Frequency and Type of Chromosomal Abnormalities in Childhood Acute Lymphoblastic Leukemia Patients in Kuwait: A Six-Year Retrospective Study

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Key Words
Childhood acute lymphoblastic leukemia · Genotype · Chromosome anomalies

Abstract
Objective: To characterize the frequency of genetic profiles in pediatric acute lymphoblastic leukemia (ALL) patients in Kuwait. Subjects and Methods: This review presents the general cytogenetic characteristics of 164 pediatric patients diagnosed as having ALL in a 6-year period. Chromosomal and fluorescence in situ hybridization studies were made on bone marrow aspirates at diagnosis and during different stages of the disease. Results: Recurring aberrations, observed in 123 (75%) patients, included hyperdiploidy (n = 68, 41%), tetraploidy (n = 12, 7.3%), hypodiploidy (n = 2, 1.2%), TEL-AML1 fusion (n = 11, 7%), mixed-lineage leukemia rearrangement (n = 6, 3.6%), t(9;22) (n = 4, 2.4%), t(1;19) (n = 3, 1.8%), t(8;14) or t(8;22) (n = 2, 1.2%), +21 (n = 2, 1.2%), del(6) (n = 2, 1.2%) and miscellaneous abnormalities (n = 9, 5%). The highest observed numerical chromosome abnormality was high hyperdiploidy in 89 patients (54%) with abnormal karyotype while the TEL-AML fusion was the highest observed structural abnormality. Conclusion: This study showed that clonal anomalies detected in pediatric ALL have shown correlations between specific abnormalities and clinicobiological characteristics of the patients.

Introduction
Acute lymphoblastic leukemia (ALL) is a malignant disorder of the bone marrow in which a lymphoid progenitor cell becomes genetically altered. It is the most common malignancy of childhood with an annual incidence rate of 3–4 cases per 100,000 children. Although there are few identified factors associated with an increased risk of developing ALL such as genetic, parental and environmental factors, the etiology of the disease remains largely unknown [1, 2]. The disease has a bimodal distribution: a sharp peak in incidence among children aged 2–5 years [3], and the incidence is substantially higher in white than black children [4–6].

Molecular genetic techniques as well as better cytogenetic techniques revealed that the disease is heterogeneous in its pathogenesis. Numerous genetic alterations have been and continue to be discovered in ALL, and it has been repeatedly shown that specific genetic abnormalities are present in the majority of successfully karyotyped patients with ALL [3–6]. This study presents the general cytogenetic characteristics of pediatric patients diagnosed as having ALL within a 6-year period.

Subjects and Methods
Between 2002 and 2007, diagnostic karyotypes of 164 pediatric ALL patients (median age 5.9 years, range 0.4–15) were investigated at Kuwait Cancer Control Center. Cytogenetic studies
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were performed on bone marrow aspirates on unstimulated direct and/or short-term cultures, using the G-banding technique. Whenever possible, 20–30 metaphases were studied, and karyotypes were described according to the International System of Human Cytogenetic Nomenclature [7]. To identify the cryptic t(12;21)(p13;q21), the locus-specific identifier dual-color TEL-AML1 probe (Vysis, Downers Grove, Ill., USA) was applied to 40 patients with B-cell ALL according to the manufacturer’s protocol. The event-free survival (EFS) was calculated from the date of diagnosis to the first of any event, or time from registration to last contact. Survival of cases without events was calculated from the date of diagnosis to the time of last contact.

**Results**

The incidence was higher in males (n = 104, 63%) than females (n = 60, 37%). Of the 164 ALL patients, 143 (87%) had B-cell and 21 (13%) T-cell leukemia. The median white blood cell (WBC) count for the study population was 31.7 × 10⁹/l (range 0.5–300) and the median hemoglobin level was 9.9 g/dl (range 2.7–13.9). The majority had (142, 86%) thrombocytopenia and presented with platelet counts less than 150 × 10⁹/l (table 1). Clonal chromosome abnormalities were detected at presentation in 123 (75%); 41 patients (25%); 29 males, 12 females; median age 6.4 years) had apparently normal karyotypes (fig. 1).

### Table 1. Characteristics of the study population

| Subgroup       | Cases, n (%) | Sex ratio (M/F) | Median age, years | Median WBC, × 10⁹/l | Median Hb, g/dl | Median platelets, × 10⁹/l | 3-year EFS, % |
|----------------|--------------|-----------------|------------------|---------------------|-----------------|---------------------------|---------------|
| Down syndrome  | 12 (7.3)     | 3               | 7.5              | 8.4                 | 6.5             | 62.7                      | 50            |
| B-cell ALL     | 143 (87)     | 1.5             | 6.2              | 40.7                | 7.4             | 46.9                      | 75            |
| T-cell ALL     | 21 (13)      | 6               | 7.7              | 78.1                | 11.2            | 75.3                      | 62            |
| Normal         | 41 (25)      | 1.5             | 6.4              | 48.6                | 9.6             | 60.7                      | 78            |
| Abnormal       | 123 (75)     | 1.6             | 6.2              | 26.3                | 10.0            | 62.3                      | 68            |
| Total          | 164 (100)    | 1.7             | 5.9              | 31.7                | 9.9             | 66.6                      | 72            |

Hyperploidy     | 68 (41.5)    | 1.5             | 4.6              | 15.4                | 9.7             | 58.1                      | 82            |

Tetraploidy     | 12 (7.3)     | 1               | 6.5              | 81.4                | 11              | 75.4                      | 60            |

Hypodiploidy    | 2 (1.2)      | 1               | 5.5              | 4.4                 | 8.4             | 57.0                      | 0             |

TEL/AML1        | 11 (6.7)     | 0.6             | 5                | 6.2                 | 6.3             | 35.5                      | 45            |

MLL/11q         | 6 (3.6)      | 2               | 7.5              | 166.6               | 7.2             | 24.4                      | 17            |

t(1;19)         | 3 (1.8)      | 2               | 7                | 7.8                 | 4.0             | 30.0                      | 0             |

Ph              | 4 (2.4)      | 4               | 11               | 30                  | 10.6            | 83.0                      | 0             |

c-myc/8q24      | 2 (1.2)      | 1               | 11               | 35.4                | 7.4             | 70.5                      | 0             |

Others          | 15 (9.1)     | 4               | 7.8              | 29.8                | 7.7             | 65.3                      | 44            |

Hb = Hemoglobin; M = male; F = female.

Fig. 1. Distribution of specific subgroups in the study population.

The occurrence of ALL was associated with characteristic peak incidences, and there were significant differences between mean ages of different genotypes (fig. 2). The highest frequency of high-hyperdiploidy and TEL-AML1 fusion was found between the ages of 2 and 7, while the frequencies for other cytogenetic abnormalities such as t(1;19), mixed-lineage leukemia (MLL) rearrangement...
and Philadelphia (Ph) were low in this age group and evenly distributed during childhood. It was noteworthy that MLL rearrangement was not restricted to infancy and young age, as 50% of cases in this group were detected between the ages of 9 and 15.

**Numerical Chromosomal Alterations**

Numerical chromosome abnormalities included: low hyperdiploidy (modal number 47–50), high or massive hyperdiploidy (>50), hypodiploidy (46 and lower), near-tetraploidy and gain or loss of a single chromosome. Modal chromosome numbers of ≤45 were rare findings in our study population (n = 2, 1%). Hyperdiploidy without specific translocations was present in 68 (41%) patients. All cases were of precursor B-cell type associated with moderate WBC counts at presentation, thrombocytopenia and Fab L₁ or L₂ morphology. The most frequently acquired numerical changes were +21 (n = 48), +X (n = 47), +14 (n = 43), +17 (n = 40), +6 (n = 38), +18 (n = 37), +4 (n = 35), +8 (n = 34) and +10 (n = 32). Among ploidy groups, 12 patients (14%) had hyperdiploidy with modal numbers of 47–50 with most frequently acquired numerical abnormalities of chromosomes 21 and X. The complete remission rate for the total hyperdiploid group was 90%, the median response duration of 43 months with a 3-year EFS rate of less than 60%.

**TEL-AML1 Rearrangement**

The TEL-AML1 (now known as ETV6-RUNX1) rearrangement was the most commonly observed structural abnormality identified by fluorescence in situ hybridization in 7% of the study population and 27% of the studied cases; 45% of cases had deletion of the remaining TEL allele. Additional anomalies were observed in 55% of cases (table 2). Children without additional anomalies tended to be younger (median age 4 vs. 6.2 years), had lower hemoglobin levels (median 5.4 vs. 9.9 g/dl) and platelets (median 26.6 vs. 115.5 × 10⁹/l) than children with additional karyotypic changes. Of 11 children, 5 relapsed within 1–3 years (median 32 months) including 4 of 5 children with additional anomalies (table 2).

![Fig. 2. Age distribution of the study population.](image)

**t(1;19)(q23;p13), t(9;22)(q34;q11) and MLL Rearrangements**

The t(1;19)(q23;p13) translocation which involves fusion of the TCF3 gene on chromosome 19 to the PBX1 gene on chromosome 1 occurred in 1.8% of patients. All cases were pre-B ALL with positive CD10, CD19 and CD22 expression. This group of patients presented with
low WBC counts, and with the lowest median hemoglobin level (median 4 g/dl) and platelets (30 × 10⁹/l) among subgroups. The median EFS rate was 8 months, and ultimately all patients failed therapy within 2 years.

The Ph chromosome t(9;22) translocation creating a hybrid BCR-ABL gene was present in approximately 2% of children. Ph-positive ALL cases showed an absolute male predominance, and all patients in this group had a short remission duration, and ultimately all cases relapsed within 3 years including both patients who underwent bone marrow transplantation.

The MLL gene located at band 11q23 was altered in 3.6% of cases. In 4 out of 6 cases the anomaly was associated with complex karyotypes. The complete remission rate was 67%, with a median EFS of 10 months, and at present only 1 patient remains in remission.

Miscellaneous Abnormalities
Nonrecurring anomalies were detected in 15 patients (9%) with abnormal karyotypes; 3 patients are still in remission and the remaining 4 patients relapsed within 4–48 months (median 19.7 months) indicating that children with miscellaneous chromosome anomalies tended to have an unfavorable clinical outcome.

T-Cell ALL. The diagnosis of T-cell ALL was made in 21 cases (13%) associated with older age and a higher proportion of boys than in other karyotype groups (18 males, 3 females). Rearrangements in the 14q11–13 region, in which the T-cell receptor chain genes are located, were present in 2 cases. Further structural anomalies included: del(1)(p32), del(6q) and a Ph translocation associated with high-hyperdiploidy and marker chromosomes. The median EFS was 23 months with 3-year EFS rates of 44% of those with an abnormal karyotype and 75% of those with a normal karyotype.

Down Syndrome. Of the 164 patients, 12 were identified with Down syndrome and ALL (ALL-DS). There was a clear male predominance, and DS patients were more likely to have normal cytogenetic results when compared with total patients (58 vs. 25%). The DS patients tended

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Table 2. Clinical characteristics of patients with additional karyotypic abnormalities

| Age/sex | WBC, ×10⁹/l | Chromosome abnormality | EFS months |
|---------|-------------|------------------------|------------|
| **TEL** |             |                        |            |
| 8/F     | 1.9         | 48–51,XX,der(1)t(1;?)(q23;?) | 2+         |
| 8/F     | 14.4        | 49–55,XX,add(6)(p25)     | 43         |
| 3/M     | 10.2        | 46,XY,dup(1)(q21q44),add(4)(p16),del(5)(q?),−9,del(11)(q14),add(17)(p13),−18,+21c,+mar | 32         |
| 8/F     | 11          | 46,XX,t(3;?)(q27–28;?),del(6)(q21),t(8;?)(q21;?),del(12)(p11p13),add(14)(q32),+mar | 41         |
| **MLL** |             |                        |            |
| 9/M     | 208         | 46,XY,t(4;11)(q21;q23)/47,XX,t(4;11)(q21;q23),+17 | 5          |
| 14/M    | 205         | 53,XY,+3,t(4;11)(q21;q23),+6,+7,+13,+16,+19,+mar/53,XY,idem,t(1;7)(q31;p22),+mar1−2/56,XY,dup(1)(q11q43),t(4;11)(q21;q23),+6,+16,+18,+19,+20,add(21)(q21),+22,+mar1−4 | 5          |
| 15/F    | 171         | 45,XX,t(4;11)(q21;q23),−21/44,XX,idem,t(17;18)(p11;q11)/45,XX,idem,t(1;5)(q32;q35)/44,XX,idem,t(9;13)(q11;q34),t(17;18)(p11;q11) | 4          |
| 4/M     | 36          | 46,XY,(5;11;17)(q31;q23;q21–22),i(7)(q10),del(12)(p13) | 8          |
| t(1;19) |             |                        |            |
| 14/M    | 5.0         | 47,XY,t(1;18)(q;p?)+,t(13;?):(q13;?),der(19)(q23q13),+mar | 1          |
| 6/F     | 12.5        | 46,XX,del(11)(q23),add(12)(p13),der(19)(t1;19)(q23p13),+8,del(11)(q23),add(12)(p13)x2,der(19)(t1;19)(q23p13) | 23         |
| **t(9;22)** |          |                        |            |
| 4/M     | NA          | 52–55,XY,t(9;22)(q34;q11) | 14 BMT     |
| 14/M    | 30          | 51–54,XY,del(6)(21),t(9;22)(q34;q11) | 35 BMT     |
| 14/M    | 80          | 51–54,XY,t(9;22)(q34;q11),+mar1−4 | 17 T-cell  |
| 12/M    | NA          | 47,XY,t(9;22)(q34q11),+mar | 13         |
| **c-myc** |            |                        |            |
| 11/F    | 56          | 46,XX,t(8;22)(q24q11),der(15)(t8;15;22)(q13q26q11−12) | 4+         |
| 11/M    | 14.9        | 46,XY,t(8;14)(q24q32)/46,XY,idem,der(7)(t1;7)(q10:p10) | 10         |

BMT = Bone marrow transplantation; NA = not assessed; + = EFS without event.
to be older, and no ALL-DS patients were less than 2 years of age. Of the 7 patients with abnormal karyotype, 3 had near-triploidy/tetraploidy and in 4 miscellaneous chromosome anomalies were observed including 1 TEL/AML1-positive patient with multiple numerical and structural anomalies involving chromosomes 1, 4, 5, 9, 11, 17 and 18 (table 2). No translocations associated with adverse outcome such as t(9;22), t(4;11) or t(1;19) were presented. A notable finding in this group was a TEL-AML1 fusion in a 3-year-old male and complex karyotype as TEL-AML1 rearrangements (table 2) which appear to be uncommon in the ALL-DS population. The DS patients were more likely to suffer leukemic relapse than non-DS patients as 6 of 12 patients (50%) relapsed with a median of 16 months. ALL-DS patients were more likely to have normal cytogenetic results when compared with total patients (58 vs. 25%). ALL-DS patients were more likely to suffer leukemic relapse than ALL-non-DS patients as 6 of 12 patients (50%) relapsed with a median of 16 months (range 4–32 months).

Discussion

While the cause in most cases of childhood leukemia is not known, certain factors such as susceptibility or environmental influences have been suggested. Evidence in support of this theory comes from the observation that the type and frequency of ALL vary by geographic region [4–6] and ethnicity [8].

From January 2002 to 2007, a diagnosis of ALL was made in 205 children (34 cases/year). While the population of Kuwait is about 3.05 million (1.92 million males; 1.13 million females; sex ratio 1.7), only about one quarter (n = 692,000; sex ratio 1.05) of the population are children [9], making the annual incidence rate of childhood ALL about 5 cases (4.9) per 100,000 children. The presenting ages at diagnosis in our study group did not differ substantially from those of children with ALL reported in previous studies. A notable exception was the older age of patients in the group with MLL rearrangement as 5 out of 6 patients were older than 1 year at diagnosis (table 2). The observed 75% frequency of genetic abnormalities in our study population was consistent with those of previous reports [10–14]. The difference between the findings of this study and previous reports is the relatively higher frequency of hyperdiploid cases (41.5%) seen in this cohort, as in most of the reported series. A decreased prevalence of hyperdiploidy in patients with DS is consistent with recent reports [15–19] that showed a significantly lower frequency of hyperdiploid cases in ALL-DS patients suggesting this may contribute to a poorer outcome of patients in this group. Further differences were observed in the clinical outcome of children with TEL-AML1 rearrangement (table 2). While only few reports [20–22] questioned the favorable prognosis for TEL-AML1-positive ALL, in our patients, 5 out 11 children relapsed, most likely due to the presence of additional chromosome anomalies. The current study confirmed the specific clinical features of patients with t(9;22), MLL and c-myc rearrangements including high leukocyte counts and older age at diagnosis. The clinical outcome for patients in all 4 subgroups has been very poor indicating that the treatment for this category of patients remains a challenge. Maybe, ongoing cytogenetic analysis can identify new nonrandom chromosome aberrations and uncover a previously unrecognized diversity among patients [23–25].

Conclusion

The results of this study confirmed that clonal abnormalities detected in childhood ALL have shown correlations between specific recurrent chromosomal abnormalities and clinicobiological characteristics of the patients.

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