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ORIGINAL ARTICLE

Factors associated with fluoxetine and norfluoxetine plasma concentrations and clinical response in Mexican patients with mental disorders

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Abstract

Over the last few years, fluoxetine has been one of the most prescribed medications for the treatment of diverse psychiatric conditions in Mexico. Fluoxetine therapeutic effect is consequence of the joint action of the parent drug and its active metabolite, norfluoxetine. However, the clinical efficacy of fluoxetine, can be affected due to diverse factors, such as drug-drug interactions and the large interindividual variability in the pharmacokinetics of this drug. The aim of this study was to determine the factors associated with variability in plasma concentrations of fluoxetine and norfluoxetine and its association with the therapeutic response. Fluoxetine and norfluoxetine plasma concentrations were quantified by liquid chromatography in 81 Mexican patients with mental disorders; 25% of the patients had no medication adherence and 40% were below the reference range of fluoxetine plus norfluoxetine plasma concentrations. The results showed that concentrations can be affected by fluoxetine metabolism caused by CYP2D6 phenotype and the concomitant administration of olanzapine. Furthermore, CYP3A5 and CYP2C19 phenotype were associated with lower anxiety and depression control during treatment with fluoxetine. This study can be a starting point to elucidate the causes of fluoxetine variable response in Mexican patients with mental disorders, as well as to detect and support medication adherence.

KEYWORDS
fluoxetine, genetic factors, norfluoxetine, pharmacokinetics, plasma concentrations

Abbreviations: ESI, electrospray ionization; HWE, Hardy–Weinberg equilibrium; MRM, multiple reaction monitoring; SNP, single nucleotide polymorphism; SSRI, serotonin reuptake inhibitor; TDM, therapeutic drug monitoring.

1 | INTRODUCTION AND BACKGROUND

Epidemiological data show that mental disorders, such as major depression, obsessive-compulsive disease, anxiety, acute depressive episodes and other psychiatric conditions, have a reported prevalence of 450 million people and are the cause of 33% of disability around the world.[1–7] In Mexico, 18% of the population between 15–64 years old experience some kind of affective disease; just 20% of the people with a mental disease in the country receives treatment and only half of them receive an adequate treatment.[7,8]

In several countries including Mexico, fluoxetine is one of the first-line drugs used in adult and pediatric treatment of the most prevalent psychiatric disorders.[1–5] Fluoxetine is a drug classified as a selective serotonin reuptake inhibitor (SSRI), whose action is explained by decreasing presynaptic serotonin reuptake, increasing the concentration of 5-Hydroxytryptamine.[1,2,5] Fluoxetine is metabolized by N-demethylation to the active metabolite norfluoxetine and several CYP450 enzymes, including CYP2D6, CYP2C19, CYP2C9, and CYP3A5 play an essential role in this conversion.[1–4,9]

Despite its extensive use, from 30% to 40% of patients in treatment with fluoxetine do not present an adequate therapeutic response[1] and 50% of the patients diagnosed with major depressive disorder fail to initial SSRI therapy.[2] The factors that cause this non expected response to fluoxetine have not been widely evaluated in Mexican population. However, previous studies have sought to elucidate diverse variables that may influence the therapeutic response to treatment with other SSRIs and to predict an adequate therapeutic response.[10,11]

There are several factors that modify fluoxetine and norfluoxetine pharmacokinetics, resulting in an inadequate exposure to this drug. The quantification of plasma concentrations will allow to identify patients with risk of therapeutic failure or lack of adherence.[1] Therefore, the aim of this work was to determine the possible factors that modify fluoxetine and norfluoxetine plasma concentrations and its association with treatment response in Mexican patients with mental disorders treated with standard dose of fluoxetine.

2 | MATERIALS AND METHODS

The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies.[12]

This study was approved by the Research and Ethics Committee from the Central Hospital "Dr. Ignacio Morones Prieto" (HCIMP, from its Spanish acronym), in San Luis Potosí, Mexico (Registration number 46-17). All patients provided written informed consent prior to study enrollment. The study was conducted in the Pharmacy Laboratory from Faculty of Chemical Sciences at Autonomous University from San Luis Potosí (FCQ—UASLP from its Spanish acronym) and Psychiatric Service at HCIMP from January 2018 to December 2019.

2.1 | Patients and study design

Patients over 18 years old with a clinical diagnosis of mental disorder and under fluoxetine treatment during at least 4 weeks were consecutively included. Patients were diagnosed according to ICD-10 by psychiatry physicians. The 21-item Beck Depression Inventory and Beck Anxiety Inventory, were used to evaluate depressive and anxiety symptoms; depression symptoms were considered moderate to severe for a score ≥17 and anxiety symptoms were considered moderate to severe for a score ≥22, respectively.

Patients who self-reported alcohol consumption of ≥3 standard alcoholic drinks per day at least twice weekly, were considered patients with regular alcohol consumption.

Adherence in patients was determined by the quantification of plasma concentrations of the analytes. Due to fluoxetine and norfluoxetine remain at detectable concentrations in plasma several days post-ingestion, patients with undetectable concentrations of fluoxetine and norfluoxetine (<1.45 and <2.15 ng/ml) were classified as patients with non-adherence.

The patients' clinical information was compiled from the clinical records after authorization and during the interview.

2.2 | Sample collection and handling

A single blood sample was obtained from each patient in a 4 ml EDTA vacutainer® tube during their attendance at the psychiatry service. All patients were sampled at least after 28th dose, assuming steady-state plasma level. The blood samples were centrifuged at 179 g, for 20 min at 4°C; then, plasma was separated and stored at −80°C until the analysis.

The methodology reported by Domingues and cols.[13] was adapted for fluoxetine and norfluoxetine quantification. Plasma samples (200 μl) were extracted with 400 μl of mass grade acetonitrile containing 100 ng/ml of indomethacin. After centrifugation at 14 000 rpm for 20 min at 4°C, supernatant was brought to dryness in a vacuum concentrator (Vacufuge plus, Eppendorf®); 100 μl of mobile phase (ammonium acetate 5 mmol/L containing 0.1% formic acid and acetonitrile (60:40 v/v)), was used to reconstitute the dried extract. For analysis, 20 μL of solution was injected into the liquid chromatography/tandem mass spectrometry (UPLC-MS/MS) system.

Fluoxetine and norfluoxetine concentrations in plasma were measured on an Acquity UPLC class H system, including a quaternary solvent manager, sample manager flow through needle and column heater coupled to a Xevo-TQD triple quadrupole mass spectrometer equipped with an electrospray ionization (ESI) source (Waters Corporation®).

The chromatographic separation was performed on an Acquity UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) from Waters, including a pre-column of the same characteristics, at 40°C. Sample manager cooling unit was set at 10°C. The mobile phase consisted of ammonium acetate solution 5 mmol/L (acidified with 0.1% formic acid), and mass grade acetonitrile (60:40, vol/vol). The isocratic flow rate was

set to 0.4 ml/min to elute fluoxetine, norfluoxetine, and indomethacin as the internal standard with a final run time of 5.5 min.

Mass spectrometry conditions consisted of multiple reaction monitoring (MRM) with a positive ESI (ESI+) mode. The optimized operating parameters were as follows: capillary voltage, 0.8 kV; desolvation temperature, 500°C; desolvation gas flow, 1000 L/h. The collision gas used was Argon (99.9% purity). To determinate fluoxetine, the cone voltage was set to 24 V, mass transitions for fluoxetine identification and quantification were *m/z* 310.16 > 43.9 and 310.16 > 148.02 with corresponding collision energies of 10 and 8 V, respectively. For norfluoxetine determination the cone voltage was set to 18V, transitions were *m/z* 296.16 < 29.9 for quantification and *m/z* 296.16 < 134 for identification with collision energies of 7 and 4 V, respectively. Finally, for indomethacin determination the mass transition was *m/z* 358.03 > 138.88 with a cone voltage of 34 V and collision energy of 18 V. The software MassLynx® v.4.2 from Waters Corporation® was used for data acquisition and processing.

Bioanalytical method was validated in accordance with Mexican regulation, NOM-177-SSA1-2013 which is consistent with FDA guidelines. The detection limits of fluoxetine and norfluoxetine were 1.45 and 2.15 ng/ml, respectively. The LC–MS/MS method was linear in the range of 10–800 ng/ml for both analytes. The accuracy of the method for fluoxetine ranged from 88.4% to 112.2% and for norfluoxetine ranged from 89.2% to 109.5%. The intra-assay imprecision CV values for fluoxetine and its metabolite ranged from 1.7% to 6.7% and 3.5% to 14.9%. Inter-assay imprecision CV values for fluoxetine and norfluoxetine ranged from 1.9% to 10.2% and 2.3% to 14.8%, respectively.

2.3 | Genotyping

Genomic DNA was obtained from peripheral blood (Wizard® Genomic DNA purification kit, Promega) to analyze polymorphisms associated to fluoxetine metabolism. CYP2C19*2 (rs4244285), CYP2C19*17 (rs12248560), CYP3A5*3 (rs776746), CYP2D6*4 (rs3892097), and CYP2D6*10 (rs1065852) were determined by real-time polymerase chain reaction (PCR), through the device CFX®, Bio-rad®, using TaqMan® assays for single nucleotide polymorphism (SNP) genotyping (Thermofisher scientific®). The Hardy–Weinberg equilibrium (HWE) test was performed by χ^2 goodness-of-fit test. For CYP2D6 and CYP2C19 analysis, patients' phenotypes were assigned as CYP2D6 extensive (*1/*1, *10/*10, *1/*4, *1/*10), intermediate (*10/*4) and poor (*4/*4) metabolizers and as CYP2C19 ultrarapid (*17/*17, *1/*17), extensive (*1/*1), intermediate (*1/*2, *17/*2) and poor (*2/*2) metabolizers based on Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6 and CYP2C19 genotypes.[2]

2.4 | Pharmacokinetic parameters estimation

Individual pharmacokinetics parameters were determined based on Bayesian estimation using ADVAN2 TRANS2 subroutines in NONMEM® v7.4 software (ICON Software development) and based on previously published population pharmacokinetic model for fluoxetine.[14] The Ka was fixed according to the previous value reported of 0.3 h^{-1} by P. Pauchad and cols in 2011.[14] A one-compartment open model was also implemented for norfluoxetine pharmacokinetics, considering the estimation of the fraction metabolized (Fm).

Interindividual variabilities were assessed by exponential error in fluoxetine and norfluoxetine pharmacokinetics models. In order to describe the intra-individual variability, an additive structure was used. Model parameters for base models were estimated by the zero-order conditional estimation (FO) with post hoc analysis.

2.5 | Statistical analysis

Individual parameters (clearance and half-life time) and plasma concentrations of fluoxetine and norfluoxetine were associated with clinical, anthropometric, and genetic information. Analysis of the continuous data using the Kolmogorov–Smirnov normality test showed that the data collected were non-parametric data, and therefore their subsequent analysis was carried out through the Spearman´s correlation and Mann–Whitney *U* test.

The analysis of the data was performed using SPSS® Statistical software v.26.

3 | RESULTS

A total of 81 patients were included in the current study. Twenty-one of the patients showed undetectable plasma concentrations of fluoxetine and norfluoxetine, which was considered as lack of adherence; consequently, in the further analyses, these patients were not included. Clinical and demographics features of the patients are summarized in Table 1. The main clinical diagnoses presented by the population were major and moderate depressive disorders, generalized anxiety, alone or combined, but depressive diagnoses were predominant; 82% had daily doses of 20 mg of fluoxetine and only 7% of patients used the reference drug Prozac®.

The quantification of fluoxetine and norfluoxetine plasma concentrations was performed after a median 1 year (interquartile range, IQR: 90–1004 days) under treatment, a median time after last dose of 11.0 (3.9–24.7) h, and showed a median concentration (IQR) of fluoxetine plus norfluoxetine of 168 (74.7–287.8) ng/ml, with a ratio of concentrations norfluoxetine/fluoxetine of 0.9 (0.6–1.2). The results showed that 41.6% of fluoxetine plus norfluoxetine concentrations were outside of the therapeutic reference range recommended by the therapeutic drug monitoring (TDM) group on neuropsychopharmacology (Arbeitsgemeinschaft fuer Neuropsychopharmakologie and Pharmakopsychiatrie, AGNP) (120–500 ng/ml).[15] The Figure 1 illustrates the specific plasma concentrations in each patient versus time after last dose. The quantification of fluoxetine and its metabolite plasma concentrations allowed to identify 24 patients who were

TABLE 1 Clinical and demographics features of Mexican patients treated with fluoxetine

Variable [units; statistics]	Value
Sex [*n* (%)]	
Female	38 (63.33%)
Male	22 (36.67%)
Age [years; mean ± SD]	43.33 ± 17.07
Weight [kg; mean ± SD]	70.30 ± 12.64
Height [m; mean ± SD]	1.61 ± 0.08
BMI [kg/m^2; mean ± SD]	27.04 ± 4.03
Diabetes mellitus [*n* (%)]	10 (16.67%)
Arterial hypertension [*n* (%)]	16 (26.67%)
Overweight and obesity [*n* (%)]	43 (71.67%)
Smokers [*n* (%)]	7 (11.67%)
Regular alcohol consumption [*n* (%)]	8 (13.33%)
Epilepsy [*n* (%)]	8 (13.33%)
Fluoxetine medication [*n* (%)]	
Prozac	4 (6.67%)
Generic	56 (93.33%)
Concomitant drug free [*n* (%)]	10 (16.67%)
Concomitant olanzapine treatment [*n* (%)]	18 (30%)
21-item BDI [score; median (IQR)]	4 (2 a 11)
21-item BAI [score; median (IQR)]	8 (2.25–13)
Plasma concentration of fluoxetine [ng/ml; median (IQR)]	81.35 (34.63–156.78)
Plasma concentration of norfluoxetine [ng/ml; median (IQR)]	70.7 (36.52–108.15)
Ratio norfluoxetine/fluoxetine [median (IQR)]	0.86 (0.57–1.23)
Patients inside the therapeutic range [*n* (%)]	35 (58.33%)

Abbreviations: BAI, Beck anxiety inventory; BDI, Beck depression inventory; BMI, body mass index; IQR, interquartile range; SD, standard deviation.

underdosed and one patient with concentrations over the upper limit of the therapeutic reference range.

Additionally, the evaluation of depression and anxiety scores in patients through the Beck's 21-item inventories, showed 11.7% of patients had moderate to severe levels of depression while the other 88.3% had intermittent depression or mild mood disturbances. The Beck anxiety inventories indicated the presence of moderate to severe levels of anxiety in 15% of the patients and low levels of anxiety for the rest of the patients. Furthermore, a strong association was found between the presence of higher anxiety scores and higher depression scores ($r = .626$, $p < .001$).

The individual pharmacokinetic parameters obtained for fluoxetine and norfluoxetine, were a median (IQR) fluoxetine clearance of 10 (6.4–25.7) L/h with a median (IQR) half-life of 44.6 (17.4–70.2) h. The median (IQR) norfluoxetine clearance was 0.9 (0.6–1.6) L/h with a median (IQR) half-life of 31.3 (18.1–47.2) h. The summary of the pharmacokinetic parameters determined based on Bayesian estimation for fluoxetine and its metabolite are shown in Table 2.

The interindividual variability (reported as the coefficient of variation, (CV%)) associated to fluoxetine clearance was 87.4% and 56.4% for its metabolite clearance.

Fluoxetine plasma concentrations were positively correlated with norfluoxetine concentrations ($r = .753$, $p < .01$). The statistics tests showed a positive relation between the dose of fluoxetine and plasma concentrations of norfluoxetine ($p = .044$), without finding an association with the parent drug concentrations. The patients who used Prozac, despite being a small number ($n = 4$), showed lower depressive and anxiety scores than patients under treatment with generic medication ($p < .05$, Figure 2).

The results of genetic analysis did not show significant deviations from HWE in the genetic variants analyzed ($p > .05$). Results also revealed that 63.3% of the patients carried CYP3A5*3/*3 genotype, these individuals are considered CYP3A5 non expressor, due to metabolize some CYP3A substrates less rapidly than CYP3A5 expressor genotypes (CYP3A5*1/*1 and *1/*3).[16] Also 80% of the participants were CYP2D6 extensive metabolizers, and 66.7% were CYP2C19 extensive metabolizers. The complete genetic information of CYP3A5, CYP2C19, and CYP2D6 in the population of study is summarized in the Table 3. Patients who were CYP3A5 expressors ($n = 22$), had a lower distribution of Beck´s anxiety scores than CYP3A5 non expressors (3.5 (1–11.5) vs. 9 (5–15), $p = 0.037$); the difference found between groups of CYP3A5, CYP2C19, and CYP2D6 are shown in the Figure 3. Furthermore, the results show that patients with CYP2C19 ultrarapid metabolism had depression scores lower than CYP2C19 intermediate metabolizers (1.5 (0.25–3.5) vs. 5 (4–17.25), $p < .01$). The effect of CYP3A5 and CYP2C19 phenotype on anxiety and depression scores cannot be statistically related to sum concentrations of fluoxetine and norfluoxetine in CYP3A5 expressors and CYP2C19 ultrarapid metabolizers.

Patients with CYP2D6 extensive metabolizer phenotype ($n = 48$) had lower plasma concentrations of fluoxetine (78.9 (31.7–133.9) ng/ml) than intermediate metabolizers (153 (66.5–235.2) ng/ml) ($p = .026$). Also, CYP2D6 extensive metabolizers had almost twice the value of norfluoxetine/fluoxetine ratios (0.9 (0.7–1.7)) than patients with CYP2D6 intermediate metabolism (0.5 (0.3–0.8)), ($p < .01$).

The concomitant use of olanzapine increased norfluoxetine clearance (1.3 (0.9–2.1) L/h; $p = .045$), compared with norfluoxetine clearance in olanzapine-free patients (0.8 (0.6–1.4) L/h).

Regular alcohol consumption seemed to be relevant in fluoxetine plasma concentrations, 37.6 (8.4–87.4) ng/ml compared with 88.9 (36.4–172.4) ng/ml in patients without regular alcohol consumption ($p = .039$); this habit also increased fluoxetine clearance (20.4 (10–60) vs. 9.7 (6.1–23.4) L/h) and reduced half-life time of the parent drug (26 (8.1–45.1) h vs. 46.1 (19.2–72.8) h), respectively ($p = .041$). Modifications on the pharmacokinetics parameters of fluoxetine due to alcohol habit are illustrated in the Figure 4. Alcohol consumption also was associated with higher Beck´s depression scores (11.5

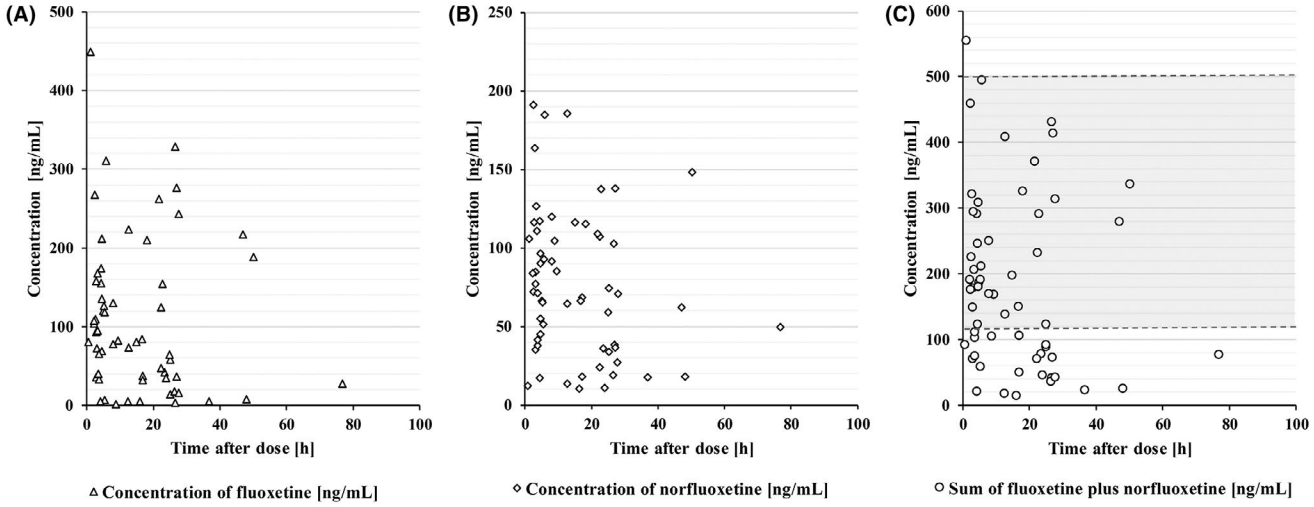

FIGURE 1 Plasma concentrations determined in Mexican patients with mental disorders ($n = 60$). The figure shows the specific plasma concentrations in each patient versus the time after last dose for: (A) fluoxetine; (B) norfluoxetine; (C) the sum of fluoxetine plus norfluoxetine. The highlighted area indicates the therapeutic reference range, 120–500 ng/ml, considering the sum of fluoxetine plus norfluoxetine concentrations

TABLE 2 Initial parameters estimates used to determine individual parameters of fluoxetine and norfluoxetine trough Bayesian estimation

Pharmacokinetic parameter	Initial estimates
Fluoxetine	
Ka, h^{-1}	0.3 (Fixed)
CL, L/h	8.31
V_d, L	645
ω_{CL}, %	87.40
σ, ng/ml (additive)	0.099
Norfluoxetine	
Ka, h^{-1}	0.3 (Fixed)
CL, L/h	0.873
V_d, L	41.5
Fm, %	7.61
ω_{CL}, CV%	56.39
σ, ng/ml (additive)	0.1

Abbreviations: CL, clearance; Fm, fraction metabolized, ω_{CL}, interindividual variability associated to total clearance; Ka, absorption rate constant; V_d, volume of distribution; σ, residual error as standard deviation.

(6–19.5)) than same scores in patients without regular consumption of alcohol (4 (2–8)) ($p = .011$).

As expected, lower fluoxetine clearance was related with fluoxetine concentration in plasma ($r = -.950$; $p < .01$) and, in consequence, lower fluoxetine clearance also increased the sum of the parent drug plus norfluoxetine concentrations ($r = .910$; $p < .01$). Higher fluoxetine half-life time was translated in higher fluoxetine concentrations ($r = .950$; $p < .01$) and higher norfluoxetine half-life time ($r = .745$; $p < .01$).

4 | DISCUSSION

In spite of the absence of knowledge about the precise fluoxetine´s mechanism of action, its relevance and wide use into the psychiatric field are unquestionable.[3] However, it is necessary to focus on the clinical use of fluoxetine. In this study the main diagnoses found in the population were mood disorders; this data agrees with the previous information about the prevalence of the psychiatric disorders in Mexico.[17] A report from 2011 shows that 28% of mental diseases were affective disorders[18] and, according to the Pan American Health Organization (2017), the most prevalent mental illnesses were depressive and anxiety disorders.[19] More than 60% of patients included in this study were women; this was expected since it is known that in Mexico more than a half of the outpatients who attend for mental health care usually are females.[18]

Even when the patients included were under chronic treatment with fluoxetine, the background indicates that the average time between the onset of any mental disease and its diagnosis ranges from 4 to 20 years, and only 50% of patients receive an adequate treatment.[7,8,19] This fact can be attributed to environmental conditions and particular situations of each patient, including habits, activities, and genetic variability.[1]

Taking into account the previous mentioned variable response, in this study it was quantified fluoxetine and norfluoxetine concentrations in plasma of Mexican patients. The results were associated and related with factors that potentially modifies the pharmacokinetics and therapeutic response to fluoxetine treatment. The analysis also allowed to identify non-adherence based on undetectable plasma concentrations, as well as underdosed patients with plasma concentrations of fluoxetine plus norfluoxetine below the therapeutic reference range (120–500 ng/ml).[1] Significantly higher prevalence of moderate to severe depression was found in non-adherent patients (25%) compared to patients with adherence (12.7%, $p = .033$).

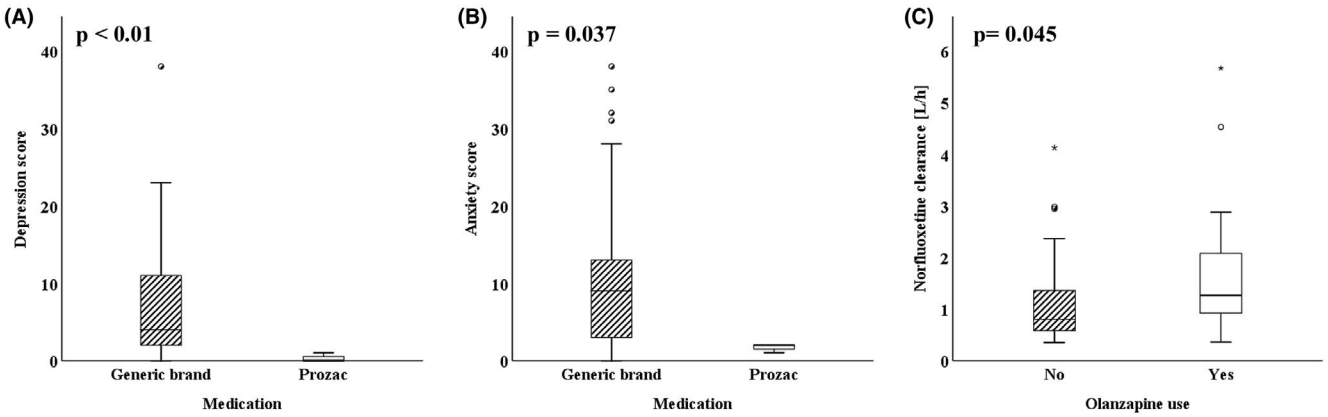

FIGURE 2 Graphical comparison of the estimated norfluoxetine clearance, anxiety, and depression scores between groups of fluoxetine medication and olanzapine comedication. The figure shows the box plots in which a significant difference was found in (A) depression scores and (B) anxiety scores between groups according to brand of fluoxetine used (Prozac, $n = 4$ and generic formulation $n = 56$); (C) norfluoxetine clearance between groups with concomitant treatment of fluoxetine and olanzapine (patients in treatment with olanzapine $n = 18$) and without it ($n = 42$). The box plots show the median, first and third quartile, and the minimum and maximum value of the data

The results also indicate, that after at least 1 month under fluoxetine therapy, 40% of patients were below the therapeutic reference range; this result is higher than in previous papers, that report from 16% to 33% of patients with plasma concentrations under the therapeutic reference range.[1,13] Moreover, fluoxetine and norfluoxetine concentrations in plasma were lower than those reported by Da Silva and cols in 2018,[1] in which fluoxetine concentrations were 18.4–517.9 ng/ml and norfluoxetine concentrations were within 25.3–328 ng/ml, after a pharmacotherapy greater than 6 months with fluoxetine. However, the concentrations found in this study were in the same order of magnitude as the FDA report which indicates that, after 30 days of daily administration of fluoxetine 40 mg, the plasma concentrations are expected to range from 91 to 302 ng/ml and from 72 to 258 ng/ml for norfluoxetine plasma concentrations.[20] Despite that was twice the usual dose and for a longer administration period, it is recognized that fluoxetine´s metabolism is not proportional to the dose and besides, the steady-state after prolonged dosing are similar to concentrations found at 4–5 weeks.[4]

The concentration ratio of norfluoxetine/fluoxetine helps to understand the transformation relation from fluoxetine to norfluoxetine; the expected value reported for this ratio, was between 0.7 and 1.9,[21] which was similar to the norfluoxetine/fluoxetine ratio obtained in this population.

The initial estimates for the parameters obtained by the pharmacokinetic analysis, were similar to the previously reported by Panchaud and cols in 2011.[14]

It was determined that the individual clearance for the patients included, exhibited a high interindividual variability, greater than the 50% for both analytes. This is a situation that has been proven in previous studies, with variabilities reported between 20 and over 50%.[1–3,22]

In order to determine possible causes that modify the pharmacokinetic of fluoxetine and norfluoxetine and their effect in the patients' response, personal information was collected, as well as anxiety and depressive scores using a depression and anxiety scales. The findings show a direct association between depression and anxiety levels. Anxiety and depressive-like behaviors have been associated with increases in 5-Hydroxytryptamine[5] and then, this finding seems to be expected.

Despite the small number of patients who used Prozac® versus other generic medications, it is relevant to mention that the patients with this product had significantly lower scores of depression and anxiety. However, more studies are required in this regard, and additionally, it would be necessary to identify the different brands of generic fluoxetine available.

Since fluoxetine is extensively metabolized by the cytochrome P450,[3,5] it makes sense the positive and direct correlation found between the fluoxetine and its metabolite concentrations.

The direct relation between fluoxetine dose and the norfluoxetine concentration in blood, without finding this association with the parent drug concentration, responds to the fact that norfluoxetine appears to show linear pharmacokinetics but not fluoxetine.[20]

Furthermore, there is a substantial variability in the response that can possibly be explained by changes in the patients´ genotype.[23] At the moment, there is not a genetic predictor of depression, anxiety, suicide behavior, or any other gene-based dosing recommendations for fluoxetine use.[2] However, the present findings show that patients CYP3A5 non expressors had higher anxiety scores than CYP3A3 expressors. CYP3A5 is one of the isoenzymes that have been described by commonly present individual variation; the polymorphic allele CYP3A5*3 presents diminished enzymatic activity consequent to a single base substitution in intron 3 at position 6986 A>G.[23] Nevertheless, this result cannot be statistically related with higher sum levels of fluoxetine and norfluoxetine in patients with CYP3A5*1, despite that 13 of the 22 patients CYP3A5 expressors, were into the therapeutic reference range. This lack of association between CYP3A5 genotype with the concentrations of the analytes can be a consequence of the small number of patients with the genotype or other undefined factors such as possible differences between concentrations of norfluoxetine enantiomers, since

TABLE 3 Genetic information of CYP3A5, CYP2C19, and CYP2D6 in Mexican patients treated with fluoxetine

CYP3A5		
CYP3A5*3 allele frequency, %		79.16
CYP3A5 genotype	CYP3A5 phenotype	*n* (%)
CYP3A5 *1/*1	Extensive metabolizer	3 (5)
CYP3A5 *1/*3	Extensive metabolizer	19 (31.67)
CYP3A5 *3/*3	Poor metabolizer	38 (63.33)
CYP2C19		
CYP2C19*2 allele frequency, %		14.17
CYP2C19*17 allele frequency, %		6.67
CYP2C19 genotype	CYP2C19 phenotype[a]	*n* (%)
CYP2C19 *1/*1	Extensive metabolizer	40 (66.67)
CYP2C19 *1/*2	Intermediate metabolizer	11 (18.33)
CYP2C19 *1/*17	Ultrarapid metabolizer	4 (6.67)
CYP2C19 *2/*17	Intermediate metabolizer	2 (3.33)
CYP2C19 *2/ *2	Poor metabolizer	2 (3.33)
CYP2C19 *17/*17	Ultrarapid metabolizer	1 (1.67)
CYP2D6		
CYP2D6*4 allele frequency, %		10.83
CYP2D6*10 allele frequency, %		15
CYP2D6 genotype	CYP2D6 phenotype[a]	*n* (%)
CYP2D6 *1/*1	Extensive metabolizer	46 (76.67)
CYP2D6 *1/*10	Extensive metabolizer	2 (3.33)
CYP2D6 *4/*10	Intermediate metabolizer	11 (18.33)
CYP2D6 *4/*4	Poor metabolizer	1 (1.67)

[a]Patients' phenotypes were assigned based on Clinical Pharmacogenetics Implementation Consortium Guideline for CYP2D6 and CYP2C19 Genotypes.[2]

fluoxetine´s enantiomers have the same activity, but S-norfluoxetine enantiomer has 5 to 20 times higher activity of R-norfluoxetine and R/S-fluoxetine.[3]

As well as CYP3A5, CYP2D6 helps to convert fluoxetine into R/S-norfluoxetine enantiomers; people with less activity in CYP2D6 have been demonstrated to possess significantly higher fluoxetine plasma concentrations than individuals with normal CYP2D6 activity. However, this has not seemed to affect the sum of fluoxetine plus norfluoxetine concentrations in plasma.[2] There is evidence that the fluoxetine and its metabolite norfluoxetine are substrate and inhibitors of CYP2D6,[9] which may lead to alterations in the pharmacokinetics of this drug.[24] Variations in CYP2D6 activity may result in greater or lower exposure to fluoxetine.[2,3] Congruent with this, current analysis proves the existence of differences between fluoxetine concentrations and the norfluoxetine/fluoxetine ratio between CYP2D6 phenotypes.

Similar to CYP2D6, CYP2C19 polymorphisms, result in the possibility of predisposing patients to poor therapeutic outcomes due to alter the transformation of fluoxetine.[2] Current results showed higher depression scores in CYP2C19 intermediate metabolizers than CYP2C19 ultrarapid metabolizer patients.

The fact that genetic variants change fluoxetine and norfluoxetine concentrations and response, is reinforced by the finding that patients in the study with concomitant olanzapine treatment had higher clearance of norfluoxetine, compared to patients without this therapy. It is known that olanzapine is metabolized in the liver, mainly through CYP1A2, but also trough CYP2D6 and CYP3A5,[25] enzymes that, as previously mentioned, participate in the transformation of fluoxetine to norfluoxetine by sharing the metabolic route. Thus, less norfluoxetine could be formed, causing lower metabolite concentrations in plasma, which may be the cause of the apparent increase in norfluoxetine clearance. In the study of Domingues and cols in 2016,[13] patients treated in combination with fluoxetine and olanzapine had fluoxetine concentrations below the therapeutic reference range. Here we found 66.7% of patients treated just with fluoxetine were within the reference range, compared with the 44.4% patients within the reference range that had concomitant olanzapine treatment.

Finally, current results indicate that alcohol consumption modifies the fluoxetine pharmacokinetics, causing lower fluoxetine plasma concentrations and higher fluoxetine clearance. The inductor role of ethanol is widely recognized[26,27]; previous studies have shown that humans with constant alcohol use exhibit chronic alterations in gene expressions.[28] Furthermore, this disorder has been related with higher aldosterone concentrations,[29] and this hormone plays a key regulator role in maintaining blood pressure and the body fluid balance through the electrolyte homeostasis.[30,31] The effects of high aldosterone concentrations include an increase in urinary excretion,[32] which can be responsible for the higher fluoxetine clearance, and its consequent lower concentration.

The presence of depression has been reported to be more frequent in people with alcohol dependence than in the rest of the population.[33] This is consistent with the positive association found in this study between alcohol consumption and higher depressive scores. Moreover, around 20% of the patients with depressive disorders may be refractory to antidepressants at doses considered adequate.[33] As we mentioned, alcohol consumption has been previously associated with higher concentrations of aldosterone, which at the same time have been associated with stress sensitization in the central nucleus of amygdala,[29] and it is known that the stress is one of the main depression components.[34]

This study has some limitations that need to be recognized. First, the sample size is smaller than previously SSRI studies published[35,36]; nevertheless, considering the outpatient condition of the patients included, the sample size resulted adequate compared to similar antidepressants studies.[13,37] Second, the outpatient

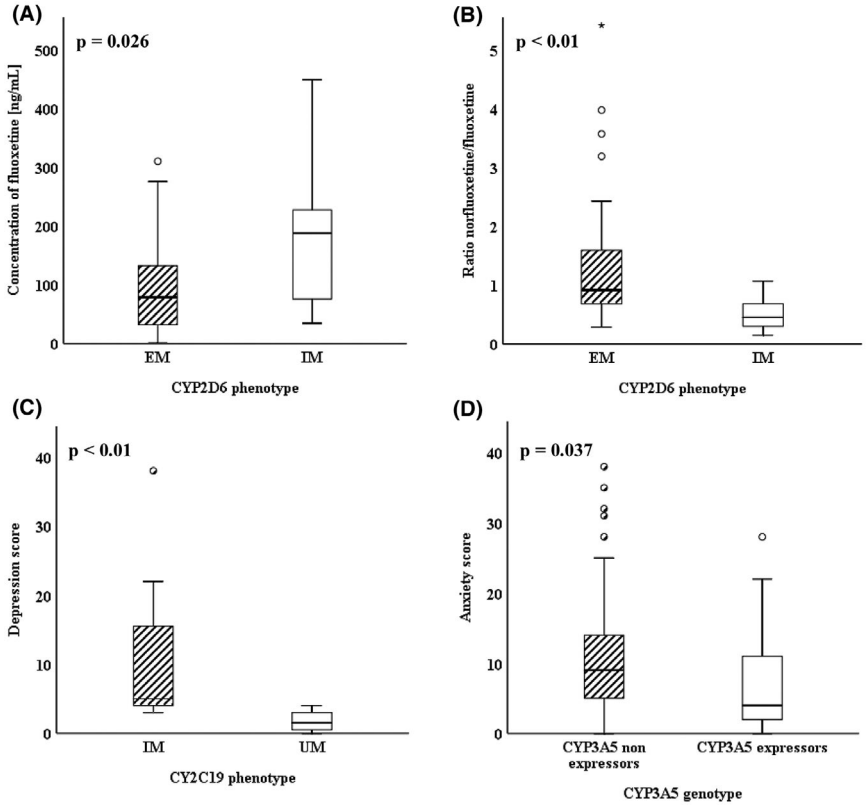

FIGURE 3 Graphical comparison of the estimated fluoxetine concentrations and anxiety and depression scores between groups of CYP3A5, CYP2D6, and CYP2C19. Abbreviations: EM, extensive metabolizer; IM, Intermediate metabolizer; UM, Ultrarapid metabolizer. The figure shows the box plots, in which a significant difference was found in (A) concentration of fluoxetine and (B) Ratio norfluoxetine/fluoxetine, between groups of CYP2D6 phenotype (EM $n = 48$ and IM $n = 11$); (C) depression scores between CYP2C19 phenotype (IM $n = 13$, UM $n = 5$) and (D) anxiety scores between CYP3A5 genotype (CYP3A5 expressors $n = 22$, CYP3A5 non expressors $n = 38$). The box plots show the median, first and third quartile, and the minimum and maximum value of the data

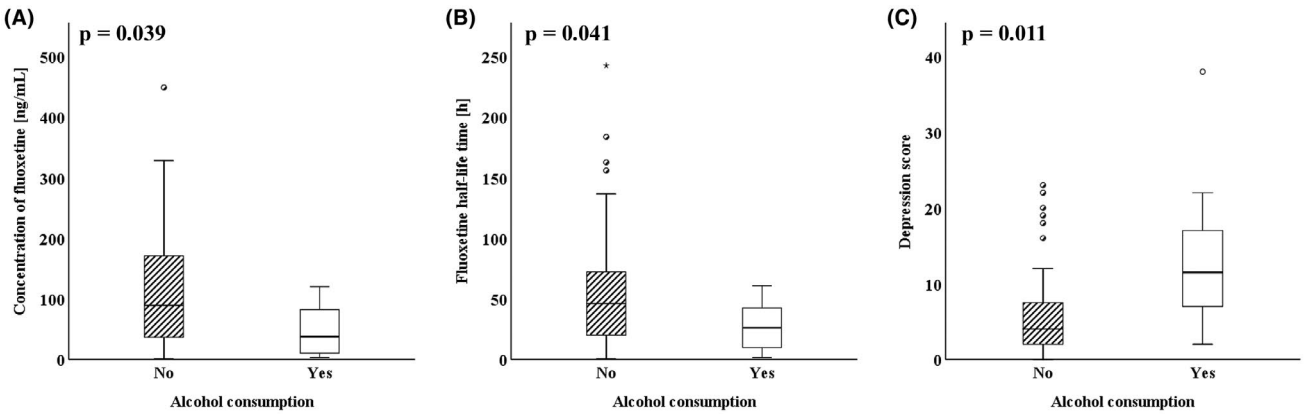

FIGURE 4 Graphical comparison of the estimated fluoxetine pharmacokinetics parameters and depression scores between groups of alcohol consumption habit. The figure shows the box plots, in which a significant difference was found in (A) concentration of fluoxetine; (B) fluoxetine half-life time; and (C) depression scores, between groups of patients with alcohol consumption habit ($n = 8$) and without it ($n = 52$). The box plots show the median, first and third quartile, and the minimum and maximum value of the data

setting condition did not guarantee medication adherence due to the unsupervised administration of the drugs and there is knowledge that factors like difficulty in accepting the need for long-term treatment and preference for the psychological management, make frequent high non-adherence rates in psychiatric clinical practice.[38] The outpatient setting, also generated a lack of information about patients´ laboratory results, such as creatinine clearance, hepatic function, and plasma protein levels, which have a key role in the distribution, metabolism, and excretion of fluoxetine.[4,13] Further, some of the patients received psychotherapy and others just pharmacological treatment; psychotherapy was able to modify the clinical evolution of the patients, without being related with the fluoxetine and norfluoxetine concentrations in plasma. Also, fluoxetine and norfluoxetine were determined as a racemic mixture, which did not allow to elucidate if the results obtained are due to changes in the concentrations of the R or S, fluoxetine and norfluoxetine enantiomers. Finally, clinical factors, such as the presence of other concomitant treatments with lower prevalence in the study population, medication interactions and other untested genetic variants, can be important for the pharmacokinetics

characterization of fluoxetine and norfluoxetine and its therapeutic effect.[39,40]

In summary, the findings of this study showed the influence of CYP2D6 phenotype, concomitant olanzapine treatment and alcohol consumption in the plasma concentrations and the pharmacokinetic parameters of fluoxetine and norfluoxetine.

Although more studies are needed in this field, the findings of this analysis contribute to understand the causes of fluoxetine variable response in Mexican patients with mental disease in the clinical practice.

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DISCLOSURE

The authors have no conflict of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request; preserving patient confidentiality. Requests should be made to the corresponding author and will require the approval of the co-authors and the institutions involved: Autonomous University from San Luis Potosí and Central Hospital "Dr. Ignacio Morones Prieto."

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