Acta Med. Okayama, 2011
Vol. 65, No. 4, pp. 225–230

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Postmortem Changes in Myoglobin Content in Organs

Masanobu Miura[a], Toru Naka[a,b], and Satoru Miyaishi[a]*

[a]*Department of Legal Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama 700–8558, Japan, and* [b]*Department of Physical Therapy, School of Health Sciences, Suzuka University of Medical Science, Suzuka, Mie 510–0293, Japan*

Postmortem changes in myoglobin concentrations in blood and organs were investigated using an enzyme immunoassay by animal experiments in combination with immunohistochemical staining of human cases. Blood myoglobin concentrations were found to increase drastically within a very short time after death. Those in striated muscle, however, did not change by day 14 postmortem. Myoglobin content in the liver and kidney increased slightly by day 5 postmortem, and more obviously by day 7 or later. However, almost no change was observed by day 5 in the kidney when the renal artery and vein had been ligated just after death. In the thyroid gland and the lung, the myoglobin content markedly increased by day 7 postmortem, with the logarithmical values rising nearly linearly as the time after death passed. In the thyroid gland, concentrations reached the level of the striated muscle. The mechanisms of postmortem myoglobin increase in organs are thought to be direct diffusion from the striated muscle and/or distribution through the blood. To estimate the postmortem interval, the determination of myoglobin content in the thyroid gland or the lung appears to be useful.

Key words: myoglobin, postmortem diffusion, postmortem distribution, postmortem interval

To estimate the postmortem interval, macroscopic findings of postmortem changes are generally used. Many biochemical substances used for this purpose have been determined, *e.g.* C3 (the third component of complement) in blood, potassium in cerebrospinal fluid, glutamic oxaloacetic transaminase (GOT) and other enzymes in pericardial fluid, and free amino acids in vitreous humor [1–10]. Wehner *et al.* have reported the postmortem changes of insulin in pancreatic β-cells or thyroglobulin in the thyroid gland using immunohistochemistry and have referred to the possibility of estimating the postmortem interval [11, 12]. However, the relation between observed morphological findings in the organs and concentrations of the target substances in the blood have not been described in these previous reports.

Myoglobin is a hemoprotein that exists profusely in the striated muscle and only a little in the blood of the living body. It is known that myoglobin concentrations in blood increase extraordinarily postmortem, and previous studies have found that this substance is exuded into blood from the striated muscle after death [13–15]. Although we have reported that organs as autopsy material contain only a small amount of myoglobin comparing with skeletal muscle based on qualitative/semiquantitative analysis [16], postmortem changes of myoglobin in the organs have not yet been elucidated in detail. In the present study we investigated postmortem changes of the myoglobin content in various organs together with heart blood and evaluated the applicability of these changes to diagnosis of the postmortem interval.

Received October 15, 2010; accepted December 17, 2010.
*Corresponding author. Phone:+81–86–235–7194; Fax:+81–86–235–7201
E-mail:miyaishi@md.okayama-u.ac.jp (S. Miyaishi)

Materials and Methods

Animal experiments. In the investigation of postmortem changes of blood myoglobin concentrations, 3 male rabbits approximately 3,000 g in weight were sacrificed using carbon dioxide after auricular venous blood had been taken. These rabbits were fixed on the back and left at room temperature. Approximately 0.5 ml of left heart blood was obtained at 15 min and at 1, 2, 3, 6, and 12 h and 1, 3, 5, and 7 days after death.

To investigate the myoglobin content in organs/tissues, small pieces (approximately 1 cm^3) of the brain, left lung, liver, left and right kidneys, heart, and longissimus muscle (belly) were taken from a rabbit at approximately 30 min and on 1, 3, 5, 7, and 14 days postmortem. The thyroid gland was removed as a whole organ in the same time course. Specimens of the lung and liver were taken from the region not covered by skeletal muscle (*e.g.* diaphragm). The renal artery and vein were ligated only on the right side immediately after death. For each postmortem interval, five rabbits were used.

These experiments met the standards and principles of animal care, and ethical approval was obtained from the Department of Animal Resources, Advanced Science Research Center, Okayama University.

Sample preparation and determination of the myoglobin content in blood and organs. Blood myoglobin concentrations were determined using the obtained supernatant after centrifugation at 20,000G for 10 min (24℃) by the in-house developed enzyme immunoassay modified for rabbit-myoglobin [13, 16]. For the quantitative analysis of myoglobin in organs/tissues, 0.1 g was removed from the obtained small pieces and homogenized with 0.9 ml phosphate-buffered saline containing 1% bovine serum albumin using an ultrasound homogenizer. After the centrifugation under the same conditions for the blood, myoglobin concentrations in the supernatant were determined and its content in the organ was calculated. The data are presented as the mean ± S.D. on the graph. Statistical analyses were performed using the *t*-test for comparison between groups according to the postmortem interval. Differences were considered significant at $p<0.05$.

Histological investigation of postmortem myoglobin exudation. The thyroid gland was removed together with the surrounding muscles in 5 autopsy cases (3 males and 2 females, postmortem interval was between 8 h and 2 days, causes of death were drowning, blood loss, ligature strangulation, and fire). The specimens were fixed in 10% formalin and embedded in paraffin. For the myoglobin detection, polyclonal anti-human myoglobin antibody and ENVISION + (DAKO) were used. The detection of specific antibody bonding succeeded with diaminobenzidine, and postmortem exudation of myoglobin was morphologically evaluated.

Results

Myoglobin concentrations in the left heart blood began to increase just after death and rose very rapidly. Concentrations were 20 times higher than those in antemortem auricular venous blood, which were only 0.0029 −0.0074 μg/ml, in 15 min and reached levels approximately 6,600 times higher by day 1 after death (Table 1).

Myoglobin content in the striated muscles was approximately 1 mg/g or more and it showed almost no change after death except for between day 0 (within 30 min after death) and day 14 in the myocardium. In the comparison between the myocardium and the skeletal muscle (longissimus muscle), the former contained somewhat higher levels than the latter (Fig. 1).

In the left kidney, a slight increase in myoglobin content was observed by day 5 postmortem, while

Table 1 Postmortem increase in myoglobin concentrations in left heart blood

Postmortem interval	Ratio of myoglobin concentration in postmortem blood to antemortem blood*
15 min	2.2×10
1 h	6.1×10
2 h	3.8×10^2
3 h	6.3×10^2
6 h	2.7×10^3
12 h	5.8×10^3
1 day	6.6×10^3
3 days	6.1×10^4
5 days	9.6×10^4
7 days	8.0×10^4
14 days**	—

*Mean of 3 animals
**Blood could not be obtained

there was almost no change in the right kidney during this period (Fig. 2). On day 7 postmortem and later, an obvious increase in myoglobin content occurred on both sides. In the liver, it also increased slightly by day 5 and more obviously by day 7 and later (Fig. 3A). Myoglobin content in the brain showed a similar increase to that in the liver or left kidney by day 5. On day 7 postmortem or later, however, it was more obviously increased (Fig. 3B). In contrast, myoglobin content in the thyroid gland had increased markedly by day 1 postmortem. Such an increase was still maintained by day 7 postmortem, when the myoglobin content in this organ reached almost the same level as that in the striated muscle. From day 7 to 14, no increase was observed (Fig. 3C). The postmortem myoglobin content in the lung also increased rapidly as in the thyroid gland, but it reached a lower level than that in the striated muscle on day 14 (Fig. 3D).

In the morphological investigation of postmortem exudation of myoglobin, myoglobin was stained in the part of the thyroid gland close to the skeletal muscle in all cases. The farther the distance from the skeletal muscle, the weaker the myoglobin was stained. The connective tissue between the skeletal muscle and

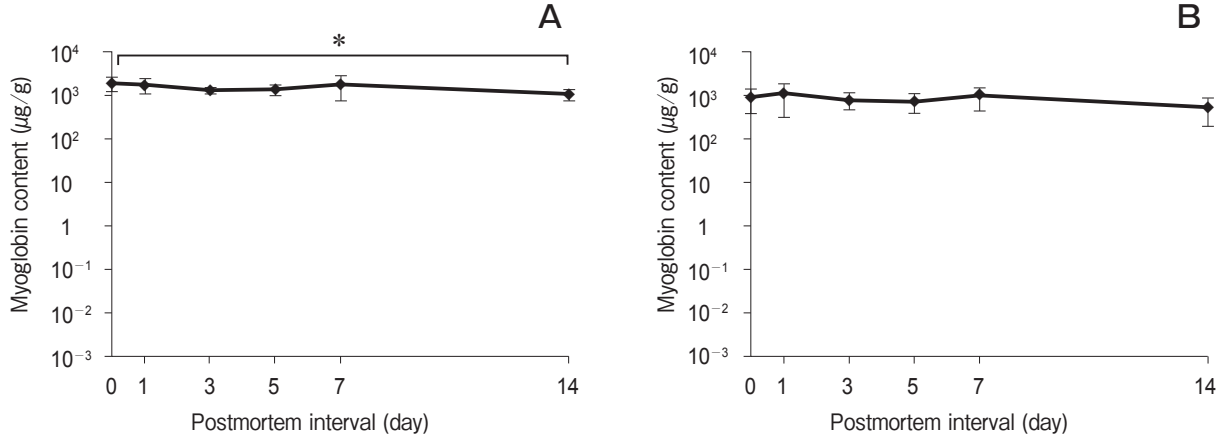

Fig. 1 Postmortem change in myoglobin content in the striated muscles (mean ± S.D.). **A**, myocardium; **B**, skeletal muscle (M. longissimus). A significant difference was observed only between day 0 and day 14 in myocardium (*t*-test, $^*p < 0.05$).

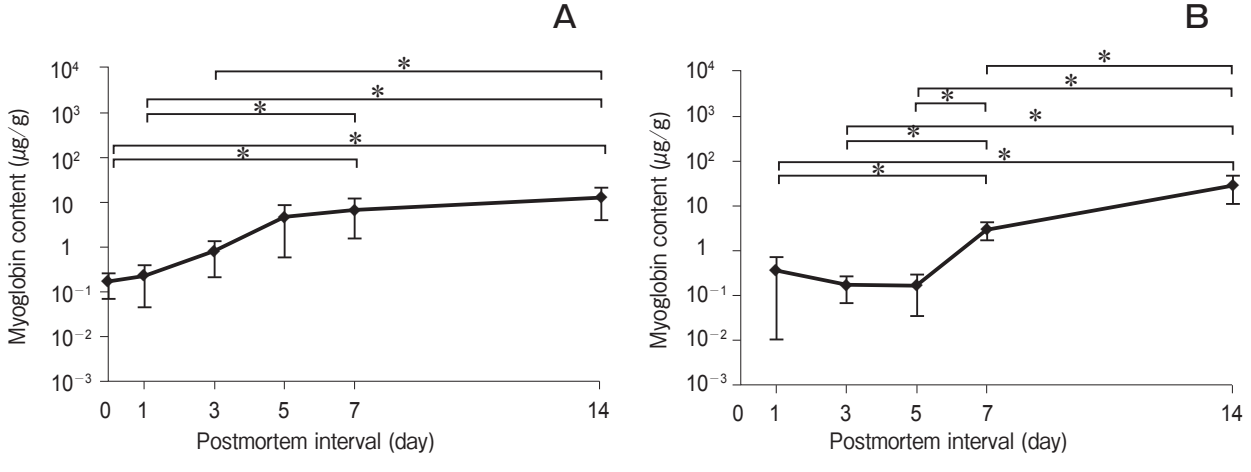

Fig. 2 Effect of the blockage of postmortem blood distribution on the myoglobin content in the kidney (mean ± S.D.). **A**, left kidney; **B**, right kidney. The right renal artery and vein were ligated immediately after death. Significant differences were observed between the following days; days 0-7, 0-14, 1-7, 1-14, 3-14 in the left kidney, and days 1-7, 1-14, 3-7, 3-14, 5-7, 5-14, 7-14 in the right kidney (*t*-test, $^*p < 0.05$).

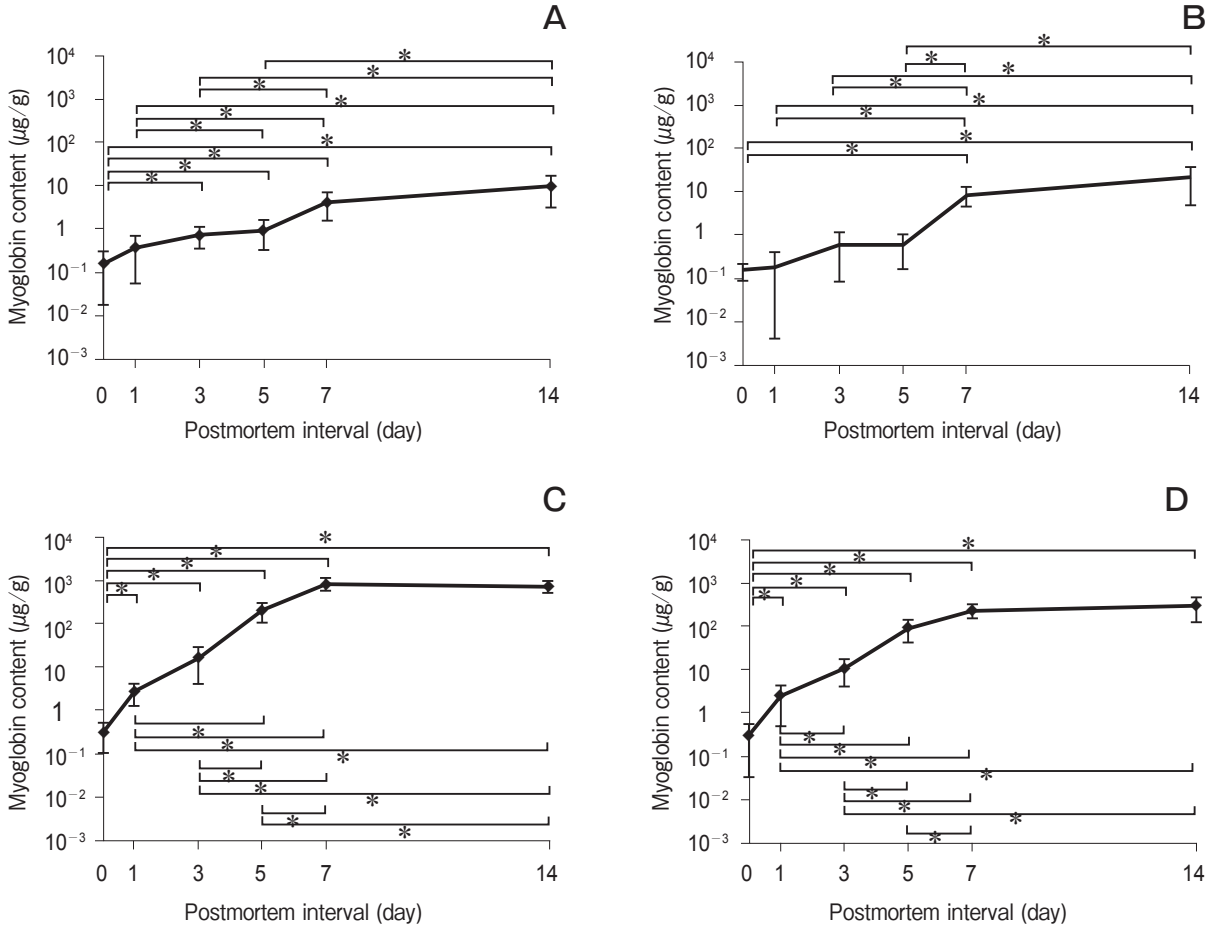

Fig. 3 Postmortem changes in myoglobin content in A, the liver; B, the brain; C, the thyroid gland; D, the left lung (mean ± S.D.). Significant differences were observed between days 0-3, 0-5, 0-7, 0-14, 1-5, 1-7, 1-14, 3-7, 3-14, 5-14 in the liver, days 0-7, 0-14, 1-7, 1-14, 3-7, 3-14, 5-7, 5-14 in the brain, days 0-1, 0-3, 0-5, 0-7, 0-14, 1-5, 1-7, 1-14, 3-5, 3-7, 3-14, 5-7, 5-14 in the thyroid gland, and days 0-3, 0-5, 0-7, 0-14, 1-3, 1-5, 1-7, 1-14, 3-5, 3-7, 3-14, 5-7 in the left lung (*t*-test, $^*p < 0.05$).

the thyroid gland was strongly stained, as was the skeletal muscle itself (Fig. 4).

Discussion

It is known that blood myoglobin concentrations increase markedly after death [13–15]. However, there have been no reports of changes in the very early postmortem period. Our animal experiment, however, has demonstrated that postmortem blood myoglobin concentrations increase very rapidly. In the present study, they reached approximately 30 μg/ml on day 1, which corresponded to 6,600 times the antemortem blood levels. This result corresponded to previously reported findings based on the use of human materials [13, 14]. In contrast, almost no decrease in myoglobin levels was observed in the striated muscles, from which the protein exuded into blood. These results, however, are not paradoxical. The striated muscle contained approximately 1 mg/g of myoglobin or more. When the postmortem decrease in myoglobin in the striated muscle was evaluated based on the amount of muscle and blood [17, 18], it was calculated to be less than approximately 10% theoretically.

When the postmortem changes in myoglobin content in both kidneys were compared with each other, that in the left increased by day 5, while that in the right, in which the renal artery and vein had been ligated immediately after death, was maintained at the same

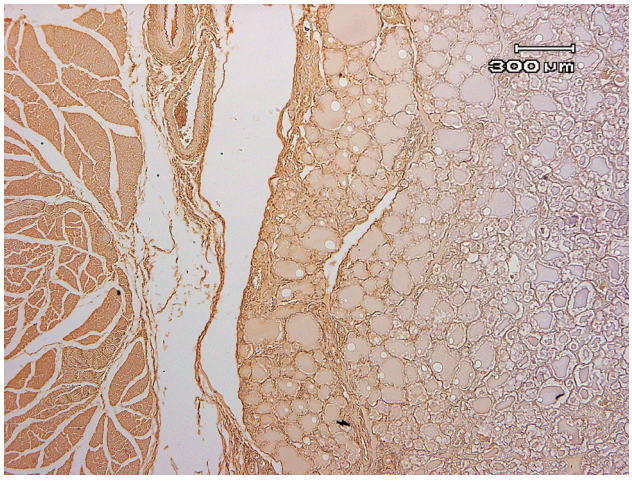

Fig. 4 Morphological feature of postmortem diffusion of myoglobin from the skeletal muscle into the thyroid (8 years old, female, cause of death was drowning, postmortem interval was approximately 2 days).

level 30 min after death. These results suggest that myoglobin was distributed through the blood into organs from the striated muscle after death. The myoglobin content in the liver increased after death as well as in the left kidney, although the specimen was taken from a region far from the diaphragm. This result can also be explained by the postmortem distribution of myoglobin throughout the blood, as mentioned above. The increase in myoglobin content in the investigated abdominal organs became obvious on day 7 or later. It is thought that the diffusion of myoglobin from the fluid of decomposition in the abdominal cavity contributed to such an increase. According to our experience with a human case [19], such fluid contains large amounts of myoglobin. In the present study, the fluid of decomposition was not found by day 5 after death, but appeared on day 7 in most of the animals used. We found that the myoglobin concentrations in the fluid observed in our experiment were 10 μg/ml or more, which was many times higher than that in the organs.

The myoglobin content in the brain on day 7 or later was higher than that in the abdominal organs. It is difficult to explain such an increase by the diffusion from the fluid of decomposition, as the cranial cavity, unlike the abdominal cavity, was not surrounded by skeletal muscle. One possible explanation is the difference in myoglobin levels between the sites of the blood. According to some previous reports, myoglobin level differ between the central and peripheral blood [16–18].

In contrast with the organs mentioned above, a great increase in the myoglobin content of the thyroid gland was observed by day 7 after death. Its logarithmical value rose almost linearly as the time after death passed, reached the level of that in the striated muscle on day 7. It was difficult to interpret such a marked increase based only on the postmortem distribution through the blood, but could be explained by direct diffusion from the striated muscle. There are some skeletal muscles, Mm. sternohyoideus, sternothyroideus, *etc.*, directly on the ventral side of the thyroid gland. It is thought that myoglobin may be exuded directly into this organ from the skeletal muscles, which has clearly been showed by immunohistochemical investigations in human cases. As revealed in Fig. 4, a strong positive reaction in the peripheral region of the thyroid gland near the skeletal muscle demonstrated a direct diffusion of myoglobin. This interpretation was also supported by the result that the myoglobin content in this small organ reached a plateau at the level of that in the striated muscle.

In the lung, the postmortem changes in myoglobin content were similar to those in the thyroid gland. However, the myoglobin content in the lung was approximately 1/3 that of the thyroid gland on days 7 and 14. One of the reasons for this difference, and especially for the quicker increase than that in the abdominal organs and the brain, is thought to be the difference in the amount of blood. The concentrations of free hemoglobin in the homogenate of the lung were much higher than those of the other organs (lung: 0.58 g/dL, liver: 0.22 g/dL, kidney: 0.24 g/dL, mean of 5 samples taken on day 1 postmortem). Another reason might be the direct diffusion from the surrounding skeletal muscles (intercostal muscles, diaphragm, and the heart), though the myoglobin content was lower than in the thyroid gland. This difference between the 2 organs was caused by their size. The specimen of the lung was taken from the part far from the skeletal muscle. The logarithmical value of myoglobin content in the lung also rose nearly linearly, similar to that in the thyroid gland. It was suggested that the myoglobin content in these organs after death could be a parameter for estimation of the postmortem interval.

In conclusion, It was confirmed that myoglobin content in the organs increased after death. Its mechanisms were thought to be the postmortem direct diffusion from the striated muscle and/or the distribution through the blood, in which the myoglobin concentrations rose to a level approximately 6,600 times that of antemortem blood in 1 day after death. Myoglobin content in the thyroid gland or the lung therefore appears to be a useful parameter for estimating the postmortem interval.

References

1. Kominato Y, Harada S, Yamazaki K and Misawa S: Estimation of postmortem interval based on the third component of complement (C3) cleavage. J Forensic Sci (1988) 33: 404–409.
2. Querido D: Double logarithmic, linear relationship between plasma sodium/potassium concentration ratio and postmortem interval during the 6-96-h postmortem period in rats. Forensic Sci Int (1990) 44: 125–134.
3. Murray EF and Hordynsky W: Potassium levels in the cerebrospinal fluid and their relation to duration of death. J Forensic Sci (1958) 3: 480–485.
4. Franschini F, Müller E and Zanoboni A: Post-mortem increase of potassium in human cerebrospinal fluid. Nature (1963) 198: 1208.
5. Aoki T: Studies on the estimation of time after death. Jikeikai Med J (1965) 1: 3–18.
6. Madea B, Henssge C, Hönig W and Gerbracht A: References for determining the time of death by potassium in vitreous humor. Forensic Sci Int (1989) 40: 231–243.
7. Sturner WQ: The vitreous humour: postmortem potassium changes. Lancet (1963) 13: 807–808.
8. Muñoz JI, Suárez-Peñaranda JM, Otero XL, Rodríguez-Calvo MS, Costas E, Miguéns X and Concheiro L: A new perspective in the estimation of postmortem interval (PMI) based on vitreous. J Forensic Sci (2001) 46: 209–214.
9. Girela E, Villanueva E, Irigoyen P, Girela V, Hernández-Cueto C and Peinado JM: Free amino acid concentrations in vitreous humor and cerebrospinal fluid in relation to the cause of death and postmortem interval. J Forensic Sci (2008) 53: 730–733.
10. Coe JI: Postmortem chemistry update. Emphasis on forensic application. Am J Forensic Med Pathol (1993) 14: 91–117.
11. Wehner F, Wehner HD, Schieffer MC and Subke J: Delimitation of the time of death by immunohistochemical detection of insulin in pancreatic β-cells. Forensic Sci Int (1999) 105: 161–169.
12. Wehner F, Wehner HD, Schieffer MC and Subke J: Delimitation of the time of death by immunohistochemical detection of thyroglobulin. Forensic Sci Int (2000) 110: 199–206.
13. Miyaishi S: An enzyme immunoassay for human myoglobin and its application to forensic medicine. Jpn J Leg Med (1990) 45: 6–25 (in Japanese).
14. Puschel K, Lockemann U and Bartel J: Postmortem investigation of serum myoglobin levels with special reference to electrical fatalities. Forensic Sci Int (1995) 72: 171–177.
15. Suzuki T, Kashimura S and Umetsu K: Postmortem permeation of myoglobin into the blood. Z Rechtsmed (1983) 90: 297–301 (in German).
16. Kitao T, Miyaishi S and Ishizu H: Identification of human skeletal muscle from a tissue fragment by detection of human myoglobin using a double-sandwich ELISA. Forensic Sci Int (1995) 71: 205–214.
17. Long C: Biochemists' Handbook, 1st ED E & F N Spon Ltd, London (1961) pp 665.
18. Ernest Beutler: Williams Hematology, 5th Ed, New York, Tokyo, McGraw-Hill (1995) pp 2699.
19. Miyaishi S, Moriya F, Yamamoto Y, Kitao T and Ishizu H: Discrimination between postmortem and antemortem blood by a dot-ELISA for human myoglobin. Jpn J Leg Med (1994) 48: 433–438 (in Japanese).