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The Relation between Multidrug Resistance Gene (MDR1) Polymorphism and Response to Nilotinib in Egyptian Patients with Chronic Myeloid Leukemia (CML)

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ABSTRACT

Background: For the past 10 years, the tyrosine kinase inhibitors (TKI), first generation Imatinib and second-generation Dasatinib and Nilotinib have been the standard treatment of chronic phase (CP) CML. However, a subset of patients does not respond even to the 2nd generation TKIs. One of the possible mechanisms of resistance to TKIs is the inappropriate expression of the multidrug resistance (*MDR1*) gene encoding the P glycoprotein (Pgp).

Patients and Methods: Thirty-one upfront Ph+ve CML patients, planned to receive Nilotinib, were included in this study. Detection of *MDR1* gene polymorphism C3435T, using PCR Restriction Fragment Length Polymorphisms (PCR-RFLP) was done initially for every patient. We prospectively followed up the patients between February 2012 and February 2014 with PCR for *BCR-ABL1* transcripts every 3 months. The molecular response to Nilotinib, according to the level of *BCR-ABL1* by PCR, was correlated to the different MDR1 3435 genotypes.

Results: Fifteen/31 patients (48.4%) carried the CC genotype, 9 (29.1%) carried TT genotype, while 7 (22.5%) carried CT genotype. Molecular response was optimal in 56%, 60% and 80% of the patients at month 3, 6 and 12 months respectively. Patients carrying *MDR1* 3434CT genotype showed a higher, yet insignificant, molecular response to Nilotinib.

Conclusion: In our study, the *MDR1*-C3435T genotype did not significantly affect the molecular response to Nilotinib. Further studies in larger series of patients are needed to define the genetic polymorphisms with therapeutic relevance in patients on Nilotinib among Egyptians.

Key Words: *Chronic myeloid leukemia (CML) – Multidrug resistance (MDR1) gene polymorphism – Molecular response – Nilotinib.*

INTRODUCTION

Chronic Myeloid Leukemia is one of the first neoplasms that are linked to genetic aberration namely the Philadelphia (Ph) chromosome. Ph chromosome results from a translocation between chromosome 9 and 22 with fusion of *RBC* gene on chromosome 22 to the *ABL1* gene on chromosome 9, with formation of the *BCR-ABL1* chimeric oncogene. This oncogene codes for a constitutively active cytoplasmic tyrosine kinase, which is implicated in the development of CML and has become a primary target for the treatment of this disorder **[1]**.

CML is the first human Cancer that responds to molecular target therapy. Imatinib, a member of TKIs works through competitive inhibition at the adenosine triphosphate (ATP) binding site of the BCR-ABL1 protein, which results in the inhibition of phosphorylation of proteins involved in BCR-ABL1 signal transduction. The BCR-ABL1 inhibition results in apoptosis of the malignant cells that express BCR-ABL1 without affecting the normal cells **[2]**.

However, some patients develop Imatinib-resistant disease or intolerance to Imatinib because of toxicities **[3]**.

Nilotinib (AMN107), a second generation TKI, represents viable alternative to Imatinib with approximately 30 folds more potency **[4]**.

These products were initially launched for use as second line therapies and were approved for first line use by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) in 2010 on the basis of the results from two ongoing multinational RCTs **[5]**.

In newly diagnosed CP-CML patients, Nilotinib 600mg and 800mg daily were compared to Imatinib 400mg daily in ENESTnd clinical trial. Nilotinib was statistically superior at one year on the same end points for all comparisons **[5]**.

However, despite their excellent efficacy, a subset of patients does not respond to TKIs, and are deemed to have resistance to the drug **[6]**.

Currently, resistance to TKIs is believed to be a consequence of the interaction of multiple factors such as treatment compliance, bioavailability, pharmacodynamics, genetic changes, *BCR-ABL1* kinase domain mutations, or combinations of these **[7]**.

Multi drug resistance gene (*MDR1*) [*ABCB1* (ATP-binding cassette, sub-family B (*MDR* /ATP), member 1)] product is an ATP-driven efflux pump contributing to the pharmacokinetics of drugs that are P-glycoprotein (P-gp) substrates. The generally accepted action of MDR1 is to reduce intracellular drug accumulation through Pgp-mediated efflux, thus hampering the achievement of effective drug levels at the target site **[8]**.

The inappropriate expression of the *MDR1* gene has been frequently implicated in resistance to different chemotherapeutic drugs as *MDR1* single nucleotide polymorphisms (SNPs) are associated with drug clearance **[9]**.

Nilotinib had been identified as a substrate of P-gp in Nilotinib-resistant cell lines **[10]**.

More than 50 single nucleotide polymorphisms (SNPs) have been identified concerning the *MDR1* gene, and SNP polymorphisms may affect the expression and function of the P-gp. The SNPs T1236C, G2677T/A, and C3435T are the most common variants in the coding region of *ABCB1* SNPs in *MDR1* gene and have the potential to alter protein function and could also influence the efficiency of absorption or elimination **[8]**.

PATIENTS AND METHODS

Between February 2012 and March 2013, 31 chronic phase CML patients who were consecutively admitted to clinical hematology unit, kasr Alainy Hospital or visited its outpatient clinic were enrolled in this study. The study was approved by the Ethical Committee of Kasr Alain and all patients signed an informed consent before enrollment. The patients were prospectively followed for 12 months to evaluate their response to TKI Therapy.

A total of 31 patients, aged 18 years or older, were eligible. They were 15 males (48.3%) and 16 females (51.7%) with an age range of 18 to 78 with a median of 38 years. All had Ph-positive chronic-phase CML. The diagnosis of CML was based on standard clinical data and confirmed by cytogenetics and molecular analysis.

Chronic-phase (CP) CML was defined according to WHO criteria, by the presence of less than 10% blasts, less than 20% basophils, and a platelet count of $\geq 100x10^9/L$ with no extramedullary involvement **[11]**.

Patients with the following criteria were excluded: Patients under the age of 18 years, pregnant females, Philadelphia negative CML and Patients treated before with interferon or underwent autologous bone marrow transplantation.

Initial assessment included: Detailed Medical history recording, complete Physical examination, complete blood count and examination of peripheral blood film, estimation of Sokal and Hasford risk scores, quantitative measurement of *BCR-ABL1* transcripts using RQ-PCR at diagnosis and every 3 month after staring TKIs and the detection of *MDR1* gene polymorphism C3435T, using PCR Restriction Fragment Length Polymorphisms (PCR-RFLP).

Treatment:

All patients were planned to receive nilotinib 400 mg od.

Follow-up:

We prospectively followed-up the patients between February 2012 and February 2014. Patients were regularly monitored on an outpatient basis; biweekly Physical examinations, Blood counts, and biochemistry were obtained during the first month of TKI Therapy and then monthly until a complete hematological response was achieved, and then every 3 months with RQ-PCR for *BCR-ABL1* there after.

Response to treatment:

It was evaluated according to ELN criteria 2013 (Table 1) using CBC at month 3 to assess hematological response and *BCR-ABL1* transcripts % at month 3, 6 and 12 to assess molecular response [12].

Table (1): Molecular Response According to ELN criteria 2013.

Time point	Optimal	Warning (Suboptimal)	Failure
3 months	*BCR-ABL1* ≤10%	*BCR-ABL1* >10%	Non-CHR
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%

Drug toxicity:

It was evaluated according to the common Toxicity Criteria for Adverse events (CTCAE) version 4.3.

RNA extraction and cDNA synthesis:

Total RNA extraction was carried out from fresh peripheral blood leucocytes according to the initial silica extraction method described by Boom et al., [13], using QIAmp RNA Blood minikit (Qiayen Hilslen, Germany). For cDNA synthesis, 1 µg total RNA was used to synthesize first-strand cDNA according to the manufacturer protocol (Fermentas).

BCR-ABL1 transcripts measurement:

Real-time quantitative polymerase chain reaction (RQ PCR) was used for detection of *BCR-ABL1* transcripts level.

MDR1 C3435T genotyping:

MDR1 C3435T polymorphism was detected using a PCR-RFLP assay. The following primers were used: 5’-GCTGG TCCTGAAGTTG ATCTGTGAAC-3’ as forward and 5’-AC ATT-AGGCAGTGACTCGATG AAGGCA-3’ as reverse primer. The PCR mixture included: 1µM primer, 200µM of each dNTP (Sigma), Taq DNA polymerase, 1X buffer with 1.5mM MgCl2, and 2.5 units Taq polymerase (5U/µL, Sigma). The PCR product (248bp in size) was digested for 3h at 37°C with 2U *MboL* restriction enzyme. The expected fragment sizes are: a 238-bp fragment for TT genotype, 172-and 60-bp fragments for the CC genotype, and 238, 170 and 60bp for the CT genotype. DNA fragments generated were analyzed by electrophoresis on a 2% agarose gel, stained with ethidium bromide, and observed with an ultraviolet transilluminator.

Statistical analysis:

Pre-coded data was entered on the computer using “Microsoft Office Excel Software” program (2010) for windows. Data was transferred to the Statistical Package of Social Science Software program, version 21 (SPSS). Comparison between groups was performed using independent sample *t*-test or one way ANOVA with Tukey’s post hoc test for quantitative variables and Chi square or Fisher’s exact test for qualitative ones. Repeated measures were tested using Friedman test and pairwise Wilcoxon test with Bonferroni adjustment of P values. Spearman correlation coefficients were calculated to get the association between different quantitative variables. *p*-values less than 0.05 were considered statistically significant, and less than 0.01 were considered highly significant.

RESULTS

The present study included 31Ph positive CML patients in chronic phase of the disease. Patient’s age ranged from 18 to 78 with a median of 38 years. Male patients were 24 (48.3%) and female patients were 19 (51.7%).

Drug toxicities leading to discontinuation of treatment:

Three patients (10%) stopped Nilotinib treatment after 6 months because of drug toxicity (1 had persistent Grade 2 hepatotoxicity; 2 had Grade 4 hematological toxicity).

Response to treatment:

At month 3, molecular response was optimal (*BCR-ABL1%* ≤10%) in 56%, warning zone was identified (*BCR-ABL1%* >10%) in 41% and 3% failed to achieve hematological response.

At month six, 60% achieved optimal molecular response, warning zone was identified (*BCR-ABL1%* >10%) in 20% and molecular failure occurred in 20% of patients.

At month 12, 80% achieved major molecular response, warning zone was identified (*BCR-ABL1%* >10%) in 15% and molecular failure occurred in 5% of patients.

Progression of the disease:

According to WHO definition of blastic transformation of CML [11], no patient on Nilotinib arm transformed into acute leukemia.

Progression of BCR-ABL1 transcripts % by time after treatment with Nilotinib:

BCR-ABL1 transcripts % significantly decreased at month 3 and 12 in comparison with the previous *BCR-ABL* transcripts % (p=<0.001, and 0.004 respectively). There was statistically significant reduction in *BCR-ABL1* transcripts % in relation to the baseline *BCR-ABL1* transcripts (p=<0.001, <0.001, <0.001 and <0.001 at month 3, 6, 9 and 12 respectively, Table 2).

Distributions of genotypes among study group:

The overall frequency of different *MDR-1* 3435 genotypes among CML patients enrolled in our study showed that the majority, 15 patient (48.4%) carried the CC genotype, 9 patients (29.1%) carried TT genotype, while 7 patients (22.5 %) carried CT genotype.

The Relation between the MDR C3435T Genotypes and Response to TKIs:

Although patients carrying *MDR1* 3434CT genotype showed a higher molecular response to Nilotinib, there was no statistically significant difference between *MDR*- C3435T genotypes and the molecular response to treatment with Nilotinib according to ELN 2013 criteria of response (Table 3).

Table (2): Kinetics of *BCR-ABL1* by time after Nilotinib therapy in 31 chronic phase CML patients.

Time point	*BCR-ABL1*	Transcript level		*p*-value	
	Mean±SD	Median	Range	Baseline	Stepwise
At Diagnosis	138.6±126.2	97.0	0.0–465.0	–	–
Month 3	38.8±78.7	8.0	0.0–382.0	< 0.001	<0.001
Month 6	34.6±71.5	1.0	0.0–225.0	<0.001	0.26
Month 9	6.4±16.4	0.3	0.0–55.0	<0.001	0.255
Month 12	1.2±4.0	0.1	0.0–18.0	<0.001	0.004

Table (3): The Relation between the *MDR* C3435T Genotypes and Response to Nilotinib.

Response		*MDR* Genotype			*p*-value
		CC	CT	TT	
% Response at 3 months (n=30):	Failure	7.1	0.0	0.0	0.8
	Suboptimal	42.9	28.6	44.4	
	Response	50.0	71.4	55.6	
% Response at 6 months (n=26):	Failure	23.1	16.7	28.6	0.6
	Suboptimal	23.1	0.0	28.6	
	Response	53.8	83.3	42.9	
% Response at 12 months (n=20):	Failure	10.0	0.0	0.0	0.7
	Suboptimal	20.0	0.0	16.7	
	Response	70.0	100.0	83.3	

DISCUSSION

Despite the excellent efficacy of TKIs in treatment of chronic myeloid leukemia, a subset of patients does not respond to TKIs, and are deemed to have resistance to the drug. Resistance to BCR-ABL1 TKIs has become a pressing challenge in the treatment of CML. Thus, studies on the mechanisms of resistance to TKI have been driven by the need to improve response and prevent or overcome drug resistance **[6]**.

Although point mutations in the *BCR-ABL1* kinase domain is the most common mechanism, several mechanisms can play a role in the resistance to TKIs but the possible importance of drug-transporter proteins has been only recently appreciated with the demonstration that TKIs is a substrate of P-glycoprotein (Pgp), the product of *MDR1* gene. The generally accepted action of MDR1 is to reduce intracellular drug accumulation through Pgp-mediated efflux, thus hampering the achievement of effective drug levels at the target site **[14]**.

P-glycoprotein is encoded by the multidrug resistance *ABCB1* gene, and the functional variation in this gene could explain, at least in part, variable responses to this drug **[6]**.

(SNPs) in *ABCB1* genes have the potential to alter protein function and could also influence the efficiency of absorption or elimination. The up-regulation of drug transporters (ABCB1-ABCG2) is one of specific causes of resistance to Imatinib **[15]**.

In our study, genotype distribution revealed elevation of CC genotype frequency in CML patients (48.4%), followed by TT genotype (29.1%) and CT genotype (22.5%).

It has been found that the distribution of *MDR1*-C3435T polymorphism is significantly influenced by ethnicity. It is clear that people of African origin carry predominantly the wild-type (CC) allele and not the homozygous allele (TT). Ameyaw et al., reported high frequency of CC allele in Ghanian, Kenyan, African American and Sudanese populations (83%, 83%, 84% and 73% respectively) compared with British Caucasian, Portuguese, south-west-Asian, Chinese, Filipino and Saudi populations who showed lower frequencies of the C allele (48%, 43%, 34%, 53%, 59%, and 55%, respectively) **[16]**.

In Caucasian people, the frequency of CC and TT alleles is approximately the same. However, the TT is the predominant genootype among Asian and Indian populations **[17]**; in the Indian population, the frequency of the homozygous TT variant was 43%, CC 18% and C/T 39% **[18]**.

In our study, patients carrying *MDR1*-3434 CT genotype showed a higher response at month 3, 6 and 12 compared to CC and TT genotypes with no statistical significance.

To the best of our knowledge, no study addressed the impact of *MDR* C3435T polymorphism on the molecular response to Nilotinib. However some studies revealed a significant relation between molecular response to Imatinib and *MDR* C3435T polymorphism.

Dulucq et al., reported overall frequency of the *MDR1* 3435 CC, CT, and TT genotypes of 18.9%, 51.1%, and 30%, respectively in 90 French CML patients treated with imatinib; the haplotype (1236C-2677G-3435C) was statistically linked to less frequent major molecular response (70% vs 44.6%; $p<0.021$) **[19]**.

Deenik et al., studied *ABCB1* gene single nucleotide polymorphisms (SNPs), C1236T, G2677T/A, and C3435T, with respect to molecular response in a cohort of 46 early chronic phase CML patients, in Nederland, receiving high-dose imatinib (800mg); patients homozygous for 3435T and 2677T showed lower probabilities to obtain a major molecular response (MMR) and complete molecular response CMR **[20]**.

Vivona et al., investigated the relation between *ABCB1* polymorphisms c.1236C>T, c.3435C>T and c.2677G>T/A with markers of response to Imatinib in patients with CML in 118 Brazilian patients initially treated with a standard dose of Imatinib for 18 months. In the responder group, the frequency of *ABCB1* 1236CT/2677GT/3435CT haplotype was higher in patients with MMR than in patients without MMR (51.7% vs. 8.3%, p=0.010). Furthermore, carriers of this haplotype had increased probability of reaching the MMR compared with the non-carriers (OR: 11.8; 95% CI: 1.43-97.3, p=0.022) **[21]**.

Conclusion:

In our study, although patients carrying *MDR1*- 3434CT genotype showed a higher response to Nilotinib at month 3, 6 and 12 compared to CC and TT genotypes, statistical significance was not achieved.

In view of the significant influence of *MDR1*-C3435T genotypes on the response to Imatinib in CML patients previously reported, we would expect a similar impact on response to Nilotinib. This discrepancy may be due to racial differences or to the small number of patients in our cohort. Further studies on larger series of patients with a longer follow-up period (2 years) are needed to verify. Other mechanisms of resistance such as point mutation of *BCR-ABL1* gene, OCT level, etc should also be incorporated in such studies.

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