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Role of Pre-Therapeutic Expression of Human Organic Cation Transporter1 (*SLC22A1*) Gene in Predicting Clinical Outcome in Imatinib Treated CP-CML Patients

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ABSTRACT

Background: Human organic cation transporter1 (hOCT1, SLC22A1), an influx transporter,is responsible for the uptake of Imatinib into chronic myelod leukemia (CML)cells. Some patients fail to achieve optimal molecular response to Imatinib, defined as major molecular response (MMR) i.e.BCR-ABL 1 ≤ 0.1% within 12 months of therapy. Pretherapeutic expression of hOCT1 may be beneficial in predicting the response to imatinib in CML patients. **Methodology:** 30 newly diagnosed BCR-ABL positive CML patients in chronic phase&30 healthy control subjects, all ethnic Indians ,were recruited in the study. hOCT1 gene expression in PBMCs was quantified by SYBR Green based qRT-PCR, using the 2-DDCt method. After initiation of imatinib therapy, hematological response was monitored at regular intervals, and molecular response (BCR-ABL1/ABL1 ratio) assessed after 6 or 12 months. **Results & discussion:** The cases were divided into two groups, high expression (n=15) and low expression (n=15) groups, based on the median value of fold change in hOCT1 gene expression. (Median =5.6). 11 (73.33%) patients with low expression achieved CHR by the end of 3 months, whereas 4(26.66%) did not. On the contrary, all 15 patients (100%) with high hOCT1 gene expression achieved CHR by the end of 3 months. (p=0.10).It was also observed that the mean THR (time to CHR) in low expression group was higher than in high expression group.(p=0.046)It was seen that while all 15 patients with high expression had an optimal response , only 13.33 % (n=2) patients with low expression had it. In the low expression group, 40% patients had treatment failure(n=6)while remaining 23.33 % (n=7)were categorized as warning.(p=0.000)(ELN 2013 guidelines) **Conclusions:** Hence it was concluded that high expression of hOCT1 gene leads to early achievement of CHR. In case of molecular response, it was observed that high expression of hOCT1 gene was significantly associated with achievement of on optimal response to imatinib. These findings emphasize that knowledge of pretherapeutic level of hOCT1 could be a useful marker to predict imatinib therapy outcome in CML patients, and the prospects of personalized therapy in such patients.

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INTRODUCTION

Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder of pluripotent stem cells which is characterized by the Philadelphia (Ph) chromosome.[1] This chromosome is formed due to a balanced reciprocal translocation between chromosome 9 and 22 t(9;22)(q34.1;q11.2)[2]. The incidence of CML varies around the world,with the lowest incidence being in Sweden and China (approximately 0.7 per 100,000 persons),and the highest incidence being in Switzerland and the United States (approximately 1.5 per 100,000 persons).[3] In India, CML is the commonest adult leukaemia , with the annual incidence ranging from 0.8–2.2/100,000 population in males and 0.6–1.6/100,000 population in females.[4] Imatinib was established as the standard of care for patients with CP-CML based on the results of the International Randomized Study of Interferon and STI571 (IRIS) trial. Imatinib , marketed by Novartis as Gleevec (Canada, South Africa and the USA) or Glivec (Australia, Europe and Latin America), and sometimes referred to by its investigational name STI-571, is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes. It works by binding close to the ATP binding site of bcr-abl, locking it in a closed or self-inhibited conformation, and therefore inhibiting the enzyme activity of the protein semi-competitively.[5] Response to imanitib treatment is measured in terms of hematologic, cytogenetic, and molecular parameters, as proposed by the European leukemia Net (ELN).Although the prognosis of chronic myeloid leukemia patients treated with imatinib is good, many fail to develop an optimal response or lose one .Studies on both primary cells and resistant cell lines have identified a number of mechanisms by which resistance to imatinib arises, including both bcr-abl dependant and independent mechanisms. Influx transporters are classified into families which broadly reflect their preferred substrates.[6] The main transporters with the ability to translocate organic cations across the plasma membrane belong to the solute carrier family 22A (general gene symbol SLC22A).[7] Imatinib is weakly cationic at physiological pH and in chronic myeloid leukemia primary cells and cell lines, its uptake has been shown to be dependent on organic cationic transporter -1(OCT1). The degree of OCT1 expression has been suggested to be a useful biomarker to predict the success of imatinib-based therapy in leukemia patients, and, also CML patients who had higher OCT1 expression levels have been shown to have better response to the drug.

AIMS AND OBJECTIVES

The present study aimed to study, the hOCT1 (*SLC22A1*) gene mRNA expression. in peripheral blood leukocytes of Chronic Myeloid Leukemia patients and to correlate it with the hematological and molecular response to Imatinib of Chronic Myeloid Leukemia patients.

METHODOLOGY

The study included thirty newly diagnosed CML patients, in the age group 18-80 years, with diagnosis confirmed by qualitative PCR for BCR-ABL1 fusion gene , who were to be initiated on Imatinib therapy. Any patients with Chronic MyeloMonocytic Leukemia (CMML) , BCR-ABL1 positive adult ALL , other myeloproliferative disorders and patients who had previously undergone any treatment for chronic myeloid leukemia were excluded. Age and sex matched 30 normal healthy volunteers were selected as controls. On inclusion in the study the patients underwent detailed clinical examination (relevant findings like spleen size were noted) and hematological laboratory tests including complete blood count (Hb , TLC, DLC ,total platelet count) were recorded. A peripheral blood sample, 5mL from cases and 3mL from controls was collected for molecular studies, in an EDTA vial by venipuncture, after taking informed consent, which was used for molecular analysis.RNA Extraction from CML patient samples was done using TRIZOL RNA extraction method was used. RNA from control samples was extracted by using Total RNA Mini Kit (Blood/Cultured Cell) from Gene Aid Biotech Ltd., Taiwan using protocol as per manufacturer's instructions. Extracted RNA was reverse transcribed to c-DNA by RT-PCR. cDNA was synthesized using the Verso cDNA synthesis kit (Thermo Scientific, EU) using protocol as per the manufacturer's instructions. A parallel PCR was performed on each sample using primers specific for the constitutively expressed β-actin gene which was used as endogenous control.

RESPONSE CRITERIA

Complete Hematologic Response (CHR)	Platelets < 450 x10^9/L, AND White cells < 10 x10^9/L, AND No circulating immature myeloid cells, AND < 5% basophils on differential, AND No palpable splenomegaly

TABLE 1: CRITERIA FOR HEMATOLOGICAL RESPONSE TO IMATINIB

Duration of treatment	OPTIMAL	WARNING	FAILURE
6 months	BCR-ABL1 < 1%	BCR-ABL1 = 1-10%	BCR-ABL1 >10%
12 months	BCR-ABL1 ≤ 0.1 %	BCR-ABL1 > 0.1-1%	BCR-ABL1 > 1%

TABLE 2: CRITERIA FOR MOLECULAR RESPONSE TO IMATINIB

MOLECULAR ANALYSIS

1. Multiplex RT –PCR for detection of BCR-ABL1 fusion gene transcripts:

Diagnosis of CML was confirmed by Multiplex RT-PCR which allows simultaneous detection of all the BCR-ABL1 fusion gene transcripts in addition to normal BCR gene as an internal control. cDNA synthesized from the total RNA(as described previously) was used in multiplex PCR .The sequence of oligonucleotide primer sequences used for this multiplex PCR . The expected band size for different BCR-ABL1 fusion transcripts were: 808bp - normal BCR,481 bp - e1a2,385 bp - b3a2 ,310 bp - b2a2,103bp – b2a3 & 209bp – b3a3 as shown in figure 1.

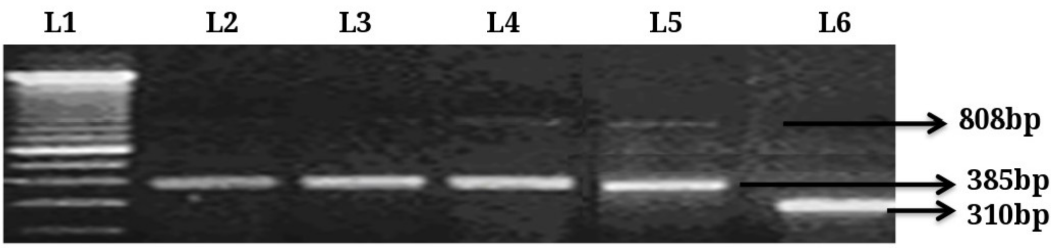

FIGURE 1: Ethidium Bromide stained gel electrophoresis image of BCR-ABL transcripts by multiplex RT-PCR. L1.100bp ladder, L2, L3, L4, L5. b3a2 transcript (385 bp). L6. b2a2 transcript (310bp).

2. Expression of hOCT1 gene in CML patients and controls

After cDNA quality was checked it was used to study the expression of hOCT1 gene in patient and control samples by quantitative Real Time PCR using Rotor Gene Q (Qiagen) analyzer. Thermo Scientific Maxima SYBR Green qPCR master mix was used for quantative real-time PCR which contains Hot start Taq polymerase , SYBR Green qPCR buffer , SYBR Green I dye, reference dye and dNTP. βactin gene was used as internal control. In this PCR , amplification of hOCT1 gene was compared with the amplification of β actin gene and relative expression was calculated. Melting curve analysis was done in the temperature range 35°C to 95°C for assessment of homogeneity of the qPCR products. Gene expression levels were calculated based on the $\Delta\Delta C_t$ method. Primers used were 5′-GGGCAGCCTGCCTCGTCAT-3′(Forward) & 5′-ACCTCCCTCAGCCTGAAGAC-3′ (Reverse).

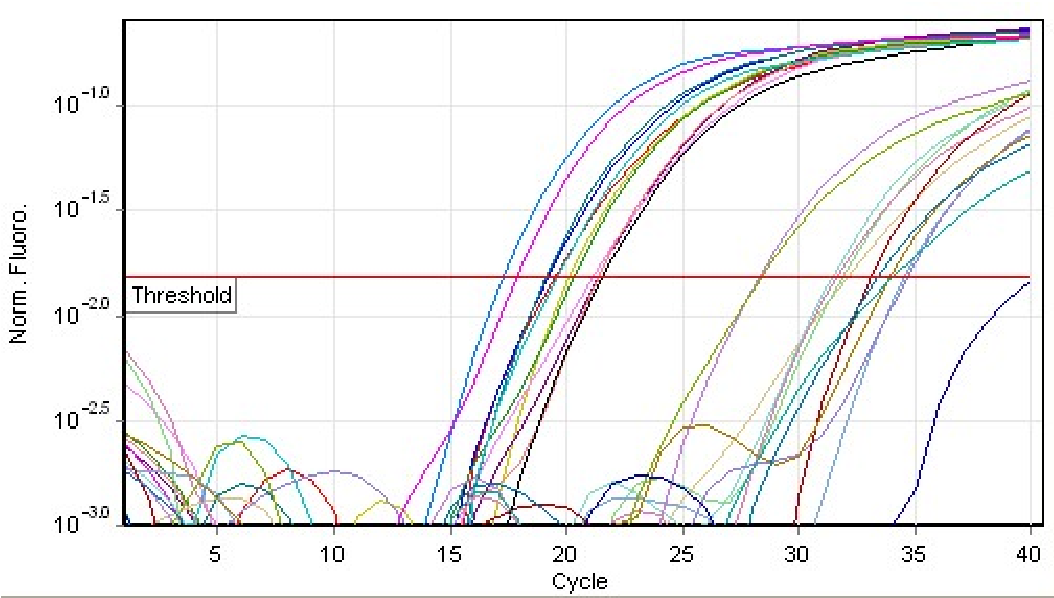

Figure 2: Gene expression curve for hOCT1 gene.

3. Quantitative REAL-TIME PCR for BCR-ABL1 fusion gene

A peripheral blood sample was collected at follow-up either at 6 months or at 12 months after initiation of imatinib therapy to assess the molecular response. Molecular response was assessed by calculating BCR-ABL1/ABL1 ratio. This was done by quantification of BCR-ABL1 p210b2a2 or b3a2 transcripts. *Ipsogen* BCR-ABL1 Mbcr kit(from QIAGEN, Netherlands,) was used for this purpose and protocol followed was as per the manufacturer's instructions. For each gene (ABL1 and BCR-ABL1), raw Ct values obtained from plasmid standard dilutions were plotted according to the log copy number (3,4 and 5 for C1,C2 C3 ; and 1,2,3,4,5 for F1, F2, F3, F4, F5).The ABL1 standard curve equation was used to transform raw Ct values for the unknown samples into ABL1 copy numbers ($ABL1_{CN}$)The BCR-ABL1 standard curve equation was used to transform raw Ct values for the unknown samples into BCR-ABL1 copy numbers ($BCR\text{-}ABL1Mbcr_{CN}$). Normalized copy number (NCN) was calculated using the formula: NCN = [$BCR\text{-}ABL1Mbcr_{CN}$ / $ABL1_{CN}$] * 100

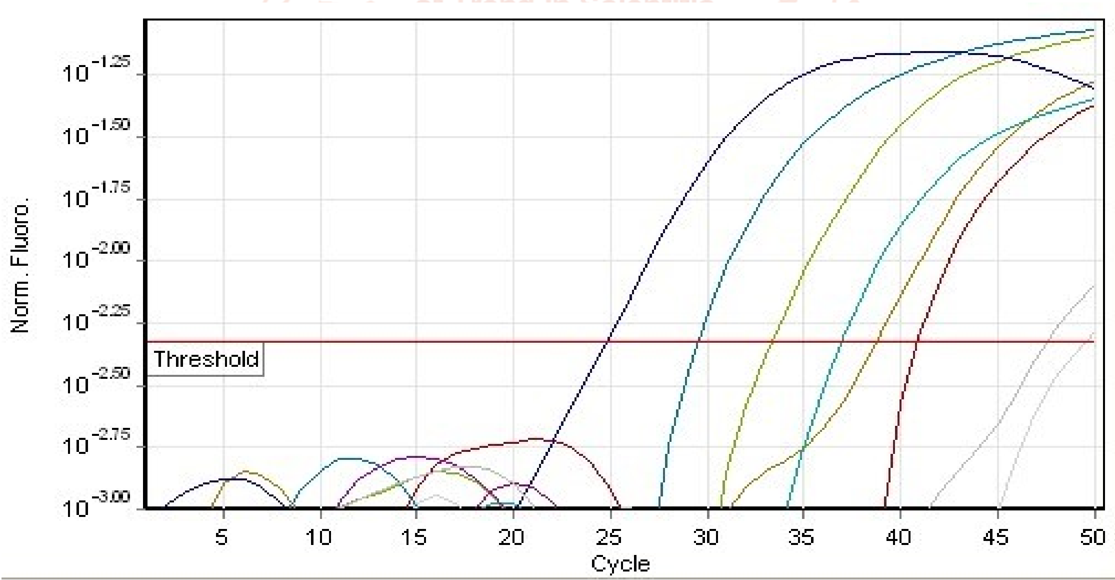

Figure 3: Standard amplification curve for BCR/ABL for Quantitative REAL-TIME PCR for BCR-ABL1 fusion gene

FOLLOW-UP & RESPONSE MONITORING: After initiation of Imatinib therapy, follow-up and response monitoring of patients was done for 6 months to 12 months i.e. during the duration of the study, depending on the time point at which the patient was recruited in the study. **Hematological response** (Hb, TLC, DLC and platelet count) was assessed at regular intervals during the duration of the study. **Molecular Response (BCR-ABL1/ABL1 %)** was assessed once, either at 6 months or at 12 months after beginning of imatinib therapy, depending on the time of recruitement of patient in the study.

STATISTICAL ANALYSIS: All statistical analysis was performed using SPSS software22.0. Variables were presented as range, Mean ± SD and median value. Fisher exact, Mann Whitney-U and Kruskal Wallis tests were used to estimate the statistical significance of differences observed between the groups. $p<0.05$ was taken as statistically significant.

RESULTS

The cases comprised of 22 males and 8 females with mean age being 39.70 ± 18.04 yrs .Baseline investigations including clinical and hematological parameters were analyzed at baseline and at follow up are shown in table 3.

TABLE 3: CLINICAL AND HEMATOLOGICAL PARAMETERS

Parameter	Mean±S.D. at baseline (n=30)	Mean±S.D.after 6 months of imatinib therapy (n=30)	Mean±S.D.after 12 months of imatinib therapy (n= 10)
1. Hb (gm%)	11.01±2.08	10.61±1.73	10.29±1.46
2.TLC(*10^9/L)	203±176.73	6.82±1.63	6.47± 2.21
3.PLATELET COUNT (*10^9/L)	318.30±166.75	166.40±42.16	148.20±43.27
4. SPLEEN SIZE (cms below costal margin)	11.93±2.98	Not palpable	Not palpable
5. BLASTS(%)	3.73±1.20	00±0.000	0.30±0.95
6. BASOPHILS(%)	2.23±0.89	1.63±0.85	1.90±0.87
7. EOSINOPHILS(%)	4.03±1.84	2.73±0.82	1.70±0.67

Study of hOCT1 gene expression was done by quantitative real time PCR in 30 CML patients with respect to 30 healthy controls. The fold change in expression of hOCT1 gene ranged from 0.02 to 96.33 (Table 4).On the basis of the median value for hOCT1 fold change in expression(5.60), the cases were divided into 2 groups – high expression(n=15) and low expression(n=15).

TABLE 4: CLINICO-PATHOLOGICAL FEATURES OF CML PATIENTS AND HOCT1 GENE EXPRESSION.

Variables	Fold change ↓ (Mean + SD)	Fold change (Range)	MEDIAN	p value
GENE				
Hoct1		0.026- 96.335	5.6	-
GENDER				
Males	17.17±28.25	0.07-96.33	4.81	0.5967
Females	13.39±14.64	0.02-41.93	9	
AGE GROUP (in years)				
<40 years	21.75±30.58	0.10-96.33	6.72	0.2454
>40 years	8.85±13.41	0.02-41.93	1.16	
Bcr-Abl TRANSCRIPTS				
b3a2		0.1-96.33	8.69	0.4726
b2a2		0.02-41.93	3.03	
b2a2/b3a2		0.07-39.67	0.36	

The relation of hOCT1 gene expression with hematological to Imatinib was assessed as shown in table 5 . The mean time to achieve CHR (THR) was significantly lower in the patients with high expression (p=0.046) as depicted in table 5.

TABLE 5: ASSOCIATION OF HOCT1 GENE EXPRESSION WITH HEMATOLOGICAL RESPONSE TO IMATINIB

HEMATOLOGICAL RESPONSE				
hOCT1 gene expression		CHR at 3 months present ,n=	CHR at 3 months absent,n=	p-value
High		15	0	0.10
Low		11	4	
TIME TO CHR (THR)				
GROUP	THR Range (months)	Mean THR ±SD (months)	Median THR (months)	p-value
High expression	1-3	1.93± 0.62	2.0	0.046*
Low expression	1-4.5	2.67± 1.06	2.5	

Morever, high expression of hOCT1 gene was also significantly associated with achievement of optimal molecular response.(p= 0.0012) (Table 6)

TABLE 6: ASSOCIATION OF HOCT1 GENE EXPRESSION WITH MOLECULAR RESPONSE TO IMATINIB

hOCT1 gene expression	No.of cases with Optimal response, n=	No. of cases with Warning response, n=	No. of cases with Failure response, n=	p-value
High	15	0	0	0.0012*
Low	2	7	6	

DISCUSSION

There is currently enough evidence to support the influence of hOCT1 gene expression on intracellular drug concentration and studies have shown that the pretherapeutic expression of hOCT1may be beneficial in predicting the response to imatinib in CML patients.[8] In the present study, expression of hOCT1 gene was calculated with respect to the controls using RT-PCR. The cases were divided into two groups, high expression(n=15) and low expression (n=15) groups, based on the median value of fold change in hOCT1 gene expression. (Median =5.6).The association of expression of hOCT1 gene with hematological and molecular response was assessed. It was seen that 11 (73.33%) patients with low expression achieved CHR by the end of 3 months, whereas 4(26.66%) did not. On the contrary, all 15 patients (100%) with high hOCT1 gene expression achieved CHR by the end of 3 months. However, this difference was not found to be statistically significant. It was also observed that the mean THR (time to CHR) in low expression group was significantly higher than in high expression group.Hence it was concluded that high expression of hOCT1 gene leads to early achievement of CHR.In case of molecular response, it was observed that high expression of hOCT1 gene was significantly associated with achievement of on optimal response to imatinib. It was seen that while all 15 patients with high expression had an optimal response , only 13.33 % (n=2) patients with low expression had it. In the low expression group, 40% patients had failure while remaining 23.33 % were categorized as warning.

Luciana Nardinelli et al[9], have reported that pretherapeutic expression of the hOCT1gene predicts a complete molecular response to Imatinib mesylate in chronic-phase CML. Wang L et al [10], reported that expression of hOCT1 is an important clinical determinant of the reponse to imatinib in chronic myeloid leukemia. They found that imatinib uptake into a CML cell line with high hOCT1 expression was greater than those with modest or low expression. Sailaja Kaigita et al[11] reported that the response to imatinib was significantly correlated with hOCT1gene expression. Crossman LC et al[12] reported that hOCT1 gene expression is a good predictor of clinical outcome and pre-treatment hOCT1 levels were found to be 8 times higher in responders compared to non-responders.[13]The findings of the present study are in accordance with previous studies and it can be concluded that hOCT1 gene expression alters the response to imatinib and high gene expression is associated with a better hematological and molecular response as compared to low gene expression. hOCT1 is an influx transporter which causes increased entry of imatinib into the cells.[14] This could be the possible underlying mechanism by which response is altered.

STRENGTHS & LIMITATIONS

Although previously work has been done on role of hOCT1 gene expression in response to Imatinib, this study is especially important in context with the Indian population. With CML being the most common adult leukemia in India, analysis of expression levels of hOCT1 gene could help in judicial use of Imatinib as a treatment option in CML patients, resulting in its selective use in responsive patients only. However, we also recognize that the present study is limited in that it considered hoct1 mRNA expression levels instead of protein expression which could have been more informative. Moreover, the sample size of thirty cases is also a limitation and the findings need to be confirmed in a bigger sample size , including patients in accelerated phase and blast-crisis also. We also acknowledge the fact that our study was limited to a single tertiary care hospital in north India and the results can be better assessed if patients from multiple health-care centres are included.

CONCLUSIONS

A significant correlation between achievement of complete hematological response (CHR) and optimal molecular response to Imatinib treatment and high *hOCT1* mRNA expression was seen. This study therefore, confirms the findings of previous studies and emphasizes the significance of pre-therapeutic high expression of *hOCT1* gene as a predictor of favorable response to Imatinib in the treatment of CML patients. It is also thereby suggested that upregulation of hoct1 gene could be used to get better clinical outcome in CML patients treated with Imatinib.

REFERENCES:

[1] Sawyers CL. Chronic myeloid leukemia. N Engl J Med. 1999; 340:1330–1340.

[2] Amabile M, Giannini B, Testoni N et al. Real-time quantification of different types of bcr-abl transcript in chronic myeloid leukemia. *Haematologica.*2001; 86: 252–259.

[3] Marin D, Hedgley C, Clark RE, Apperley J, Foroni L, Milojkovic D, Pocock C, Goldman JM, O'Brien S. Predictive value of early molecular response in patients with chronic myeloid leukemia treated with first-line dasatinib. Blood. 2012a; 120:291–294.

[4] Marin D, Ibrahim AR, Lucas C, Gerrard G, Wang L, Szydlo RM, Clark RE, Apperley JF, Milojkovic D, Bua M, Pavlu J, Paliompeis C, Reid A, Rezvani K, Goldman JM, Foroni L. Assessment of BCR-ABL1 transcript levels at 3 months is the only requirement for predicting outcome for patients with chronic myeloid leukemia treated with tyrosine kinase inhibitors. J Clin Oncol. 2012b; 30:232–238.

[5] Preudhomme C, Guilhot J, Nicolini FE, Guerci-Bresler A, Rigal-Huguet F, Maloisel F, Coiteux V, Gardembas M, Berthou C, Vekhoff A, Rea D, Jourdan E, Allard C, Delmer A, Rousselot P, Legros L, Berger M, Corm S, Etienne G, Roche-Lestienne C, Eclache V, Mahon FX, Guilhot F. Imatinib plus peginterferon alfa-2a in chronic myeloid leukemia. N Engl J Med. 2010; 363:2511–2521.

[6] Yaghmaie M, Ghaffari SH, Ghavamzadeh A, Alimoghaddam K,Jahani M, Mousavi SA, *et al.* Frequency of BCR‑ABL fusion transcripts in Iranian patients with chronic myeloid leukemia. Arch Iran Med 2008; 11:247‑51.

[7] Kagita S, Jiwtani S, Uppalapati S, Linga VG, Gundeti S,Digumarti R. Early molecular response in chronic myeloid leukemia patients predicts future response status. Tumour Biol 2014; 35:4443‑6.

[8] Kagita S, Uppalapati S, Jiwatani S, Linga VG, Gundeti S,Nagesh N, *et al.* Incidence of Bcr‑Abl kinase domain mutations in imatinib refractory chronic myeloid leukemia patients from South India. Tumour Biol 2014; 35:7187‑93.

[9] Lichtman MA, Lieveld JL. Chronic myelogenous leukemia and related disorders. In: Beutler E, Lichtman MA, Coller BS,Kipps TJ, editors. Williams Hematology. 6th ed. New York: McGraw‑Hill; 2001. p. 1125‑36.

[10] Rabinowitz I, Larson RS. Chronic myeloid leukemia. In:Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F,Glader B, editors. Wintrobe's Clinical Hematology. 11th ed.Philadelphia: Lippincott Williams & Wilkins; 2004.p. 2235‑58.

[11] Deininger MW. Milestones and monitoring in patients with CML treated with imatinib. Hematology Am Soc Hematol Educ Program 2008:419‑26.

[12] Vardiman JW, Melo JV, Baccarani M, Thiele J. Myeloproliferative neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, *et al.*, editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press; 2008. p. 32‑7.

[13] Ou J, Vergilio JA, Bagg A. Molecular diagnosis and monitoring in the clinical management of patients with chronic myelogenousleukemia treated with tyrosine kinase inhibitors. Am J Hematol 2008; 83:296‑302.

[14] Udomsakdi‑Auewarakul C, U‑Pratya Y, Boonmoh S,Vatanavicharn S. Detection of molecular variants of BCR‑ABLgene in bone marrow and blood of patients with chronic myeloid leukemia by reverse‑transcriptase polymerase chain reaction (RT‑PCR). J Med Assoc Thai 2000; 83:928‑35.