CHARACTERISTICS OF IMMUNOLOGIC MARKERS IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA WITH GENETIC MUTATION AT NATIONAL INSTITUTE OF HEMATOLOGY AND BLOOD TRANSFUSION FROM 2016 TO 2018

Hoang Thi Hong[1], Mai Lan[1], Nguyen Quang Tung[1],
Nguyen Trieu Van[1], Bach Quoc Khanh[1]

ABSTRACT

Determining characteristic of immune classification and genetic mutations plays an important role in the diagnosis, treatment and prognosis of pediatric ALL.

Objective: *Research on characteristic of cluster of differentiation in pediatric ALL with genetic mutations in National institute of Hematology and Blood transfusion from 2016-2018.*

Methods: *Cross-sectional descriptive on 189 pediatric patients aged 1-15 years old with newly diagnosis ALL.*

Results*. Frequency of fusion genes was 26.9% (fusion gene TEL-AML1 13.2%, BCR-ABL 8.5%, E2A-PBX1 2.6%, MLL-AF4 2.6%). B - ALL was prevalent with 82.0%; T - ALL accounted for 16.4%. 97,8% of the patients with genetic mutation were in group of B-ALL. CD45 showed strong positive expression in most of the groups; The rate of HLA-DR imprints was high in genetically modified groups. The incidence of CD34 patients was highest in the BCR-ABL1 fusion gene group. The E2A-PBX1 gene mutation group was negative for CD34. The presence of CD19, CD79a markers was high in pediatric patients. CD10 (+) was low in the MLL-AF4 group. The incidence of CD20 was low in the groups. The incidence of myeloid CD was highest in BCR-ABL1 (37.5% positive for CD33), without the presence of myeloid CD in the pediatric patients with the E2A- PBX1 and MLL-AF4 fusion gene.*

I. INTRODUCTION

Acute lymphoblastic leukemia (ALL) accounts for about 25% of childhood cancers and about 1% of adult cancers. About 60-70% of ALL have genetic changes. The presence of genetic alterations and characteristics of immunologic markers are important in prognosis, evaluating the therapeutic response for ALL [1], [2], [3]. In Vietnam, the relationship between genetic variation and immunological traits has not been studied extensively. So we conducted this research with the aim:

- *Research characteristics of immunologic markersin pediatric all with genetic mutation in national institute of hematology and blood transfusion from 2016 to 2018.*

II. SUBJECTS AND METHODS

2.1. Subjects of Study

189 pediatric patients who were diagnosed with acute lymphoblastic leukemia newly according to the WHO 2008 standard, treated

1. National Institute of Hematology and Blood transfusion

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- Corresponding author: Hoang Thi Hong
- Email: hoanghong.nihbt@gmail.com. Tel: 0983885350

in Pediatric Department, National Institute of Hematology and Blood Transfusion from 01/8/2016 to 30/4/2018.

2.2. Research Methods

- Study Design: Cross-sectional descriptive study

- Criteria for selecting patients:

• Patients diagnosed with acute lymphoblastic leukemia, aged 1-15.

• No previous chemotherapy or corticosteroids.

• Be fully tested

• The family agrees to participate in the study.

- Steps of Study:

• Do bone marrow aspirate.

• PCR assay for mutation of TEL / AML1, E2A / PBX1, BCR / ABL, MLL / AF4 fusion gene.

• Immunization tests by flow cytometry with panel of the NIHBT.

• Analyze characterization of immunologic markers with genetic variations.

III. RESULTS

3.1. Characteristics of age and gender

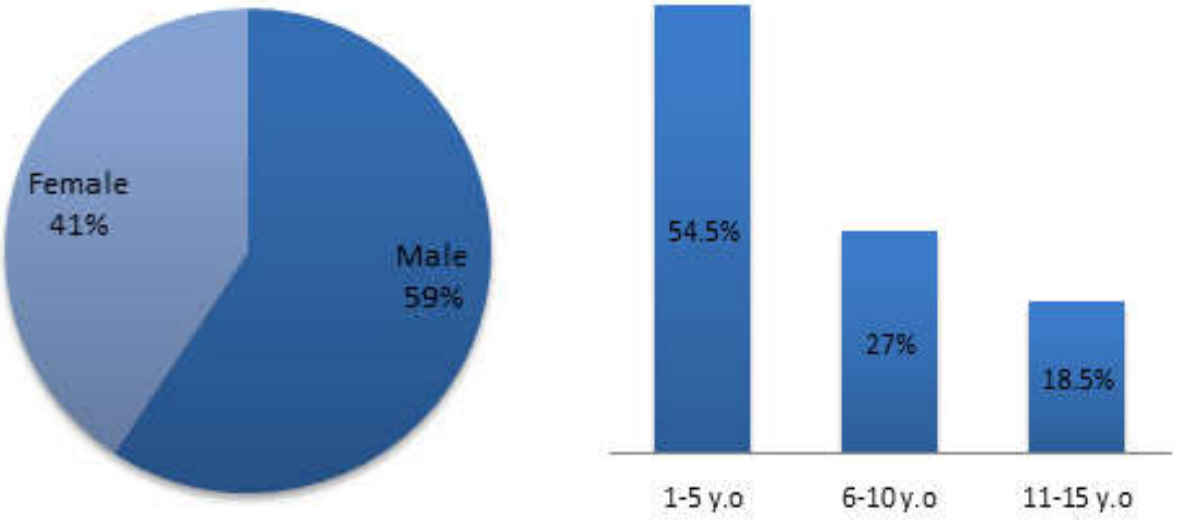

Figure 3.1. Distribution of pediatric patients by sex and age group (n=189)

Male patients in the study group were higher than the female patients. The ratio of male / female was 1.45 / 1. Age group 1-5 represented the highest proportion with 54.5%.

3.2. Distribution of pediatric patients by immunological classification

Immunological classification	n	%
B – ALL	155	82.0
T – ALL	31	16.4
Mixed B/T – ALL	1	0.5
Mixed acute lineage leukemia	2	1.1
Total	189	100

Table 3.1. Distribution of pediatric patients by immunological classification (n=189)

B - ALL was prevalent with 82.0%. T - ALL accounted for 16.4%. The study also had low level (1.6%) mixed acute lineage leukemia

3.3. Rate of genetic variations in the study

Table 3.2. Rate of genetic variations in the study (n=189)

Genetic variations		n	%
No gene mutations detected		138	73.1
Fusion genes detected (n=46, 26.9%)	TEL-AML1	25	13.2
	BCR-ABL1	16	8.5
	E2A-PBX1	5	2.6
	MLL-AF4	5	2.6
Tổng		189	100

The detection rate of fusion genes which research in the study was low (26.9%). The emergence of the TEL-AML1 fusion gene accounted for the highest rate of 13.2%. 8.5% of pediatric patients had the BCR-ABL1 fusion gene. Patients with E2A-PBX1 and MLL-AF4 fusion genes accounted for the same proportion (2.6%).

3.4. Classification of immunity by fusion gene groups

Table 3.3. Classification of immunity by fusion gene groups (n=46)

Immune phenotype / Fusion gene	B-ALL	T-ALL	Total
TEL-AML1	25 (100%)	0 (0%)	25 (100%)
BCR-ABL1	15 (93,8%)	1 (6,2%)	16 (100%)
E2A-PBX1	5 (100%)	0 (0%)	5 (100%)
MLL-AF4	5 (100%)	0 (0%)	5 (100%)
Total	45 (97,8%)	1 (2,2%)	46 (100%)

97.8% of patients with four fusion genes belonged to the B-ALL group. Only one pediatric patient (2.2%) had BCR-ABL1 fusion gene in the T-ALL group.

3.5. Characteristic of CD45 expression in fusion gene groups

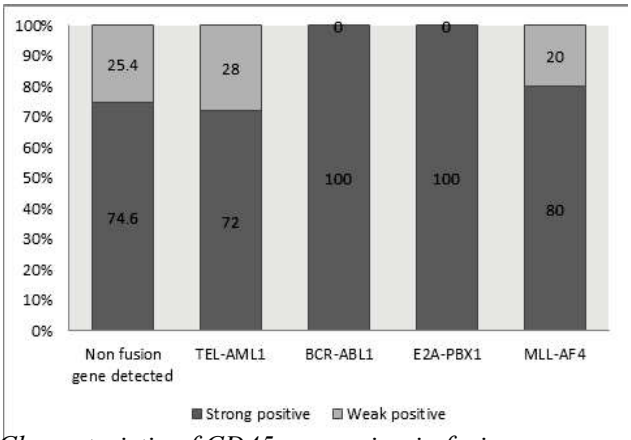

Figure 3.2. Characteristic of CD45 expression in fusion gene groups (n=189)

Most pediatric patients had a strong CD45 level of expression. 100% of patients with BCR-ABL1 and E2A-PBX1 fusion gene had positive with CD45 strongly. The incidence of strong CD45-positive patients in the non-fusion gene group was 74.6%, TEL-AML1 fusion gene group was 72% and MLL-AF4 group was 80%.

3.6. Characteristic of CD 34 and HLA-DR expression in fusion gene groups

Table 3.4. Characteristic of CD34 and HLA-DR expression in fusion gene groups (n=189)

CD / Fusion genes	HLA-DR		CD34	
	Negative	Positive	Negative	Positive
No fusion genes detected	31.2%	68.8%	38.4%	61.6%
TEL-AML1	4.0%	96.0%	24%	76%
BCR-ABL1	18.8%	81.2%	6.2%	93.8%
E2A-PBX1	0%	100%	100%	0%
MLL-AF4	0%	100%	20%	80%
	p<0,05		p<0,01	

- The rate of HLA-DR expression was high in fusion gene goups.

- There was high incedence of CD 34 in groups of BCR-ABL1; MLL-AF4 and TEL-AML1 fusion gene. All patients with E2A-PBX1 fusion gene were negative with CD34.

3.7. Characteristic features of B- cell phenotype in fusion gene groups

Table 3.5. Characteristic features of B- cell phenotype in fusion gene groups (n=46)

	TEL-AML1	BCR-ABL1	E2A-PBX1	MLL-AF4
CD10 (+)	100%	93.8%	100%	20%
CD19 (+)	100%	87.5%	100%	100%
CD20 (+)	12.0%	25.0%	0%	20%
CD79a (+)	100%	93,8%	100%	100%

CD19 and CD79a had been shown to appear at high rates in fusion gene groups. Few patients with MLL-AF4 had positive with CD10 (20%). The incidence of CD20 positive patients was low in fusion gene group and did not appear in the E2A-PBX1 fusion gene group.

3.8. Characteristics of abnormalities immune marker in fusion gene groups

Table 3.6. Characteristics of abnormalities immune marker in fusion gene groups

	TEL-AML1	BCR-ABL1	E2A-PBX1	MLL-AF4
CD13 (+)	4.0%	0 %	0 %	0 %
CD33 (+)	4.0%	37.5%	0%	0%
CD56 (+)	4.0%	6.2%	0%	0%

The rate of patients who had abnormalities immune marker was the highest in the BCR-ABL1 fusion gene groups, with CD33 (+) in 37.5% and CD56 (+) in 6.2%. Myeloid imprints did not appear in pediatric patients with E2A-PBX1 and MLL-AF4 fusion gene.

IV. DISCUSSION

4.1. Characteristics of age and gender

Our research is similar to that of Mai Lan (2015), Tran Quynh Mai (2016), the ratio of male was higher than female, the age group 1-5 represented the highest proportion in pediatric ALL patients of NIHBT.

4.2. Classification of pediatric patients according to immune markers.

The proportion of B-cell ALL patients is dominant with 82%, meanwhile the percentage of T-cell ALL patients is 16,4% and the rest is the level

of hybrids (Table 3.1). This result is well suited to various domestic and international researches. The proportion of ALL fluctuates around 85%, T-cell ALL is about 15%. The study of author Hoang Chi Cuong about classification of immunity in pediatric lymphoblastic leukemia showed 78.7% B-cell ALL, while 17% T-cell ALL. According to our research, the percentage of the mixed acute lineage leukemia (1,6%) is lower than in the Hoang Chi Cuong study (4,3%). The mixed acute lineage leukemia is rare in Vietnam and the world.

4.3. Incidence of the investigated genetic mutations.

In our study, 189 ALL patients were examined the genetic expression and got the results that the genetic mutation group was 26.9%, the TEL / AML1 gene was 13.2%, BCR / ABL 8.5%, E2A / PBX1 2.6%, MLL / AF4 2.6% (Table 3.2).

Table 4.1. Comparing the rate of mutated gene detection with some domestic and international studies.

Authors	BCR/ABL (%)	TEL/AML1 (%)	E2A/PBX1 (%)	MLL/AF4 (%)
Yanming Zhang-Michelle M Le Beau	5	25	5	5-7
Terzah M Horton-C Philip Steuber	3-4	20-25	5	5
Karen Rabin-Judich Margolin	3	25	6	8
C.H.Pui	2-3	20-25	4	2
Cheryl L. Willman	2	28	6	4
P.T.D. An	3	25	5	8
T.Q.Mai (n=104)	10	14	4	2
Our study (n=189)	8.5	13.2	2.6	2.6

Consequently, our proportion of mutated gene detection is still relatively low compared to research in the world. Our research is similar to that of Tran Quynh Mai, the proportion of patients with BCR-ABL1 fusion gene was higher compared with other studies. This may be due to the fact that many patients were transferred to the National Institute of Hematology and Blood Transfusion in high risk groups, so the frequency of fusion gene detection is higher than in other studies.

4.4. Immune marker characteristics in the mutated gene group

Table 3.3 illustrates that 97.8% pediatric patients which were mutated the surveyed genes is in the B-cell ALL group. Because majority of ALL is B-cell as well as almost surveyed genes are featured for the B- cell ALL. Especially, the study encountered a case of T-cell ALL with BCR-ABL1 fusion gene. The literature also reported several instances of T-cell ALL with BCR-ABL1 p190 fusion gene [8]. The research on the characteristics of several immune markers in the group with genetic mutation which have also recorded initially some results:

- In terms of the level of CD45 expression, the study found that the CD45-high positive rate (over 75% of the Blast population is positive with CD45) in most groups, especially in the BCR-ABL1 and E2A-PBX1 fusion gene group. According to the study of author Hoang Chi Cuong, CD45 expression is strong positive with 68.9% B-cell ALL, statistically meaningful lower than with T-lymphocytes (95.7%). In our study, the majority of patients with genetic mutation belonged to the B-cell ALL group, therefore the overall result of strong positive CD45 expression level was similar to that of the B-cell ALL. In our study, it was found that CD45 had a significantly higher level of positive expression in the at normal risk population (TEL-AML1) than in

the high risk group. However, due to the low number of pediatric patients, the difference was not statistically significant.

- Our results present that blast cells in the TEL-AML1, BCR-ABL1 and MLL-AF4 genetic complex group are high level positive with young markers: HLA-DR and CD34 markers. This result is similar to the study by Ludwig WD et al. (1997) on B-cell ALL, with a HLA-DR strong positive ratio of 100% (57/57 patients), CD34 positive proportion is 58% (29/50 patients). Other studies have also shown that HLA-DR (+) in all subtypes of B-cell ALL (100%), and CD34 positive in 70% of patients [8]. Pediatric patient group with the E2A-PBX1 fusion gene had a high positive rate with HLA-DR (100%) but were negative for CD34. Several studies have also shown that patients with E2A-PBX1 fusion gene are generally positive for CD19, CD10, CD79a, HLA-DR but negative for CD34 [10].

- Among the examined hallmarks of B lymphocytes, the CD19, CD79a markers had a high positive rate. CD 20 had a lower positive rate than other B-type imprints because these mutated gene groups were mostly in B-cell or pro B-cell groups. CD10-positive rates were found low in the group with the MLL-AF4 fusion gene, high in the groups with the other mutated gene. This is consistent with many studies. The MLL-AF4 genetic complex appears with a high proportion in less than 6 months of age and older infants, and several studies have also shown that most patients with MLL-AF4 fusion gene have no CD10 on there surface [9].

- Occurrence of myeloid markers in ALL may be observed at rates ranging from 13 to 28% in some studies. In our research, the incidence of myeloid markers was not high, but found primarily that the incidence of myeloid markers is highest in BCR-ABL1, especially CD33 was positive in 37.5% of cases. Several studies have also found that the incidence of myeloid imprints may be as high as 30% in cases of BCR-ABL1 [9].

V. CONCLUSION

In a study of 189 children with acute lymphoblastic leukemia at the NIHBT, we had some conclusions:

- B-cell ALL accounted for the majority (82%); 97.8% of patients with genetic variation were in the B-cell ALL group.

- A rare case of BCR-ABL1 fusion gene belonged to T-cell ALL.

- CD45 expression was high in most groups with genetic modification.

- The rate of HLA-DR imprints was high in genetically modified groups

- The CD34-negative count in the E2A-PBX1 group was high in the remaining groups.

- The CD19, CD79a (+) levels was high in all the groups. CD10 (+) was low in the MLL-AF4 fusion gene. The incidence of CD20 was low in all the groups.

- Incidence of myeloid CD was highest in BCR-ABL1 (37.5% positive for CD33).

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