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Research Paper

Correlation of Pretreatment Hoct1 Expression Levels on Imatinib Response in Chronic Myeloid Leukemia Patients

Medical Science

KEYWORDS : Chronic myeloid leukemia, Imatinib, Response, hOCT1, Real-Time PCR

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ABSTRACT

Suboptimal responses to imatinib in chronic myeloid leukemia (CML) depend on either Bcr-Abl kinase domain mutations or varied intra cellular drug concentration. The hOCT1 gene mediates the intake of imatinib and maintains intracellular levels. To study the association of hOCT1 expression in newly diagnosed CML patients. One hundred and nine pretreatment CML samples were analyzed for hOCT1 mRNA expression using real-time PCR. Patients were grouped into low- and high- hOCT1 expressing groups based on median value as cut off point and correlated with different variables. Patients having high hOCT1 levels are significantly associated with chronic phase (p=0.001), low EUTOS risk (p=0.01) and achievement of complete cytogenetic response at 6 months after imatinib initiation (p=0.03). We could not found any significant association with low hOCT1 levels. Hence the present study suggests that pretreatment hOCT1 levels might be used as prognostic marker in predicting the clinical response to imatinib in CML patients.

Introduction:

Imatinib is one of the frontline therapies used for chronic myeloid leukemia (CML) management. Most of the CML patients achieve major responses. However, over the course of time, a substantial number 25-35% acquire resistance to imatinib [1]. The reasons for such suboptimal responses could be several. While the main cause appears to be kinase domain mutations in BCR-ABL [2], variable metabolizing enzyme activities [3], different influx & efflux transporter activities [4], poor medication compliance [5] and drug-drug interactions [6] could be the other reasons.

The active transport of imatinib is mediated by the influx pump (hOCT1 gene) and efflux pump (MDR1 gene) [7,8]. Transporters play a key role in drug absorption, distribution and elimination in vivo at the cellular level [9]. Inter-individual variations in expression patterns or activity of transporter genes may affect imatinib efficacy, leading to pharmacokinetic drug resistance and disease progression in CML patients [7,10].

The human organic cation transporter, hOCT1, regulates the intake of imatinib. It encodes for a solute carrier family 22 gene (SLC22A1) on chromosome 6q26 [10]. Alterations in hOCT1 gene may affect the response to IM in CML patients. Earlier studies reported that CML patients with high pre-therapeutic hOCT1 expression levels had higher probability of achieving a cytogenetic or molecular response and better survival [5,11,12]. White et al in 2007 reported that patients with lower hOCT1 activity had less chance of achieving major molecular response (MMR) [4]. Hence, we aimed to study the pre-treatment hOCT1 expression levels and to explore its association with imatinib resistance in CML patients.

Materials and Methods:

A total of 109 samples from newly diagnosed patients of CML were included in the study. Patient samples were collected from department of medical oncology, Nizams Institute of Medical Sciences, Hyderabad from January to December of 2011. The study was approved by the institutional ethics committee and an informed consent was obtained from every patient. The median age at presentation was 35 years (range 9-80 years). Of the 109 cases, 60 (55.04%) were men and 49 (44.9%) were women.

RNA extraction, cDNA synthesis and Quantitative mRNA analysis: RNA was extracted from 5 ml of peripheral blood, after the lysis of red blood cells, using TRIzol method (Invitrogen, Karlsruhe, Germany). Total RNA (1µg) was reverse transcribed into complementary DNA using high capacity reverse transcription kit (Applied Biosystems, Foster city, CA). Commercially available primers and Taqman probe from Applied Biosystems (Taqman Pre-Developed Assay Reagents for gene expression) was used for quantification of hOCT1 mRNA expression (Applied Biosystems, Foster city, CA). Actin ß gene as endogenous control and control cDNA were included in the RT-PCR. The reaction mixture of 20 µL contains: 0.25µL of primer (250nmol/L) and probe (150 nmol/L), 10.0 µL of expression assay mix, 7.0µL nuclease free water and 2µl of cDNA. The thermal cycling conditions were as follows: 2 min at 50°C, 5 min at 95°C, followed by 45 cycles 95°C for 15 seconds and 60°C for 1 minute. All the samples were run in duplicate to minimize handling errors. Assays included negative controls in all stages of reactions to assess specificity and to rule out contamination. Relative quantification was performed by the ΔΔCT method and fold change of transcript levels were measured relative to the reference gene in terms of cycle threshold (CT) [13,14].

Statistical analysis: Two-sided unpaired Student t-test and ANOVA tests were performed to determine the statistical significance of experimental results. Probability (p) values <0.05 are considered statistically significant. These tests were used to find out the correlation of hOCT1 mRNA levels with different hematological and clinical variables. All statistical analysis was performed using Graphpad Prism (version 6.0).

Results:

One hundred and nine samples were analysed for hOCT1 mRNA expression using Real-Time PCR. Patient hematological and clinical features were presented in Table 1. At the time of analysis 82.56% of patients were in chronic phase and 17.43% were in advanced phases of disease (accelerated and blast crisis). After 6 months of imatinib therapy, 55.96% achieved complete cytogenetic response (CCyR), 20.18% had partial cytogenetic response (PCyR), 11.0% died and 12.84% lost follow-up (LFU). Among 22 partial responders, 7 had drug toxicity, dose hiked for 10 patients and 5 were on the same IM dose. The minimum duration of follow-up was 24 months.

Patients were grouped into low- and high- hOCT1 expression based on a median expression of 119.50% (range 0 - 4757.79) as the cut-off value. Out of 109, 50.45% (55/109) of patients had high expression and 49.54% (54/109) had low expression. The hOCT1 mRNA expression levels were correlated with different

hematological and clinical variables, represented in Table 2 & Table 3.

Correlation of hOCT1 expression levels with hematological and clinical variables

The mean total leucocyte count and platelet count were significantly lower in patients having high hOCT1 levels (P=0.04 and P=0.03) compared to patients with low hOCT1 levels. No significant difference was observed in regard to haemoglobin between the two groups of patients with either high or low hOCT1 levels (Table 2).

With respect to phase of disease, among high hOCT1 expression group, patients in chronic phase had elevated levels compared to those in advanced phases of CML (p=0.001). No variation was observed with low expression group and phase of the disease (p=0.38). Sokal risk scores did not correlated with either high (p=0.75) or low hOCT1 expression group of patients (p=0.53). When EUTOS risk was considered, in high hOCT1 expressing group, patients with low EUTOS risk had significantly higher levels than to high EUTOS risk patients (p=0.01), whereas no significant correlation was observed between low hOCT1 expression and EUTOS risk (p=0.26). When duration of symptoms (less than 6 months and more than 6 months) before starting treatment was compared with expression, no significant difference was found in both high (p=0.82) or low expression groups (p=0.70). When cytogenetic response at 6 months after imatinib initiation was considered, in high hOCT1 expression group, patients having CCyR had higher levels compared to those having PCyR (p=0.03). Whereas with low hOCT1 expression group, there was no variation with expression between the patients having either CCyR or PCyR (p=0.63) (Table 3).

Basing on the median Bcr-Abl levels, patients were grouped into two categories: less than median and more than median and compared with hOCT1 expression. In both high and low hOCT1 expression groups, patients having less than median BCR-ABL levels showed slightly higher hOCT1 expression versus patients having more than median BCR-ABL levels (p=0.84; p=0.73) (Table 3).

Discussion:

Most of the CML patients achieve major cytogenetic responses to standard dose imatinib. But, a proportion of them develop resistance. These suboptimal responders may be benefit with either dose escalation of imatinib or early changeover to second generation tyrosine kinase inhibitor therapy [15,16]. Several studies had reported that hOCT1expression affects intracellular drug concentration and further results in excessive toxicity or suboptimal anticancer effect [10,12,17]. In some patients, imatinib resistance depends on intracellular concentration of the drug; insufficient doses might be due to lower hOCT1 or higher MDR1 expression or variations in metabolizing genes [10,18]. Several studies have suggested that early achievement of high imatinib intracellular concentrations may be a crucial determinant of cytogenetic response. McWeeney SK et al. in 2010 demonstrated that gene expression approach is a better predictor of response than morphologic criteria [19]. The present study revealed that lower mean total leucocyte count & platelet count were significantly associated with patients having high hOCT1 expression levels, which might reflect preferential activation of common signaling cascade in those with high hOCT1 levels.

In the present study, patients with high hOCT1expression levels significantly correlated with CCyR at 6 months after imatinib initiation (p=0.03). Our results are in accordance with earlier reports by Wang L et al. reported that high baseline hOCT1 mRNA levels were associated with better cytogenetic response at 6 months and prolonged progression-free and overall survival [11]. Crossman LC et al. 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 [12]. Other earlier studies found no significant correlation between the patients with high hOCT1 expression and response CCyR/CHR [14,20,21]. Leukemic cells with low hOCT1 expression/activity might lead to poorer outcomes, as imatinib is transported into the cells by hOCT1 [12].

We found significant association between phase of disease and high hOCT1 expression group; patients in chronic phase had higher levels compared to those in advanced phases of CML (p=0.001). Recent study by Solali S et al. did not find any significant difference between the hOCT1 expressions and phase of CML.[21] Our results are similar to earlier reports by White DL et al. who reported that hOCT1 expression levels did not correlated with either high or low Sokal risk score groups, our results are similar to earlier reports.[4] Among high hOCT1 expressing group, EUTOS low risk was significantly associated with high levels (p=0.01), as the EUTOS score predicts CCyR at 18 months after initiation of TKI therapy.[22]

Conclusion:

In conclusion we found a significant correlation between pretreatment hOCT1 expression levels and complete cytogenetic response at 6 months of imatinib initiation. Hence pretherapeutic high hOCT1 levels might be helpful in predicting clinical response to imatinib in CML patients.

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Tables

Table 1 Basic hematological and clinical features of patients

Total number of patients	n=109
Haemoglobin	
Mean ± SD (gm/dL)	10.11±2.14
Total leucocyte count	
Mean ± SD (mm^3)	166688.77±98094.79
Platelets	
Mean ± SD (lakh/mm^3)	4.32±2.80
Spleen	
Mean ± SD (cm)	11.00±8.33
Phase	
Chronic	90 (82.56%)
Advanced	19 (17.43%)
Sokal risk	
High	39 (35.77%)
Intermediate	39 (35.77%)
Low	31 (28.44%)
EUTOS risk	
High	33 (30.27%)
Low	76 (69.72%)
Duration of Symptoms before therapy	
< 6 months	84 (77.06%)
> 6 months	25 (22.93%)
Cytogenetic Response at 6 months after IM initiation	
CCyR	61 (55.96%)
PCyR	22 (20.18%)
Died	12 (11.0%)
LFU	14 (12.84%)

EUTOS, European treatment and outcome study; IM, Imatinib; CCyR, Complete cytogenetic response; PCyR, Partial cytogenetic response; LFU, Lost Follow-Up

Table 2 Correlation of hOCT1 expression levels with hematological variables

	High hOCT1 group (n=55)	Low hOCT1 group (n=54)	p-value
Total leucocyte count			
Mean ± SD	1,59,909 ± 86,084	1,73,728 ± 1,09,593	0.04
Platelets			
Mean ± SD	13.81 ± 2.39	4.86 ± 3.11	0.03
Haemoglobin			
Mean ± SD	10.44 ± 1.92	9.76 ± 2.31	0.09

Table 3 Correlation of hOCT1 expression levels with clinical variables

	High hOCT1 (n=55)	Total	p-value	Low hOCT1 (n=54)	Total	p-value
	Mean ± SD			Mean ± SD		
Phase						
Chronic	406.0±46.49	46	0.001	24.52±4.04	44	0.38
Advanced	289.9±22.08	9		16.68±6.00	10	
Sokal risk						
High	371.1±59.25	19	0.75	19.83±4.11	20	0.53
Intermediate	332.9±32.81	19		21.74±6.49	20	
Low	321.8±46.11	17		29.58±7.91	14	
EUTOS risk						
High	371.4±35.50	13	0.01	17.99±4.71	20	0.26
Low	367.3±47.60	42		26.05±4.76	34	
Duration of Symptoms						
< 6 months	379.0±44.05	39	0.82	22.47±3.76	45	0.70
> 6 months	397.4±79.19	16		26.01±9.55	9	
Cytogenetic Response at 6 months after IM initiation						
CCyR	512.4±127.9	33	0.03	25.58±5.23	28	0.63
PCyR	412.0±104.5	11		24.01±6.04	11	
BCR-ABL expression						
< Median	392.7±69.11		0.84	20.85±4.95		0.73
> Median	376.3±49.26			18.73±3.47		

EUTOS, European treatment and outcome study; IM, Imatinib; CCyR, Complete cytogenetic response; PCyR, Partial cytogenetic response

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