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RESEARCH ARTICLE

Numerical Analysis of the Change in Skin Color due to Ecchymosis and Petechiae Generated by Cupping: A Pilot Study



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acupoint; cupping; ecchymosis; petechiae; pigmentation

Abstract

Cupping is one of the various treatment methods used in traditional oriental medicine. Cupping is also used as a diagnostic method and it may cause skin hyperpigmentation. Quantitative measurements and analysis of changes in skin color due to cupping are critical. The purpose of this study is to suggest an optical technique to visualize and identify changes in skin color due to cupping. We suggest the following analysis methods: digital color spaces [red, green, and blue (RGB) and L*a*b], the Erythema Index (E.I.), and the Melanin Index (M.I.). For experiments, we selected and stimulated 10 acupoints at 80 kilopascals (kPa) per minute. The RGB and L*a*b color spaces were observed to be decreased (p < 0.05) after cupping. The E.I. and M.I. were observed to be increased significantly (p < 0.05) after cupping. To assess various changes in skin color, we observed the changes for 72 hours. We also obtained the color changes by using the recovery pattern during the recovery period (p < 0.01). We propose that this method can be useful for visual identification and as a way to improve the identification of skin color changes.

1. Introduction

Oriental medicine is a medical science that is used to cure internal nature through controlling life energy (Qi). Various

studies have been carried out on acupuncture, moxibustion, and cupping as representative treatments for controlling Qi [1-3]. However, research on objectification of the condition of meridians and acupoints is lacking. Some

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representative studies reported hypodermic phenomena such as perspiration and induration occurring after meridian. Other studies proceeded to objectify the relationship between meridians, nervous systems, and body fluid factors [4,5]. In addition, many researches were reported as an attempt to visualize meridian and acupoints using image processing through infrared filming and ultrasonic filming [6–9]. Recently, several studies were undertaken to secure an objective diagnostic area by observing situations of meridians and changes in the cerebral cortex using positron emission tomography (PET), functional magnetic resonance imaging (fMRI), or autoradiography [10,11].

However, studies to date have not suggested enough evidence to illustrate the exact mechanism that corresponds to the meridian theory. It is difficult to acquire the exact mechanism of interaction between the internal organs and the meridian in which the symptoms of disorders occur. Thus, there is an urgent need to develop biomedical instruments functionally similar to the five senses—the traditional diagnostic methods used in Oriental medicine—that can be widely used to identify the state of acupoints incurred with different disorders. In this regard, cupping is also one of the diagnostic method that needs to propose a new method. Cupping has been used as treatment for chronic pain, including lower back pain, and indigestion [12-16]. Recently, some papers reported its therapeutic effect on dermatologic conditions including acne when applied to the face [17,18]. Moreover, cupping has commonly been applied to the back and abdomen in order to diagnose ecchymoses and petechiae as skin ailments where a cup was placed [19]. Cupping has been utilized in diagnosis for observing the skin hemoglobin response, blister response, and response of an oppressive pain. Among these, the hemoglobin response has been widely used to identify extravasated blood. In addition, the pigmentation response, which is divided into four visual steps, assesses the state of internal organs indirectly [20]. Because cupping as a diagnostic method is still subjective, there is a need to suggest a new numerical analysis as an alternative to the visual diagnostic method. Thus, this paper suggests a new numerical analysis using optical techniques in order to detect the changes in skin color incurred from cupping. There are two representative methods of quantitatively assessing the human skin surface. The first method is parallel-polarized light photography, which enhances the surface reflectance components. Parallel-polarized light photography is widely used to examine the skin texture and elevation simply by obtaining reflected light. The second method is crosspolarized light photography, which eliminates the regular reflectance of human skin and enhances the subsurface reflectance components. Cross-polarized light photography has been widely used as a technique for visual examination of subsurface characteristics, such as pigmentation and erythema, by obtaining the back-scattering light [21-23]. There are two ways to adjust the color balance by illumination. One of them is the auto-white balance, which cannot respond to the different photography condition, because the camera automatically adjusts to the changing illumination levels. The custom white balance uses an achromatic color tool, such as a gray card, for balancing the color. However, the achromatic color tool poses some problems including some color-related damage and the degree of the color change [24]. Hence, the tri-stimulus colorimeter was used to detect various changes in the skin color such as the hemoglobin response and the pigmentation response. To confirm the significance, we selected the five acupoints of the bladder (BL) meridian as a stimulating points (left/right BL13, BL15, BL18, BL20, and BL23). Then, the detected skin color was analyzed by XYZ coordinates.

The red, green, and blue (RGB) color space represents from 0 (black) to 255 (white). After cupping, the skin color changes to a blackish color. Hence, it is necessary to analyze the RGB color space of the skin color. Moreover, it is possible to distinguish the skin color by using the L*a*b* color space, which was calculated by using XYZ coordinates. The L*a*b* color space, standardized by the Commission Internationale de l'Eclairage (CIE), has been used as the estimation index to determine information on color. L* represents the light intensity within the range from 0 (black) to 100 (white), whereas a* and b* represent the color saturation within the range from -60 to 60. In the case of a*, -60 and 60 signifies green and red, respectively; in the case of b*, -60 and 60 signifies blue and yellow, respectively [25]. As a result of the change in the skin after cupping, L* decreases to 0 (black) and a* increases to 60 (red). Thus, it is possible to utilize the change in L* and a* as an objective index.

In the study of Kouskoukis and Leider [26], during the cupping the toxins in the blood were emitted outside of the body and the alkalescent was neutralized. The numbers of red blood cells and white blood cells were increased by means of blood purification mechanisms; hemoglobin was contained in vast amounts within the red blood cells. Hence, the indirect detection of the amount of hemoglobin is an important element in color diagnosis. Moreover, cupping leads to changes in skin color such as the pigmentation response—this is caused by the melanin inside the capillaries beneath the skin. Thus, it is necessary to detect indirectly the amount of melanin. Hence, we selected the Erythema Index (E.I.) and the Melanin Index (M.I.) as our methods of analysis. The M.I. represents the amount of melanin pigmentation, and the E.I. represents the amount of hemoglobin [27,28].

For these reasons, skin color in our experiment was analyzed by the RGB and L*a*b* color spaces and the E.I. and M.I. methods. The aim of our study was to confirm that it is possible to numerically detect and analyze various changes in skin color after cupping. Thus, this paper reports a new numerical analysis method in order to objectify the diagnostic method of cupping.

2. Materials and methods

2.1. Selection of participants and acupoints

We chose 30 male college students (age, 24 ± 2 years) who voluntarily agreed to participate in the clinical test. In order to avoid potential adverse effects of acupoints, smoking, alcohol, and coffee were forbidden 12 hours prior to the clinical test. A written consent form was obtained from each participant after they were informed of the nature and requirements of this experiment. We also checked skin lesions due to burns around acupoints to

reduce experimental error. Typically, cupping has been used to attach cupping glass to the Bladder Meridian (BL) or the Governor Vessel Meridian (GV). We considered relieving the pain caused by cupping, thus we selected acupoints in the BL, which is positioned in the back muscle. In order to detect the change in skin color as a result of cupping, the left/right BL13, BL15, BL18, BL20, and BL23 were selected. The reason for the selections was that the 10 acupoints were the special points that represent the five viscera of heart, liver, spleen, lungs, and kidneys of the BL.

2.2. Analysis using optical techniques

To observe the change in skin color such as the pigmentation response and hemoglobin response after cupping, we used the CR-400 (Konica Minolta, Osaka, Japan) as a tristimulus colorimeter, which was standardized by illuminant Dc. The tri-stimulus colorimeter detected the amount of light reaching the skin via three filtered photodiodes and the XYZ coordinates were determined from the state of the reflected light [29]. The colorimetric data can be classified from the characteristics of the illuminant. The RGB and L*a*b* color spaces were standardized by illuminant D50 [30]. The XYZ coordinates of illuminant Dc were converted to illuminant D50 using the Bradford Matrix [31] [Equation 1]:

$$\begin{bmatrix} X \\ Y \\ Z \end{bmatrix}_{D50} = \begin{bmatrix} 1.0377 & 0.0154 & -0.0583 \\ 0.0171 & 1.0057 & -0.0189 \\ -0.0120 & 0.0204 & 0.6906 \end{bmatrix} \begin{bmatrix} X \\ Y \\ Z \end{bmatrix}_{Dc}$$
 (1)

The Color Checker chart has been used in the photographic and video fields. The chart consists of 24 color patches and is used to assess color values. The RGB color spaces of the Color Checker were divided into four different spaces [32,33]. Each RGB color space has a different color gamut. The sRGB has been commonly used in cathode ray tube (CRT) monitors, but it is problematic due to the loss of green and blue [34,35]. The Adobe RGB has a wider color gamut than the sRGB and can overcome the loss of color. The XYZ coordinates were converted to the Adobe RGB color space, which was standardized by illuminant D50 using Equation 2 [31]. We calculated the L*a*b* space using Equation 3.

$$\begin{bmatrix} R \\ G \\ B \end{bmatrix} = \begin{bmatrix} 1.9624274 & -0.6105343 & -0.3413404 \\ -0.9787684 & 1.9161415 & 0.0334540 \\ 0.0286869 & -0.1406752 & 1.3487655 \end{bmatrix} \begin{bmatrix} X \\ Y \\ Z \end{bmatrix}_{D50}$$
(2)

$$L^* = 116 \times \left(\frac{Y}{Y_n}\right)^{\frac{1}{3}} - 16$$

$$a^* = 500 \times \left[\left(\frac{X}{X_n} \right)^{\frac{1}{3}} - \left(\frac{Y}{Y_n} \right)^{\frac{1}{3}} \right]$$
 (3)

$$b^* = 200 \times \left[\left(\frac{Y}{Y_n} \right)^{\frac{1}{3}} - \left(\frac{Z}{Z_n} \right)^{\frac{1}{3}} \right]$$

where,

$$\frac{X}{X_n} > 0.008856$$
 else $\frac{X}{X_n} = 7.787 \times \frac{X}{X_n} + \frac{16}{116}$

$$\frac{Y}{Y_n} > 0.008856$$
 else $\frac{Y}{Y_n} = 7.787 \times \frac{Y}{Y_n} + \frac{16}{116}$

$$\frac{Z}{Z_n} > 0.008856$$
 else $\frac{Z}{Z_n} = 7.787 \times \frac{Z}{Z_n} + \frac{16}{116}$

$$X_n = 96.422, Y_n = 100, Z_n = 82.521$$

To analyze the E.I. and M.I., we used Equation 4 and Equation 5:

$$E.I. = 100 \times \log\left(\frac{R_r}{R_o}\right)$$
 [4]

$$M.I. = 100 \times \log\left(\frac{1}{R_a}\right)$$
 [5]

The "r" and "g" denote the red color and the green color, respectively. The $W_{r,g}$ is the average red and green with a value of 99% diffuse reflectance white standard using the reference of normalization, and $S_{r,g}$ is the detected red color and green color at the acupoint. The $R_{r,g}$ was calculated by $S_{r,g}/W_{r,g}$. Hence, the $R_{r,g}$ is the normalized red color and green color at the acupoint. Matlab version 7.1 was used to calculate the RGB and L*a*b* color spaces, the E.I., and the M.I. in order to obtain information on skin color.

2.3. Selection of negative pressure intensity on cupping and stimulation methods

It has been revealed that various bio-responses are incurred depending on the degree of intensity of negative pressure on cupping [36]. Thus, there is a need for an accurate diagnosis of constant negative pressure. It was reported that when it is below 34 kilopascals (kPa) [37], linear changes could be observed in the tissue deformation at stimulated areas. However, studies have reported that it is more appropriate to use 70–80 kPa negative pressure in Chinese medicine [38,39]. Therefore, the constant pressure of 80 kPa for 1 minute was chosen.



Figure 1 ColorChecker 2005.

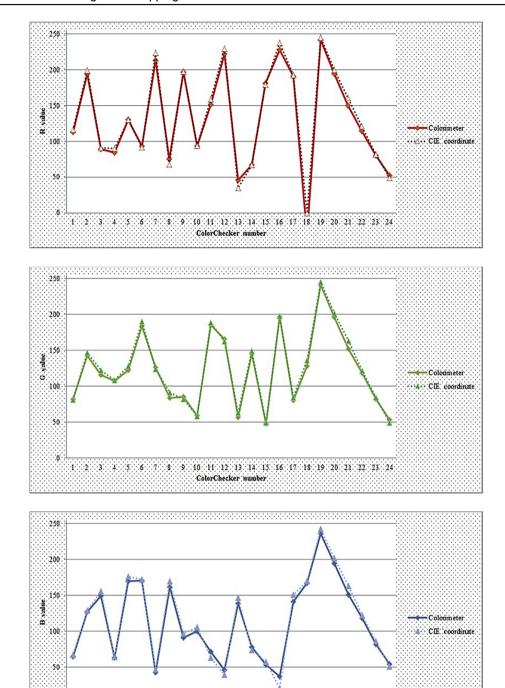


Figure 2 Measurement accuracy between colorimeter and CIE coordinate (R: red, G: green, B: blue).

ColorChecker number

7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

2.4. Measurement method

Because the dryness/moisture of the skin responds to the environmental temperature of the experiment, this experiment was conducted in an optical test laboratory, a dark room where a constant temperature of 24°C at a humidity of 40% could be maintained. In order to relieve the discomfort caused by the fixed posture and to decrease the reflected light error caused by the angle of the irradiation on the emitted light,

3. 4. 5. 6

participants were laid down on a flat bed. The detection of skin color were measured after fixing the tri-stimulus color-imeter vertically and repeated with 10 sets in a dark room in order to minimize the interference from other light sources. In order to confirm the changes in skin color during the process, we observed the RGB and L*a*b* color spaces and the E.I. and M.I. for 72 hours. This experiment was approved by the Yonsei University Wonju Campus Human Subjects Institutional Review Board (Wonju. Republic of Korea).

Table 1	Comparison of measured roughness data.	
	Prior to cupping	After cupping
R value	100	71.16 ± 13.98
G value	100	63.82 ± 1.19
B value	100	65.96 ± 20.56
L* value	100	67.25 ± 15.73
a* value	100	66.51 ± 95.10
E.I	100	180.93 \pm 43.11
M.I	100	158.72 ± 34.09
B = blue; E.I. = Erythema Index; G = green; M.I. = Melanin Index; R = red.		

2.5. Statistical analysis

A simple linear regression analysis was used to confirm the accuracy of the measurements carried out by the Adobe RGB color space (standardized by the CIE) and the RGB color space at Color Checker 2005.

All participants had different skin colors at each acupoint. Therefore, the skin color variance after cupping was influenced by skin color prior to cupping. Hence, it is necessary to normalize the measured skin color from the normal skin color. For this purpose, the measurement values before cupping are considered as a reference value and other measurement values at after cupping, after 24 hours, 48 hours and 72 hours are divided by reference value in order to normalization. So, RGB, L*a*b* color space, E.I and M.I before cupping was considered as 1. All indexes that indicated the skin color were normalized. After normalizing, all indexes were converted to a percentage. We conducted the paired t test on all indexes to assess the reflection of the changes in skin color after cupping. We selected one participant that was representative of the various change in skin color at each acupoint. To assess the reflection of various changes in skin color, the contrast test based on the one-way repeated measures analysis of variance (ANOVA) was conducted. Moreover, we conducted the contrast test based on the one-way repeated measures ANOVA to confirm the reflection of any tiny change in skin color from the normal state. The significance level of the

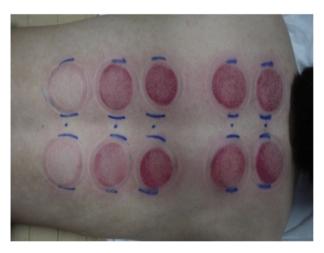


Figure 3 The change in skin color after cupping (Participant 1).

statistical analysis was set at p < 0.05. All data were represented on a quadratic regression curve and spline in order to express general changes in skin color (Fig. 1).

3. Results

3.1. The accuracy

A simple linear regression analysis indicated a high linearity of 0.996, 0.996, and 0.937 in each R square of RGB color space (Fig. 2).

3.2. Analysis of the changes in RGB, L*a*b*, E.I., and M.I. prior to and after cupping

Because the physiological and pathological conditions of the participants were different, various changes in skin color after cupping were generated. Hence, the RGB, L*a*, E.I., and M.I. differed from individual to individual. Moreover, all indexes were observed diversely by different values at each acupoint. However, both RGB and L*a* color spaces were commonly observed to decrease after cupping on results of the paired t test (p < 0.05) on all acupoints in all the participants except in a few cases. Asian skin color has the proximate negative or positive range from 0 to a *. The a * also changed the positive range because of the increase toward 60 (red) saturation after cupping. Hence, after normalizing, we observed that a* was lower than its level prior to cupping. Likewise, E.I. and M.I. commonly increased after cupping on the results of the paired t test (p < 0.05). Table 1 illustrates the changes in average RGB, L*a*, E.I., and M.I. after cupping. We confirmed that all indexes had the various ranges of expression in accordance with the skin color (Table 1).

3.3. Analysis of the changes in RGB color space prior to and after stimulation on special cases

The contrast test based on the one-way repeated measures ANOVA indicated that the detected RGB color space was significantly decreased (p < 0.01) after cupping except with the left/right BL23. The RGB color space at the left BL23 significantly increased after cupping (p < 0.05). Moreover, the R and B values at the right BL23 significantly increased after cupping (p < 0.05) and the G value decreased slightly (p < 0.05). There were significant differences at the left/ right BL13, BL15, and BL18 when visually assessing the change in skin color. Moreover, the significant differences were not visually found at the left/right BL20 and BL23. Hence, the RGB color space at the left BL13, BL15, and BL18 was significantly lower compared with the left BL21 and BL23. Moreover, there were no significant differences at the left BL20 and BL23 by comparison with other left acupoints. Only the RGB color space at the left BL23 increased compared with its level prior to cupping. There were significant differences at the right BL13, BL15, and BL18 compared to other acupoints visually, but the RGB color space at the right BL18 was higher than its level at the right BL20. The RGB color space at the right BL20 was similar to its level at the right BL15. Moreover, a significant increase

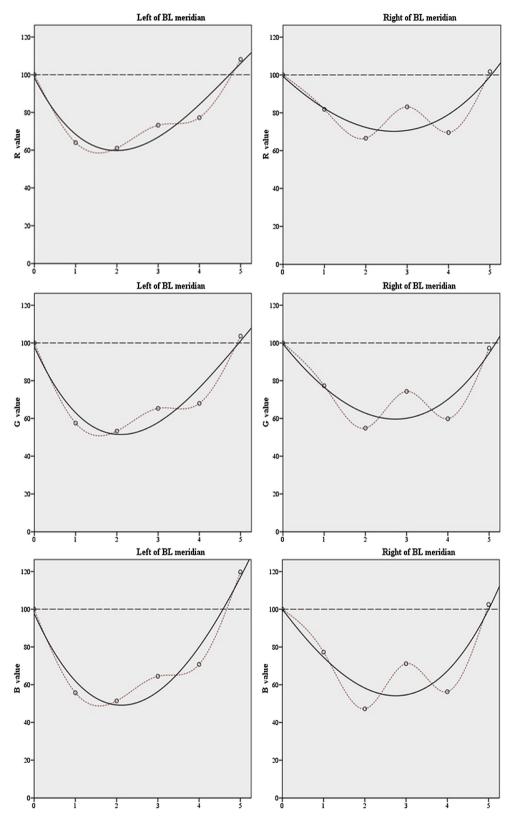


Figure 4 The change in the RGB color space after cupping. 0 = normalized reference; 1 = BL13; 2 = BL15; 3 = BL18; 4 = BL20; 5 = BL23; B = blue; G = green; B = color; $B = \text{col$

Left BL	average RGB color space after cupp R value	G value	B value
Prior to cupping	100	100	100
After cupping (BL13)	63.97 ± 1.09	57.53 ± 1.19	55.71 ± 1.04
After cupping (BL15)	61.07 ± 1.10	53.31 ± 0.96	51.39 ± 1.25
After cupping (BL18)	$\textbf{73.22}\pm\textbf{0.89}$	65.61 \pm 0.80	64.46 ± 0.93
After cupping (BL20)	77.25 \pm 1.05	67.97 ± 1.23	70.76 ± 1.83
After cupping (BL23)	108.13 \pm 1.83	103.66 \pm 1.68	119.75 \pm 2.73
Right BL	R value	G value	B value
Prior to cupping	100	100	100
After cupping (BL13)	81.81 \pm 0.82	77.33 ± 0.78	77.31 ± 1.16
After cupping (BL15)	66.56 ± 1.32	54.86 \pm 1.31	47.22 ± 1.52
After cupping (BL18)	83.13 ± 0.44	$\textbf{74.32}\pm\textbf{0.33}$	$\textbf{71.17} \pm \textbf{0.34}$
After cupping (BL20)	69.55 ± 2.80	$\textbf{59.86}\pm\textbf{2.67}$	$\textbf{56.29} \pm \textbf{3.62}$
	101.74 \pm 1.02	97.28 ± 0.97	102.57 ± 1.47

compared with the RGB color space prior to cupping was found at only the right BL23 (Figs. 3 and 4; Table 2).

3.4. Analysis of the changes in L*a* color space prior to and after stimulation on special cases

The contrast test based on the one-way repeated measures ANOVA indicated that the detected L* value significantly decreased (p < 0.01) after cupping except at the right BL23. Moreover, the contrast test based on the one-way repeated measures ANOVA results indicated that the detected a * value significantly decreased (p < 0.01) after cupping. The L* value at the right BL23 showed a tiny difference after cupping (p = 0.048). We observed that the L* was lower because of the change in the blackish skin after cupping except at the left/right BL23. Moreover, after normalizing, we observed that a* was lower than its level prior to cupping. The L*a* color space at the left BL13, BL15, and BL18 was significantly lower compared with the left BL20 and BL23 and these results corresponded with visual estimation. There were no significant differences at the left BL20 and BL23 by comparison with other left acupoints. Only the L* value at the left BL23 increased compared with its level prior to cupping. There were significant differences at the right BL13, BL15, and BL18 compared with other acupoints visually, but the L* value at the right BL18 was more increased than its level at the right BL20. The L* value at the right BL20 was similar to its level at the right BL15. Moreover, there were no significant differences at the right BL23 prior to cupping and after cupping (Fig. 5; Table 3).

3.5. Analysis of the changes in E.I. and M.I. prior to and after stimulation on special case

The contrast test based on the one-way repeated measures ANOVA results indicated that the detected E.I. and M.I. significantly increased (p < 0.01) except for M.I. at the left BL23. We observed that both the E.I. and the M.I. were generally increased after cupping. There were significant

differences at all acupoints. In contrast to results for the RGB color space and L*a* color space, the E.I. at the left BL18 was significantly more different than its level at the left BL20. However, the M.I. at the left BL18 was slightly higher than its level at the left BL20, which was identical to the results of the RGB color space and the L*a* color space. Both E.I. and M.I. at the left BL20 showed no significant differences compared with other acupoints. Both E.I. and M.I. at the right acupoints had a similar pattern when considering the results of RGB color space and L*a* color space. Although visually we observed the higher change in skin color at the right BL18 than the right BL20, both E.I. and M.I. at the right BL18 were more decreased than at the right BL20. In contrast to the result of the visual estimation, both E.I. and M.I. at the right BL20 were similar to the levels at the right BL15. In addition, there were no significant differences at the right BL23 compared with other acupoints prior to cupping and after cupping (Fig. 6; Table 4).

3.6. Analysis of the changes in RGB, L*a*b*, E.I., and M.I over time

Fig. 5 shows the changing patterns of the RGB color space over a period of 72 hours. The RGB color space decreased after cupping and increased during the recovery period. It started to recover quickly at R, G values between after cupping and 24 hours after. Moreover, it was observed that the R, G value slowly changed from after cupping and 72 hours after. The B value decreased after cupping and increased slowly until 72 hours after. The contrast test based on the one-way repeated measures ANOVA results indicated that there were significant differences between RGB color space after cupping and its level during the recovery period (p < 0.01; Fig. 7; Table 5).

Fig. 6 illustrates the changing patterns of the L*a* color space up to 72 hours after. The L*a* color space resulted in a decrease after cupping and increased during the recovery period. It was observed that the L*a* color space recovered quickly between after cupping and 24 hours after. A slight change was observed between 24 hours and 72 hours. The contrast test based on the one-way repeated measures

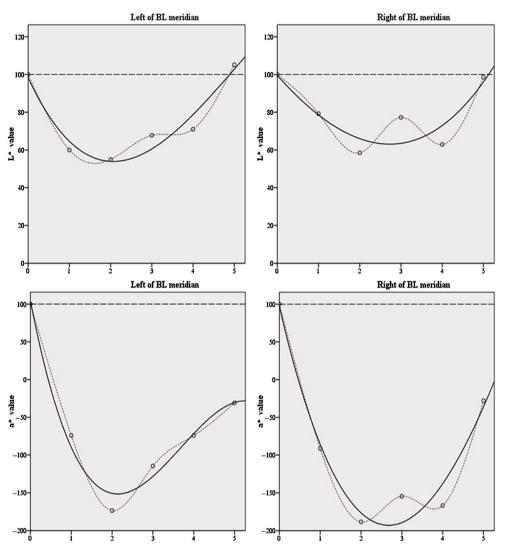


Figure 5 The change in the L*a* color space after cupping. 0 = normalized reference; 1 = BL13; 2 = BL15; 3 = BL18; 4 = BL20; 5 = BL23.

Table 3 The changes in the average L*a* color space after cupping.

Left BL	L* value	a* value
Prior to cupping	100	100
After cupping (BL13)	59.99 ± 1.14	-74.09 ± 4.70
After cupping (BL15)	54.96 ± 1.06	-173.41 ± 15.59
After cupping (BL18)	67.77 ± 0.83	-114.61 ± 6.51
After cupping (BL20)	71.02 ± 1.19	-74.13 ± 4.01
After cupping (BL23)	105.17 \pm 1.76	-30.87 ± 5.57
Right BL	L* value	a* value
Right BL Prior to cupping	L* value	a* value
Prior to cupping	100	100
Prior to cupping After cupping (BL13)	100 79.21 ± 0.78	100 -91.30 ± 8.02 -188.38 ± 11.68
Prior to cupping After cupping (BL13) After cupping (BL15)	100 79.21 ± 0.78 58.46 ± 1.32	100 -91.30 ± 8.02 -188.38 ± 11.68 -154.51 ± 13.34
Prior to cupping After cupping (BL13) After cupping (BL15) After cupping (BL18)	$\begin{array}{c} 100 \\ 79.21 \pm 0.78 \\ 58.46 \pm 1.32 \\ 77.28 \pm 0.83 \end{array}$	100 -91.30 ± 8.02

ANOVA results indicated that there were significant differences between the L*a* color space after cupping and its level during the recovery period (p < 0.01; Fig. 8; Table 6).

Fig. 7 illustrates the changing patterns of the E.I. and M.I. up to 72 hours after cupping. The E.I. and M.I. increased after cupping and decreased during the recovery period. Both E.I. and M.I. recovered to approximately normal state during the recovery period. The recovery of E.I. and M.I. within after cupping and 24 hours was faster than at other times. Moreover, slight changes in both E.I. and M.I. between 24 hours and 72 hours were observed. The contrast test based on the one-way repeated measures ANOVA results indicated that there were significant differences between E.I. and M.I. after cupping and its level during the recovery period (p < 0.01; Fig. 9; Table 7).

4. Discussion

Because the RGB color space represents 0 (black) to 255 (white), a consistent result in the decrease in RGB color

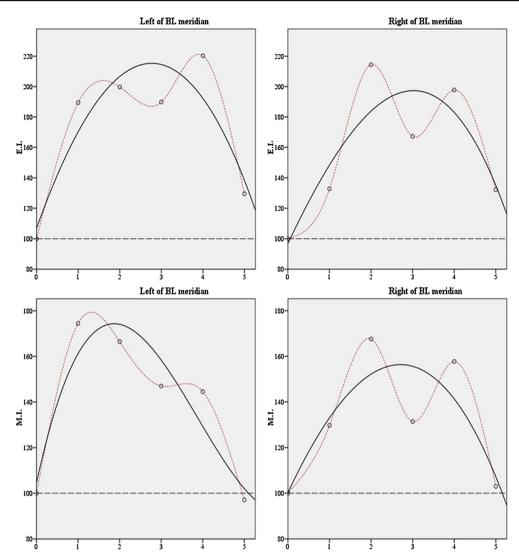


Figure 6 The change in E.I. and M.I. after cupping. 0 = normalized reference; 1 = BL13; 2 = BL15; 3 = BL18; 4 = BL20; 5 = BL23; E.I. = Erythema Index; M.I. = Melanin Index.

Table 4 The changes cupping.	in average E.I.	and M.I. after	
Left BL	E.I.	M.I.	
Prior to cupping	100	100	
After cupping (BL13)	189.47 ± 5.63	174.44 ± 4.23	
After cupping (BL15)	199.72 ± 3.04	166.47 ± 2.13	
After cupping (BL18)	189.90 ± 1.29	146.96 ± 1.62	
After cupping (BL20)	220.25 ± 6.82	144.53 ± 2.36	
After cupping (BL23)	129.48 ± 1.67	97.11 \pm 1.27	
Right BL	E.I.	M.I.	
Prior to cupping	100	100	
After cupping (BL13)	132.78 ± 0.95	129.74 \pm 1.30	
After cupping (BL15)	214.40 ± 3.97	167.56 ± 3.67	
After cupping (BL18)	167.28 ± 1.37	131.41 ± 0.95	
After cupping (BL20)	197.77 ± 3.72	157.72 \pm 4.82	
After cupping (BL23)	$\textbf{132.19} \pm \textbf{2.20}$	102.99 \pm 1.10	
E.I. = Erythema Index; M.I. = Melanin Index.			

space due to the changing blackish color after cupping was observed. Moreover, since the L* represents light intensity within the range 0 (black) and 100 (white), L* was observed to decrease for the same reason. In addition, the a* represents color saturation within the range -60(green) and 60 (red). Hence, we observed that a* increased toward 60 after cupping. Asian skin color has the proximate negative or positive range from 0 in a*. The a* also changed to the positive range because of the increased 60 (red) saturation after cupping. Hence, after normalizing, we observed that a * was lower than its level prior to cupping. Moreover, studies report that cupping leads to changes in skin such as pigmentation and blood purification mechanisms in the body. Hence, the facts that the number of red blood cells and white blood cells were increased and melanin pigmentation was generated were reported. Both E.I. and M.I. increased after cupping corresponding with previous studies [19,20]. The special cases that increased R, G, B, and L* values were observed. There were significant differences at the left/right BL13, BL15, BL18, and BL20 visually when assessing the change in skin color in a

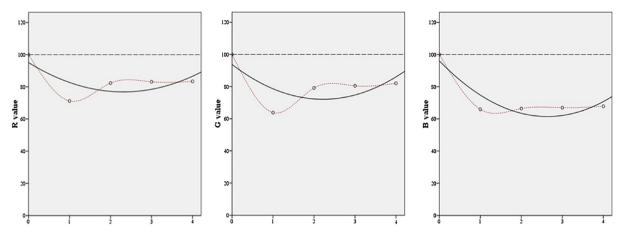


Figure 7 The change in the RGB color space over time. 0 = prior to cupping; 1 = after cupping; 2 = after 24 hours; 3 = after 48 hours; 4 = after 72 hours; B = blue; G = green; R = red.

Table 5 The change in the RGB color space over time.			
	R value	G value	B value
Prior to	100	100	100
cupping			
After cupping	71.19 ± 13.97	63.82 ± 1.19	$\textbf{65.96}\pm\textbf{20.56}$
After 24 hours	$\textbf{83.32} \pm \textbf{5.96}$	$\textbf{79.14} \pm \textbf{6.18}$	$\textbf{66.41}\pm\textbf{11.88}$
After 48 hours	$\textbf{83.12} \pm \textbf{5.96}$	$\textbf{80.48} \pm \textbf{6.18}$	66.97 ± 11.88
After 72 hours	$\textbf{83.39} \pm \textbf{5.30}$	$\textbf{82.05}\pm\textbf{5.00}$	$\textbf{67.79}\pm\textbf{9.44}$
$\overline{B = \text{blue}; G = \text{green}; R = \text{red.}}$			

special case. By contrast, there were no significant differences at the left/right BL23 compared with other left acupoints. The RGB color space at the left/right BL23 was close to its level prior to cupping or was increased. In addition, the L* value at the left/right BL13 was similar to

its level prior to cupping or was increased. The results signified that skin color changed toward white color after cupping. By contrast, a*, E.I., and M.I. maintained the difference, but had lower differences than other acupoints. This result shows that skin color changed toward red saturation. We observed the special case after cupping on skin color changing toward white and red color such as vitiligo. Moreover, there were significant differences visually at the right BL13, BL15, and BL18 rather than other acupoints, but the RGB, L*a*, E.I., and M.I. at the right BL18 were more closely than their levels at the right BL20. The CR-400 tri-stimulus colorimeter has a measuring diameter of 8 mm. Therefore, we measured the skin color at the acupoint up to a 4-mm maximum. The skin color turned blackish at the outline but not at the center of the acupoint when observing the changes in skin color after cupping; we concluded that the problem was generated by the narrow measuring diameter of the tri-stimulus

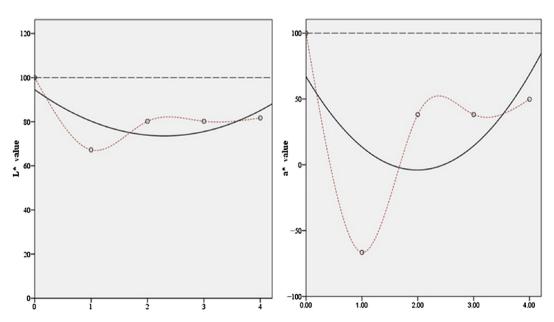


Figure 8 The change in the L*a* color space over time. 0 = prior to cupping; 1 = after cupping; 2 = after 24 hours; 3 = after 48 hours; 4 = after 72 hours.

Table 6 The char	The change in the L*a* color space over time.		
	L* value	a* value	
Prior to cupping	100	100	
After cupping	67.25 ± 15.73	-66.51 ± 95.10	
After 24 hours	$\textbf{80.19}\pm\textbf{9.94}$	38.21 ± 33.26	
After 48 hours	$\textbf{80.19}\pm\textbf{9.95}$	38.21 ± 31.21	
After 72 hours	$\textbf{81.72}\pm\textbf{6.24}$	49.77 ± 5.00	

colorimeter. Cupping is reported to affect the blood through gas exchange and purification, and it changes the acid/base balance of body fluid. When we analyzed the deep skin-colored part and the light skin-colored part after cupping, it was reported that the deep-skin colored part has higher levels of white blood cells, polymorphonuclear neutrophil leukocytes, red blood cells, hemoglobin, mean corpuscular hemoglobin, and hematocrit compared with the light-skin colored part. By contrast, the light skincolored part was reported to have higher numbers of lymphocytes, monocytes, mean corpuscular volume, and platelets [40]. Among the results of previous studies, red blood cell and hemoglobin levels in the deep skin-colored part after cupping have a high correlation with the increases in the E.I. and the darkish level of skin color. The lighter the skin color gets after cupping, the higher the RGB and L*a* color spaces are compared to the dark part; this result correlated with the levels of monocytes and platelets and the mean corpuscular volume.

In oriental medicine, cupping has been used for various treatments and for diagnosis through the hemoglobin response, the blister response, and the oppressive pain response. In particular, the pigmentation response incurred a hemoglobin response in certain areas (acupoints) and was used to diagnose irregularities in the functions of internal organs, and extravasated blood was

Table 7 The change in E.I. and M.I. over time.			
	E.I.	M.I.	
Prior to cupping	100	100	
After cupping	185.93 \pm 43.11	158.72 ± 95.10	
After 24 hours	146.99 ± 14.55	130.41 ± 33.26	
After 48 hours	129.86 \pm 16.58	131.52 ± 19.77	
After 72 hours	125.80 ± 16.65	131.93 \pm 14.34	
E.I. = Erythema Index; M.I. = Melanin Index.			

considered a major factor. The rate of the pigmentation response was divided to detect the existence of disorders. but it is still difficult to judge the state of skin color objectively using visual estimation. Therefore, it has limitations in quantitative and objective diagnosis. This study was aimed at assessing the possibility of quantitative measurement and analysis of changes in skin color such as pigmentation and hemoglobin response using a tri-stimulus colorimeter. Accordingly, cupping was applied at a consistent negative pressure (80 kPa), and the XYZ coordinates were measured. Because the skin color changed to a blackish color after cupping, the XYZ coordinates were converted into RGB and L*a*b* color spaces in order to confirm the change objectively. Moreover, we calculated the M.I. as the amount of melanin pigmentation and the E.I. as the amount of hemoglobin. In conclusion, it was confirmed that these methods made it possible to quantitatively distinguish the various changes in skin color following cupping. However, we confirmed the necessity for a wide measuring diameter of the tri-stimulus colorimeter in order to reflect more of the surroundings of an acupoint. In addition, it is important that not only analysis of skin color change but also relativeness for understanding between the extravasated blood and skin color. For this reason, we conclude that the comparison after cupping

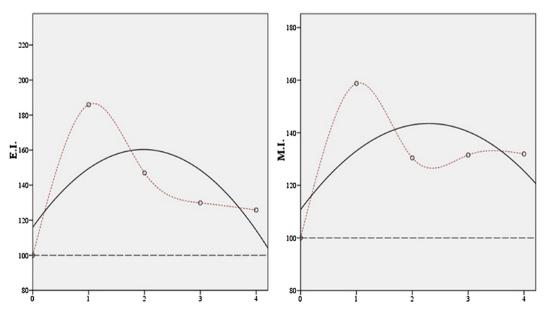


Figure 9 The change in E.I. and M.I. over time. 0 = prior to cupping; 1 = after cupping; 2 = after 24 hours; 3 = after 48 hours; 4 = after 72 hours; E.I. = Erythema Index; M.I. = Melanin Index.

between the proposed indexes and the results of blood tests are essential.

Disclosure statement

The author affirms there are no conflicts of interest and the author has no financial interest related to the material of this manuscript.

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