Chapter 9

Application of CO-oximeter for Forensic Samples

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Abstract

CO-oximeter is routinely used in clinical practice, and it has been applied in the field of forensic medicine. It is a simultaneous and nondestructive technique for the analysis of total hemoglobin (Hb) and various Hb species, such as oxyhemoglobin, reduced hemoglobin, carboxyhemoglobin, and methemoglobin. It automatically measures the proportion of each species of Hb and oxygen contents. This is an easy, rapid, and convenient way as the laboratory test. Since there are many advantages such as no necessity of sample preparation, easy handling, and portability, it may provide valuable information for forensic diagnosis. In the present paper, we discuss about the diagnostic application of CO-oximeter in the field of forensic medicine.

Keywords: CO-oximeter, forensic diagnosis, carbon monoxide poisoning, hypothermia, methemoglobin

1. Introduction

CO-oximeter is widely used in clinical practice [1–5]. It would be measurable total hemoglobin (Hb) and various Hb species, such as oxyhemoglobin (O_2Hb), reduced hemoglobin (HHb), carboxyhemoglobin (COHb), and methemoglobin (MetHb), simultaneously. This is an easy, rapid, and convenient way as the laboratory test, and it provides valuable information for clinical diagnosis. It is widely used in clinical laboratory, emergency department, intensive care unit, or cardiac catheterization laboratory for the evaluation of general status including the ability of oxygenation or ventilation [1–5].

It has been reported that CO-oximeter is applied in a field of forensic diagnosis [6–30]. In the present chapter, we discuss about the diagnostic application of CO-oximeter in the field of forensic medicine.

2. Principle of the CO-oximeter

The CO-oximeter is a spectrophotometer that determines hemoglobin derivatives in blood by measuring absorbance at selected wavelength [1, 2, 31, 32]. The Hb solutions obey the Lambert-Beer Law, and the absorbance measured at selected wavelength is the sum of the absorbance of each Hb derivative [31–36]. The wavelength is selected by the combination of absorption maxima and isosbestic points [37]. The concentration of four Hb derivatives (O_2Hb, HHb, COHb, and MetHb) is determined by measuring absorbance at four wavelengths [33].

The CO-oximeter is included in the routine toxicological examination in daily forensic practice, as it is not necessary for the sample pretreatment or any reagent.

3. Forensic application of CO-oximeter

Since the O_2Hb in the postmortem heart blood was usually very low (under 10%) in most of the cases, postmortem blood gas analysis is less valuable for interpretation of cause of death [13]. However, the composition of Hb provides valuable information for forensic diagnosis.

We have been using the AVOX4000 (AVOX; International Technidyne Corporation, NJ, USA), which monitors seven wavelengths (488.4, 520.1, 562.4, 585.2, 597.5, 621.7, and 671.7 nm) in the visible region for the determination of various Hb species [24]. This portable CO-oximeter (**Figure 1**) requires 50 μl of blood for single measurement, and it may be a valid option in case of difficult blood sampling due to severe blood loss. Since there are many advantages such as no necessity of sample preparation, easy handling, and portability, it is suitable for forensic practice. It automatically analyzes the proportion of each species of Hb and oxygen contents.

3.1. Carbon monoxide (CO) poisoning

CO is an odorless, colorless and nonirritable gas, mainly produced by incomplete combustion of fuels or carbon compounds [38, 39]. CO is second most common cause of death among non-medical poisonings in United States [40], and the leading cause of poisoning death in Japan [41, 42]. It includes accidental or suicidal poisoning, and the postmortem investigation for CO poisoning and its related death are important for forensic practice.

CO binds to hemoglobin and forms COHb (represented as a percentage of the total Hb) following inhalation of CO gas [39, 43, 44]. As the affinity of CO for Hb is 200–300 times greater than that for oxygen, the toxicity of CO is mainly thought to be the decrease the capacity of oxygen transport, and it causes impairment of oxygen supply in tissue level [39, 43–46]. In a recent study, it may be involved interference with ferroproteins such as myoglobin and cytochrome oxidases [43, 45, 46].

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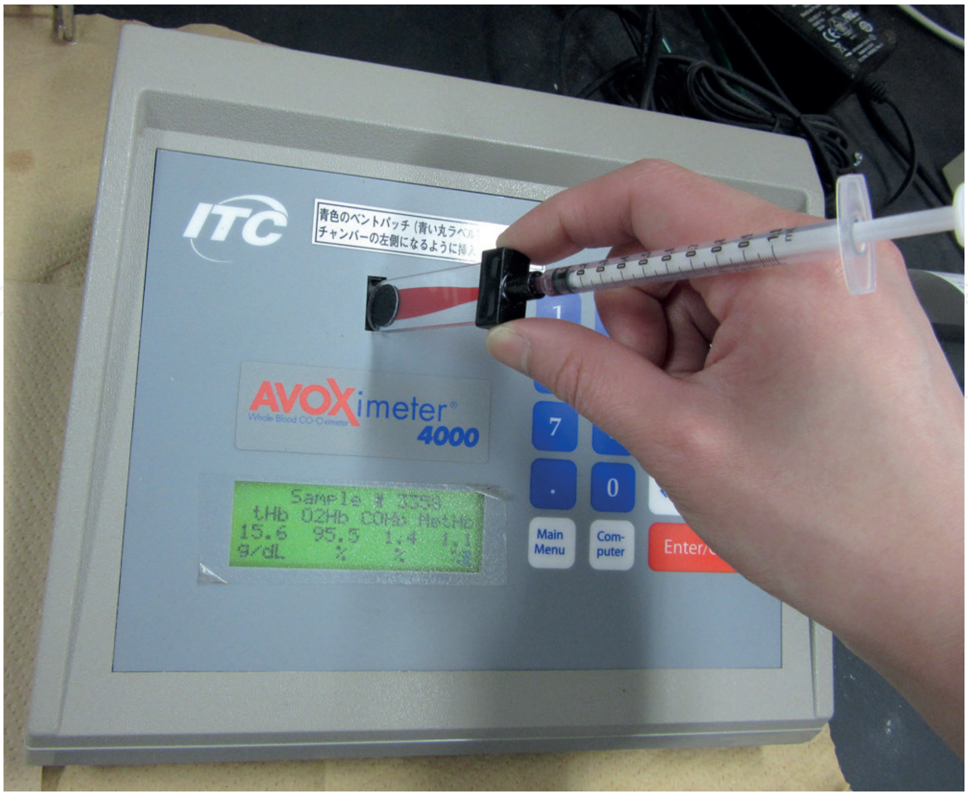

Figure 1. Portable oximeter (AVOX 4000).

CO-Hb (%)	Clinical symptoms
<1-2	Normal range (due to endogenous production)
<10	Smoker's blood (no symptom)
10–20	Headache
20–40	Headache, nausea, vomiting
40-	Severe symptoms
50–60	Coma, convulsions
60-	Cardiorespiratory depression or failure, often fatal

Table 1. Level of carboxyhemoglobin (COHb) and symptoms.

Table 1 shows the relationship between toxic symptoms and COHb levels [39, 46]. However, it is not an absolute reference value, and large individual difference was observed in fatal level. Severe symptoms such as convulsion, deep coma, and cardiovascular failure are observed around 50% of COHb level. The fatal COHb levels are more than 50–60%, and its values are important for the diagnosis of fatal CO poisoning [46]. However, lethal level has large variation. Elderly people may die at relatively low concentration in some cases [46]. It may be involved to pathological status such as anemia, coronary artery disease, and respiratory insufficiency [46]. Autopsy findings indicate that blood, organs, and muscle will be cherry red color, as a result of COHb formation. Other findings such as pulmonary edema and generalized organ congestion are also observed [46].

The application of oximeter in fatal CO poisoning case has been reported [6, 7, 10–13, 15–20, 22–29]. Various methods such as spectrophotometric method and gas chromatography have been also employed for the identification and quantification of CO [43, 47]. There was a good correlation between the COHb values obtained by AVOX and by the conventional method [23]. No arterial-venous difference of COHb concentration was observed at the level less than 75% of COHb [23] and in animal experiments [48].

In a forensic practice, we sometimes treat various kinds of denatured blood samples such as from the putrefied bodies or thermo-coagulated one. As the COHb is relatively stable under the storage in cool and dark conditions, it would be accurately measurable less than 2 years under the storage in fridges [22, 49], and it is possible for rough estimation of COHb levels in thermo-coagulated blood with CO oximeter [6, 12]. In recent study, it has been reported that splenic blood, which is obtained by manual squeezing, is applicable for alternative matrix for COHb measurement using AVOX [29].

3.2. Fire-related cases

The victims by fire will die not only from the thermal injury but also from inhalation of the toxic substances such as CO, cyanide, nitric oxide, phosgene, and others and reduction of the atmospheric oxygen [46]. When the organic material burn but access of oxygen is limited in fire, large amount of CO is produced by the incomplete combustion. The COHb level in fire-related cases is an important aspect [46]. The presence of CO in circulating blood and carbon particle in the air passages indicates that the victim was alive after the fire began [46]. It is a valuable indicator in fire-related cases. The COHb levels in blood of the fire victim depend on various factors such as CO concentration in atmosphere, time of exposure, and oxygen contents [46].

It has been reported that marked arteriovenous and centroperipheral difference of COHb were observed in the group of above ca.70% of COHb [11]. It seems to be that inhalation of CO-rich air immediately causes acute heart failure [11]. On the other hand, COHb levels in victim were lower in flash fires or open-air petrol involved cases. The quick measurement of COHb using CO-oximeter in victim of fire-related case provides valuable information for forensic diagnosis.

3.3. Hypothermia

There is little or no diagnostic findings in cases of fatal hypothermic death [14, 28, 30, 46, 50–55]. It has been reported that the blood in left cardiac chamber is bright red compared to that in right cardiac chamber [28, 30, 50, 51, 53, 56]. This color difference between left and right heart blood is a common characteristic sign of hypothermic death. This finding was observed in approximately 95% of hypothermic death cases [30, 50, 53]. This color difference is formed by many factors such as decrease of body temperature, binding of oxygen to Hb, and inhalation of cold air before death [30, 50]. The blood in left heart has usually higher oxygen contents than that in right heart. The lower body temperature keeps the antemortem composition of Hb following death, and it also enhances the oxygen binding to Hb [30, 50, 53].

The O_2Hb saturation level could increase as a result of cardiopulmonary resuscitation or administration of oxygen. We have to exclude the following cases such as subjected to body rewarming, long postmortem interval, and received cardiopulmonary resuscitation, for forensic diagnostic application of CO-oximeter [30].

It has been proposed that the diagnostic criterion of hypothermic death, designating O_2Hb in left cardiac blood ≥36% as a basic condition, with the difference in the O_2Hb saturation level between left and right heart blood ≥13% or O_2Hb saturation ratio between left and right heart blood ≥1.8, as a complementary condition [30]. This finding reflects the final balance of oxygen uptake and consumption in the dying process, and the pathophysiological status of the victim would be obtained by the application of CO-oximeter for forensic diagnosis.

3.4. Evaluation of MetHb

MetHb is a form of Hb in which ferric iron (Fe^{3+}) is carried in its heme group [57]. It is formed by the exposure to oxidizing agents such as nitrates, nitrites, or chlorates [57–59]. MetHb may also arise from genetic, dietary, or idiopathic etiologies [57–59]. It causes impairment of O_2 and CO_2 transport, leading to tissue or cellular hypoxia [57–59]. High levels of MetHb have been observed in cases of fire and poisoning by various oxidizing agents such as vehicle exhaust (containing nitric oxide and nitrogen dioxide), nitrate, and chlorate [60–64]. We should also take into consideration about the stability of MetHb. The formation of MetHb by postmortem oxidation of heme-protein has been reported [8]. On the other hand, high COHb containing blood is considered to have heat resistant properties, and the formation of MetHb is lower [65]. Spontaneous reduction of MetHb in blood sample due to the enzyme activity has also been reported [66]. We have to consider them for the interpretation of the MetHb value.

Measurement of MetHb using the conventional spectrophotometric method [67] is relatively complicated procedure. On the other hand, CO-oximeter is routinely used in clinical practice [2, 3, 5] and has also been applied in forensic practice [8, 64]. **Table 2** shows

MetHb (%)	Clinical symptom
<1–2	Normal range (no symptom)
10–15	Cyanosis
20-	Headache, dyspnea, tachycardia, tachypnea
40–50	Mental derangement, metabolic acidosis
50-	Coma, convulsion
70-	Death

Table 2. Level of methemoglobin (MetHb) and symptoms.

the relationship between toxic symptoms and MetHb concentration [57–59, 68]. The blood MetHb concentration is less than 1–2% in normal healthy subjects, and fatal concentration of MetHb in blood has been reported higher than 70% [57–59, 68]. Blood MetHb concentration provides useful toxicological information for forensic diagnosis. From these results, additional toxicological examination for oxidizing agent may be requested to the forensic toxicologist.

4. Conclusion and future perspective

We have discussed about the application of CO-oximeter in forensic practice. The postmortem oximetric profiles may be considered to reflect the final balance of oxygen uptake and consumption in the dying process [13]. Those data may be valuable for interpretation in some cause of death and provides valuable information for forensic diagnosis. Further applications in the fields of forensic practice can be expected.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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