Hair Analysis for Morphine in Heroin Addicts in Thailand

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Morphine analysis in the hair of heroin addicts in Thailand by radioimmunoassay (RIA) was performed. Samples of hair were taken from 50 patients who had been treated for heroin addiction at the Northern Narcotic Control Center, Mae Rim, Chiang Mai University. The hair samples were cut at the ankle and immersed in a pronase solution for 3 hours. The concentration of morphine in the hair was determined by RIA. The results showed that the morphine concentration in the hair ranged from 0.066 to 0.48 mg/g, with a mean of 0.22 mg/g. The cut-off level for morphine was 0.051 mg/g. The results indicate that hair analysis is a reliable method for detecting morphine in the hair of heroin addicts.

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Hair analysis for morphine was studied in heroin addicts by using radioimmunoassay (RIA). About 50 mg of hair was cut from each heroin abuser who had come to the Northern Narcotic Control Center for drug detoxification. Different methods of hair washing and drug dissolution protocols were compared. Washing hair with distilled water (DW) three times followed by acetone and enzyme pronase hydrolysis yielded the highest morphine level. The range of morphine in the hair of heroin addicts was 0.48–11.48 ng/mg of hair and the mean ± SEM was 4.47 ± 0.33 ng/mg of hair. While the morphine level of control hair was 0–0.051 ng/mg of hair and the mean ± SEM was 0.011 ± 0.004 ng/mg of hair. The cut off level calculated from the mean + 3SD of the control group was 0.066 ng/mg of hair. The mean morphine level of addicts was about 400 times higher than control and also higher than the cut off level detected in our assay system. There was no linear correlation between urine and hair morphine levels. There was no linear correlation between the hair morphine levels and the time-dose profile of heroin abuse. This study showed that hair analysis for morphine could be detected in heroin abusers by RIA with no false negative, compared to morphine urinalysis that gives about an 8 % false negative.

Key words: morphine, analysis, radioimmunoassay, hair, heroin addict
Introduction

Heroin abuse is still an important problem in Thailand, with an estimated 214,180 abusers in 1993 (Poapongsakorn et al., 1995). The prevalence rate of heroin addiction was approximately 4 addicts per 1000 population. Heroin, when administered into the body, is metabolized to 6-monoacetylmorphine (6-MAM) and subsequently morphine. Morphine is then conjugated in the liver and excreted in urine as morphine 3- or 6-glucuronides (Way et al., 1960). The detection of free or conjugated morphine in blood or urine is an indicator of heroin consumption. Due to a very short half-life, heroin is present in the circulation for only a few hours (Jenkins et al., 1994). Heroin metabolites, 6-MAM and morphine, also disappear from the circulation within several hours after the last use (Jenkins et al., 1994).

The identification of free or conjugated morphine in urine is an ordinary way for the diagnosis of heroin abuse. However, these metabolites cannot be found in urine 96 hours after the use of heroin or morphine (Yeh et al., 1976). Hair analysis for morphine is an alternative way to identify heroin addicts (Dupont and Baumgartner, 1995). The detection of morphine in hair has been reported since 1979 (Baumgartner et al., 1979). Since then, many studies have been conducted (Staub, 1995). Hair analysis for drugs of abuse has many advantages (Dupont and Baumgartner, 1995). It can detect heroin metabolites for weeks or months after use. This wide surveillance window makes hair analysis a better method of identifying heroin abusers. Pretreatment of hair can eliminate some external contamination, which cannot be done with urine samples. Finally, in terms of specimen collection, gathering hair is not invasive and much easier than collecting urine. If supervision is necessary while collecting specimens, cutting hair will not embarrass subjects as much as urine collection. In Thailand, the detection of heroin metabolites in hair has not yet been studied. In this study, the detection of morphine in the hair of heroin addicts admitted to the Northern Narcotic Control Center is reported. The radioimmunoassay technique was used for the analysis of morphine.

Materials and Methods

This study was approved by the Research Ethical Committee, Faculty of Medicine, Chiang Mai University, and supported by the Faculty of Medicine Endowment Fund for Medical Research, Faculty of Medicine, Chiang Mai University.
Subjects and specimens

Subjects were heroin addicts who came to the Northern Narcotic Control Center for drug detoxification. Patients were informed about the study by attending physicians at the center. On admission, about 50-100 milligrams of hair strands was cut as close as possible to the scalp of the posterior vertex of each subject, then kept in a clean plastic container. A urine samples was also collected on the admission day. Their history of drug of abuse was recorded following the center’s protocol. Control hair was collected from the staff of this research who had no history of drug abuse. Hair and urine samples were brought to the Department of Forensic Medicine, Chiang Mai University and kept in a -4 °C refrigerator until analysis.

Chemicals

Dithiothreitol, sodium dodecylsulfate (SDS), Tris buffer and enzyme pronase were obtained from the Sigma company. Acetone was purchased from the Merck company. Phosphate buffer was prepared in the laboratory by adding 400 ml of 0.05 M Na₂HPO₄ in 100 ml of 0.05 M K₂HPO₄ and adjusted pH to 7.6 by adding diluted HCl. Radioimmunoassay Coat-A-Count Serum Morphine and RIA Coat-A-Count Urine Morphine were purchased from the Diagnostic Product Corporation, Los Angeles, CA, USA. The RIA Coat-A-Count Serum Morphine is very specific to free morphine and it shows less than 1% cross reactivity to monoacetylmorphine, morphine-glucuronide or other opiates (Cassani and Spiehler, 1993).

Effect of different washing and extraction on hair analysis for morphine

Hair specimens from 10 heroin addicts and 7 control subjects were used. For comparison of different methods in the same subject, approximately 100-mg of hair from each individual was divided into two parts (50 mg each) then subjected to two different washing processes.

Hair washing with 0.1% SDS and distilled water (SDS/DW): The washing procedure was conducted in accordance with the method described by Nakahara et al. (1992) with some modifications. Briefly, the whole strands of hair weighed 50-mg were kept in a 10-ml screw-capped glass tube. Then 5 ml of 0.1 % SDS were added. The tube was sonicated for 1 min and then the washed solution was decanted. This step was repeated three times. Then the hair was washed for the last time with 5 ml of distilled water.
Hair washing with distilled water and acetone (DW/acetone): Following the method described by Moeller & Mueller (1995), 50-mg of hair strand was kept in a 10-ml screw-capped glass tube. The hair was then washed and sonicated for 1 min with 5 ml of distilled water three times and with 5 ml of acetone for the last wash.

After washing, the hair was dried in a 50 °C oven for about one hour and cut into small pieces (about 1 mm in length or less). Hair in the same washing process was divided into two groups (about 20 mg in each) and subjected to two different methods of drug extraction.

Enzymatic hydrolysis (Offidani et al., 1993; Strano-Rossi et al., 1995): Five hundred μl of 6 mg/ml dithiothreitol in Tris buffer pH 7.2 were added to each hair sample and incubated at 40 °C for two hours. Then, 500 μl of 2 mg/ml of pronase in Tris buffer pH 7.2 were added. The tubes were vortexed and incubated overnight at 40 °C. After incubation, the tubes were centrifuged at 3,000 rpm for 2 min. The supernatants were used for morphine analysis.

Direct phosphate buffer dissolution (Moeller and Moeller, 1995): One ml of phosphate buffer (pH 7.6) was added to each hair sample and incubated overnight at 40 °C. The samples were then centrifuged at 3,000 rpm for 2 min and the supernatants were used for morphine analysis.

Detection of morphine in hair extracts by RIA

Morphine in hair extract was detected using radioimmunoassay Coat–A– Count Serum Morphine that we used previously to measure morphine level in addicted animal (Sribanditmongkol et al., 1994). Briefly, 25 μl of standards or samples were added to a morphine antibody coated tube. Then 1 ml of 125I-labeled morphine was added. The tube was vortexed for 1 min and incubated for 60 min. The reaction was stopped by decanting the solution. The radioactivity was counted by a gamma counter for 1 min. The levels of morphine in different groups of hair washing and extraction were compared by a repeated ANOVA, using Graphpad Instat software.

Comparison of hair and urine morphine analysis in heroin addicts and controls

Hair specimens from 65 heroin addicts and 17 control subjects were used. About 30 mg of hair from each individual were washed with distilled water and acetone following
the method mentioned previously. After drying, the hair was cut and weighed at 20 mg and then subjected to enzymatic hydrolysis. Morphine was detected in hair extracts using RIA. The level of morphine in the hair of addicts was compared to the level in that of controls by the nonparametric Mann–Whitney test. Urine samples were analyzed for morphine by using RIA Coat–A–Count Urine Morphine that was performed previously (Offidani et al., 1993). The relationship between morphine levels in hair and urine and the relationship between the levels of morphine in hair and the time–dose of heroin abuse were compared by linear regression analysis.

Results

Effect of different washing and dissolution on hair analysis for morphine

Morphine was measured in hair extracts subjected to different washing and extraction protocols. Using a repeated ANOVA followed by a post–hoc test, there was a significant difference of morphine levels in these protocols (p=0.0281, Figure1). The result showed that hair subjected to a DW/aceticone wash followed by enzyme pronase hydrolysis yielded the highest level of morphine in the extracts. Therefore, this protocol was used in the subsequent experiment.

![Figure 1](image.png)

**Figure 1** Comparison of different hair washing and dissolution of hair morphine levels.

- SDS/DW : Hair washed with 0.1% SDS 3 times followed by distilled water
- DW/Acetone : Hair washed with distilled water 3 times followed by acetone
- Pronase : Hair subjected to enzyme pronase extraction
- Phosphate : Hair subjected to phosphate buffer dissolution

*p<0.05 compared to DW/Acetone/Pronase*
Comparison of hair and urine morphine analysis in heroin addicts and controls

There were 65 heroin addicts (62 males and 3 females) who came to the Northern Narcotic Control Center for cessation of addiction. The subjects were between 16–57 years old (Tables 1). All of them abused heroin, either by intravenous injection, inhalation or both. The duration of addiction was from 1 month to 25 years. Almost one half of them had enrolled in the detoxification program before, but became addicted to heroin again. Most of the subjects had consumed heroin a few hours before coming to the center. For the negative control, there were 17 subjects (7 males and 10 females). All of them had no history of drug abuse (Table 1).

Table 1 Summary of the characteristics of groups studied

<table>
<thead>
<tr>
<th></th>
<th>Addiction</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>65</td>
<td>17</td>
</tr>
<tr>
<td>Sex</td>
<td>male: 62; female: 3</td>
<td>male: 7; female: 10</td>
</tr>
<tr>
<td>Mean ± SEM of age (year)</td>
<td>32 ± 1.32</td>
<td>29 ± 2.24</td>
</tr>
<tr>
<td>Range of age (year)</td>
<td>16 – 57</td>
<td>17 – 46</td>
</tr>
<tr>
<td>Drug of abuse</td>
<td>heroin (inhalation, intravenous injection)</td>
<td>none</td>
</tr>
<tr>
<td>Mean ± SEM of average daily heroin use (gm)</td>
<td>0.57 ± 0.04</td>
<td>none</td>
</tr>
<tr>
<td>Range of average daily heroin use (gm)</td>
<td>0.15–1.2</td>
<td>none</td>
</tr>
<tr>
<td>Duration of addiction</td>
<td>1 mo – 25 yr</td>
<td>none</td>
</tr>
<tr>
<td>Duration of the last heroin use</td>
<td>1 – 48 hr</td>
<td>none</td>
</tr>
</tbody>
</table>

Morphine detected from hair of all heroin addicts was 0.48–11.48 ng/mg of hair. The mean ± SEM was 4.47 ± 0.33 ng/mg of hair. On the other hand, only 8 of 17 control hair extracts were positive for morphine by RIA. The range of morphine detected in the control was 0–0.051 ng/mg of hair and the mean ± SEM was 0.011± 0.004 ng/mg of hair. By using the nonparametric Mann–Whitney test, the levels of morphine in the hair of both groups were significantly different (p<0.0001, Table 2). For urine analysis, all urine specimens from heroin addicts were positive for morphine measured by RIA. The range of urine morphine was 3.4–105,960 ng/ml and the mean ± SEM was 14,863 ± 2,383
ng/ml. Compared to the control group, the urine morphine was between 0–10.7 ng/ml and the mean ± SEM was 7.12 ± 1.067 ng/ml. The levels of urine morphine in both groups were significantly different (p<0.0001, Table 2).

Table 2 Comparison of hair and urine morphine in addiction and control groups

<table>
<thead>
<tr>
<th></th>
<th>Addiction</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>65</td>
<td>17</td>
</tr>
<tr>
<td>Number of hair morphine positive results</td>
<td>65</td>
<td>8</td>
</tr>
<tr>
<td>Range of hair morphine (ng/mg hair)</td>
<td>0.48 - 11.48</td>
<td>0 - 0.051</td>
</tr>
<tr>
<td>Mean ± SEM of hair morphine (ng/mg hair)</td>
<td>4.47 ± 0.33*</td>
<td>0.011 ± 0.004</td>
</tr>
<tr>
<td>Number of urine morphine positive results</td>
<td>64 (of 64)</td>
<td>9 (of 10)</td>
</tr>
<tr>
<td>Range of urine morphine (ng/ml)</td>
<td>3.4 - 105,960</td>
<td>0 - 10.7</td>
</tr>
<tr>
<td>Mean ± SEM of urine morphine (ng/ml)</td>
<td>14,863 ± 2,383*</td>
<td>7.12 ± 1.067</td>
</tr>
</tbody>
</table>

*p<0.0001 compared to control by the Mann-Whitney test

The relationship between urine and hair morphine in the same subjects is shown in Figure 2. Also the relationship between hair morphine and the amount and duration of heroin use (gm-month) was demonstrated in Figure 3. Using least square analysis, the slopes of regression line of both correlation were not significantly different from zero (p = 0.2521 and p = 0.1259, respectively). The result showed that there was no linear correlation between the level of hair morphine and the level of morphine in urine and the amount and the duration of heroin abused.

Figure 2 Correlation between urine morphine and hair morphine in heroin addicts

Figure 3 Correlation between hair morphine and dose-duration of heroin abuse
Discussion

Hair is a uniformed, but very complex and stable structure (Harkey, 1993). It is also not easily decomposed (Harkey and Henderson, 1989). So, it can be used to detect drugs even in decomposed remains. The main compositions of hair are keratin, which is approximately 65–95% of total hair contents, water (15–35%), and lipid (1–9%) (Harkey, 1993). Hair originates from hair follicle that is embedded in the epidermal epithelium of the skin and surrounded by capillary plexuses and secretory glands. During growth, substances from the circulation can be deposited in hair and transported (Bost, 1993). Many drugs and substances, therefore, can be detected in human hair (Harkey and Henderson, 1989). In past decade, drugs abuse such as cocaine, opiates and amphetamines have been identified in hair (Cone and Jufer, 1995; Moeller et al., 1993; Moeller and Moeller, 1995; Strano-Rossi et al., 1995; Staub, 1995). For heroin abuse, the detection of morphine in hair was first reported by Baumgartner and his colleagues in 1979 (Baumgartner et al., 1979). Unlike in urine, monoacetylmorphine is the main metabolite of heroin detected in hair. It is approximately 2–3 times higher than free morphine presented in hair (Bermejo-Barrera and Strano-Rossi, 1995; Cassani and Spiehler, 1993; Moeller et al., 1993). Heroin itself is also identified in the hair but in smaller amount than morphine (Goldberger et al., 1991; Moeller and Moeller, 1995; Strano-Rossi et al., 1995). The mechanism by which heroin metabolites are deposited in hair is not clear. It is proposed that these drugs incorporate into hair either by simple passive diffusion or complex multicompartment mechanisms (Harkey and Henderson, 1989; Henderson, 1993). The drug, however, seems not to bind strongly to the sulphydryl group of hair matrix like heavy metals (Baumgartner and Hill, 1993).

Morphine can be detected in hair approximately a week after a single use (Cone, 1990) and can be detected for a period of weeks to months (Dupont and Baumgartner, 1995). However, about 50% of drugs presented in hair is decreased after 5 months due to normal hair hygiene (Blank and Kidwell, 1995).

After heroin administration, its metabolites also present in scalp, pubic and axillary hair (Kintz and Mangin, 1993). However, hair from the scalp seems to be more appropriate for collection. The posterior vertex of the scalp is the recommended site of hair collection, since its growth rate is quite consistent and shows less individual variation (Harkey and Henderson, 1989). The recommended hair use is 10–30 mg (Baumgartner and Hill, 1993). In this experiment, the whole strands of hair from posterior vertex were
used for analysis. After optimizing the analysis condition, only 20 mg of hair were used and this amount of cut hair was cosmetically insignificant.

The main concern of hair analysis is external contamination, which causes an evidentiary false positive (Blank and Kidwell, 1995). The decontamination of hair is an important step (Baumgartner and Hill, 1993). The purpose of hair washing is to eliminate external drug contamination that may form from air or direct contact. The idea is to remove as much external contamination as possible, but not affect the drugs incorporated in hair shafts. The process of decontamination varies from using distilled water to some detergents (Baumgartner and Hill, 1993). The best decontamination protocol is not conclusive. In this experiment, two different washing methods, SDS/DW and DW/acetone, were compared in hair from the same subjects. After washing, the hair was subjected to the step of drug dissolution and extraction. In this step, hair can be digested by alkaline or acidic solution or by enzymatic hydrolysis (Harkey, 1993). Moeller and Mueller (1995) reported direct dissolution of drugs from hair by a buffer solution, but they pulverized the hair before use. In this present study, two different methods, enzymatic hydrolysis and direct phosphate buffer dissolution, have been compared in the hair of the same subjects. However, instead of pulverization, the hair was cut into small pieces of about 1 mm in length or smaller. After overnight incubation, the samples were centrifuged and morphine was measured in the supernatant using the RIA technique. The result showed that hair washed with DW/acetone and hydrolyzed by pronase yielded the highest morphine level (Figure 1). Baumgartner and Hill (1993) proposed that drugs might incorporate to a rope-like macroprotein structure of hair. Therefore, the liquefaction of hair by an enzyme can dissolve drugs better than other extraction procedures. This protocol, therefore, is applied to the detection of morphine in the hair of addicts.

Many analytical methods have been used to detect morphine in hair. The most common and recommended technique is gas chromatography/mass spectrometry (GC/MS) (Staub, 1995). Radioimmunoassay (RIA) is the second most common method used, since it is rapid, easy to use and inexpensive compared to GC/MS. Other methods such as FPIA (fluorescent polarization immunoassay), HPLC (high pressure liquid chromatography), MS-MS (mass spectrometry-mass spectrometry), TLC (thin layer chromatography), and capillary electrophoresis (CE) have been reported in hair analysis for opiates (Staub, 1995; Tagliaro et al., 1995). The RIA Coat-A-Count Morphine is very specific to free morphine and it shows less than 1% cross reactivity to other opiates or conjugated morphine (Cassani
and Spiethler, 1993). Although, this Coat-A-Count Morphine is prepared for serum, which is different from the matrix in hair extracts, it has been shown that this matrix did not interfere with an RIA antibody (Offidani et al., 1993; Strano-Rossi et al., 1995).

The objective of this study is to determine the method and technique for the detection of morphine in hair. All the subjects that came to the Northern Narcotic Control Center admitted that they were heroin abusers (Table 1). They wanted to abstain, but before coming to the center this time, most of them used drugs again to avoid the abstinence syndrome. These addicts had been using heroin for a long period of time, either by intravenous injection or inhalation or both. Morphine was detected in the hair of all these addicts. The range of hair morphine level in the addiction group was 0.48–11.48 ng/mg of hair by RIA, and the mean ± SEM was 4.47 ± 0.33 ng/mg of hair. On the other hand, hair morphine was detected in only 8 of 17 control subjects. The morphine level detected in the control hair was between 0–0.051 ng/mg of hair and the mean ± SEM was 0.011 ± 0.004 ng/mg of hair. Using the mean + 3 SD of the control group (Baumgartner et al., 1989), the cut off level in our study was 0.066 ng/mg of hair. The levels of morphine detected in drug-free subjects in this study were lower than this cut off level. Staub (1995) reviewed that the level of morphine in the hair of heroin addicts was 0.08–27.10 ng/mg of hair. Using the RIA technique, Cassani and Spiethler (1993) reported that the level of morphine was 0.1–10 ng/mg of hair and the cut off level, 0.08 ng/mg of hair. In this present study, the level of morphine in the hair of heroin addicts fell between the range of previous reports and was higher than the cut off level. The lowest hair morphine level detected in the addiction group was about 10 times higher than the highest hair morphine level in the control group. This difference allows hair analysis to discriminate between heroin addicts and non-heroin users.

For urinalysis, morphine was detected in all urine samples of heroin addicts. Following the National Institute on Drug Abuse (NIDA) criteria, the cut off level of urine morphine is 300 ng/ml (Cassani andSpiethler, 1993). The levels of urinary morphine in five addicts (8% of addiction) were lower than the urine cut off level, and thus considered negative. But the levels of morphine in the hair of all addicts were higher than the hair cut off level. This supports that hair analysis is more sensitive than urinalysis in identifying heroin abuse.
To investigate the relationship between the levels of urine and hair morphine in the addiction group, there was no linear correlation between both (Figure 2). The lack of correlation may be due to urine morphine representing a short-term exposure and reduced level following a time profile, while hair morphine represented long-term exposure and showed some level of accumulation. Also the main metabolites of heroin detected in hair and urine are different.

Since the hair morphine represented long-term exposure, the correlation between hair morphine and the time-dose profile of heroin abuse was compared (Figure 3). There was no linear correlation between the levels of hair morphine and the amount and duration of self-reported use ($r = 0.2070$, $p = 0.1259$). The lack of correlation might be due to the inaccuracy of amount of drugs reported by addicts. It is quite difficult for abusers to recall how many drugs they used in the past. This result is similar to previous report that the concentration of opiates in hair was not correlated to the dose of heroin reported use (Harkey and Henderson, 1989). Notwithstanding, there was a good correlation between the morphine in hair and the total dose of heroin in the controlled heroin-maintainance subjects (Kintz et al., 1998) and the animal study (Baumgartner et al., 1989).

**Summary**

Morphine could be identified in the hair of heroin addicts by using the RIA technique. The mean level of hair morphine in heroin addicts was about 400 times higher than the mean level of the control group. The lowest hair morphine level detected in the addiction group was about 10 times higher than the highest level in the control group. All the hair morphine levels of the addiction group were higher than the calculated cut off level and the levels reported elsewhere. None of the hair morphine levels in the control subjects were higher than the cut off level. This difference allowed hair analysis to discriminate between heroin addicts and non-heroin users. Compared to urine morphine analysis, there was no false negative in hair analysis while about 8% of urine tests reported a false negative. This study is useful for the detection of heroin use and identification of heroin addicts. A field study should be conducted using hair analysis.
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References


Dupont RL. and Baumgartner WA. 1995. Drug testing by urine and hair analysis: complementary features and scientific issues. Forensic Sci Inter 70 : 63-76.


