**HUE UNIVERSITY**

**UNIVERSITY OF MEDICINE AND PHARMACY**

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**RESEARCH OF GENETIC ABNORMALITTIES IN DIAGNOSIS AND TREATMENT OF CHILDHOOD ACUTE LEUKEMIA AT HUE CENTRAL HOSPITAL**

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DISSERTATION LAYOUT

The dissertation is presented in 145 pages (references and appendix not include). It is structured with Introduction (2 pages), Literature review (39 pages), Material and Methods (21 pages), Results (37 pages), Discussion (43 pages), Conclusion (2 pages), and Recommendation (1 page).

The dissertation consists of 57 tables, 16 diagrams, 12 figures, and 193 references (12 in Vietnamese and 181 in English).

Appendix includes publications, references, technical procedures appendices, questionaires, patient list, some genetic analysis images and patient images.

INTRODUCTION

Acute leukemia is the most common cancer in children and adolescents, accounting for 25% of all new cancers diagnosed in children under 15 years of age. It is a disease of the hematopoietic system caused by uncontrolled proliferation of one or more malignant immature cell lines.

Diagnosis and classification of acute leukemia is based on clinical features combined with morphological malignancies, cytochemical staining, immunocytochemical markers, and genetic tests.

Regarding the treatment of childhood acute leukemia, the application of chemotherapy by risk group and genetic modification has significantly improved the survival rate of childhood patients, especially in acute lymphoblastic leukemia. In developed countries, the overall survival rate for acute lymphoblastic leukemia from 10% in the 1960s has increased to more than 90% in recent years. However, the long-term survival rate for acute myeloid leukemia remains challenging. Although the rate of remission after inductionphase of treatment is up to 94.6%, the 5-year disease-free survival rate still accounts for a modest rate of about 50 - 61.6%, recurrence still remains in the range of 20% to 41%. Therefore, the constant search for progressive treatments is essential in acute leukemia. In order to have advanced treatment methods, studying the molecular genetic characteristics of childhood acute leukemia lead to accurate diagnosis, classification of risk groups and improvement of treatment.

During the treatment of acute leukemia, drug metabolism in each patient plays an important role in drug dose adjustment and appropriate drug selection. Polymorphisms of the NUDT15 and TPMT genes have been shown to be involved in the metabolism of 6-mercaptopurine (6-MP), one of the main drugs of the acute lymphoblastic leukemia regimen.

In Vietnam, the implementation of gene tests on acute leukemia patients is carried out at a number of large centers, with the test to detect 4 types of common gene fusions by RT-PCR technique. The performance of NUDT15 and TPMT genetic tests on new acute lymphoblastic leukemia patients is carried out at a few centers.

The Pediatric Center of Hue Central Hospital was established in 2013 on the basis of the original pediatric department. Children with blood cancer have been treated in Hue since 2005. The implementation of genetic tests has been conducted since 2012 and the pharmacogenetic analysis has been carried out since 2018. Therefore, we conducted a study on the topic: "**Research on genetic abnormalities in the diagnosis and treatment of childhood acute leukemia at Hue Central Hospital**", with the following three objectives:

*1. Describe clinical and laboratory characteristics of childhood acute leukemia.*

*2. Determine genetic abnormalities in childhood acute leukemia.*

*3. Investigate the correlation between gene abnormalities and the treatment results of childhood acute leukemia*

Chapter 1: LITERATURE REVIEW

* 1. INTRODUCTION OF CHILDHOOD ACUTE LEUKEMIA

Acute leukemia is the most common cancer in children. The incidence of childhood acute leukemia is about 46.7 cases per million in Europe, ranging from 30 to 60 cases per million. For acute lymphoblastic leukemia (ALL), the peak incidence is at aged 2-5 years. For acute myeloid leukemia (AML), it does not have peak incidence. Boys get the disease more often than girls.

The exact cause of childhood acute leukemia is still undefined. Hypotheses on the pathogenesis focus mainly on two factors: genetic mutations and environmental effects

1.2. CLINICAL AND PARACLINICAL CHARACTERISTICS OF ACUTE LEUKEMIA

Clinically, patients present with syndromes: anemia, hemorrhage, infiltration, infection. In terms of subclinical, whole blood count changes (increase or decrease in white blood cell count, thrombocytopenia, low hemoglobin), biochemical tests (high uric acid level, high LDH level, elevated liver enzymes, kidney failure, electrolyte imbalance),the myelogram show blast cell infiltration.

1.3. GENE MUTATIONS OF CHILDHOOD ACUTE LEUKEMIA

Genetic abnormalities are mainly caused by structural chromosomal mutations, which can be caused by chromosomal translocations, deletions, inversions, gene reconstructions or point mutations. Among them, chromosomal translocation is the most common. In ALL, the most prevalent gene fusions are in the order: *TEL/AML1* gene fusion due to t(12;21)(p13;q22), *E2A/PBX1* gene fusion due to t(1;19) (q23;p13), *BCR/ABL1* gene fusion by t(9;22)(q34;q11), *MLL/AF4* gene fusion by t(4;11)(q21;q23), *SET/NUP214* fusion. translocation t(8,14)(q24;q32) and some genetic changes in T-cell lymphoblastic leukemia. In AML, common gene fusions include: *AML1/ETO* gene fusion due to t(8,21)(q22; q22), *PML/RARA* gene fusion due to t(15,17)( q22;q21), inversion of chromosome 16, inv(16)(p13;q22), *MLL/AF6* gene fusion by t(6;11)(q27;q23), *KMT2A/MLLT10* gene fusion by t( 10;11)(p12;q23). Gene fusions play an important role in diagnosis and prognostic risk grouping.

1.4. PROGNOSIS FACTORS OF ACUTE LEUKEMIA

In ALL, prognostic factors include: age, sex, race, genetic variation, white blood cell count, immune phenotypes, nutritional status, and treatment response. For AML, prognostic factors include: clinical manifestations, laboratory tests, genetic variations, and treatment response.

1.5. RISK FACTORS, DIAGNOSIS AND MANAGEMENT

In ALL, the risk group according to the United State National Cancer Institute classification criteria (age, white blood cell count), divided into three groups: normal risk, high risk, and acute lymphoblastic leukemia in infants.

Diagnosis of acute leukemia is based on clinical evidence, myelogram (blasts ≥ 20%), cytochemistry, and immunocytochemistry.

Regarding treatment, for ALL, modified CCG 1881 and 1882 protocols are being applied. For AML, 7 & 3 protocol is being applied (particularly for acute promyelocytic leukemia, separate treatment protocol are used).

**1.6. GENES RELATED TO METABOLISM OF 6-MERCAPTOPURINE**

*NUDT15* and *TPMT* polymorphisms play an important role in the metabolism of 6-MP, a medication that patients with ALL have taken for 2 to 3 years. The identification of drug metabolism based on two *NUDT15* and *TPMT* polymorphisms helps clinicians apply appropriate drug doses for patients.

**1.7. ASSESSMENT OF TREATMENT RESPONSE**

Assessment on remission after induction phase not only helps us to predict but also helps us to select the next treatment. Before that, we evaluate remission basing on morphology. Nowadays, we could evaluate on minimal residual disease (MRD).

Chapter 2: MATERIALS AND METHOD

2.1. MATERIALS

The study materials were 118 children diagnosed with acute lymphoblastic leukemia and acute myeloid leukemia, treated at Pediatric Oncology - Hematology – Bone Marrow Transplant Department, Pediatric Center - Hue Central Hospital, from 11/2017 to 6/2022.

* + 1. **Inclusion criteria**
* The patient was diagnosed with acute lymphoblastic leukemia or acute myeloid leukemia for the first time.
* Age < 16
* All patients were done multiplex RT-PCR genetic analysis, with the Hemavision 28N kit, 28 basic genetic mutations in acute leukemia were detected.

**Criteria for diagnosis of acute leukemia:** Clinical: Systemic symptoms: fever, fatigue, poor appetite. Anemia, bleeding under the skin or mucous membranes. Symptoms of extramedullary infiltrates: liver, spleen, lymphadenopathy, gingival hypertrophy, subcutaneous papules, central nervous system infiltrates, mediastinal infiltrates, or testicular infiltrates.

Peripheral blood count: There is usually a decrease in hemoglobin (Hb), the white blood cell count may be elevated, normal or decreased, but often there is a severe decrease in neutrophils, and peripheral bleeding may be visible or not. The platelet count is usually reduced.

Myelogram: Blast cells in the marrow ≥ 20%. This is the gold standard for the diagnosis of acute leukocytosis

**Diagnostic criteria for childhoodnacute lymphocytic leukemia**

Cytochemistry: PAS (+), Soudan-black (-), Peroxydase (-).

Diagnostic criteria based on cytoplasmic CDs

**B cell:** CD19 is strongly positive with ≥ 1 strongly positive markers for: CD79a, CD22, CD10 or CD19 is weakly positive with ≥ 2 strongly positive markers such as: CD79a, CD22, CD10.

**T cell:** CD3 positive and at least one of the markers CD2, 5, 7, or 8.

**Diagnostic criteria for childhood acute myeloid leukemia**

Cytochemistry: Usually positive for MPO, Soudan - Black, negative for PAS.

Immune markers consistent with acute myeloid leukemia: MPO, CD13, CD33, CD117, ±CD14. CD15 [61]..

* + 1. **Exclusion criteria**
* Pediatric patients with secondary or relapsed acute leukemia
* The child and the representative did not agree to participate in the study.
  1. RESEARCH METHOD
     1. **Research design**

Descriptive, prospective longitudinal study. The longitudinal follow-up time to the end of the study was June 1, 2022.

* + 1. **Sampling method**

Convenient sample size: All new pediatric patients diagnosed with acute lymphocytic leukemia or acute myeloid leukemia were hospitalized during the study period.

* + 1. **Research content**
       1. ***Description of clinical and subclinical characteristics of childhood acute leukemia***

- General characteristics of the research group: Age, gender, geography, classification of diseases according to immunity, classification of subgroups and classification of risk groups.

- Describe the clinical features: symptom onset, time from onset to hospital admission, common and uncommon clinical manifestations in acute leukemia.

Describe the paraclinical characteristics: complete blood count (white blood cells, granulocytes, platelets, hemoglobin), immature white blood cells in the peripheral blood, bone marrow changes. The biochemical changes: LDH, uric acid, liver function, kidney function. Changes in blood clotting function.

* + - 1. ***Identification of genetic variations in childhood acute leukemia***

- Determine the rate of some significant gene fusion mutations in childhood acute leukemia. Based on 118 pediatric patients, multiplex-RT-PCR tests were performed (kit capable of detecting 28 gene mutations). Detect gene mutations in each disease group and divide gene mutations according to diagnosis, subclassification.

- Identification of *NUDT15* and *TPMT* polymorphisms in 72 acute lymphocytic leukemia patients. Allele and genotype frequency analysis of *NUDT15* and *TPMT* gene polymorphisms*.*

***2.2.3.3. Investigating the relationship between gene mutations and treatment outcomes for childhood acute leukemia.***

- Determine the rate of remission, relapsed rate, the overall survival rate and the event free survival rate in childhood acute leukemia

+ Conduct to determine the rate of remission, the rate of relapse in each group of ALL and AML.

+ Calculate the overall survival and event free survival rates in each group of ALL and AML for a period of 3 years.

- Find the relationship between gene mutations and treatment results:

+ Relationship between gene mutation and treatment results after induction phase.

+ Find the relationship between gene mutations and relapse in each disease group.

+ Find the relationship between gene mutations and the overall survival rate and the event free survival rate in each disease group.

+ Find out the relationship between *NUDT15* and *TPMT* gene polymorphisms with the overall survival rate and the event free survival rate in the acute lymphoblastic leukemia.

* 1. **EVALUATION CRITERIA**

- Assess for complete remission: granulocyte count > 1.5x109/l, platelet count > 100.000x109/l. The marrow is normal, with moderate or reduced cell density, < 5% blast cells. No recurrence within 4 weeks. Non remission: myelogram still present ≥ 20% blast cells. Evaluation of partial remission: 5-20% blasts are present in the myelogram.

**-** Bone marrow relapse: after achieving remission over 4 weeks, the patient appeared anemia, fever, hepatosplenomegaly. Lumbar puncture shows blast cells ≥ 20%. Central nervous system relapse: with or without headache, nausea. Cerebrospinal fluid shows more than 5 leukocytes/mm3, and blast cells, or intracranial tumors, or cranial nerve damage. Testicular relapse: testicular swelling, pain, fine-needle testicular biopsy showed blast cells.

**Chapter 3. RESULTS**

3.1. CLINICAL AND PARACLINICAL CHARACTERISTICS OF ACUTE LEUKEMIA

3.1.1. General characteristics

- Mean age of childhood acute lekemia: 5.8 ± 4.0 years old.

- B-ALL: 70 (59.3%), T-ALL: 13 (11.0%), AML: 35 (29.7%). For ALL, the standard risk is 51 (61.4%), high risk: 32 (38.6%), the age group from 1-5 years old accounts for the highest rate is 57.8%. In contrast, for AML, there is no peak incidence.

- Male patients: 71 (60.2%), female patients: 47 (39.8%).

- The majority of patients live in rural areas, accounting for 68.6%.

3.1.2. Clinical presentations in childhood acute leukemia

- The onset of symptoms in patients with acute leukemia include: fever (57.6%), pale skin 32.2%, hemorrhage 21.2%, lymphadenopathy 18.6%, joint pain 15.3%. These reasons account for a different proportion between the two groups of acute lymphoblastic leukemia and myeloid leukemia, but there is no statistical significance (p>0.05). Except for joint pain, which was more common in the acute lymphoblastic leukemia group than in the myeloid leukemia group, 20.5% vs 2.9% respectively, there was statistical significance (p < 0.05).

- Mean time from first symptom onset to hospital admission for acute leukemia group was 14 days. Mean time for ALL and AML was 14.0 and 10.0 days, respectively. The difference was not statistically significant (p>0.05).

- The most common clinical manifestations in acute leukemia were anemia (87.3%), hepatomegaly (48.3%), lymphadenopathy (44.9%), fever (44.9%), spleen large (41.5%), hemorrhage (39.8%) and bone pain 921.2%). Less common symptoms include: testicular involvement (2.8%), gingival hyperplasia (1.7%) and leukemia cutis (0.7%).

3.1.3. Paraclinical characteristics of childhood acute leukemia

- Whole blood count showed that 28.8% of patients had leukocyte count ≥ 50x109/l, 43.2% of patients had neutrophil count < 500/μl. Hb concentration in the range of 7-11 g/dl accounted for the highest rate, 62.7%. Platelets < 20x109/l accounted for 24.6%. The ratio of levels of leukocytes, neutrophils, Hb levels as well as platelet counts between the two disease groups was not statistically significant (p>0.05).

- There were 20.3% of acute leukemia patients without presence of blast cells in peripheral blood, 29.7% of patients had blast cell rate from 1 to < 25%, 21.2% of patients had blast cell rate 25-50% and 28.8% of patients had a ratio of blast cells > 50%. The difference in the distribution of blast cells in peripheral blood between two groups of ALL and AML was not statistically significant (p>0.05).

- There were 65,3% patients with the number of blast cells rate in bone marrow ≥ 50%, and 34,7% patients with that rate from 20 - <50%.

- The median of bone marrow cells in acute leukemia, ALL and AML were 100.0 x109/l, 100.0 x109/l and 60.0 x109/l respectively. The difference of that in ALL and AML group was not statistically significant (p>0.05).

**-** There were 89.4% patients with increased LDH, 16.8% of patients with increased uric acid, 35.7% of patients with elevated liver enzymes, 0.9% of patients with renal failure and 25.0% of patients with elevated CRP.

3.2. DETERMINE GENETIC ABNORMALITIES IN CHILDHOOD ACUTE LEUKEMIA

**3.2.1. Genetic variants in childhood acute lymphoblastic leukemia**

Table 3.1. Common genetic variants in ALL

|  |  |  |
| --- | --- | --- |
| **Gene fusions** | **Number** | **Percentage (%)** |
| *TEL/AML1*- t(12;21)(p13;q22) | 10 | 12,1 |
| *BCR/ABL*- t(9;22)(q34;q11) | 4 | 4,8 |
| *E2A/PBX1* – t(1;19)(q23;p13) | 3 | 3,6 |
| *MLL/AF4*- t(4;11)(q21;q23) | 2 | 2,4 |
| *SET/NUP214*- t(9;9)(p34;q34) | 1 | 1,2 |
| Unexpressed | 63 | 75,9 |
| Total | 83 | 100,0 |

- The results of the analysis with multiplex PCR showed that there were 24.1% patients with genetic abnormalities in the following order: *TEL/AML1* (12.1%), *BCR/ABL1* (4.8%), *E2A/PBX1* (3.6%), *MLL/AF4* (2.4%) and *SET/NUP214* (1.2%).

- Prognostic grouping according to genetic mutation: good prognosis (12.1%), intermediate prognosis (80.7%), poor prognosis (7.2%).

- The difference in the distribution rate of gene fusion between the two groups of standard and high risk, or between the two groups B-ALL and T-ALL was not statistically significant. (p>0,05).

**3.2.2. Genetic variants in childhood acute myeloid leukemia**

Table 3.2. Common genetic variants in AML

|  |  |  |
| --- | --- | --- |
| **Gene fusion** | **Number** | **Percentage (%)** |
| *AML1/ETO* – t(8;21)(q22;q22) | 5 | 14.2 |
| *AML1/ETO + BCR/ABL1* | 1 | 2.9 |
| *PML/RARA* – t(15;17)(q22; q22) | 3 | 8,6 |
| *MLL/AF6*- t(6;11)(q27;q23) | 2 | 5,7 |
| *KMT2A/MLLT10* – t(10;11)(p12;q23) | 1 | 2,9 |
| Unexpressed | 23 | 65,7 |
| Total | 35 | 100,0 |

- The most common gene fusions were in the following order: *AML1/ETO* (14.2%), *PML/RARA* (8.6%), *MLL/AF6* (5.7%), *KMT2A/MLLT10* (2.9%), *AML1/ETO* and *BCR/ABL1* ( 2.9%).

- Prognostic grouping according to genetic mutation: good prognosis (22.9%), intermediate prognosis (65.7%) and poor prognosis (11.4%).

**3.2.3. Pharmacogenetic variants during the treatment of acute lymphoblastic leukemia:**

- There were 72 patients with ALL tested for *NUDT15* and *TPMT*. The test results showed that 18.1% of patients with *NUDT15* polymorphism. The detection rate of *NUDT15* polymorphism in the two groups B-ALL and T-ALL was 20.0% and 8.3%, respectively. This difference was not statistically significant (p>0.05). In addition to allele *NUDT*15\*1 (90.2%), other alleles were also detected such as: NUDT15\*3 (6.3%), NUDT15\*6 (2,1%), NUDT15\*2 (0.7%) and NUDT15\*5 (0,7). There were genotypes in the following order: *NUDT15*\*1/\*1 (81,9%), *NUDT15*\*1/\*3 (11,1%), *NUDT15*\*1/\*6 (4,2%), *NUDT15*\*1/\*2 (1,4%) and *NUDT15*\*3/\*5 (1,4%).

- There were 6.9% patients with *TPMT* polymorphism, in which the frequency of TPMT\*1 allele was the majority (96.5%), TPMT\*3C allele (3.5%). In addition to the TPMT\*1/\*1 genotype (93.1%), only one TPMT\*1/\*3C genotype (6.9%) was detected.

**Table 3.1. 6-MP metabolism based on NUDT15 and TPMT gene polymorphism**

|  |  |  |
| --- | --- | --- |
| **6-MP metabolism** | **Number** | **Percentage (%)** |
| Normal metaboliser | 55 | 76,4 |
| Intermediate metaboliser based on 1 gene polymorphism | 12 | 16,6 |
| Possible intermediate metaboliser based on 1 gene polymorphism | 1 | 1,4 |
| Intermediate metaboliser based on 2 gene polymorphisms | 1 | 1,4 |
| Indeterminate | 3 | 4,2 |
| Total | 72 | 100 |

**3.3.** **SURVEYING THE RELATIONSHIP BETWEEN GENETIC VARIATIONS AND CHILDHOOD ACUTE LEUKEMIA TREATMENT OUTCOME**

**3.3.1. Determine the rate of remission, relapse, the overall survival and even free survival in childhood acute leukemia**

- Results showed that 95% of ALL patients recovered based on blast cell count < 5% after induction phase. The remission rate for the standard risk group is usually 100%, while the figure for the high-risk group is 86.7%. The difference was statistically significant (p<0.05).

**-** Regarding AML group, the results showed that 73.1% of patients achieved remission, 15.4% of patients had partial remission and 11.5% of patients did not.

- The relapsed rate in the acute leukemia group was 19.9%, in which the bone marrow relapse rate was 11.9%, the central nervous system relapse was 6.0%, and the combination of central nervous and medulla reslapse were 1.0%, and soft tissue recurrence was 1.0%. There was a difference in the recurrence rate between the two groups. The AML group had a high relapsed rate, 40.9%, compared with the ALL group of 14.0%. AML has a higher rate of bone marrow relapse than ALL, 36.4% compared with 5.1%. The ALL group had a higher rate of CNS relapse than AML, 7.6% compared to 0%. This difference is statistically significant (p<0.05).

- Out of total 118 patients, 30 patients died during the study period. The number of patients alive was 88, accounting for 74.6%. The mean overall survival time during follow-up was 40.8 ± 2.1 months. At 1 year, 2 years, and 3 years, the overall survival rate for acute lekemia patient was 82.2 ± 3.6%, 75.1 ± 4.5%, and 67.4 ± 5.5%, respectively.

- At 3 years, the overall survival rate of B-ALL, T-ALL and AML was: 77.6 ± 6.7%, 68.4 ± 15.8%, and 45.5 ± 10.2%, respectively. P<0.05 was considered statistically significant.

- At the time of 1 year, 2 years, and 3 years, the event free survival rate of childhood acute leukemia was 81.1 ± 3.7%, 71.1 ± 4.8%, and 69.0 ± 5.1%, respectively.

- At the time of 3 years, the event free survival rate of B-ALL, T-ALL and AML was 79.5 ± 6.1%, 70.3 ± 14.8% and 44.6 ± 10.0%, respectively. There was a statistically significant correlation between them, p<0.05.

**3.3.2. The correlation between genetic abnormalities and the rate of remission, relapse, the overall survival and event free survival rate in childhood acute leukemia**

***3.3.2.1.******The correlation between genetic abnormalities and the rate of remission in childhood acute lymphoblastic leukemia***

**-** Remission rates varied between groups, in which the poor prognosis group accounted for the lowest (66.6%), 98,4% for the intermediate prognosis group, and 90,0% for the good prognosis group. The difference was statistically significant (p<0.05).

- The group with good prognosis and intermediate prognosis had the rate of MRD<10-4 after induction therapy was 88.9% and 92.2%, respectively, while this rate was only 50% in the group with poor prognosis. There was statistically significant difference between the three groups (p<0.02).

***3.3.2.2. The correlation between genetic abnormalities and the rate of remission in childhood acute myeloid leukemia***

**-** The poor prognosis had the lowest remission rate (33.3%). That rate was 66.7% in the intermediate prognosis and 100% in the good prognosis group. There was not a statistically significant correlation between them, p>0.05.

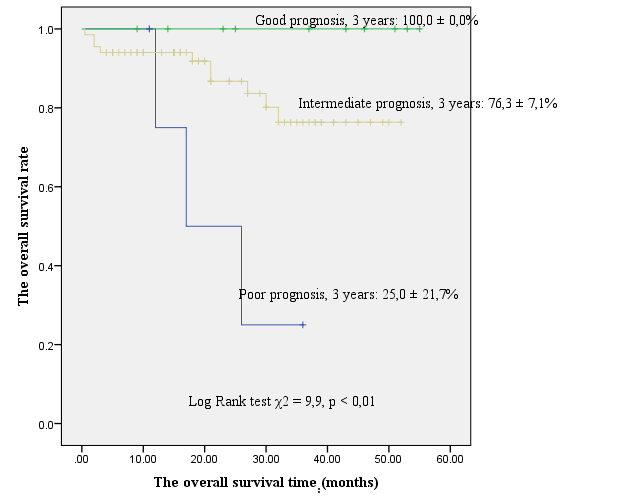
***3.3.2.3. Evaluation of acute lymphoblastic leukemia relapse rate basing on genetic risk groups***

- There was a difference in the relapse rates between the genetic risk groups, in which this rate of the poor prognosis group was highest (66.7%), followed by the intermediate prognosis group (11.1%), while the group with good prognosis did not show any recurrence. The difference was statistically significant (p<0.05).

***3.3.2.4. Evaluation of acute myeloid leukemia relapse rate basing on genetic risk groups***

- The relapse rates were different between the genetic risk groups. The poor prognosis group had the highest relapse rate (66.7%), while the figures for the intermediate prognosis group and good prognosis group were 66,7% and 26,8%, respectively. The difference was not statistically significant (p>0.05).

***3.3.2.5. Evalution of the overall survival rate according to genetic risk group in acute lymphoblastic leukemia***

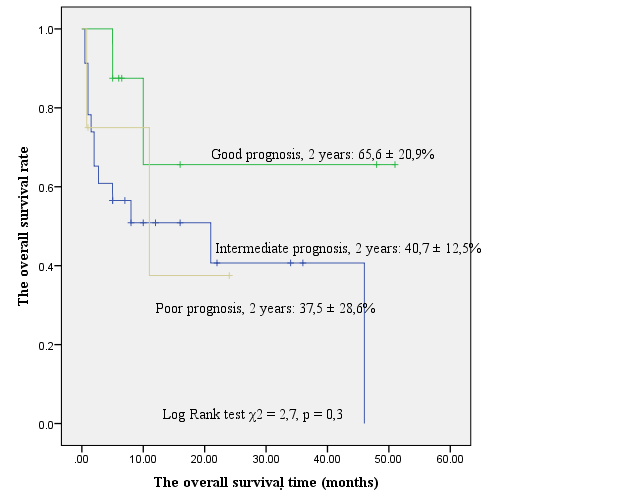


|  |  |  |  |
| --- | --- | --- | --- |
|  | 1 year | 2 years | 3 years |
| Poor prognosis | 75.0 ± 21.7% | 50.0 ± 25.0% | 25.0 ± 21.7% |
| Intermediate prognosis | 94.0 ± 2.9% | 86.7 ± 4.9% | 76.3 ± 7.1% |
| Good prognosis | 100.0 ± 0.0% | 100.0 ± 0.0% | 100.0 ± 0.0% |

**Figure 3.1**. The overall survival curve by genetic risk group in ALL

- At the end of the study, out of a total of 83 patients with ALL, there were 13 patients died, of which 3/6 patients belonged to the poor prognosis group, 10/67 patients belonged to the intermediate prognosis group and no patient in the good prognosis died. The number of surviving patients is 70 patients, accounting for 84.3%. At 3 years, the overall survival for the group with good, intermediate, and poor prognosis were 100.0 ± 0.0%, 76.3 ± 7.1%, and 25.0 ± 21.7%, respectively. The difference was statistically significant (p<0.05).

***3.3.2.6. Evalution of the overall survival rate according to genetic risk group in acute myeloid leukemia***

****

|  |  |  |  |
| --- | --- | --- | --- |
|  | 1 year | 2 years | 3 years |
| Poor prognosis | 37.5 ± 28,6% | 37.5 ± 28.6% | - |
| Intermediate prognosis | 56.5 ± 10.3% | 40.7 ± 12.5% | 40.7 ± 12.5% |
| Good prognosis | 65.6 ± 20.9% | 65.6 ± 20.9% | 65.6 ± 20.9% |

Figure 3.2. The overall survival curve by genetic risk group in AML

- At the end of the study, out of a total of 35 AML patients, there were 17 patients died. The number of surviving patients is 18, accounting for 51.4%. At 2 years, the poor prognosis group had the overall survival rate of 37.5 ± 28.6%, the figure for intermediate and favorable prognosis group were 40.7 ± 12.5% and 65.6 ± 20.9% respectively. At 3 years, the overall survival rate in the intermediate prognosis group was 40.7 ± 12.5%, and the good prognosis group was 65.6 ± 20.9%. In the poor prognosis one, no patient survived to 3 years. However, the difference was not statistically significant (p>0.05).

***3.3.2.7. The event free survival rate bases on genetic risk group in childhood acute lymphoblastic leukemia***

- At 2nd years, the event free survival rate in the groups with good, intermediate and poor prognosis were: 100 ± 0.0%, 81.5 ± 5.8%, and 27.8 ± 23.2, respectively. The difference was statistically significant (p<0.05).

***3.3.2.8. The event free survival rate bases on genetic risk group in childhood acute myeloid leukemia***

- At 1st year, the event free survival rate of the poor-prognostic group was 37.5 ± 28.6%, and that of the intermediate prognosis group and the good prognostic group were 38.0 ± 12.3% and 65.6 ± 20.9%, respectively. However, this difference was not statistical significance (p>0.05).

***3.3.2.9****.* ***The overall survival rate and the event free survival rate with pharmacogenetic changing***

- At 3rd year, the overall survival rate and the event free survival rate of the *TPMT* polymorphism group and the group without *TPMT* polymorphism were 66.7 ± 27.2%, 75.0 ± 21.7% and 84.8 ± 5.9%, 86.0 ± 5.5%. Similarly, at the 3rd year, the overall survival rate and the event free survival rate of the *NUDT15* polymorphism group and the group without *NUDT15* polymorphism were 90.0 ± 9.5%, 90.0 ± 9.5% and 81.7 ± 7.1%, 83.0 ± 6.7%. These differences were not statistical significance (p>0.05).

Chapter 4

DISCUSSION

4.1. CLINICAL AND PARACLINICAL CHARACTERISTICS OF ACUTE LEUKEMIA

**4.1.1. General characteristics**

The mean age was 5.8 ± 4.0 years old, which is similar to Robazzi’s result, the mean age was 6,18 years old. For ALL, children aged 1 to 5 accounted for 57.8%. For AML, it does not have peak incidence. These results are similar to those of local and foreign researchers.

The male to female ratio was 1.5:1, similar to Robazzi’s study which showed that male accouted for 59.4% and female accounted for 40.1%. 68,6% patients came from rural areas. According to Cha, children who live nearby farm or exposure with pesticide have higher incidence of cancer. Beside that, people is living in central zone of Vietnam where the war was happened in the past, exposured with dioxin and agent orange which could cause cancer.

There was 70.3% patients with ALL and 29.7% patients with AML. According to Robazzi and Sinigaglia, the percentages of ALL were 77.1% and 83.6% respectively, the percentages of AML were 22.9% and 16.3% respectively. For ALL, B-ALL accounted for 84.3% and T-ALL accounted for 15.7, which is similar to Shalal’s result. ALL-standard risk that accounted for 61.4% was higher than ALL-high risk with 38.6%. Our result is similar to Jonavoska’s research.

4.1.2. Clinical presentations in childhood acute leukemia

The onset of symptoms were fever (57.6%), pallor (32.2%), hemorrhage (21.2%), enlarged lymph nodes (18.6%) and bone pain (15.3%). These are similar to Shahab’s result.

The mean time from symptom onset to hospital admission was 14 days, similar to Nwannadi’s.

The most common symptoms were anemia (87.3%), hepatomegaly (48.3%), enlarged lymph nodes (44.9%), fever (44.9%), splenomegaly (41.5%), hemorrhage (39.8%) and bone pain (21.2%). In our research, bone pain. In our result, the percentage of bone pain in ALL was higher of that in AML (26.5% vs. 8,6%). The difference has statistical (p<0,05). Our result is similar to Colby-Graham’s result. Testicular involvement accounted for 2.8%. According to foreign researchers, testicular involvement accounted for 1.1-2.4% at the time of diagnosis. There were some uncommon symptoms such as: gingival hyperplasia (1.7%) and leukemia cutis (0.8%).

**4.1.3. Paraclinical characteristics of childhood acute leukemia**

Regarding peripheral blood, there were 28.8% of patients with white blood cell count ≥ 50x109/l, similar to some other local and foreign researchers, white blood cell count accounted from 19.4 to 34.5%. Hb from 7-11 g/dl accounted for 62.7%. According to Barbosa, Hb < 11 g/dl accounted for 88%. Moreover, 24.6% of patients had PLT < 20 x109/l, which is similar to Shalal’s result, 25.5% of ALL patients had PLT < 20 x109/l.

The percentage of blast cells in peripheral blood ≥ 25% accounted for 50%. According to Dai, B-ALL patient with low blast cells in peripheral blood has longer term survival than that who have higher blast cells. In contrast to T-ALL, patient who has low blast cells in peripheral blood has shorter term survival.

The median of bone marrow cells was 100,0 x109/l and there was not any difference between ALL and AML patients. Our result is similar to Nguyen Tu Hung’s result.

89.4% of patients showed increased LDH, 16.8% of patients had elevated uric acid level. According to Sivinir, there was 12.6% of patient with elevated uric acid level. In our result, 35.7% of patients had elevated liver enzyme. Blast cells could infiltrate liver and cause elevated liver enzyme. Renal failure that was diagnosed in one case accounted for 0.9%. The most common cause of kidney failure is tumor lysis syndrome, where cancer cells die on their own in large number, or under the influence of chemotherapy. Beside that, some medicines or sepsis shock could cause renal failure. 25% of patients had elevated CRP. CRP is a marker to evaluate infection.

4.2. DETERMINE GENETIC ABNORMALITIES IN CHILDHOOD ACUTE LEUKEMIA

4.2.1. Genetic variants in childhood acute lymphoblastic leukemia

The results of the analysis with multiplex PCR showed that there were 24.1% patients with genetic abnormalities including 12.1% of patients had *TEL/AML1* fusion, 4.8% of patients had *BCR/ABL1* fusion, 3.6% of patients had E2A/PBX1 fusion, 2.4% of patients had MLL/AF4 fusion and 1.2% of patients had SET/NUP214 fusion. With the multiplex-PCR, our result found new fusion comparing with other previous results in Vietnam. It was *SET/NUP214* fusion which is rare fusion gene and often occurs in T-ALL, at the aged 10-11. The patient with *SET/NUP214* will have high percentage of relapse. Until now, *SET/NUP214* is still poorly understood.

Basing on genetic abnormalities, some authors such as Inaba, Pui classify patients into three risk genetic group: favorable prognosis, intermediate prognosis and poor prognosis. In our result, there were 12.1% patients with favorable prognosis, 80.7% patients with intermediate prognosis and 7.2% patients with poor prognosis.

4.2.2. Genetic variants in childhood acute myeloid leukemia

The results of the analysis with multiplex PCR showed that there were 34.3% patients with genetic abnormalities including 14.2% patients with *AML1/ETO*, 8.6% patients with *PML/RARA*, 5.7% patients with *MLL/AF6*, 2.9% patient with *KMT2A/MLLT10*. New point in our result was that one patient had two fusion genes: *AML1/ETO* and *BCR/ABL1*. This was a special case and reported the first time in Vietnam. For AML patient, the presence of *BCR/ABL1* fusion gene is rare. And the combining of *AML1/ETO* and *BCR/ABL1* is extreme rare. According to foreign literatures, there is a few case having of *AML1/ETO* and *BCR/ABL1*. And these patients will have poor prognosis. Beside that, our result also found two fusion genes which haven’t reported in children yet. They were *MLL/AF6* fusion and KMT2A/MLLT10 fusion genes.

Similar to ALL patients, basing on genetic abnormalities, Quessada classify patients into three risk genetic group: favorable prognosis, intermediate prognosis and poor prognosis. In our result, there were 22.9% patients with favorable prognosis, 65.7% patients with intermediate prognosis and 11.4% patients with poor prognosis.

**4.2.3. Pharmacogenetic variants during the treatment of acute lymphoblastic leukemia**

In ALL group, there were 72/83 patients who were done *NUDT15* and *TPMT* analysis at the same time. The results showed that 18.1% patients have *NUDT15* polymorphisms, 6.9% patients have TPMT polymorphisms 1.4% patients have both *NUDT15* and *TPMT* polymorphisms. This is a new point in our results.

The table 3.3 described the capacity of 6-MP metabolism and when we know the capacity of each phenotype, we will indicate the dose for patients. So, the definition of *NUDT*15 and *TPMT* polymorphisms plays an important role for each patient during treatment.

**4****.3. SURVEYING THE RELATIONSHIP BETWEEN GENETIC VARIATIONS AND CHILDHOOD ACUTE LEUKEMIA TREATMENT OUTCOME**

**4.3.1. Determine the rate of remission, relapse, the overall survival and even free survival in childhood acute leukemia**

The rate of remission after induction phase for ALL and AML are 95% and 73.1% respectively. The rate of ALL remission is similar to that of other researchers all over the world, the rate achieves 90-98%. Regarding AML, our rate is lower than that of Bui Thi My Huong, the rate achieved 89,4%.

The relapsed rate in childhood acute leukemia was 19,9%, in which, the relapsed rate for ALL and AML were 14% and 40.9% respectively. According to Nguyen Thi Mai Huong, the relapsed rate for ALL-high risk was 16,7%. According Pedram, the relapsed rate for AML patient was 34,0%. Therefore, for AML, the relapsed rate is still high, and how to improve the survival rate for patient is still a challenge.

The overall survival rate for childhood acute leukemia after 3 years were 67.4 ± 5.5%, in which, the overall survival rate for B-ALL, T-ALL and AML were 77.6 ± 6.7%, 68.4 ± 15.8% and 45.5 ± 10.2% respectively. The difference has statistical (p<0,05).

According to Escherich, the overal survival for ALL with COALL 92 and 97 protocol after 5 years were 81.1 ± 1.8% và 85.4 ± 1.4%. For AML group, our overal survival rate was lower than that of Waack, the overall survival rate after 3 years were 82 ± 3%.

The event free survival rate for childhood acute leukemia after 3 years were 69.0 ± 5.1%, in which, the overall survival rate for B-ALL, T-ALL and AML were 79.5 ± 6.1%, 70.3 ± 14.8% and 44.6 ± 10.0% respectively. The difference has statistical (p<0,05).

According to Nguyen Thi Mai Huong, the event free survival rate for ALL-high risk after 5 years were 46.0 ± 5.0%. According to Escherich, the event free survival for ALL with COALL 92 and 97 protocol after 5 years were 73.2 ± 2.0% and 76.7 ± 1.7% respectively. For AML group, our event free survival rate was lower than that of Waack, the event free survival rate after 3 years with AML-BFM2012 were 69 ± 4%. So, we have to improve the quality of treatment.

**4.3.2. The correlation between genetics abnormalities and the rate of remission, relapse, the overall survival and even free survival in childhood acute leukemia**

***4.3.2.1. The correlation between genetics abnormalities and the rate of remission in childhood acute lymphoblastic leukemia***

The result showed that the percentage of remission differed from three risk genetic group. The rate of remission for the poor prognosis group was 66.6%, the favorable prognosis group achieved 90.0% and the intermediate prognosis group was 98.4%. The difference has statistical (p<0.05). Our results are similar to some studies by Pui, Lin, Toksvang and Cimino. So, genetic abnormalities made a tremedous impact on the result of treatment.

The good prognosis group had 88.9% MRD < 10-4 after induction phase and that percentage was just 50% for the poor prognosis group and that was 92.2% for the intermediate prognosis group. The difference has statistical (p<0.05). Our results are similar to the study by Borowitz. The percentage of MRD < 10-4 after induction phase in patients with *TEL/AML1* fusion gene is 87,9%, however, that percentage is only 70,7% for patients with MLL rearrangement.

***4.3.2.2. The correlation between genetic abnormalities and the* *rate of remission in childhood acute myeloid leukemia***

The rate of remission in the poor prognosis group, the intermediate prognosis group and the good prognosis group were 33.3%, 66.7% and100%. However, the difference is not statistically significant (p>0.05). That result illustrated that beside the impact of genetic abnormalites, supportive care and infection control play an important role in AML treatment.

***4.3.2.3. Evaluation of acute lymphoblastic leukemia relapse rate basing on genetic risk group***

The poor prognosis group had the highest relapsed percentage, 66.7%, the intermediate prognosis group had 11.1% relapse and the good prognosis group had no relapse. The difference has statistical (p<0.05). Our results are similar to the study by Sanchez, the poor prognosis has the highest relapsed rate. 23% patients with *BCR/ABL1* relapsed after achieving remission, in which, there is 10% of CNS relapse or CNS and bone marrow relapse.

***4.3.2.4. Evaluation the relapsed rate in childhood acute myeloid leukemia basing on genetic risk group***

The relapsed rate of the poor prognosis group, the intermediate prognosis group and the good prognosis group were 66.7%, 41.7% and 28.6%. The difference does not has statistical significance (p>0,05). According to Henris, there are some factors that predict relapse in AML after transplant are: the condition of bone marrow and patient before transplant, genetic abnormalities, type M4/M5.

***4.3.2.5. Evalution of the overall survival rate according to genetic risk group in acute lymphoblastic leukemia***

The overall survival rate for the poor prognosis, intermediate prognosis and good prognosis risk group after 3 years were 25.0 ± 21.7%, 76.3 ± 7.1% and 100.0 ± 0.0%. The difference is statistically significant (p<0,05). Our results are similar to the study by Pui, the overall survival rate for patients with *BCR/ABL1* fusion gene was 48,0 ± 2,0%, and the patients without *BCR/ABL1* fusion genes was 94,5 ± 1,8%.

***4.3.2.6. Evalution of the overall survival rate according to genetic risk group in acute myeloid leukemia***

The overall survival rate for childhood acute myeloid leukemia with intermediate prognosis risk group and favorable prognosis risk group after 3 years were 40.7 ± 12.5% and 65.6 ± 20.9% respectively. However, there was not any patient with poor prognosis risk group that was still alive. The difference is not statistically significant (p>0,05). Our results differed from the study by Cho, Pui and Rubnitz.

***4.3.2.7. The event free survival rate bases on genetic risk group in childhood acute lymphoblastic leukemia***

The event free survival rate for the poor prognosis, intermediate prognosis and favorable prognosis risk group after 2 years were 27.8 ± 23.2%, 81.5 ± 5.8% and 100.0 ± 0.0%. The difference is statistically significant (p<0,05). Our results are similar to the study by Jeha, Pui.

***4.3.2.8. The event free survival rate bases on genetic risk group in childhood acute myeloid leukemia***

The event free survival rate for the poor prognosis, intermediate prognosis and favorable prognosis risk group after 1 year were 35.7 ± 28.6%, 38.0 ± 12.3% and 65.6 ± 20.9%. However, the difference has no statistical (p>0,05).

Our results differed from the study by Cho, Pui and Rubnitz who illustrated that there is a corelation between genetic risk group and the event free survival. Our result demonstrated that AML treatment is so complicated, and supportive care plays an important role, how to control infection and reduce mortality is very necessary.

***4.3.2.9. The overall survival time and the event free survival time with pharmacogenetic changing***

*NUDT15* and *TPMT* polymorphisms do not affect the overall survival time and the event survival time in ALL. These results are similar to the study Liang, there is not a statistically significant difference between *NUDT15* and *TPMT* wide type and *NUDT15* and *TPMT* variants.

CONCLUSION

Based on research on 118 childhood acute leukemia including 83 acute lymphoblastic leukemia and 35 acute myeloid leukemia at Hue Pediatric Center- Hue Central Hospital, we reach these conclusions:

1. **Clinical presentations and laboratory findings in childhood acute leukemia**

* The mean age was 5,8 ± 4,0. For ALL, the peak incidence was at the aged 1-5 (57.8%). For AML, it doesnot have peak incidence. The male to female ration was 1.5:1
* AML accounted for 29.7% and ALL accounted for 70.3%, in which, the percentage of B-ALL is higher than the percentage of T-ALL (84.3% vs. 15.7%).
* The most common symptoms in childhood acute leukemia were: anemia (87.3%), hepatomegaly (48.3%), enlarged lymph nodes (44.9%), fever (44.9%), splenomegaly (41.5%), hemorhage (39.8%), bone pain (21.2%). There were some uncommon symptoms such as: testicular involvement (2.8%), gingival hyperplasia (1.7%) and leukemia cutis (0.8%).
* Whole blood count showed that 28.8% of patients had white blood cell count ≥ 50x109/l, 43.2% of patients had neutrophil count < 500/μl. Hb concentration < 7 g/dl accounted for 29.7%. Platelets < 20x109/l accounted for 24.6%.
* Biochemical features showed that there were 89.4% patients with increased LDH, 16.8% of patients with increased uric acid, 35.7% of patients with elevated liver enzymes, 0.9% of patients with renal failure and 25.0% of patients with elevated CRP.

**2. Determine genetic abnormalities in childhood acute leukemia**

* In acute lymphoblastic leukemia, the results of multiplex PCR tests showed that there were 24.1% patients with genetic abnormalities, in which, 12.1% of patients had *TEL/AML1* fusion, 4.8% of patients had *BCR/ABL1* fusion, 3.6% of patients had E2A/PBX1 fusion, 2.4% of patients had MLL/AF4 fusion and 1.2% of patients had SET/NUP214 fusion. There were 12.1% patients with favorable prognosis, 80.7% patients with intermediate prognosis and 7.2% patients with poor prognosis
* In acute myeloid leukemia, there were 34.3% patients with genetic abnormalities, in which, 14.2% patients with *AML1/ETO*, 8.6% patients with *PML/RARA*, 5.7% patients with *MLL/AF6*, 2.9% patient with *KMT2A/MLLT10* and 2.9% patient with AML1/ETO and BCR/ABL1.
* Pharmacogenetic test showed that the percentage of NUDT15 and TPMT polymorphisms were 18.1% and 6.9% respectively, and there is 1.4% patient having NUDT15 and TPMT polymorphisms. Finding NUDT15 and TPMT polymorphisms guide 6-MP dose for patients.

**3. Surveying the relationship between genetic variations and childhood acute leukemia treatment outcome**

* The rate of remission after induction phase for ALL and AML were 95.0% and 73.1% respectively. The relapsed rate for acute leukemia were 19.9%, in which, that for ALL and AML were 14.0% and 40.9%. The overall survival and event free survival rate for B-ALL, T-ALL and AML were 77.6 ± 6.7%, 79.5 ± 6.1%; 68.4 ± 15.8%, 70.3 ± 14.8%; and 45.5 ± 10.2%, 44.6 ± 10.0% respectively
* For ALL, there were statistical correlations between the remission rate, the relapsed rate with the genetic risk group. The poor prognosis group had the lowest remission rate (66.6%) and the highest relapsed rate (66.7%). The remission rate and relapsed rate in the intermediate prognosis group and the good prognosis group were 98.4%, 11.1% and 90.0% and 0%. There were statistical correlations between the overall survival rate, the event free survival rate with the genetic risk group. The overall survival rate and the event free survival rate for the poor prognosis, intermediate prognosis and good prognosis risk group after 2 years were 50.0 ± 25.0%, 27.8 ± 23.2%; 86.7 ± 4.9%, 81.5 ± 5.8% and 100.0 ± 0.0%, 100.0 ± 0.0%.
* For AML, the remission rate for the poor prognosis group was the lowest (33.3%), and the relapsed rate was the highest (66.7%). However, there were not statistical correlations between the remission rate, the relapsed rate, the overall survival rate and the event free survival rate with the genetic risk group (p>0,05). The overall survival rate and the event free survival rate for the poor prognosis, intermediate prognosis and good prognosis risk group after 1 year were 37.5 ± 28.6%, 37.5 ± 28.6%; 56.5 ± 10.3%, 38.0 ± 12.3% ; 65.6 ± 20.9%, 65.6 ± 20.9%.
* There were not statistical correlations between NUDT15 and TPMT polymorphisms and the overall survival time and the event survival time in ALL.

**RECOMMENDATIONS**

- Continuing to do genetic tests for childhood acute leukemia. Beside that, we should analyse karyotype. So we could classify genetic prognosis group more exactly.

- We should do *NUDT15* and *TPMT* tests for all acute lymphoblastic leukemia, so we could have the evidence to adjust 6-MP dose, reducing toxicity and side effects.

- For AML, the death rate in induction phase is high, we should improve the quality of supportive care, and set up isloated room for AML patients to reduce infection.

PUBLICATIONS

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7. Clinical, laboratory features and some genetic abnormalities in childhood acute leukemia at Hue Central Hospital, Vietnam. Will publish at The Journal of Medicine and Pharmacy, Hue University of Medicine and Pharmacy, 12/2022.