METHADONE PLASMA LEVELS IN HEROIN ADDICT PATIENTS DURING SUBSTITUTION THERAPY

ROBERT DANIEL VASILE[1], DANIELA BACONI[1*], CRISTINA HUDIȚĂ[2], MARIA BÂRCĂ[1], CRISTIAN BĂLĂLĂU[3], ANNE-MARIE CIOBANU[1]

[1]University of Medicine and Pharmacy "Carol Davila", Faculty of Pharmacy, Traian Vuia 6, Bucharest, Romania
[2]"Sf. Stelian" Centre for Evaluation and Treatment of Addictions for Young People, Cristian Pascal 25-27, Bucharest, Romania
[3]University of Medicine and Pharmacy "Carol Davila", Faculty of Medicine, Sf. Pantelimon Emergency Hospital, Sos Pantelimon 340 – 342, Bucharest, Romania

*corresponding author: daniela_baconi@yahoo.com

Abstract

Opiates, especially heroin, continue to be the main problem in terms of drug abuse worldwide, as reveal the statistics for the treatment demand. In general, current concerns in the field of diagnosis and treatment of heroin addiction are materialized both in assessing the prevalence and patterns of problem drug use and analytical diagnosis of the consumption, overdoses treatment and establishment the methadone substitution treatment procedures. During the substitution treatment with methadone, it is noticed the need for therapeutic intervention following a well-established treatment plan, correlation, coordinating and adapting the treatment to the specific particularities of each patient.

This study aimed the quantification of methadone in plasma of the patients during methadone substitution treatment to assess the possible relationship with methadone doses. The patients selected for the study were long-term heroin addicts (n = 28) volunteering for substitution therapy with methadone (a medium dose of about 60 mg/day), 20 – 43 years old, monitored at the Centre for Evaluation and Treatment of Addictions for Young People "St. Stelian" Bucharest, Romania. Methadone plasma concentrations were determined by a GC-MS method using diphenylamine as internal standard.

A large distribution of methadone plasma levels was registered, with an average plasma methadone levels of 303.35 ± 140.33 ng/mL (range 123 – 808 ng/mL); the results are consistent with other studies indicating that plasma methadone levels between 150 and 600 ng/mL are required to counter for the craving effect of opioid addicts. In addition, a positive significant correlation between methadone doses and the plasma levels has been shown.

The study suggests that monitoring methadone substitution therapy by analytical methods is useful in assessing compliance and detecting patients with extra-consumption of methadone; also it contributes significantly to avoiding the relapse in drug use and in occasional heroin consumption.

Rezumat

La nivel mondial, opiaceele şi în special heroina, rămân principala problemă în ceea ce priveşte abuzul de droguri, după cum o demonstrează statisticile referitoare la cererea de tratament. În general, preocupările actuale în domeniul diagnosticului şi tratamentului dependenţei de heroină se concentrează asupra evaluării prevalenţei şi a tiparelor de consum de droguri, precum şi asupra diagnosticului analitic al consumului, tratamentului supradozărilor şi stabilirii procedurilor de tratament de substituţie cu metadonă. În timpul tratamentului de substituţie cu metadonă, se remarcă necesitatea unei intervenţii terapeutice conform unui plan de tratament bine stabilit, corelarea, coordonarea şi adaptarea tratamentului la particularităţile specifice fiecărui pacient.

Acest studiu a avut ca scop cuantificarea metadonei din plasma pacienţilor aflaţi în tratament de substituţie cu metadonă pentru a evalua posibila corelaţie cu dozele de metadonă.

Pacienţii selectaţi pentru studiu sunt dependenţi de heroină pe termen lung (n = 28), care au intrat voluntar în tratamentul de substituţie cu metadonă (cu doză medie de 60 mg/zi), cu vârstă cuprinsă între 20 – 43 de ani, monitorizaţi la Centrul de Evaluare şi Tratament al Toxicodependenţelor pentru Tineri "Sfântul Stelian" Bucureşti, România. Nivelele plasmatice de metadonă au fost determinate printr-o metodă GC-MS, folosind difenilamină ca standard intern.

A fost obţinută o distribuţie mare a concentraţiilor plasmatice de metadonă, cu un nivel mediu de 303,35 ± 140,33 ng/mL (interval 123-808 ng/mL); rezultatele sunt în concordanţă cu alte studii care indică faptul că nivele plasmatice de metadonă între 150 şi 600 ng/mL sunt necesare pentru a contracara *craving-ul* dependenţilor de opiacee. În plus, a fost demonstrată o corelaţie pozitivă semnificativă între dozele de metadonă şi nivelele sale plasmatice.

Studiul sugerează că monitorizarea terapiei de substituţie cu metadonă prin metode analitice este utilă pentru evaluarea complianţei la tratament şi detectarea pacienţilor cu extra-consum de metadonă. De asemenea, aceasta contribuie în mod semnificativ la evitarea recăderilor în consumul de droguri şi în consumul ocazional de heroină.

Keywords: heroin addiction, methadone substitution therapy, methadone plasma levels

Introduction

Opiates and opioids are the first on the list of problematic drugs that cause most diseases and drug-related deaths worldwide [18].

Drug addiction treatment is a long-term process that implies multiple interventions until achieving abstinence. Addiction treatment, including diagnosis, medical assistance, and social reintegration of addicts, has the purpose to improve the health status and the quality of life. This can be achieved by diminishing the drug use, the morbidity and mortality due to the addiction, and by facilitating the access to public services and full social reintegration.

The use of methadone in the substitution treatment of opiate addict patients is well-documented [17]. Clinical success in the methadone substitution treatment depends on the methadone dosage suitable to maintain blood levels in the pharmacologically effective range. Many studies highlighted the insufficient methadone dosage as a major cause of the treatment failure. Several evidences suggest a relationship between methadone dose and plasma methadone concentration in addicted patients during substitution therapy.

Individualization of methadone dose is necessary for optimal clinical response, due to the differences in methadone pharmacokinetics.

Methadone is rapidly absorbed after oral administration; it is widely distributed in the tissues, with high levels in the liver, lungs and kidney.

Biotransformation of methadone, mainly catalysed by CYP3A4, results in 2-ethylidiene-1,5-dimethyl-3,5-diphenylpyrrolidine (EDDP - major urinary metabolite), and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline (EMDP). Both metabolites are inactive.

The methadone plasma concentrations evaluated 24 hours after the methadone daily doses have been proposed as a measure of efficacy of the treatment [9]. The aim of this study was to quantify the plasma methadone levels in patients during methadone substitution therapy to determine the correlation with methadone dosage.

Materials and Methods

The study group consisted of 28 heroin addict patients, 20 – 43 years old, voluntarily enrolled in the methadone substitution treatment and monitored at ”Sf. Stelian” Centre for Evaluation and Treatment of Addictions for Young People in Bucharest, Romania. The inclusion criteria in the substitution treatment were the following: addiction diagnostic (ICD-10), analytic diagnostic of opiate abuse, one year of heroin dependence, polydrug abuse, comorbidity (C and B hepatitis). The study was approved by the Ethical Committees of the Centre and was carried out in accordance with the Declaration of Helsinki [19]. The informed consent for the participation in study was obtained from all patients.

The data for socio-demographic, substance use history, methadone treatment courses and clinical characteristics was collected from the medical records of the patients. The following parameters were assessed: age, gender, level of education, marital status, criminal history (detention period), age at the beginning of heroin consumption, years of heroin consumption, other drugs use, comorbidities, urine tests for relapse evaluation.

Venous blood samples were taken before administration of the daily methadone dose, approximately 24 hours after the previous intake of methadone dose, and plasma has been separated. All patients had negative urine analyses for heroine at that time.

A GC-MS method, using diphenylamine as internal standard and a liquid-liquid extraction procedure (with hexane:i-propanol 97:3) was applied for methadone quantification in plasma [2]. The analysis was performed on a Focus DSQ II GC-MS, and the separation was achieved on TR-5MS capillary column (15m×0.25mm I.D., 0.25 μm film thickness). The injection port (split mode) was set to 220°C. The column temperature had been initially held at 150°C for 1 min; later it was increased to 280°C (10°C/min), then held at 280°C for 1 min. The carrier gas was helium at 1 mL/min.

Sample extraction: to 1 mL plasma sample, 250 μL diphenylamine 0.01% in methanol (internal standard), 500 μL of 2 M kalium hydroxide, pH 10, and 4 mL of mixture n-hexane:2-propanol (97:3) were added. The extraction was performed in a shaker for 15 min., then the samples were centrifuged 10 min at 3000 rpm; the organic layer was then transferred into a separate tube and evaporated under nitrogen stream at 40°C. The residue was reconstituted in 100μL of methanol and 1μL of sample was analysed by GC.

Biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT) activity, glucose, cholesterol, and triglycerides were determined using commercial kits (Randox, UK). The haemoglobin concentration and leukocyte count were determined using an automated haematology analyser.

Statistical analysis. Descriptive statistics, frequency distribution, and comparison were performed with SPSS Statistics ver. 21; data for different parameters are presented as the mean ±standard deviation (SD). The correlations between the investigated parameters, using correlation coefficients (Pearson, Spearman and Kendall), were also evaluated. All tests were statistically significant at threshold $p_1 = 0.05$ and $p_2 = 0.01$.

Results and Discussion

The socio-demographic, substance use history, and clinical characteristics

Twenty-eight heroin addicts of 20 – 43 years old, stabilized in the methadone maintenance treatment (MMT) programme at "Sf. Stelian" Centre Bucharest have been selected for the study. It can be noticed the prevalence of the male in the study group, the male/female ratio being 2.50 (Table I). The average age was 32.89 (± 5.32) years (range 20 – 43) for the

whole group. The distribution of the patients according to their age group indicates the most frequent age groups 30 – 35 years (39%), 35 – 40 years (28%), and 25 – 30 years (14%) (Table I, Figure 1).

Table I describes the socio-demographic, substance use history and clinical characteristics of the study group.

Table I.

The socio-demographic, substance use history, and clinical characteristics for the study group

| Total cases	28
Gender distribution	20 men, 8 women
Men/women ratio	2.50
Mean age (years)	32.89 ($\pm$ 5.32) (range 20 – 43)
Frequent age groups	30 – 35 years (39%); 35 – 40 years (28%); 25 – 30 years (14%)
Duration of heroin use (years)	8.62 ($\pm$4.96) (range 1 –17)
Other drug use	NPS (New Psychoactive Substances) 28.57% (8/28); Marijuana 10.71% (3/28); Benzodiazepines 35.71% (10/28); Cocaine 7.14% (2/28); Ketamine 7.14% (2/28) 10.71% declare daily consumption of alcohol
Level of education	1-4 grades 14.29%; 5-8 grades 14.29%; 9-12 grades 32.14%; higher education 3.57%; unschooled 7.14%; undeclared 28.57%
Marital status	Married 17.86%; unmarried 82.14%; married, with children 14.29%; married, no children 3.57%; unmarried, with children 32.14%; unmarried, no children 50.00%
Detention history	Yes 14.29%; no 85.71%
History of screening urine tests	Positive for Opiates 14.29%; positive for Benzodiazepines 14.29%
Comorbidities – positive HCV (number of patients)	27 (96.43%)
Other comorbidities	HBV 14.29% (4/28); HIV 7.14% (2/28); tuberculosis 7.14% (2/28); hepatitis A 3.57% (1/28); Moderate mental retardation 3.57% (1/28); Bipolar affective disorder 3.57% (1/28)
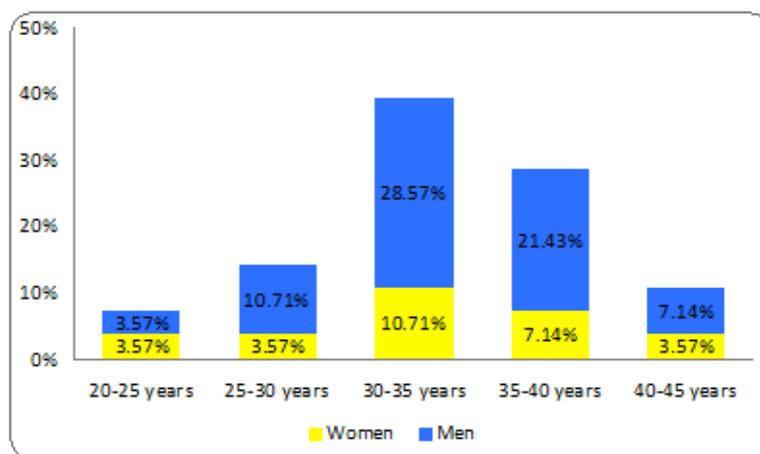
Figure 1.
The distribution of the patients according to gender and their age group

The majority of the patients are long-term heroin addicts, the duration of heroin use being approximately 9 years (Table I). Most of the patients are polydrug users, new psychoactive substances (NPS, improperly named „ethnobotanicals") and benzodiazepines being the most frequent drugs used along with heroin (Table I, Figure 2). These results are consistent with the official data published in the national report on drugs, indicating that about half of the people who were admitted for substitution treatment in 2013 for illegal drug use and NPS were opiate users. Of these beneficiaries, about half have had previous episodes of treatment with methadone or other opiates [15].

Negative urine analyses for heroine at the time of methadone quantification have been shown, but the screening urine tests during the methadone treatment revealed occasionally positive test for opiates (14.29% cases) and for benzodiazepines (14.29% cases) (Table I).

The majority of patients had secondary education and high school education level (46.43%); only one patient has higher education. The majority of them were unmarried (82.14%), but 32.14% of these unmarried patients, had children. Few patients (14.29%) had detention history (Table I).

The most frequent comorbidity was the hepatitis C virus (HCV) infection, diagnosed in over 95% of patients (27/28 patients). Many studies reported higher incidence rates of HCV infection in patients who were under methadone substitution treatment [13]. In addition, there were 4 cases with hepatitis B virus (HBV) infection and 2 cases with human immunodeficiency virus (HIV) infection. The data are consistent with the prevalence of drug related infectious diseases showed in the national report on the drug situation. Thus, in 2012 significant increases of HBV and HCV (exceeding European average), and alarming increases of HIV (exceeding European average) cases have been shown [16].

The patients were stabilized in methadone maintenance therapy at an average daily dose of 59.10±11.71 mg (range 30 – 85 mg). It can be noticed that for methadone maintenance the effective daily doses generally reported of 60 – 80 mg, but they vary from 30 – 120 mg. Doses higher than 120 mg/day may be necessary in some patients, with a rapid methadone metabolism, while few patients can be maintained effectively on doses less than 60mg/day [7]. In our study, 20 patients (40.8%) were given oral methadone doses between 50 – 65 mg, 3 patients less than 50 mg, and 5 patients were given doses higher than 70 mg. Significant statistical differences between these subgroups were found in respect to both methadone doses and plasma concentrations of methadone.
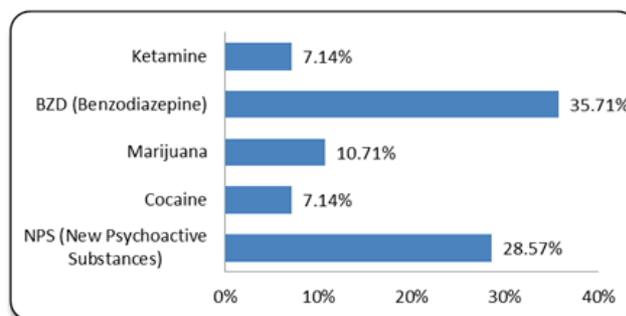
Figure 2.
The distribution of the patients according to other drugs use

A profile of the patient enrolled in the methadone substitution therapy reveals a man who is 30 - 40 years old, unmarried, with secondary or high school education and who has a long time history of heroin consumption (about nine years), as single drug or as polidrug abuse. The consumption of heroin in combination, mainly by men, has been also shown as the predominant model of drug abuse, based on the emergency room admission in Romania [11].

Quantitative analysis. Methadone plasma levels and the relationship with methadone doses

Methadone plasma levels were determined by a slightly modified published GC-MS method. Diphenylamine has been used as internal standard, and liquid-liquid extraction technique has been applied. Methadone has been determined in SIM (selected ion monitoring) mode (at m/z 72); the internal standard has been also evaluated in SIM mode (at m/z 163). Working in the full scan mode, the analytical diagnostic has no revealed relapse in heroin or other drugs consumption. A typical chromatogram for the quantification of methadone in a patient sample is presented in Figure 3. Methadone was identified at a retention time of 11.29 min., and the internal standard at a retention time of 6.28 min.
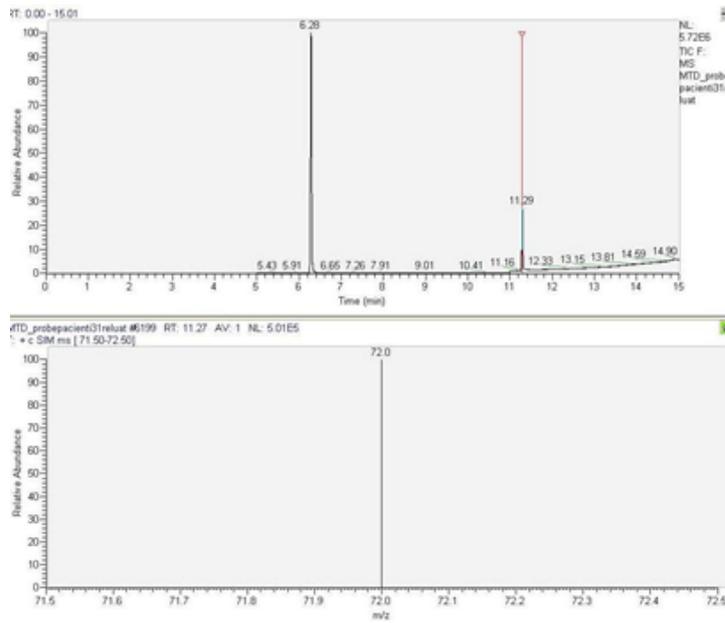
Figure 3.
Typical chromatogram for quantification of methadone in plasma patients

A large distribution of methadone plasma levels was shown, in the range of 123 – 808 ng/mL (average 303.35±140.33 ng/mL) (Figure 4). The average methadone dose was 59.10 mg ±11.71 (range 30 – 85 mg). Methadone doses in our study ranged between 30 mg to 85 mg with an average methadone dose of 59.51±11.71 mg. Mean plasma methadone concentration was 303.35±140.33 ng/mL; data analysis indicates significant inter-individual variability; some individual data could suggest a possible methadone extra-consumption.

By now there are findings indicating that during methadone maintenance treatment, there are often significant fluctuations in plasma concentrations from day to day. In addition, a decrease in plasma methadone concentration as dispositional tolerance develops, has been shown [8].
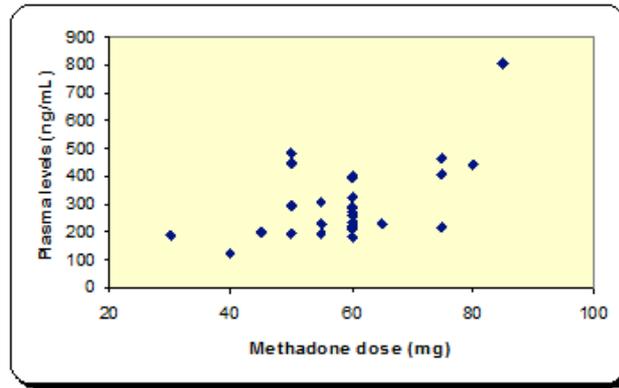
Figure 4.
Relationship between methadone dose and plasma concentrations

The normal unimodal distribution curve has been shown in Figure 5, for methadone dosage, which signifies a normal distribution.
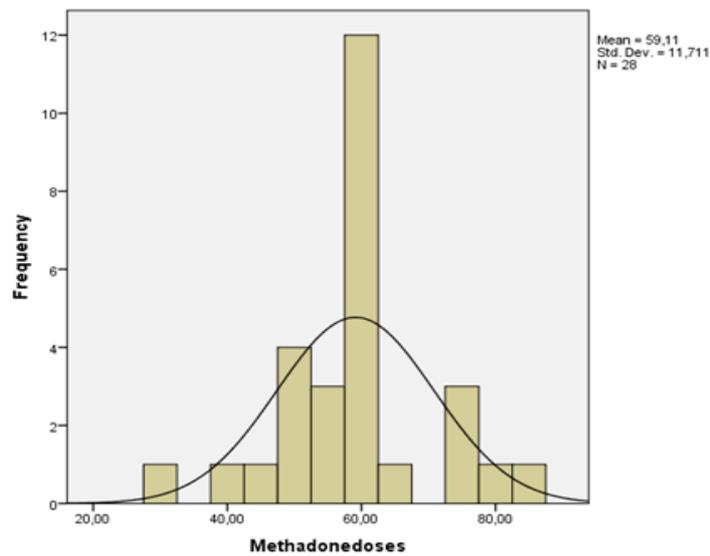

Figure 5.
Histogram for the distribution of methadone dose

The results on plasma methadone levels are in accordance with literature data which indicate plasma methadone levels between 150 and 600 ng/mL to counter for the craving effect of opioid addicts. However, based on the methadone concentration in plasma, inter-individual variability exists in response to methadone. In a review regarding the metabolism,

pharmacokinetics and interactions of methadone, Ferrari and al showed that the pharmacokinetics of methadone varies greatly from person to person and the administration of the same dose could result in considerably different concentrations [6]. Recent data indicate that HCV infection influences the plasma concentrations of methadone; therefore, it has been shown that the average total plasma methadone and methadone R enantiomer concentrations were higher in the HCV antibody-positive than in the HCV antibody-negative patients [13]. Studies on methadone pharmacokinetics indicate a large inter-individual variability in the activity of CYP enzymes implied in methadone biotransformation as responsible for the variability in methadone kinetics and plasma levels [3].

The distribution of methadone plasma levels *versus* methadone doses showed statistic significant positive correlations (Pearson $r = 0.567$, $p_2 = 0.002$); weak positive Kendall and Spearman correlations have been found ($r = 0.309$, $p_1 = 0.031$ and $r = 0.395$, $p_1 = 0.037$).

The possible correlations between methadone doses, methadone plasma concentrations and different biochemical and haematological parameters (descriptive statistics is shown in Table II) have been also evaluated. No correlations have been found between methadone plasma levels and biochemical and haematological parameters. AST was significantly correlated with ALT and GGT (Pearson $r = 0.934$, $p_2 = 0.0001$, and $r = 0.489$, $p_1 = 0.029$).

Table II.

Descriptive statistics for biochemical, haematological and analytical parameters

Parameter	Minimum	Maximum	Mean	Std. Deviation
Methadone dose (mg)	30.00	85.00	59.10	11.71
Methadone plasma levels (ng/mL)	123.00	808.00	303.35	140.33
AST (IU/L)	11.00	225.90	54.85	52.92
ALT (IU/L)	4.70	262.30	70.90	72.52
GGT (IU/L)	4.00	370.00	66.70	92.71
Glucose (mg/dL)	63.00	144.00	89.15	19.59
Cholesterol (mg/dL)	95.00	202.00	143.45	29.56
Triglycerides (mg/dL)	55.00	287.00	129.25	55.65
Haemoglobine (g/dL)	11.70	14.70	13.32	1.10
Leucocyte count	4600.00	6180.00	3320.01	1416.15

Results from our study showed a positive, significant correlation between methadone doses with its plasma levels. These results are consistent with other studies indicating that the plasma methadone levels are fairly correlated with methadone doses. The correlation coefficients reported in different studies were between weak and strong: $r = 0.20$ ($p < 0.05$) [1]; $r = 0.55$, ($p < 0.01$) [5]; $r = 0.82$, ($p < 0.001$) [12]. Although the correlation

between methadone doses and plasma levels was highlighted in many studies, it is suggested that a true significant linear association is very difficult, as the plasma level depends on different factors. Specific cytochrome P-450 gene polymorphisms might determine slow metabolizers, rapid metabolizers, and ultra-rapid metabolizers [4]. It has been suggested that the correlation is higher when the patient has a complete abstinence from illicit drug [9]. In addition, there are findings suggesting that the linear relationship between methadone dose and its serum concentration in lower doses (below 80 mg) cannot be extrapolated to higher doses [10].

Conclusions

The results of our study are in agreement with the current view of a fairly relationship between methadone dose and plasma methadone concentrations in patients with methadone maintenance treatment.

The study suggests therapeutic drug monitoring during methadone substitution therapy as a useful tool to assess the treatment compliance and to detect the patients with extra-methadone consumption. In addition, evaluating plasma methadone concentrations could contribute to a personalized methadone therapy approach, by establishing the optimum dose of methadone and reducing the unwanted side effects.

References

1. Charlier C., Dessalles M.C., Plomteux G., Methadone maintenance treatment: is it possible to adapt the daily doses to metabolic activity of the patient? *Ther. Drug Monit.*, 2001; 23(1): 1-3.
2. Ciobanu A.M., Baconi D.L., Barca M., Negrei C., Cristescu I., Balalau D., GC–MS method for methadone quantification in plasma. *Toxicol. Lett.*, 2009; 189S: S62.
3. Crettol S., Déglon J.J., Besson J., Croquette-Krokar M., Hämmig R., Gothuey I., Monnat M., Eap C.B., ABCB1 and cytochrome P450 genotypes and phenotypes: Influence on methadone plasma levels and response to treatment. *Clin. Pharmacol. Ther.*, 2006; 80(6): 668-681.
4. Eap C.B., Bertschy G., Baumann P., Finkbeiner T., Gastpar M., Scherbaum N., Interindividual Variability of Methadone Enantiomer Blood Levels to Dose Ratios. *Arch. Gen. Psychiatry*, 1998; 55(1): 89-90.
5. Eap C.B., Déglon J.J., Baumann P., Pharmacokinetics and Pharmacogenetics of Methadone: Clinical Relevance. *Heroin Add. & Rel. Clin. Probl.*, 1999; 1(1): 19-34.
6. Ferrari A., Rosario Coccia C.P., Bertolini A., Sternieri E., Methadone - metabolism, pharmacokinetics and interactions. *Pharmacol. Research*, 2004; 50: 551-559.

7. Maremmani I., Metteo P., Pier P.P., Basics on Addiction. A Training Package for Medical Practitioners or Psychiatrists who treat Opioid Dependence. *Heroin Add. & Rel. Clin. Probl*., 2011; 13(2): 5-40.
8. Moffat A.C., Osselton M.D., Widdop B. (eds) – Clarke's analysis of drugs and poisons, fourth edition, Pharmaceutical Press, London, Chicago, 2011: 1649.
9. Mohamad N., Mohd Salehuddin R., Ghazali B., Abu Bakar N.H., Musa N., Abdulkarim Ibrahim M., Adnan L.H.M., Rashidi A., Ismail R., Plasma Methadone Level Monitoring in Methadone Maintenance Therapy: A Personalised Methadone Therapy. New Insights into Toxicity and Drug Testing, chapter 10, InTech, 2013.
10. Okruhlica L., Valentova J., Devinsky F., Formakova S., Klempova D., Methadone serum concentration and its relationship to methadone dose revisited. *Heroin Add. & Rel. Clin. Probl*., 2005; 7(4): 49-58.
11. Vasile R.D., Baconi D., Bârcă M., Ciobanu A.M., Bălălău C., Emergency room admission in polydrug consumers: one year survey in Romania. *Farmacia*, 2013, 61(3): 551-557.
12. Wolff K., Sanderson M., Hay A.W.M., Raistrick D., Methadone concentrations in plasma and their relationship to drug dosage. *Comment in: Clin. Chem*., 1991; 38(3): 438-439.
13. Wu S.L., Wang S.C., Tsou H.H., Kuo H.W., Ho I.K., Hepatitis C Virus Infection Influences the S-Methadone Metabolite Plasma Concentration. *PLoS ONE*, 2013; 8(7): e69310.
14. *** - Drugs and Human Performance Fact Sheets. National Highway Traffic Safety Administration (www.nhtsa.gov)
15. *** - 2013 National Report Romania (www.ana.gov)
16. *** - 2012 National Report Romania (www.ana.gov)
17. *** - Principles of Drug Addiction Treatment – A Research -Based Guide. www.nida.gov
18. *** - World Drug Report 2014. (www.unodc.org)
19. www.wma.net/e/ethicsunit/helsinki.htm

_____

Manuscript received: April 2014