DOI: 10.5455/bcp.20120131045705

Individual Differences in The Pharmacokinetics of Clozapine in Healthy Chinese Adults

Yiping Liu[1], Huande Li[1], Miao Yan[1]

ABSTRACT:
Individual differences in the pharmacokinetics of clozapine in healthy Chinese adults

Introduction: Differences in response to clozapine among patients have received considerable attention. Individual pharmacokinetic differences are a major reason.
Objective: The aim of this study was to investigate the pharmacokinetic variability in the blood levels ofclozapine among young, healthy, Chinese male volunteers.
Methods: A total of 18 volunteers who received 12.5mg of clozapine participated in the study. Blood samples were measured by using High-Performance Liquid Chromatography-Electrospray ionization tandem mass spectrometry (LC-MS/MS). The major pharmacokinetic parameters were calculated from the plasma concentration-time curve and individual variability was assessed.
Results: The findings indicated that the $AUC_{0\text{-}48h}$, C_{max} and $t_{1/2}$ varied 2.9, 2.6, and 2.0 fold, respectively among the individuals even with similar physiological status.
Conclusions: Individual differences were obvious among healthy volunteers and greater differences could have been observed if more subjects had been included in the study. Psychiatrists and pharmacists may need to have more patience and pay more attention to high variability in response to clozapine among schizophrenic patients with differences in weight, age, liver function, renal function, and pharmacogenetics.

Key words: Clozapine, pharmacokinetics, individual differences, different response, variability

Bulletin of Clinical Psychopharmacology 2012;22(1):17-22

[1]Clinical Pharmacy & Pharmacology Research Institute, the Second Xiangya Hospital of Central South University, 139# Middle Renmin Road, Changsha, Hunan 410011, China

Yazışma Adresi / Address reprint requests to: Huan-De Li, Clinical Pharmacy & Pharmacology Research Institute, the Second Xiangya Hospital of Central South University, Renmin Middle Road 139, Changsha, Hunan 410011, China

Elektronik posta adresi / E-mail address: lihuande1953@126.com

Gönderme tarihi / Date of submission: 04 Aralık 2011 / December 04, 2011

Kabul tarihi / Date of acceptance: 31 Ocak 2012 / January 31, 2012

Bağıntı beyanı:
Y.L., H.L., M.Y.: Yazarlar bu makale ile ilgili olarak herhangi bir çıkar çatışması bildirmemişlerdir.

Declaration of interest:
Y.L., H.L., M.Y.: The authors reported no conflict of interest related to this article.

INTRODUCTION

Clozapine is an 'atypical' antipsychotic drug. It clearly has been shown to be more effective in reducing symptoms of schizophrenia than other antipsychotics and remains the gold standard for antipsychotic treatment. Clozapine is used only in patients who have failed to respond adequately to standard drug treatment of schizophrenia given for an adequate duration due to the risk of agranulocytosis and seizures. Differences in clinical response have been observed in schizophrenic patients given clozapine treatment and one of the potential reasons is pharmacokinetic variability (1,2). Many factors (i.e. age, body weight, gender, liver function, and renal function) can affect pharmacokinetic parameters (3,4), which may result in insufficient effectiveness or intolerable adverse reactions. This study was carried out to investigate the extent of pharmacokinetic variation among healthy volunteers with similar age, gender, body weight, and body surface area (BSA). We hope to provide essential information for psychiatrists, which may help them to optimize the therapeutic effects of clozapine and prevent adverse reactions in clinical practice.

METHODS

Study Design

This study was a prospective, single-centre investigation of the pharmacokinetics of clozapine in healthy Chinese volunteers. The protocol was approved by the Ethics Committee of the Second Xiangya Hospital, Central South University and all volunteers signed informed consent before the study.

Volunteers

The study included 18 volunteers aged between 21 with 26 years (23.1±1.5 years), with body weight ranging between 54.0 and 70.0 kg (61.6±4.6 kg) and height ranging between 160.0 and 177.0 cm (169.6±4.7 cm). Their health status was assessed by clinical evaluation including physical examination and the following laboratory tests: albumin, alkaline phosphatase, ALT, AST, blood glucose, creatinine, BUN, total cholesterol, protein, total bilirubin, Hb, Hct, total and differential white cell counts, and routine urinalysis. During the trial period, the volunteers were hospitalized at the Clinical Pharmacology Lab, Second Xiangya Hospital of Central South University at 18:00 P.M.

Drug Administration and Blood Sample Collection and Preparation

All the volunteers had an evening meal before 20:00 P.M. After overnight fasting, they received a single dose of clozapine tablets (Novartis Pharma Ltd, Switzerland) 12.5 mg at 8:00 A.M. with 250 mL of water. The volunteers were then seated for at least 1 hr. and then fasted for an additional 4 hrs. Standard lunches and evening meals were provided 4hrs. and 10 hrs. after the clozapine dose. Liquid consumption was allowed ad libitum after lunch except liquids containing xanthine and acidic beverages, including tea, coffee, and cola. At 0, 2, 4, 8, 10, 24, and 48 hrs. after the clozapine dose, blood pressure, heart rate, and body temperature were recorded. Blood samples (4 mL) were withdrawn from a suitable antecubital vein by indwelling catheters into heparin-containing tubes before, and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, and 48 hrs. after, dosing. The blood samples were centrifuged at 2500 × g for 10 min. at room temperature and plasma was stored at -70 °C until analysis. A 1.0 mL aliquot of human plasma sample was put into a 1.5 mL Eppendorf tube, and 80 μL of carbamazepine (internal standard, IS) working solution (0.28μg/mL of IS) was added. The mixture was vortex-mixed and loaded into a disposable strata-X solid-phase extraction (SPE) cartridge (Phenomenex, USA). The cartridge was washed with 2.0 mL of water and 1.0 mL 8% methanol, respectively, and then eluted by 0.5 mL of the mobile phase. The elution solvents were transferred into 150 μL autosampler inserts And a 2 μL of sample was injected into the LC-MS/MS system.

Analytical Methods

Clozapine concentrations in plasma were analysed by a specific and validated LC-MS/MS method. The chromatography was performed on the ACQUITY™ UPLC system (Waters Corp., Milford, MA, USA) with cooling autosampler and column oven enabling temperature control of the analytical column. Triple-quadrupole tandem mass spectrometric detection was carried out on a Micromass® Quattro micro™ API mass spectrometer (Waters Corp.) with an electrospray ionization (ESI) interface. The ESI source was set in the positive ionization mode. The analytical column was a MG-II C_{18} (100mm ×2.0mm, 3.0 μm; Shiseido Co., Japan). The mobile phase consisted of 15 mM ammonium acetate (containing 0.125% formic acid) and acetonitrile (60:40, v/v), its flow rate was 0.25 mL/min and the injection volume was 2 μL. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 327.00→269.90 for clozapine and m/z 236.90→193.90 for the IS respectively, with a scan time of 0.10 s per transition. The optimal MS parameters were as follows: capillary 0.75 kV, cone 30.0 V, extractor 4.0 V, source temperature 120°C, desolvation temperature 400°C, cone gas flow 50 L/h, desolvation gas flow 750 L/h, dwell time 0.05 s. Nitrogen was used as the desolvation and cone gas. The optimized collision energy of clozapine and the IS were 23.0 and 20.0 eV. All data collected in centroid mode were acquired and processed using MassLynx™ NT 4.1 software with QuanLynx™ program (Waters Corp.). No significant interference at the retention times of clozapine or the IS was observed in the MS chromatograms of blank plasma under the aforementioned LC-MS/MS conditions. Calibration curves were based on peak area ratios of clozapine to the IS for seven calibration standards over the range of 0.154–77.000 ng/mL for clozapine in human plasma analyzed in duplicate. Linearity was determined to assess the performance of the method. Linear least-squares regression with a weighting index of $1/x^2$ was performed on the peak area ratios of clozapine /IS vs. clozapine nominal concentrations of the seven plasma standards (0.154, 0.385, 1.155, 3.080, 9.240, 30.800 and 77.000 ng/mL) in duplicate to generate a calibration curve. Accuracy and precision were based on assays of five replicates of quality control samples analyzed on three different days. Recovery was determined by expressing the mean result of

recovery sample analysis as a percentage of the nominal concentration.

Pharmacokinetics and Variability Analysis

C_{max} and t_{max} were obtained directly from the plasma concentration-time curve. Ke was calculated from the slope of regression line of the last four natural log-transformed plasma concentrations-time curve. The $t_{1/2}$ was calculated as 0.639/Ke. $AUC_{0\text{-}48h}$ was calculated by the linear trapezoidal rule. The correlation between $AUC_{0\text{-}48h}$, C_{max}, weight, and BSA were evaluated.

RESULTS

Data were obtained from 18 young, healthy Chinese volunteers with normal liver function and renal function. The main characteristics of the 18 volunteers are shown in Table 1. The CV% of age, body weight, and BSA were within 10%, which showed that the volunteers were in similar a physiological state and were fit for variability research.

The concentration-time curve of the 18 volunteers is shown in Figure 1. The major pharmacokinetic parameters of clozapine and the corresponding CV% are summarized in Table 2, showing that C_{max} was 33.4±9.5ng·ml^{-1} (CV%: 28.4%; range: 19.6~50.5ng·ml^{-1}), t_{max} was 1.4±0.7h (CV%: 50%; range: 1~4h), $AUC_{0\text{-}48h}$ was 321±91ng·ml^{-1} (CV%: 28.3%; range: 190~550ng·ml^{-1}), Ke was 0.05±0.011h^{-1} (CV%: 25%, range: 0.036~0.072h^{-1}), and $t_{1/2}$ was 14.5±2.9h (CV%: 20%, range: 9.6—19.2h). The maximum values of $AUC_{0\text{-}48h}$, C_{max}, t_{max}, Ke, and $t_{1/2}$ were 2.9, 2.6, 4, 2.0 and 2.0 fold higher than the minimal value, respectively.

The relationships between $AUC_{0\text{-}48h}$, C_{max}, andweight

Table 1: Main characteristics of 18 volunteers

Patient	Age (years)	Height (cm)	Weight (kg)	BSA (m^2)[a]
1	23	175	60	1.73
2	22	167	60	1.67
3	22	160	61	1.63
4	24	175	65	1.79
5	21	170	60	1.69
6	22	177	70	1.86
7	24	170	58	1.67
8	23	166	54	1.59
9	26	172	58	1.68
10	22	170	60	1.69
11	22	175	65	1.79
12	22	170	60	1.69
13	24	165	63	1.69
14	23	172	60	1.71
15	23	172	70	1.83
16	21	170	68	1.79
17	25	162	63	1.67
18	26	165	54	1.59
Mean	23.06	169.61	61.61	1.71
SD	1.51	4.68	4.64	0.08
%CV	6.56	2.76	7.53	4.42
Median	23	170	60	15.33
Minimum	21	160	54	14.29
Maximum	26	177	70	16.98
Ratio[b]	1.24	1.11	1.30	1.19

[a]Calculated according to the Dubois formula, BSA = (W 0.425 x H 0.725) x 0.007184
[b]Ratio: Maximum/Minimum

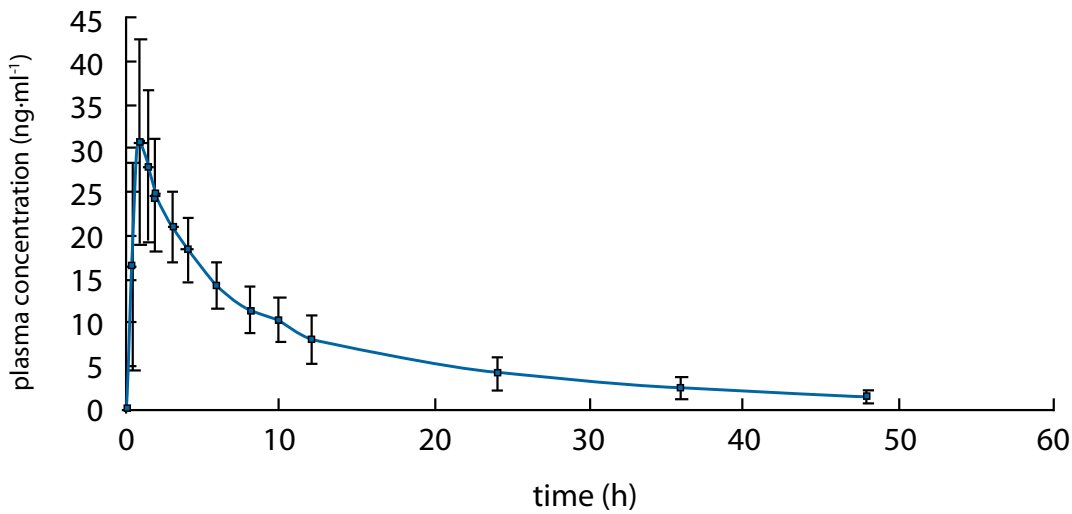

Figure 1: Mean plasma concentration-time curves of 18 volunteers after a single dose clozapine 12.5 mg.

Table 2: Major pharmacokinetic parameters of clozapine in 18 volunteers

Patient	AUC_{0-48} (ng×h·mL^{-1})	t_{max} (h)	C_{max} (ng×h·mL^{-1})	Ke (h^{-1})	$t_{1/2}$ (h)
1	250	1.5	26.2	0.039	18
2	287	1	28.3	0.044	15.9
3	416	1	47.4	0.038	18.3
4	262	1	36.3	0.052	13.4
5	190	1.5	19.6	0.071	9.7
6	261	1	39.5	0.063	11
7	258	1.5	22.9	0.040	17.2
8	325	1	41.9	0.041	16.8
9	219	1	33.5	0.072	9.6
10	368	1.5	41.9	0.050	14
11	550	1	50.5	0.056	12.3
12	288	1	27.0	0.055	12.7
13	407	1	42.9	0.044	15.9
14	344	2	32.6	0.043	16.2
15	283	4	21.7	0.051	13.5
16	467	1.5	39.0	0.036	19.2
17	312	1	30.2	0.057	12.1
18	298	1	20.3	0.043	16.2
Mean	321	1.40	33.4	0.050	14.50
SD	91	0.70	9.5	0.011	2.90
%CV	28.3	50.0	28.4	0.25	20.0
Median	293	1	33.05	0.047	14.95
Minimum	190	1	19.6	0.036	9.6
Maximum	550	4	50.5	0.072	19.2
Ratio	2.9	4	2.6	2	2

Ratio: Maximum/Minimum

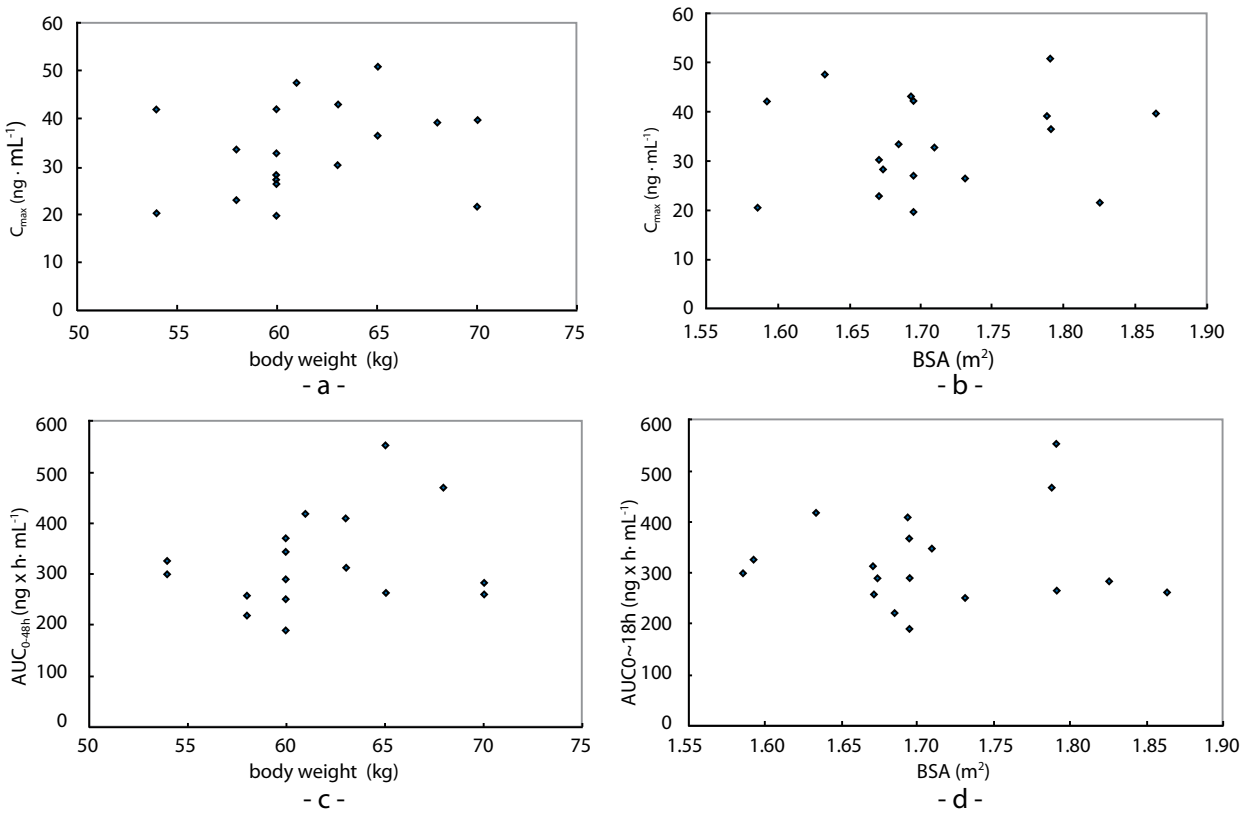

Figure 2: a: Correlation between C_{max} and body weight, (r=0.225, p=0.368)
b: Correlation between C_{max} and BSA, (r=0.133, p=0.599)
c: Correlation between AUC_{0-48} and body weight, (r=0.250, p=0.317)
d: Correlation between AUC_{0-48} and BSA, (r=0.109, p=0. 666)

and BSA are presented in figure 2. The correlation coefficients were no more than 0.3 and the p values were more than 0.05, which demonstrated that there were not any significant correlations between body weight/BSA and these pharmacokinetics parameters.

DISCUSSION

Pharmacokinetic variability may result in different individual responses (5,6).

A study of Lee et al. has revealed a large inter-individual variation that was affected by dose, age, smoking habits, and sex. They also found there was a wide inter-ethnic variance. The pharmacokinetic profiles of Koreans were similar to those observed in Asians but quite different from those in Caucasians (7). Other research also has confirmed that the factors mentioned above may alter the absorption, distribution, metabolism, and elimination and affect the pharmacokinetic profile of clozapine (8-10). In the present study we investigated pharmacokinetic variability by using limiting factor analysis. Limiting factor analysis is an approach which is used to assess individual differences by controlling the most common affecting factors. The advantage of this design is to suppress the interference of other factors. In our study, only volunteers with similar age, body weight, BSA, race, and physiological state were enrolled. As shown in Table 1 all the CV% of age, body weight, and BSA were within 10%. The results showed that the main characteristics of none of 18 volunteers were palpably different from the other healthy Chinese volunteers and the CV% for $AUC_{0\text{-}48h}$, C_{max}, and $t_{1/2}$ were 28.3%, 28.4%,0.25%, 20.0%, respectively. However, it is important for us to pay attention to the differences between the maximal value and the minimal value of $AUC_{0\text{-}48h}$, C_{max}, and $t_{1/2}$. As shown in Table 2, the maximum values were 2.9-, 2.6-, and 2.0 fold higher than the minimal value, respectively. Thisvariability can produce significant differences in clinical responses. Due to the significant risk of agranulocytosis and seizures associated with its use, a study of the multiple dose pharmacokinetic variability of clozapine among healthy volunteers was terminated. Fortunately, the degree of variability can be estimated using pharmacokinetic principles. According to the pharmacokinetic equations, $Css= R*C_0e^{-kt}$ (R is cumulative coefficient, $R=1/(1-e^{-kj})$). The ratio of Css could vary over 3 fold if volunteers were given repeated similar doses of clozapine in a long-term study. Due to the diversity and complexity of patients, greater differences would have been produced in clinical practice. A larger sample size study among 193 Chinese inpatients with schizophrenia demonstrated that the concentrations of clozapine were up to 8 fold different at a given dose (11).

The correlations between $AUC_{0\text{-}48h}$, C_{max}, andbody weight, and BSA were also investigated to clarify the reasons for variation. Lower cross-correlations were observed between $AUC_{0\text{-}48h}$, C_{max}, and body weight, and BSA in Figure 2. They showed that weight and BSA were not major factors resulting in differences in the present study. Due to the limitations of the experimental conditions, the intrinsic reasons for variation were unclear. An in vivo investigation carried out by Raedler and et al. has illustrated a high degree of inter-subject and intra-subject variability in the metabolism of clozapine (12). Lee and et al. found that clinical response had a positive correlation with the level of norclozapine (NDMC), which showed that the metabolic enzyme was the major reason for the difference (7). Other scholars have speculated that the variability may result from the absorptive process and/or CYP1A2 polymorphism (13). Thus, further studies are necessary to investigate the potential mechanisms of variation and to elucidate the major influential factors to help us make full rational use of medications and minimize the risk of adverse reactions.

Compared to the studies in the literature, we carried out a prospective investigation in healthy volunteers with similar physiological status, not in schizophrenic patients. Our original aim was to investigate the degree of variability though the study was designed to be similar to pharmacokinetic procedures of new drugs entering the market. Only 18 volunteers participated in the study, and yet there were 2.9, and 2.6 fold differences in $AUC_{0\sim48}$ and C_{max}, respectively. Greater differences could have been observed if more subjects were included in the study. This article reminds us that pharmacokinetic variability is a universal phenomenon and persons even with similar physiological statuses may need different dosages. This is why the same antipsychotics at the same doses result indifferent responses, such as some patients may show ideal response, some may have no effect, and yet some others may suffer from adverse drug reactions.

CONCLUSIONS

In this study, we have developed a high performance liquid chromatography-electrospray tandem mass spectrometry (LC-MS-MS) to measure the plasma concentration of clozapine with higher sensitivity and lower detection limits compared to other methods(14). Our results showed there were large inter-individual variations within the normal population with respect to pharmacokinetic parameter analysis. Our findings should remind psychiatrists and pharmacists to have more patience and to pay more attention to the variability of the response to clozapine in schizophrenic patients due to differences in weight, age, liver function, renal function, P450 enzyme function, transporters, and receptors. For most antipsychotics with narrow therapeutic indicies, therapeutic drug monitoring (TDM) is a feasible and practical method to ensure the safety of patients and to optimize the efficacy of medications.

References:

1. Agelink MW, Sayar K, Klieser E. Usefulness of heart rate variability (HRV) for monitoring clozapine plasma levels. Pharmacopsychiatry 2003;36(4):166-7.
2. Eschweiler GW, Bartels M, Langle G, Wild B, Gaertner I, Nickola M. Heart-rate variability (HRV) in the ECG trace of routine EEGs: fast monitoring for the anticholinergic effects of clozapine and olanzapine. Pharmacopsychiatry 2002;35(3):96-100.
3. Rajji TK, Uchida H, Ismail Z, Ng W, Mamo DC, Remington G, et al. Clozapine and global cognition in schizophrenia. J Clin Psychopharmacol 2010;30(4):431-6.
4. McKean A, Vella-Brincat J, Begg E. Prescribing and monitoring clozapine in Christchurch. Australas Psychiatry 2008;16(4):263-7.
5. Yasuda SU, Zhang L, Huang SM. The role of ethnicity in variability in response to drugs: focus on clinical pharmacology studies. Clin Pharmacol Ther 2008;84(3):417-23.
6. Sternieri E, Pinetti D, Coccia CP, Leone S, Bertolini A, Ferrari A. Pharmacokinetics of sumatriptan in non-respondent and in adverse drug reaction reporting migraine patients. J Headache Pain 2005;6(4):319-21.
7. Lee ST, Ryu S, Nam HJ, Lee SY, Hong KS. Determination of pharmacokinetic properties of clozapine and norclozapine in Korean schizophrenia patients. Int Clin Psychopharmacol 2009;24(3):139-44.
8. Qiu XW, Fu PX, Wang CY, Liu M, Zhou TY, Lu W. Population pharmacokinetics research of clozapine in Chinese schizophrenic patients. Yao Xue Xue Bao 2009;44(7):785-92.
9. Raedler TJ, Hinkelmann K, Wiedemann K. Variability of the in vivo metabolism of clozapine. Clin Neuropharmacol 2008;31(6):347-52.
10. Hagg S, Spigset O, Mjorndal T, Dahlqvist R. Effect of caffeine on clozapine pharmacokinetics in healthy volunteers. Br J Clin Pharmacol 2000;49(1):59-63.
11. Tang YL, Mao P, Li FM, Li W, Chen Q, Jiang F, et al.Gender, age, smoking behaviour and plasma clozapine concentrations in 193 Chinese inpatients with schizophrenia. Br J Clin Pharmacol 2007;64(1): 49-56.
12. Raedler TJ, Hinkelmann K, Wiedemann K. Variability of the in vivo metabolism of clozapine. Clin Neuropharmacol 2008;31(6):347-52.
13. Balibey H, Basoglu C, Lundgren S, Babaoglu MO, Yasar U, Herken H, et al. CYP1A2*1F polymorphism decrease clinical response to clozapine in patients with schizophrenia. Klinik Psikofarmakoloji Bulteni - Bulletin of Clinical Psychopharmacology 2011;21(4):93-9.
14. Wohlfarth A, Toepfner N, Hermanns-Clausen M, Auwarter V. Sensitive quantification of clozapine and its main metabolites norclozapine and clozapine-N-oxide in serum and urine using LC-MS/MS after simple liquid-liquid extraction work-up. Anal Bioanal Chem 2011;400(3):737-46.