Application of portable oximeter for the determination of methemoglobin in forensic practice

Naoko Tanaka¹, Kiyoshi Ameno¹, Mostofa Jamal¹, Asuka Ito¹, Nobuyuki Miyatake², Mitsuru Kumihashi¹, Shoji Kimura¹, Kunihiro Tsutsui³, Hiroshi Kinoshita¹*

Abstract: The concentration of methemoglobin (Met-Hb) in blood provides useful information in cases of exposure to oxidizing agents such as nitrates, nitrites or chlorates. In the present study, we determined Met-Hb concentrations in blood samples using a portable AVOX 4000 oximeter (AVOX) and compared the results to the conventional spectrophotometric method. There was good correlation between the values obtained by oximeter and those obtained by the conventional method, in the range of 0-70% Met-Hb. Because Met-Hb is unstable, it is important to analyze it soon after sampling. The concentrations of Met-Hb in samples stored at 4°C gradually decreased over time. We applied forensic sample obtained from fire-related deaths, and carboxyhemoglobin (CO-Hb) was also determined simultaneously by AVOX. AVOX is a new tool that is suitable for Met-Hb determination because of its easy handling and it requires a smaller sample size than the conventional method.

Key Words: oximeter, methemoglobin, stability, fire-related death.

INTRODUCTION

Methemoglobin (Met-Hb), is a form of hemoglobin in which ferric iron (Fe³⁺) is carried in its heme group [1]. It is formed by exposure to oxidizing agents such as nitrates, nitrites or chlorates [1, 2]. It causes impairment of O₂ and CO₂ transport, leading to tissue or cellular hypoxia [1]. High levels of Met-Hb have been observed in some forensic cases such as fire and poisoning by various oxidizing agents [3-6]. Because a normal Met-Hb level is less than 1% in healthy subjects [1], blood Met-Hb concentration provides useful information for forensic diagnosis.

Measurement of Met-Hb is usually performed by the spectrophotometric method [7]; however, the procedure is relatively complicated. On the other hand, an oximeter is routinely used in clinical practice [8, 9] and has also been applied in forensic practice [10-17]. It simultaneously determines the total hemoglobin content, which includes other hemoglobin species, such as oxyhemoglobin and carboxyhemoglobin (CO-Hb), by a simple procedure. We used an AVOX 4000 oximeter (AVOX, International Technidyne Corporation, NJ) for the measurement of Met-Hb and compared the results to the conventional spectrophotometric method, and we also measured Met-Hb concentrations in forensic cases in which exposure to oxidative gas agents was speculated.

MATERIALS AND METHODS

Preparation of Met-Hb standard and evaluation of its stability

Human blood donated from a healthy volunteer was used. Preparation of Met-Hb blood samples was
performed in accordance with the previous report [19]. Briefly, 0.145 M sodium nitrite solution was added to the blood and incubated overnight at 4°C. This process resulted in the conversion of all of the hemoglobin to Met-Hb. Following incubation and washing with saline, the Met-Hb blood sample was diluted with normal blood to prepare a sample with a Met-Hb concentration in the range of 0-70%, and the stability of Met-Hb was investigated using the diluted blood sample kept at 4°C.

Measurement of Met-Hb and CO-Hb
The measurement of Met-Hb was performed on the day of sample preparation. Measurement of Met-Hb in blood was performed by both an AVOX oximeter and by the conventional spectrophotometric method. The operation of the oximeter and the preparation of the samples were in accordance with the manufacturer's specifications [17, 18]. Briefly, an approximately 50 µL blood sample was injected into the cuvette and inserted into the AVOX oximeter. The sample was analyzed automatically within 10s. The CO-Hb was simultaneously measured by this method [17,18]. Conventional spectrophotometric measurement of Met-Hb was employed using Sato's method [7].

Forensic samples
Femoral venous blood and left and right cardiac blood samples were collected from 30 medicolegal autopsy cases (90 blood samples). Twenty fire-related death cases were divided into two groups according to CO-Hb concentration. The high CO-Hb group included samples with a CO-Hb ≥50% (8 males, 2 females, mean age 66.5±16.4), and the moderate CO-Hb group included samples with a CO-Hb <50% (7 males, 3 females, mean age 74.5±16.0). Control samples were collected from 10 cases (2 trauma, 2 asphyxia, 2 drowning, 2 natural death, and 2 hypothermia; 7 males, 3 females, mean age 62.4±17.7). Postmortem intervals were all within 48 h. This autopsy study was approved by the Ethics Committee of Kagawa University and was conducted according to the Declaration of Helsinki Principle.

Statistical analysis
Simple regression analysis was used to measure the association between Met-Hb levels measured by the AVOX and those measured by the conventional method. Comparisons among the three groups were performed using the Kruskal-Wallis test. A p value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION
The relationships of Met-Hb concentrations between the AVOX and conventional methods are shown in Figure 1. The concentrations determined by AVOX were almost equivalent to those obtained by the conventional method (regression equation of $y = 1.0528x - 0.4561$; $r = 0.995$). It demonstrated excellent correlation in the range of Met-Hb concentrations of 10-70%. These results indicate that AVOX is a useful and valuable tool for Met-Hb determination.

The stability of Met-Hb in blood kept at 4°C is shown in Figure 2. The concentration of Met-Hb gradually decreased over time. It has been reported that spontaneous reduction of Met-Hb due to enzyme activity could not negligible when the sample is kept at 4°C [20]. Our results support the previous study, which indicate that we should measure Met-Hb soon after sampling.

Met-Hb concentrations in our forensic cases are summarized in Table 1. The Met-Hb concentrations in those cases were less than 1%. No elevation of Met-Hb concentration in fire-related fatalities was observed in our small number of groups, as has been described previously [21]. There were no significant differences
in Met-Hb concentrations among the three groups. The high concentration of Met-Hb may be dependent on the oxidizing capability of the inhaled gas species or the degree of heating in fire-related fatalities [21,22]. The Met-Hb levels in blood samples did not differ among the three sampling sites (femoral venous blood and left and right cardiac blood samples). These data suggest that sample site differences in Met-Hb concentration are negligible.

The AVOX is a portable oximeter that automatically analyzes not only the Met-Hb concentration, but also the total hemoglobin content including oxyhemoglobin, CO-Hb, and oxygen contents in a small amount of samples [17, 18, 23]. It is a suitable new method that can be used in forensic practice and has many advantages, such as its portability, fast and easy operation, and small sample requirement in comparison with the conventional method.

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

### References


### Table 1. Methemoglobin (Met-Hb) concentrations (%) in femoral venous blood and left and right cardiac blood samples from autopsy cases. Fire-related death cases were divided into two groups based on carboxyhemoglobin (CO-Hb) concentration (CO-Hb ≥50% or CO-Hb <50%)

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>CO-Hb ≥50%</th>
<th>CO-Hb &lt;50%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Met-Hb (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left cardiac blood</td>
<td>0.19±0.48 (0-1.5)</td>
<td>0.02±0.06 (0-0.2)</td>
<td>0.20±0.43 (0-1.2)</td>
</tr>
<tr>
<td>Right cardiac blood</td>
<td>0.41±1.10 (0-3.5)</td>
<td>0.07±0.22 (0-0.7)</td>
<td>0.88±1.49 (0-3.6)</td>
</tr>
<tr>
<td>Femoral venous blood</td>
<td>0.13±0.41 (0-1.3)</td>
<td>0.02±0.06 (0-0.2)</td>
<td>0.38±0.70 (0-1.9)</td>
</tr>
<tr>
<td>CO-Hb (%) in femoral blood</td>
<td>2.35±1.60</td>
<td>68.6±7.80</td>
<td>24.1±3.3</td>
</tr>
</tbody>
</table>