

Analysis of 6-Acetylmorphine, Morphine, and Codeine in the Uncut Hair, Cut Hair and Nails of Heroin Abusers after Different Withdrawal Times

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Abstract

As hair and nail are powerful tools for drug exposure history investigation, it is not only used to investigate whether an individual "used" drug, but also used to investigate whether the drug is "withdrawal" in recent years. Hair and nail samples from 69 subjects with different heroin withdrawal times have been analysed in this study, for evaluating how 6-acetylmorphine, morphine, and codeine residual in hair (cut or uncut) and nail. Pulverized under liquid nitrogen environment and ultrasonic bath were used for extraction. A statistical analysis was performed to evaluate the mean residual "half-lives" of target compounds in uncut hair, which were determined to be 1.10 ± 0.39 months, 1.68 ± 0.44 months, and 1.52 ± 0.45 months, respectively. In contrast, nail analysis did not correlated with the change of drug taking. These results provide meaningful data in hair analysis to investigate drug withdrawal.

Keywords: Forensic science; Hair; Nails; 6-acetylmorphine; Morphine; Haircut

Introduction

Hair and nails are becoming internationally recognized specimen in drug exposure investigation [1,2]. Once incorporated into growing hair, most drugs can be detected over a significant period of time, as long as the hair is not cut. Therefore, segment hair analysis is used for the detection of drug exposure [3] and is based on the stable growth rate of hair (approximately 1 cm/month) [4,5]. In recent years, the use of hair to investigate withdrawal (detoxification) is becoming a hot topic. This

may be useful in workplace testing, the licensing of drivers, clinical compliance control and a number of forensic issues (e.g., drug abstinence control, the evaluation of drug-induced diminished responsibility and drug facilitated crimes) [6]. However, many complex factors affect the results from segmental hair analysis [7,8], like what was found in our previously study, ketamine and norketamine were found not only in the 0- to 0.5-cm segments obtained 1 week after administration but also in other segments[9].

The Residual of target drugs along a *cut hair* shaft is more complicated. Conversely, the time of the last haircut is important in the interpretation of results. How the time of the last haircut influences the results of segmental analysis of hair is still also not clear. Unfortunately, there was a lack of information on the analysis of *cut hair* after drugs were taken. For a better interpretation of the results from the analysis of hair, especially the influence of haircut, one question that is urgent to understanding the results is the decline of the concentrations of drugs in hair after abstinence.

Nails have been used in substance analysis in recent years [10]. Drugs and other deleterious substances remain in nails for long periods of time [11], thereby providing information about an individual's history of exposure to these substances [12,13]. To the best of our knowledge, the window of time in which a drug is in the nails after withdrawal from a long-term history of taking the drug has not been studied.

The main aims of this study were threefold. First, the study aimed to perform a comparative study on the differences in the Residuals of 6-acetylmorphine,

morphine, and codeine in cut hair and uncut hair after the discontinuation of heroin. The simplest haircut condition (the hair was all shaved after drug discontinuation) was used in this study as cut hair. Second, this study aims to investigate the relationship between the concentrations of 6-acetylmorphine and morphine in hair and nails. Lastly, the study aims to evaluate the possibility of using nails to prove history of drug consumption.

Materials and Methods

Samples

Forty-three healthy women (ages 20–52 years) and twenty-six healthy men (ages 23–47 years) with different drug withdrawal times, were recruited for this study. They had ceased consuming heroin for 1–4 months voluntarily before collection of the hair sample. The abstinence was been monitored by random urine test. The demographic data and sample information for each volunteer are shown in Table 1. Informed consent was obtained from all individual participants included in the study. All protocols were approved by an institutional review committee.

Withdrawal time	Sex	Num	Age
<1 month	Male	17	23-44
1-2 month	Female	19	22-55
	Male	4	27-47
2-3 month	Female	17	20-45
	Male	3	35-42
3-4 month	Female	7	24-43
	Male	2	36-44

Table 1: Demographic data and sample information of the subjects.

Approximately 200 hairs of each female subject were cut by an ordinary stainless steel scissors from the posterior vertex as close as possible to the scalp. The length of those hair samples ranged from 4 cm to 10 cm. For the male subjects, all hairs were shaved off their heads and collected; the lengths of their hair samples were all shorter than 1cm. Nail samples were obtained from all fingers of each subject by commercially available cosmetic nail clippers. Hair and nail samples were stored independently in a clean paper bag at room temperature in a dry and light proof place until analysis. Sample collection procedures were in accordance with the ethical standards of the responsible regional committee.

Drug-free hair and nails were obtained from six laboratory personnel and pooled for quality control (QC).

Reagents

6-acetylmorphine, morphine, codeine, 6-acetylmorphine-d6, and morphine-d3 were all purchased from Cerilliant (Round Rock, TX, USA). Acetonitrile, methanol, and 2-propanol, all of HPLC gradient grades, were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC solvent grade chloroform was purchased from Baker (Baker, USA). HPLC grade ammonium acetate and formic acid (Puriss, 50 %) were purchased from the Fluka Chemical Co. (Buchs, Switzerland). Ultra purified water prepared by a Milli-Q system (Millipore, Bedford, MA, USA) was used.

Sample Preparation and Analysis

The hair and nail samples were prepared and analyzed

by a previously published method [14, 15]. Briefly, the hair samples were cut into segments of 1 cm each. Then, each hair segment or nail sample was decontaminated and pulverized by a 6700 freeze mill instrument (Freezer/Mill, SPEX CertiPrep). Powered hair or nail was extracted in an ultrasonic bath and analyzed by the liquid chromatography–tandem mass spectrometry (LC–MS/MS) system.

The LC–MS/MS analyses were completed on an Acquity UPLC (Waters, Milford, MA) coupled with an API 4000 QTRAP mass spectrometer (Applied Biosystems/MSD Sciex, Foster City, CA). Chromatographic separation was achieved with a PFP Propyl column (100×2.1 mm, 5 µm i.d. Restek, Bellefonte, PA, USA) fitted with a C18 guard column (12.5×2.1 mm, 5 µm i.d. Restek, Bellefonte, PA, USA). The mobile phase consisted of acetonitrile (phase A) and an ammonium acetate buffer (phase B).

The mass spectrometer was operated in the positive electro spray ionization (ESI) mode, and the multiple reaction monitoring (MRM) mode was used to monitor the target compounds. The ion transitions were monitored by the following mass/charge (m/z) ratios: m/z 328.3>211.2 and m/z 328.3>165.3 for 6-acetylmorphine, m/z 286.1>201.2 and m/z 286.1>165.2

for morphine, m/z 300.2>199.2 and m/z 300.2>165.2 for codeine, m/z 334.3>211.3 for 6-acetylmorphine-d6, and m/z 289.2>201.1 for morphine-d3.

The analytical method for the hair [15] and nail [14] samples has been fully validated, the limits of detection and of quantification were 0.02 ng/mg for all analytes.

Results

Female Hair (Uncut Hair) Segment Analysis Results

Assuming a rate of hair growth of 1 cm/month, the female subjects hair samples were cut into segments of 1 cm each and analysed. The results of the segmental hair analyses from thirty-two subjects are shown in Tables 2-4. The coloured cells in Tables 2-4 indicate the hair segment that corresponded to the month in which the subject underwent heroin withdrawal. The concentration of 6-acetylmorphine in those hair segments that were grown before subjects underwent heroin withdrawal ranged from 0.2 to 96.6 ng/mg (mean =18.4 ng/mg), while the concentration of morphine was 0.4 to 33.6 ng/mg (mean =5.8 ng/mg), and the concentration of codeine was 0.3 to 34.1 ng/mg (mean =7.8 ng/mg).

Num	Hair segment							
	1	2	3	4	5	6	7	8
	(0-1cm)	(1-2cm)	(2-3cm)	(3-4cm)	(4-5cm)	(5-6cm)	(6-7cm)	(7-8cm)
1	0.8	0.9	5.3	7	8.8	9	9.8	9.1
2	0.1	-	0.2	-	0.8	10	8.6	8.4
3	-	-	-	6.5	21.1	19.8	22.9	
4	0.5	0.4	0.9	49	52.3	45.9	42.9	40.3
5	1.9	13.9	16.4	19.5	19.9	17.8	19.6	
6	3.3	17.3	45.3	57.3	49.7			
7	-	-	21	19.6	29.1	24.1	23.8	
8	1.6	3.8	9.5	11.4	8.1	9.1	12	13.2
9	-	3.1	9.2	25.4	16.7	25.8	19.9	
10	1.5	1.6	7.9	9.9	9	10.4	8.2	9.3
11	5.8	5	18.7	16.2	15.6	16.8		
12	1.5	1.8	18.7	59.9	79.9	73.3		
13	-	-	13.8	13.6	13	13.7		
14	-	3.2	7.3	26.1	80.3	96.6	81	
15	0.1	3.7	18.9	12.9	13.9	14.7	13.5	14.4
16	-	7.4	13.6	23.7	17.8			
17	-	8.7	32	44.1	44.5	45.6		
18	-	19.2	19.5	16				
19	-	-	7.5	14.3	12.3	17.2	19.7	19.9
20	-	-	0.3	4.5	7.6	6.5		
21	-	-	5.8	15.4	15.7	14.4	18.8	17.8

22	-	-	-	19.1	46.6	47.5		
23	-	0.9	4.7	4.3	36.4	38.4		
24	-	-	-	0.7	6.1	21.8	30.9	30.8
25	0.5	3.5	3.5	9.8	11.9	14.6	13.7	18.8
26	0.6	1.1	18	40.1	42	49.4		
27	-	-	-	-	10.7	14.9	14.4	
28	-	-	5.7	37.7	40.7	43.4		
29	1.8	3.2	3.2	10.9	43.1	47.4		
30	5.1	9.5	35.2	31.9				
31	0.5	0.6	0.3	4.9	5	10.7	12.6	12.9
32	-	-	-	1.8	7	6.1		
33	0.3	0.4	5.3	24.5	31.1	35.9		
34	-	-	-	2.6	24.3	23.7		
35	-	6.7	7.3	8.6	19.3	20.2	22.2	25.8
36	-	6.4	22.1	31.9	25.6			
37	-	-	-	-				
38	0.5	1.1	3.8	4.7	3.1	38.1	40	40.8
39	0.6	0.8	8.3	16.6	27.7	23	27.8	
40	0.3	0.5	0.6	1.7	3.7	9.3	7.6	
41	-	-	-	6	11.7	11	10	11.7
42	-	-	2.2	20.5	27.7	29		
43	-	-	4.6	4.7				

Table 2: Concentrations of 6-acetylmorphine (ng/mg) in the hair segments of female subjects after different opiate withdrawal times.

"-" means not detected

Num	Hair segment							
	1	2	3	4	5	6	7	8
	(0-1cm)	(1-2cm)	(2-3cm)	(3-4cm)	(4-5cm)	(5-6cm)	(6-7cm)	(7-8cm)
1	-	1.2	1	6.6	6.6	7.7	7.5	7
2	-	-	-	0.5	0.5	0.5	0.5	0.5
3	-	-	-	4.5	4.1	4	4.3	
4	-	-	0.9	2.9	3.4	3.6	4.1	3.8
5	-	-	0.4	1.7	4.6	4.2	4.8	
6	1.4	15.4	18.6	18.5	18.1			
7	-	-	2.9	5.3	7.7	8.3	9	
8	1	5.8	11	24.9	26	29.2	31.3	33.6
9	-	0.4	2.4	6.8	6.6	6.8	6.2	
10	-	-	0.4	0.6	1.3	1	1.5	1.9
11	-	-	1.7	1.7	1.1	1.1		
12	1	1.3	3.3	7.4	6.8	7.4		
13	-	0.3	0.2	0.8	0.8	1.2		
14	0.7	0.8	3.8	7.2	7.6	6.3	7.3	
15	-	-	4	18.8	19.3	19.1	20.1	20.7
16	-	0.3	14.3	14.3	15.7			
17	-	-	1.6	3.8	3.3	5.9		
18	-	0.4	0.4	0.4				
19	-	1.2	3.1	9.4	11	11.2	14.6	16.7
20	-	-	0.8	1.3	1.6	3.5		
21	-	-	-	1.1	1.2	0.9	1.2	0.9

22	-	-	-	1.3	4.7	4.6		
23	-	0.4	0.6	3.1	3.4	3		
24	-	-	-	-	1.9	6.4	9.1	9.9
25	0.5	0.5	3.4	3.9	17.2	14	17.6	15.2
26	0.3	1	0.7	2.5	7.7	7.4		
27	-	-	-	-	-	1.7	1.7	
28	-	-	-	3.4	12.4	13.6		
29	-	-	-	0.6	6.9	8.1		
30	-	-	2.2	8.4				
31	-	-	-	-	2.1	1.8	1.9	1.7
32	0.8	0.6	0.6	0.8	2.5	2.8		
33	0.7	0.8	0.8	1.7	1.9	2.3		
34	-	-	-	-	4.7	5.1		
35	-	-	-	-	1.4	1.7	5.3	2.4
36	-	-	4.4	4.6	4.2			
37	-	-	-	-				
38	-	-	-	-	0.3	1.4	3.1	2.9
39	-	-	-	-	1.3	5.4	5.4	
40	-	-	-	-	0.3	3.1	3.4	
41	-	-	-	-	2.3	2.3	2.1	2.2
42	-	-	-	-	3.3	3.3		
43	-	-	-	-				

Table 3: Concentrations of morphine (ng/mg) in the hair segments of female subjects after different opiate withdrawal times.

"-" means not detected

Num	Hair segment							
	1	2	3	4	5	6	7	8
	(0-1cm)	(1-2cm)	(2-3cm)	(3-4cm)	(4-5cm)	(5-6cm)	(6-7cm)	(7-8cm)
1	-	1.2	1.6	1.3	13.8	12.4	12.2	12.2
2	-	-	-	-	-	1	1	1.3
3	-	-	-	0.6	3	3.9	3.7	
4	-	-	-	9.4	10.2	8.8	13.9	15.3
5	-	2.8	7.8	7.5	7.9	7.3	7.6	
6	0.6	0.8	6.3	7.2	8.3			
7	-	-	2	5.7	5.8	3.8	3.6	
8	-	-	4.1	21.2	34.1	33	29.2	31.3
9	-	-	-	7.8	18.6	21	19.9	
10	-	-	11.6	11.9	18.2	25.2	21.8	26.7
11	3.2	3.3	4.8	5.2	6.8	4.8		
12	0.5	1	2.8	8.8	9.6	10.4		
13	-	-	-	-	1.4	4.2		
14	-	-	3.7	4.6	9.3	10.4	12.5	
15	-	0.4	3.3	3.6	15.2	26.4	23.6	34
16	0.1	0.7	18.5	18.8	18.5			
17	-	-	2.7	4	6.8	6.9		
18	-	2.6	2.6	2.4				
19	-	1.5	6.9	6.7	5.7	11.2	19.5	17.4

20	-	-	1.2	11.3	5.3	6		
21	-	-	-	0.5	0.6	0.4	0.4	0.4
22	-	-	-	-	1.6	1.5		
23	1.1	0.7	0.8	2	2.3	2.3		
24	-	-	-	-	0.6	3.4	6.1	4.8
25	-	-	0.8	8.9	12.5	16.4	18.5	14.3
26	-	-	-	0.6	3.5	3.5		
27	-	-	-	-	0.3	1.1	0.9	
28	-	-	0.5	4.4	6	5.2		
29	-	-	-	2.9	20.7	21		
30	-	-	0.7	6.6				
31	-	-	-	-	4.6	4.6	4.5	4.9
32	-	0.1	0.2	4.1	3.3	5.7		
33	-	1	4.4	22.8	15	15.9		
34	-	-	-	0.1	3.3	1.8		
35	-	-	-	-	0.2	0.7	1.5	1.6
36	-	1.1	5.6	9.6	6.8			
37	-	-	-	-				
38	-	-	-	-	0.6	0.3	0.4	0.3
39	0.4	0.6	1.2	3.2	8.3	7.8	7.1	
40	-	-	-	1.8	1	1.3	1	
41	-	-	-	-	0.9	0.5	1.2	1.3
42	-	-	0.3	1.3	2.4	4.4		
43	-	1.5	3.8	3.8				

Table4: Concentrations of codeine (ng/mg) in the hair segments of female subjects after different opiate withdrawal times.

"-" means not detected

Male hair (cut hair) analysis results

The hair of 26 male subjects was analysed. Because the hair lengths of all of the samples were shorter than 1 cm (the growth time of hair shorter than 1 month), no segmentation was performed. The time of withdrawal of the seventeen subjects was not longer than one month. All of these seventeen hair samples contained detectable 6-acetylmorphine (ranging from 0.4 to 4.4 ng/mg, mean = 1.4 ng/mg) (Table 5). However, only three samples

contained detectable morphine, and the concentrations were 0.5 ng/mg, 0.7 ng/mg and 1.2 ng/mg (Table 5). Codeine was detected in five of the seventeen samples (ranging from 0.2 to 1.7 ng/mg, mean = 1.1 ng/mg) (Table 5). Negative results were obtained in the other nine hair samples, whose withdrawal times were longer than one month and the hair had been shaved more than once (Table 5).

Withdrawal time	6-acetylmorphine	morphine	codeine	Withdrawal time	6-acetylmorphine	morphine	codeine
<1 month	3.6	0.7	1.6	<1 month	1.9	-	-
<1 month	1.8	-	-	<1 month	2.5	0.5	-
<1 month	0.6	-	-	<1 month	1.1	-	-
<1 month	0.4	-	-	<1 month	0.5	-	1.7
<1 month	0.4	-	-	>1 month	-	-	-
<1 month	0.7	-	0.2	>1 month	-	-	-
<1 month	0.4	-	0.4	>1 month	-	-	-
<1 month	1.6	-	1.5	>1 month	-	-	-

<1 month	1.6	-	-	>1 month	-	-	-
<1 month	0.6	-	-	>1 month	-	-	-
<1 month	0.6	-	-	>1 month	-	-	-
<1 month	0.8	-	-	>1 month	-	-	-
<1 month	4.4	1.2	-	>1 month	-	-	-

Table5: Concentrations of 6-acetylmorphine, morphine, and codeine (ng/mg) in male hair samples.

"-" means not detected

Nail analysis results

The results of nail analyses from all of the subjects are shown in Table 6. The concentrations of 6-acetylmorphine and morphine in the nails collected from the subjects whose withdrawal times were less than two months ranged from 2.2 to 15.3 ng/mg (mean = 14.6 ng/mg) and 10.0 to 75.4 ng/mg (mean = 30.7 ng/mg), respectively.

The concentrations of 6-acetylmorphine and morphine in those nails collected from the subjects whose withdrawal times were more than two months were significantly lower than the samples from individuals with shorter withdrawal times. 6-acetylmorphine, morphine, and codeine were undetectable in some subjects whose withdrawal times were more than two months.

Withdrawal times	6-acetylmorphine	morphine	codeine	Withdrawal times	6-acetylmorphine	morphine	codeine
<1 month	2.2	15.1	3.5		8.4	30.8	2.1
	5.7	15.7	1		7.7	10.9	2.5
	4.3	43.4	1.1		6.2	14.3	2.5
	5.9	25	1.2		9.4	50.3	1.8
	6	19.8	4	Positive rate (%)	100	100	100
	3.4	16.8	1.4				
	5.6	13.5	0.6	2-3 month	17.9	40.1	0.7
	7	17.3	0.6		17.1	13.7	0.7
	4.4	32.2	4.7		5.6	46.5	2.4
	4.2	16.3	1.9		0.8	8.3	1.9
	4.8	33.8	1.9		3.4	12.5	0
	6.7	43.9	0.9		3.7	35.4	0
	3.4	35.2	1		5.2	22	0
	14.5	75.4	1.1		0.9	6.8	0
	12	75.2	1.2		0.7	8.4	0
	5.2	18.8	1.6		11.6	13	0
	15.3	33.2	1.8		0.5	5.8	0
Positive rate (%)	100	100	100		13.8	77.9	0
					3.7	25.9	0
1-2 month	6	23.4	0.9		12.6	60.6	0
	5.3	22.5	1		3.4	36.6	0
	3.4	18.8	1.2		9.2	28.1	0
	4.6	17.7	1.2		3.6	10.4	0
	7.3	37	1.2		3	15.1	0.8
	2.6	15.3	1.2		6.9	45.3	0.5

	3	10	1.5		3.7	14.9	0.5
	11.7	60.3	1.9	Positive rate (%)	100	100	35
	12.8	70.9	2				
	4	20.4	2.3	3-4 month	3.4	15.2	0
	3.9	17.8	3.3		5	26.8	0
	5.2	19.1	1.4		4.3	21.9	0
	12.8	75.3	1		8.1	39.3	0
	4.5	16.7	1.8		0.6	10	0
	13.4	67.1	8.3		6.7	34.5	0
	9.9	32.9	1.2		2.8	38.5	0
	13.1	34.3	1.5		3.6	32.4	1.8
	6.2	10.8	1.1		+	+	0.7
	6.7	21.4	1.7	Positive rate (%)	89	89	22

Table 6: Concentrations of 6-acetylmorphine, morphine, and codeine (ng/mg) in nail samples. "+" means detected, but lower than LOQ (0.05ng/mg)

Discussion

Residual of 6-Acetylmorphine, Morphine and Codeine in Female Hair (Uncut Hair) Segments

Target compounds in hair segments that were grown before withdrawal: The concentrations of the three target compounds in hair, which was grown before drug withdrawal, showed no significant differences between different segments and were in agreement with the references. All of the hair segments have been classified as grown before or after drug withdrawal assuming an average hair growth rate of 1 cm per month [12,16]. The concentrations of 6-acetylmorphine in hair segments that were grown before drug withdrawal were 0.2-96.6 ng/mg (mean =18.4 ng/mg), while the morphine concentrations were 0.4-33.6 ng/mg (mean =5.8 ng/mg), and the codeine concentrations were 0.3-34.1 ng/mg (mean =7.8 ng/mg). These results are similar to those resulting from a statistical analytical [17]. According to the variance-threshold t-test score, there is no significant difference between different segments of hair that were grown before drug withdrawal. These results reflect that the subjects have a long-term history of heroin abuse [18].

Another interesting phenomenon is that the concentration of each compound in the hair of subjects whose time since withdrawal was shorter is higher. According to the variance-threshold t-test, the concentrations of 6-acetylmorphine and morphine in the hair segments that were grown before withdrawal were

inversely proportional to the withdrawal time. These changes in the concentrations may relate to the daily cleaning treatment for all of the tested compounds [19] but more research is needed.

Target compounds in hair segments that were grown after withdrawal: The concentrations of the three target compounds in hair that was grown after drug withdrawal decreased at a certain rate as the withdrawal time become longer. This phenomenon shows that the concentrations of the three target compounds in the hair decreased. The decreasing trends of 6-acetylmorphine and morphine were synchronous, but the decreasing trend of codeine was not regular, which may be because of the complicated sources of codeine in hair (seeds, pharmaceutical preparations, impurities or other substances) [20].

The decreases rates were calculated by the concentrations of the target compounds in five consecutive hair segments from those females whose withdrawal time was longer than 2 months. In order to show the trend of this decreases in a better way. The DAS version 2.1.1 software was used (Mathematical Pharmacology Professional Committee of China, Shanghai, China). A statistical analysis of the 6-acetylmorphine, morphine and codeine in hair was performed using a first-order kinetic model. Assuming a hair growth rate of 1 cm/month, a 1-cm-long segment represented approximately 1 month of growth. The decreases rate

constant (K_e) was calculated with linear regression analysis of the terminal points of the semi-log plot of the hair concentration against time. The decreases "half-life" was calculated by $0.693/K_e$.

The results of the calculations are shown in Table. 7. It is shown that the "half-lives" of 6-acetylmorphine, morphine, and codeine were 1.10 ± 0.39 months,

1.68 ± 0.44 months, and 1.52 ± 0.45 months, respectively. Based on the variance-threshold t-test, these results were similar to our previous data (0.88 ± 0.40 months, $t=0.79$; 0.73 ± 0.24 months $t=0.38$, and 0.61 ± 0.22 months, $t=0.19$, respectively) [21]. Through the analysis of our data, the "half-lives" of 6-acetylmorphine, morphine, and codeine were all approximated as 1 month.

Num#	Half-life(month)			Elimination rate constants (ng/mg/month)		
	6-acetylmorphine	morphine	codeine	6-acetylmorphine	morphine	codeine
Range	0.59-2.36	0.94-2.39	0.83-3.84	0.70-171.66	0.20-29.89	0.19-36.15
Mean	1.10	1.68	1.70	59.64	6.84	8.84

Table 7: The "half-lives" (in months) and elimination rate constants of 6-acetylmorphine, morphine, and codeine (a hair growth rate of 1cm/month was assumed in the calculations) in female hair samples.

Residual and residual of 6-acetylmorphine, morphine and codeine in male hair (cut hair): The target compounds were hardly detectable in the male hair (*cut hair*) samples. 6-acetylmorphine could only be detected in the hair obtained from males whose withdrawal time was less than 1 month, but the concentration was significantly lower than mean level in female hair samples (1.4 ± 0.8 ng/mg vs. 4.5 ± 3.2 ng/mg, $t=|-2.47| > 1.70$). Morphine and codeine were only detected in the hair samples of a few males whose withdrawal times were less than 1 month. All of the hair analysis results of those males whose withdrawal time was longer than 1 month were negative for the analytes.

The hair of the males was cut when heroin withdrawal began. Therefore, only a few target drugs remained in the stubble and sebaceous gland. Sebocyte's cytoplasm distends with lipid droplets during its differentiation, which will coalesce and finally rupture the cell approximately after 3 weeks. Contents will then "contaminate" the hair. This is one reason that small amounts of drug were still detectable in hair. This was considered to be the reason that only a low level of 6-acetylmorphine was detected in the *cut hair* of subjects whose withdrawal times were less than 1 month. Even in *uncut hair*, morphine and codeine had lower levels, which made it harder for them to be detected in *cut hair*. Males whose withdrawal time was longer than 1 month had their haircut at least twice and the newly grown hair contained no history of heroin use. Consequently, there were negative results in those subjects' hair analyses.

Residual of 6-acetylmorphine, morphine and codeine in nails: Nails were obtained and analysed from all of the

subjects with different withdrawal times. According to the variance-threshold t-test, there were no significant differences between the nails of individuals with different withdrawal times ($t < 2.04$). In our previous work, a long-term follow-up analysis has been used to understand the concentration change in nails after a single drug dose [22]. According to this discussion, the drugs secrete into the nails mainly from the nail-beds and the matrix of the nails. Therefore, for a heroin abuser, the target compounds are continuously secreted into the nail during his/her abuse. The newly grown nail after withdrawal still does not grow to the hyponychium (the growth rate of nails is approximately 1mm/week, and the mean length of the nail plate is approximately 10mm [23-29]). And on the other hand, the length of nails is generally more than 7-10 mm and depends on the specific finger. In this study, the nails from all fingers of the same individual have been analyzed together. Consequently, relatively stable levels of the target compounds in the nails showed nail maybe not good enough in "negative testification".

Conclusion

The "half-lives" of opiates in *uncut hair* are approximately 1 month, but the Residuals of opiates in *cut hair* are completely different. Under the simplest condition, the concentrations of opiates in *cut hair* (which was cut after the discontinuation of opiate abuse) are significantly lower than mean levels in *uncut hair* and cannot be detected after a secondary cut. This suggests that the time of the last haircut is important in the interpretation of results. For a better understanding of the hair analysis results, the haircut history of the subject should be considered. Nails still cannot prove a "clear"

history of drug consumption, but a relatively stable level of the target compounds in nails can indicate a long-term history of drug contact.

Ethical Approval

Necessary ethical approval was obtained from the ethics committee of Institute of Forensic Science, Ministry of Justice. And Informed consent was obtained from all patients.

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