Preliminary Report
Clinical Characteristic, Hematologic Response and Gene Mutation of Patients with Chronic Phase Chronic Myeloid Leukemia (CML) to Imatinib at Cipto Mangunkusumo National Hospital (RSUPN CM)

A. HARRYANTO REKSODIPUTRO[1], HILMAN TADJOEDIN[2], IKHWAN RINALDI[1], ARIF B. WITARTO[3]

[1] Division of Medical Oncology-Hematology Department of Internal Medicine University of Indonesia

[2] Division of Medical Oncology-Hematology Dharmais National Cancer-Centre Hospital

[3] Indonesian Institute of Science (LIPI)

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ABSTRACT

Chronic Myeloid Leukemia (CML) is caused by the BCR-ABL tyrosine kinase, the product of the Philadelphia chromosome. Imatinib mesylate is a selective inhibitor of this kinase. Data about clinical characteristic of patients with chronic phase CML, hematologic response to Imatinib, and gene mutation of BCR-Abl is still rare in Indonesia. Design and methods: This is a cross sectional study using retrospective medical record of patients with BCR-ABL positive chronic phase CML at policlinic of Teratai Department of Internal Medicine Cipto Mangunkusumo National Hospital during January-December 2009.

Results : In a period of 1 year, we included 20 positive BCR-ABL patients with chronic phase CML. The median age was 36 years (13-62 years). Males were slightly more frequent than females (12 vs 7) with ratio of 1.7:1. Seven patients (36.8%) were from javanese ethnic. The features of patients were 15 (78.9%) chronic phase, 3 (15.8%) accelerated phase and 1 (5.3%) blast crisis. 12 (63.2%) of patients had splenomegaly. Median of hemoglobin level were 9.9 g/dL (5-14 g/dL), median of white-cell count were 73.000/uL (4.100-332.000/uL), and median of platelet count was 481.000/uL (263.000-1.116.000/uL). Median of basophils was 1% (0%-10%) and median of peripheral blood blasts was 1% (0-22%). A 3 months Complete Hematologic Response (CHR) was achieved in 10/19 (52.6%) patients during the study, including 1 accelerated phase patient and 1 blast crisis patient. 18/19 (94.7%) had been treated by hydrea before treated by imatinib, 1/19 (5.3%) never treated by any drugs for CML. 5/11(45.4%) are in low risk of Sokal score.

Conclusions: The median age of patients with chronic phase CML was 36 years with slightly more frequent in male. Sixty three point two percent of patients had splenomegaly. Median of white-cell count was 73.000/uL (4.100-332.000/uL) and median of peripheral blood blasts was 1% (0-22%). A 3 months Complete Hematologic Response (CHR) was achieved in 52.6% patients.

Keywords: *Chronic phase CML, imatinib, hematologic response, mutation.*

KORESPONDENSI:
dr. Hilman Tadjoedin, SpPD KHOM,
SMF Hematologi-Onkologi Medik RS. Kanker "Dharmiais".
Email: hom_rskd@yahoo.com

ABSTRAK

Leukemia Granulositik Kronik (LGK) disebabkan oleh gen BCR-Abl domain *tyrosin kinase*, produk dari kromosom Philadelphia. Imatinib mesylate merupakan inhibitor selektif terhadap kinase tersebut. Di Indonesia, data mengenai karakteristik pasien LGK fase kronik, respons hematologi terhadap imatinib, dan mutasi gen masih jarang ditemukan. Metode dan desain: studi potong lintang ini menggunakan data rekam medik pasien yang didiagnosis sebagai LGK fase kronik dengan BCR-ABL positif yang berobat ke Poliklinik Teratai Departemen Ilmu Penyakit Dalam Rumah Sakit Cipto Mangunkusumo selama Januari – Desember 2009.

Hasil: dalam periode 1 tahun studi, peneliti mengikutsertakan 20 pasien LGK fase kronik yang memiliki BCR-ABL positif dengan median umur 36 tahun (13-62 tahun). Pasien laki-laki lebih banyak dibandingkan dengan perempuan (12 vs 7) dengan rasio 1,7: 1. Sebanyak tujuh pasien (36,8%) berasal dari suku Jawa. Dilaporkan juga karakteristik pasien adalah

15 orang berada pada fase kronik (78,9%); 3 pasien berada pada fase akselerasi (15,8%) sementara 1 pasien mengalami krisis blast (5,3%); 12 pasien (63,2%) ditemui adanya splenomegali; dan 5 dari 11 pasien dilaporkan memiliki skor Sokal yang rendah. Berdasarkan hasil laboratorium didapati nilai median hemoglobin 9,9 g/dL (5-14 g/dL); leukosit 73.000/uL (4.100-332.000/uL) dan nilai median trombosit 481.000/uL (263.000/uL-1.116.000/uL); nilai median kadar basofil di darah perifer 1% (1-10%) dengan nilai median sel blast di perifer adalah 1% (0-22%). Selama studi, respons hematologik komplet dalam 3 bulan dicapai oleh 10 dari 19 pasien (52,6%), termasuk di antaranya 1 pasien yang mengalami fase akselerasi dan 1 pasien yang lain mengalami krisis blast. Sebanyak 18 pasien (94,7%) telah diobati dengan hydrea sebelum mendapat terapi Imatinib, sementara 1 pasien (5,3%) tidak pernah mendapatkan pengobatan apapun sebelumnya.

Kesimpulan: didapati nilai median usia pasien LGK fase kronik adalah 36 tahun, sebagian besar adalah laki-laki. Sebanyak enam puluh tiga persen pasien memiliki splenomegali. Dilaporkan juga nilai median leukosit adalah 73.000/uL (4.100-332.000/uL) dengan nilai median sel blast di darah perifer sebanyak 1% (0-22%). Respons hematologik komplet dalam 3 bulan dicapai oleh 52,6% pasien.

Kata kunci: LGK fase kronik, imatinib, respons hematologik, mutasi.

INTRODUCTION

Chronic Myeloid Leukemia (CML) is a clonal myeloproliferative disorder, characterized by acquisition of the Philadelphia chromosome (Ph) in leukemic stem cells and their progeny.[1,2] The abnormal Ph chromosome is the result of a reciprocal translocation between chromosomes 9 and 22. The major consequence of this translocation is the fusion of the ABL gene to the BCR gene on chromosome 22.[3] The BCR-ABL fusion gene encodes a new protein of 190, 210 or 230 kd, depending on the breakpoint on the BCR gene.[4,5] All these BCR-ABL fusion proteins have enhanced tyrosine kinase activity, which is crucial for the development of the disease.[6]

Imatinib mesylate is highly effective in the treatment and management of Ph-positive CML.[7,8] This drug is thought to bind competitively to the adenosine triphosphate (ATP)-docking site of tyrosine kinase proteins, including ABL itself and the hybrid BCR/ABL proteins.[9]

Point mutations, which impair imatinib binding by interrupting critical contact point or by inducing a conformation to which imatinib binding is reduced, were identified as an important mechanism of acquired imatinib resistance.[10]

To date, more than 50 different point mutations encoding for more than 40 different amino acid substitutions in the BCR-ABL kinase domain have been described in CML patients after relapse due to resistance to imatinib.[11] Some mutants, such as T315I and E255K, are insensitive to imatinib at clinically achievable doses, whereas others, such as M315T or Y253F, retain immediate levels of sensitivity to imatinib.[12] The probability of finding a mutation increases with disease stage.[10] Nevertheless, it was reported that mutation may be detected even before initiation of treatment with imatinib.[13]

There is a large variation in the previously reported frequency of mutations found in association with imatinib resistance, ranging from 26 to 90% of the patients.[12,14]

To the best of our knowledge, there have been no data regarding the BCR-ABL KD mutation in Indonesian patients, therefore this study was designed to characterize pattern of mutation who obtained a CHR after imatinib mesylate treatment, in our division.

DESIGN AND METHODS

Patients

Between January-December 2009, 20 patients were recruited from Teratai Policlinic, Cipto Mangunkusumo General Hospital, from those results, 3 of them could not be read because of technical problem, and 4 were doubtful. We suceeded in sequencing of 13 patients, 9 were normal results and 4 mutated. From 4 mutated patients, 1 patient with new paradigm and 3 were clasically like the same as literatures (Table 1).

Subjects were considered as chronic phase (CP) if they have less than 15% blasts, less than 30% plus promyelocytes, less than 20% basophils in peripheral blood (PB) or bone marrow (BM), and no extramedullary infiltration of leukemic blasts other than liver and spleen.

Also platelets should be greater than or equal to 100 x 10^9/L. Accelerated phase (AP) was defined by the prescence of any of the following criteria in PB or BM : at least blast 15-30%, more than 30% blasts plus promyelocytes (with less than 30% blasts alone), more than 20% basophils, or less than 100 x 10^9 platelets/L. Blasts crisis (BC) was defined by the presence of more than 30% bast in PB or BM, or extramedullary disease.

Complete Hematologic Response (CHR) : based on ELN (European Leukemic Net) guidelines, which consist of : WBC ‹ 10 x 10^9/L, basophils ‹ 5%, no : myelocytes, promyelocytes, myeloblasts in the differential, platelets count ‹ 450 x 10^9/L, spleen nonpalpable.[15] Sokal's score defined as (11 x age + 35 x spleen + 89 x blasts + 0,4 x platelet – 550)/1000.

Sequencing Methods

Blood withdrawn from PB or BM, after that we isolated mononuclear cells (MNC's) with RBC lysis buffer. Total

Table 1: Patients characteristics at onset of Imatinib (IM) therapy*

Characteristic (N=21)	No.	%
1. Age, years :		
- Median	36,38	
- Range	13-62	
2. Sex :		
- Male	12	63,2
- Female	7	36,8
3. Ethnics :		
- Javanese	7	36,8
- Sundanese	2	10,5
- Balinese	0	0
- Batak	2	10,5
- Chinese	1	5,3
- Others	7	36,8
4. Sokal risk group :		
- Low	5	26,3
- Intermediate	4	21,1
- High	10	52,6
5. Splenomegaly :		
- Spleen size ≥ 10 cm below the costal margin	7	36,8
6. Hemoglobin : (g/dl)		
- Median	9,9	
- Range	5-14	
7. Leukocyte : ($x10^9$/L)		
- Median	73	
- Range	4,1-332	
8. Peripheral-blood blasts : (%)		
- Median	1	
- Range	0-22	
9. Peripheral-blood basophils : (%)		
- Median	1	
- Range	0-13	
10. Trombocyte : ($x10^9$/L)		
- Median	481	
- Range	263-1116	

** Source : Dharmais National Cancer Centre and Ciptomangunkusumo National General Hospital*

Table 2: Patient responses (N=19)

Response	No.	%
CHR :		
- No	7	36,8
*Progression		
-Yes	10	52,6
*Loss of CHR		
*Progression		

Abbreviations : CHR : Complete Hematologic Response. Progression : accelerated (AP) or blastic crisis (BC).

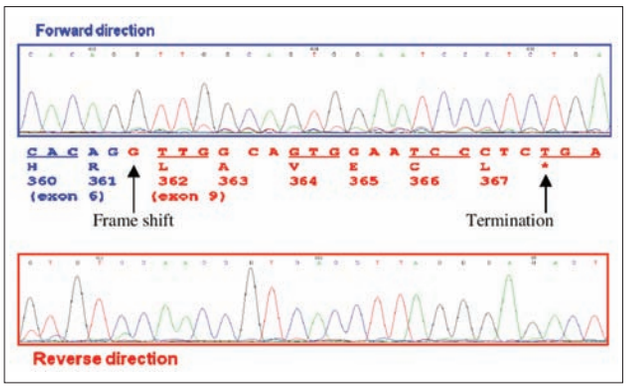

Figure 1: Deletion of exon 7 & 8 resulted in frame shift and premature termination (ABS)

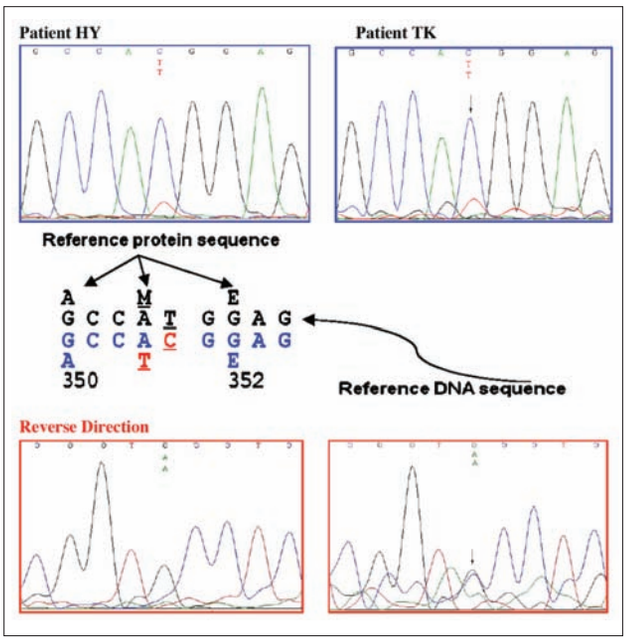

Figure: 2 M351T mutations (HY & TK)

amount of RNA were extracted with Trizol® reagent (Invitrogen), after that we synthesized first strand cDNA using random hexamer primer – M-MLV reverse transcriptase (Invitrogen), and 100 ng of RNA template. For nested PCR, we used KOD Hot Start DNA polymerase (Toyobo).

For first PCR we're using primer B2A and A10R1; cDna template; PCR product 1,754 bp. For second we're using A4F and A10R2; PCR product template ; PCR product 905 bp.

RESULTS

In this study, we performed clinical characteristic, hematologic evaluation, and mutation screening of 20 Indonesian CML patients. Characteristic of patients are

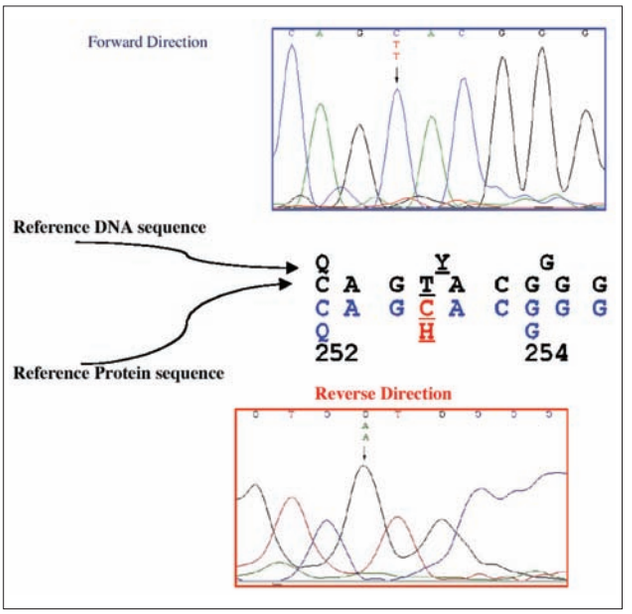

Figure 3: Y253H (P-loop) mutation. Patient MSS

Table 3: Mutation form

No.	Patient's Name	Mutation Form
1.	ABS	Deletion of exon 8,9 (388 nnt)
2.	HY	M351T (GAG › GAA)
3.	MSS	Y253H (TAC › CAC), P-loop
4.	TK	M351T (GAG › GAA)

eventually be achieved but is not yet very predictive of progression-free survival (PFS).

Figure 1 shows that from sequece reference, there was deletion at exon no. 7 and 8, no evidence of imatinib influencing.

DISCUSSION

We have enrolled 20 CML BCR-ABL (+) patients, whom evaluated for CHR and gene mutations. From those results, 3 of them could not be read because of technical problem (purifications were not good), 4 were doubtful (forward and reverse direction were not the same). We

Table 4 : Clinical responses patients with mutation

No.	Initial	Phase	Sokal's score	Duration of Illness	Response	Mutation
1.	K	chronic	intermediate	> 1 yr	CHR (+) in 3 mo	normal
2.	HT	chronic	low	< 1 yr	CHR (+) in 3 mo	normal
3.	S	accelerated	high	< 1 yr	CHR (+) in 6 mo	normal
4.	AR	chronic	low	< 1 yr	CHR (+) in 3 mo	normal
5.	RS	accelerated	high	< 1 yr	CHR (+) in 3 mo	normal
6.	U	chronic	low	< 1 yr	CHR (-) in 3,6,9 mo	normal
7.	ABS	chronic	low	< 1 yr	CHR (+) in 3 mo	deletion of exon 8,9 (338 nt)
8.	AM	chronic	high	< 1 yr	CHR (+) in 3 mo	normal
9.	NL	accelerate	high	< 1 yr	Just 1 mo in imatinib	normal
10.	HY	chronic	high	> 1 yr	CHR (-) in 3,6,9,12 mo	M351T (GAG>GAA)
11.	MSS	chronic	high	> 1 yr	CHR (+) in 6 mo CHR (+) in 3 & 6 mo	Y253H (TAC>CAC), P-loop
12.	SP	chronic	low	< 1 yr	CHR (-) in 9 & 12 mo	normal
13.	TK	chronic	low	< 1 yr	CHR (+) in 6 mo	M351T (GAG>GAA)

shown in table 1. We carried out direct sequencing for all patients to detect mutation in BCR-ABL KD.

A baseline PCR test is needed to confirm the type of BCR-Abl transcripts that are expressed to enable proper interpretation of subsequent results. There is no proven prognostic value for the actual baseline BCR-Abl level.

Twice monthly blood count monitoring for the first few months is valuable to document achievement of CHR, which is nearly always achieved in the first 3 months. RQ-PCR can be done monthly for the first 3 months, but its value at this stage is unproven. The result at 3 months provides an indication of the probability that MMR will

suceeded in sequencing of 13 patients, 9 were normal, 4 mutated (ABS, HY, TK and MSS). From 4 mutated patients, 3 of them reached CHR (ABS,MSS and TK). One patient (HY) didn't. Although 3 patients succeded in CHR , but the time to reach it were not the same (ABS : 3 mo's, MSS and TK : 6 mo's). We conclude that patients with mutations still have good clinical responses. ❖

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