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TECHNICAL NOTE

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A Comparison of Carboxyhemoglobin Saturation Values in Postmortem Heart Blood and Peripheral Blood Specimens

ABSTRACT: The following is a study conducted to determine whether there was any significant difference in carboxyhemoglobin (COHb) saturation levels between the heart blood and blood collected from a peripheral site. The average heart blood to peripheral blood COHb saturation level ratio in the 42 cases studied was 1.09. Sixty-two percent (26 of 42) of the cases had a heart blood to peripheral blood ratio between 0.9 and 1.1; 74% (31 of 42) had a ratio between 0.8 and 1.2. Eighty-three percent (35 of 42) had a ratio between 0.7 and 1.3. There were four cases where the heart blood to peripheral blood ratio was either below 0.6 or greater than 1.4. The differences between the two sites were not statistically significant.

KEYWORDS: forensic science, carbon monoxide, postmortem blood, site dependence

The primary mission of the postmortem forensic toxicologist is to assist the medical examiner or coroner in the determination of cause and manner of death. This assistance usually involves the analysis of specimens obtained from the decedent for alcohol, drugs, or other toxic substances. One underlying assumption in these analyses has been that the measurements made on these specimens some time after death accurately reflects the condition at death. However, a number of studies over the past 20 years have indicated that for a number of substances, this assumption may not be true. For instance, differences in blood concentration as a function of time after death and as a function of blood source have been well documented for a number of drugs (1–4). In fact, this topic has been the subject of a number of review articles (5,6).

Carbon monoxide (CO) remains a commonly analyzed substance in postmortem forensic toxicology laboratories. CO remains a major factor in fire deaths, aircraft accidents, and intentional exposure to auto exhaust. Since arson fires are classified as homicides, the analysis and interpretation of carboxyhemoglobin (COHb) saturation values can have significance in criminal proceedings. Moreover, even in cases where CO is not the causative agent in a death, the analysis of CO may also have civil ramifications, such as "pain and suffering" issues.

The following is a study conducted on cases investigated by the Office of the Chief Medical Examiner, State of Maryland, where elevated levels of COHb were detected. The purpose of the study was to determine whether there was any significant difference in COHb saturation levels between the heart blood and blood collected from a peripheral site. The data indicated that there was no difference in heart blood to peripheral blood ratios as a function of heart blood COHb saturation levels.

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Materials and Methods

Specimen Collection

Specimens were obtained from cases investigated by the Office of the Chief Medical Examiner, State of Maryland. For each case, blood from the heart and blood from the subclavian vein was collected.

Specimen Analysis

Blood specimens were analyzed on an IL-682 CO-Oximeter. The instrument was operated according to the manufacturer's specifications (7). Blood specimens were pretreated as follows: to 4 mL of blood were added 2 mL CO-Oximeter diluent and 50 mg sodium hydrosulfite (dithionite). The sample was then vortexed for 30 s and centrifuged at 3000 RPM for 10 min. The supernatant was aspirated into the instrument.

Blood specimens with a COHb saturation level greater than 12% were confirmed and quantitated by gas chromatography (8). Briefly, 1 mL of blood is placed in a headspace vial. A 10% solution of potassium ferricyanide is added to the vial to release the CO from the Hb. A sample of headspace is introduced into the gas chromatograph, passed through a 5A molecular sieve column, catalytically reduced to methane and detected using a flame ionization detector. This measures CO content in the specimen. CO capacity is measured by saturating another aliquot of specimen with CO using a IL 237 Tonometer and then preparing as above. The percent saturation of CO was calculated: CO content/CO capacity $\times$ 100%.

Statistical Analysis

The mean heart blood to peripheral blood COHb saturation level ratios were compared using a two-tailed *t*-test for independent samples of equal variance. The difference between the means was also investigated by calculating the two-tailed *p*-value of the *z*-test ($\alpha = 0.05$) to determine statistical significance.

Results

Data that were collected in 42 cases are displayed in Table 1.

Discussion

In this laboratory, COHb is screened in blood specimens by spectrophotometry. All COHb values greater than 12% are quantitated by gas chromatography. One advantage of the chromatographic method is the ability to measure both CO content and CO capacity, thus removing the need to measure hemoglobin. This may be important when measuring CO in postmortem specimens where a large variety of hemoglobin concentrations may be encountered. In this study, normalizing each specimen for hemoglobin ensured that measured differences between the heart blood and peripheral blood were not due to significant differences in hemoglobin between the two specimens. In addition, all paired blood specimens were analyzed within the same batch to eliminate any between-run variability.

TABLE 1—*Heart (H) and peripheral (P) blood COHb saturation levels in the presented cases.*

	% CO-Hb Saturation Level		
No.	H. Blood	P. Blood	H/P
1	11	14	0.79
2	12	4.2	2.86
3	14	12	1.17
4	16	16	1.00
5	18	20	0.96
6	18	33	0.55
7	20	19	1.05
8	23	24	0.96
9	24	38	0.63
10	26	29	0.90
11	26	26	1.00
12	27	27	1.00
13	28	29	0.97
14	29	21	1.38
15	30	29	1.03
16	31	28	1.11
17	34	29	1.11
18	35	34	1.03
19	35	37	0.95
20	37	32	1.16
21	38	36	1.06
22	38	31	1.23
23	39	43	0.91
24	42	38	1.10
25	45	46	0.98
26	45	54	0.83
27	46	48	0.96
28	49	35	1.40
29	52	52	1.00
30	57	52	1.10
31	59	55	1.07
32	61	55	1.11
33	64	52	1.23
34	64	61	1.05
35	66	68	0.97
36	67	62	1.08
37	68	48	1.42
38	69	67	1.03
39	72	58	1.24
40	73	71	1.03
41	77	50	1.54
42	79	69	1.14

The average heart blood to peripheral blood ratio in the 42 cases was 1.09. Sixty-two percent (26 of 42) of the cases had a heart blood to peripheral blood ratio between 0.9 and 1.1; 74% (31 of 42) had a ratio between 0.8 and 1.2. Eighty-three percent (35 of 42) had a ratio between 0.7 and 1.3. The heart blood and peripheral blood values were compared statistically to determine whether there were differences between the two groups. In the presented study, the average heart blood CO concentration was 42 (range = 11 − 79; standard deviation = 19.95; median = 38); the average peripheral blood concentration was 39 (range = 4.2 − 71; standard deviation = 17.07; median = 37). The difference between the two means was found not to be statistically significant ($t' = 0.51$, $df = 51$, $\alpha = 0.05$, $t_{\alpha/2} = 2.01$, $p > 0.05$).

The data presented in this study are consistent with a previously reported study. Dalpe-Scott et al. (2) reported an average heart blood to peripheral blood ratio of 1.0 in 35 cases examined. The ratios in these cases ranged from 0.9 to 1.5. The specific COHb saturation levels in these cases were not provided.

Although the data, when examined in their entirety, failed to demonstrate any statistically significant differences between the blood sites, it was possible that within a given subset of the data, such as either low or high COHb saturation levels, differences between the two sites may exist. As a result, the data was subdivided according to ranges of heart blood COHb saturation levels. The mean, median, and range for three sub-categories are shown in Table 2. The table clearly indicates that there is no difference in heart blood to peripheral blood ratios as a function of heart blood COHb saturation levels.

Of the 42 cases studied, four (case numbers 2, 6, 37, and 41) had a heart to peripheral blood ratio either below 0.6 or greater than 1.4. These cases were reviewed in greater detail to determine whether there was some aspect to the cases that could account for these differences. This included a review of the analytical data for differences in hemoglobin and a review of the case history. In three cases (2, 37, and 41) there was greater than 50% difference in the total hemoglobin between the two specimens as indicated by the CO capacity.

Case 2 was a 26-year old, Caucasian female involved in a motor vehicle accident where her car was struck from behind by a tractor-trailer and was immediately engulfed in flames. This was an acute death which would account for not only the relatively low COHb values but also the almost 3-fold difference between

TABLE 2—*Mean, median and range of heart blood to peripheral blood ratios at different heart blood COHb saturation levels.*

	Heart Blood COHB Saturation Level (%)		
	11–30	31–50	>50
Mean ratio	1.08	1.06	1.07
Median ratio	1	1.06	1.1
Ratio range	0.55–2.86	0.91–1.40	0.97–1.54

heart and peripheral concentrations since the blood would not have had time to circulate to the periphery before cessation of cardiac function.

Case 6 was a 2-year old African American female removed from a house fire. This child was immediately intubated by ambulance personnel who then initiated oxygen therapy and CPR. These procedures were continued at the hospital, resulting in resuscitative efforts lasting almost 1 h before the code was "called." This child had a prolonged exposure to CO (relative to case 2) accounting for the elevated peripheral COHb concentration. The 1-h of resuscitative efforts with circulation only being accomplished by the CPR effort could explain the H/P value of 0.55.

In two cases (37 and 41), the difference in the COHb between the two specimens was insignificant in terms of interpretive aspects. Case 37 was a 21-year-old Caucasian male who committed suicide by running a hose from the exhaust pipe on his car through the rear window. This individual was dead at the scene (no resuscitative efforts were instituted) and, as in Case 2, the H/P ratio of 1.42 may be indicative of a relatively short survival time. Case 41 was a 54-year-old Caucasian female who was found dead at the scene in the back seat of her car that was still running in a closed garage. Similarly to case 37, the H/P ratio of 1.54 may be an indication of a relatively acute death.

From this study we conclude that although there may be differences between heart blood and peripheral blood COHb saturation levels in isolated cases, the general trend is that there are no significant differences in COHb saturation levels between the two specimen sources. This is different than what is observed with a number of drugs such as tricyclic antidepressants (5,6).

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