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Article

Analysis of Adherence to Fluoxetine Treatment through its Plasma Concentration

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Depression plays an important role in non-adherence to medical recommendations. Fluoxetine is a first line of depression treatment. This study aimed to evaluate adherence to drug therapy in fluoxetine users by different methods. A cross-section study was conducted with 53 depressed patients on fluoxetine for at least six months. Drug therapy adherence was assessed by validated questionnaires [Brief Medication Questionnaire (BMQ) and Morisky-Green test (MG)] and by the blood concentration of fluoxetine and its active metabolite norfluoxetine. Blood samples were taken before the daily first dose of fluoxetine. The plasmatic concentration of fluoxetine and norfluoxetine indicated that 58.5% volunteers were within the recommended therapeutic range and thus considered adherent to drug therapy. However, questionnaires indicated a non-adherent majority: 41.5% patients had a high degree of adherence in MG and only 13.2% were adherent to pharmacological treatment in BMQ. Most fluoxetine users showed a plasma concentration of fluoxetine and norfluoxetine within the therapeutic range, despite the low adherence to the drug therapy evaluated by the questionnaires. Thus, we suggest that plasma levels of fluoxetine and norfluoxetine could be used as the main method to check adherence to treatment.

Keywords: Adherence to treatment. Fluoxetine. Norfluoxetine. BMQ. Morisky-Green. Plasma concentration.

INTRODUCTION

Fluoxetine (FLU; Prozac®), a selective serotonin reuptake inhibitor (SSRI), is a first-line of depression treatment. FLU elevates the process of serotonergic neurotransmission by neural inhibition of serotonin reuptake by presynaptic neurons. Chronic inhibition of serotonin reuptake leads to down-regulation of 5HT1 (serotonin) inhibitory presynaptic autoreceptors, thus increasing the release of serotonin in the synaptic cleft (Nevado *et al.*, 2005). For the initial treatment of depression, a single dose of 20 mg/day FLU in the morning is recommended. In case of no clinical improvement, it is considered to increase doses up to 80 mg/day in two daily administrations (Djordjevic *et al.*, 2005). Fluoxetine use is estimated at 2.4% in the Brazilian population (Machado, 2018).

FLU is almost completely absorbed after oral administration, due to first-pass hepatic metabolism, bioavailability is about 70%, with peak plasma concentration between 6 and 8 hours after administration and extensive tissue accumulation (Catterson, Preskorn, 1996; Hiemke, Hartter, 2000). Thus, like other lipophilic drugs, fluoxetine has a wide volume of distribution, between 12 and 97 L/Kg, which indicates high accumulation in tissues (Hiemke, Hartter, 2000). It undergoes extensive metabolic conversion, being the CYP2D6 isoenzyme of the cytochrome P450 family responsible for N-demethylation that generates the active metabolite, norfluoxetine (NFLU) (Hiemke, Hartter,

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2000). NFLU is believed to be responsible for a significant part of the therapeutic activity of FLU (Kecskeméti *et al.*, 2005) and is therefore of interest to determine this metabolite in therapeutic drug monitoring. Fluoxetine has a half-life ($T_{1/2}$) from 1 to 4 days, while NFLU, between 7 and 15 days (Lundmarck *et al.*, 2000). Due to the long half-life of the drug and its metabolite, it takes up to twenty-two weeks to reach steady state (Harvey, Preskorn, 2001). FLU is mainly eliminated via urine and its renal clearance ranges from 0.6 to 0.83 mL/min. After chronic administration, fluoxetine clearance drops to approximately 30% baseline (0.18 to 0.25 mL/min) (Altamura *et al.*, 1994) due to CYP2D6 self-inhibition.

Plasma concentration of FLU and NFLU as well as the NFLU/FLU ratio are used for therapeutic drug monitoring (TDM). The *Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie* (AGNP) classifies this TDM as recommended, since approximately 30 - 40% patients do not present a satisfactory response to FLU treatment (Blazquez *et al.*, 2012). On the other hand, plasma concentrations are related to clinical effects, and adverse effects are more frequent at concentrations above the suggested therapeutic range (Hiemke *et al.*, 2011). The AGNP Consensus Guideline for TDM in Psychiatry recommends a therapeutic reference range for the sum of FLU and NFLU plasma concentrations of 120 – 500 ng/mL with an expected NFLU/FLU ratio of 0.7 - 1.9. Measurement of FLU and NFLU plasma concentrations may help to identify non-adherence, while the NFLU/FLU ratio can identify the metabolizing phenotype of CYP2D6 (Hiemke *et al.*, 2011).

According to WHO (2017), adherence to treatment is the extent to which a person behavior meets the recommendations of a health care professional whether it is taking their medication, following their diet and/or changing their lifestyle. Depression plays an important role in non-adherence to medical recommendations. The depressed patient is three times more likely do not follow the medical regimen than the non-depressed one (Dimatteo, Lepper, Croghan, 2000). The strong relationship between depression and non-adherence indicates that these patients should undergo frequent screening.

Several methods were used to evaluate adherence to pharmacological treatment. They are usually classified as direct method, such as the laboratory dosage of the drug and its metabolites and indirect method, such as the application of questionnaires (Hawkshea, Krousel-Wood, 2007). Several methods have been applied to assess adherence to drug therapy, such as self-report, pill count, drug withdrawal in pharmacies, laboratory dosages of drugs or their metabolites and questionnaires. Despite the low sensitivity and accuracy, questionnaires are the most used because they have a relatively low cost and feasible application in large populations. These instruments can be useful in differentiating between low adherence and non-response to treatment when used in combination with other methods (Zeller, Schroeder, Peters, 2007), such as the blood dosage of drugs (Leite, Vasconcelos, 2003).

The Morisky-Green (MG) method characterizes the degree of adherence and its intentionality (Morisky, Green, Levine, 1986). In addition to this, the Brief Medication Questionnaire (BMQ) can also be used (Svarstad *et al.*, 1999), identifying barriers to adherence to treatment from a patient perspective. Structured questionnaires are the most used due to easy application and low cost. However, these instruments can overestimate adherence, because once again the patient can hide from the interviewer or doctor on how the treatment is actually performed (Milstein-Moscati, Persano, Castro, 2000). In fact, direct methods are based on analytical techniques that check whether the drug was administered or taken at the required dose and frequency by identifying the drug's metabolites or chemical markers with longer permanence in the body (Milstein-Moscati, Persano, Castro, 2000). In this context, this study aimed to evaluate adherence to drug therapy in fluoxetine users by different methods: the plasmatic concentration of FLU and NFLU and the administration of MG and BMQ questionnaires.

METHODS

Study design

A cross-sectional study was performed with 53 patients with depressive disorder, diagnosed as per the Diagnostic and Statistical Manual of Mental Disorders

IV (DSM-IV) criteria, and users of fluoxetine for at least six months. All volunteers are patients of CIES - FEEVALE, Integrity Center of Health Specialties of FEEVALE University, Novo Hamburgo, Southern Brazil, and are at least 18 years old. The same was approved by the Research Ethics Committee of FEEVALE University (CAAE 44035115.0.0000.5348), and carried out according to resolution 466/2012 of the Brazilian National Council of Education and Research and the 1964 Declaration of Helsinki. All volunteers signed the informed consent.

All volunteers were interviewed to record their socioeconomic profile (schooling, family income) and their clinical characteristics [comorbidities, body mass index (BMI), systolic blood pressure (SBP), diastolic blood pressure (BPD), medications in use] were collected from their medical records. Likewise, they answered the questions of the MG and BMQ instruments to assess their degree of adherence to pharmacological treatment and collected blood to determine plasma FLU and NFLU concentrations.

Patient characteristics

The clinical characteristics of the 53 fluoxetine users of this study are listed in Table I. The mean age was 57 years, with the predominance of female patients (86.8%). The most commonly treated comorbidities in these volunteers are hypertension (56.6%), hypercholesteremia (20.7%) and diabetes (17%). In the studied group, there was a predominance of nonsmokers (83%) and non-alcoholics (100%). Still, 66% volunteers did not complete basic education and 90.6 % had a family income of up to three minimum wages. Most patients (84.9%) used 20 mg fluoxetine daily.

TABLE I - Clinical and social demographic characteristics of fluoxetine users

Characteristics	Fluoxetine users (n = 53)
Age (years)	57 ± 11
Sex	
Female	46 (86.8 %)
Male	7 (13.2 %)
BMI (kg/m²)	29.9 ± 6.5
SBP (mmHg)	125 ± 17.8
DBP (mmHg)	81.7 ± 14.1
Smoking	
No	44 (83.0 %)
Yes	9 (17.0 %)
Alcoholism	
No	53 (100 %)
Education	
Incomplete elementary education	35 (66.0 %)
Complete elementary education	7 (13.2 %)
Incomplete high school	6 (11.3 %)
Complete high school	4 (7.5 %)
Incomplete higher education	1 (1.9 %)
Family income	
From 0 to 3 minimum wages	48 (90.6 %)
Above 3 minimum wages	5 (9.4 %)
Comorbidities	
Hypertension	30 (56.6 %)
Diabetes	9 (17.0 %)
Hypercholesterolemia	11 (20.7 %)
Hypothyroidism	7 (13.2 %)
Arthritis	6 (11.3 %)
Osteoporosis	6 (11.3 %)
Others	7 (13.2 %)
Daily FLU dose	
20 mg	45 (84.9 %)
40 mg	6 (11.3 %)
60 mg	2 (3.8 %)

BMI: body mass index. SBP: systolic blood pressure. DBP: diastolic blood pressure. FLU: fluoxetine. Others comorbidities: lupus, disc herniation, esophageal reflux, glaucoma, and circulatory problems. The frequency attributed to comorbidities is greater than 100% since a patient may have one or more of them.

Measurement of treatment adherence questionnaires

The characterization of "adherent" by the MG Adherence Scale (Morisky, Green, Levine, 1986) is given by a psychometric scale with four items to which patients

respond in a dichotomous manner (yes/no), thus YES is considered to be 0 (zero) point, while NOT equals 1 (one) point. Thus, a user with a high degree of adherence is the user whose answers are all negative, totaling 4 points. For moderate degree of adherence, when the total answers are 3 points and low degree of adherence when 1 or 2 points are totaled. The classification of "non-adherent" occurs when all the answers are positive, totaling 0 points (Morisky, Green, Levine, 1986). Moreover, patients are classified as adherent (score equal to 4) or non-adherent (score between 0 and 3) (Milstein-Moscati, Persano, Castro, 2000). In patients considered non-adherent in this classification, the positivity observed in at least one of the three or four questions classifies the patient as intentional non-adherent. If they have these two negative questions, they are classified as non-adherent and unintentional. Such analysis made it possible to discern whether the low-adherence behavior was intentional or unintentional (Tavares *et al.*, 2016).

The BMQ is divided into three screens, including 11 questions, that identify adherence barriers to a drug regimen, beliefs and recall regarding medication treatment from the patient perspective. The first domain evaluates the behavior of the patient in relation to adherence to the prescribed treatment regimen; the second domain assesses the patient belief in treatment efficacy and opinions on unwanted side effects; and the third domain identifies problems with remembering to take the medication (Svarstad *et al.*, 1999; Ben, Neumann, Mengue, 2011). Adherence to treatment was analyzed in two ways: by the score found in each of the three domains (beliefs, regimen and recall), and according to the total number of positive responses: adherent (none), probable adherence (1), probable low adherence (2) and low adherence (3 or more) in any domain (Mantovani *et al.*, 2015).

FLU and NFLU plasma concentration

Blood samples were drawn into EDTA tubes before the daily first dose of fluoxetine. These were centrifuged for 10 min at 2,500 rpm for plasma separation, which was stored in an ultra-freezer at -80°C. Determination of the plasma concentration of FLU and its NFLU metabolite was obtained by high performance liquid chromatography tandem mass spectrometry (LC-MS/MS). This analysis was preceded by a liquid-liquid extraction with organic phase, according to the method proposed by Da Silva *et al.* (2018).

FLU and deuterated FLU (FLU-D6) solutions were acquired from Cerilliant (Round Rock, USA) and NFU was from Toronto Research Chemicals (NorthYork, Canada). Acetonitrile, methyl-tert-butyl ether (MTBE), n-hexane, methanol and formic acid were purchased from Merck (Darmstadt, Germany) and tris(hydroxymethyl) aminomethane (TRIS) was acquired from Sigma Aldrich (Saint Louis, USA). NFLU stock solution, at the concentration of 2 mg/mL, was prepared by dissolution in methanol. FLU and FLU-D6 stocks were purchased at the concentrations of 1,000 and 100 µg/mL, respectively. FLU, FLU-D6 and NFLU intermediate solutions, at the concentration of 10 µg/mL, were prepared by dilution in methanol. Combined working solutions of FLU and NFLU were prepared at concentrations 20 times higher than calibration and control levels, also by dilution in methanol. TRIS buffer pH 10.0 was prepared by dissolution of TRIS in water to obtain a 10 mM solution, with pH adjustment with a sodium hydroxide solution. Mobile phase A consisted of purified water with 0.1% formic acid and mobile phase B was acetonitrile with 0.1% formic acid.

Samples were analyzed using an Ultimate 3000 XRS UHPLC coupled to a TSQ Quantum access triple quadrupole mass spectrometer purchased from Thermo Scientific (San Jose, USA). Separation was performed in an Accucore C18 (100 × 2.1 mm, p. d. 2.6 µm) column, also from Thermo Scientific. The column temperature was 40 °C, and eluent flow rate was fixed at 0.4 mL/min. Initial eluent composition was 80% A, for 1.0 min, followed by a linear 5.0 min ramp to 50% A, which was held for 1.0 min, returning to the initial composition at 7.5 min. Column equilibration time was 2 min. The MS conditions were as follows: ESI positive mode, capillary voltage of 4.5 kV; sheath gas, nitrogen, 40 arb; auxiliary gas, nitrogen, 15 arb; collision gas, argon, 1.5 mTor; vaporizer temperature, 380 °C; ion transfer temperature 210 °C. MRM transitions were: FLU m/z 310 → 44 (quantification); 310 → 42 and 310 → 117 (qualification); NFLU m/z 296 → 134 (quantification);

296 → 105 and 296 → 30 (qualification); FLU-D6 m/z 316 → 44 (quantification); 316 → 187 and 316 → 42 (qualification). Collision energies were 13, 79 and 34 eV for FLU; 5, 16 and 12 eV for NFLU and 13, 52 and 70 eV for FLU-D6.

The therapeutic concentration range (CR) of fluoxetine recommended for depression treatment is 120 – 500 ng/mL, and these values represent the sum of the concentrations of FLU and NFLU. For analyses, patients were classified as below the CR (plasma concentration < 120 ng/mL), within the CR (plasma concentration between 120 – 500 ng/mL) and above the CR (plasma concentration > 500 ng/mL). Another classification used refers to the metabolizing capacity of CYP2D6, obtained by the ratio of the NFLU concentration to FLU concentration. This should be between 0.7 and 1.9, with an average of 1.3 (Reis *et al.*, 2009). Thus, patients were classified as poor (ratio < 0.7), intermediate (ratio between 0.7 - 1.9) or rapid metabolizers (ratio > 1.9).

Statistical analysis

The therapeutic range and the NFLU/FLU metabolic ratio grouped according to adherence levels were tested by Pearson Chi Square. The relationship between concentrations of FLU or NFLU and daily dose of FLU was tested by Pearson correlation. Data were expressed as mean ± standard deviation (SD) or median and percentile 25 and percentile 75, as appropriate. *P* values < 0.05 were considered statistically significant. The software SPSS 24.0 (SPSS, Chicago, IL) was used for statistical analysis.

RESULTS

FLU and NFLU plasma concentration

Table II lists the results of plasmatic concentration of FLU and its main metabolite, NFLU. The median of FLU plasma concentration was 105.5 (41.6 – 186.7) ng/dl, NFLU was 118.8 (66.6 – 194.5) ng/dl, FLU + NFLU was 216.4 (116.7 – 392.4) ng/dl and the NFLU/FLU ratio was 0.9 (0.5 – 1.2).

These data indicated that 58.5% volunteers were within the expected therapeutic range for patients receiving FLU. However, 26.4% have not yet reached the same level and 15.1% were above this level. Using the NFLU/FLU metabolic ratio, the intermediate metabolizer phenotype of CYP2D6 was attributed to 52.8% FLU users, the rapid metabolizer phenotype, to another 34%, and poor metabolizer, to the other 13.2% patients.

In this study, we found a positive and moderate correlation between daily dose and concentration of FLU (r = 0.293; p = 0.033), NFLU (r = 0.394; p = 0.004) and FLU + NFLU (r = 0.357; p = 0.009).

TABLE II - Classification of FLU users according to therapeutic range and CYP2D6 phenotype

Characteristics	Fluoxetine users (n = 53)
Concentration (ng/mL)	
FLU	105.5 (41.6 – 186.7)
NFLU	118.8 (66.6 – 194.5)
FLU + NFLU	216.4 (116.7 – 392.4)
NFLU / FLU	0.9 (0.5 – 1.2)
Therapeutic range	
Below	14 (26.4 %)
Inside	31 (58.5 %)
Above	8 (15.1 %)
CYP2D6 phenotype	
Slow	7 (13.2 %)
Below	2
Inside	2
Above	3
Intermediary	28 (52.8 %)
Below	7
Inside	16
Above	5
Fast	18 (34.0 %)
Below	5
Inside	13
Above	0

FLU: fluoxetine; NFLU: norfluoxetine. These concentrations of FLU and NFLU, FLU + NFLU and NFLU/FLU are expressed by the median (25th percentile and 75th percentile) of the obtained data. Therapeutic range: concentration of FLU + NFLU are used (Below: < 120 ng/mL; Inside: 120–500 ng/mL; Above: >500 ng/mL). CYP2D6 phenotype: ratio of NFLU/FLU are used (Slow: < 0.7; Intermediary: 0.7–1.9; Fast: >1.9).

Treatment adherence

The classification according to the degree of adherence, proposed by MG, indicated that 41.5% patients studied have a high degree of adherence. The authors' approach, which classifies the non-adherent patient as "intentional" and "unintentional", pointed out that, out of the 58.5% who are not adherent, 51.6% are intentional. On the other hand, the BMQ instrument classified as adherent to pharmacological treatment only 13.2% volunteers and 50.9% are assigned a low adherence behavior. The main barrier to adherence was forgetting to take the medication (75.5%), followed by regimen (52.8%), omissions of days or doses, and beliefs associated with the use of drugs and adverse effects (39.6%) (Table III).

Table IV shows the analyses of the adherence level and daily dose of FLU *vs* the therapeutic range. According to MG, 70% patients with "low adherence" are in the expected therapeutic range (FLU + NFLU = 120 - 500ng/mL), as well as 50% of those classified as non-adherent. Likewise, 68% patients with "probable low adherence" and 59% patients with "low adherence", according to BMQ, are in the therapeutic range. Considering the daily dose of FLU, 62% patients who use 20 mg/day are in the recommended range, while 60% of those who use 40 mg/day are higher than expected (FLU + NFLU > 500 ng/mL).

TABLE III - Classification of FLU users according to their adherence levels (using Morisky-Green and BMQ classifications)

Characteristics	FLU user (n = 53)	
	Frequency	Percent (%)
Morisky-Green [1]		
Non-adhesion	2	2.8
Low adhesion	20	37.7
Moderate adhesion	9	17.0
High adhesion	22	41.5
Morisky-Green [2]		
Adherent	22	41.5
Non-adherent	31	58.5
Intentional	16	51.6
Non-intentional	15	48.4
BMQ		
Regimen screen		
Score = 0	25	47.2
Score ≥ 1	28	52.8
Beliefs screen		
Score = 0	32	60.4
Score ≥ 1	21	39.6
Recall screen		
Score = 0	13	24.5
Score ≥ 1	40	75.5
Adherence		
Adherent	7	13.2
Likely low adhesion	19	35.8
Low adhesion	27	50.9

BMQ: Brief Questionnaire for Medication Compliance. [1]Classification according to degree of adhesion; [2]Classification according to degree of non-adherence and intensity.

TABLE IV - Distribution of the classified fluoxetine users according to their adherence level (using Morisky-Green and BMQ classifications) and therapeutic range (FLU + NFLU)

Characteristics	Therapeutic range			Significance *p – value*
	Below (n=14)	Inside (n=31)	Above (n=8)	
Morisky-Green [1]				
Non-adhesion (n = 2)	1 (50 %)	1 (50 %)	0	1.000
Low adhesion (n = 20)	3 (15 %)	14 (70 %)	3 (15 %)	0.002
Moderate adhesion (n = 9)	2 (22 %)	6 (67 %)	1 (11 %)	0.097
High adhesion (n = 22)	8 (36 %)	10 (45 %)	4 (19 %)	0.280

TABLE IV - Distribution of the classified fluoxetine users according to their adherence level (using Morisky-Green and BMQ classifications) and therapeutic range (FLU + NFLU)

Characteristics	Therapeutic range			Significance *p – value*
	Below (n=14)	Inside (n=31)	Above (n=8)	
Morisky- Green [2]				
Adherent (n = 22)	8 (36 %)	10 (45 %)	4 (19 %)	0.280
Non adherent (n = 31)	6 (19 %)	21 (61 %)	4 (20 %)	< 0.001
Intensional (n = 16)	3 (19 %)	13 (81 %)	0	0.012
Non-intensional (n = 15)	3 (20 %)	8 (53 %)	4 (27 %)	0.247
BMQ				
Adherence (n = 7)	3 (44 %)	2 (28 %)	2 (28 %)	0.867
Likely low adhesion (n = 19)	4 (21 %)	13 (68 %)	2 (11 %)	0.004
Low adhesion (n = 27)	7 (26 %)	16 (59 %)	4 (15 %)	0.013
Daily dose				
20 mg (n = 45)	13 (29 %)	28 (62 %)	4 (9 %)	< 0.001
40 mg (n = 6)	1	2 (40 %)	3 (60 %)	0.607
60 mg (n = 2)	0	1 (50 %)	1 (50 %)	1.000

BMQ: Brief Questionnaire for Medication Compliance. [1]Classification according to degree of adhesion; [2]Classification according to degree of non-adherence and intensity. Therapeutic range: concentration of FLU + NFLU are used (Below: < 120 ng/mL; Inside: 120–500 ng/mL; Above: > 500 ng/mL). The Person Chi Square was used in statistical analysis.

DISCUSSION

The predominance of women with a mean age of 57 years in the studied group can be justified by the fact that women access health services more frequently due to the different disease prevention programs directed to them (Hwang, Kim, 2007; Caiaffo *et al*., 2016) and the gender-related subtypes of depression are suggested to exist, with the plethora of sex differences in brain structure, function, and stress responsivity, as well as differences in exposure to reproductive hormones, social expectations and experiences (Kuehner, 2017). The presence of pre-obese patients (BMI between 25.0 and 29.9 kg/m^2), obese grade I (BMI between 30.0 and 34.9 kg/m^2) and obese grade II (BMI between 35.0 and 39.9 kg/m^2) in depressed patients represents an increased risk for the development of chronic diseases (ABESO, 2009), such as hypertension, diabetes and hypercholesterolemia, also evidenced in this study.

The findings of this study did not show a direct relationship between plasma concentration and adherence levels evaluated by the MG and BMQ questionnaires. In this sample of fluoxetine users, we found a predominance of low adherence assessed by the questionnaires, while the evaluation of adherence by plasma concentration (FLU + NFLU) points to greater adherence to therapy. Most non-adherent patients, according to the MG and BMQ, presented plasma concentration within the therapeutic range. These data can be explained by three approaches: (i) the bias inherent to the structured interviews, (ii) the pharmacokinetic characteristics of fluoxetine, (iii) the presence of polymorphisms in CYP2D6 metabolizing enzyme.

According to the instrument created by Morisky *et al.* (1986), to measure non-adherence, one of these reasons explain this: forgetfulness, improvement of symptoms, worsening or carelessness. In this study, 51.6% patients classified as non-adherent are intentional, i.e., non-adherence is conscious and is not a consequence of forgetfulness or cognitive deficiency. Low schooling and family income are important factors to be considered in this intentionality. Do not having the precise knowledge of the risks of not adhering to treatment or not having access to medication, due to financial limitations; appear

to be decisive for this nonconformity. This is because the side effects, often cited as the main cause of drug discontinuation, have not been consistently associated with the discontinuation of antidepressant medication, as shown by Mitchell (2006). Non-adherence to the treatment, classified as non-intentional, also evidenced in our study, was associated with forgetfulness (Barfod *et al.*, 2006) and common cognitive impairment among the elderly (Ayalon, Areán, Alvidrez, 2005) as prominent causes.

Regarding adherence to pharmacological treatment based on the analysis of the BMQ total score, most patients were categorized as "low adherence", followed by "probable low adherence". This result corroborates other studies in our group (Mineto *et al.*, 2018; Kasper *et al.*, 2017). The main barrier to adherence was forgetting to take the medication, followed by regimen, omissions of days or doses, and beliefs associated with the use of drugs and adverse effects. Therefore, multiple-dose regimens and the difficulty to remember taking medications (recall barrier) are important factors that contribute to low adherence in the group studied (Fritzen, Motter, Paniz, 2017).

The assessment of adherence by the BMQ questionnaire showed a higher rate of low adherence (52.3% vs 38.6%) than that found in the MG method. However, similarly, most patients were within the therapeutic range. As a bias of the questionnaires, we first emphasize that they were not filled by the volunteers, but rather by the interviewers, who recorded the answers given. This is based on what we have pointed out as the main limitation of this study: the high age group of the volunteers and their low level of schooling. These two characteristics of the studied group contributed to reduce the effectiveness of the indirect method, since the interpretation given by the interviewer to the answers may have influenced the results found. Secondly, we highlight the characteristics of each instrument. MG has a simple, practical and easy-to-apply model of four questions, while the BMQ allows to assess adherence to more possibilities, revealing where the main obstacles are in tracking treatment. In addition, it should be noted that self-report is subjected to recall errors and implies some imprecision in the estimates obtained (Guilarducci *et al.*, 2016).

The measurement of plasma concentrations of FLU and NFLU could help to identify non-adherence, as well as the metabolizer phenotype of CYP2D6 (Hiemke *et al.*, 2011). The pharmacokinetic characteristics of FLU and its NFLU metabolite may also be influencing the discrepancies found. Due to the high $T_{1/2}$ FLU (1 to 4 days) and NFLU (7 to 15 days) (Hiemke, Hartter, 2000), small or moderate dose change or discontinuation may not influence plasma concentration in the short term. Another reason that could explain this finding is the high rate of CYP2D6 polymorphism that alter the metabolic ratio of NFLU to FLU, leading to changes in their plasma concentrations (Hiemke *et al.*, 2011). It may also be noted that chronic administration of fluoxetine creates a complex inhibition system, in which FLU, NFLU and its stereoisomers act as inhibitors of CYP2D6, decreasing the clearance of fluoxetine to approximately 30% baseline values, 0.18 to 0.25mL/min (Altamura *et al.*, 1994) and, consequently increases the plasmatic concentrations of FLU and NFLU.

In addition, we may consider the possible presence of CYP2D6 polymorphism that alter the NFLU/FLU metabolic ratio, leading to changes in their plasma concentrations (Hiemke *et al.*, 2011). CYP2D6 activity varies from poor to ultra-rapid, due to the occurrence of a large number of allelic variants of the CYP2D6 gene, causing absent, decreased or increased enzyme activity in relation to the wild type functional allele (Sachse *et al.*, 1997; Bertilsson *et al.*, 2002). Adding the genetic alterations to the inhibition caused by the chronic administration of FLU, we obtain a large variability in therapeutic results.

As for the NFLU/FLU ratio, according to the therapeutic range, we also identified divergences. Out of the 28 patients classified as intermediate metabolizer phenotype (NFLU/FLU ratio between 0.7 and 1.9), 7 are below the therapeutic concentration and 5 are above. This demonstrates the non-adherence to the pharmacological treatment evidenced by the questionnaires. On the other hand, the non-adherence to the treatment evidenced by the indirect method (questionnaires) did not reproduce the actual therapeutic condition of the volunteers, because although classified as such, the majority were within the recommended therapeutic range.

On the other hand, although measurement of drug serum levels is an objective direct method for testing compliance, it can be distorted by "white-coat adherence" or by variations in drug elimination. Sima *et al*. (2017) demonstrated that assessment of adherence to medication reinforced with therapeutic drug monitoring and pharmacokinetic simulations is proposed as an optimal method by reducing disadvantages of simple drug concentration measurements.

In view of this, it is suggested that the measurement of FLU plasma concentration and its main metabolite, NFLU, is recognized as the best strategy to evaluate adherence to treatment and, consequently, to the therapeutic monitoring of this drug. This direct method of laboratory dosage of the drug concentration can be honorable and limit its implementation. Nevertheless, recently, our group developed and validated the dosage methodology of these substances using dry blood spots (DBS) (Da Silva *et al*., 2018). This method is low cost and easy to apply, proving useful for the therapeutic monitoring of FLU.

CONCLUSION

Most fluoxetine users have a plasma concentration of FLU and NFLU within the therapeutic range, despite having low adherence to the drug therapy evaluated by the MG and BMQ questionnaires. Thus, we suggest that plasma levels of FLU and NFLU could be used as the main method to check adherence to their treatment. The differences found between the methods (questionnaire and plasma dosages) may be due to the pharmacokinetic characteristics of fluoxetine, and the presence of polymorphisms in the CYP2D6 metabolizing enzyme.

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