    | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Positive  
2. Positive  
3. Positive  
4. Negative |
| 25   | 14  | Male   | No Karyotype | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Positive  
2. Negative  
3. Positive  
4. Negative |
| 26   | 28  | Male   | 46, XY, t(9;22)(q34;q11.2) | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Positive  
2. Negative  
3. Positive  
4. Negative |
| 27   | 39  | Female | 46, XY    |                     |
| 28   | 05  | Female | 46, XX    | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Negative  
4. Positive |
| 29   | 30  | Female | No Karyotype | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Positive  
4. Negative |
| 30   | 76  | Female | 46, XX    |                     |
| 31   | 13  | Female | 46, XX    | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Negative  
4. Negative |
| 32   | 23  | Male   | 46, XX    |                     |
| 33   | 05  | Male   | 46, XY, t(10;11)(p12;23) | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Positive  
4. Negative |
| 34   | 24  | Male   | 46, XY, t(2;5)(q35;q35), t(9;11)(q34;q23), t(12;13)(q11;12), +21[13]/ 46,XY[07] | 1. BCR-ABL  
2. E2A  
3. MLL  
4. TEL-AML  
1. Negative  
2. Negative  
3. Positive  
4. Negative |
| 35   | 04  | Male   | 46, XY    |                     |
Fig.1: G-banded karyotype of a male ALL patient showing 55-61, XY, del(1p), +4, del(6q), +8, +10, +11, +12, +14, +17, +18, +21, +21, +22.

Fig.2: FISH analysis using LSI BCR/ABL dual colour probe (Vysis, Abbott Laboratories). A. Normal Cell: Two green and two orange signals representing the normal BCR/ABL loci. B. Abnormal Cell: One green, one orange, and one green-orange (yellow) fusion signal, indicating a fusion oncogene BCR-ABL.
Fig. 3: FISH analysis using a dual colour LSI MLL probe (Vysis, Abbott Laboratories). A. Normal Cell: Two green-orange fusion signals representing the two normal MLL loci. B. Abnormal Cell: One green, one orange, and one green-orange (yellow) fusion signal, indicating a chromosome break in the MLL locus.

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