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Original Article

Translocation ETS leukemia-acute myeloid leukemia 1 (TEL-AML1) gene fusion in childhood acute lymphoblastic leukemia

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Abstract

Background Acute lymphoblastic leukemia (ALL) in children is a heterogeneous disease with different subtypes based on their cellular and molecular characteristics. This condition would influence the treatment outcome and subsequent risk for relapse. Accurate assignment of individual patients to risk groups is a critical issue for better outcome. TEL-AML1 gene fusion is the most frequent in childhood ALL.

Objective The aim of this study was to investigate the incidence of TEL-AML1 children with ALL in Sardjito Hospital.

Methods This was a cross sectional study. In this preliminary study, we used nested reverse-transcriptase polymerase chain reaction (RT-PCR) to analyze the present of TEL-AML1 gene fusion in bone marrow sample of childhood ALL patients.

Results We analyzed 41 samples. Out of these, 30 (73%) were amplified. Twenty three out of 30 ALL patients with good medical record were analyzed for this gene fusion. Out of 30 patients, there were five patients (17%) with TEL-AML1-positive gene fusion and 25 (83%) were TEL-AML1-negative. Among five patients with TEL-AML1-positive gene fusion, four patients (80%) were one year to less than 10 year old. All of the patients (100%) were with leukocyte < $50x10^9$/L.

Conclusions TEL-AML1 gene fusion was found in 17 % of samples. This gene fusion was more frequent in standard risk group (based on age and leukocyte). These data must be clarified with more samples. RT-PCR must be apply in all center as one part of improving diagnostic quality, especially in managing leukemia patients. **[Paediatr Indones. 2009;49:270-5]**.

Keywords: *acute lymphoblastic leukemia-TEL-AML1*

Genetic molecular analysis on leukemia cell has provided the basic knowledge of pathogenesis and prognosis in acute lymphoblastic leukemia (ALL). Molecular mechanism of leukemia resulted from aberrant of proto-oncogen expression and chromosome translocation leading to gene fusion promotes more active of kinase and also increases gene transcription. ALL in children is a heterogeneous disease with different subtypes based on their intracellular and molecular features. This condition will influence the treatment response and risk for relapse.[1] Thus, a good early stratification is needed to achieve outcome. Low risk patients can be managed with minimal regiments to minimize the side effects, but on the other side, high risk patients should receive more intensive treatments.

Nowadays, genetic and molecular analyses have been widely used in practice to support the clinical criteria which have been used. The routine gene fusion

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examinations, used to screen ALL with B-lineage, are t(9;22) *(BCRABL)*, t(1;19) *(E2A-PBX1)*, t(12;21) *(TEL-AML1)*, *MLL* rearrangement in chromosome 11q23, and also hyperdiploid karyotype with chromosome number > 50.[2] Chromosome structure aberration in acute leukemia was firstly found in routine G-banding method, then fluorescent in situ hybridization (FISH) and molecular technique have been proven in analyzing specific genetic.[3] Although FISH is good enough to detect specific site of fusion, it is not efficient to be used for individual samples due to the expensive marker. This may lead the difficulties in developing countries with limited resources. Reverse transcription-polymerase chain reaction (RT-PCR) method has been developed to detect gene fusion resulted from chromosome translocation in acute leukemia. The chromosome examination with banding technique more frequently results in false negative or difficult to analyze due to lack of metaphase picture making poor chromosome morphology.[4] The same result was found in ALL patients in Sardjito Hospital, Yogyakarta. Out of 35 patients, good chromosome spreading was only found in 50%.[5]

At present molecular diagnosis has not been used for patient services in most developing countries including Indonesia. Many centers used clinical examination and cytology to diagnose and stratify the patients.[6]

Methods

Nested RT-PCR was done to detect blast cell taken at early diagnosis from 41 patients with ALL in Sardjito Hospital, Yogyakarta, Indonesia. Diagnosis was based on the clinical classification (French American British criteria and cytochemistry staining). Informed consent was done prior to bone marrow aspiration. The leukemia cell line REH and HL60 were used as an external positive and negative control as well as a PCR assay validation.[4]

Trizol reagent (Life Technologies, Gibco, Gaithersburg, MD) was used for RNA extraction according to the manufacture's instruction. One microgram of RNA was reversed transcribed using MMLV reverse transcriptase protocol from pediatrics oncology group Singapore (POG(S)) Central Molecular Laboratory containing Buffer RT, 1.0 uM dNTP mix, 1.0 ug random hexamere, 20 U RNAse inhibitor, and 200 U MMLV at 37^{o}C for 60 minutes.

The integrity of isolated RNA and the correct synthesis of the cDNA were verified using positive internal control. A 690 bp segment of the ubiquitously expressed transcription E2A mRNA was co-amplified. Nested RT-PCR was performed after cDNA synthesis to detect TEL-AML1 gene fusion. The first PCR included a final volume of 25 ul with 10x PCR buffer, 200 uM DNTP, 5% DMSO, 0.2 ul of forward and reverse primer of E2A, 0.5 of each primer pair, and 0.2 ul (2.5U) Hot Start Tag^{tm} DNA polymerase. We used Gene Amp PCR system 9700 machine.

PCR activation of the polymerase was at 95^{o}C for 15 minutes, followed by 30 cycles of PCR amplification (annealing at 60^{o}C for 30 seconds, elongation at 72^{o}C for 45 seconds and denaturation at 95^{o}C for 30 seconds). Following the first PCR, 1ul aliquots for each of the first PCR product were transferred to second round mixtures that were similar with the first one. The primer sequences are noted in the **Table 1**. Ten microliter of the PCR products were electrophoresed in a 2% agarose gel for 45 minutes at 120 V and visualized by ethidium bromide staining.

Results

The aim of this study was to determine TEL-AML1 gene fusion in childhood ALL patients. Performance

Table 1. Primers used in the Nested RT-PCR protocol[4]

Fusion transcript	1st round primers (5' to 3')	Fusion transcript	2nd round primers (5' to 3')
TEL:87IU23	CACTCCGTGGATTTCAAACAGTC	TEL:944U23	CTCATCGGGAGCAGAGGAAGTTG
AML1A:1891L23	AGCCGAGTAGTTTTCATCATTGC	AML1A:1772L21	AGCACGGAGCAGAGGAAGTTG
E2A:1075U21	TTCTCGTCCAGCCCTTCTACC	E2A:1173U19	CTACGACGGGGGTCTCCAC
E2A:1883L22	TTTTCCTCTTCTCGCCGTTTCA	E2A:1884L19	AGGTTCCGCTCTCGCACTT

of PCR assay was teste d by detection of this fusion in to cell line REH (TEL-AML1) which was used as an external positive control. This gene fusion was detected in **Figure 1**. PCR products were determined by theirs fragment sizes on an agarose gel.

Out of 41 nested RT-PCR, 30 (73%) were amplified. Out of 30 samples, only 23 samples had clinical data. TEL-AML1-positive gene fusion was found in five (16.7 %) patients. Four (80%) patients with TEL-AML1-positive gene fusion come from a standard risk group based on age (1 year to < 10 year old) and total leukocyte < $50x10^9$/L. Most of male patients (80%) had TEL-AML1-positive, while all patients with white blood cell ≥ $50x10^9$/L had TEL-AML1-negative gene fusion (**Table 2**).

The PCR products were clearly demonstrated (**Figure 2&3**), A was 100bp leader, B was external positive control of TEL-AML1 using cell line REH (320 bp), C was external negative control using cell line HL60, C was blank (H2O). Some PCR products were depicted in the Arabic number. Collumn number 1 and 6 were showed no amplified of cDNA. These

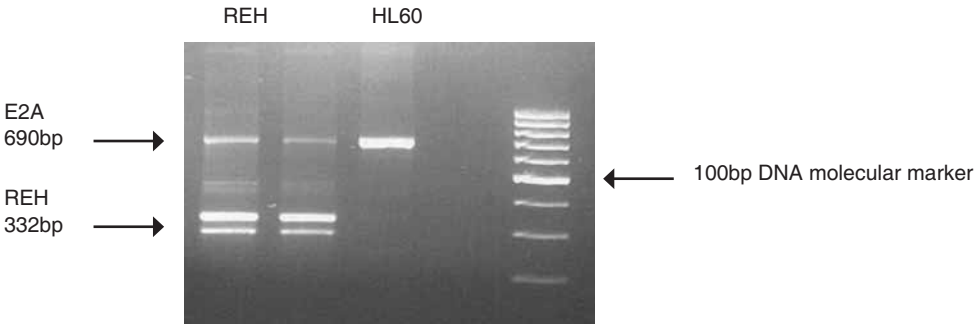

Figure 1. REH fragment as a positive control has 2 band, REH and E2A band as an internal control with 390 bp and 690 bp, respectively. HL60 is a cell line, must be containt E2A band, as an internal positive control.

Table 2. Clinical and Nested RT-PCR data from 23 ALL patients:

No	Clinical characteristic		Nested RT-PCR finding T(12;21) /TEL-AML1
	Sex/ Age (year)	Leukocyte (x109/L)	
1.	F/3.6	4.0	negative
2.	M/13.03	10.29	T(12;21) /TEL-AML1-positive
3.	M/3.05	5.8	T(12;21) /TEL-AML1-positive
4.	M/2.1	4.88	negative
5	M/6.11	7.2	negative
6	M/7	9.6	negative
7	F/11.4	5.5	negative
8	F/ 0.6	186.86	negative
9	M/10.4	47.2	negative
10	F/12.06	16.14	negative
11	F/9.4	5.3	T(12;21) /TEL-AML1-positive
12	F/7	1.0	negative
13	M/7.1	7.0	negative
14	F/8.3	226.34	negative
15	M/2	3.12	T(12;21) /TEL-AML1-positive
16	F/10.9	144.3	negative
17	M/3.8	1.2	negative
18	M/2.1	3.6	T(12;21) /TEL-AML1-positive
19	F/2.2	6.0	negative
20	M/10.3	115.0	negative
21	F/14	12.6	negative
22	M/1.4	3.5	negative
23	M/11.7	184.0	negative

problems may result from the poor quality of cDNA. Number 2, 3, 5, 7-9, 11 and 12, 14-23 were described the patients with TEL-AML1-negative with E2A band in 690bp which appropriate with external negative control. The patients were on number 4, 6, 13 with TEL-AML1-positive which had 2 bands (TEL-AML1 and E2A as internal control).

Discussion

In our center, in routine services, we used clinical pictures (such as age, metastasis of tumor, total leukocyte) and morphological leukemia blast cell in bone marrow aspiration. The patient risk prior to treatment was based on those criteria. The outcome

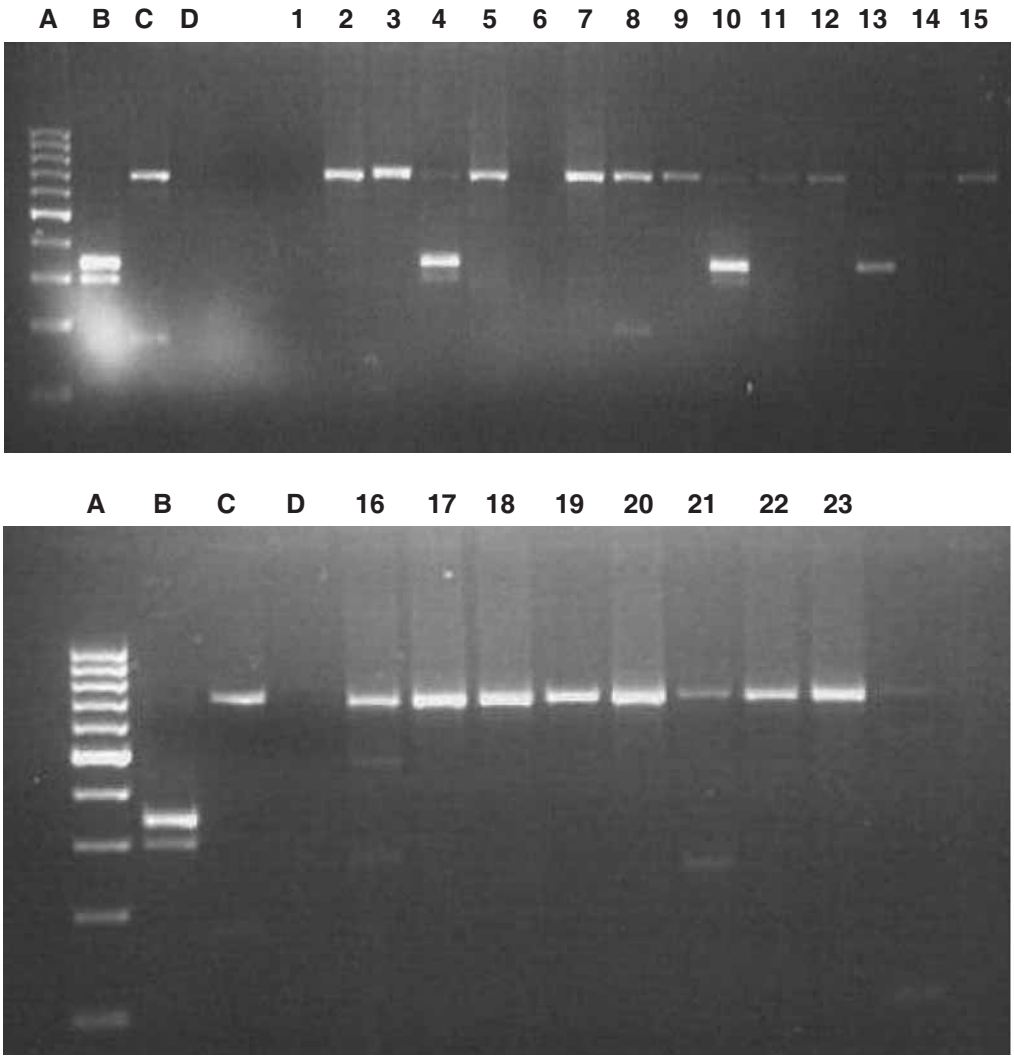

Figure 2 & 3. Representative of patients with TEL-AML-positive and TEL-AML-negative
A : 100bp DNA molecular marker
B : REH, a cell line positive control
C : Hl60, a cell line negative control
D : H2O (Blank)
1 & 6 : cDNA were not amplified
2, 3, 5, 7-9, 11&12, 14-23 : TEL-AML1-negative
4, 6, 13 : TEL-AML1-positive

of treatment was still lower compared with developed countries. Many patients were died due to the toxicity of drugs. The background of this study was to improve our diagnostic quality for stratifying new childhood ALL which would lead to better outcome. The main of this study was to introduce the molecular method in our center to improve the quality of clinical diagnosis. In addition, in the preliminary report we want to determine TEL-AML1 gene fusion which is most frequent in childhood ALL at Sardjito Hospital, Yogyakarta, Indonesia. Our result was demonstrated that molecular-based assay can be performed successfully on 73% patients.

This study demonstrated 16.7 % of childhood ALL patients in our hospital had TEL-AML1-positive. The higher incidences were found previously by Liang et al[7] showing 18% t (12; 21)/TEL-AML1-positive among childhood ALL in a center, Taiwan , where as study by Martinez-Climet[8] in other center found 18% TEL-AML1 positive in standard risk ALL patients. In China the incidence of TEL-AML1 fusion gene in cases of ALL was repoted 21.05%.[9] In Thailand, it was found higher TELAML1-positive 16/63 (25.4%) among childhood ALL patients.[10] We conclude that the incidence of TEL-AML1 gene fusion is comparable with the other countries; even in Western countries, but in the future with more samples should be done.

In this study we found all patients with TEL-AML1 gene fusion had initial leukocyte less than $50x10^9$/L. In Taiwan, 4 of 30 patients with TEL-AML1 had initial leukocyte counts greater than $100x10^9$/L with one of them as high as $300x10^9$/L.[7] In a Japanese study, three of 14 patients had white blood cell count greater than $100x10^9$/L with one of them at $200x10^9$/L.[11] In the reports from Western countries, only few cases with TEL-AML1 had leukocyte counts greater than $100x10^9$/L.[11-14]

In conclusion we reported that nested RT-PCR method successfully detects TEL-AML1 gene fusion in 73% childhood ALL patients. The reason of this apparent difference in leukocyte counts between patients from this study and those from the Western coutries remains to be studied. These inconsistent results may be attributed to the variation of ethnicity, genetic susceptibility, and environmental exposure. Therefore, future study with larger samples is needed to verify our results.

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