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# Postmortem Blood Free and Total Morphine Concentrations in Medical Examiner Cases

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**ABSTRACT:** This purpose of this study was to determine the relationships between postmortem free morphine and total morphine levels in a large series of medical examiner morphine and heroin related deaths. Free morphine, total morphine, and 6-monoacetylmorphine (6-MAM) concentrations were measured by gas chromatography-mass spectrometry (GC-MS) in 87 medical examiner cases over 20 months. The mean total morphine concentration, mean free morphine concentration, and mean percent free morphine for all cases were: 2.3 mg/L (SD 5.2 mg/L), 0.5 mg/L (SD 1.6 mg/L), and 19.4% (SD 22.8%); respectively. Regression analyses showed weak correlations between total and free morphine concentrations over the entire concentration range (0 to 36.6 mg/L, $r = 0.603$, $n = 91$) and over a subset concentration range of 0 to 1.0 mg/L ($r = 0.369$, $n = 54$). Twenty-three out of 56 (41%) tested positive for 6-MAM, indicative heroin abuse cases. Lower total and free morphine concentrations and a higher percent free morphine were found in individuals with detectable 6-MAM. Comparing blood concentrations for cases with and without detectable 6-MAM demonstrated mean total morphine concentrations of 0.9 mg/L versus 2.1 mg/L ($p = 0.05$), mean free morphine concentrations of 0.3 mg/L versus 0.4 mg/L ($p = 0.21$), and mean percent free morphine of 34.7% versus 13.7% ($p < 0.003$), respectively. Our findings demonstrate higher free to total morphine ratios in individuals with detectable 6-MAM than in individuals without 6-MAM. The database established in this study may assist medical examiners in the evaluation of postmortem blood opiates regarding the cause of death in opiate related ingestion cases.

**KEYWORDS:** forensic science, forensic toxicology, morphine, free morphine, heroin, death

Both heroin and morphine are common drugs of abuse (1,2). Heroin, after being injected, is rapidly converted to 6-monoacetylmorphine (6-MAM) in human blood as a result of hydrolysis by cholinesterase and as a result of spontaneous hydrolysis. Its half-life is three to six min. 6-MAM is further deacetylated to morphine with a half-life of 5 to 25 min. Morphine, whether obtained from direct ingestion or as a metabolic product of heroin, is then converted to the inactive morphine-3-glucuronide and active morphine-6-glucuronide, with the former produced in larger concentrations than the latter (3). Although the detection of 6-MAM is indicative of recent heroin exposure (4), differentiating heroin ingestion from other opiates, particularly morphine, is difficult when only morphine or its metabolites are detected (5,6). This is due to the very short half-lives of heroin and 6-MAM.

Interpretation of postmortem blood morphine concentrations can be difficult as blood morphine concentrations in heroin related deaths overlap with those found in nondrug-related deaths such as homicides, and analgesic concentrations of morphine overlap with those reported in opiate-related deaths (7). The ratio of free morphine to total morphine may provide information about the time of exposure to the opiate. Garriot and Sturner were the first to show a relationship between free morphine and survival interval following heroin injection in 22 individuals (8). Several authors since have shown higher mean percent free morphine concentrations in rapid deaths following heroin or morphine ingestion, with percent free morphine ranging from 51% to 75% of the total morphine (9–11).

In this study, we measured blood free and total morphine concentrations on all requests for postmortem opiate quantitation received over a 20 month period from medical examiner cases. Review of the available case histories was performed in relation to the cause of death and complete toxicology, and correlated with the free and total morphine concentrations and percent free morphine.

## Methods

### *Cases and Specimen Collection*

Beginning in August 1998, both free and total morphine concentrations were determined for each postmortem blood opiate quantitation request from medical examiner cases. As part of routine toxicology evaluation, urine and/or blood drug screens were performed by immunoassay (Microgenics Corporation, Pleasanton, CA) and high pressure liquid chromatography (Remedi HS Drug Profiling System, Bio-Rad Diagnostics Group, Hercules, CA). If 6-MAM, morphine or morphine metabolites were identified by the drug screen, total and free morphine were quantitated by gas chromatography-mass spectrometry (GC-MS). Additionally, one case was identified by detecting 6-MAM on a postmortem nasal swab and three cases were identified by detecting 6-MAM in materials found next to the body (syringe or spoon). 6-MAM, which identifies cases of recent heroin exposure, was not quantitated in this study. Whole blood samples were collected in gray top tubes containing 17.5 mg NaF, and stored at 4°C until analysis. Review of the investigative reports was performed to gather demographic information, cause and manner of death, and results of toxicologic screens and quantitations. All requests during the study period were included except those for which insufficient quantity of blood was available for both free and total morphine quantitations.

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*Analysis*

Total and free morphine concentrations were quantitated in whole blood by GC-MS on a Hewlett-Packard 5972 mass selective detector following chromatography on a 5890 gas chromatograph equipped with a 30 m DB-5 capillary column (Agilent Technologies, Palo Alto, CA). A Unix-based Target Thru-Put operating software computer system was used for data compilation. Standards and deuterated internal standards were obtained from Radian Corp (Austin, TX). Using the total morphine concentration measurements as an example, 1 mL of whole blood (appropriate standards, controls, and case) was mixed with 50 μL of morphine-d3 (10 ug/mL, internal standard), to which was added 1 mL 20% TCA and 0.5 mL concentrated HCl. The mixture was vortex mixed and centrifuged for 5 min. The supernatent was then placed in a heating block at 120°C for 20 min. After cooling to room temperature, the pH was adjusted to between 7.0 and 8.0. The sample was then vortex mixed for 30 s after adding 6 mL of 0.1 M phosphate buffer and centrifuged for 15 min at 2000 rpm. Supernatant was poured into the extraction column preconditioned with methanol and water (Bond Elut columns, Varian, Harbor City, CA) and allowed to run through for 2 min. Each column was rinsed with 3 mL water, 3 mL 0.1M acetate buffer, and 2 mL methanol. Methylene chloride/isopropanol (80:20, 2 mL) with 2% ammonium hydroxide was subsequently passed through the column and collected. Each tube was evaporated at 30 to 40°C with nitrogen using a Pierce evaporator. The residue from evaporation was reconstituted with 0.5 mL of ethyl acetate and reevaporated under nitrogen. 50 uL BSTFA (with 1% TCMS) and 30 uL DMF were added to the tubes, and were then vortex mixed and capped. Samples were derivatized at 60 to 70°C for 20 min. After cooling, samples were transferred to the autosampler. The MS was operated in the select ion monitoring mode (SIM), and the following ions were scanned: morphine quantitating ion 429, qualifier ions 414 and 430; morphine-d3 quantitating ion 432, qualifier ions 417 and 433. Standard curves were derived for each analyte. Area ratios for unknowns were used to calculate the corresponding analyte concentrations. Quantitation of free and total morphine was based upon ratios of integrated ion areas to the corresponding deuterated internal standard analogues for each analyte. Ion ratios were calculated by dividing the area of the qualifyer ion by the area of the quantitative ion. Analytes were identified based upon comparison of retention time and ion ratios with the corresponding values of calibration standards assayed in the same run. Limit of detection, limit of quantitation, and limit of linearity were 75, 75, and 1500 μg/L. Values less than 75 μg/L were presented as zero. Any value greater than 1500 μg/L were reanalyzed after dilution. At 0.139 mg/L and 0.405 mg/L, the CV% was 15.1% and 6.9%, respectively. Completeness of hydrolysis of glucuronides were verified using a control containing morphine glucuronide. The procedure for free morphine quantitation was the same with the omission of the acid hydrolysis step.

## Statistics

All calculations were performed on a Compaq Desk Pro computer equipped with a Pentium II chip, using Microsoft Excel Windows Version 4.0 and Statview for Windows Version 5.0.1. Correlation coefficients were calculated by the Pearson Product-Moment method. Statistically significant differences between means were evaluated by the paired t-test. A probability ($p$) of $<0.05$ was considered significant.

## Results

Over a 20 month period, 151 medical examiners cases had requests for free and total opiate concentrations to be measured, for which 91 cases had detectable free and/or total morphine. The mean values (SD, range) for total morphine, free morphine, and percent free morphine in all 91 cases were 2.21 mg/L (SD 4.99 mg/L; 0.11 to 36.6 mg/L), 0.42 mg/L (SD 1.58 mg/L; 0 to 14.5 mg/L), and 18.6% (SD 22.5%; 0 to 83.3%), respectively. Figure 1 shows the correlation graph for total morphine versus free morphine for all 91 cases. Regression analysis showed a modest correlation between total and free morphine ($y = 0.19x + 0.005$, $r = 0.603$). Figure 2 shows the correlation graph of a subset of cases between total morphine and free morphine in which total morphine was $<1.0$ mg/L ($n = 54$). Regression analysis showed a weak correlation between total and free morphine ($y = 0.18x - 0.00$, $r = 0.354$). If the 21 cases with undetectable free morphine concentrations were removed from Fig. 2, there was a better correlation between the free and total morphine concentrations ($y = 0.31x + 0.04$, $r = 0.5$)$^2$ graph not shown).

Fifty-seven of the 91 (63%) cases with detectable blood morphine were available for case review. There were a total of 46 men and 11 women, with a mean age of 47.0 years. Twenty-three out of 57 (40%) had detectable blood 6-MAM by screening, of which all were suspected heroin users. As shown in Table 1, men were predominant in cases with 6-MAM detected (heroin users) and without 6-MAM detected (morphine users), with a mean age lower for those cases with 6-MAM present. Table 1 also shows the mean (SD, range) concentrations and percent free morphine values for cases with and without detectable 6-MAM. The mean total morphine concentration with 6-MAM present was significantly less (two-fold) than in cases without 6-MAM present (0.93 mg/L versus 2.0 mg/L, $p = 0.05$). While the mean free morphine concentration with 6-MAM present tended to be less (70%) than in cases

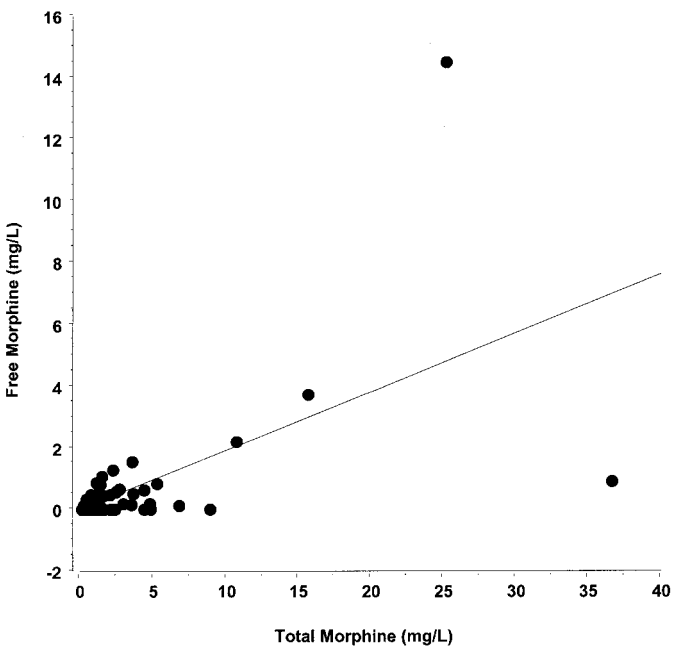


FIG. 1—*Relationship between postmortem blood total morphine and free morphine concentrations in 91 medical examiner cases. The line demonstrates the correlation of* y = *0.19*x + *0.005;* r = *0.603.*

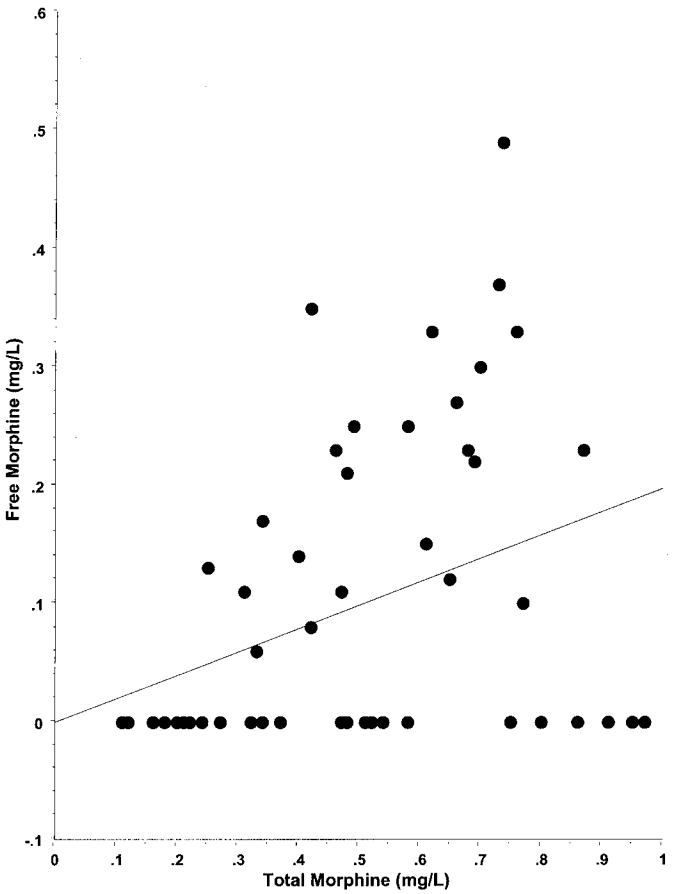


FIG. 2—*Relationship between postmortem blood total morphine and free morphine concentrations in a subset of cases* (n = *54) for morphine concentrations < 1.0 mg/L. The line demonstrates the correlation of* y = *0.18*x − *0.00;* r = *0.354.*

TABLE 1—*Demographic information and postmortem total morphine, free morphine, and percent free morphine values in 57 medical examiner cases with and without detectable blood 6-MAM.*

| | Heroin Exposure Cases with 6-MAM | Morphine Exposure Cases without 6-MAM | *P* value |
|---|---|---|---|
| Men/Women | 20/3 | 26/8 | |
| Mean age in years (range) | 36.4 (20 to 50) | 54.1 (23 to 89) | NS |
| Total morphine (mg/L) | | | |
| Mean (SD) | 0.93 (0.68) | 2.0 (3.2) | 0.05 |
| Range | 0.31 to 2.96 | 0.1 to 15.7 | |
| Free morphine (mg/L) | | | |
| Mean (SD) | 0.26 (0.21) | 0.37 (0.78) | 0.21 |
| Range | 0 to 0.87 | 0 to 3.73 | |
| Percent (%) free morphine | | | |
| Mean (SD) | 34.7% (24.6%) | 12.9% (17.2%) | 0.0001 |
| Range | 0 to 83.3% | 0 to 58.6% | |

NS = not significant.

TABLE 2—*Total morphine, free morphine and percent free morphine values according to causes of death categories.*

| | Opiate Toxicity as COD (*n* = 15) | Mixed Opiate-Polydrug Toxicity as COD (*n* = 22) | COD Unrelated to Opiates (*n* = 20) |
|---|---|---|---|
| Total morphine (mg/L) | | | |
| Mean (SD) | 2.5 (3.8) | 1.4 (2.2) | 0.99 (1.9) |
| Range | 0.34 to 15.7 | 0.22 to 10.69 | 0.11 to 5.2 |
| Free morphine (mg/L) | | | |
| Mean (SD) | 0.42 (0.94) | 0.48 (0.56) | 0.09 (0.20) |
| Range | 0 to 3.7 | 0 to 2.19 | 0 to 0.85 |
| Percent (%) free morphine | | | |
| Mean (SD) | 18.4% (23.0%) | 36.8% (22.1%) | 7.5% (11.9%) |
| Range | 0 to 83.3% | 0 to 78.4% | 0 to 31.9% |

COD = cause of death.

without detectable 6-MAM (0.26 mg/L versus 0.37 mg/L), there was no statistically significant difference ($p = 0.21$). The percent free morphine value was statistically greater in cases with 6-MAM present (34.7%) versus cases without 6-MAM present (12.9%) ($p < 0.001$).

Table 2 shows the mean (SD, range) concentrations and percent free morphine values according to each cause of death category. Fifteen of 57 cases (26%) were attributed to opiate toxicity (heroin, morphine, or both), 22 (39%) were attributed to a polydrug ingestion that included heroin and/or morphine, and in 20 cases (35%) the cause of death was not related to opiate ingestion. The differences in mean total morphine concentration was only significant in comparing the opiate toxicity cases with the opiate unrelated cases (2.5 mg/L versus 0.99 mg/L, $p = 0.05$). The mean free morphine concentrations for the opiate toxicity group and the mixed opiate-polydrug group were similar (0.42 mg/L versus 0.48 mg/L, $p = 0.41$). Although the mean free morphine concentrations for the opiate toxicity group and the mixed opiate-polydrug group were four to five times greater than the opiate unrelated group, only the mixed opiate-polydrug group vs. the unrelated group was statistically significant ($p = 0.003$), with the opiate toxicity group approaching significance ($p = 0.067$). The mixed opiate-polydrug group had the highest percent free morphine, 36.8%, which was statistically higher than both the opiate toxicity group (18.4%, $p < 0.01$), and the opiate unrelated group (7.5%, $p < 0.001$). The difference in percent free morphine between the opiate toxicity group and the opiate unrelated group was also significant ($p = 0.04$).

## Discussion

We present a database of 91 consecutive medical examiner cases investigated over 20 months for opiate related deaths, including 57 with toxicologic and cause of death information. Our findings demonstrate a large range for both total and free morphine concentrations and percent free morphine values, with a trend of lower total morphine and free morphine, but higher percent free morphine in heroin cases with 6-MAM present. Also, our results show the highest mean total morphine concentration in those cases with only morphine detected, whereas the highest mean percent free morphine was found in those cases with mixed opiate-polydrug toxicities.

Published series of free morphine concentrations in subjects who died following heroin injection vary widely. Goldberger et al. (13) found a mean free morphine concentration of 0.36 mg/L in subjects dying rapidly after heroin injection, with an overall mean free morphine concentration of 0.22 mg/L for all cases. Staub et al. (9) found a mean free morphine concentration of 0.78 mg/L in the rapid death group with mean free morphine concentration of 0.71 mg/L in all subjects. Both of these studies used GC-MS with either solid phase extraction or liquid extraction. Using a fluorometric procedure, Garriott and Sturner (8) reported a free morphine concentration range of 0.1 to 0.9 mg/L in rapid deaths following heroin injection, and Richards et al. (14) reported a range of 0 to 0.2 mg/L in fatal heroin injections. Our finding of 0.26 mg/L of free morphine in subjects with detectable 6-MAM agrees with these previously published reports.

There is less published on the use of percent free morphine in evaluating heroin related deaths, and most of this has focused on the correlation between percent free morphine and the interval between heroin exposure and death. Previous work shows a higher mean percent free morphine in rapid deaths (within 15 min) following heroin injection (9–11,13), ranging from 51% to 76%, compared to 31% to 38% in deaths after a survival period (within several hours). Although we did not specifically evaluate the time interval between the heroin ingestion and death (data was not available), by separating heroin cases with detectable 6-MAM, we have essentially selected those subjects who died within several hours of heroin exposure (4). The percent free morphine of 34% found in subjects with 6-MAM in our study was also consistent with death within hours of heroin exposure. Artificial intelligence software has been used to analyze variables such as percent free morphine, total morphine concentration and free morphine concentration in evaluating morphine-related deaths (16). Using such systems, rapid deaths (within 3 h of exposure) were characterized by percent free morphine greater than 44 to 50%.

Our study showed a lower mean free morphine concentration (0.26 mg/L) in the heroin exposed group with 6-MAM detected, compared to the morphine only exposed (0.37 mg/L) group without 6-MAM. Heroin is a relatively nonpolar molecule with high lipid and membrane solubility detected, allowing easy passage across the blood-brain barrier (3,15). Once heroin enters the brain, it is converted to 6-MAM and morphine, the latter compound interacting with the opioid receptor. Morphine is more polar compared to heroin, so that for given blood concentration of morphine, there is more morphine at the opioid receptors following heroin injection than following morphine injection. This could account for the lower blood morphine concentrations observed in heroin related deaths as compared to morphine deaths.

The use of free and total morphine concentrations in evaluating opiate related deaths is extremely difficult for several reasons. First, although there are published reports of therapeutic, toxic, and lethal ranges of morphine (7,17), significant overlap exists between these three groups as to the extreme variability in tolerance to opiates. Second, existing studies have differed methodologically. Several published reports of blood morphine concentrations in heroin fatalities are, in fact, free morphine measurements, whereas a commonly cited study that addresses fatal morphine overdoses uses total morphine measurements (18). Using free and total morphine concentrations in arriving at a cause of death is further complicated by the recent observation that morphine-6-glucuronide has agonist activity at the opioid receptor (19,20). This metabolite may play a role in opiate toxicity in subjects with renal failure or with repeat exposure to opiates.

The stability of morphine in pharmacologic preparations has been well-characterized, and shows varying degrees of degradation, particularly in aqueous solutions, with the formation of pseudomorphine, morphine-N-oxide, and apomorphine (21). This degradation is accelerated in the presence of oxygen and high pH. Hadidi and Oliver (22) demonstrated long-term stability of morphine in postmortem blood stored between −20 and 25°C, with at least 85% of morphine recovered up to one year later. Moriya and Hashimoto (23) showed both free and conjugated morphine to be stable in post-mortem blood stored at temperatures ranging from 4 to 37°C. These two studies did not specifically address the formation of degradation products observed in aqueous solutions, which is an area that may need further study. An interesting finding in the latter study was the hydrolysis of conjugated morphine in the liver, such that the free morphine measurement obtained from the liver increases over the postmortem interval. A limitation of the current study is the fact that while the presence of 6-MAM clearly indicated recent heroin exposure prior to death, one can not be definitive whether the absence of 6-MAM in the morphine related deaths truly indicates morphine exposure only.

In conclusion, the current study adds to the growing database of heroin/morphine related toxicity cases and demonstrates a wide distribution for free and total morphine concentrations in postmortem blood, with trends toward higher percent free morphine and lower total and free morphine in individuals recently exposed to heroin and mixed drug ingestions. This study also showed substantial overlap in morphine concentrations for various causes of death, which underlies the need for complete data correlation, including full toxicologic data in a forensic death investigation.

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