DETECTION OF THE COMMON GENETIC ABNORMALITIES BY REVERSE TRANSCRIPTASE-POLYMERASE CHAIN REACTION (RT-PCR) IN CHILDREN WITH ACUTE LEUKEMIA AT HUE CENTRAL HOSPITAL

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ABSTRACT

Background: *Acute Leukemia (AL) is a heterogenerous disease. Recent advances in cytogenetics and molecular genetics have made it possible to identify the genetic abnormalities with prognostic and therapeutic significance. In Vietnam, the diagnostic molecular screening for AL is still limited.*

Objective: *To detect the genetic abnormalities among the children with AL admitted to Hue Central Hospital.*

Methods: *This is a single institutional prospective study of 50 children newly diagnosed with AL from May 2012 to January 2015 at Hue Central Hospital. The molecular genetic screening test using Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) was performed to detect gene fusions.*

Results: *Of 50 patients analyzed, there were 38 children with ALL and 12 children with AML, median age was 3.9 year (range 2 months to 15 years). Regarding ALL The frequencies of the genetic abnormalities were as follow: 9(23.7%) with t(12;21)/TEL-AML1, 2(5.3%) with t(9;22)/BCR-ABL , 3(7.9%) with t(4;11)/ MLL-AF4, 4 (10.5%) with t(1,19)/E2A-PBX1. Regarding AML The frequencies were: 1(8.3%) with t(8;21)/ AML1-ETO, 2(16.6%) with t(15;17)/PML-RARA, 1(8.3%) with t(16,16)/CBFB-MYH11, 2 (16.6%) with t(9;11)/ MLL-AF9. 15,8% of ALL and 25,0 % of AML had been chromosomal abnormalities. Hyperdiploidy were 5,3 % of ALL and 16,6 % of AML.*

Conclusion: *The frequencies of the genetic abnormalities of our patients are higher than that in the reported literatures. Our sample size is small; therefore, further study with larger sample size will be necessary to have the true frequencies. RT-PCR is a useful and rapid tool to detect genetic abnormalities to improve the ability to accurately and rapidly risk-stratify children with AL.*

Key words: *Genetic abnormalities, Acute Leukemia (AL)*

I. BACKGROUND

Acute Leukemia is a hematology malignant heterogenerous disease and the most common childhood cancer included Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leukemia (AML).

In the past 40 years, in the world, there were many achievements in the diagnosis and treatment for leukemia. Most patients of ALL achieved complete remission after a course of induction and 5 years survival is about 75- 80%, most of them can be considered a complete recovery. The

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results obtained as above, is not only the significant progress in the clinical trials, but also the great scientific breakthroughs in hematology, biochemistry, immunology and genetics.

Advances in cytogenetic and in molecular biology we can detect abnormalities of cellular and molecular characteristics in leukemia disease. These characteristics influence diagnosis and accurate classification of disease as well as the choice of the appropriate treatment regimen. In Vietnam, the molecular diagnostic screening for AL is still limited. The goal of this study was to detect the genetic abnormalities among the children with AL admitted to Hue Central Hospital by The molecular genetic screening test: Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR).

II. MATERIALS AND METHODS

2.1. Patient enrollment

This is a single institutional prospective study of 50 children newly diagnosed with acute leukemia from May 2012 to January 2015 at Hue Central Hospital. The diagnosis was confirmed by morphological FAB criteria, cytochemistry and immunophenotype

2.2. Methods

- All patients were aspirated 1 ml of bone marrow to culture the marrow chromosomes

- RT-PCR technique by using the Hemavision Full Kit from DNA Technology A / S company, Denmark following these steps:

* *take a sample*
* *Separated extract RNA*
* *Synthesis of cDNA (complementary DNA)*
* *Gene amplification reaction*
* *Check the quality of cDNA*

- *Screening of 4 common gene fusion in ALL and AML*

cDNA after reverse transcription of RNA will be used as a template for PCR to detect the gene fusions. In ALL, we focused to screen 4 common gene fusions: *E2A / PBX1, MLL / AF4, BCR / ABL* and *TEL / AML1*. For AML we focused to screen 4 common gene fusions: AML1/ *ETO, PML / RARA, CBFB / MYH11* and *MLL / AF9*.

III. RESULTS

3.1. Cytogenetic Characteristics in acute leukemia

All the bone marrow samples of the patients were cultured but only 19 samples (38%) were successful, the result of chromosome analysis as follows:

Table 3.1. Chromosome abnormalities in two group of Leukemia

Group	Total patients with DNA culture		Positive culture		Patients with chromosome abnormalities		Patients with normal chromosome	
	n	%	n	%	n	%	n	%
ALL	38	76.0	12	63.2	6	66.7	7	70.0
AML	12	24.0	7	36.8	3	33.3	3	30.0
Total	50	100	19	100	9	100	10	100

In 19 patients with positive results: 10 patients had normal chromosome, 9 patients had abnormal chromosomes (6 with ALL and 3 with AML)

3.2. Abnormalities of chromosomal ploidy in bone narrow

Table 3.2. Abnormalities of chromosomal ploidy in bone narrow

Number of chromosomes	ALL		AML	
	n	%	n	%
Hypodiploidy	2	33.3	1	33.3
Trisomy 21	2	33.3	0	0
Hyperdiploidy	2	33.3	2	66.7
Total	6	100	3	100

Abnormal chromosome is mainly in ALL group (6 patients) and AML group has 3 patients with abnormalities. Hypodiploidy and hyperdiploidy account for 33.3% respectively in ALL group. Two patients with trisomy 21 are in ALL group while there is no child with Down 's syndrome in AML group.

3.3. Detect gene fusions in ALL group

3.3.1. Four gene fusions in newly diagnosed patients with ALL

Table 3.3. Four gene fusions in newly diagnosed patients

Gene fusions	n	%
TEL/AML1	9	23.7
BCR/ABL	2	5.2
MLL/AF4	3	7.9
E2A/PBX1	4	10.5
No expression	20	52.6
Total	38	100

In 38 patients with ALL: 9 patients with TEL/AML1 (23.7%), 2 patients with BCR/ABL (5.2%), 3 patients with MLL/AF4 (7.9%), and 4 patients with E2A/PBX1 (10.5%). 20 patients (52.6%) have normal gene.

3.3.2. Classification into prognosis groups with 4 gene fusions in ALL

Table 3.4. Classification into prognosis groups with 4 gene fusions in ALL

Prognosis	Gene fusion	n	%
Good	TEL/AML1	9	23.7
Poor	BCR/ABL MLL/AF4 E2A/PBX1	9	23.7
Unclassified		20	52.6
Total		38	100

Good prognosis group has 9 cases (23.7%), and poor prognosis group also has 9 cases (23.7%). Unclassified group accounted for 52.6%.

3.3.3. The relationship between gene fusions and other factors in ALL

Table 3.5. Distribution of ALL according to gene fusions and 3 age groups

Age group	Gene fusion					Total
	TEL/AML1	BCR/ABL	MLL/AF4	E2A/PBX1	No expression	
Under 1 year	1	0	1	0	3	2 (5.2%)
1-10 years	6	1	2	3	12	12 (31.6%)
Over 10 years	2	1	0	1	5	4 (10.4%)
Total	9(23.7%)	2(5.2%)	3(7.9%)	4(10.5%)	20(52.6%)	20

Gene expression have difference frequency in 3 group. The highest frequency expresses in 1-10 years group (31.6%), and the lowest is under 1 year (5.2%).

3.4. Detect gene fusions in AML group

3.4.1. Four gene fusions in patients with AML by RT - PCR

Table 3.6. Four gene fusions in newly diagnosed patients AML

Gene fusions	n	%
AML1/ETO	1	8.3
PML/RARA	2	16.7
CBFB/MYH11	1	8.3
MLL/AF9	2	16.7
No expression	6	50.0
Total	12	100

In 12 cases with AML: 1 case has AML1/ETO, 2 cases have PML/RARA, 1 case has CBFB/MYH11 and 2 cases have MLL/AF9. There are 6 patients (50%) have normal gene.

3.4.2. Classification into prognosis groups with 4 gene fusions in AML

Table 3.7. Classification into prognosis groups with 4 gene fusions in AML

Prognosis	Gene	n	%
Good	*AML1/ETO* *PML/RARA* *CBFB/MYH11*	1 2 1	33
Poor	MLL/AF9	2	17
Unclassified		6	50
Total		12	100

Good prognosis accounted for 33% and poor prognosis accounted for17%.

IV. DISCUSSION

4.1. Genetic Characteristics in acute leukemia

Nowadays, cytogenetic plays an important role in the diagnosis and prognosis of acute leukemia. The cytogenetic abnormalities reported in acute leukemia involve both chromosomal ploidy and structural rearrangement. With limited conditions, the rate of successful chromosome culture in this study was only 38% (19 cases), this result is equivalent to that of Bui Ngoc Lan's study was 41.2% (40 / 97) and Nguyen Cong Khanh was 40.3% (67/142) [1]. In our study, there were 9 patients (18%) had abnormal chromosome compared to Nguyen Cong Khanh 's study was 40.3%.

4.2. Detect gene fusionss in ALL

For ALL, chromosomal abnormalities at diagnosis is an independent prognostic fator that helps to decide the treatment protocol. There are 4 common types of chromosomal translocation in ALL; the translocation of t (12; 21), t (9; 22), t (4; 11), t (1; 19) correspond with 4 types of the gene fusion as folow *TEL / AML1, BCR / ABL, MLL / AF9* and *E2A / PBX1* . Among these chromosomal translocation, the t (12; 21) is good prognosis group and 3 remaining types are poor prognosis group.

In the result, we detected 9 patients with expression of *TEL / AML1* (23.7%), 2 patients with *BCR / ABL* (5.2%), 3 patients with *MLL / AF4* (7.9%), and 4 patients with *E2A / PBX1* (10.5%). Yang L reported 148 children with ALL in Taiwan, the result was *TEL/AML1* (17.6%), *BCR/ABL*(8%), *MLL / AF4* (2%), and *E2A / PBX1* (4%) [7]. In Southeast Asia, Sri M studied a sample of 67 children with ALL in Indonesia, the result of gene fusion were *TEL / AML1* (22%), *BCR / ABL* (6%), *MLL / AF4* (0%), and *E2A / PBX1* (9%) [4]. In Vietnam, a study by Phan Nguyen Thanh Van using RT-PCR method on 179 patients with ALL at Hochiminh Hematology and Blood Transfusion Hospital (134 children and 45 adults) gave the results as follow: *TEL/ AML1* (9.5%), *BCR/ABL* (8.9%), *MLL/AF4* (2.8%), and *E2A/PBX1* (4.5%). [2]

4.3. Detect gene fusions in AML

For AML, chromosomal abnormalities at diagnosis has prognostic and therapeutic significance 4 types of common chromosomal translocation in AML are the translocation of t (8; 21) (q22, q22), t (15; 17) (q22; q11), inv (16) (p13; Q22), and t (9; 11) (p21-22; q23) correspond with 4 types of the gene fusion as follow: *AML1 / ETO, PML / RARA, CBFB / MYH11, MLL / AF9* . Among these chromosomal translocation, the t (8; 21) (Q22, Q22), t (15; 17) (Q22; q11), inv (16) (p13; Q22) are good prognosis group and t (9; 11) (p21-22; q23) is the poor prognosis group.

Using RT-PCR to detect the common genetic abnormalities in our study, we found the gene *MLL / AF9 (poor prognosis group)* accounting for 16.7%. This result is higher than in the report of Mrozek K et al with the rate was only 2.1% [3].

V. CONCLUSION

The frequencies of the genetic abnormalities of our patients are higher than that were found in the reported literature, so further study with larger sample size will be necessary to explore this further. RT-PCR is a useful and rapid tool to detect genetic abnormalities to improve the ability to accurately and rapidly risk-stratify children with ALL in our hospital. However, the price of RT-PCR test is too expensive and not covered by insurance. With the funding from Asian Children's Care League, we performed RT-PCR for the children with acute leukemia for 2 year.

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