RESEARCH ARTICLE

Characteristics of Korean patients with methamphetamine use disorder based on the quantitative analysis of methamphetamine and amphetamine in hair

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Abstract Methamphetamine (MA) is a highly addictive central nervous system stimulant. MA use disorder is characterized by a chronic, relapsing brain disease that is enhanced by a dynamic process of repeated use and withdrawal. The analysis of MA and its metabolite, amphetamine (AM), in hair is routinely performed in forensic laboratories for illegal MA use determination. However, few studies regarding the clinical application of hair analysis have been conducted to monitor the treatment of MA use disorder. Herein, the characteristics of Korean patients with MA use disorder were investigated based on drug abuse screening instruments and quantitative analysis of MA and AM in hair. A HPLC–MS/MS method for the quantification of MA and AM in hair was validated and clinically applied to healthy subjects (HS, n = 30, male) as well as current (CP, n = 33, male) and former (FP, n = 22, male) MA use disorder patients. The validation results of the hair analysis method showed high selectivity, accuracy, and precision with acceptable linearity within the calibration range (0.05–5.0 ng/mg). The limit of detection (LOD) and limit of quantification for both MA and AM were 0.05 ng/mg. The concentrations of MA and AM ranged from ≤ LOD to 166 ng/mg and from not detected (ND) to 9.15 ng/mg in the CP group and from ND to 6.14 ng/mg and from ND to 0.32 ng/mg in the FP group, respectively. No correlation was observed between the hair MA concentrations and the NIDA-modified ASSIST, DUDID extended, or DAST scores in both groups. The hair MA concentrations showed advantages for differentiating the CP and FP groups compared with the scores provided by the above-mentioned drug abuse screening instruments.

Keywords Methamphetamine · Hair · HPLC–MS/MS · Drug use disorder

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Introduction

According to the 2019 World Drug Report published by the United Nations Office on Drugs and Crime, drug users comprise approximately 5.5% of the adult population worldwide (aged 15–64), of which people with drug use disorder account for approximately 13% as of 2017 (global overview of drug demand and supply). The clinical diagnosis of this disease is mainly based on self-reported drug-seeking behavioral and psychological conditions using drug abuse screening instruments, including the National Institution of Drug Abuse alcohol, smoking and substance involvement screening test (NIDA-ASSIST), drug use disorder identification test (DUDIT), and drug abuse screening test (DAST). However, characterization of drug use disorder is equivocal due to the lack of peripheral diagnostic biomarkers. The reliability and validity of the drug abuse screening instruments remain under consideration for use in pregnancy, primary care, or emergency settings (Hildebrand 2015; Giguere et al. 2017; Tiet et al. 2017; Oga et al. 2020).

For the forensic purposes, the analytical results of drugs and metabolites in biological specimens such as urine or hair are often used to prove current or past drug use (Lee et al. 2012; Shin et al. 2014; Bough and Pollock 2018; Truong et al. 2018). Particularly, the severity of drug use is often evaluated using hair drug analysis. Statistical evaluations,

where the low, medium, and high ranges for hair drug concentrations are defined using the minimum to the 25th percentile, 25th to 75th percentile, and 75th percentile to the maximum, respectively, are typically performed to identify heavy heroin (Lee et al. 2009a), cocaine (Cordero et al. 2010), and methamphetamine (MA) (Lee et al. 2009b) users. To improve the diagnostic potential of hair analysis for alcohol dependence, hair analysis results were interpreted in conjunction with diagnostic questionnaire answers. It was previously demonstrated that AUDIT scores and the analytical results of ethyl glucuronide in hair increased the diagnostic performance for problematic alcohol use (Kim et al. 2018; Lendoiro et al. 2018).

MA is traditionally the most abused drug, despite the recent rapid influx of new designer drugs (Kong et al. 2018), and the amount of seized MA surprisingly increased by a factor of > 3 between 2017 and 2018 in Korea (Kim et al. 2019). The analysis of MA and its metabolite, amphetamine (AM), in hair is routinely performed in forensic laboratories to identify illegal MA use. The detection of MA in hair after abstinence was previously studied to facilitate interpretation of hair analysis results for forensic purpose (Suwannachom et al. 2015). However, few studies regarding the clinical application of hair analysis have been conducted to monitor the treatment of MA use disorder. Herein, the characteristics of Korean patients with MA use disorder were investigated based on commonly used drug abuse screening instruments as well as quantitative analysis of MA and AM in patients' hair.

Materials and Methods

Chemicals

($\pm$)-MA, ($\pm$)-AM, ($\pm$)-MA-d$_5$, and ($\pm$)-AM-d$_5$ (1 mg/mL methanol solution for each) were purchased from Cerilliant (Round Rock, TX, USA). The certified concentrations $\pm$ associated uncertainties of ($\pm$)-MA, ($\pm$)-AM, ($\pm$)-MA-dd$_5$ and ($\pm$)-AM-d$_5$ were 1.000 ± 0.006, 1.000 ± 0.005, 1.000 ± 0.005, and 1.000 ± 0.005 mg/mL, respectively. The mixed working standard solutions (100 ng/mL) were prepared and serially diluted from stock solutions (100 µg/mL). The mixed working internal standard solution (100 ng/mL) was also prepared from stock internal standard solutions (100 µg/mL). All stock and working solutions were diluted in methanol and stored at -80 °C. Hydrochloric acid (37%, American Chemical Society grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents and chemicals for high performance liquid chromatography tandem–mass spectrometry (HPLC–MS/MS) were of HPLC grade.

Clinical Study

The clinical study was approved by the Institutional Review Board of Bugok National Hospital (Gyeongsangnam-do, Republic of Korea, Approval Number BNH-2018-03, approval date 26 April 2018). Three groups of healthy subjects (HS, n = 30, male), current patients with MA use disorder (CP, n = 33 male), and former patients with MA use disorder (FP, n = 22, male) were included. All participants were recruited from a national drug addiction treatment hospital, Bugok National Hospital in Korea. Male adults previously diagnosed with MA use disorder were enrolled for the patient groups. The patients currently under treatment were included in the CP group and rehabilitated patients who recovered from MA dependence at least a year before the time of recruitment were included in the FP group. The age and gender matched healthy male adults were also recruited for the HS group. The cigarette and alcohol use as well as previous drug history was investigated for all participants. In particular, alcohol use status was classified according to the drinking levels defined by the National Institute on Alcohol Abuse and Alcoholism in the US (drinking levels defined). MA dependence was accessed using three drug abuse screening tools, the Korean versions of the NIDA-Modified ASSIST, DUDID Extended, and DAST methodologies. Participants were excluded from the study if they presented with a current or previous substance use disorder other than MA or any general medical condition. Hair samples from the participants were collected as close to the scalp as possible from three separate areas of the back of the head. The 3-cm section of hair strands from the root were used for analysis.

Hair Sample Preparation

MA and AM in hair were extracted as previously described (Lee et al. 2010, 2012) with a minor modification. First, the hair was washed with 2 mL of methanol followed by 2 mL of distilled water and again 2 mL of methanol. The hair was then cut, weighed accurately (approximately 10 mg), and incubated in 1% HCl in methanol for 16 h at 40 °C. Subsequently, 10 µL of 100 ng/mL MA-d$_5$ and AM-d$_5$ were added as internal standards before incubation. The extract was evaporated to dryness under a stream of nitrogen at 40 °C. The residue was reconstituted in 50 µL of methanol and mobile phase A (1:9) and subsequently filtered using a 0.45 µm polyvinylidenefluoride microporous membrane (Millipore, Bedford, MA, USA). Finally, 5 µL of the extract was injected into the HPLC–MS/MS system.

HPLC–MS/MS Analysis

The HPLC–MS/MS instruments used for the analysis were an Agilent 1260 Infinity LC and 6460 triple quadrupole MS/

MS (Agilent Technologies, Santa Clara, CA, USA). The guard column was a Poroshell 120 UHPLC Guard EC-C18 (3.0 mm × 5 mm, 2.7 μm, Agilent) and a Poroshell EC C-18 (3.0 mm × 50 mm, 2.7 μm, Agilent) was used as the analytical column maintained at 45 °C. The mobile phase consisted of 2 mM ammonium formate/0.2% formic acid in water (A) and acetonitrile (B). The gradient conditions were as follows: 0–1 min, 95% (A); 1–5.5 min, 10% (A); 6.5–6.6 min, 95% (A); 6.6–11.5 min, 95% (A) at a flow rate of 300 μL/min. The temperature of the column oven was 45 °C and that of the auto sampler was 4 °C. The total run time was 11.5 min.

The MS system was operated using electrospray ionization (ESI) in positive mode. The optimum conditions were as follows: gas temperature, 350 °C; gas flow, 10 L/min; nebulization pressure, 35 psi; sheath gas temperature, 250 °C; sheath gas flow, 5 L/min; and capillary voltage, 4.5 kV. Two multiple reaction monitoring (MRM) transitions were chosen for each analyte and one MRM transition was chosen for MA-d$_5$ and AM-d$_5$. The MRM transitions, retention times, and conditions are listed in Table 1. Data were processed using Masshunter software (B.04.00, Agilent Technologies).

Method Validation

The analytical method was validated using spiked drug-free hair pooled from five different volunteers, as described previously (Matuszewski et al. 2003; Peters et al. 2007; Yu et al. 2018). Selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), matrix effect, recovery, precision, and accuracy were evaluated. To demonstrate linearity, five sets of calibrators (0.05–5.0 ng/mg hair for both MA and AM) were prepared and analyzed. The analyte concentration showing a signal-to-noise ratio of > 3 was designated as the LOD and that with < 20% coefficient of variation (CV) for precision and < ± 20% for bias was defined as the LOQ. The matrix effect and recovery were determined by comparing the analyses of five neat standards, five extracts of blank hair spiked with analytes after extraction, and five extracts of blank hair spiked with analytes before extraction

at low (0.05 ng/mg), medium (0.5 ng/mg), and high (5.0 ng/mg) concentrations of MA and AM. Method precision and accuracy were examined by analyzing drug-free hair samples spiked at the same concentrations levels. The five sets of each sample were analyzed on five different days to account for inter-day variation.

Analysis of Clinical Hair Samples

The hair strands measuring 3 cm from the root of each hair sample were analyzed. The samples were washed with methanol and distilled water, cut, and weighed accurately (approximately 10 mg). Subsequently, MA and AM in the hair were extracted and analyzed as described in "Hair Sample Preparation" section.

Statistical Analysis

Statistical evaluations of the MA concentrations in the hair and scores of drug abuse screening tools were performed via Student t-test and Pearson correlation test. To investigate significant differences, values below the LOQ and not detected (ND) MA concentrations were replaced with LOQ/2.

Results

Characteristics of Korean Patients with MA Use Disorder

The characteristics of the subjects in the study groups, HS, CP, and FP, are provided in Table 2. The average age of the three groups were 44.7 ± 7.7, 48.2 ± 8.1, and 50.6 ± 9.1 years, respectively; the body mass indices were 24.7 ± 2.4, 24.3 ± 2.9, and 25.4 ± 3.3 kg/m^2, respectively (mean ± standard deviation). Compared to the HS group (33% and 37%), the percentages of never- or former smokers were lower for both the CP (6% and 18%) and FP groups (0% and 27%). The majority of subjects in the

Table 1 Multiple reaction monitoring transitions, retention times, and other conditions for methamphetamine, amphetamine, and internal standards

Compound name	Precursor ion (*m/z*)	Product ion (*m/z*)	t$_R$ (min)	Frag (V)	CE (V)
Methamphetamine	150.2	<u>91.1</u>	4.7	75	20
		119.1			8
Amphetamine	136.1	91.1	4.6	65	18
		<u>119.1</u>			4
Methamphetamine-d$_5$	155.2	<u>92.1</u>	4.7	75	20
Amphetamine-d$_5$	141.1	<u>124.1</u>	4.6	65	4

The underlined transitions were used for quantification

t$_R$ retention time, *Frag* fragmentation voltage, *CE* collision energy

Table 2 Characteristics of the subjects in the study groups

	Healthy subjects (HS, n = 30, male)	Current patients with methamphetamine use disorder (CP, n = 33, male)	Former patients with methamphetamine use disorder (FP, n = 22, male)
Age (years)	44.7 ± 7.7	48.2 ± 8.1	50.6 ± 9.1
Height (cm)	173 ± 4.6	174.3 ± 6.2	172.4 ± 4.9
Weight (kg)	74 ± 8.4	73.9 ± 11.6	75.5 ± 10.7
Body mass index (kg/m^2)	24.7 ± 2.4	24.3 ± 2.9	25.4 ± 3.3
Cigarette smoking status			
Never-smoker (n, %)	10 (33)	2 (6)	0 (0)
Former smoker (n, %)	11 (37)	6 (18)	6 (27)
Current smoker (n, %)	9 (30)	25 (76)	16 (73)
Alcohol use status			
Non-drinker (n, %)	2 (7)	14 (42)	8 (36)
Moderate drinker (n, %)	2 (7)	0 (0)	1 (5)
Low-risk drinker (n, %)	3 (10)	4 (12)	2 (9)
Binge drinker (n, %)	23 (76)	15 (46)	11 (50)
Types of drug use			
Methamphetamine only (n, %)	0 (0)	15 (47)	6 (27)
More than 2 types of drugs including methamphetamine (n, %)	0 (0)	17[a] (53)	16[b] (73)
Scores of drug abuse screening tools (Korean versions)			
National Institution of Drug Abuse Modified-The alcohol, Smoking and Substance Involvement Screening Test			
0–3 (Lower risk, n, %)	NA	0 (0)	0 (0)
4–26 (Moderate risk, n, %)	NA	7 (21)	3 (14)
27+ (High risk, n, %)	NA	26 (79)	19 (86)
Drug Use Disorders Identification Test Extended			
Low motivate (− 2 to 3)	NA	3 (9)	1 (5)
Medium motivate (4 to 9)	NA	14 (42)	6 (27)
High motivate (10 to 14)	NA	8 (24)	7 (32)
Outlier (> 14)	NA	8 (24)	8 (36)
Drug Abuse Screening Test			
0 (Healthy, n, %)	NA	0 (0)	0 (0)
1–2 (Risky, n, %)	NA	3 (9)	1 (5)
3–5 (Harmful, n, %)	NA	8 (24)	4 (18)
6+ (Severe, n, %)	NA	22 (67)	17 (77)

NA not analyzed

[a]Cannabis (n = 13), cocaine (n = 3), opium (n = 2), inhalers (e.g., toluene, butane etc., n = 3), sedative/anesthetic drugs (e.g., diazepam, lorazepam etc., n = 6), hallucinogens (e.g., 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide etc., n = 2), prescription stimulants (e.g., phentermine, methylphenidate etc., n = 2), prescription opioids (e.g., morphine, codeine etc., n = 2)

[b]Cannabis (n = 13), cocaine (n = 3), opium (n = 1), inhalers (e.g., toluene, butane etc., n = 3), sedative/anesthetic drugs (e.g., diazepam, lorazepam etc., n = 5), hallucinogens (e.g., 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide etc., n = 6), γ-hydroxybutyrate (n = 1), prescription stimulants (e.g., phentermine, methylphenidate etc., n = 2)

CP (76%) and FP groups (73%) were current smokers. In terms of alcohol use, 42% and 36% of the CP and FP groups, respectively, were non-drinkers, while only 7% of HS did not drink. The percentage of binge drinkers in the HS group (76%) was higher than those in the CP (46%) and FP groups (50%). In terms of drug use history, 47% and 27% of the CP and FP groups, respectively, declared that they used only MA, while 17 and 16 subjects in CP and FP, respectively, reported that they previously used more than two types of drugs including MA. Cannabis was the most co-used drug for both groups (n = 13 for both), followed by sedative/anesthetic drugs (n = 6) for CP and hallucinogens (n = 6) and sedative/anesthetic drugs (n = 5) for FP. Most CP and FP participants were classified as high risk in NIDA-Modified ASSIST (79% and 86%, respectively) and severe in DAST (67% and

77%, respectively). In the DUDID Extended evaluation, > 90% of each group were identified as moderately to highly motivated.

Method Validation

Figure 1 shows representative chromatograms obtained for MA, AM, and their internal standards in a hair sample spiked at 0.05 ng/mg for MA and AM (a), a blank hair
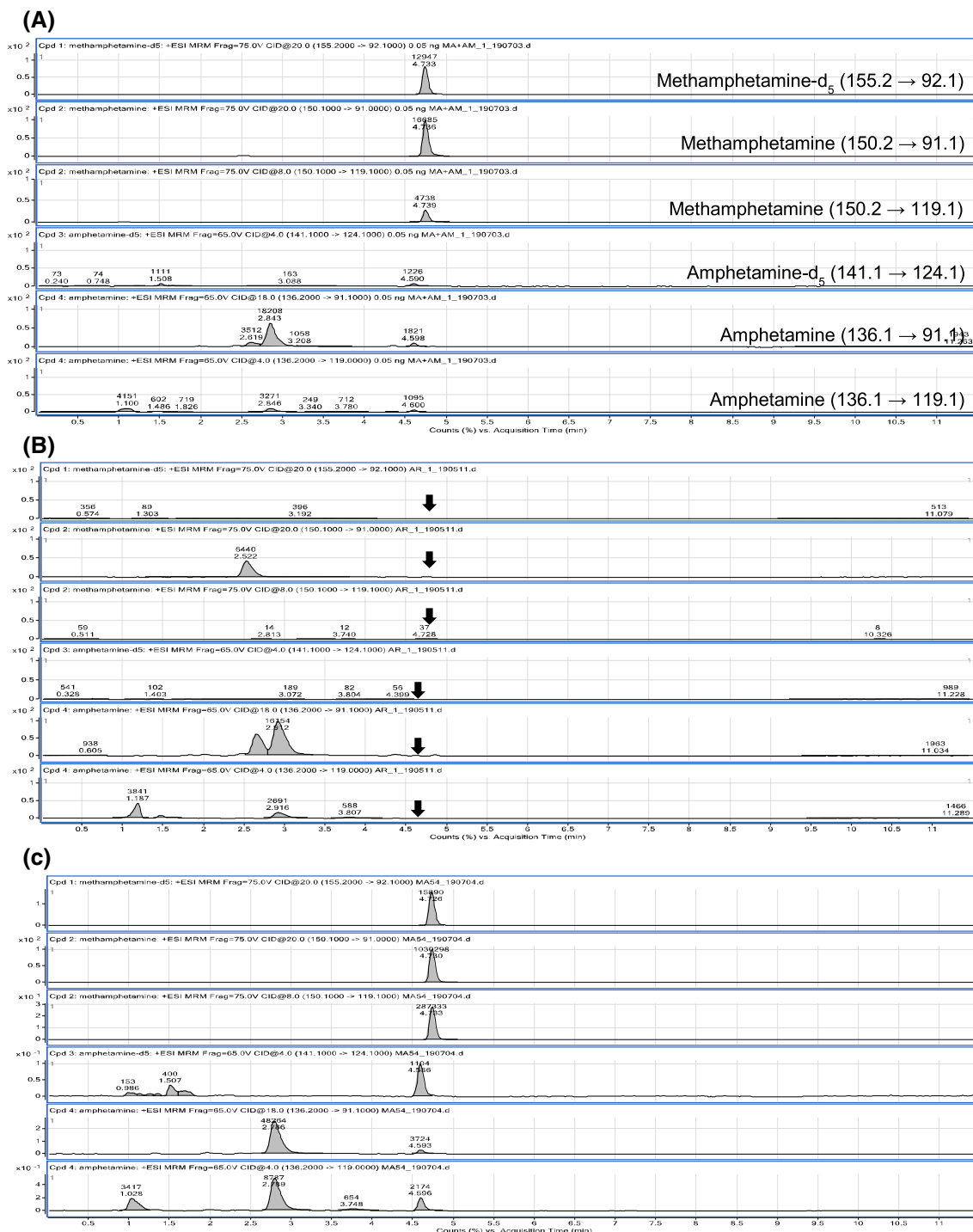

Fig. 1 Representative chromatograms of methamphetamine, amphetamine, and internal standards in a fortified hair sample (0.05 ng/mg for both methamphetamine and amphetamine (**a**), blank hair sample (**b**), and an authentic hair sample from a methamphetamine user (**c**)

sample (b), and an authentic hair sample from a MA user (c). No interference was observed near the retention times of MA, AM, and their internal standards in the blank hair samples from the five individuals. The LOD and the LOQ values were 0.05 ng/mg for MA and AM, while both analytes produced effective linearity within the wide calibration range (0.05–5.0 ng/mg) with respective slope (a), y-intercept (b), and r values as follows: a, 17.3 ± 0.24; b, 0.32 ± 0.10; r, 0.9989 ± 0.0006 for MA and a, 13.7 ± 0.29; b, 0.08 ± 0.04; r, 0.9982 ± 0.0005 for AM (n = 5).

The results of other method validation parameters are summarized in Table 3. The analytes produced different degrees of ion enhancement at the tested low, medium, and high concentration levels (> 110%). However, the obtained CV values were 14–26%, which substantially decreased to 0.1–4.3% after correction by internal standards, which is considered acceptable for deuterated internal standards. The recovery was > 79% at all concentrations for MA and AM. The repeatability and intermediate precision were < 10% at all concentrations of MA. For AM, these values were < 20% at 0.05 ng/mg and 10% at 0.5 and 5 ng/mg. The accuracy values were 17% and 9.4% at 0.05 ng/mg for MA and AM, respectively. At higher concentrations, values of $< \pm 10\%$ were obtained for both compounds. These results are acceptable, given the guidelines for analytical method validation [precision, < 20% CV near lower limit of quantification (LLOQ) and < 15% at higher concentration; accuracy, within $\pm 20\%$ bias near LLOQ and $< \pm 15\%$ bias at higher concentrations].

Hair Analysis Results of Korean Patients with MA Use Disorder

Table 4 shows the hair analysis results for MA and AM in the CP and FP groups. The MA and AM concentrations in hair from the CP group ranged from below LOQ to 166 ng/mg and from ND to 9.15 ng/mg, respectively. The MA and AM concentrations in hair from the FP group were ≤ 6.14 and ≤ 0.32 ng/mg, respectively. The MA concentrations in hair samples obtained from the CP group were significantly higher than those from the FP (p < 0.05), as shown in Fig. 2. However, no significant differences in the NIDA-Modified ASSIST, DUDID Extended, and DAST scores were observed between the groups. Pearson correlation coefficients between the scores of the drug abuse screening tools and MA concentrations in hair indicated no correlation between the hair MA concentrations and the NIDA-Modified ASSIST, DUDID Extended, and DAST scores for both groups (r = 0.1727, 0.2200, and 0.0638 for the CP group; r = 0.2892, 0.3885, and 0.0121 for the FP group, respectively).

Discussion

Illegal drug users or drug addicts show poor reliability in their answers to questionnaires related to drug use experience (Han et al. 2015; Ou et al. 2020). Therefore, the specific information regarding cigarette smoking status, alcohol use, types of drug use, and scores in the drug abuse screening tools provided by the subjects in the study groups were presented in several large scopes (Table 2). Different cigarette smoking status, alcohol use severity, or types of drug use did not have any significant influence on the onset or progress of MA use disorder. Even though the current and former patients were only diagnosed with MA use disorder, more than half of the patients reported the use of > 2 types of drugs including MA. Cannabis was the most co-used drug, in agreement with current drug use trends in Korea (Cho et al. 2019).

Table 3 Summary of the validation data

| Compound name	Concentration (ng/mg)	Matrix effect (%)			Recovery (%)	Repeatability[c] (CV, %)	Intermediate precision[d] (CV, %)	Accuracy (bias, %) CV[b]
		Mean	CV[a]	CV[b]	Mean			Mean
Methamphetamine	0.05	110	26	0.1	108	4.9	5.0	17
	0.5	202	18	2.4	116	7.0	8.2	3.6
	5	253	26	3.9	99	2.7	2.9	− 0.9
Amphetamine	0.05	297	26	4.2	79	17	18	9.4
	0.5	174	14	4.3	116	1.8	2.0	− 1.9
	5	216	15	3.3	98	5.4	8.7	− 1.5

[a]Coefficient of variation

[b]Coefficient of variation of values corrected by internal standards

[c]Intra-day variation

[d]Combination of intra- and inter-day variation

Fig. 2 Comparison of hair methamphetamine concentrations (**a**) and drug abuse screening tool scores (**b**) between current and former patients with methamphetamine use disorder
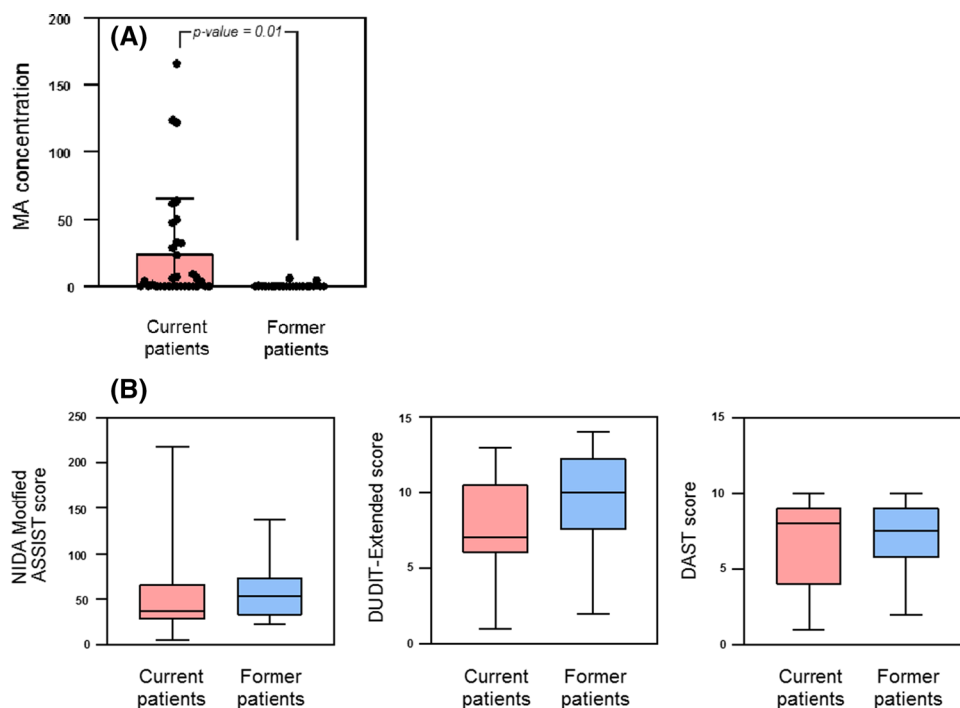
The original version of the NIDA-Modified ASSIST method consisted of eight questions regarding the types, frequency, desire, problems, and other issues related to drug use over the subject's lifetime or during the past three months (Questions 1–8 of the NIDA-Modified ASSIST V2.0). The Korean NIDA-Modified ASSIST questionnaire was the same as the original, except drug use in lifetime or during the past year. The Korean versions of the DUDID Extended and DAST tests were the same as the original versions. The DUDID Extended test consisted of questions such as "What is positive for you about using drugs?", "What is negative for you about using drugs?", and "What are your thoughts about drugs?" (Berman et al. 2007). The DAST is a 10-question screening tool for behavioral and psychological problems related to drug use experiences (Skinner 1982; Lam et al. 2015). The reliability of these self-report instruments were examined in groups of drug users in forensic or clinical settings such as drug-accusers or pregnant women (Berman et al. 2007; Lam et al. 2015; Tiet et al. 2016). For drug-accusers, biased results could be obtained due to the psychological burden of social criticism and punishment for illegal drug use (Berman et al. 2007). The patients included in this study were already legally sentenced and currently under (CP) or had completed treatment (FP) at the time of recruitment. The majority of both groups scored highly in all three drug screening tools used herein. The results showed that these scores were effective to diagnose MA use disorder but were not useful for treatment monitoring of MA use disorder. From these tests, it was not possible to discriminate between current and former patients, probably because the

questionnaires rely on self-reporting of drug use severity as well as psychological and behavioral status and related opinions. However, it is difficult to accurately examine the period of drug use, and no questionnaires are available to assess the development or prognosis of drug addiction.

The method validation results demonstrated high selectivity, accuracy, and precision with acceptable linearity within calibration ranges (0.05–5.0 ng/mg). The LOD and LOQ for both MA and AM were 0.05 ng/mg, falling below the recommended LOQ by the Society of Hair Testing (Recommendations for Hair Testing in Forensic Cases).

The distribution of the MA and AM concentrations were reported previously for forensic cases in the Korean population. The concentration ranges for MA and AM were 0.5–608.9 and 0.1–41.4 ng/mg in hair from illegal MA users (2355 segments, with a variety of hair lengths up to 12 cm from 2070 cases) (Lee et al. 2009b). The hair MA and AM concentrations reported herein were included in these ranges. Nevertheless, we expected much lower concentration ranges of MA and AM in hair from the CP and FP groups because they had stopped using MA. Therefore, the calibration range was set for the low ranges up to 5 ng/mg according to the previous study (Lee et al. 2009b). However, the higher concentrations of MA and AM in some hair samples. Thus, we compared the linearities of the calibration curve up to 5 ng/mg with that up to 200 ng/mg. Any significant difference between the slopes of those calibration curves was not shown. The slope (a), y-intercept (b), and r values of the extended calibration curve were as follows: a, 17.1 ± 0.36; b, 0.87 ± 0.22; r, 0.9992 ± 0.0008 for MA and a, 15.1 ± 0.25;

Table 4 Hair analysis results for methamphetamine and amphetamine

	Current patients with methamphetamine use disorder (CP, n=33, male)			Former patients with methamphetamine use disorder (FP, n=22, male)		
Subject No.	Methamphetamine (ng/mg)	Amphetamine (ng/mg)		Subject No.	Methamphetamine (ng/mg)	Amphetamine (ng/mg)
1	[a]< LOQ	ND		1	< LOQ	ND
2	< LOQ	ND		2	< LOQ	ND
3	< LOQ	ND		3	< LOQ	ND
4	< LOQ	ND		4	4.70	0.32
5	< LOQ	ND		5	< LOQ	ND
6	49.83	2.86		6	6.14	0.27
7	7.12	0.84		7	< LOQ	ND
8	4.03	0.23		8	< LOQ	ND
9	< LOQ	ND		9	< LOQ	ND
10	61.53	1.56		10	< LOQ	ND
11	ND	ND		11	< LOQ	ND
12	0.21	ND		12	< LOQ	ND
13	< LOQ	ND		13	< LOQ	ND
14	6.89	0.37		14	0.25	ND
15	121.89	9.15		15	ND	ND
16	63.87	1.67		16	ND	ND
17	33.14	2.61		17	0.06	ND
18	26.36	1.70		18	0.05	ND
19	1.63	0.10		19	ND	ND
20	166.17	8.74		20	< LOQ	ND
21	32.15	1.73		21	< LOQ	ND
22	47.35	2.55		22	ND	ND
23	28.63	2.33				
24	0.18	ND				
25	0.11	ND				
26	0.12	ND				
27	< LOQ	ND				
28	[b]< LOD	ND				
29	0.12	ND				
30	124.05	3.95				
31	9.24	0.71				
32	3.70	0.15				
33	6.25	0.22				
Range	< LOD-166	ND-9.15		Range	ND-6.14	ND-0.32

ND not detected

[a]Detected but below the LOQ

[b]Detected but below the LOD

b, 0.23 ± 0.30; r, 0.9981 ± 0.0009 for AM (n=3). The range of the hair MA and AM concentrations were much wider than those of hair samples from MA abusers in black-hair Chinese populations at rehabilitation centers reported by Ou et al. In that study, the MA and AM concentrations in hair strands of approximately 3–10 cm length ranged from ND to 57.51 ng/mg and ND to 7.51 ng/mg, respectively (total, n=563; male, n=466; female, n=73; not specified, n=19).

No correlation of MA and AM concentrations were observed with respect to age, gender, hair length, or hair color (Ou et al. 2020).

Neither group in this study contained current MA users, but the majority of the current patients were MA and AM positive. The patients in the CP group were those currently under treatment for MA use disorder and those in the FP group were previously rehabilitated with a recovery date

at least one year before recruitment. The last time that MA was used was not reported. However, the severity of previous MA abuse was determined by the quantitation of MA and AM in the hair samples. Unexpectedly, both MA and AM were higher than the LOQ in hair from 2 of the 22 subjects in the FP group. A previous study that focused on the duration of detection of MA in hair after abstinence reported that MA could still be detected in hair after 120 days of abstinence (16% of the study population of n = 63), but all samples were negative after 152 days of abstinence (Suwannachom et al. 2015). Therefore, MA and AM in all hair samples from the FP group should be ND, considering the hair growth rate (approximately 1 cm/month). The hair growth cycle can be divided into the anagen phase (4–8 years), the catagen phase (2 weeks), and the telogen phase (10 weeks). Approximately 10–20% of hair in the catagen or telogen phase was suggested as a reason for the positive result even after discontinuation of drug use. Another reason may be that the tissue deposits of the scalp store the drug and continue to release it into the hair after discontinuation (Pragst and Balikova 2006; Shen et al. 2013).

To date, hair drug and/or metabolite concentrations have been used as a definite diagnostic marker in identifying drug abusers in forensic toxicology. The clinical diagnosis of drug addiction was mostly performed based on scores obtained for drug abuse screening tools, such as NIDA-ASSIST, DUDID, and DAST. Herein, both hair analysis and questionnaire investigations using common drug abuse screening tools were conducted. The hair MA concentrations demonstrated advantages for differentiating the CP and FP groups. Previously, the determination of ethyl glucuronide in hair in conjunction with AUDIT was successfully applied for detecting high-risk alcohol consumption (Kim et al. 2018). By integrating the drug abuse screening tool scores and determined hair drug concentrations, higher performance can be obtained for the diagnosis and prognosis of drug addiction.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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