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Frequency of Cytogenetic Findings and its Effect on the Outcome of Pediatric Acute Lymphoblastic Leukemia

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ABSTRACT

Introduction: Acute lymphoblastic leukemia (ALL) is one of the most common cancers in children and accounts for about ⅓ of cancers in children. The annual incidence of ALL is 4 patients per 100,000 children. Their peak age is between 2-5 Year. One of the most important prognostic factors is cytogenetic abnormalities which are very effective in determining treatment policy. **Aim:** To determine the frequency of cytogenetic findings and its effect on the outcome of children with ALL. **Materials and Methods:** This retrospective cross-sectional analytical study was conducted on children with ALL who their disease was diagnosed between 2001and 2009. Furthermore, 206 patients with ALL were examined by referring to Clinic of Ali Asghar Hospital in Tehran. Data was collected from medical records and analyzed by SPSS16 software. **Results:** 206 children with ALL were enrolled in the study. The estimated event-free survival rate of all enrolled patients was more than 70%. There was a significant relationship between type of cytogenetic disorder and clinical outcome of patients (P<0.0001), where the highest mortality was observed in patients with t (9;22) and t (4;11). There was no significant correlation between the sex and age with the clinical outcome of the patient (P = 0.064; p=0.322). There was a statistically significant relationship between mediastinal mass and clinical outcome (P = 0.002), indicating that the presence of cells growth in an involuntary way can be cause of the cancer. A significant association was found between the clinical outcome of patients and radiotherapy (P = 0.043), indicating that radiotherapy is effective in improving cancer. **Conclusion:** The findings demonstrated that the average survival rate without recurrence in children was at level of the European countries. However, the strong chemotherapy weakened the role of many prognostic factors in ALL patients, but some translocations are prognostic factors in predicting death in patients with ALL. Therefore, patients with this factor need to receive more confident treatment policy. Comprehensive studies are required by focusing on more samples because of low number of relapses and deaths in the present study.

Keywords: Acute lymphoblastic leukemia, Children, Cytogenetics, Child.

1. INTRODUCTION

Cancer is an uncontrolled growth of the body's cells, the most common form of which is in children as hematologic malignancies, accounting for 44% of the cancers diagnosed in children (33% leukemia and 11% lymphoma) (1). Acute Lymphoblastic Leukemia (ALL) accounts for about 75% of leukemia cases, and is considered as a clonal malignancy of bone marrow in which early lymphoid precursors proliferates, leading to replacement of them with normal hematopoietic cells in the bone marrow (2).

The annual ALL incidence is estimated to be 4 case per 100,000 children aged 2 to 5 years old (3). Despite the high prevalence of ALL in children, the percentage of complete remission has increased from 10% in the years before 1970 to about 100% (4. 5). Clinical suspicion of leukemia can be increased via clinical symptoms such as fatigue, fever, petechiae, ecchymosis, nose and gum bleeding, bone pain, and distributed lymphadenopathy, as well as central nervous system symptoms such as headache, vomiting and paralysis (6). The prognosis of patients with ALL is evaluated based on several factors. One of these factors is cytogenetic findings. The relationship between prognosis and karyotype at diagnosis time of ALL is first introduced by Sedker Walker in the year 1978 and continues until today (7).

In diagnosis, a cytogenetic evaluation, including chromosomal trans-

location, and hyperdiploidy, is performed simultaneously with patient sampling.

Chromosomal abnormalities are found in most patients with ALL. These abnormalities may show abnormal number of chromosomes, chromosomal translocation and chromosomal removal, which provides valuable information about the prognosis of the disease (8, 9). Despite the low prevalence of leukemia in children of Iran in comparison with Western countries, but ALL is the most common malignancy in Iranian children (10). Considering the above evaluations, there is no comprehensive data on other cytogenetic disorders. Moreover, studies on acute lymphatic leukemia in children in Iran are very limited.

2. AIM

Aim of this study was to evaluate the cytogenetic findings and its effect on prognosis ALL patients to evaluate the relationship between recurrence and cytogenetic disorders and other biological data and to determine the factors affecting the prognosis of the disease for providing new therapies.

3. MATERIALS AND METHODS

This retrospective cross-sectional study was conducted on children with ALL who their disease was diagnosed between 2001and 2009. The medical records of these patients were selected by referring to the archives of Ali Asghar Hospital Tehran-Iran. Demographic data (age and gender), karyotype, cytogenetic information, therapeutic regimen and clinical outcome of patients were extracted and then recorded in the relevant checklist.

The data were analyzed using SPSS 16 software; demographic data were analyzed descriptively and Chi-square, Log-Rank and Kaplan–Meier tests were used for survival analysis. P-value less than 0.05 was statistically significant.

Ethical approval was obtained from Ali-Asghar Children Hospital, Tehran, Iran.

4. RESULTS

Total amount of 206 children with ALL were included in the study. Based on the age range, 7 (3.4%) patients belonged to the age group of less than one year, followed by one to ten years (171, 83%) and more than 10 years (28 people, 13.6%). Of the 206 children enrolled in the study, 116 cases (56.3%) were boys and 90 (43.7%) were girls. Morphologically, 1 patient (0.5%) belonged to type L3. For FAB classification, 95 cases (46.1%) were classified in the Early pre B group, followed by pre-B (79 cases, 38.8%), T Cell (27 cases, 12.7%) pro B Cell, (4 cases, 1.9%) and Mature B cell (1 case, 0.5%).

The frequency of types of markers was evaluated in Table 1. CD19 (73.8%) was the most frequent marker, and both CD4 and CD22 had the least frequency among markers (6.3%).

The findings showed that 10 (4.85%) had mediastinal mass breast and 196 (95.15%) had no mass. In terms of white blood cell count, at the beginning of the study, 172 patients (83.5%) showed WBC < 50,000, followed by WBC between 50000-100,000 (17 patients, 8.3%) and WBC <100,000 (17 patients, 8.3%). Table 2 shows the level of primary LDH in patients.

Flow cytometric findings	Frequency	Frequency percentage
CD10	139	67.5%
CD19	152	73.8%
CD20	55	26.7%
CD22	13	6.3%
CD5	22	10.7%
CD2	30	14.6%
CD3	30	14.6%
CD7	31	15%
CD34	63	30.6%
CD45	84	40.8%
HILADR	124	60.2%
CD4	13	6.3%
CD8	15	7.3%

Table 1. Frequency of markers

Primary LDH	Frequency	Frequency percentage
> 500	84	40.8%
1000-500	101	49%
1500-1000	16	7.3%
< 1500	6	2.9%

Table 2. Prevalence of primary LDH in patients

Cytology type	Frequency	Frequency percentage
normal	122	59.2%
t (9,22)	6	2.9%
t (4,11)	8	3.9%
t (1,19)	7	3.4%
t (12,21)	3	1.5%
Hyperdiploidy	34	16.5%
Other cases*	23	11.2%
Down syndrome	3	1.5%

Table 3. Cytogenetic disorders t(9;7), t(2;16), t(14;20), t(19,14), t(2;4), t(2;16), t(8;14), t(10;11)*

Cytogenetic disorders of patients are shown in Table 3. Out of 206 patients, 122 cases (59.2%) were normal for genetic disorders, and 84 patients (40.8%) had a variety of cytogenetic disorders.

According to the treatment protocol, the most treatments were related to the BFMIC 2002 protocol (106 cases, 51.9%), followed by Conventional BFM (74 cases, 35.9%) and NY-1 (26 cases, 12.2%). Of course, children with T-cell ALL were treated. The median duration of treatment was determined as 48 months with a standard deviation of 31.6.

Of the 206 patients under study, 148 patients (71.8%) survived for 48 months. In this period, 58 cases of relapse were reported, of which 12 (5.9%) were related to the deaths, and 46 (22.3% for other factors. BM relapse was observed in 41 patients, followed by relapse in CNS (16 cases) and 1 patient relapse in the testes.

The median time of relapse rate in this study was 41.15 with a standard deviation of 26.6 months. Regarding the location of the second relapse in the patients, 5

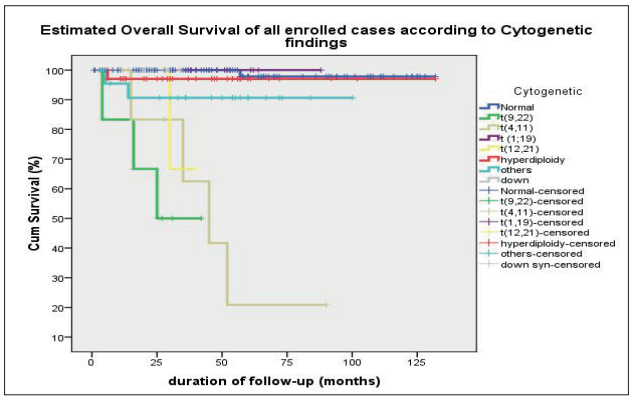

Figure 1. Kaplan Meier analysis of patient survival with regard to death

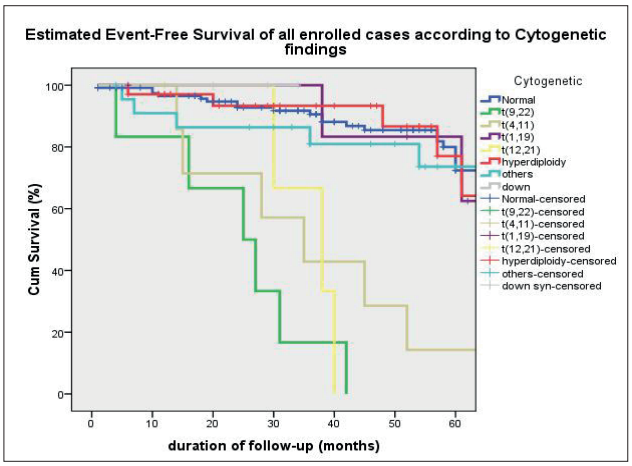

Figure 2. Kaplan Meier analysis of patient survival with regard to death and recurrence

cases (33.3%) showed relapse in BM, followed by 6 cases (40%) of recurrence in CNS and 4 cases of (26.6%) testes recurrence. The third relapse was reported in CNS (1 case) and testes (one case). Furthermore, 37 patients (18%) had radiotherapy in this study, of which 21 were alive, 14 were relapsed and 2 were died.

In Table 4, the clinical outcome of patients was evaluated based on cytogenetic type. There was a statistically significant relationship between cytogenetic disorder and clinical outcome of patients (P-valu<0.0001), with the highest death rate in patients with t (9.22) and t (4.11). In addition, only three patients had t (12.21), which recurred all three cases until the end of the study and one of them died.

There was no significant relationship between the cause of death and the type of cytogenetic impairment (P = 0.682), indicating that the type of cytogenetic disorders does not correlate with the death of patients. No significant relationship was found between white blood cell count and cytogenetic disorder (P = 0.855). There was no statistically significant relationship between the initial white blood cell count and the clinical outcome of the patient (P = 0.857). Moreover, there was no significant correlation between the sex and age with the clinical outcome of the patient (P = 0.064; p=0.322). There was a statistically significant relationship between mediastinal mass and clinical outcome (P = 0.002), indicating that the presence of cells growth in an involuntary way can be cause of the cancer.

There was no significant relationship between morphological and clinical outcome (P = 0.14). A significant correlation was found between clinical outcome and radiotherapy (P = 0.043), indicating that radiotherapy was effective in improving cancer, but there was no significant relationship between the early levels of LDH and the clinical outcome of the patients (P = 0.80).

Clinical outcome based on cytogenetic markers are presented on Table 4. Patient survival is shown in Figures 1 and 2, based on the type of cytogenetic.

				Clinical outcome	Total
		Live	Death	Recurrence	
Normal cytogenetic markers	Number	95	1	26	122
	% based on cytogenetic marker	%77.9	%0.8	%21.3	%100
	% By clinical outcome	%64.2	%8.3	%56.5	%59.2
	% Overall	%46.1	%0.5	%12.6	%59.2
t (9,22)	Number	0	3	3	6
	% based on cytogenetic marker	%0	%50	%50	%100
	% By clinical outcome	%0	%25	%6.5	%2.9
	% Overall	%0	%1.5	%1.5	%2.9
t (4,11)	Number	2	4	2	8
	% based on cytogenetic marker	%25	%50	%25	%100
	% By clinical outcome	%1.4	%33.3	%4.3	%3.9
	% Overall	%1	%1.9	%1	%3.9
t (1,19)	Number	5	0	2	7
	% based on cytogenetic marker	%71.4	%0	%28.6	%100
	% By clinical outcome	%3.4	%0	%4.3	%3.4
	% Overall	%2.4	%0	%1	%3.4
t (12,21)	Number	0	1	2	3
	% based on cytogenetic marker	%0	%33.3	%66.7	%100
	% By clinical outcome	%0	%8.3	%4.3	%1.5
	% Overall	%0	%0.5	%1	%1.5
Hyperdiploidy	Number	28	1	5	34
	% based on cytogenetic marker	%82.4	%2.9	%14.7	%100
	% By clinical outcome	%18.9	%8.3	%10.9	%16.5
	% Overall	%13.6	%0.5	%2.4	%16.5
Other cases	Number	15	2	6	23
	% based on cytogenetic marker	%65.2	%8.7	%26.1	%100
	% By clinical outcome	%10.1	%16.7	%13	%11.2
	% Overall	%7.3	%1	%2.9	%11.2
Down syndrome	Number	3	0	0	3
	% based on cytogenetic marker	%100	%0	%0	%100
	% By clinical outcome	%2	%0	%0	%1.5
	% Overall	%1.5	%0	%0	%1.5

Table 4. Clinical outcome based on cytogenetic markers

5. DISCUSSION

Leukemia refers to a group of types of cancers that usually originate from bone marrow and cause the formation of a large number of abnormal white blood cells. These white blood cells are not completely formed and they are called blast or leukemia cells (11). Symptoms include bleeding and bruising, severe fatigue, fever, and increased risk of infection. Diagnosis is performed using blood tests and bone marrow biopsy (12). Like many other cancers, children's leukemia is due to several factors that arise from interactions between various aspects of the human environment and human genetics. The largest

subgroup of leukemia is ALL, which accounts for 75% to 80% of children's leukemia (13). To investigate the effect of prognostic factors on outcomes of patients with ALL, mediastinal involvement, age, sex, type of ALL, according to cytological markers, were included in the study.

The findings of our study showed a significant relationship of translocation T (22;9) and translocation t (11;4) with the clinical outcome of patients, so that there was a significant relationship between the two translocation with death. On the other hand, translocation t (21;12) was associated with relapse of the disease, which was consistent with the study conducted by Conter et al in 2004 on ALL. In their study, 2% of the patients had t (9;22), 3.2% had t (1;19), 2.4% had t (4;11), and 38.8% had t (12;21) and hyperdiploid, which are known as the most common cytologic marker that these markers showed good prognosis and higher survival than other markers (14). Josep-Mar et al also concluded in 2002 that patients with translocation (22;9) had a poor prognosis (15), which was consistent with our findings.

In the present study, there was a significant relationship between the mediastinal mass (P = 0.002) and radiotherapy (P = 0.04) with the clinical outcome of the patient. On the other hand, no significant relationship between white blood cell levels at the time of diagnosis and the clinical outcome. Contrary findings by Hashemi et al. indicated that the WBC count of more than 50,000 was a prognostic factor in determining the prognosis of patients (16). Advani et al. in India reported an age group of 9-2 years old (63%) and male gender (64.8%) as involved groups that were consistent with our findings (17). In our study, the WBC evaluation was divided into three groups (less than 10,000, from 10,000 to 50,000 and more than 50,000), with the highest levels of WBC in the first and second groups. While Chen and colleagues demonstrated that the WBC >50,000 is the most important factor in patients suffering from ALL by Multivariate Analysis (18).

Another study in Egypt, conducted by Hussein et al on 154 ALL children, showed that central nervous system involvement, high-risk group, and slow early response to treatment are of great importance in prognosis of disease via multivariate analysis. In this study, the frequency of LDH level holders between 500 and 1000 was the highest value. On the other hand, unlike other studies, Bulky Extramedullary Disease has been identified as an important risk factor and concludes that prognostic factors in different regions of the world can be very different. Also, in this study, a significant relationship of the level of WBC, LDH, platelet, and hemoglobin level with clinical outcome was found (19).

6. CONCLUSION

The results of this study showed that the average survival rate in children with ALL was at level of the European countries. However, the strong chemotherapy weakened the role of many prognostic factors in ALL patients, but some translocations are prognostic factors in predicting death in patients with ALL. Therefore, patients with this factor need to receive more confident treatment policy. Comprehensive study is needed by focusing on more patients due to the low number of relapses and deaths.

- **Declaration of patient consent**: The authors certify that they have obtained all appropriate patient consent forms
- **Author's contribution**: G.B. and M.N. gave substantial contribution to the conception or design of the work and in the acquisition, analysis and interpretation of data for the work. Each author had role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work.
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- **Conflicts of interest**: There are no conflicts of interest.

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