Riga Stradins University

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Parkinsonism in Ephedrone Users

Doctoral Thesis Summary

Speciality – Neurology

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1. Table of abbreviations

ADL – Schwab and England Activities of Daily Living Scale

AIDS – acquired immunodeficiency syndrome

AM – anterior midbrain

CaNaDTPA – diethylenetriamine pentaacetic acid

CDC – Centers for Disease Control and Prevention

CNS – central nervous system

GP – globus pallidus

CT – computed tomography

DBS – deep brain stimulation

FLAIR – fluid attenuated inversion recovery

HCV – hepatitis C virus

HIV – human immunodeficiency virus

HYS – Hoehn and Yahr Staging of Parkinson's disease

ICD-10 – International Classification of Diseases (10th edition)

ICL – Infectology Center of Latvia

IVD – intravenous drug

$KMnO_4$ – potassium permanganate

L-dopa – levodopa

Max – arithmetic mean of the maximal values

Min – arithmetic mean of the minimum values

MMSE – Mini-Mental State Examination

Mn – manganese

MRI – magnetic resonance imaging

PET – positron emission tomography

PPN – pedunculopontine nucleus

PD – Parkinson's disease

pts – patients

RECUH – Riga East Clinical University Hospital

RelatExpr – relative expressiveness

RSU – Riga Stradins University

SD – standard deviation

SI – substantia innominata

SN – nucleus subthalamicus

STN – substantia nigra

sy – symptom

T1 – spin-lattice relaxation time

T2 – spin-spin relaxation time

UPDRS – Unified Parkinson's Disease Rating Scale

2. Introduction

Parkinsonism is an akinetic type of motor disorder syndrome caused by injury of the extrapyramidal system which clinically manifests as loss of movement (oligo- and bradykinesia) and it is accompanied in different stages of the disease by tremor, muscle rigidity, balance, vegetative and psychical disorders.

Extrapyramidal disorder is a relevant group of neurological syndromes and the most common cause for it in physician's daily life is Parkinson's disease (PD). PD is a degenerative central nervous system (CNS) disorder, predominantly seen in persons over 50 years of age and being the cause of serious functional restrictions and economical effects.

However damage of the extrapyramidal system is caused not only by degenerative CNS disorders bet also a range of different pathological conditions triggered by CNS injury, cerebrovascular diseases, neuroinfections, various toxic substances and use of certain groups of medication.

This material overviews extrapyramidal system damage syndrome in drug addicts, which occurred after homemade ephedrine or pseudoephedrine based narcotic substance – ephedrone (methcathinone) intravenous injection. Clinically this syndrome considerably differs from motor disorder seen in PD. Even though initially it was associated with neurological complications caused by HIV/AIDS due to fact that first participants of the study were HIV-positive, however, recognition of the syndrome in HIV-negative patients and subsequent thorough examination suggested that this syndrome has to be classified as parkinsonism, caused by toxic effects of manganese (Mn). Moreover the clinically depicted extrapyramidal motor disorder in ephedrone users resembles the syndrome, which is described in workers of manganese mining and manufacturing industry.

3. Research novelty

Up to now the consequences of systematic use of narcotic substances belonging to stimulant-group were mainly related to direct and indirect drug effects, for the most part on CNS, liver and immune system.[1-3] Patients portray a characteristic astenisation and development of underlying psychic diseases, toxic and infectious damage of the liver as well as hepatitis C infection and HIV/AIDS. There are less frequent reports concerning cardiovascular disorders like heart failure or stroke. Although it is well known that stimulant-group narcotic substances have a toxic impact on CNS dopamine receptors[4-8], permanent motor disorder in drug abusers are

reported on rather rare occasions. Up to year 2003, when the syndrome research in Latvia was initiated, no reports on motor disorder in ephedrone users had been published and up to this moment only separate reports, focusing mainly on motor disorder cases in particular drug abusers from the former USSR territory, have emerged[9-14], however owing to the freedom of migration and internet resources containing easily accessible information on drug production process, the first reports on several cases of motor disorder in ephedrone abusers in Turkey emerged in year 2009 including patients, who had not left the territory of the former USSR.[15]

Thus the key novelty of this study concerns depicted motor disorder research among intravenous drug addict (IVD) population, which has not been depicted yet, resulting in knowledge which would be ground for understanding extrapyramidal syndrome clinical specific features and pathogenetic mechanisms, thus building a base for developing methods that would potentially correct the existing disorder.

Taking into consideration that motor disorder caused by ephedrone use have a considerable effect on patients activities of daily life and capacity for work, the importance of the issue of the study is increased not only by social and medical interest concerning consequences caused by use of narcotic substances, but also the direct and indirect expenses for their treatment and disability, which is determined by the enormous number of drug abusers.[16-19]

4. Aim of the study, objectives of the study and hypotheses

4.1. Aim of the study

To research specific features, pathogenesis and course of motor disorder syndrome in intravenous drug – ephedrone (methcathinone) users.

4.2. Objectives of the study

1. Perform motor disorder syndrome clinical and neurological assessment in ephedrone users in a structural and detailed manner.
2. Assess association of motor disorder syndrome with paraclinic examination data: blood manganese concentration and changes in magnetic resonance imaging of the brain.
3. Assess comorbidity in ephedrone users with motor disorder syndrome.

4. Assess the course and prognosis of motor disorder syndrome in ephedrone users during follow-up evaluation of the patients.
5. Work out practical guidance for parkinsonism, which has developed in ephedrone users, early diagnostics, treatment and evaluation of prognosis.

4.3. Study hypotheses

1. Movement disorder in ephedrone abusers is related to toxic extrapyramidal system damage caused by use of homemade methcathinone (ephedrone), which has been contaminated with manganese.
2. The development of extrapyramidal syndrome is related to hypermanganemia and subsequent deposition of manganese in tissue, i.e., CNS basal nuclei, which is detected by magnetic resonance imaging as pathological intensity signal in corresponding brain structures on T1-weighted images.
3. Comorbidity in ephedrone users with motor disorder syndrome is for the most part related to intravenous drug abuse determined diseases – HIV and hepatitis C infection.
4. Further ephedrone abuse may worsen intensity of motor disorder syndrome. Despite the discontinuation of ephedrone use and/or chelation therapy implementation and manganese level depletion or even success in achieving normal manganese levels, the clinical condition of patients does not improve.

4.4. Structure of doctoral thesis and personal contribution of author

Doctoral thesis is written in Latvian language. It has a classical structure, consisting of introduction, literature review, description of materials and methods used, results, discussion, conclusions and list of references – a total of 142 pages. Appendix consists of analytical illustrative material of 16 figures, 15 tables, 16 charts, as well as four samples of assessment scales used.

Reference list comprises of 279 publication titles.

The author has independently carried out clinical evaluation of all the patients enrolled in the study, filled in specifically developed and particularly study adjusted questionnaire schemes and single-handedly performed processing of acquired results and interpretation of clinical data.

4.5. Ethical aspects

Prior to enrollment in the study patients were acquainted with the written information form concerning the study and signed informed consent in order to take part in the study. Positive approval of the study was provided by the Ethics Committee of Riga Stradins University.

5. Materials and methods

5.1. General description and patients enrolled

In cooperation with several RSU clinics and University of Oxford scientists, a prospective and descriptive study was carried out in order to establish specific characteristics of motor disorder in intravenous drug users. During the time period of year 2003 till year 2009, 28 intravenous ephedrone users were enrolled in the study, including 23 men and 5 women of 23 to 47 years of age, who had developed characteristic motor disorder syndrome with gait, speech and handwriting impairment.

Patients were acquainted with a written information form about the study and signed informed consent in order to take part in the study. Positive approval of the study was provided by the Ethics Committee of Riga Stradins University.

12 patients (43 %) who admitted having used ephedrone during the previous year were identified as “active” users, but 16 patients (57 %) were identified as „former” users. They reported cessation of ephedrone use for at least a year.

Patient examination took place in close coordination between several RSU clinics: clinical examination and laboratory tests were carried out at ICL, brain MRI imaging was performed in RECUH „Gailezers”, and blood manganese levels spectrometric analysis – at Institute of Occupational Safety and Environmental health Laboratory of Hygiene and occupational diseases.

5.2. Clinical examination

5.2.1. Neurologic examination

5.2.1.1. General neurologic examination

Patient clinical neurologic examination was performed by study author. According to general standards inquiry concerning patients’ current complaints and motor disorder, drug use and history of other diseases was obtained and recorded systematically and in a descriptive manner. Patient

neurological examination was performed according to generally accepted scheme[20]. All the disorders according to assessor's experience were graded in four classes: 0 – normal, 1 – slightly marked, 2 – moderately marked and 3 – marked disorder. Moderately marked and marked disorders were characterized as clinically significant, but slightly marked – as clinically insignificant impairments.

5.2.1.2. Clinical examination of parkinsonism

Apart from general patient neurological examination the following characteristic symptoms of parkinsonism: akinesia, rigidity, tremor at rest, postural instability and gait, speech and handwriting impairment were evaluated and graded in four classes: 0 – normal, 1 – slightly marked, 2 – moderately marked and 3 – marked disturbance.

5.2.1.3. Parkinsonism rating scales

In order to systemize the severity of disturbances established, broad range PD objective rating scales were used:

5.2.1.3.1. *UPDRS*

Unified Parkinson's Disease Rating Scale (UPDRS).[21] UPDRS consists of 31 sections, describing main PD symptoms and difficulties of activities of daily life in a well structured manner. Every section consists of 5 possible answers (0 to 4), where 0 identifies normal condition, but 4 - maximal possible disorder. Total value of score ranges form 0 to 176, the greater the score, the more marked the degree of disturbance.

5.2.1.3.2. *HYS*

Hoehn and Yahr scale (HYS).[22] HYS is a six section scale describing the stage of the disease in general. It consists of six stages from 0 to 5, where 0 indicates condition when no signs of disease are traceable.

5.2.1.3.3. *ADL*

Schwab and England Activities of Daily Living Scale (ADL).[23] This is an eleven section scale describing the level of patient's independency concerning activities of daily living in percentage scale ranging from 100 % to 0 %.

5.2.2. Mental state evaluation

In order to examine patients cognitive function impairment, Mini-mental State Examination (MMSE) scale is used.[24] It comprises of 11 subdivisions, which are combined in five sections. Maximal score possible is 30. Score below 24 indicates cognitive impairment; score below 20 indicates dementia or needs differentiation from acute consciousness disorder, schizophrenia or marked depression.[25]

5.2.3. Follow-up assessment

Of 28 study participants re-examination was possible in 18 (64 %) patients.

Follow-up assessment included systematic general neurological and parkinsonism evaluation according to scheme previously described, i.e., including parkinsonism rating scales and MMSE. For a wholesome perception of the development of symptoms patient examination video data – recorded during the first visit and following examinations – were compared.

5.3. Motor disorder co-morbidity identification

Information concerning co-morbidity was attained by structural evaluation of patient's questionnaire (history) data, primary medical documentation (case history and out-patient care data), clinical examination, magnetic resonance and laboratory test results from both – first visit examination as well as follow-up evaluation.

Diseases were divided in five groups applying International Classification of Diseases, ICD-10.[26] According to this gradation only diseases evaluated as clinically significant were classified, i.e., their effects caused or had caused long-term influence on patient's health, and/or activities of daily life or could be potentially associated with motor disorder. Clinically insignificant conditions and diseases were not recorded.

5.4. Laboratory testing

5.4.1. Blood and serum parameters

Performing clinical analysis of patient blood and serum samples, the following parameters were evaluated: anti-HIV and anti-HCV antibodies, CD4 counts, copper serum levels, ceruloplasmin, alanine transaminase (ALAT), alkaline phosphatase, albumin and prothrombin. All the parameters were measured at ICL certificated laboratory.

5.4.2. Blood manganese level detection

Patient venous blood samples were collected in tubes containing lithium-heparinate and immediately frozen and stored in -18^0C temperature till analysis. Blood manganese concentration was measured at RSU Institute of Occupational Safety and Environmental health Laboratory of Hygiene and occupational diseases.

All the measurements were performed by single specialist. The recognized upper limit of normal for manganese was 209 nmol/l (198±11).[27]

5.5. Magnetic resonance imaging of the brain

Brain MRI was performed using GE 1.0 T Signa Horizon LX High-speed system applying head reel according to standard evaluation protocol with slice thickness of 5 mm and interval of 1,5 mm. Images were analyzed by one particular neuroradiologist, who was aware of the clinical syndrome, but uninformed about whether images were from „active" or „former" ephedrone users.

In addition to general patient MRI description, pathological signal hyperintensity on T1- weighted sequence was analyzed separately as well and graded in four categories: 0 -normal, 1 – mildly hyperintense (agrees with signal intensity in capsula interna), 2 – moderately hyperintense (signal intensity between 1 and 3) and 3 – severely hyperintense (signal intensity agrees with fat tissue signal intensity).

6. Statistic methodology

Descriptive statistical methods were applied for describing the groups. Measures of central tendency: arithmetic means, arithmetic mean of the maximum and minimum values were calculated depending on the variable. Dispersion results were measured to fall within one standard deviation (SD). Parameter relative expressiveness was calculated dividing sum of parameter values with parameter frequency (number of cases).

Prior to selection of method for data analysis it was determined whether the test statistic follows a normal distribution, applying measures of asymmetry (skewness) and excess (kurtosis). If either of these parameters denied a normal distribution, non-parametric Wilcoxon Signed-Rank test was applied. If both parameters indicated normal distribution, two-sample t-test was applied to determine the equality of two population means, but for single variable determination among patients upon repeated evaluation paired t-test was applied. Z, T and p values were rounded to three decimal places. Results were interpreted as statistically significant when $p \leq 0{,}05$.

All of the calculations were performed using computerized NCSS (Number Cruncher Statistical System) 2001 and PASS (Power Analysis and Sample Size) 2002 version.

7. Results

7.1. Study population general description and individual description of patients

15 patient were identified at Infectology Center of Latvia (ICL) Departments of HIV/AIDS as all of the patients were HIV-positive and initially this syndrome was related to the neurological findings of HIV/AIDS. 13 patients were identified afterwards; six of them were HIV-negative. Two of the HIV-negative patients were forwarded to ICL general profile neurological departments, one searched for help at ICL concerning HCV infection, but two more patients were informed about the opportunity to take part in the study by previously enrolled patients.

7.1.1. Timing of ephedrone use and onset of first neurologic symptoms

12 patients (43 %) enrolled in the study admitted having used ephedrone during the period of last year and were referred to as „active" users, but 16 patients (57 %) denied having used ephedrone during the last year and they were referred to as „former" users.

Summarizing case history it was found that symptoms developed on average 6,4 ± 5,3 (Max 20; Min 0) years after initiation of ephedrone use.

In majority (22/28; 79 %) of patients the onset of morbidity set in with gait disturbances, experiencing particular difficulties when moving backwards or getting out of car, however five (18 %) patients noted voice tone alterations as the first symptom accompanied by speech impairment. One patient (Nr. 27) with slightly marked disease symptoms reported finger movement slowness and episodic cramps affecting shin and shoulder muscles. Usually disorder was of rapid progression and in the period of few months to a year 27 (96 %) patients developed both gait and speech impairment of different grades.

7.1.2. Disability at the time of diagnosis

At the point of setting diagnosis 13 (46 %) patients had marked motor disorder – falling at least once a day –including a patient (Nr. 3) who moved in a wheelchair. One of the patients (Nr. 22) was able to cycle, however noted significant difficulties in getting on and off the bicycle. Five patients (18 %) experienced speech impairment and in one of them (Nr. 5) impairment was severely marked allowing him to communicate exclusively by pointing out letters in the alphabet. Four patients (14 %) noted temporary swallowing difficulties, however compared to speech impairment difficulties to swallow were slightly marked and did not cause significant difficulties when swallowing food and/or drink.

None of the participants reported a decline in intellectual function.

7.2. Results of clinical examination

7.2.1. Neurologic examination

7.2.1.1. General neurologic profile changes

During general neurological examination pathological alterations were reported by 27 patients (96 %), however only in 13 patients (46 %) they were classified as moderately marked or marked. In rest of the patients findings were clinically insignificant.

Visual fields, eye-ball and voluntary facial movements were normal in all of the patients. Voluntary and reflex palatal movements were normal. No palmomental reflexes were present in any patient.

None of the patients presented with upper or lower extremity paresis. No clinically significant disturbance completing Romberg test or cerebellar dysmetria was found in any patient.

Upper extremity tendon reflexes were not clinically significantly altered in any patient, whereas alteration of tendon reflexes of the lower extremity were the most frequently found pathological symptom – present in 18 patients (64 %): eight patients (29 %) presented with hyporeflexia, including clinically significant in five cases, all of these patients shared a history of alcohol abuse, but ten patients (36 %) presented with hyperreflexia, including five clinically significant cases and associated with foot dystonia. Babinski reflex was not found in any patient.

Various sensation impairments were present in 14 patients (50 %), however only six of them were evaluated as clinically significant: “sock” dysesthesia was found in three patients, all of them had a history of alcohol abuse, but clinically significant vibration sensation diminishing was found in six patients, four of them had a history of alcohol abuse. Clinically significant neurological examination findings are given in Chart 1.

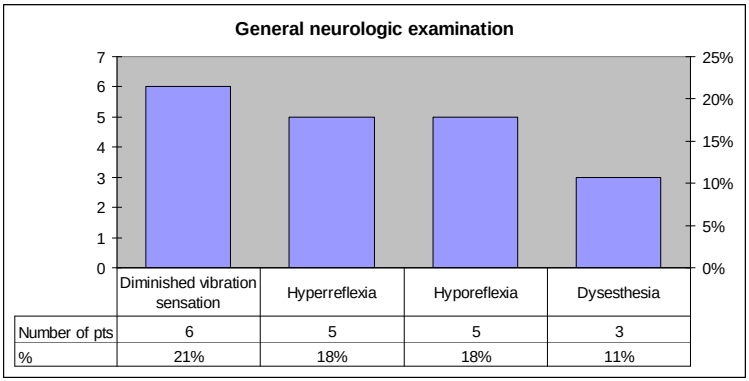


Chart 1. Clinically significant neurologic examination findings

7.2.1.2. Parkinsonism clinical profile

7.2.1.2.1. *Akinesia*

Akinesia predominantly moderately (16/28, 57 %) or slightly marked (11/28, 39 %), was the most common parkinsonism symptom present.

25 patients (89 %) had a characteristic hypomimic facial expression and generally slowed movement and speech.

All of the patients had a slightly slowed finger movements (thumb – pointing finger opposition), bet the major symptom was decrease in amplitude. More severe slowness of movements and decrease of amplitude was present when modification of test was performed and patient was asked to complete the test opposing thumb to other fingers as well (thumb – pointer, thumb – middle finger, thumb – ring finger, thumb – pinky and repeating the sequence in reverse order).

20 patients (71 %) presented with alternating hand movements with characteristic slight slowness of movements and a tendency of involving shoulder joint in abduction – adduction movements.

7.2.1.2.2. *Rigidity*

None of the patients demonstrated rigidity and associated “cogwheel” phenomenon.

7.2.1.2.3. *Tremor at rest*

No tremor at rest was present in any patient, however three patients (11 %) presented with slight postural tremor.

7.2.1.2.4. *Postural impairment and gait*

Postural and gait impairment was most strikingly present with the extrapyramidal disorder. Gait impairment was present in 27 patients (96 %) and in most cases (24/28, 86 %) impairment was marked or moderately marked and was the major cause for difficulties concerning activities of daily living.

Only one patient (Nr. 27) had retained normal ability to walk, but three more patients (11 %) had slight gait impairment.

22 patients (79 %) presented with a forward tilt of the trunk from the hips on standing but did not have a hunched shoulder posture characteristic with PD.

23 patients (82 %) held their arms slightly abducted from the sides when walking, with reduction or loss of arm swinging. 21 patients (75 %) presented with foot dystonia. When moving forward patients tended to walk on the balls of the feet seemingly falling forward into the next stride, and sometimes with inversion of the ankle. Patient gait presented with propulsions, i.e., when walking stride had a tendency to shorten with every step, if there was no support, falling was inevitable. When attempting to turn, 24 patients (86 %) took a corrective step sideways to preserve balance. 22 patients (79 %) sat down from a standing position by deliberately placing themselves in front of the seat and gently flexing their knees until they fell backward. The most severe disturbance was evident with moving backwards. 13 patients (46 %) had lost the ability to move backwards, but nine (32 %) fell backwards after the first few steps. Only five (18 %) patients were able to move backwards, using short and uncertain steps. Patient using a wheelchair (Nr. 3) had difficulties moving in reverse, although he was able to forward the wheelchair without significant difficulties.

7.2.1.2.5. *Speech impairment*

26 (93 %) patients developed speech impairment of different severity. Six patients (21 %) experienced insignificant speech disturbance which was mainly present as diminished speech volume.

14 patients (50 %) presented with moderate severity speech disturbance, but six patients (21 %) demonstrated significant speech disorder. In one of them (Nr. 5) disturbance had resulted in muteness and patient was only able to communicate by pointing out letters in the alphabet.

Speech impairment did not agree with typical pseudobulbar or cerebellar dysarthria, it however took a form of ability of delivering individual sounds, but patients were unable to link them together in a word.

Characteristic was the patients' inability of enunciating a sequence of consonants, for example, „p", „b", „m" or „l", „g", „k", even when each individual consonant sounded accurately.

7.2.1.2.6. *Handwriting impairment*

Handwriting impairments were evident in 23 patients (82 %). Six patients (21 %) had slight handwriting disturbance which mainly presented as slow writing and/or micrographia. 11 patients (39 %) experienced moderately marked writing disturbance, when slow handwriting and moderate micrographia was accompanied by frequent use of block letters or written letters each drawn separately.

When writing, patients presented typical progressive micrographia, i.e., the following letter of each word became tinier than the previous. In six patients (21 %) handwriting disturbance severity had caused loss of ability to write or their handwriting was practically illegible.

The following Chart summarizes most characteristic extrapyramidal disturbances in study patients by frequency and relative severity (Chart nr 2).

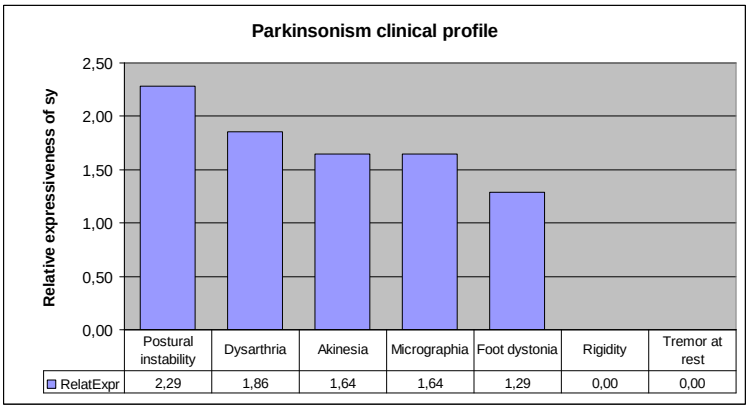


Chart 2. Relative expressiveness of extrapyramidal disturbances

7.2.1.3. Parkinsonism rating scale results

7.2.1.3.1. *UPDRS*

Syndrome severity score according to UPDRS was on average 38,8 ± 12,9 (Min 5, Max 59) which, taking into account that none of the patients

presented with disorder according to maximum score in the rigidity and rest tremor domain (UPDRS section Nr. 16, 20, 22, maximal score ranging form 0 to 44), is interpreted as moderately marked or marked movement disorder syndrome. In only four patients (14 %) UPDRS score was less than 24, corresponding to slightly marked disturbance.

UPDRS I domain "Mentation, behaviour and mood" demonstrated average score of 3,2 ± 2,1 (Min 0, Max 9) and it had the least effect on total UPDRS evaluation. The most frequent presentation was that of slight to moderate severity disturbance in domains measuring depressive mood and loss of motivation/initiation.

UPDRS II domain "Activities of daily living" demonstrated average score of 14,5 ± 4,9 (Min 1, Max 24). Although the absolute score of this domain was below III domain results, taking into consideration the relatively lowest point, this is the domain which represents the severity of disturbances. The most frequently evident impairment was related to domain measuring speech, handwriting, gait changes and falling.

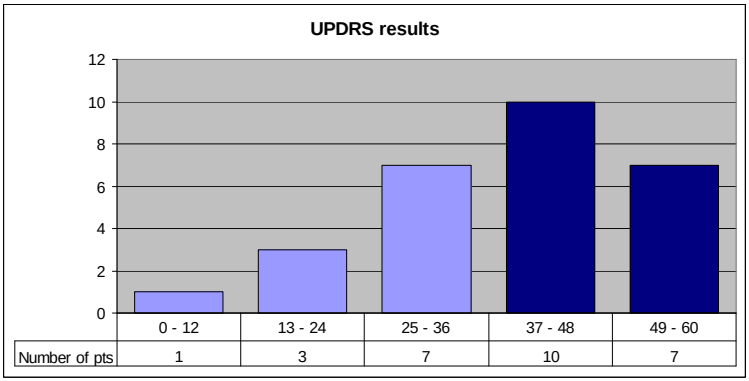


Chart 3. Patients' distribution regarding UPDRS total score

UPDRS III domain "Movement assessment" results made up more than half of the total UPDRS score and was on average 21,0 ± 8,6 (Min 2, Max 34). The most frequently evident impairments were related to sections measuring speech and agility disorder, facial expression, posture, gait and postural stability. Since patients did not present with disturbance in sections measuring rigidity and rest tremor, this information was the least helpful for reflecting the severity of disorders.

7.2.1.3.2. *HYS*

According to HYS, syndrome severity was on average 3,4 ± 0,8 (Min 2, Max 5), which is generally characterized as moderately to severe disease stage. Most of the patients (23/28, 82 %) presented with disorder corresponding to HYS stage III and IV, indicating bilateral disturbance with moderate or significant effect on activities of daily living. Three patients (11 %) presented with disturbances of severity corresponding to stage II – activities of daily living difficulties were insignificant, but two patients (7 %) experienced significant disturbance, being unable to walk without assistance (HYS stage V).

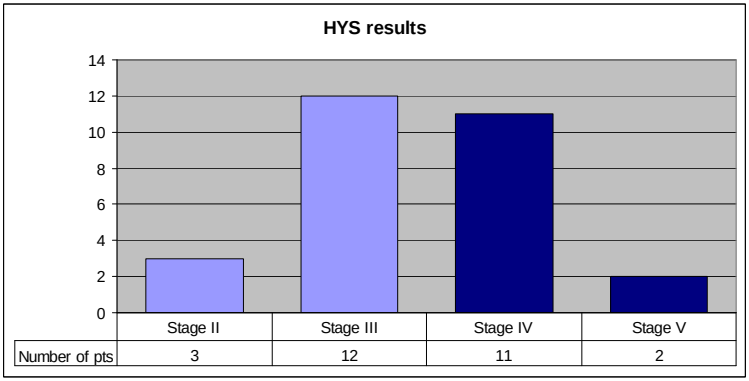


Chart 4. Patients' distribution regarding HYS

7.2.1.3.3. *ADL*

According to ADL, difficulties of daily living score was on average 59 ± 18 % (Max 90; Min 30), which suggests that the overall self care-level involves difficulties concerning all the daily activities when up to half of daily chores need to be assisted. 11 patients (39 %) were independent (ADL ≥70 %), six patients (21 %) presented with some dependency and need for slight assistance ADL =60 %), but eight patients (29 %) with ADL score of ≤40 % were practically totally dependent mainly due to gait disturbance and postural instability.

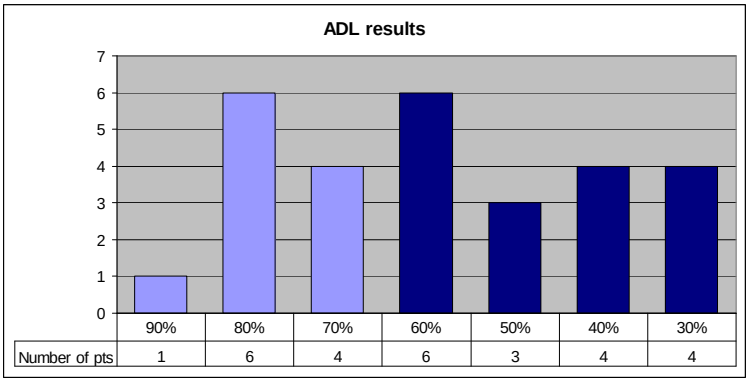


Chart 5. Patients' distribution regarding ADL

7.2.2. Mental state assessment results

None of the patients enrolled in the study presented with cognitive function worsening. 22 patients were assessed applying MMSE. Only one of them presented with cognitive deficit symptoms. According to MMSE, cognitive function score was on average 27,5 ± 2,6 (Min 18, Max 30) and indicating normal performance. One of the patients (Nr.15) had a MMSE score of 18 resulting form poor educational background (incomplete elementary education, significant reading and writing difficulties) and diminished intellectual level since childhood. No patient demonstrated disturbance in categories „Orientation", „Registration", „Recall". A few of the patients presented with inaccuracy when performing serial sevens from category „Attention and calculation", but patients with writing disturbance demonstrated inaccuracy while writing a sentence and drawing figures shown from category „Language". Although six remainder patients did not undergo MMSE, no significant disturbance in these patients was found during UPDRS evaluation in sections „Intellectual impairment" and "Thought disorder".

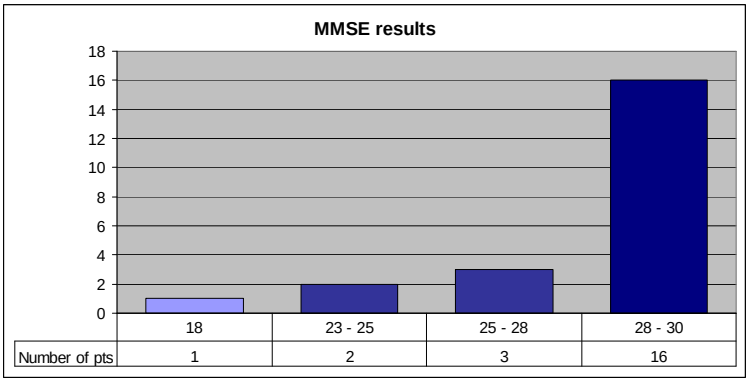


Chart 6. Patients' distribution regarding MMSE

7.2.3. Patients' condition changes throughout the study

Of 28 study patients repeated examination was applicable to 18 patients (64 %). Follow-up evaluation was possible for those patients, who searched for help at ICL repeatedly, usually based on need for HIV infection or hepatitis control or treatment. Thus patients were examined during different periods of time following initial evaluation (on average 31,5 ± 14,8 months, Min 3, Max 59) and paraclinic examination data reevaluation was not possible in all of the patients. Repeated brain MRI was performed in nine patients (9/18, 50 %), but Mn blood concentration was analyzed in 17 patients (17/18, 94 %). Examination finding results are given in respective chapter.

Of nine patients recorded as „active" ephedrone users during the initial evaluation four patients (4/9, 44 %) reported cessation of ephedrone use, but five patients admitted further ephedrone use. Nine patients having denied ephedrone use for at least a year during the first visit denied having resumed drug abuse upon repeated examination.

7.2.3.1. General neurologic profile at follow-up

Overall, significant changes in patients' general neurological status were not detected (Z=0,765, p=0,444). None of the patients presented with changes of the visual field, eyeball movements and voluntary facial movements - matching initial examination findings. Voluntary and reflex palatal movements were normal in all patients. One patient (Nr. 18) had developed a periferic type paresis of the right lower extremity associated with traumatic *n.peroneus* damage, no other patient presented with paresis of the extremities. There were no notable palmomental reflexes in any patient.

One patient (1/18, 6 %) presented with Babinski reflex, bet no other pathologic reflexes were detected in the lower extremity in any patient.

Clinically significant disturbance performing Romberg test or cerebellar dysmetria was not detected in any patient. Upper extremity tendon reflexes were not significantly altered in any of the patients, whereas abnormal tendon reflexes of the lower extremity – similar to previous findings – were the most commonly detected pathologic symptoms in study patients. Even though they were demonstrated by 13 patients (13/18, 72 %), severity of disturbance had not significantly changed compared to initial evaluation (patellar reflex T=-0,369, p=0,717; Achilles reflex T=0,000, p=1,000).

Various types of sensation impairment were detected in 10 patients (10/18, 56 %). Even though one of the patients demonstrated serious worsening in superficial sensation (Nr. 18, traumatic *n.peroneus* damage), and one more patient had a significant worsening of vibration sensation (Nr. 14, alcoholic polyneuropathy), compared with initial evaluation, overall, significant abnormalities were not observed (pain sensation Z=1,005, p=0,315; vibration sensation Z=0,709, p=0,478).

7.2.3.2. Parkinsonism symptoms at follow-up

Despite the lack of reported considerable change in motor disorder, in most of the patients (12/18, 67 %) worsening of parkinsonism symptoms by one scoring point was estimated in at least one group of symptoms and this alteration was evaluated to be statistically credible (Z=3,153, p=0,002).

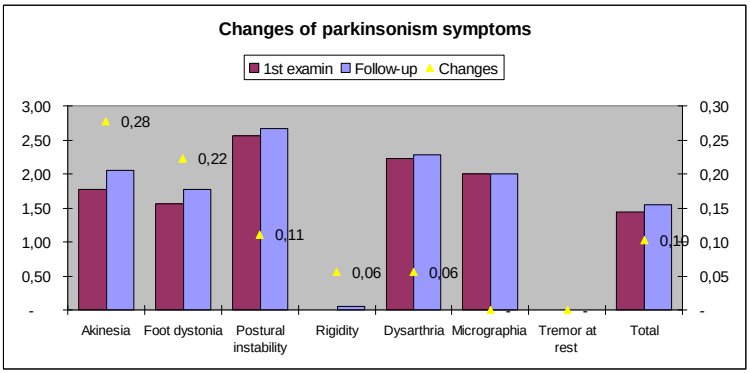


Chart 7. Parkinsonism symptoms at follow-up. Significant progression of akinesia and foot dystonia was observed

Presentation of parkinsonism symptom worsening was more common (4/5, 80 %) in patients who continued ephedrone use after initial evaluation. The

most common finding was increase of akinesia (Z=2,236, p=0,025) and foot dystonia (Z=2,000, p=0,046), less common – worsening of postural instability (T=-1,000, p=0,331) and speech impairment (Z=0,577, p=0,564), but no worsening of handwriting was notable in any patient. One of the patients (Nr. 5) demonstrated traces of rigidity, which had not been detected during initial assessment in any patient. Likewise, during first evaluation, rest tremor was not found in any patient.

7.2.3.3. Parkinsonism rating scale results at follow-up

UPDRS, HYS and ADL scale results were repeatedly evaluated in all 18 patients where follow-up examination was applicable.

7.2.3.3.1. *UPDRS results at follow-up*

Severity score of the syndrome according to repeated UPDRS was on average 43,9 ± 10,3 (Min 29, Max 60), which was on average by 0,9 ± 3,7 more per patient compared to initial evaluation result and despite failing to reach statistically credible result (T=-1,097, p=0,28), it still supports tendency of syndrome coarse worsening. In patients who carried on ephedrone use following initial evaluation, UPDRS score had a more dramatic increase – on average by 2,0 ± 3,8 (Z=0,817, p=0,414).

The greatest effect on increase of score had changes in UPDRS III domain "Motor examination" – score per patient had an average increase of 1,1 ± 2,7, but in patients who continued ephedrone use the increase was by 2,6 ± 2,8 (Z=1,697, p=0,090, $p_{unilateral}$=0,049) (Chart 8).

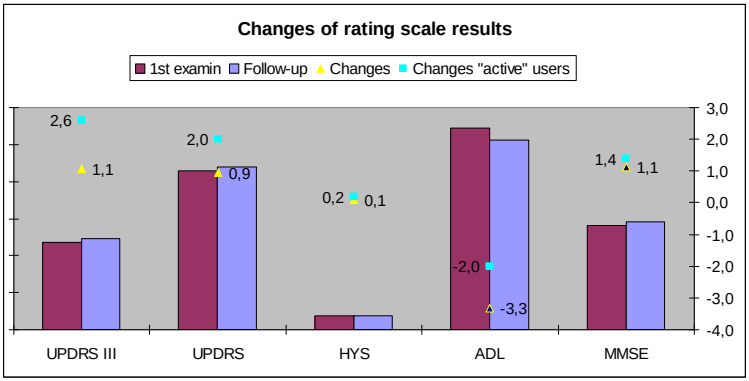


Chart 8. Rating scales at follow up. UPDRS and HYS showed tendency of syndrome coarse worsening but results did not reach statistical significance

7.2.3.3.2. *HYS results at follow-up*

Severity of parkinsonism according to repeated HYS was on average 3,8 ± 0,7 (Min 3, Max 5), which exceeded primary evaluation results by an average of 0,1 ± 0,5 and indicated increase in parkinsonism severity (T=-1,000, p=0,331). Patients who continued ephedrone use following initial evaluation had a greater increase in HYS score – on average by 0,2 ± 0,4 (Z=1,000, p=0,317) (Chart 8).

7.2.3.3.3. *ADL scale results at follow-up*

According to repeated ADL difficulties of activities of daily living score was on average 51,1 ± 16,4 % (Max 80; Min 30), which unlike UPDRS and HYS score indicated a decrease in severity of daily activity difficulties by average of 3,3 ± 8,4 % (T=1,683, p=0,111) and was interpreted as improvement of patient adaption to completion of activities of daily living. Even though in patients who continued using ephedrone after first evaluation there was an increase in scores for activity of daily living, it was less marked and according to ADL was increased on average by 2,0 ± 8,4 % (Z=0,577, p=0,564) (Chart 8).

7.2.3.4. Mental state evaluation at follow-up

Upon repeated evaluation MMSE was performed in all 18 patients, but comparative evaluation was only possible in 11 patients as seven patients did not undergo MMSE during initial examination. According to MMSE cognitive function score was on average 29,1 ± 1,5 (Min 25, Max 30), which indicates normal performance. Comparing the results, repeated score exceeded the result of initial examination on average by 1,1± 0,9 and did not indicate worsening of the cognitive function (Z=2,743, p=0,006). Similar tendency was detected in patients who continued ephedrone use following initial evaluation – MMSE score increased by an average of 1,4 ± 0,5 (Z=1,890, p=0,059, $p_{unilateral}$=0,029) (Chart 8).

7.3. Syndrome co-morbidity

All of the patients enrolled in the study beside ephedrone addiction and motor disturbance syndrome suffered from other co-morbidities as well.

7.3.1. Infectious diseases

All patients were positive for hepatitis C virus (HCV), and had acquired it by IVD use, however none of them demonstrated either clinical or paraclinical signs of liver cirrhosis.

Of 28 patients enrolled in the study 22 (79 %) were HIV-positive. According to CDC classification[28] ten of the patients (45 % of HIV-positive

patients) had AIDS, but eight patients (36 %) were asymptomatic HIV-positive patients with normal CD4 counts. The most common HIV-associated symptomatic conditions were diagnosed to be lung tuberculosis (6/22, 27 %), criptococcal meningitis (3/22, 14 %) and specific pneumonia (3/22, 14 %).

Some of the patients also had a history of pyelonephritis (1/28, 4 %), lambliasis (1/28, 4 %) and hepatitis A virus infection (1/28, 4 %).

7.3.2. Addictions

Beside ephedrone addiction eight patients (8/28, 29 %) were recognized to have other substance addiction. Seven patients (7/28, 25 %) were noted to suffer from alcohol overconsumption and one (1/28, 4 %) – from opiate addiction.

7.3.3. Psychic diseases, neurologic diseases and head injury

Five patients (5/28, 18 %) had a history of concussion of the brain. Five patients (5/28, 18 %) were found to have alcoholic polyneuropathy and one (1/28, 4 %) was diagnosed with *n. peroneus* traumatic damage.

Persistent depressive condition was recorded in one patient (1/28, 4 %).

7.3.4. Internal diseases

History of clinically significant non-infectious internal diseases was recorded in nine patients (9/28, 32 %). Five patients (5/28, 18 %) had a history of septic endocarditis in association with IVD use and four patients (4/28, 14 %) – duodenal ulcer.

One patient had a history of ileus with subsequent surgical treatment given in section „Other diseases". The most significant comorbidities are given in Chart 9.

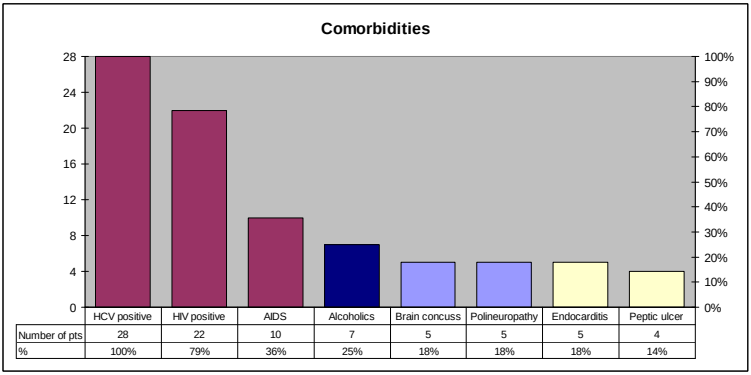


Chart 9. The most significant co-morbidities. Other diseases were rare (< 5 %)

7.4. Laboratory test results

7.4.1. Blood and serum test results

Of 28 patients enrolled in the study 22 (79 %) were positive for anti-HIV antibodies. In three of them (14 %) CD4 cell count per mm^3 was lower than 200, in nine (41 %) – it was between 200 and 499, and for the rest 10 (45 %) – 500 or more.

All patients were positive for hepatitis C virus (HCV) being infected via IVD use.

Hepatic alanine transaminase (ALAT) serum levels were detected in 27 patients and were mildly elevated (< 135 U/l, reference interval 0 – 45) in 14 (52 %) patients and moderately elevated (229 U/l) – in one patient. Alkaline phosphatase levels were slightly elevated (Max 439 U/l, reference interval 64 – 306) in two patients (2/27, 7 %).

Serum albumin level was mildly lowered (Min 24,9 g/l, reference interval 38 – 51) in two patients and measured in 27 (7 %) patients.

Prothrombin level was measured in 21 patients and was normal.

Ceruloplazmine serum levels were normal in all the patients, but copper concentration was mildly elevated in six patients (Max 31,5 μmol/l, reference 24,4).

7.4.2. Blood manganese concentration

Elevated Mn blood concentration in patients was a particularly characteristic feature – it was detected in 26 patients (93 %) and was on average 555,7 ± 466,0 nmol/l (Min 114,1, Max 2101,7), which is a significant rise above the reference interval (≤ 209 nmol/l).

Identifying Mn blood concentration, a significant difference was established (T=3,042, p=0,005) between „active" ephedrone users (12/28, 43 %) and patients who reported cessation of drug use at least a year ago (16/28, 57 %).

Eleven (11/12, 92 %) of „active" ephedrone users' Mn blood concentrations exceeded reference interval and were on average 835,5 ± 624,7 nmol/l (Min 200,9, Max 2101,7).

Of 16 „former" ephedrone users 15 patients (94 %) had elevated Mn blood concentrations but it was comparatively more than two times lower than in „active" users and was on average 345,9 ± 147,4 nmol/l (Min 114,1, Max 726,7).

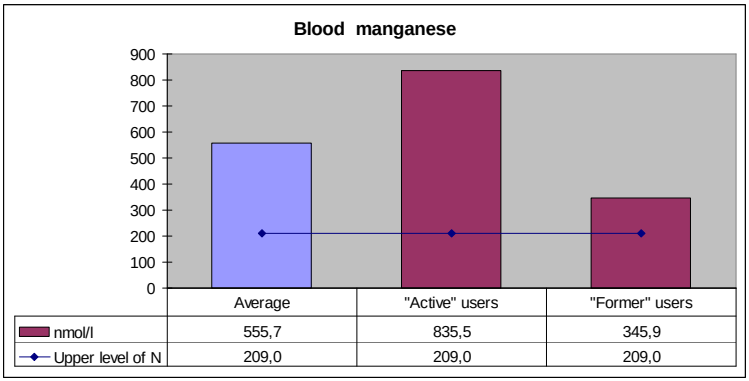


Chart 10. Mn blood concentrations. Significant difference was established between „active" users and patients who reported cessation of drug use for a year at least

7.4.2.1. Changes of blood Mn concentration at follow-up

Of 18 patients who were examined repeatedly Mn blood concentration was determined in 17 patients (94 %). Follow-up evaluation revealed significant difference in Mn blood concentration between patients who were active to continue ephedrone use and those who had stopped drug use (T=2,683, p=0,017).

In patients who denied having used ephedrone after the first examination, Mn blood concentrations had significantly decreased (on average by 545,1 ± 637,9 nmol/l) and reached a level of 388,4 ± 95,4 nmol/l (Min 311,0, Max 520,6), whereas in patients who continued ephedrone use, Mn blood concentration continued to rise (on average by 301,9 ± 663,8 nmol/l) and reached a level of 1259,3 ± 1300,7 nmol/l (Min 241,0, Max 3289,3). Of patients examined repeatedly and who had already been recorded as „former" ephedrone users during the first evaluation, none had resumed drug use and Mn blood concentration in these patients continued to reduce (on average by 127,1 ± 136,8 nmol/l) reaching a level of 242,4 ± 79,5 nmol/l (Min 115,0, Max 319,0), which was only slightly above the reference interval.

7.5. Brain MRI results

MRI imaging changes in study patients were particularly characteristic – they were detected in majority of patients (24/28, 86 %) and manifested as bilateral and symmetric signal hyperintensity (pathologic) in various structures of basal ganglia on T1-weighted sequence images. Only four

patients who had not been using ephedrone for two years and more demonstrated no changes in either basal ganglia or other structures and their MRI images were evaluated as normal. In the rest of the patients no other significant focal changes were detected beside hyperintensity in various structures of basal ganglia on T1-weighted sequence images, as well as other imaging sequences (T2- weighted sequence, FLAIR).

The most common and marked characteristic changes were observed in the globus pallidus (GP) – hyperintensity of different degrees was observed in 24 patients including severe hyperintensity in six patients, moderate – in seven, and mildly marked hyperintensity in 11 patients (Figure 1 A, C, D).

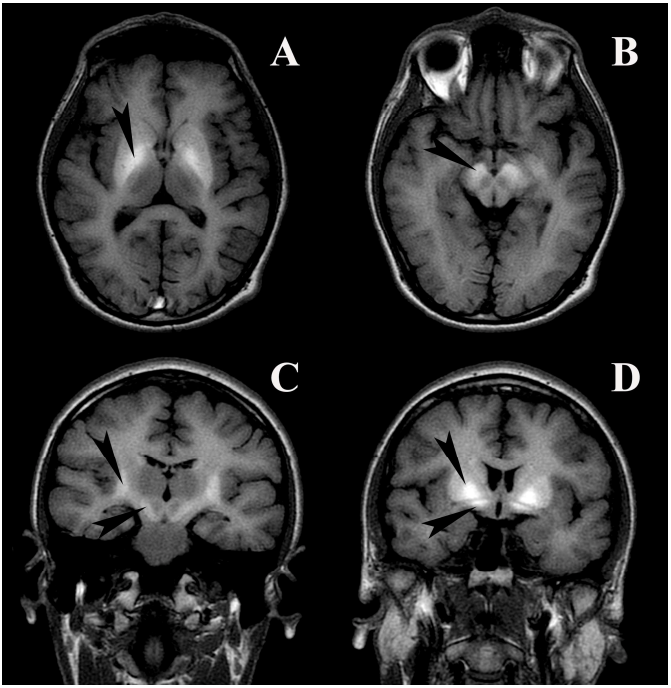


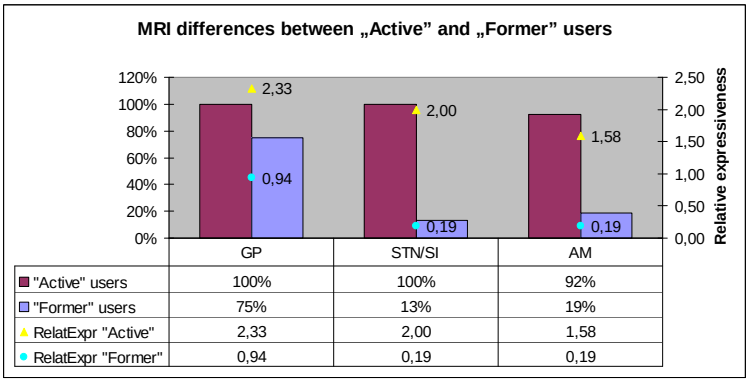
Figure 1. T1-weighted MRIs from Patient 7, an "Active" ephedrone user. Bilateral and symmetric signal hyperintensity in strctures of *globus pallidus* (GP), *substantia nigra* (STN)/*substantia innominata* (SI) and *anterior midbrain* (AM)

Although 16 patients presented not only with signal hyperintensity in GP structures but in the substantia nigra (STN)/substantia innominata (SI) and/or anterior midbrain (AM) areas as well, it was not found to be more intense than hyperintensity of the GP.

Fourteen patients demonstrated concurrent hyperintensity of GP structures and STN/SI – which was described as severe in three patients, moderate – in seven patients and mildly marked – in four patients (Figure 1 B, C, D). Twelve patients (12/14, 86 %) demonstrated a concurrent hyperintensity of the AM.

14 patients had hyperintensity of both GP and AM structures, which was relatively less severe than the intensity of STN/SI structures and in three patients it was described as severe, in two – moderate, but in nine patients – mildly marked (Figure 1 B). 12 of the previously described patents (12/14, 86 %) demonstrated a concurrent hyperintensity of STN/SI structures.

There was a significant difference between brain MRI results in patients who were „active" ephedrone users and patients who reported cessation of ephedrone use at least a year ago (T=9,216, p=0,000). Changes of GP were observed in all the „active" ephedrone users (T=5,181, p=0,000). Compared to „former" users, changes were more severe and in all patients associated with hyperintensity of STN/SI (T=7,491, p=0,000) and 92 % (11/12, 92 %) and AM structures (T=5,100, p=0,000). Whereas of 16 „former" ephedrone users hyperintensity of GP structures were observed in 12 patients (12/16, 75 %), it was less marked and only in two patients (2/16, 13 %) associated with changes of STN/SI and AM structures, but in one patient – exclusively in AM.



	GP	STN/SI	AM
"Active" users	100%	100%	92%
"Former" users	75%	13%	19%
RelatExpr "Active"	2,33	2,00	1,58
RelatExpr "Former"	0,94	0,19	0,19

Chart 11. Comparision of T1-weighted MRI between „Active" and „Former" users. "Active" showed significantly more severe hyperintensity in all locations

7.5.1. Changes of MRI at follow-up

Of 18 patients examined repeatedly, data from follow-up MRI of the brain could be evaluated in nine patients (9/18, 50 %) on average of 33,2 ± 19,1

months after initial examination. Of four patients who had been recorded as „former" ephedrone users and also in the further coarse of the study had refrained from drug use, two patients demonstrated decrease of the hyperintensity on T1-weighted sequence of GP, but hyperintensity of STN/SI and AM structures had disappeared. One patient (Nr. 3) did not demonstrate any changes from the very beginning of evaluation but another patient (Nr. 17) still retained a mild hyperintensity of GP. Of five patients initially recorded as „active" ephedrone users, two patients (Nr. 10 and 13) continued drug use and their MRI showed similar pathologic hyperintensity to the initial findings of GP, as well as STN/SI and AM. Three more patients denied having continued ephedrone use and in two of them (Nr. 7 and 22) pathologic signal intensity of all the basal ganglia structures had disappeared, but in the third patient (Nr. 1) intensity had become more severe in GP as well as STN/SI and AM.

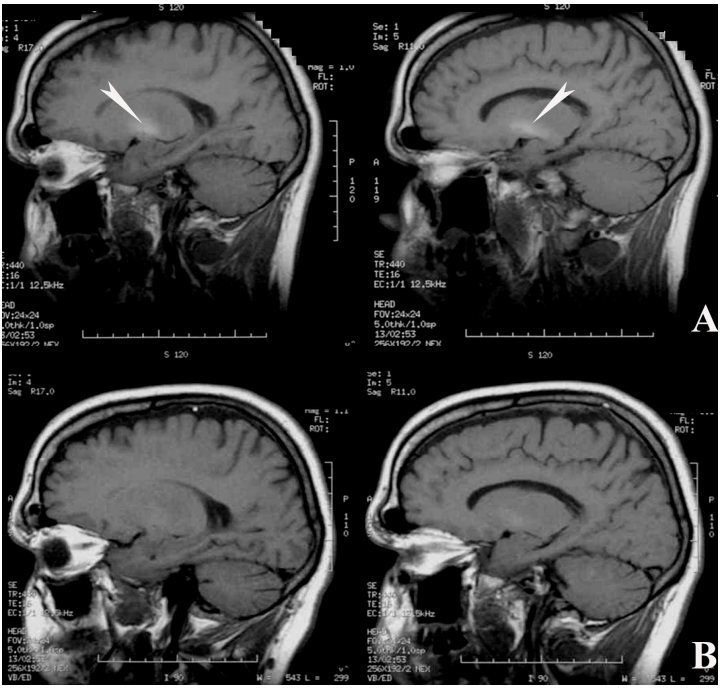


Figure 2. Sequential T1-weighted MRIs from Patient 6 as an active methcathinone user, and 14 months after cessation of use. Globus pallidus hyperintensity faded after cessation of ephedrone

In 10 patients there was a possibility of comparing MRI images with examination results from other clinical facilities in order to identify the

etiology of motor disorder prior to investigation within this study. Similar to follow-up observation results, in those four patients who had continued ephedrone use, the initially demonstrated hyperintensity had increased or there was no considerable change. Whereas of six patients who had refrained from ephedrone use, in five patients the initially determined hyperintensity of the basal ganglia either diminished or disappeared over time (Figure 2) and only in one patient (Nr. 17), who continued active ephedrone abuse for a short time after the first outpatient care MRI evaluation, hyperintensity of GP and STN/SI had increased.

8. Discussion

8.1. Study syndrome clinical features and alternative diagnoses

Key clinical manifestations indicate the syndrome described in 28 IVD ephedrone users to be related to extrapyramidal system damage. The possible toxic effects of Mn were supported by similar motor disorder described in workers of the manganese mining and manufacturing industry.[29-32] Patients having received parenteral nutrition high in Mn[33-35] as well as patients with hepatic cirrhosis causing Mn excretion disturbance presented with motor disorder.[36-39] Therefore taking into consideration that all patients enrolled in the study were users of homemade methcathinone (ephedrone), who in the course of drug preparation utilized KMnO4, the basic theory forwarded was neurotoxicity which accounts for the course of the syndrome, clinically manifesting as parkinsonism caused by the toxic effects of Mn. Furthermore this was approved by the fact that none of the alternative diagnoses agreeing with Mn neurotoxicity provided persuasive explanation for motor disorder described.

Prior to onset of symptoms patients had been using ephedrone for a wide variation of time. Thus in patient Nr. 28 gait disturbance set in already 4 months after the first ephedrone injection, whereas patient Nr. 10 presented with gait disorder 20 years after regular ephedrone abuse. The dramatic difference in duration of ephedrone use before manifestation of the first symptoms is considered to be related to different severity of ephedrone use, diverse chemical contamination of the solution as well as delayed identification of the syndrome.

The major clinical manifestations of the motor disorder syndrome described in this study were postural instability and gait disturbances which were the principal factors causing difficulties of activities of daily living. The next

most common group of symptoms was speech impairment, akinesia, micrographia and foot dystonia.

Motor disorder syndrome in ephedrone users was markedly uniform unlike syndrome found in workers of the manganese mining and manufacturing industry which usually has a less severe course and is clinically more diverse.[40-42] At the same time disease was lacking symptoms characteristic with idiopathic Parkinson's disease (PD). Unlike typical PD patients, but like welders and workers in the manganese metal industry suffering from occupational disease, study patients did not present with tremor at rest, they were noted to have a characteristic walk on the balls of the feet and had a particular difficulty to walk and experienced frequent falling with backward movement.

Symmetry of parkinsonism symptoms, specific type of speech impairment and unresponsiveness to treatment with L-dopa were also the features that marked the difference between the study syndrome and PD.[29, 43, 44]

Although progressive supranuclear palsy (PSP, Steele–Richardson–Olszewski syndrome) is characterized by postural instability, dysarthria and dystonia as well as inefficiency of L-dopa medications, study patients were not detected to suffer from ophthalmoplegia and axial rigidity – cardinal manifestations of PSP.[45, 46] No MRI changes typical with PSP were detected either.[47, 48]

Copper and ceruloplasmin serum measurements were normal in study patients and no changes characteristic of Wilson's disease were traceable on MRI.[49, 50]

Since the study syndrome initially prior to its identification in HIV-negative patients was related to manifestations of HIV/AIDS and all patients enrolled in the study were HCV-positive it was essential to analyze the causes and connections accounting for the high incidence of blood-borne infections among IVD users.

Extrapyramidal motor disorder with parkinsonism is common in HIV-positive patients, usually at the late stages of the disease when CD4 counts are markedly lowered and is related with AIDS dementia complex[51], CNS opportunistic infection[52, 53], or less commonly as an isolated HIV manifestation.[54] Even though 22 patients, including 15 HIV-positive patients and nine of who according to CDC classification[28] had AIDS, eight patients were asymptomatic HIV-positive patients with normal CD4 counts, no dementia or focal MRI and CT changes were detected in any of them. After the distinctive motor disorder syndrome was identified in HIV-negative patients as well, the primary role of HIV infection in the

progression of syndrome was ruled out. Of 28 patients described six (21 %) are HIV-negative.

Despite the fact that all of the patients enrolled in the study were HCV-positive, it has not been established that it is the cause of CNS damage with extrapyramidal disorder. Liver failure and cirrhosis which increases possible Mn neurotoxicity of respiratory genesis in case of occupational disease[55] and might be associated with extrapyramidal type of motor disorder[36, 56], were not found in study patients either. None of the patients enrolled in the study presented with clinically significant jaundice or portal hypertension symptoms. 14 patients (50 %) underwent ultrasonographic abdominal examination, which provided no evidence concerning portal hypertension; liver biopsy was performed in two more patients in order to attain a more exact view of the course of hepatitis, revealing chronic active hepatitis with no signs of liver cirrhosis.

Similar to general population of addicted patients ephedrone users were found to be alcohol abusers and presented with concurrent alcohol polyneuropathy.[57]

Medication administered in eight patients proved to be inefficient. As there have been several cases of successful chelation therapy in patients with occupation determined Mn parkinsonism[58], administration of CaNaDTPA was initiated in two „active" ephedrone users presenting with symptom progression (Nr. 10 and 24), according to instructions for use.[59] Due to poor compliance patient Nr. 10 received three doses of medication in a period of six days (one dose contained 1g of CaNaDTPA). Despite considerable decrease in Mn blood concentrations (by 28 %) no clinical improvement or worsening of the syndrome was observed. Patient Nr. 24 was administered with ten medication doses in a period of fifteen days. There was an insignificant decrease in blood Mn concentration (by 0,5 %) and no clinical improvement or worsening was detected in this patient either.

Prior to patient enrolment in the study several of them were diagnosed with PD and appropriate therapy consisting of various medications was administered. Two patients received therapy based on L-dopa medications which had no effect in the form of improving the course of the disease and was discontinued. Therapy cancellation had no effect on patients' condition - neither positive, nor negative. Inefficiency of L-dopa as a form of study syndrome treatment comprises with research results concerning its inefficiency in case of Mn parkinsonism in occupational patients.[43] Six more patients were treated with amantadine causing no effect - neither

positive, nor negative and medication was therefore cancelled. Amantadine cancellation triggered no changes in the course of the syndrome.

Inefficiency of conservative therapy demanded solutions for improving the course of clinical syndrome by means of surgical intervention methods. Complying with the conclusion of our working group[60], the method of choice is considered to be deep brain stimulation (DBS) of the brain stem pedunculopontine nucleus (PPN). DBS of PPN has been presenting with positive results in diminishing akinesia, postural instability and frequency of falling in parkinsonism patients.[61, 62]

8.2. Hypermanganemia and MRI changes

Patients described in this study especially those who continued active use of drugs demonstrated elevated Mn blood concentration and hyperintensity of basal ganglia structures – mainly GP – on MRI T1-weighted sequence.

Since manganese is a paramagnet it increases signal intensity on T1-weighted frequency images in those brain structures where metal has been deposited; these primary are GP and STN pars reticulata.[63, 64] These are characteristic changes of Mn neurotoxicity[40, 41, 44] and were detected in all the „active" ephedrone users. Similar changes but of less intensity were observed in patients who had refrained from ephedrone use for a year or more. This complies with MRI findings in manganese metal industry workers after cancellation of exposition to the toxic agent.[41] Hyperintensity of the basal ganglia has been reported in patients with liver failure and cirrhosis as well[56, 65] and these conditions can be associated with extrapyramidal type of motor disorder.[36] Parkinsonism symptoms in these patients faded after successful liver transplantation.[39, 66]

Unlike with Parkinson's disease, patients who developed motor disorder during the period of ephedrone use presented with no significant nigrostrial tract changes on PET examination.[67] This approves an undamaged dopaminergic transmission in nigrostrial system and agrees with the observation in manganese occupational patients and experimental animals were motor disorder was induced by intravenous MnCl injection.[68, 69]

At the same time there was not always an association found between hyperintensity of the basal ganglia structure and clinical manifestation severity. Despite fading or even disappearance of intensity of characteristic MRI changes in patients who had refrained from ephedrone use, their neurological condition showed no improvement; on the contrary – the majority of patients undergoing follow-up evaluation demonstrated a slight increase in the severity of parkinsonism, especially akinesia and dystonia.

This indicates permanent nerve cell damage even when Mn concentration has become normal and it is possible that hypermanganemia acts as trigger mechanism for persistent neurodegenerative process which may progress parallel to resuming normal Mn tissue concentration.[70, 71]

Functional study MRI results also suggest permanent cell damage.[72] 10 patients undergoing MRI DTI (diffusion tensor imaging) compared to 15 patients from the control group demonstrated a characteristic decrease in functional tracts involving premotor cortex areas of the right lobe and bilateral medial prefrontal cortex.

These neuroanatomical changes suggest damage mainly of the prefrontal areas without involvement of the primary and secondary motor cortex fields agreeing with the clinical observation that patients demonstrated no pyramidal system damage.

Thus the functional neuroanatomical features confirm clinical observations and suggest a higher-level motor programming disorder which is possibly related to prefrontal cortex inhibitory function disturbances.[73]

Like in patients with Mn intoxication determined occupational disease[74], the most distinctive changes in study patients were found on MRI, as for CT imaging – no changes were traced.

8.3. Syndrome pathogenesis

The source of manganese neurotoxicity was obviously associated with the use of $KMnO_4$ in the process of ephedrone preparation due to its strong oxidizing properties in order to convert ephedrine or pseudoephedrine into methcathinone. This reaction was usually performed at home and depending on availability, different medications containing ephedrine or pseudoephedrine were utilized as substrates and without any considerable precautions in order to purify ephedrone from contaminants, solution containing $KMnO_4$ and acetic acid was intravenously injected several times a day commonly for a long time.[75] Since the solution administered via parenteral route contained hundreds of times greater manganese concentration[14] compared to normal concentration with diet intake, capacity of mechanism responsible for manganese excretion was obviously insufficient and manganese deposition occurred, including CNS where the toxic effects on the basal ganglia caused the extrapyramidal system damage symptoms described in the study.

Mn is known to have an essential role in CNS development and normal function, mainly in the form of co-factor for superoxiddismutase and glutaminsintetase, however in excessive concentrations Mn demonstrates

toxic effects.[76, 77] Even though the pathogenetic mechanism of Mn caused CNS damage is unclear, after intravenous injection Mn half-life period exceeds this equivalent four times (approximately 40 days) compared to oral dose load.[11] Thus a repeated intravenous ephedrone administration results in permanent hypermanganemia and subsequent manganese deposit in the tissue, including CNS basal ganglia. These factors explain the observed differences in Mn blood concentrations and MRI changes between „active" users with mores intense changes and patients who reported cessation of ephedrone use.

Currently two main Mn neurotoxicity mechanisms have been described: cell component oxidation with ready to reduce trivalent Mn^{3+} complexes and inhibitory effect of bivalent Mn^{2+} on Ca^{2+} dependent energy metabolism (adenosine tri-phosphate [ATP] production) in mitochondria.[78] Study observations support theory of mitochondrial structure damage in study patients basal ganglia neurons and glial cells.[79]

Parenteral drug injection route and strong oxidizing action of $KMnO_4$ also share a part in distinction of study syndrome from formerly described Mn neurotoxicity cases, when the predominant mechanism was that of inhalation and absorption of Mn particles containing Mn oxides via respiratory system.[80, 81]

It is not known whether $KMnO_4$ powerful oxidizing activity is an additional factor causing uniform motor disorder syndrome in ephedrone users. It is possible that the characteristic psychostimulating effects of methcathinone, especially stimulation of dopaminergic terminals can promote Mn toxic effect on basal ganglia structures.[82]

Study described a neurological disorder in IVD users presenting with permanent extrapyramidal system damage syndrome which is credibly associated with Mn neurotoxicity which in turn is related to substrate proportions used in synthesis and failure to provide appropriate reaction conditions and excessive non-organic Mn blood concentration and subsequent deposit in the tissue, including CNS.

Study findings demonstrate consequences of uncontrolled homemade methcathinone (ephedrone) use and invites discussion of circulation regulations of widely available medications used for drug synthesis.

9. Conclusions

1. Motor disorder syndrome observed in ephedrone users is classified as a new type of manganese parkinsonism and is clinically characterized with extrapyramidal system damage symptoms of which the most distinctive are postural instability, dysarthria and akinesia.
2. Motor disorder syndrome cause is the toxic extrapyramidal system damage induced by manganese-contaminated homemade ephedrone use. The source of manganese in the injection solution is potassium permanganate used for drug synthesis to convert ephedrine or pseudoephedrine into methcathinone.
3. The study syndrome paraclinic features are hypermanganemia and hyperintense bilateral symmetric signal on T1-weighted images indicating CNS basal ganglia manganese deposits mainly affecting the globus pallidus, as well as substantia nigra/substantia innominata and anterior midbrain.
4. Paraclinic examination results correlate with severity of ephedrone use: both hypermanganemia and MRI changes are more intense in „active" users.
5. Other diseases have no direct effect on the course of motor disorder syndrome observed and co-morbidity is predominantly associated with intravenous drug use determined diseases – HIV and hepatitis C infection. Similar to general addicted patient population ephedrone users commonly demonstrate chronic alcohol overconsumption and concurrent alcoholic polyneuropathy.
6. Despite reported cessation of ephedrone use and subsequent manganese concentration decrease or even recovery of normal levels, no clinical improvement in patients is noted moreover – in some of the cases clinical worsening is observed. In patients who continue ephedrone use motor disorder syndrome severity increases.

10. Practical guidance

1. Manganese neurotoxicity is not solely a casuistic disease found in occupational patients of metal industry in the developing countries, but is an issue in Eastern Europe in association with ephedrone use among intravenous drug abusers. Therefore manganese parkinsonism has to be ruled out in any situation dealing with a patient presenting with bilateral and symmetric extrapyramidal damage and the clinical

syndrome of predominant postural instability, akinesia and dysarthria. Diagnose of manganese parkinsonism is particularly probable if patient meets the following criteria:

a. young age,
b. history of drug abuse,
c. HCV-positive,
d. HIV-positive/has AIDS.

2. To set the proper diagnose of manganese parkinsonism, brain MRI is required. Bilateral symmetric hyperintensity of globus pallidus and less commonly also of substantia nigra /substantia innominata and/or anterior midbrain on T1-weighted sequence is highly specific and indicates manganese CNS deposit. Still it must be noted that normal MRI findings do not rule out manganese parkinsonism especially in patients who report cessation of ephedrone use for at least a year. CT is not a useful diagnostic examination and it can only be used as means of differential diagnostics when MRI is not available.
3. Blood manganese concentration detection is recommended in all cases when manganese neurotoxicity is suspected as hypermanganemia is a highly specific feature of the syndrome but the use of this method is limited due to requirement for a specially equipped laboratory. Normal blood manganese concentrations do not exclude manganese parkinsonism diagnose, especially in patients reporting cessation of ephedrone use for at least a year.
4. Manganese neurotoxicity in ephedrone users demonstrates high hepatitis C virus and HIV infection comorbidity which is why it is suggested that recommendations to all patients diagnosed with manganese parkinsonism should be given to undergo corresponding examination.
5. Currently no effective conservative or surgical manganese parkinsonism treatment method is established, therefore administration of specific medication, including anti-parkinsonism preparations, apart from clinical trials, is not useful. Essential part of patient care is treatment of any existing comorbidities as well as adjusting social and psychological support arrangements.
6. Patients need to be informed that there is a high probability that the course of disease may continue worsening should they choose to continue ephedrone use. Also it must be explained to patient that despite cessation of ephedrone use motor disorder may progress, especially during the first six months after the onset of first symptoms.

7. Since study results suggest severe consequences of uncontrolled homemade ephedrone use, discussion arises concerning circulation regulation of wide medication availability, which are utilized for drug synthesis. The results also may serve for informing society about consequences of narcotic substance use.

11. Presentations and publications

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- Skuja S., Groma V., Saulite L., Stepens A., Liguts V. Ultrastructural Study of the Human Basal Ganglia in Intravenous Drug Users Exposed to Methcathinone. Baltic Morphology 2009. Kaunas, Aug 27-28. Conference presentation. Abstract book p. 25-26
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