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Estimation of Post-mortem Interval Based on Livor Mortis using a Colorimeter in Thai Populations

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ABSTRACT

OBJECTIVE Livor mortis is a helpful and widely used method of estimating postmortem interval (PMI) in Thailand. This study aimed to investigate the value of a colorimeter as a tool for estimating the PMI.

METHODS The color of livor mortis and control skin in 80 cadavers whose PMI was within 12 hours was measured by a colorimeter. The L* (brightness), a*b* (chroma and hue), and ΔE* values were compared to the control skin values. Statistical analysis was performed to determine the relationship between PMI and skin color before and after application of a specific pressure.

RESULTS The results showed that colorimetric parameters were only weakly correlated with the PMI. An univariable analysis of ΔE* values was performed and showed good discriminatory power, with an area under the ROC curve of 0.82. The recommended cut-off value of ΔE* was 14 for the discrimination between early PMI (less than 6 hours) and late PMI (6-12 hours), in which the sensitivity and specificity were 72.5% and 80%.

CONCLUSIONS The findings in this study reinforce the utility of colorimetric measurements in PMI estimation. With additional study and a larger sample size, the estimation of PMI could be established for general use in forensic practice.

KEYWORDS livor mortis, colorimeter, time since death, taphonomy, forensic pathology

INTRODUCTION

In the field of forensic science, the post-mortem interval (PMI) refers to the period between the occurrence of death and the discovery of a body. The accurate determination of the PMI is of the utmost priority, as it facilitates the identification of human remains and contributes to an extensive examination of potential causes of death. Furthermore, the establishment of the PMI assumes significance in discerning potential criminal acts and facilitating the determination of appropriate legal consequences (1-3). The estimation of the PMI relies on the examination of various post-mortem changes exhibited by a cadaver. These alterations include physical changes (body cooling and livor mortis), physicochemical processes (cadaveric stiffening), metabolic reactions (supra-vital reactions), decomposition, and the influence of insect activity (4-6).

Livor mortis is a dark-purple staining of the skin resulting from the gravitational accumulation of blood within the vascular system of the body's dependent regions (7-9). After circulatory arrest, livor mortis develops as one of the early postmortem alterations. Within one hour after death, livor mortis becomes visible as pink patches that result

in a progressive and consistent confluence with an increasing PMI, usually reaching its maximum coloration and becoming fixed at variable times after death (8). For that reason, livor mortis is a useful and popular method for estimating PMI (9), and it is a common practice in Thailand. To assess livor mortis, forensic pathologists frequently employ visual observation of its color and distribution (10). Nevertheless, it is challenging to give a precise and accurate description of the color and level of hypostasis due to the high subjectivity of visual color recognition and estimation of the progression of livor mortis. To measure the color of livor mortis more accurately, a number of objective techniques have been attempted (11-18). One technique is colorimetric analysis which is employed as an adjunctive tool to facilitate the estimation of the PMI.

Previous studies have demonstrated the utilization of a tristimulus colorimeter for assessing skin color changes caused by livor mortis (11, 12, 15, 18). Vanezis found that there was a linear relationship between the fading color of hypostasis and the time during which the measurements were carried out (11). Kaatsch and Nietert used a colorimeter to measure pressure-induced color changes in livor mortis. They highlighted the potential usefulness of colorimetric measurement in estimating the PMI based on initial measurements on cadavers, describing the regular course of color changes in livor mortis with the application of increasing pressure (12). Vanezis and Trujillo also emphasized that livor mortis is particularly useful for estimating the PMI within the first 48 hours, as the rate of color change is more pronounced during this period. After that time, the rate of change becomes reduced or non-existent, and by 72 hours, livor mortis has typically become fixed in the majority of cases (15).

Although numerous novel methods have been created which potentially provide more accurate PMI estimation and that can be applied universally (19-21), none have been designed for specific geographic regions (22). Climatic and environmental settings can influence the rate and pattern of postmortem change (22, 23). Therefore, region-specific studies should be conducted. Unfortunately, no taphonomic research focusing on the relationship between the rate of change of livor mortis and PMI has been conducted in tropical zones such as Thailand. In addition, variations in skin color exist among different ancestral populations, potentially reducing the accuracy of colorimetric measurement of livor mortis in Thai and other populations.

This study aimed to investigate the usefulness of the colorimetric measurement of livor mortis in accurately determining the time since death in Thailand, thereby giving further information to help forensic pathologists determine the PMI of bodies found in tropical countries.

METHODS

The study sample

This study site was a metropolitan area of Bangkok, the capital city of Thailand. The region experiences a tropical climate, with an average daily temperature of 28.0 °C. It is classified as Aw according to the Köppen-Geiger classification system (24). This classification means Bangkok is an equatorial savanna with a dry winter. At the time of the measurements, the average environmental temperature ranged from 26 °C to 31 °C and humidity ranged from a minimum of 74% to a maximum of 85%.

This prospective study was conducted on cadavers examined in the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand. From October 2022 to September 2023, 80 forensic autopsy cases were selected for this study. An informed consent form was obtained from legal heirs before data collection. Cadavers with the following features were excluded from this study: 1) an indeterminate time of death, 2) signs of decomposition, 3) a history of anemia or bleeding tendency, 4) not lying in a supine position, and 5) a history of significant hemorrhage from trauma or disease. Pertinent demographic information including sex, age, underlying disease, time and date of death, location of livor mortis, and posture of the cadaver were recorded. The causes of death were determined following complete autopsy examinations and extensive police investigations. The PMI data was checked with hospital records and police investigative records to help ensure the accuracy and consistency of the information.

The PMI was known for all cases and ranged between 1 hour and 12 hours. According to a previous pilot study in Thailand, the level of lightness

of postmortem livor mortis becomes fixed 12 hours after death.

Colorimetric measurement

Skin color measurements were conducted using a portable colorimeter (FRU® WR-18). The instrument was set up including a standard D65 light source, illumination mode 8/d, light mode SCI, observer = Commission Internationale de l'Eclairage (CIE) 10°, color space CIE1976 LAB, and the 8 mm diameter caliber. The instrument was calibrated using a black-and-white calibrator prior to each measurement.

In some cases, a cadaver had been left in an environment with an unknown temperature between the time of death and measurement. At the autopsy room, measurements were carried out at a temperature of 25 °C to 30 °C. Cadavers were turned from a supine position to one side, and the left and right scapulae at the areas inferior to the lateral aspect of the scapular spine were measured three times (Figure 1). These regions were selected due to their higher probability of onset of livor mortis and ease of application of the colorimeter (18). Colorimetric data were measured with minimal force at a right angle to the area of the livor mortis. This initial set of data from the area before the application of pressure was called the 'before-pressure group' or 'BP' group.

Following that, pressure was applied on the same location of livor mortis by pressing the measuring head of the dynamometer (Force Meter SF-100) at a force of 1.5 kg/cm^2 for 3 seconds. These parameters are the most appropriate values for inducing blanching in livor mortis (18). Colorimetric measurements were then taken on the pressed area. This new data set was referred to as the 'after-pressure group' (or 'AP' group). The control colorimetric data were determined by measuring normal skin at the anterior chest (Figure 1). The average data of three scans was used to create each control. The end result was 3 sets of data (control, BP, and AP) for each cadaver which were then analyzed in this study.

Color analysis

The CIE L*a*b* system was used to quantitatively evaluate the color of livor mortis in a three-dimensional space. The L* value denotes brightness with a range of 0-100, where 0 is black and 100 is white. The proportions of different colors are represented by the chroma coordinates a* and b*. A positive value of a* denotes a red component, while a negative value denotes a greenish one. A yellow color is represented by a positive value of b*, whereas a blue color is represented by a negative value. The individual typology angle determination (ITA) was caluclated using the formula: °ITA = [arctan(L*-50)/b*] × 180/3.14159. This allows classificaton skin color types into six groups, from very light to dark skin color: very light > 55°, light 55° to > 41°, intermediate 41° to > 28°, tan 28° to > 10°, brown 10° to > -30° and dark ≤ -30° (25).

In order to evaluate the colorimetry of livor mortis in all three values, L*, a*, and b* were observed before and after the application of pressure and were compared to the colorimetry of the control region. The ΔL*, Δa*, and Δb* colorimetric values were obtained by subtracting the livor mortis values from the control value. In addition, the color difference value (ΔE*) was used to encompass all color differences in a single arithmetic value. The equation used is ΔE* = [(ΔL*)2 + (Δa*)2 + (Δb*)2]1/2. The ΔE* value range is 0 to 100, with 0 indicating very little color change and 100 indicating extreme distortion. The lower the ΔE*, the harder it is to distinguish colors. The greater ΔE*, the more dissimilar two colors are (26).

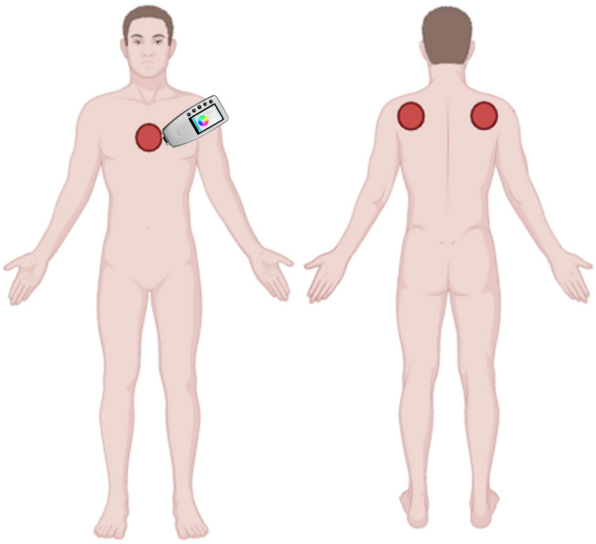

Figure 1. Anatomical landmarks on cadavers from which color measurements were obtained (red circles). This image was created with BioRender (biorender.com)

Statistical analysis

The sample data consisted of descriptive statistics, with continuous data shown as mean, standard deviation (SD), and minimum-maximum values (range). Categorical information is displayed as a number and a percentage. Normal probability plots were used to verify the normality of the colorimetric data. To investigate the relationship between measured color value and PMI, simple correlation was calculated using the Pearson correlation coefficient. Logistic regression analysis was used to analyze the association of PMI and colorimetric parameters as ROC curves and the area under the curves. A cutoff score of the most statistically significant colorimetric parameter was selected based on the sensitivity and specificity of the ROC curve.

All statistical analyses were carried out using SPSS version 25 software (IBM, USA). Statistical significance was defined as a p-value of less than 0.05.

Ethical approval was obtained from the Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand (SIRB Protocol No.652/2565 (IRB1), 8 December 2022).

RESULTS

Sample characteristics

A total of 80 cadavers were included in this study, 57 males and 23 females. The mean age of the males and females was 51.5 years (range 24-86 years, standard deviation [SD]=14.23) and 58.3 years (range 29-92 years, SD=20.31), respectively. The mean PMI was approximately 6.9 (SD=3.42) hours in males and 5.6 (SD=3.14) hours in females. Deaths were due to the following causes: sudden cardiac arrest (42.5%), coronary artery disease (31.25%), head and neck injury (6.25%), infectious diseases (6.25%), asphyxia (3.75%), thyrotoxicosis (2.5%), intracerebral hemorrhage (2.5%), senility (2.5%), and gastrointestinal diseases (2.5%).

Using L* and b* colorimetric data from the control area, the cadavers were classified into five types of skin color: very light (7.5%), light (23.75%), intermediate (43.75%), tan (18.75%), and brown (6.25%).

Colorimetric characteristics

Characteristics of color data obtained from the areas of livor mortis before and after application of pressure are illustrated in Figure 2. Values of pressure-induced changes in L*, a*, and b* were analyzed. Lower observed L* values indicated a decrease in the brightness of livor mortis as the postmortem time progressed, while the application of 1.5 kg/cm^2 of pressure made the livor mortis lighter. After 6-hours postmortem, changes in levels of brightness were still slightly positive. Similar to the L* values, the b* value also displayed a general decline over time. As time progressed, the a* values showed an increase in the reddish hue. No statistically significant difference was observed between the left and right scapular areas in any of the L*, a*, and b* data ($p > 0.05$).

Table 1 shows the Pearson correlation coefficients for the relationship between colorimetric measurements (ΔL*, Δa*, Δb*, ΔE*) and PMI. All colorimetric measurements of the control areas showed an insignificant, very weak correlation with PMI (r = -0.07 – 0.22, p = 0.13-0.54). A negative correlation was observed in the L* and b* values before and after application of pressure. In this study, all ΔL*, Δa*, Δb*, and ΔE* were found to be statistically significantly associated with the PMI ($p < 0.01$).

The ΔE* values of the right scapular area before the application of pressure showed the highest correlation with PMI (r = 0.62, $p < 0.01$) and were plotted against postmortem intervals as shown in Figure 3. The regression formula was used to find the rate of change of ΔE* during the measurement interval. Plotting the line of best fit through the points, the relationship between the BP ΔE* values and PMI was found to be as follows: PMI (hours) = 0.388 (ΔE*) + 1.038. However, BP ΔE* had only a weak positive relationship with PMI (adjusted R2 = 0.382, $p < 0.01$), so ROC curves in logistic regression were used to determine the best cut-off values in this dataset.

The area under the ROC curve for the BP ΔE* values was 0.82, 95% CI [0.7-0.9] (Figure 4). The BP ΔE* values were used to discriminate between early PMI (less than 6 hours) and late PMI (6-12 hours). The cut-off value of ΔE* of the right scapular area before the application of pressure, which allows for the best sensitivity and specificity, is 14 (sensitivity 72.5%, specificity 80%) (Table 2). A score range with higher scores indicates a greater likelihood of later PMI.

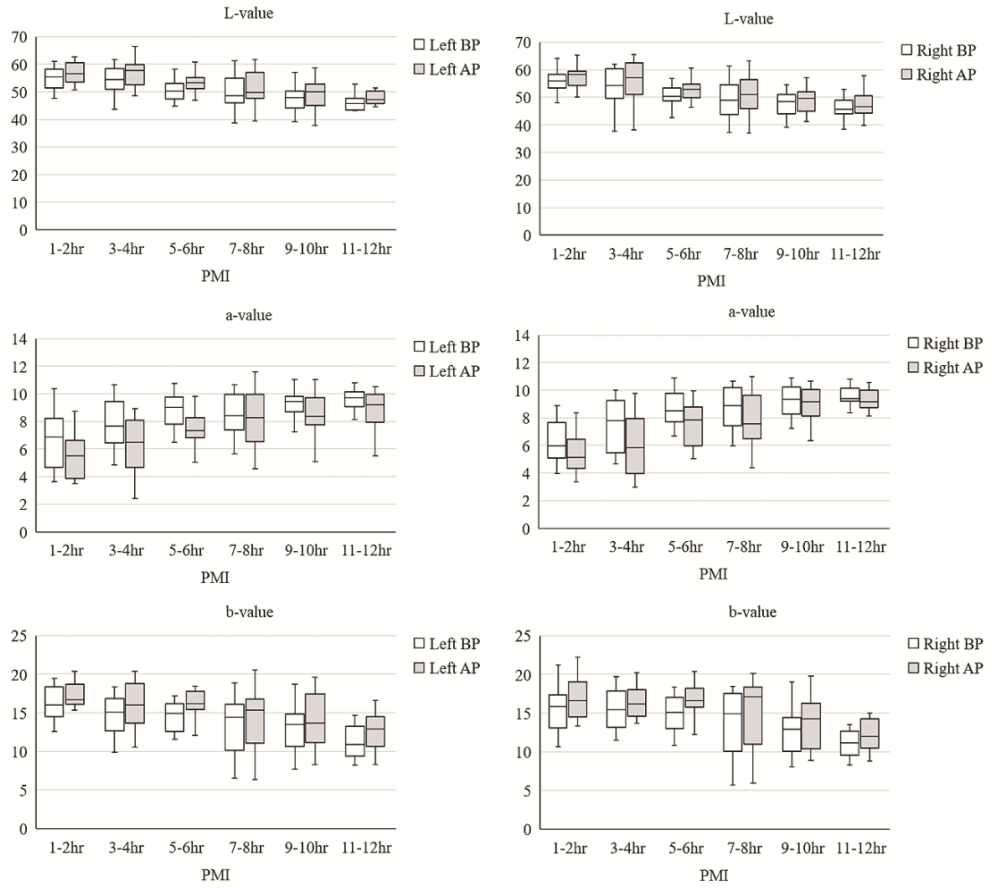

Figure 2. Characteristics of color data obtained from the area of livor mortis before (BP) and after (AP) application of pressure

Table 1. Pearson correlation for PMI and colorimetric measurement before (BP) and after (AP) application of pressure

	Right scapular area								Left scapular area							
	BP				AP				BP				AP			
	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*	ΔL*	Δa*	Δb*	ΔE*
Pearson correlation	-0.54	0.58	-0.49	0.62	-0.52	0.61	-0.47	0.60	-0.53	0.52	-0.50	0.57	-0.53	0.57	-0.46	0.60
p-value	< 0.01															

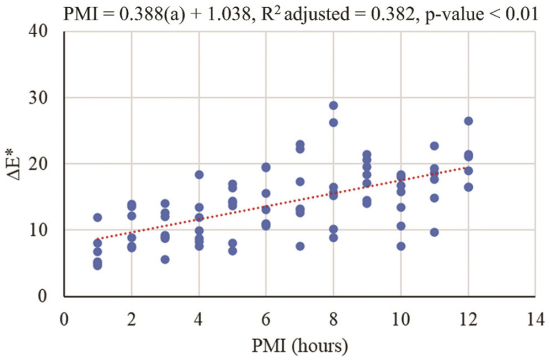

Figure 3. Color difference value (ΔE*) of the right scapular area before applying pressure with different postmortem intervals (PMI)

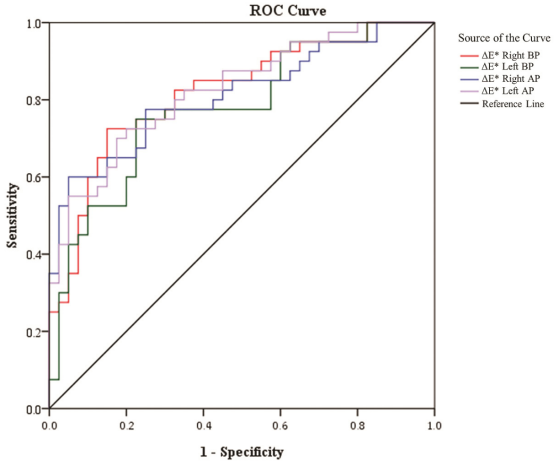

Figure 4. ROC curve associated with the PMI: area under ROC curve of ΔE* values of the right scapular area before application of pressure = 0.82

Table 2. Sensitivity and specificity of ΔE* values of the right scapular area before application of pressure

Cut point (ΔE*)	Sensitivity (%)	Specificity (%)	Accuracy (%)	Positive predictive value (%)	Negative predictive value (%)
12	85	57.5	71.25	66.67	79.31
13	82.5	65	73.75	70.21	78.79
14	72.5	80	76.25	78.38	74.42
15	67.5	85	76.25	81.82	72.34
16	60	87.5	73.75	82.76	68.63

DISCUSSION

In Thailand, livor mortis is commonly used to estimate PMI. Among forensic pathologists, direct observation remains the most popular analytical method for determining livor mortis (4, 7). This study investigated pressure-induced color changes in livor mortis under standardized conditions on a large number of cadavers with known PMI.

A wide variety of skin colors among cadavers were observed. It is considered that the color of the original skin has an impact on the color of postmortem livor mortis (17). Original skin color in this study was determined by the colorimetric measurements of the color of the skin in an area without livor mortis. The results showed a wide variety in the degree of natural pigmentation, ranging from very light to brown, with 43.75% of the cadavers being intermediate in skin color. These findings parallel a skin color study by Del Bino and Bernerd (27), which found the skin color of Caucasians to be generally light to intermediate. The authors of the present study calculated the color difference between postmortem livor mortis and the control skin to standardize the colorimetric parameters before analysis.

Several authors have attempted to use more objective methods to estimate an unknown PMI from livor mortis; however, the wide scattering of outcomes gives the results little practical value in forensic casework (11-17). It is imperative to reconsider the following factors affecting livor mortis: intensity and duration of pressure applied and the area where the pressure is applied. In a recent study of 101 cadavers, Romanelli et al. suggested that a pressure of 1.5 kg/cm^2 and a duration of 3 seconds were the most suitable conditions to make a more standardized analysis of livor mortis (18). The present study attempted to use those parameters to improve the value of livor mortis in the determining the time since death. This study found all colorimetric parameters to be statistically significant ($p < 0.01$), but the data obtained from areas of livor mortis were only weakly correlated with PMI. There is a linear relationship between colorimetric measurements and PMI during the first 12 hours with the degree of color change and brightness associated with livor mortis decreasing as the PMI increases (15). Even though ΔE* showed the highest correlation with the PMI, its regression formula has been reported to have a weak correlation coefficient of 0.382.

A number of issues were considered in conducting this study. The study had to be designed to resemble actual forensic practice, where it is sometimes difficult to find trustworthy information about the history of the deceased and the circumstances of their death. The wide variations in PMI estimation observed in this study can be explained by other variables such as antemortem physical conditions, cause of death, and antemortem and postmortem environmental factors. Due to the variability of the time before each of the remains arrived at the mortuary, environmental factors such as temperature and humidity could have affected the rate of livor mortis development. Such variables need to be considered when determining PMI using colorimetric examination.

In this study, the authors used the ΔE* value from the right scapular area prior to application of pressure to discriminate between early PMI (< 6 hours) and late PMI (6-12 hours). Even though uncontrolled factors such as antemortem status and environmental conditions were inevitably encountered, the authors decided to use a cut-off point with the most appropriate sensitivity and specificity in order to achieve the best possible accuracy. Accordingly, we recommend a cut-off point ΔE* value of 14 to differentiate between early and late PMI.

This study provides quantitative criteria that allow for a more accurate estimation of the time of death. There is a need for additional studies using a larger sample and a wider diversity of skin color characteristics. Postmortem blood concentration may be considered for its role in the formation of livor mortis. Research on how uncontrolled antemortem conditions might affect PMI need to be studied as well. Further research is needed to help develop a better understanding, e.g., studies which include subjects with a known medical history such as the percentage by volume of red cells.

CONCLUSIONS

In forensic medicine, PMI estimation in medicolegal cases is one of the most frequent and challenging topics. Numerous techniques have been developed and implemented in an attempt to improve the accuracy of determination of the PMI. In this study, color change of livor mortis under the application of pressure was evaluated based on colorimetric measurements. By offering objective standards for measuring color change, the method presented here should encourage wider use of pressure-induced blanching of livor mortis in estimating PMI.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

ADDITIONAL INFORMATION

Author contribution

S.N.: conceptualization, methodology, data collection, data analysis, writing - draft. V.V.: supervision, conceptualization, methodology, data analysis, writing – review and editing

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