Effects of Gender and Age on Plasma Levels of Clozapine and Its Metabolites: Analyzed by Critical Statistics

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Background: Previous reports concerning the effects of gender and age on steady-state plasma concentrations of clozapine and its major metabolites, norclozapine and clozapine-*N*-oxide, have been controversial. Since the frequency distribution of the plasma levels is asymmetrical and skewed to the right, the statistical methods (such as analysis of variance and regression analysis) used earlier are actually inappropriate for analyzing the effects of the variables on the concentrations and might contribute to the inconsistent results. The goal of the present study, with befitting statistics, is to measure the potential effect of dose, gender, age, and body weight on plasma levels of clozapine and its 2 major metabolites.

Method: We retrospectively analyzed data from a therapeutic drug monitoring study for steady-state plasma clozapine, norclozapine, and clozapine-*N*-oxide levels that was conducted in a large group of Chinese schizophrenic inpatients (male:female ratio = 83:79; age range, 33.8 ± 9.3 years). The daily doses of clozapine had ranged from 100 to 900 mg, with a mean ± SD value of 379.5 ± 142.2 mg. Plasma concentrations had been measured using high-performance liquid chromatography with ultraviolet detection. Multiple linear regression was adopted to quantify the effects of various factors on the plasma levels. The natural logarithm of the plasma level was used as the dependent variable to overcome the skewness problem.

Results: After adjusting the effects of gender, age, and body weight by multiple linear regression, each 1-mg increment in the daily dose could raise the clozapine level by 0.31%, norclozapine by 0.27%, and clozapine-*N*-oxide by 0.16%. Female patients had 34.9% higher clozapine levels and 36.3% higher norclozapine, with other variables being controlled. No sex differences were demonstrated for clozapine-*N*-oxide levels. Each 1-year increment in age would elevate the clozapine level by 1.1%, norclozapine by 1.0%, and clozapine-*N*-oxide by 1.0%. Body weight could not influence the levels of these compounds.

Conclusion: The present results suggest that women possess higher plasma levels (about one third higher) of clozapine and norclozapine, but not the *N*-oxide metabolite. Each addition of 1 year in age elevated clozapine and either metabolite's levels by about 1%. Furthermore, every 1-mg increase in the daily dose raised clozapine and norclozapine concentrations by approximately 0.3%. These findings could assist clinicians in optimizing clozapine dosing strategies.

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The frequency of plasma clozapine concentrations in patients receiving a given dose appears widely distributed and skewed to the right, with a minority of individuals possessing extremely high concentrations.[1–5] Clozapine is oxidized into 2 principal metabolites, norclozapine (or *N*-desmethyclozapine) and clozapine-*N*-oxide. Furthermore, the *N*-oxide metabolite is in part converted back to its parent drug.[6,7] Although the pharmacologic activity of either metabolite has been reported to be much lower than that of clozapine,[8,9] a recent study revealed that clozapine and norclozapine are both 5-HT_{1C} receptor antagonists and have similar affinities for D_2 and 5-HT_2 receptors.[10]

Several patient-related factors may increase the variability of the plasma levels. Earlier studies demonstrated that women may have higher levels of clozapine[1,2,11,12] and norclozapine[12]; however, others could not replicate this finding.[3,13,14] Fabrazzo et al.[15] suggested that the gender differences increase after 4 and 6 weeks, but vanish after 24 weeks, of treatment.[15] No sex difference has been detected with respect to clozapine-*N*-oxide levels.[12,15] Higher age was associated with higher plasma clozapine concentrations in some studies,[1,2] but was not in others.[3,13] The association of norclozapine levels with age has been indicated to be weakly negative.[3] In addition, body weight may be another factor altering clozapine levels.[1,2]

Unfortunately, since the distribution of plasma concentrations is skewed,[1–5] the statistical methods such as analysis of variance, regression analysis, and multiple linear regression, used in the above investigations,[1–3,11–14] are

actually inappropriate for analyzing the effects of prognostic factors on plasma concentrations and may partially explain the inconsistent results. These statistical methods should be applied to the data with a symmetrical distribution, such as a normal distribution.[16]

In a recent study by our group,[5] the steady-state plasma clozapine levels in 162 Chinese inpatients were about 30% to 50% higher than those reported in white individuals. Moreover, accordant with other studies,[1–3] the doses were only moderately correlated with clozapine concentrations ($r = .590$, $p = .0001$), and the distribution of clozapine levels was skewed. The present study, using the same subjects retrospectively, explored different issues and aimed to measure the potential effects of gender, age, dose, and body weight on the plasma levels of clozapine and its 2 metabolites. Since logarithmic transformation can convert the right-skewed distribution to a normal distribution (which is feasible for further statistical analyses),[16] the value of each plasma level was transformed to its natural logarithm (ln).

METHOD

We retrospectively analyzed data from a therapeutic drug monitoring program designed to measure steady-state plasma clozapine and norclozapine levels that was conducted in 162 Chinese schizophrenic inpatients during the last several years (from 1992 to 1996). Clozapine-*N*-oxide concentrations were also measured in the latter 88 patients. The facility's institutional review board had approved the project. All subjects gave informed consent to participate in the study. They met DSM-III-R diagnostic criteria for schizophrenia and were "treatment resistant" according to the guidelines proposed by Kane et al.[17] The participants were physically healthy, and all of their laboratory test results were within normal limits. Subjects were selected on the basis of the following parameters: no depot antipsychotics for at least 6 months prior to entering the study; no concomitant antidepressants and other antipsychotics; no enzyme-inducing agents (e.g., carbamazepine) or enzyme-inhibiting drugs (e.g., fluvoxamine)[18]; persons who smoked 0 to 20 cigarettes per day; and a stable clozapine dosage for a minimum of 7 days (≥ 4 weeks for most patients).

The demographic data of these subjects were as follows: male:female ratio = 83:79, mean ± SD age = 33.8 ± 9.3 years, and body weight = 59.8 ± 13.2 kg. Of all subjects, 51 men and 55 women (N = 106) consuming ≤ 5 cigarettes a day were defined as nonsmokers.[1,2] The relative frequency of nonsmokers/smokers did not significantly differ between men and women ($\chi^2 = 1.196$, $p > .05$) and was uniform across all age groups: 26/16 for ages 19 to 27 years, 45/21 for ages 28 to 36 years, 24/13 for ages 37 to 45 years, and 11/6 for ages 46 to 69 years ($\chi^2 = .461$, $p > .05$). Clozapine was given orally in 2 equally divided daily doses. The dosage, ranging from 100 mg/day to 900 mg/day (mean ± SD = 379.5 ± 142.2 mg/day), was individually titrated according to clinical efficacy and adverse effects. In most patients, blood samples were collected once a week for 2 to 6 weeks at the same dose. The plasma drug concentrations were averaged and analyzed. In patients receiving several different doses, the steady-state plasma concentrations obtained at the highest dose were taken into account.

Blood samples were taken 10 to 12 hours after the evening dose and prior to the morning dose. The venous blood was collected into an EDTA tube and centrifuged at 3000 rpm for 15 minutes. The plasma samples were stored at –60°C until assayed. Plasma clozapine and norclozapine of the former 74 subjects were measured using high-performance liquid chromatography (HPLC) with ultraviolet detection as described in detail by Chung et al.[19] The intraassay and interassay coefficients of variation were < 10% at 100 ng/mL for both compounds. The lower limit of detection for clozapine was 1 ng/mL, and for the metabolite was 2 ng/mL. Because the HPLC assay for the samples of the earlier 74 patients was unable to measure clozapine-*N*-oxide, the assay for the latter 88 was modified to that of Weigmann and Hiemke.[20] Clozapine and its 2 main metabolites were detected simultaneously. In comparing the 2 assays, the correlation coefficient was .908 for clozapine and .882 for norclozapine. The lower limits of detection were identical for clozapine and norclozapine, and for clozapine-*N*-oxide was 2 ng/mL. The intraassay and interassay coefficients of variation were < 10% for all compounds.

We hypothesized that several variables, e.g., gender, age, body weight, and dose, may have contributed to the diverse plasma levels. Accordingly, the multiple linear regression model, capable of controlling other variables, was adopted to evaluate each variable's impact on plasma levels.[21] To convert the right-skewed distribution of plasma levels[5] to a normal distribution (which is feasible for multiple regression analyses[16]), each value was measured in its ln scale prior to the statistical analysis. The Generalized Linear Interactive Modeling statistical package (GLIM, version 4.0)[22] was used to analyze the data. In some patients, the clinical assessments were based on routine observation and interviews only, without the use of standardized rating scales. Due to the retrospective, naturalistic design of this study, the correlations of concentration with clinical efficacy or side effects were not examined here.

RESULTS

As shown in Figure 1, the frequency distribution of plasma clozapine levels in all 162 subjects is skewed to the right. After the value of each plasma level was converted to its ln, the frequency of the transformed data became normally distributed (not shown). The normal

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Figure 1. The Distribution of the Steady-State Plasma Levels of Clozapine in 162 Schizophrenic Patients

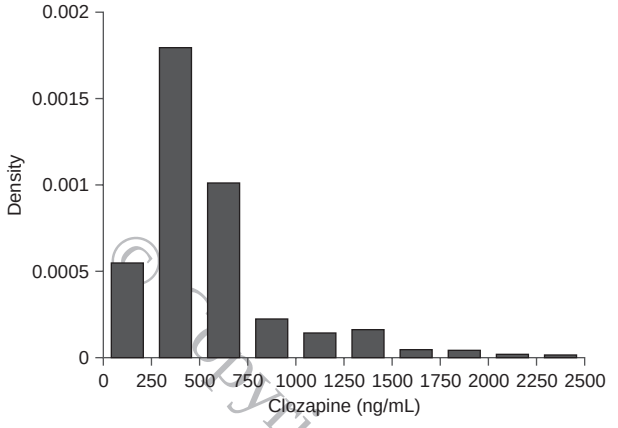
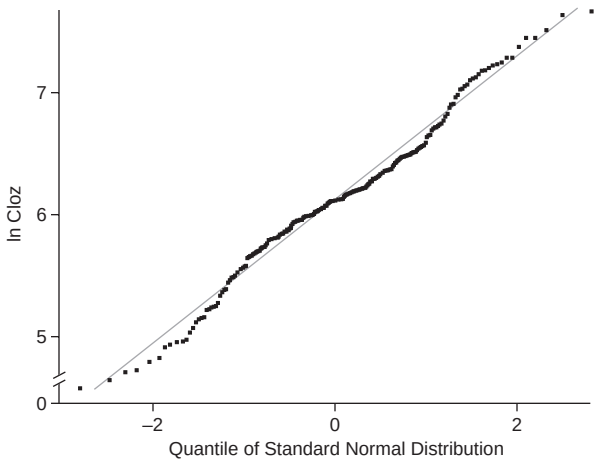


Figure 2. The Normal Quantile-Quantile Plot of the Natural Logarithms of the Values of the Steady-State Plasma Clozapine Levels (ln Cloz) in 162 Schizophrenic Patients



quantile-quantile plot (Q-Q plot) of the ln clozapine levels appeared nearly linear (Figure 2) and thus confirmed the normal distribution.[21] Likewise, the distribution of either metabolite's levels is also skewed to the right. The logarithmic transformations were also applied to the values of the concentrations of these 2 metabolites. The normal distribution of the transformed values of both metabolites was also tested by the Q-Q plot (figures not presented). Thereafter, the effects of variables (dose, gender, age, and body weight) on the ln values of each compound's plasma levels were analyzed by multiple linear regression.[21] As a result, it was found that a high dose or increased age raised the ln plasma level of each compound. Female gender elevated the ln clozapine level and the ln norclozapine level but not the ln level of the *N*-oxide metabolite. Body weight had no significant influence on the levels of the 3 molecules (Table 1).

Specifically, for those patients with same gender, age, and body weight, each unit (mg) increment in the daily dose could raise the mean ± SEM ln clozapine level (ng/mL) by .003092 ± .0002934, the ln norclozapine level by .002717 ± .0003088, and the ln clozapine-*N*-oxide level by .001587 ± .0004734 (Table 1). In other words, every 1-mg increase in the daily dose elevated the clozapine level by 0.31% ($e^{.003092} = 1.0031$), norclozapine by 0.27% ($e^{.002717} = 1.0027$), and clozapine-*N*-oxide by 0.16% ($e^{.001587} = 1.0016$).

Compared with male patients, female patients had a higher mean ± SEM ln clozapine level by .2993 ± .08802, and a higher ln norclozapine level by .3096 ± .09264, with the effects of dose, age, and body weight all being excluded. Hence, the ratio of the clozapine level for women to that of men would be 1.349 ($e^{.2993}$) and the female:male ratio in terms of norclozapine was 1.363 ($e^{.3096}$). That is, female patients had 34.9% higher clozapine levels and 36.3% higher norclozapine. Finally, each 1-year increment in age elevated the ln clozapine level by .01120 ± .004568 (or the clozapine level by 1.1%), the ln norclozapine level by .01020 ± .004808 (or norclozapine level by 1.0%), and the ln clozapine-*N*-oxide level by .01035 ± .004662 (or clozapine-*N*-oxide level by 1.0%) after adjusting other variables.

DISCUSSION

The conclusions of antecedent reports concerning the effect of gender on plasma clozapine and norclozapine levels have been debatable.[1–3,11–15] After the skewed concentration data were normalized and dose, age, and body weight effects were adjusted, the present study suggests that women have about 35% higher clozapine and norclozapine concentrations than men.[1,2,11,12,15] The incremental magnitudes in this study are near the lower limits of those previously reported for clozapine (9.8%–54.1%,[11] 36.4%–71.2%,[12] 44.3%,[2] and 70.0%–93.4%[15]) and norclozapine (69.5%–87.0%[15] and 75.8%–87.4%[12]). Noteworthily, it is likely that a suitable statistic strategy (such as that adopted in this study) could more precisely quantify the extent of the impact. No significant gender difference existed in our patients' clozapine-*N*-oxide concentrations, in line with prior results.[12,15]

Clozapine is metabolized mainly by cytochrome P450 1A2 (CYP1A2), albeit also by CYP3A and the flavin-containing monooxygenase system.[18] CYP1A2 inhibitors could diminish the conversion of clozapine to norclozapine[18] and thus perhaps curtail the risk of agranulocytosis.[23] It has been suggested that norclozapine might itself be toxic or further metabolized to an unstable compound that is toxic to hemopoietic precursors.[23] Earlier reports have postulated that females could possess less CYP1A2 activity and consequently higher clozapine concentrations.[12]

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Table 1. Multiple Linear Regression Analysis of Dose, Sex, Age, and Body Weight on the Natural Logarithm of the Plasma Levels of Clozapine and Its Metabolites

Parameter	Estimated Difference[a]	Standard Error of the Estimated Difference	p Value
Clozapine			
Dose (1-mg increment)	.003092	.0002934	.0000
Sex, (female)	.2993	.08802	.0003
Age (1-y increment)	.01120	.004568	.0071
Body weight (1-kg increment)	.003424	.003430	.1587
Norclozapine			
Dose (1-mg increment)	.002717	.0003088	.0000
Sex (female)	.3096	.09264	.0004
Age (1-y increment)	.01020	.004808	.0170
Body weight (1-kg increment)	–.002018	.003610	.2877
Clozapine-*N*-oxide			
Dose (1-mg increment)	.001587	.0004734	.0004
Sex (female)	.02596	.09389	.3917
Age (1-y increment)	.01035	.004662	.0132
Body weight (1-kg increment)	–.001937	.004463	.3336

[a]Difference in the natural logarithm of the plasma level (ng/mL).

The previous data concerning the potential impact of age on clozapine disposition have also been controversial.[1–3,13] Parallel to the studies by Haring et al.,[1,2] we found that, after removing interference from other factors, aging can raise the plasma level of clozapine. The influence of age on clozapine metabolism may be a result of the decreasing hepatic enzyme activity in older patients.[2] However, the aging process does not significantly alter the pharmacokinetics of sertindole and quetiapine (both not metabolized mainly by CYP1A2).[18] These differences might be attributable to the greater susceptibility to the aging process of the CYP1A2 pathways.[18] Additionally, according to our knowledge, this study is the first to demonstrate that the aging process could also increase norclozapine and clozapine-*N*-oxide in plasma. Each 1-year increment showed elevated levels of clozapine and either metabolite of about 1% in our subjects.

Compatible with earlier research,[1–3,15] our report indicates that high doses lead to high concentrations of clozapine and its metabolites. Each milligram added heightened clozapine and norclozapine levels by approximately 0.3%, and clozapine-*N*-oxide by approximately 0.15%. On the other hand, in contrast to prior findings,[1,2] body weight did not seem to modify our subjects' plasma clozapine levels. This discordance remains to be elucidated.

Smoking has been suggested to lower plasma clozapine levels in men, but not in women.[1,2] On the contrary, no significant differences in clozapine or norclozapine concentrations between male or female smokers and nonsmokers were found in other investigations.[3] It has been implicated that passive smoking might also alter clozapine levels and reduce the smoking-effect difference between smokers and nonsmokers.[3] Since our subjects were liable to be exposed to passive smoking, the possible smoking impact on the plasma levels of the 3 molecules was not assessed. Notwithstanding, the relative frequency of smokers versus nonsmokers was similar across gender and all ages. Another drawback in this study is that we changed our laboratory assays once during the several-year study period. The correlation coefficient of the 2 assays was around .9. This discrepancy might fractionally account for the variety of our plasma level data set. Prospective studies (that utilize the present statistical strategies and enroll smokers, nonsmokers without passive smoking and, if possible, passive smokers) could further evaluate the effects of smoking (and passive smoking) and other variables on plasma levels of clozapine and its metabolites.

While Chinese individuals may possess higher clozapine levels than Caucasians,[5] a recent study demonstrated that 17 Korean American patients had lower mean clozapine levels than 7 Caucasians.[24] Large-scale rigorous studies may be needed to ascertain the probable differences in clozapine metabolism among the 3 populations and whether the findings of the present study, recruiting only Chinese individuals, could be extrapolated to other ethnic individuals.

Several groups of researchers have reported relationships between clozapine levels and treatment responses.[3,4,14,25–27] It has been indicated that if an equally divided dosing regimen is being used, a clinician should attempt to raise the level to at least 250 to 300 ng/mL in any patient who is not showing a good response.[4,26] If most of the dose is given in the evening, a level of 350 to 420 ng/mL should be attained.[3,14,25,27] Nonetheless, some clozapine-associated adverse effects, such as seizures, cardiac toxicity, and other autonomic reactions, are liable to emerge with high medication concentrations.[8]

The present study suggests that women have higher plasma levels (about one third higher) of clozapine and norclozapine, but not clozapine-*N*-oxide. The aging process could also raise plasma levels of clozapine as well as its 2 major metabolites. Each 1-year increase elevates the plasma level of each compound by approximately 1%. Furthermore, every 1-mg increment in the daily dose heightens clozapine and norclozapine levels by approximately 0.3%. These factors should be taken into consideration for efficient and safe clozapine dosing tactics.

Drug names: carbamazepine (Tegretol and others), clozapine (Clozaril), fluvoxamine (Luvox), quetiapine (Seroquel).

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