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# Genotyping and phenotyping of *CYP2D6* and *CYP3A* isoenzymes in patients with alcohol use disorder: correlation with haloperidol plasma concentration

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**Abstract**

**Background:** Haloperidol is used for the treatment of alcohol use disorders in patients with signs of alcohol-related psychosis. Haloperidol therapy poses a high risk of adverse drug reactions (ADR). Contradictory data, which include the effects of genetic polymorphisms in genes encoding the elements of haloperidol biotransformation system on haloperidol metabolism rate and plasma drug concentration ratio, are described in patients with different genotypes. The primary objective of this study was to investigate the effects of *CYP2D6* and *CYP3A5* genetic polymorphisms on haloperidol equilibrium concentration in patients with alcohol use disorder.

**Methods:** The study included 69 male patients with alcohol use disorder. Genotyping was performed using the allele-specific real-time PCR. *CYP2D6* and *CYP3A* were phenotyped with HPLC-MS using the concentration of endogenous substrate of the enzyme and its urinary metabolites [6-hydroxy-1,2,3,4-tetrahydro-β-carboline(6-HO-THBC) to pinoline ratio for *CYP2D6* and 6-β-hydroxycortisol to cortisol ratio for *CYP3A*]. The equilibrium plasma concentration was determined using LC-MS-MS.

**Results:** Results indicated that both C/D indexes and equilibrium concentration levels depend on *CYP2D6* genetic polymorphism, but only in patients receiving haloperidol intramuscular injections [0.26 (0.09; 0.48) vs. 0.54 (0.44; 0.74), p = 0.037].

**Conclusions:** The study demonstrates that *CYP2D6* genetic polymorphism (*1846G>A*) can affect haloperidol concentration levels in patients with alcohol use disorder.

**Keywords:** adverse drug reactions; alcohol dependence; biotransformation; haloperidol; pharmacogenetics; therapeutic drug monitoring.

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## Introduction

Haloperidol is a typical antipsychotic medication with a strong antipsychotic effect mediated by the blockade of the dopaminereceptors in the mesolimbic system. According to the recommendations, haloperidol therapy is indicated for patients with signs of alcohol-related psychosis [1, 2]. Haloperidol use is associated with high risk of severe adverse drug reactions (dyskinesia, low blood pressure, postural hypotension, cardiotoxicity).

During its metabolism, haloperidol is reduced in the liver by cytosolic carbonyl reductase to reduced form, which has 10%–20% of the parent molecule activity. *CYP3A4* catalyzes the transformation of haloperidol to 1,2,3,6-tetrahydropyridine, which can be further metabolized to haloperidol pyridinium by both *CYP3A4* and *CYP2D6* [3]. *CYP3A4* and *CYP2D6* are also responsible for the N-dealkylation of haloperidol [3]. The N-dealkylation

of reduced haloperidol is catalyzed only by *CYP3A4* [3]. In addition, *CYP3A4* also catalyzes the oxidation of reduced haloperidol back to haloperidol [3]. The results of the clinical study performed by Ghosh et al. [4] showed that *CYP3A4* is expressed not only in hepatocytes, but also in cerebral neurons.

Isoenzyme *CYP2D6* synthesis is encrypted by the gene of the same name located on chromosome 22 [5]. The *CYP2D6* gene has a high level of polymorphism: to date, 100 allele variants of genotype have been proposed (www.cypalleles.ki.se). Depending on the possible effects of allele variants on *CYP2D6* isoenzyme activity and, therefore, on biotransformation rate, patients were divided into the following groups [6]: poor metabolizers, intermediate metabolizers, extensive metabolizers, and ultrarapid metabolizers.

A replacement of guanine by adenine at position 1846 of the *CYP2D6* (*CYP2D6*4*, *CYP2D6 1846G>A*, *rs3892097*) gene resulted in a splicing defect and decreased activity of *CYP2D6* isoenzyme, which should lead to lower rates of the isoenzyme substrate elimination from the body [7].

The correlation between *CYP2D6* activity and haloperidol biotransformation rates has been shown in several studies in patients with schizophrenia [8–10]. At the same time, however, some articles refute the existence of such correlation. The studies performed in patients with alcohol use disorder have shown a statistically significant correlation between *CYP2D6* genetic polymorphism, isoenzyme activity and haloperidol efficacy, and safety [11, 12].

The articles reported in 1999–2001 evaluated the effects of *CYP3A4* on haloperidol metabolism and the development of adverse drug reactions [3, 13, 14]. Some data also indicated the absence of correlation between the development of adverse drug reactions in patients using haloperidol and genetic variations [15]. The *CYP3A4*22* polymorphism research shows that heterozygous individuals do not experience the increased serum levels of antipsychotic drugs (including haloperidol) metabolized by both *CYP3A4* and *CYP2D6* [16]. The latest research on *CYP3A5* polymorphism revealed a lack of materials and studies on this topic. The role of *CYP3A5* should be investigated in more detail as there are data showing that it catalyzes the alternative metabolic pathways. Such a discovery could lead to the emergence of the intermediate metabolites with pharmacological properties that are not yet known, and limit the bioavailability of the drug undergoing first-pass metabolism. In addition, *CYP3A5* expression may overcome *CYP3A4* drug interaction [17]. In our previous works, we investigated the effects of *CYP3A4* isoenzyme activity on haloperidol efficacy and safety indicators in patients with alcohol use disorder [18], along with the effects of concominant carbamazepine use on the isoenzyme activity [19].

The objective of this study was to evaluate the correlation between *CYP2D6* and *CYP3A5* genetic polymorphisms, *CYP2D6* isoenzyme and CYP3A subfamily member activity, ratio of equilibrium concentration levels and haloperidol dose, as well as efficacy and safety profiles of haloperidol in patients with alcohol use disorder during the period of pathological craving actualization.

## Materials and methods

### Patients

The study included 69 male patients (average age of 37.52 ± 6.73 years) with alcohol use disorder who received treatment at the Moscow Research and Practical Centre on Addictions of the Moscow Department of Healthcare. During the exacerbation of compulsive alcohol use, patients received haloperidol in tablets (OOO Ozon, Ghigulevsk, Russia) at a dose of 4.46 ± 1.49 mg per day (38 patients, single use) and injections (ZAO Bryntsalov-A, Moscow, Russia) at a dose of 5.71 ± 2.09 mg per day (31 patients, single use). Inclusion criteria were 5-day haloperidol therapy in tablets or injections and absence of concomitant mental illness in anamnesis. Exclusion criteria were presence of any other antipsychotics in treatment regimen, except haloperidol, creatinine clearance values <50 mL/min, creatinine concentration in plasma ≥1.5 mg/dL (133 mmol/L), body weight less than 60 kg or greater than 100 kg, age of 75 years or more, and presence of any contraindications for haloperidol use.

### Phenotyping

Isoenzyme *CYP2D6* and *CYP3A4* activity were evaluated by determining the urinary concentration of endogenous substrate of the enzyme and its metabolite, 6-hydroxy-1,2,3,4-tetrahydro-β-carboline (6-HO-THBC) to pinoline ratio for *CYP2D6* [20, 21] and 6-β-hydroxicortisol to cortisol ratio for total CYP3A isoenzyme group activity [22], using high-performance liquid chromatography-mass spectrometry (HPLC-MS) carried out on an Agilent 1200 LC/MS. High ratio levels reflect the high level of isoenzyme activity. The results of the isoenzyme evaluation are demonstrated in arbitrary units.

### Genotyping

Venous blood samples collected in vacuum tubes VACUETTE® (Greiner Bio-One, Austria) on the sixth day of the haloperidol therapy were used for genotyping. Real-time polymerase chain reaction (RT-PCR) was performed using DNA amplifiers “Dtlite” of DNA Technology (Moscow, Russia), CFX96 Touch Real Time System with CFX Manager software of Bio-Rad Laboratories Inc. (Hercules, CA, USA) and sets “SNP-screen” of “Syntol” (Moscow, Russia). This was

used to determine single-nucleotide polymorphism (SNPs) *1846G>A* of the gene *CYP2D6* (*rs3892097*) and *6986A>G* of the gene *CYP3A5* (*rs776746*). In every "SNP-screen" set, two allele-specific hybridizations were used, which allowed two alleles of the studied polymorphism to be determined separately on two fluorescence channels.

### Therapeutic drug monitoring

Samples for TDM were collected by venipuncture using vacuum tubes with K3EDTA after day 5 of haloperidol therapy (tablets, injections, drops), i.e. after five periods of haloperidol half-life and establishment of fixed concentration before administration of another medication dose on an empty stomach. Blood samples were centrifuged in centrifuge with refrigeration, plasma was collected into 1.5 mL plastic disposable tubes, frozen at –80 °C and delivered to the laboratory for analysis.

### Methodology of haloperidol measuring for therapeutic drug monitoring using LC-MS-MS method in blood plasma

The plasma calibration standards (St) and quality control samples (QC) were made from stock solution prepared by consistent dissolving of substance amounts in methanol with subsequent dilution to the relevant concentrations.

Calibration curve was created using 0.5, 1, 1.25, 2.5, 5, 10, 25, and 50 ng/mL calibration standards and 1.5 (low QC), 7.5 (medium QC), and 12.5 ng/mL (high QC) quality control samples (QC). Anastrozole concentration in samples was used as the internal standard.

**Sample preparation:** Samples were prepared using liquid–liquid extraction method. A 5 mL tube was filled with 500 μL of analyzed plasma sample, after which 50 μL of methanol (for volume compensation) and 2 mL of methyl tert-butyl ether (MTBE) were added. The mixture was shaken on a horizontal shaker for 15 min, then samples were centrifuged, placed on –80 °C drained organic plums in tubes for vaporization, vaporized using a concentrator, and resuspended in 250 μL of mobile phase. Samples were analyzed using high-performance liquid chromatography system Agilent 1200 conjugated with mass spectrometer Agilent 6410-2A (USA).

**Conditions of chromatographic analysis:** Stationary phase threaded column Zorbax SB-C18 (particle size 5 μm, 150 mm × 4.6 mm, Agilent, USA). Mobile phase: A – 0.2% formic acid in water, B – acetonitrile. Gradient started at 60% of phase A and was increased to 98% of B, with flow rate of 0.6 mL/min. Column temperature was 30 °C. Volume of the inserted sample was 5 μL. Whole time of distillation was 7 min.

**Conditions of mass-spectrometry determination:** The detector (electrospray ESI) worked in positive ionization mode MRM, voltage on capillary was 4 kV, temperature of the desiccant gas was 300 °C, nitrogen flow was 7 L/min ($N_2$), and nebulizer pressure was 30 psi. The detector registered following MRM-transions: 294.2 → 225.1 for anastrazole and 376.2 → 165.0 for haloperidol, voltage on phragmentor was 130 V for two analytes, collision energies were 30 V and 25 V. Time of haloperidol output within the conditions was 4.34 ± 0.2 min and 5.55 ± 0.2 min for the internal standard.

Methodology used in the study met all standards of FDA analytical methods. Calibration dependence was linear for diaoasone at 0.5–50 ng/mL, accuracy (CV, %) for quality control samples was less than 15%, and accuracy for all QC in inter- and intra-day ranged from 85%–115%. The haloperidol recovery rate was approximately 80%. Matrix effect had no influence.

### Statistical data analysis

Statistical analysis of the results was performed with non-parametric methods using the "Statsoft Statistica v. 10.0" (Dell Statistica, Tulsa, OK, USA). The normality of sample distribution was evaluated using the Shapiro-Wilk test and taken into account when choosing a method. The differences were considered as statistically significant at $p < 0.05$ (power in excess of 80%). To compare two independent groups, Mann-Whitney U-test was used. To determine the correlation between quantitative characteristics, Spearman rank correlation coefficient ($r_s$) was calculated. Correlation coefficient ($r_s$) values from 0.3–0.7 ($p < 0.05$) indicated moderate positive, but significant correlation between the characteristics, whereas $r_s > 0.7$ ($p < 0.05$) indicated strong and significant correlation. Moreover, negative values of $r_s$ indicated inverse correlation. Regression analysis was performed in "multiple regression module" to determine the effects of *CYP2D6* activity (measured by 6-hydroxy-1,2,3,4-tetrahydro-β-carboline to pinoline ratio) and *CYP3A4* activity (measured by 6-β-hydroxicortisol to cortisol ratio) on the index of C/D. Research data are presented as median and interquartile range (M [Q1; Q3]).

### Ethical issues

Ethical approval was granted by the Local Ethics Committee of the Russian Medical Academy of Postgraduate Education of the Ministry of Health of the Russian Federation.

## Results

The *CYP2D6* genotyping by polymorphic marker *1846G>A* (*rs3892097)* performed in 69 patients with alcohol use disorder revealed the following data:

- The number of patients with no mutant *CYP2D6* (genotype *GG*) accounted for 52 (75.36%), of whom 22 (42.31%) received haloperidol in injections and 30 (57.69%) received haloperidol in tablets.
- The number of patients with heterozygous polymorphism *1846G>A* of *CYP2D6* gene (genotype *GA*) accounted for 17 (24.64%), of whom 9 (52.94%) received haloperidol in injections and 8 (47.06%) received haloperidol in tablets.
- There were no patients with homozygous polymorphism *1846G>A* of *CYP2D6* gene (genotype *AA*).

The *CYP3A5* genotyping on polymorphic marker *6986A>G* (*rs776746*) performed in 69 patients with alcohol use disorder revealed the following data:

– There were no patients with homozygous wild type genotype *AA* on polymorphic marker *6986A>G* of *CYP3A5* gene (genotype *AA*).
– The number of patients with heterozygous polymorphism *6986A>G* of *CYP3A6* gene (genotype *AG*) accounted for 3 (4.35%), of whom 2 (66.7%) received haloperidol in injections and 1 (33.3%) received haloperidol in tablets.
– The number of patients with the mutant gene *CYP3A5* (genotype *GG*) accounted for 66 (95.65%), of whom 29 (43.94%) received haloperidol in injections and 37 (56.06%) received haloperidol in tablets.

The distribution of genotypes corresponded to the Hardy–Weinberg equilibrium in the European population for *CYP2D6 1846G>A* ($\chi^2 = 1.36$; p = 0.24) and *CYP3A5* 6986A>G ($\chi^2 = 0.034$; p = 0.85).

The haloperidol doses were the same in groups with different genotypes, as described below.

1. Haloperidol for intramuscular injections
   – *CYP2D6 1846G>A: GG* – 5.00 [5.00; 6.00], *GA* – 5.00 [5.00; 5.00], (p = 0.737)
   – *CYP3A5 6986A>G: AA* – 5.00 [5.00; 5.00], *AG* – 7.50 [5.00; 10.00], (p = 0.436)
2. Haloperidol in tablets
   – *CYP2D6 1846G>A: GG* – 5.00 [3.00; 6.00], *GA* – 3.00 [3.00; 5.00], (p = 0.337)
   – *CYP3A5 6986A>G: AA* – 5.00 [3.00; 6.00], *AG* – 3.00 [3.00; 3.00], (N/A)
3. All patients
   – *CYP2D6 1846G>A: GG* – 5.00 [3.00; 6.00], *GA* – 5.00 [3.00; 5.00], (p = 0.726)
   – *CYP3A5 6986A>G: AA* – 5.00 [3.00; 6.00], *AG* – 5.00 [3.00; 10.00], (p = 0.816)

**Table 1:** The results of HPLC-MS/MS on the determination of pinoline and 6-HO-THBC concentration in urine for evaluation of *CYP2D6* activity.

| Group | n (%) | Concentration of pinoline, pg/mL | Concentration of 6-HO-THBC, pg/mL | Ratio 6-HO-THBC/ pinoline, a.u. |
|---|---|---|---|---|
| Haloperidol in injections | 31 (44.93) | | | |
| M | | 1887.885 | 1943.025 | 0.913 |
| Q1 | | 1234.440 | 1346.520 | 0.566 |
| Q3 | | 2710.360 | 2527.180 | 1.868 |
| Haloperidol in tablet | 38 (55.07) | | | |
| M | | 1548.300 | 1704.880 | 0.962 |
| Q1 | | 1012.820 | 958.000 | 0.735 |
| Q3 | | 2585.700 | 2645.850 | 1.811 |
| Total group | 69 (100) | | | |
| M | | 1599.000 | 1913.980 | 0.923 |
| Q1 | | 1136.770 | 1127.000 | 0.708 |
| Q3 | | 2585.700 | 2558.580 | 1.811 |

**Table 2:** The results of HPLC-MS/MS on the determination of cortisol and 6-β-hydroxycortisol concentration in urine for evaluation of *CYP3A* activity.

| Group | n (%) | Concentration of cortisol, pg/mL | Concentration of 6-β-hydroxycortisol, pg/mL | Ratio 6-β-hydroxycortisol/ cortisol, a.u. |
|---|---|---|---|---|
| Haloperidol in injections | 31 (44.93) | | | |
| M | | 165.800 | 657.726 | 5.000 |
| Q1 | | 97.022 | 353.125 | 3.656 |
| Q3 | | 262.300 | 1420.455 | 6.392 |
| Haloperidol in tablet | 38 (55.07) | | | |
| M | | 228.133 | 1281.534 | 4.877 |
| Q1 | | 124.500 | 445.300 | 3.306 |
| Q3 | | 404.444 | 2594.900 | 6.263 |
| Total group | 69 (100) | | | |
| M | | 189.689 | 910.511 | 4.984 |
| Q1 | | 101.156 | 397.727 | 3.337 |
| Q3 | | 286.800 | 1560.000 | 6.263 |

**Table 3:** Rates of equlibrium concentration index of haloperidol and concentration/dose index.

| Group | Concentration, ng/mL | Concentration/dose, a.u. |
|---|---|---|
| Total group | 1.435 [0.62; 2.432] | 0.329 [0.129; 0.557] |
| Haloperidol in injections | 1.944 [0.852; 3.7] | 1.141 [0.605; 2.18] |
| Haloperidol in tablet | 0.328 [0.17; 0.648] | 0.33 [0.121; 0.522] |

**Table 4:** Rates of equilibrium haloperidol concentration index and concentration/dose index depending on patient genotype by polymorphic markers *CYP2D6* (*1846G>A*) and *CYP3A5* (*6986A>G*).

| Group | Concentration, ng/mL | | | Concentration/dose, a.u. | | |
|---|---|---|---|---|---|---|
| | ***GG*** | ***GA*** | **p-Value** | ***GG*** | ***GA*** | **p-Value** |
| *CYP2D6* (*1846G > A*) | | | | | | |
| Total group | 1.34 [0.37; 2.29] | 2.44 [1.07; 3.7] | 0.031436 | 0.24 [0.09; 0.48] | 0.54 [0.28; 0.84] | 0.03735 |
| Haloperidol in injections | 1.53 [0.28; 2.35] | 2.71 [2.2; 5.13] | 0.085805 | 0.26 [0.09; 0.48] | 0.54 [0.44; 0.74] | 0.03736 |
| Haloperidol in tablet | 1.14 [0.4; 2.08] | 1.37 [0.74; 2.63] | 0.190287 | 0.24 [0.11; 0.48] | 0.46 [0.22; 0.86] | 0.34681 |
| | ***AG*** | ***GG*** | **p-Value** | ***AG*** | ***GG*** | **p-Value** |
| *CYP3A5* (*6986A > G*) | | | | | | |
| Total group | 2.33 [1.85; 2.42] | 1.34 [0.6; 2.44] | 0.282328 | 0.48 [0.18; 0.78] | 0.33 [0.12; 0.55] | 0.51118 |
| Haloperidol in injections | 2.13 [1.85; 2.42] | 1.94 [0.85; 3.7] | 0.650869 | 0.33 [0.18; 0.48] | 0.33 [0.17; 0.65] | 0.74650 |
| Haloperidol in tablet | 2.33 [2.33; 2.33] | 1.11 [0.5; 2.13] | – | 0.78 [0.78; 0.78] | 0.31 [0.12; 0.5] | – |

Data on isoenzyme activity collected during the evaluation of *CYP2D6* and *CYP3A* phenotyping are presented in Tables 1 and 2, respectively.

After the pharmacokinetic examination of haloperidol and plasma concentration/dose ratio determination in patients (Table 3), the following data were obtained:

We compared the obtained rates in patients with different genotypes of polymorphic markers *CYP2D6* (*1846G>A)* and CYP3A5 (*6986A>G)* using the Mann–Whitney U-test (Table 4, Figure 1). The values of concentration/dose index in patients with GG and GA genotypes of polymorphic marker *CYP2D6* (*1846G>A)* are shown in Figure 1. Further, using the Spearman correlation analysis, we calculated the correlation between the rates of phenotyping results and the results of pharmacokinetic examination (Table 5).

Linear regression analysis also showed lack of statistically significant regression model between the rates of phenotyping results and results of pharmacokinetic examination. In view of the large data set for linear regression modeling results, we are reporting only the data for patients who received haloperidol in intramuscular injections and the metabolic rate of HO-THBC/pinoline due to the statistical relevancy of this model.

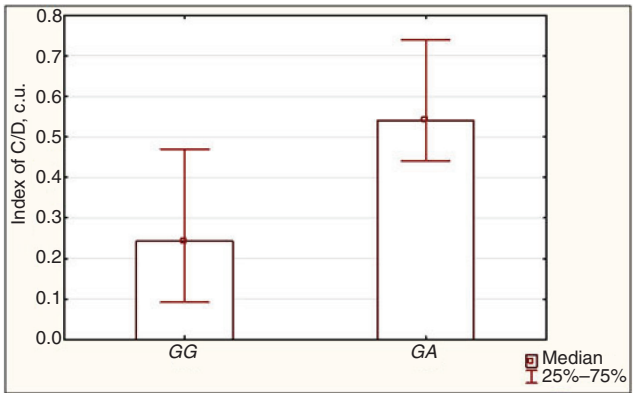


**Figure 1:** Concentration/dose index in patients with *GG* and *GA* genotypes on polymorphic marker *CYP2D6* (*1846G>A*).

$$\text{HO-THBC/pinoline (c.u.)} = a + b * \text{index of C/D (c.u.)}$$

$b = 0.007205$ ($p = 0.8518$)

$a = 0.4088$

Standard error of estimate = 0.35

**Table 5:** Rates of Spearman's correlation coefficient between phenotyping results and the results of pharmacokinetic examination.

| Ratio | Concentration of haloperidol, ng/mL | | | Index of C/D, c.u. | | |
|---|---|---|---|---|---|---|
| | Total group | Haloperidol in injections | Haloperidol in tablet | Total group | Haloperidol in injections | Haloperidol in tablet |
| Cortisol, ng/mL | −0.064 | 0.057 | −0.251 | −0.125 | −0.014 | −0.281 |
| 6-β-Hydroxicortisol, ng/mL | −0.119 | −0.095 | −0.186 | −0.127 | −0.101 | −0.205 |
| 6-β-Hydroxicortisol/cortisol, c.u. | −0.091 | −0.205 | 0.097 | −0.032 | −0.168 | 0.122 |
| Pinoline, pg/mL | −0.162 | −0.098 | −0.226 | −0.073 | 0.027 | −0.139 |
| HO-THBC, pg/mL | −0.220 | −0.295 | −0.118 | −0.186 | −0.189 | −0.157 |
| HO-THBC/Pinoline, c.u. | −0.040 | −0.228 | 0.111 | −0.086 | −0.208 | −0.025 |

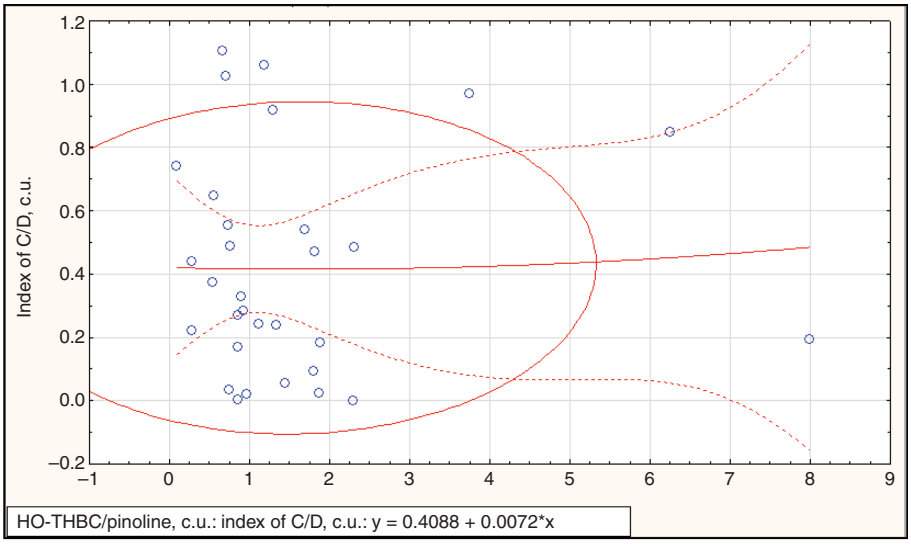


**Figure 2:** Graphic display of the linear regression model between the rates of concentration/dose index and *CYP2D6* evaluation index in patients who received haloperidol in intramuscular injection form.

The linear regression model between the rates of concentration/dose index and *CYP2D6* evaluation index in patients who received haloperidol in intramuscular injections is shown in Figure 2.

## Discussion

We studied the effects of *1846G>A* polymorphisms of the *CYP2D6* gene (*rs3892097)* and *6986A>G* polymorphisms of the *CYP3A5* gene (*rs776746*) on C/D rate in patients with alcohol use disorder during the exacerbation of compulsive alcohol use.

Findings showed that C/D rate and equilibrium concentration level correlate with *CYP2D6* genetic polymorphism only in patients receiving haloperidol in intramuscular injections [0.26 (0.09; 0.48) vs. 0.54 (0.44; 0.74), p = 0.037]. These data overlap with the clinical data obtained from our previous study [11], which examined the effects of *CYP2D6* genetic polymorphism on therapy efficacy and safety, and showed that the performance indicators were statistically significantly higher in patients with *GA* genotype (polymorphism *1846G>A* of *CYP2D6* gene), although efficacy indicators deteriorated. The main difference between the previous study data and the results of this study is that this study revealed no statistically significant effect of the *CYP2D6* genetic polymorphism on haloperidol pharmacokinetics in patients receiving haloperidol in tablets [C/D rate in patients with *GG* and *GA* genotypes: 0.24 (0.11; 0.48) vs. 0.46 (0.22; 0.86), p = 0.347]. The most likely reason may be that the bioavailability of dosage in intramuscular injections is higher than in oral tablets, and therefore, larger quantities of haloperidol exposed to biotransformation (besides, haloperidol dosage in patients receiving it in injections is higher than in patients receiving it in tablets: 5.71 ± 2.09 mg/day vs. 4.46 ± 1.49 mg/day).

**Table 6:** Comparison of genotype distribution data and *CYP3A5* gene alleles by polymorphic marker *6986A>G* in the studied population and different ethnic groups of previously conducted studies [23].

| Population | n | *GG* genotype | *GA* genotype | *AA* genotype | *G* allele | *A* allele | Comparison with the results of our research | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $\chi^2$ | p-Value | OR (95%, CI) |
| Russian | 443 | 204 (46.05%) | 25 (12.25%) | 0 (0%) | 838 (94.6%) | 48 (5.4%) | 2.39 | 0.12 | 2.46 (0.75; 8.02) |
| Tatars | 517 | 124 (23.98%) | 16 (12.9%) | 0 (0%) | 975 (94.3%) | 59 (5.7%) | 2.74 | 0.10 | 2.60 (0.80; 8.42) |
| Bashkirs | 280 | 99 (35.36%) | 21 (21.21%) | 1 (4.76%) | 512 (91.5%) | 48 (8.5%) | 6.21 | 0.01 | 4.03 (1.23; 13.14) |

Therefore, our research reinforces the evidence of previous studies in patients with schizophrenia [8–10], explaining their results that in intermediate metabolizers (*GA*), the slowdown of biotransformation and haloperidol elimination from the body occurs; in turn, plasma concentration of drugs increases and medication reaches the target receptors in higher amounts than in patients with "wild type" genotype (*GG*). The lack of statistically significant difference in equilibrium concentration index and C/D in patients with *AG* and *GG* genotypes of polymorphic marker *CYP3A5* (*6986A>G*) probably related to small number of patients with *AG* genotype and a lack of patients with "wild type" genotype *AA*. The present allele distribution follows the Hardy–Weinberg law ($\chi^2 = 0.034$; $p = 0.85$) and is similar to the results of population studies on that polymorphism in Russian and other populations residing within the Russian Federation territory [23]: in the Tatar population, *G*-allele of the *CYP3A5*3* gene has been identified with a frequency of 94.3% [OR = 2.60 (95% CI, 0.80; 8.42), $p = 0.10$], in the Bashkir population with a frequency of 91.5% [OR = 4.03 (95% CI, 1.23; 13.14), $p = 0.01$], and in the Russian population with a frequency of 94.6% [OR = 2.46 (95% CI, 0.75; 8.02), $p = 0.12$] (Table 6).

The results of our previous study [18] showed a weak statistically significant correlation between *CYP3A* activity indicator (6-β-hydroxycortisol/cortisol metabolic rate level in urine) and efficacy and safety indicators only in the group of patients receiving haloperidol in injections. Yet in this study, no correlation exists between the C/D and *CYP3A* activity indicators both in the group receiving haloperidol in intramuscular injections (Spearman's coefficient of correlation = −0.168, $p > 0.05$) and in the group receiving haloperidol in tablets (Spearman's coefficient of correlation = 0.122, $p > 0.05$). This corresponds to data obtained in the research investigating the effects of *CYP3A4*22* polymorphism on the level of haloperidol equilibrium concentration in patients with schizophrenia [16], as well as data obtained in the research investigating the correlation between the *CYP3A* gene subfamily polymorphism and frequency of ADR development in patients with schizophrenia [15]. Lack of statistically significant data of correlative and regressive analyses between C/D index and *CYP2D6* activity index is probably connected with the small sample size, because there is a weak correlation in the group of patients receiving haloperidol in injections (Spearman's coefficient of correlation = −0.208, $p > 0.05$), suggesting that low *CYP2D6* activity correlates with the low biotransformation rates, the haloperidol elimination from the body, and the high level of haloperidol equilibrium concentration in plasma.

## Conclusions

Haloperidol concentration in patients with alcohol use disorder depends on *CYP2D6 1846G>A* genetic polymorphism and not on *CYP3A5 6986A>G*. This distinction must be considered during the administration of haloperidol for such patients in order to decrease the risk of ADR development and increase therapeutic efficacy.

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