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ORIGINAL ARTICLE

Detection of FLT3 Gene Mutations In Patients With Acute Myeloid Leukemia In Surabaya, Indonesia: A Single-Center Study

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ABSTRACT

Background: FLT3 gene mutation contributes worse prognosis in patients with acute myeloid leukemia (AML). Almost 67% of patients with AML with FLT3 gene mutation cannot reach complete remission after induction therapy, and they are also at high risk of relapse. We aimed to investigate the FLT3-ITD, -TKD (D835) gene mutation prevalence in patients with AML at Surabaya and their association with leukocyte and bone marrow blast cell count.
Methods: 20 de novo patients with AML were recruited during February–July 2018. They were investigated through routine AML check-up and detection for FLT3-ITD, -TKD (D835) gene mutation.
Results: Four patients with de novo AML (20%) had FLT3-ITD gene mutation, and one patient had FLT3-TKD (D835) gene mutation. Median leukocyte count in patients with AML with FLT3-ITD mutation was higher than wild type patients (146.3×10^3/ µL vs 16.4 x 10^3 / µL, P=0,002). The mean bone marrow blast cell count was higher in patients with AML with FLT3-ITD mutation than wild type patients (86.5% vs 57.9%, P=0,047). The difference in leukocyte and bone marrow blast cell count in patients with FLT3-TKD (D835) mutation could not be analyzed since there was only one patient with this mutation.
Conclusion: The prevalence of FLT3-ITD gene mutation in patients in AML in Surabaya was 20% and FLT3-TKD (D835) was 5%. Patients with FLT3-ITD gene mutation was associated with leukocyte and bone marrow blast cell count.

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Introduction

Acute myeloid leukemia (AML) is a hematological malignancy with heterogenous clinicopathological picture and basic genetic abnormalities. Based on the data from the National Cancer Institute, the incidence and mortality of AML in the world is 4.2 and 2.8/100.000/year, respectively.[1] In Surabaya, Indonesia, approximately 36 new cases of AML has been reported annually.[2]

The treatment of AML has a trend to be personalized based on the cytogenetic and molecular abnormalities. Clinical guidelines classify AML into 3 risk groups; favorable risk, intermediate risk and poor risk. Patients with AML with FLT3 gene mutation are categorized as poor risk with low complete remission rate, high relapse rate and low overall survival rate.[3]

There are two different mutations in FLT3 gene, the first is internal tandem duplication (FLT3-ITD) which affects the juxtamembrane domain (JM domain) of the FLT3 receptor which can be found in almost 25% of patients with AML.[4] The second mutation in FLT3 is a point mutation involving tyrosine kinase domain (FLT3-TKD). The second mutation can be found in almost 13% of the patients.[5] Both mutations cause autophosphorylation and activation of tyrosine kinase receptor which results in uncontrolled proliferation of leukemic blast cells.[4]

The aims of this study was to know the prevalence of FLT3-ITD and -TKD gene mutations in patients with AML at Surabaya and to investigate the association

between FLT3 gene mutation and leukocyte bone marrow blast cell count.

Materials and Methods

Bone marrow aspirates from 20 newly diagnosed de novo patients AML were collected from patients referring to a private hospital in Surabaya. Standard diagnostic procedures to establish the diagnosis of AML and investigation for FLT3-ITD, -TKD (D835) gene mutation was performed. Patients with therapy-related AML were excluded. This research was approved by the Ethics Commitee in the Faculty of Medicine, Airlangga University Surabaya.

DNA Extraction

DNA was extracted from bone marrow aspirates using QIAmp DNA blood mini kit QIAGEN based on standard protocol. For detection of FLT3-ITD and -TKD (D835) gene mutation, 1-2 µL of DNA was extracted followed by hot start (95 °C for 5 minutes), 40 cycles of the next steps: denaturation 95 °C for 30 seconds, annealing (56 °C for 45 seconds) and extension (72 °C for 30 seconds). The final extension was done at 72 °C for 10 minutes. Primers for FLT3-ITD gene mutation were forward primers: 14F: 5'-GCA-ATT-TAG-GTA-TGA-AAG-CCA-GC-3' and reverse primers: 15R: 5'-CTT-TCA-GCA-TTT-TGA-CGG-CAA-CC-3'.[5] FLT3-ITD type will produce a single band of 329 bp, while mutant FLT3-ITD produced additional band larger than 329 bp. We used the same PCR protocol for detection of FLT3-TKD (D835) mutation. Primers for FLT3-TKD (D835) were forward primers; 20F 5'-CCG-CCA-GGA-ACG-TGC-TTG-3' and reverse primer 20R: 5'-GCA-GCC-TCA-CAT-TGC-CCC-3'.[6] After PCR amplification, 10 µL of the product was incubated and digested with EcoRV enzyme at 37 °C for 2 hours.

Undigested 114 bp product can be found in mutant FLT3-TKD (D835) and the digested fragment of 68 bp and 46 bp can be found in wild type AML patient.[6] The site for enzymatic digestion and fragment products are illustrated in figure 1.

EDTA peripheral whole blood was analyzed with ADVIA 2120 in hematology analyzer (Siemens) as a routine procedure for complete blood count analysis.

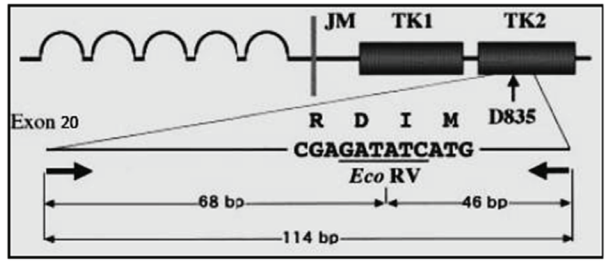

Figure 1: Scheme of digested fragment of PCR for detection of FLT3-TKD (D835) mutation.[6]

Bone Marrow Blast Cell Count

Blast cell counts for diagnosis of AML were enumerated among 500 nucleated bone marrow cells with the agreement of two hematopathologists. AML classification was based on the FAB classification criteria and cut-off of 20% confirmed AML (non-M3 subtype).

Statistical Analysis

Mann-Whitney U test was used to investigate the difference of leukocyte counts between patients with mutant and wild type of FLT3. Independent *t* test was used to investigate the difference between blast cell counts in mutant and wild types of FLT3. $P<0.05$ was considered statistically significant. SPSS software version, 22 software (Chicago, IL, USA) was used for statistical analysis.

Results

There were 20 de novo patients with AML with characteristics shown in table 1. Four patients (20%) were heterozygotes for FLT3-ITD mutation based on PCR results. The PCR results of FLT3-ITD gene mutation in AML patients are presented in figure 2,3,4.

For FLT3-TKD (D835) mutation, there was only one patient with mutation. The PCR result of FLT3-TKD(D835) gene mutation is presented in figure 5.

Discussion

FLT3 gene mutations are observed in approximately

Table 1: Distribution of the FLT3-ITD and -TKD (D835) gene mutation

	No. of patients	Mutant_FLT3-ITD No	Mutant FLT3-TKD (D835) No
Age (yr)			
< 30	7	1	0
> 30	13	3	1
Sex			
Male	11	1	0
Female	9	3	1
FAB classification			
M0	1	0	0
M1	1	0	0
M2	8	2	0
M3	7	2	0
M4	0	0	0
M5	2	0	1
M6	0	0	0
M7	1	0	0

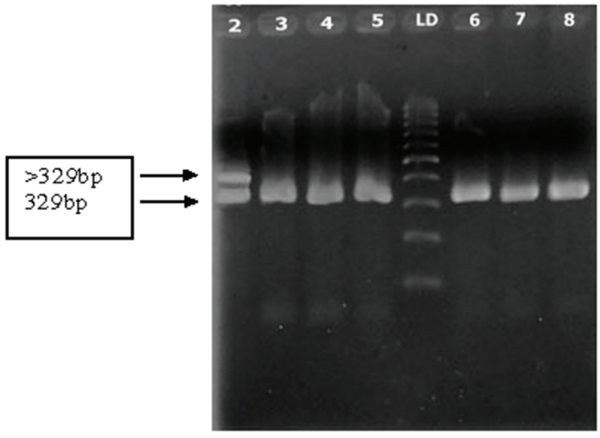

Figure 2: Detection of FLT3-ITD gene mutation by PCR. LD: DNA ladder 100 bp, positive results of heterozygote mutant FLT3-ITD in patients number 2 (2 bands of 329 and > 329 bp), negative result (wild type) in patients number 3,4,5,6,7 and 8 (single band of 329 bp).

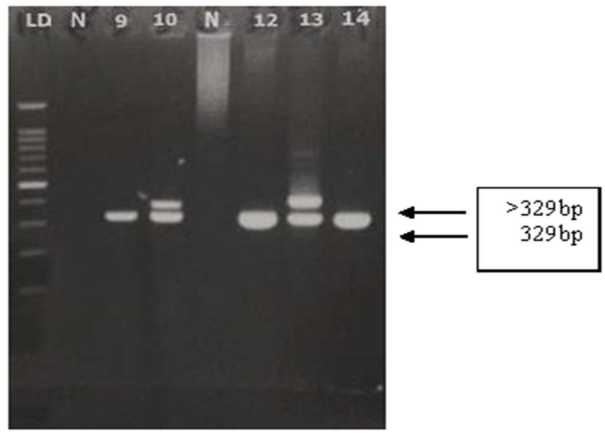

Figure 3: Detection of FLT3-ITD gene mutation by PCR. LD: DNA ladder 100 bp, positive results of heterozygote mutant FLT3-ITD in patient number 10,13 with 2 bands of 329 and > 329 bp, negative result (wild type) in patients' number 9, 12 and 14 (single band of 329 bp).

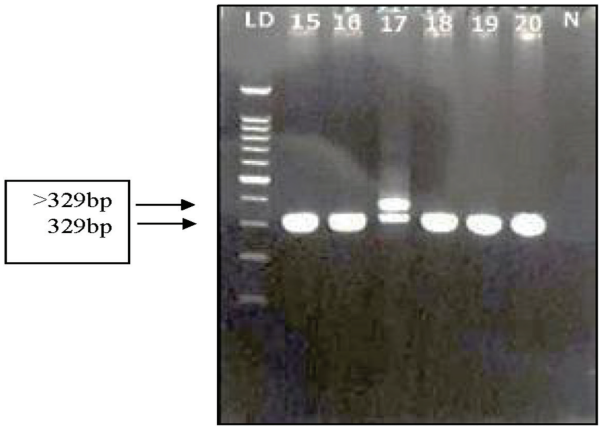

Figure 4: Detection of FLT3-ITD gene mutation by PCR. LD: DNA ladder 100 bp, positive results of heterozygote mutant FLT3-ITD in patients number 17 with 2 bands of 329 and > 329 bp, negative result (wild type) in patients number 15,16,18,19, and 20 (single band of 329 bp).

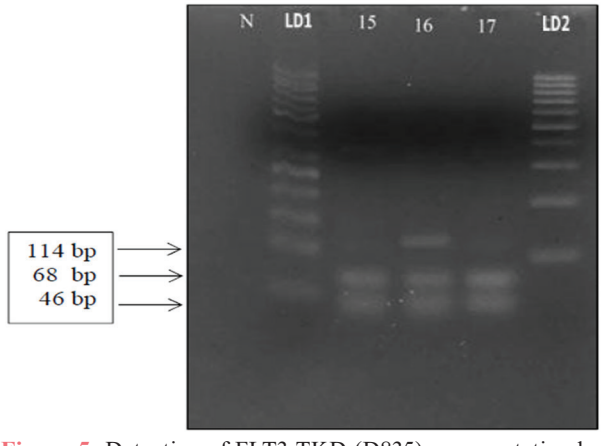

Figure 5: Detection of FLT3-TKD (D835) gene mutation by PCR. LD1: DNA ladder 50 bp, LD2 : DNA ladder 100 bp, positive results of heterozygote mutant FLT3-TKD (D835) in patient number 16 with 3 bands of 114bp, 46 bp and 68 bp, negative result (wild type) in patients number 15 and 17 with 2 bands of 46 bp and 68 bp digested products.

30% of all AML cases. Previous studies reported the frequency of 21-24% and 5-10% for ITD and TKD (D835), respectively.[7] A study from China has reported the frequency of FLT3-ITD mutation to be 19.7%.[8] There were no statistics from its frequency in Southeast Asia.

In this small study, the frequency of FLT3-ITD and -TKD (D835) in patients with AML at Surabaya was 20% and 5%, respectively. Both of the mutations were commonly found in women older than 30 years old. It was different from the study in China where the mutant FLT3 was more commonly found in men with a median age of 50 years.[8] In Iran, FLT3-ITD was more common at a younger age with a mean of 29.6 years and 52.4% of the patients were women.[9] In our study, FLT3-ITD was more common in patients with M2 and M3 subtypes, while the FLT3-TKD (D835) mutation was found in one patient with AML with M5 subtype. Mills and colleagues reported that FLT3-ITD was reported more commonly in M2 and M4 subtypes.[5] Wang and co-workers reported that FLT3-ITD was more observed in patients with AML-M1 (42,8%).[8]

We observed that four patients with FLT3-ITD mutation had significantly higher median leukocyte counts than patients with the wild type (146.3×10^3/µL *vs* 16.4×10^3/µL, P=0.002). Since there was only one patient with FLT3-TKD (D835) mutation, we could not draw any conclusion regarding the difference in their leukocyte and bone marrow blast counts. In patients with FLT3-ITD mutation, the percentage of bone marrow blast counts were significantly higher than those of wild type (86.5% *vs* 57.9%, P=0.047).

Our results were similar to the studies from Japan, China and Saudi Arabia, but different from those reported from Iran.[6, 8, 10, 11] Yamamoto and co-workers reported that FLT3-TKD (D835) mutation was not associated with leukocytosis, but was associated with lower disease free survival rate.[6] Leukocyte count at diagnosis is an important prognostic factor in patients with AML.[3] FLT3 gene mutation causes increase of autophosphorylation in tyrosine kinase receptors which in turn results in activation of multiple cytoplasmic molecules, stimulation of cell proliferation, disruption of differentiation process and blockade in apoptosis through activation of PI3K/Akt pathway, Ras/MAPK and STAT5 pathways.[4] On

the other hand, FLT3-ITD gene mutation blocks SMRT (silencing mediator of retinoic acid and thyroid hormone receptor), that interacts with PLZF (promyelocytic leukemia zinc finger) and ETO (eight twenty one) which ends up in uncontrolled cell proliferation.[4] As a result of all these mechanisms, high peripheral blood leukocytes are expected to occur more commonly in patients with AML with FLT3-ITD gene mutation.

Conclusion

The prevalence of FLT3-ITD gene mutation in patients in AML in Surabaya was 20% and FLT3-TKD (D835) was 5%. Patients with FLT3-ITD gene mutation was associated with leukocyte and bone marrow blast cell count.

This was a very small study which needs to be performed with larger sample size and more meticulous design to be able to reach a definite conclusion.

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Conflict of Interest: None declared.

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