Imatinib and Norimatinib Plasma Levels Analyses of Chronic Myeloid Leukemia Patients

Kronik Miyeloid Lösemi Hastalarında İmatinib ve Norimatinib Plazma Düzeyi Ölçümleri

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ABSTRACT Objective: Imatinib is the first line treatment in chronic myeloid leukemia (CML). Imatinib metabolism depends primarily on the presence of cytochrome P450 enzymes, its active metabolite being norimatinib (N-desmethyl imatinib). Norimatinib accumulates in plasma 10-30%. The aim of this study was to analyze certain parameters which maintain the importance in clarifying the pharmacologic effects of imatinib. **Material and Methods:** To investigate plasma concentrations, 41 CML patients on minimum 400 mg imatinib daily were included to the study. Pharmacokinetic analysis was performed by tandem mass spectrometry (LC-MS/MS). Mean plasma imatinib and norimatinib levels were statistically calculated as 4.038±1.290 ppm (min. 0.576 ppm, max. 6.795 ppm) and 1.150±0.733 ppm (min. 0.08 ppm, max. 3.835 ppm), respectively. **Results:** Limit of detection (LOD) values for imatinib and norimatinib were 30 ppb and 25 ppb, whereas limit of quantification (LOQ) values were 99 ppb and 82.5 ppb, and recovery of imatinib and norimatinib were 98.45% and 92.47%, respectively. Average percentage of imatinib transformation to norimatinib was 22.16%. Variation of this percentage from one patient to another suggested the possibility of interindividual differences in drug response. **Conclusion:** Individual results have shown that pharmacologic effects of imatinib may differentiate among patients. In this study, we standardized and justified the applicability of our analysis method. Patients' plasma drug levels can be measured using this method. Thus optimal treatment efficacy can be ensured.

Key Words: İmatinib; pharmacokinetics; drug monitoring; leukemia, myeloid, chronic-phase; tandem mass spectrometry

ÖZET Amaç: İmatinib, kronik miyeloid lösemi tedavisinde ilk tercih edilen tedavi yöntemidir. İmatinib metabolizması temelde sitokrom P450 enzimleri üzerinden, aktif metaboliti norimatinibe (N-desmetil imatinibe) dönüşme şeklinde yürür. Norimatinib plazmada %10-30 aralığında birikme gösterir. Bu çalışmanın amacı, imatinibin farmakolojik etkilerini aydınlatmaya yardımcı olan parametrelerin analiz edilmesidir. **Gereç ve Yöntemler:** Plazma konsantrasyonlarının araştırılmasında, çalışmaya, günde en az 400 mg imatinib tedavisi gören 41 KML hastası dahil edildi. Plazma analizleri, tekrarlayan kütle spektrometrisi (LC-MS/MS) ile gerçekleştirildi. Ortalama plazma imatinib ve norimatinib düzeyleri istatistiksel yöntemlerle sırasıyla 4,038±1,290 ppm (min. 0,576 ppm, maks. 6,795 ppm) ve 1,150±0,733 ppm (min. 0,08 ppm, maks. 3,835 ppm) olarak hesaplandı. **Bulgular:** İmatinib ve norimatinib için saptama sınırı (LOD) değerleri sırasıyla 30 ppb ve 25 ppb, miktar tayini (LOQ) değerleri ise sırasıyla 99 ppb ve 82,5 ppb ve imatinib ile norimatinib için yüzde kazanım sırasıyla %98,45 ve %92,47 bulundu. İmatinibin norimatinibe ortalama dönüşüm yüzdesi %22,16 olarak hesaplandı. Bu yüzdenin bireyler arası farklılık göstermesi, ilaca oluşan yanıtta bireyden bireye farklılıklar olabileceğine işaret etmiştir. **Sonuç:** Bireysel sonuçlara bakıldığında, imatinib farmakokinetiğinin bir hastadan diğerine farklılık gösterdiği anlaşılmıştır. Bu çalışmada kullanılan yöntem standardize edilmiş ve uygulanabilirliği kanıtlanmıştır. Uyguladığımız analiz yöntemiyle, hastaların plazma ilaç düzeylerinin ölçümü ve dolayısıyla ilaç kullanımında en etkili tedavinin sağlanması temin edilebilir.

Anahtar Kelimeler: İmatinib; farmakokinetik; ilaç takibi; lösemi, miyeloid, kronik-faz; ardışık kitle spektrometrisi

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Chronic myeloid leukemia (CML) is a serious, relatively rare type of cancer with myeloproliferative characteristics. CML results in hyperplasia in the bone marrow. In order to treat CML, molecules with the ability to inhibit tyrosine kinase - tyrosine kinase inhibitors (TKIs) - have been developed. When we look at individualized therapy, it is crucial to identify plasma levels of the drug in question and its active metabolite. It is possible to titrate individual therapeutic doses by means of an analytical method to measure drug plasma concentrations. It will then be possible to avoid doses which fall under clinical efficacy. Many studies have been conducted to understand the relation of imatinib plasma concentrations with clinical response.[1-3]

Imatinib is a targeted, potent and competitive TKI; it is designed to selectively interfere with key signal transduction pathways.[4-6] It performs its therapeutic activity on CML by competitive inhibition at the ATP-binding site.[4,7,8] This way, tyrosine kinase phosphorylation of proteins acting in Bcr-Abl signal transduction is inhibited.[7-9] In the clinical setting, Bcr-Abl positive cells are affected by the inhibition while normal cells are left unharmed.[8] The primary aim of imatinib use in CML management is the achievement of a prolonged life span.[10] It is considered to be a clinically successful molecule due to its effectiveness and high tolerance profiles in CML patients.[6]

Imatinib is administered orally as first line therapy to adult, chronic phase CML patients at daily doses of 400 mg, 600 mg or even 800 mg.[7,9] Dosage titration is usually planned according to the hematological responses which are monitored in the various phases of the disease and to the adverse reactions which are related to the patient and treatment regime. Imatinib yields close concentration/effect levels as regards to other cytotoxic agents.[7] Clinical studies show that the optimal response is attained at 1000 ng/ml of imatinib plasma concentration and is maintained at concentrations above this threshold.[6,10]

Imatinib metabolism depends primarily on the presence of cytochrome P450 enzymes, whose active metabolite is norimatinib. Norimatinib accumulation in plasma is 10-30% of imatinib. The aim of this study was to analyze certain parameters which are important in clarifying the pharmacologic effects of imatinib.

MATERIAL AND METHODS

The tandem mass spectrometry (LC-MS/MS) method was adapted to the present laboratory conditions of the study from previous studies in order to perform imatinib therapeutic drug monitoring (TDM) in CML patients.[11-14] Study trials were performed with nilotinib as well as imatinib.

SAMPLE COLLECTION

Blood samples were required to achieve the aim and purpose of the study. They collected from 41 CML patients (18 female, 23 male) who were treated at Ankara University School of Medicine Hematology Department. All patients included in the study were using a dose regimen of at least 400 mg daily. Two patients (4.88%) were on 600 mg whereas none were on 800 mg daily dosage regimen. All patients were informed in detail about the study, and all undersigned informed consent. Ethics committee approval for the study was received from Ankara University Clinical Trials Ethics Committee on 27 May 2013.

From each patient, within the 24 hour interval after the drug use, a 4 ml blood sample each was collected in an EDTA tube. Then, blood samples were transferred to Eppendorf tubes. Plasma was separated from blood samples by Heraeus Sepatech Labofuge 200 centrifuge working at 3500 rpm for 5 minutes. Blood samples from 41 patients were collected between April 2013 and April 2014.

STANDARD AND SAMPLE PREPARATION

For sample preparation, 250 µl plasma of each patient sample were transferred to a different Eppendorf tube. 40 µl nilotinib was added onto the sample from each patient. Finally, 1 µl protein aggregation solution (50% methanol-50% acetonitrile) was added. This mixture was vortexed for 30 seconds. The vortexed solutions were centrifuged at 1200 rpm in cold centrifuge of 4°C. Supernatant

was collected to sample vials. Each 50 µl were drawn and introduced to the LC-MS/MS device.

CHEMICALS AND STOCK SOLUTION PREPARATION

The pure active ingredient, imatinib mesylate, used during laboratory analyses was provided by Deva Holding, Inc. Also, norimatinib and the internal standard, nilotinib, were provided by Jinlan Pharm-Drugs Technology Co., Ltd. 8 mg imatinib mesylate (ref no: KL12-IMM-01(01)) dissolved in 100% methanol (1000 ppm) was stored at -20ºC. Afterwards, 8 mg norimatinib (ref no: 404844-02-6) dissolved in 100% methanol (1000 ppm) was stored at -20ºC. Eight milligrams of nilotinib (ref no: 641511-10-1) dissolved in 100% methanol (1000 ppm) was stored at -20ºC. Acetonitrile (ref no: 1.00030.2500), methanol (ref no: 1.06008.2500), HPLC Water (ref no: 1.15333.2500) and formic acid (ref no: 1.00264.1000) were also used. 50% methanol-acetonitrile solution was prepared and nilotinib was added to the solution until it becomes 10 mg/L (10ppm). During sample study, 2 mobile phases of A and B were used.

LABORATORY CONDITIONS

While studying plasma samples, study solutions containing nilotinib were prepared. For calibration, 1.5 ml (PTFE), 250 µl control blood and 250 µl patient blood were added to Eppendorf tubes. 1 ml study solution was added to the mixture and vortexed for 30 seconds. Afterwards the mixture was centrifuged at 1200 rpm for 15 minutes. Four hundred micro liters of pure supernatant was separated and mixed with 400 µl distilled water in glass tubes. Glass tubes were then vortexed for 30 seconds. The resulting solutions were injected into the LC-MS/MS device. Chromatograms were shown in Figure 1.

Mobile phase A: 100% HPLC Water + 0.05% formic acid; mobile phase B: 100% acetonitrile + %0.05 formic acid. Five micrometers, 150 mm × 4.6 mm C18 silica colon (Agilent Zorbax HPLC Colon RX-C18 5µm 4.6 × 150mm, ref no: 883.967-902; colon temperature: 40ºC, LC-MS/MS device: Shi-

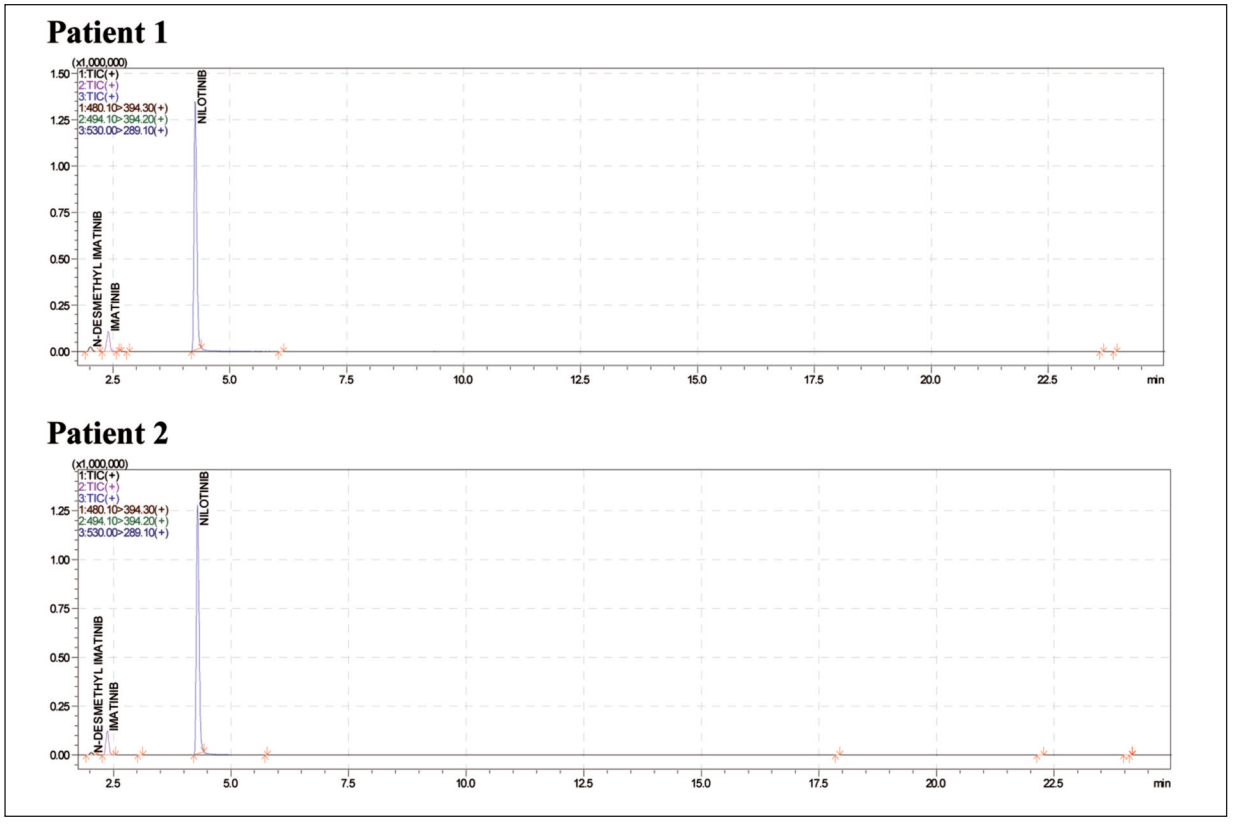

FIGURE 1: Chromatogram and mass spectrum peaks of patient 1 and 2.

madzu LC MS MS 8030) was used. With the help of the mobile phases, samples were sent through the colon to the ion source at flow rate of 1 ml/min with a certain gradient (Table 1). Here, substances were ionized and separated inside the detector in terms of mass/burden (m/z) (norimatinib m/z: 480.10>394.3; imatinib m/z: 394.10>294.20; nilotinib m/z: 530.0>289.10).

SENSITIVITY, EFFICIENCY, RECOVERY STUDIES

Imatinib and norimatinib standards of 0, 2, 5, 10 and 20 ppm each was prepared. Samples with a defined imatinib and norimatinib value were compared with the 20 ppm standard solution.

Sensitivity and efficiency were measured. LOQ levels for imatinib and norimatinib were 99 ppb and 82.5 ppb, respectively. LOD levels for the same substances were 30 ppb and 25 ppb, respectively (Table 2).

Water-based imatinib and norimatinib values were 0.062 ppm and 0.046 ppm, respectively. Water + 10 ppm standard solution-based imatinib and norimatinib values were 9.845 ppm and 9.247 ppm, respectively. Water-based imatinib and norimatinib recoveries were 98.45% and 92.47%, respectively. Imatinib and norimatinib recovery values for the last patient were 93.87% and 91.56%, respectively.

TABLE 1: Gradient scheme.

Duration (min)	Mobile phase A (%)	Mobile phase B (%)	Flow rate (ml/min.)
0.01	75	25	1
0.1	75	25	1
8	25	75	1
8.1	2	98	1
15	2	98	1
15.1	75	25	1
20	75	25	1
25	Stop	Stop	Stop

TABLE 2: Sensitivity estimations of imatinib and norimatinib.

	Imatinib	Norimatinib
Highest calibration standard	20 ppm	18 ppm
Limit of Quantification (LOQ)	0.099 ppm	0.0825 ppm
Limit of Detection (LOD)	0.030 ppm	0.025 ppm

TABLE 3: Repeatability study values for imatinib and norimatinib.

Study No.	Imatinib (ppm)	Norimatinib (ppm)
1	2.33	2.58
2	2.41	2.26
3	2.55	2.42
4	2.28	2.52
5	2.39	2.35
Average value	2.39	2.43

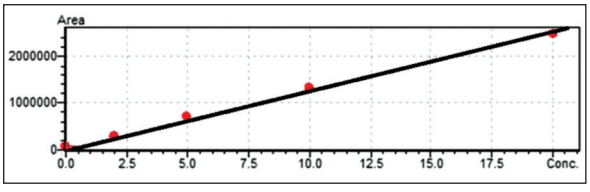

FIGURE 2a: 5-point imatinib linearity with respect to 0, 2, 5, 10 and 20 ppm standard solutions.

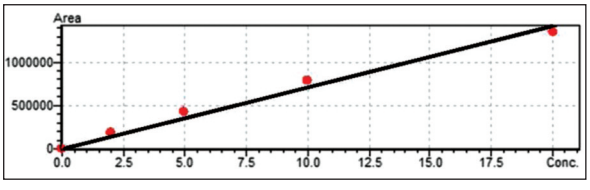

FIGURE 2b: 5-point norimatinib linearity with respect to 0, 2, 5, 10 and 20 ppm standard solutions.

REPEATABILITY AND ACCURACY

Two and a half parts per million standard solution for imatinib and norimatinib was studied 5 times each. Repeatability was good for each of the 5-time tests (Table 3).

r^2 and r values were calculated for accuracy. For imatinib, the r^2 and r values were 0.9995219 and 0.9990441, respectively (Figure 2.a). For norimatinib they were 0.9905497 and 0.9952636, respectively (Figure 2.b). Accordingly, the study was found to be accurate.

TABLE 4: Mean values of age, period of drug use, drug and active metabolite levels.

Parameters	Mean±SD	n
Age (year)	46.975±13.150	41
Period of treatment (years)	4.292±3.156	41
Plasma imatinib level (ppm)	4.038±1.290	41
Plasma norimatinib level (ppm)	1.150±0.733	41

STATISTICAL ANALYSES

Statistical analyses were performed by using statistical package for the SPSS 16.0 version package program. In the exploratory analysis, data showed a normal distribution (by Shapiro-Wilk statistics); therefore, in order to compare two independent groups, Student's t-test was used. Pearson correlation was used to compare metric data. Data were shown as mean±standard deviation (SD). $p<0.05$ was considered as statistically significant.

RESULTS

Imatinib and norimatinib levels in plasma samples of 41 CML patients (18 female, 23 male) who received at least 400 mg imatinib in the last 24 hours were analyzed in terms of ppm by LC-MS/MS. The same plasma samples were also analyzed for percentage transformation of imatinib to norimatinib. Mean age of patients was 46.975±13.150 (min. 23, max. 73), mean period of imatinib use was 4.292±3.156 years (min. 1 year, max. 13 years). Mean plasma imatinib and norimatinib levels were statistically calculated as 4.038±1.290 ppm (min. 0.576 ppm, max. 6.795 ppm) and 1.150±0.733 ppm (min. 0.08 ppm, max. 3.835 ppm), respectively (see Table 4). Imatinib percentage transformation to norimatinib was calculated as 22.16%.

As a result of the sigmoidal 2-tailed Pearson correlation test, significant correlation was found only in unchanged imatinib and norimatinib levels (Pearson correlation; p=0.001). No other significant correlation was found between other parameters (Table 5).

Imatinib and norimatinib plasma levels were compared within genders (Table 6). No statistical significance of plasma levels of these substances was found between different genders (Student's t-test; $p>0.05$). Percentage of norimatinib transformation was calculated as 24.42% and 20.43% in female and male, respectively.

Imatinib and norimatinib plasma levels were compared according to period of treatment with imatinib (Table 7). No statistical significance of plasma levels of these substances was found between patients using imatinib for less than 5 years and those using the same drug for 5 years or more (Student's t-test; $p>0.05$). Percentage of norimatinib transformation in patients using imatinib for less than 5 years was 20.28% whereas in those

TABLE 5: Descriptive statistical data (age, period of drug use, drug and active metabolite levels). Pearson correlation was used to compare metric data.

Comparative parameter		Age	Period of imatinib use	Plasma imatinib level	Plasma norimatinib level
Age	r	1.000	0.163	0.094	0.043
	p		0.309	0.559	0.790
	n	41	41	41	41
Period of treatment	r	0.163	1.000	-0.211	0.132
	p	0.309		0.186	0.411
	n	41	41	41	41
Plasma imatinib level	r	0.094	-0.211	1.000	0.582
	p	0.559	0.186		0.000*
	n	41	41	41	41
Plasma norimatinib level	r	0.043	0.132	0.582	1.000
	p	0.790	0.411	0.000*	
	n	41	41	41	41

*p<0.05.

TABLE 6: Comparison of imatinib and norimatinib plasma levels between genders. Student's t-test was used in order to compare age, period of treatment, plasma imatinib level and plasma norimatinib levels between female and male groups (p>0.05).

Comparison between genders	Gender	n	Mean±SD	p
Age	Female	18	48.117±11.263	0.646
	Male	23	46.166±14.520	
Period of treatment	Female	18	4.588±3.183	0.620
	Male	23	4.083±3.188	
Plasma imatinib level	Female	18	4.120±1.271	0.738
	Male	23	3.980±1.327	
Plasma norimatinib level	Female	18	1.331±0.732	0.187
	Male	23	1.021±0.722	

TABLE 7: Comparison of plasma imatinib and norimatinib levels as regards period of treatment groups (less than 5 years or 5 years or more). Student's t-test was used in order to compare plasma imatinib level and plasma norimatinib levels between period of treatment groups (p>0.05).

Comparison as regards period of treatment	Period of treatment groups	n	Mean±SD	p
Plasma imatinib level	1-4 years	25	4.221±1.165	0.263
	≥5 years	16	3.753±1.457	
Plasma norimatinib level	1-4 years	25	1.073±0.563	0.411
	≥5 years	16	1.269±0.949	

TABLE 8: Comparison of imatinib and norimatinib levels of patients with 2-fold age difference. Student's t-test was used in order to compare plasma imatinib level and plasma norimatinib levels between ages with 2-fold (1, 2) groups.

Comparison of ages with 2-fold difference in between each other	Age coefficients	n	Mean±SD	p
Plasma imatinib level	1	8	3.615±1.015	0.576
	2	20	3.852±0.990	
Plasma norimatinib level	1	8	1.374±0.800	0.035*
	2	20	0.904±0.337	

*p<0.05.

using imatinib for 5 years or more the same percentage was 25.27%.

Imatinib and norimatinib plasma levels between 28 patients who had 2-fold age difference in between each other were compared (Table 8). Statistically significant difference was found between norimatinib plasma levels and age (Student's t-test; *p*=0.035). Accordingly, when age increased 2-fold plasma imatinib levels increased by 6.54%, imatinib decreased by 34.17%. Imatinib transformation percentage to norimatinib was 27.54% in younger patients whereas the same percentage decreased to 19.02% in elderly patients of 2-fold age.

Patients on 400 mg daily dose of imatinib presented mean imatinib and norimatinib levels of 4.045±1.312 ppm and 1.145±0.751 ppm, respectively. Those on 600 mg daily dose of imatinib had imatinib and norimatinib plasma levels of 3.906±1.026 ppm and 1.247±0.207 ppm, respectively (Table 9).

DISCUSSION

The rapeutic drug monitoring is a crucial tool in treatment of CML patients. In fact, plasma concentration analysis of leukemia drugs could be an effective method in determination of patients' compliance to their daily oral therapies, potential drug-drug interactions, and efficiency of the treatment and assessment of adverse reactions. There are a number of studies in which imatinib concentration in biological fluids were determined using high performance liquid chromatography coupled to ultraviolet detection (HPLC-UV) or LC-MS/MS.[1,2,10-12,15-21]

Birch et al. found plasma imatinib levels of >1 mg/L in patients achieving complete molecular response, which is over the suggested target response.[15,20] Streit et al. reported imatinib through

TABLE 9: Imatinib and norimatinib levels of patients on a daily dose of 400 mg and 600 mg.

	400 mg daily dose of imatinib (n:39)			600 mg daily dose of imatinib (n:2)		
	Mean±SD	Minimum	Maximun	Mean±SD	Minimum	Maximun
Plasma imatinib level (ppm)	4.045±1.312	0.58	6.80	3.906±1.026	3.18	4.63
Plasma norimatinib level (ppm)	1.145±0.751	0.08	3.84	1.247±0.207	1.10	1.39

concentrations falling more than 10% below the suggested target response.[19] Lankheet et al. found only 26.8% of the calculated trough plasma concentrations of imatinib reaching 1.1 ppm.[21] Streit et al. and Lankheet et al. attributed this outcome to possible drug-drug and drug-diet interactions and patient-related factors.[19,21] In our study, all patients but one reached plasma imatinib levels exceeding 1 mg/L.[11] This patient with a level of 0.576 ppm also revealed a plasma norimatinib level of 0.08 ppm. Nevetheless, norimatinib transformation was 12.20%, within the expected range.[5,6,10] He was on concomitant losartan treatment at the date of sample collection. Losartan is expected to increase exposure to imatinib.[22] In this case, imatinib did not act as expected. This unexpected outcome might be due to genetic and additional external factors which will be discussed in future studies.

In our study, to be able to interpret imatinib efficiency by the help of plasma imatinib levels, imatinib and norimatinib were analyzed by the LC-MS/MS method in plasma samples of 41 CML patients (18 female, 23 male) on at least 400 mg oral imatinib treatment. According to the plasma levels obtained, drug-concentration-effect and drug-comorbidity interactions were discussed by considering the pharmacokinetic properties of imatinib. Investigations were performed according to age and gender, and to the period of imatinib treatment.

Bakhtiar et al. have suggested that the sampling period up to 24 hours after daily intake exhibits an excellent linearity from 4 to 10 ppm in human plasma.[16] Imatinib therapy in CML requires chronic drug intake. A patient receives their daily dosage regimen every 12 or 24 hours depending on the limitation of the daily dose they are clinically assigned to. In our study, samples were taken from each patient right before their daily intake. This corresponds to the 24-hour time period after the previous daily intake. As each patient included in this study had been using imatinib for at least a year during sampling, it was possible to attain optimal plasma levels of imatinib and norimatinib and hence have accurate results. During the period of sample collection, 41 patients out of 53 were willing to attend the study. Twelve patients refrained from sample collection due to the claim that they were already exhausted from chronic therapy and clinic visits. A longer period of sample collection and a sample size above 41 patients may result in even more accurate results.

Generally, imatinib levels of all patients were found to exceed the optimal levels that were reported in previous studies.[9,13] The results suggested good response of patients to imatinib treatment. To interpret the study results more accurately, we also estimated the imatinib transformation percentages to norimatinib. Therefore, the analysis was performed in accordance with the 10-30% interval discussed in various studies.[5,6,10]

Statistical analysis resulted in a mean age of 46.975±13.150 and 4.292±3.156 years of mean period of treatment. Mean plasma imatinib and norimatinib levels were 4.038±1.290 ppm and 1.150±0.733 ppm, respectively. As anticipated, there was statistical significance between plasma imatinib and norimatinib through concentrations. Mathematical average of imatinib transformation percentage to norimatinib was 22.16%, which aggress with the interval reported in previous studies.[5,6,10] Some patients revealed either high or low results. Differences in plasma drug and active primary metabolite levels showed a possible variability of the pharmacokinetic properties of imatinib from one patient to the other. This possibility helped emphasize the significance of TDM in the clinical setting.

No statistically significant difference was found in conclusion of plasma analysis between female and male. These results are consistent with information given by Di Gion et al. Besides, no statistically significant difference between female and male was observed in percentage transformation of imatinib to norimatinib.[9] 28 patients of the total of 41 had 2-fold age difference (8 patients within 20-30 years of age, 20 patients within 40-60 years of age). When age increased 2-fold, imatinib levels increased by 6.54% whereas norimatinib decreased by 34.17%. According to Di Gion et al., when age increases 2-fold, imatinib clearance may increase by 16%, which is contradictory to our findings.[6]

The contradiction of our results with that of Di Gion et al. may be explained by genotypical difference of one patient population to the other. Further studies are necessary to comprehend the underlying actual cause. Our study revealed increase in imatinib levels. Our results also suggested decrease in percentage transformation of imatinib to norimatinib as age increased by 2-fold. This dramatic decrease of norimatinib formation can be explained by the potential effects of age difference on imatinib metabolism. However, it would be necessary to perform detailed clinical monitoring on patients of 50 years and above to be able to interpret whether such changes are directly involved with imatinib clearance. Moreover, imatinib disposition increases by 12% in patients of 65 years and above.[9,23] Therefore, it would be possible to perform daily dosage titration of this drug which has nearly absolute bioavailability in favor of a decrease in elderly patients. In our study, number of patients over the age of 65 was not sufficient to interpret such a result. Young patients and elderly patients in the same group revealed 27.54% and 19.02% percentage transformation of imatinib to norimatinib, which falls within the range of results obtained in previous studies.[5,6,10]

In our study, 5 years of treatment period was considered as a threshold in comparing short and long period results. Imatinib treatment period was not found to be statistically significant in relation to the imatinib and norimatinib levels. However as imatinib treatment period was increased, percentage transformation of imatinib to norimatinib slightly increased. According to Duckett and Cameron, as a result of adverse events related to and resistance to imatinib treatment, imatinib treatment is generally ceased at the first five years.[24] In our study, the linear increase in percentage transformation of imatinib to norimatinib suggested a potential risk of increase in adverse events due to the prolonged effects of imatinib through its active metabolite. Therefore patients who have been undergoing imatinib treatment for over 5 years should be monitored much carefully for any emerging adverse events.

Titier et al. found that for patients with 400 mg once daily, mean plasma trough concentrations were 1.166 ± 0.579 ppm (mean±SD, n=50).[17] For patients with 600 mg once daily, mean plasma though concentrations were 2.094 ± 0.931 ppm (mean ± SD, n=19).[17] Götze et al. found plasma concentrations of 400 mg imatinib daily use ranging between 0.491 ppm and 1.562 ppm.[20] In our study, patients on 400 mg and 600 mg daily dose of imatinib presented mean imatinib plasma levels of 4.045±1.312 ppm (mean±SD, n=39) and 3.906±1.026 ppm (mean±SD, n=41), respectively. Results obtained in our study showed higher plasma through concentrations of imatinib in patients using imatinib 400 mg and 600 mg daily. Higher concentrations in our study address additional factors influencing increased drug response. In our study, no concomitant drug use was reported for the patients receiving 600 mg daily therapy. Most of the drugs reported to have been used along with patients' CML treatment do not have any effects on imatinib pharmacokinetics. However, concomitant use of levothyroxine, atorvastatin and verapamil are known to cause increase in imatinib effects.[22] Such concomitant drug use which was reported for some patients (1 patient on concomitant levothyroxine, 2 patients on concomitant atorvastatin and 1 patient on concomitant verapamil) could be the reason for high trough concentrations found in our study.

In conclusion, statistical results generally complied with other recent studies performed in the relevant field.[5,6,10] In general, imatinib plasma levels and pharmacokinetic profiles resulted within anticipated limits. However, dramatic differences were observed when patients' results were analyzed individually. Further studies are necessary to explain the reasons of this variability.

Concomitant drug use is an important factor affecting plasma levels of imatinib. Imatinib use was accompanied by at least two more drugs including those classified as alimentary tract and metabolism, cardiovascular system, hormone preparations, musculoskeletal system, nervous system, respiratory system and genitourinary tract. Information on probable and attributable adverse events was individually observed as well. Informa-

tion on probable and attributable adverse events were also separately observed. To be able to address these interindividual differences, results of each patient shall be interpreted by its own and interpretations shall be supported by further clinical and genetic analyses.

Results of the statistical analysis of our study were in parallel with that of other recent analytical studies which involve imatinib. However, imatinib plasma levels presented interindividual discrepancies from one patient to another one. This shows different pharmacological effects related to the individual characteristics. By means of pharmacokinetics, it is possible to detect therapeutic benefit/side effects related to drug use. Even if its therapeutic window is considered highly safe, imatinib use frequently results in resistance to the drug. This leads to null benefit from imatinib therapy hence inappropriate drug use. Pharmacokinetic analysis makes it possible to understand imatinib effects at early stages of drug use. Clinics can now be able to detect effectiveness or resistance to imatinib even before its chronic use. This is a crucial contribution to the clinical management of CML.

Pharmacokinetic analyses and clinical data of a small population with 41 patients may not be enough to reach an accurate conclusion of the interindividual effects of imatinib on the patients included in our study. There is need for further studies to support the collected data and the interpretations made under the scope of our study. Pharmacogenetic studies, which investigate individual characteristics at genetic level, and detailed studies, which are performed on a comprehensive and widespread population to understand environmental factors, may help the understanding of imatinib's interindividual variability of its metabolic transformation in the human organism.

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REFERENCES

1. Awidi A, Ayed AO, Bsoul N, Magablah A, Mefleh R, Dweiri M, et al. Relationship of serum imatinib trough level and response in CML patients: long term follow-up. Leuk Res 2010;34(12):1573-5.
2. Golabchifar AA, Rezaee S, Ghavamzadeh A, Alimoghaddam K, Dinan NM, Rouini MR. Population pharmacokinetics of imatinib in Iranian patients with chronic-phase chronic myeloid leukemia. Cancer Chemother Pharmacol 2014;74(1):85-93.
3. Koren-Michowitz M, Volchek Y, Naparstek E, Gavish I, Levi I, Rowe JM, et al. Imatinib plasma trough levels in chronic myeloid leukaemia: results of a multicentre study CSTI571AIL11TGLIVEC. Hematol Oncol 2012;30(4):200-5.
4. Burger H, Nooter K. Pharmacokinetic resistance of imatinib mesylate: role of the ABC drug pumps ABCG2 (BCRP) and ABCB1 (MDR1) in the oral bioavailability of imatinib. Cell Cycle 2004;3(12):1502-5.
5. Boddy AV, Sludden J, Griffin MJ, Garner C, Kendrick J, Mistry P, et al. Pharmacokinetic investigation of imatinib using accelerator mass spectrometry in patients with chronic myeloid leukemia. Clin Cancer Res 2007;13(14):4164-9.
6. Filppula AM, Neuvonen M, Laitila J, Neunoven PJ, Backman JT. Autoinhibition of CYP3A4 leads to important role of CYP2C8 in imatinib metabolism: variability in CYP2C8 activity may alter plasma concentrations and response. Drug Metab Dispos 2013;41(1):50-9.
7. Schleyer E, Pursche S, Köhne CH, Schuler U, Renner U, Gschaidmeier H, et al. Liquid chromatographic method for detection and quantitation of STI-571 and its main metabolite N-desmethyl-STI in plasma, urine, cerebrospinal fluid, culture medium and cell preparations. J Chromatogr B Analyt Technol Biomed Life Sci 2004;799(1):23-36.
8. Arellano C, Gandia P, Lafont T, Jongejan R, Chatelut E. Determination of unbound fraction of imatinib and N-desmethyl imatinib, validation of an UPLC-MS/MS assay and ultrafiltration method. J Chromatogr B Analyt Technol Biomed Life 2012;907:94-100.
9. Di Gion P, Kanefendt F, Lindauer A, Scheffler M, Doroshyenko O, Fuhr U, et al. Clinical pharmacokinetics of tyrosine kinase inhibitors: focus on pyrimidines, pyridines and pyrroles. Clin Pharmacokinet 2011;50(9):551-603.
10. Takahashi N, Miura M. Therapeutic drug monitoring of imatinib for chronic myeloid leukemia patients in the chronic phase. Pharmacology 2011;87(5-6):241-8.
11. Haouala A, Widmer N, Duchosal MA, Montemurro M, Buclin T, Decosterd LA. Drug interactions with tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib. Blood 2011;117(8): e75-87.
12. Parise RA, Ramanathan RK, Hayes MJ, Egorin MJ. Liquid chromatographic-mass spectrometric assay for quantitation of imatinib and its main metabolite (CGP 74588) in plasma. J Chromatogr B Analyt Technol Biomed Life Sci 2003;791(1-2):39-44.
13. Marull M, Rochat B. Fragmentation study of imatinib and characterization of new imatinib metabolites by liquid chromatography-triple-quadrupole and linear ion trap mass spectometers. J Mass Spectrom 2006;41(3): 390-404.
14. Furlong MT, Agrawal S, Hawthorne D, Lago M, Unger S, Kreuger L, et al. A validated LC-MS/MS assay for the simultaneous determination of the anti-leukemic agent dasatinib and two pharmacologically active metabolites in human plasma: application to a clinical pharmacokinetic study. J Pharm Biomed Anal 2012;58:130-5.

15. Birch M, Morgan PE, Handley S, Ho A, Ireland R, Flanagan RJ. Simple methodology for the therapeutic drug monitoring of the tyrosine kinase inhibitors dasatinib and imatinib. Biomed Chromatogr 2013;27(3):335-42.
16. Bakhtiar R, Lohne J, Ramos L, Khemani L, Hayes M, Tse F. High-through put quantification of the anti-leukemia drug STI571 (Gleevec) and its main metabolite (CGP 74588) in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci 2002;768(2):325-40.
17. Titier K, Picard S, Ducint D, Teilhet E, Moore N, Berthaud P, et al. Quantification of imatinib in human plasma by high-performance liquid chromatography-tandem mass spectrometry. Ther Drug Monit 2005;27(5):634-40.
18. Mičová K, Friedecký D, Faber E, Polýnková A, Adam T. Flow injection analysis vs. ultra high performance liquid chromatography coupled with tandem mass spectrometry for determination of imatinib in human plasma. Clin Chim Acta 2010;411(23-24):1957-62.
19. Streit F, Binder L, Hafke A, Brandhorst G, Braulke F, Haase D, et al. Use of total and unbound imatinib and metabolite LC-MS/MS assay to understand individual responses in CML and GIST patients. Ther Drug Monit 2011;33(5):632-43.
20. Götze L, Hegele A, Metzelder SK, Renz H, Nockher WA. Development and clinical application of a LC-MS/MS method for simultaneous determination of various tyrosine kinase inhibitors in human plasma. Clin Chim Acta 2012;413(1-2):143-9.
21. Lankheet NA, Hillebrand MJ, Rosing H, Schellens JH, Beijnen JH, Huitema AD. Method development and validation for the quantification of dasatinib, erlotinib, gefitinib, imatinib, lapatinib, nilotinib, sorafenib and sunitinib in human plasma by liquid chromatography coupled with tandem mass spectrometry. Biomed Chromatogr 2013;27 (4):466-76.
22. Haouala A, Widmer N, Duchosal MA, Montemurro M, Buclin T, Decosterd LA. Drug interactions with the tyrosine kinase inhibitors imatinib, dasatinib, and nilotinib. Blood 2011;117(8):e75-87.
23. Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 2005;44(9):879-94.
24. Duckett RD, Cameron MD. Metabolism considerations for kinase inhibitors in cancer treatment. Expert Opin Drug Metab Toxicol 2010;6(10):1175-93.