A New Method For Measuring Acupoint Pigmentation After Cupping Using Cross Polarization

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Objectives: Skin color deformation by cupping has been widely used as a diagnostic parameter in Traditional Korean Medicine (TKM). Skin color deformation such as ecchymoses and purpura is induced by local vacuum in a suction cup. Since existing studies have relied on a visual diagnostic method, there is a need to use the quantitative measurement method. Methods: We conducted an analysis of cross-polarization photographic images to assess the changes in skin color deformation. The skin color variation was analyzed using L*a*b* space and the skin erythema index (E.I.). The meridian theory in TKM indicates that the condition of primary internal organs is closely related to the skin color deformation at special acupoints. Before conducting these studies, it is necessary to evaluate whether or not skin color deformation is influenced by muscle condition. Hence, we applied cupping at BL13, BL15, BL18, BL20 and BL23 at Bladder Meridian (BL) and measured blood lactate at every acupoint. Results: We confirmed the high system measurement accuracy, and observed the diverse skin color deformations. Moreover, we confirmed that the L*, a* and E.I. had not changed after 40 minutes ($p > 0.05$). The distribution of blood lactate levels at each part was observed differently. Blood lactate level and skin color deformation at each part was independent of each other. Conclusions: The negative pressure produced by the suction cup induces a reduction in the volumetric fraction of melanosomes and subsequent reduction in epidermal thickness. The relationship between variations of tissue and skin properties and skin color deformation degree must be investigated prior to considering the relationship between internal organ dysfunction and skin color deformation.

Key words: negative pressure, skin deformation, erythema, cross-polarization, cupping

Introduction

Cupping is an important diagnostic and therapeutic method used in a variety of countries. In Traditional Korean Medicine (TKM), there are 2 types of cupping as follows: wet cupping and dry cupping. Before cupping, wet cupping
conducts some scarification of the skin. Simply, dry cupping includes only a vacuum stimulation with a suction cup on different body areas. In TKM, cupping has been used as a means of body fluid purification by removing extravasated blood, promoting blood circulation and purging toxin. The negative pressure of cupping involves pulling the skin into a suction cup and breaking superficial blood vessels in the papillary dermis. This negative pressure induces several skin changes such as ecchymoses and purpura in a circular pattern\(^1\,^2\). It has been reported that the vacuum of the suction cup generates a negative pressure that can induce heat production that alters tissue perfusion and metabolism\(^3\,^4\).

A review study, which investigated all clinical studies between 1959 and 2008, reported that two types of cupping had treatment effects on pain conditions, herpes zoster and other diseases. Several studies had reported treatment effects including the alleviation of low back pain\(^5\,^6\), as well as the treatment of dermatologic ailments, migraines and menstrual symptoms\(^7\,^10\). Ongoing study continues to investigate the treatment effects of cupping, but there is still a dearth of studies aimed at quantifying the diagnostic method instead of relying on a visual diagnostic method.

There are two methods of polarized light photography that may be used to assess the human skin surface quantitatively. These two types of polarized light photography are classified by the direction between incident and detected planes of polarization. One is parallel-polarization, which enhances surface reflectance components using paralleled planes of polarization; by enhancing the simply reflected light, one can better understand skin surface characteristics. This method is useful for visual examination including evaluation of skin texture, elevation and scale. The other method is cross-polarization, which eliminates the simply reflected light and obtains subsurface information such as vascularization, erythema and pigmentation. Cross-polarization can block simply reflected light and focus on back-scattered light, which contains information on subsurface structures. This method involves placing incident and detected planes of polarization perpendicularly\(^1\)\(^11\,^16\).

We conducted an analysis of cross-polarization photographic images to assess quantitatively the changes involved in inducing circularly-shaped skin ecchymoses and purpura by breaking superficial blood vessels in the papillary dermis via cupping. Previous studies have demonstrated that RGB and L\(^*a^*b^*\) color space is useful in providing objective measurements of skin color\(^16\,^17\). The L\(^*a^*b^*\) color space, standardized by the Commission Internationale de l’Eclairage (CIE), has been used to represent the degree of skin erythema and pigmentation. The L\(^*\) describes the light intensity within the range from 0(black) to 100(white). Both the a\(^*\) and b\(^*\) describe the color saturation within the range from \(-60\) to 60. The a\(^*\) varies from \(-60\) for green to 60 for red. Regarding the b\(^*\), \(-60\) refers to blue and 60 signifies yellow. Hence, it has been reported that L\(^*\) and a\(^*\) are suitable for representing the degree of skin pigmentation or erythema\(^18\,^20\).

We analyzed the L\(^*\) and a\(^*\) converted from RGB space of cross polarization photographic images. As skin color is primarily influenced by melanin and hemoglobin, the erythema index(E.I.), which represents the indirect amount of hemoglobin, is used as a parameter to evaluate skin color variation\(^18\,^21\). Hence, we analyzed the E.I. of the RGB space from cross polarization photographic images.

Prior to investigating the relationship between primary internal organ dysfunction and skin color deformation as manifested by ecchymoses and purpura caused by cupping, it is necessary to evaluate whether or not skin changes are influenced by muscle conditions at a specific acupoint. Thus, blood lactate was measured at every acupoint and was...
compared to our skin color variance parameter.

Materials and Methods

1. Cross-polarized diffuse reflectance imaging system

A digital color camera (Model EOS 600D, Canon Ctd, Tokyo, Japan) was used to acquire cross polarization photographic images. The sensor dimensions of the digital color camera were 5,184×3,456 pixels with 8 bits per color channel for RGB images. To acquire identical images, the camera was set to the same parameters (shutter speed: 1/60 second; aperture size: f/11; ISO 800). Lighting was obtained by using a Canon flash (Model SPEEDLITE 430EXII, Canon Ctd, Tokyo, Japan). The polarization planes were orthogonally mounted on both the digital color camera and flash. There are two ways to adjust the color balance by illumination. One includes auto-white balance, which cannot respond to different photography conditions because the camera automatically adjusts to the changing illumination levels. To perform a custom white balance test, an achromatic color tool must be used, such as a gray card for color balancing. However, the achromatic color tool poses some problems, including color-related damage and a degree of color change. In order to detect exactly skin color changes, we conducted both an auto-white balance and a custom white balance test. Moreover, we measured the 24 color-checker chart, which is an imaging measurement evaluation ubiquitously used in the photographic and video fields. We interpolated digital color camera measurement values with reference values from the 24 color-checker chart (Fig. 2).

2. Participant and acupoint selection

We chose 15 male college students (mean age 24±2 years) who voluntarily agreed to participate in this clinical study. In order to avoid potential adverse effects of acupoints, smoking, alcohol and coffee were forbidden 12 hours prior to clinical testing. A written consent form was submitted from each participant after they received information about the nature and requirements of this experiment. We also checked for skin lesions due to burns around the acupoints to reduce experimental error. Typically, cupping is performed by attaching a suction cup to a Bladder Meridian (BL) or a Governor Vessel Meridian (GV). In order to limit pain caused by cupping, we selected acupoints at BL which is positioned in the back muscle. Hence, the left and right BL13, BL15, BL18, BL20, and BL23 which represent the five viscera of the heart, liver, spleen, lungs, and kidneys were selected.

3. Color space conversion

To determine skin color variation objectively using the CIE L*a*b* color space metric, the RGB color space of each image was converted to XYZ coordinates using a conversion matrix [Eq. (1)].

\[
\begin{bmatrix}
X \\
Y \\
Z
\end{bmatrix} =
\begin{bmatrix}
0.412453 & 0.357580 & 0.180423 \\
0.212627 & 0.715160 & 0.072169 \\
0.019334 & 0.11919 & 0.950227
\end{bmatrix}
\begin{bmatrix}
R \\
G \\
B
\end{bmatrix}
\]

(1)

The \(X_n, Y_n, Z_n\) of the reflectance plate were computed as calibration references. The \(L^*a^*b^*\) color space was calculated using XYZ coordinates from RGB color space of skin color deformation [Eq. (2)].

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

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

\[
b^* = 500 \times \left[ \left( \frac{Y}{Y_n} \right)^{\frac{1}{3}} - \left( \frac{Z}{Z_n} \right)^{\frac{1}{3}} \right]
\]

(2)
Where,

\[
\frac{X}{X_n} > 0.008856 \quad \text{else} \quad \frac{X}{X_n} = 7.787 \times \frac{X}{X_n} + \frac{16}{116}
\]

\[
\frac{Y}{Y_n} > 0.008856 \quad \text{else} \quad \frac{Y}{Y_n} = 7.787 \times \frac{Y}{Y_n} + \frac{16}{116}
\]

\[
\frac{Z}{Z_n} > 0.008856 \quad \text{else} \quad \frac{Z}{Z_n} = 7.787 \times \frac{Z}{Z_n} + \frac{16}{116}
\]

The 'r' and 'g' variables denoted the red color and green color, respectively. \(W_{rg}\) was the average red and green of the calibration reference which was computed using an image of a 99% diffuse reflectance plate (Model SRT-99-200, Labsphere, North Sutton, NH, USA). \(S_{rg}\) denoted the red and green colors detected at the acupoint. \(R_{rg}\) was calculated by \(S_{rg}/W_{rg}\), and therefore \(R_{rg}\) included the normalized red color and green color at the acupoint. The E.I. was calculated by the ratio of \(R_r\) to \(R_g\):

\[
\text{E.I.} = 100 \times \left( \frac{R_r}{R_g} \right)
\]

4. Selection of negative pressure intensity on the cupping and measurement methods

It has been shown that various bio-responses occur depending on the degree of intensity of the negative pressure during cupping\(^{22}\). Thus, there is a need for an accurate diagnosis in constant negative pressure. It has been reported that when the negative pressure is below 34 kPa\(^{23}\), linear changes were observed in tissue deformation at the stimulated areas. However, existing studies on cupping in TKM reported that it is more appropriate to use 70~80 kPa negative pressure as the diagnostic negative pressure intensity\(^{24,25}\). Therefore, a constant pressure of 80 kPa for 1 minute was chosen.

Because the dryness/moisture of the skin responds quickly to the temperature of the surrounding environment, this experiment was conducted in an optical test lab, which was a dark room where a constant temperature of 24°C and a humidity of 40% were maintained.

To avoid any discomfort caused by remaining in a fixed position, the measurement of blood lactate was performed as the patients lied down on a flat bed. After confirming the hemostasis at each acupoint, all acupoints were medically sterilized. Negative pressure was applied locally at each acupoint for 1 minute using electric cupping (Model Muteicare LMC1000, Leadersmeditec ltd, Yongin, Korea). After detaching the suction cups, all participants were requested to sit up straight, and were photographed. The distance from digital camera to participant was 70 cm. To confirm the amount time required to achieve no further change in skin color deformation, each skin color deformation was measured by the digital color camera for 1 hour. The images were analyzed by dividing the boundary line into three equal parts (Fig. 3).

5. Statistical analysis

A simple linear regression was performed to confirm the accuracy of the measurement between standard 24 sRGB color values by the Commission Internationale de l’Eclairage (CIE) and measured 24 RGB color values using ColorChecker 2005. To assess the time required to achieve no further skin color change, the contrast test based on the one-way repeated measures analysis of variance (ANOVA) was conducted. Moreover, we conducted the one-way ANOVA and post hoc analyses to assess the significant differences in blood lactate, \(L^*\), \(a^*\) and E.I. at every acupoint. A Pearson correlation analysis was conducted to analyze the linearity of
the relationships between blood lactate and each skin color variance parameters.

Results

1. The evaluation of the accuracy of color measurement

The results of a simple linear regression indicated a high degree of linearity of each R-square between detected and standardized RGB values (0.998, 0.997 and 0.999, respectively; (Fig. 4)), and of each R-square between detected and standardized L*a*b* values (0.999, 0.998 and 0.998, respectively; (Fig. 5)).

2. Analysis of the changes in L*, a* and E.I. over time

Since the physiological and pathological conditions of the participants were different, various changes in skin color deformation after cupping were generated. Hence, the L*, a* and E.I. differed from individual to individual. Moreover, we observed all skin color variance parameters had diversely different values at each acupoint. The results of the one-way ANOVA & post hoc analysis indicated that L*, a* and E.I. measured at identical acupoints had the significant differences in each participant (p<0.05). Moreover, there were significant differences at each acupoint in identical participant (p<0.005). As a result of the contrast test of the one-way repeated measures ANOVA, we determined that the L*, a* and E.I. demonstrated significant differences up to 38
minutes after cupping ($p<0.05$) and there were no significant differences after 40 minutes ($p>0.05$; Fig. 6). We therefore determined that 40 minutes is the significant amount of time required to achieve no further change in skin color deformation after cupping.

3. Correlation between the distribution of blood lactate and the distribution of $L^*$, $a^*$ and E.I.

As a result of the one-way ANOVA and post hoc analysis, the blood lactate at each acupoint showed significant differences ($p<0.05$). As the time required to achieve no further skin color deformation was determined to be 40 minutes, the distribution of $L^*$, $a^*$ and E.I. were computed after 40 minutes. The distribution of $L^*$, $a^*$ and E.I. at each part was collected at each acupoint along with the distribution of blood lactate levels (Figs. 7 and 8). However, the result of Pearson correlation indicated that blood lactate showed no connection with $L^*$, $a^*$ or E.I. As a result of the one-way ANOVA and post hoc analysis, there are no significant differences at each part ($p<0.05$).

Discussion

Melanocytes are major cells in the body that produce the pigmentation within the epidermis. The separation between
Fig. 6. The changes in L*, a* and E.I. over time.

Fig. 7. The distribution of blood lactate levels at each acupoint.
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The distribution of $L^*$, $a^*$ and E.I. at each acupoint.

We therefore observed that the distribution of the $L^*$, $a^*$ and E.I. differed at each acupoint in identical participant. Since each participant has different physiological and pathological conditions, the $L^*$, $a^*$ and E.I. also differed from individual to individual.

Blood lactate is influenced by multiple ionic changes generated across the sarcolemma, transverse(t-) and tubular membranes. In other words, the blood lactate is closely associated with multiple ionic changes($K^+$, $Na^+$, $Ca^{2+}$, $Cl^-$, $H^+$, $HCO_3^-$, $Mg^{2+}$, $H_2PO_4^-$, $PCr$). When blood lactate increases,
the extracellular [Na$^+$]/([Na$^+$]_o) is decreased, and extracellular [K$^+$]/([K$^+$]_o) is increased$^{30}$. Due to the effect of increased extracellular [K$^+$]/([K$^+$]_o), the Na$^+$-K$^+$ pump increased the intracellular [Na$^+$]/([Na$^+$]_i). Likewise, the intracellular [Cl$^-$]/([Cl$^-$]_i) is increased and extracellular [Cl$^-$]/([Cl$^-$]_o) is decreased. Moreover, a decrease in pH(incresed [H$^+$]), increased extracellular [HCO$_3^-$]/([HCO$_3^-$]_o) and increased [Ca$^{2+}$]/([Ca$^{2+}$]_o) are observed$^{31-35}$.

The skin color deformation incurred from cupping had no significant correlation with blood lactate levels. We demonstrated that L*, a* and E.I., which represent the degree of skin color deformation by cupping, were not associated with the state of the distribution multiple ions at the acupoints.

In TKM, skin color deformations are visually divided by 5 steps. In order to objectify skin color deformation as a means of diagnostic method, several researches were conducted to establish the relation between the degree of skin color deformation and blood compositions such as white blood

Fig. 9. The distribution of L*, a* and E.I. at each part.

<table>
<thead>
<tr>
<th>A part</th>
<th>B part</th>
<th>C part</th>
</tr>
</thead>
<tbody>
<tr>
<td>L*</td>
<td>-2.4</td>
<td>-0.305</td>
</tr>
<tr>
<td>a*</td>
<td>0.81</td>
<td>0.05</td>
</tr>
<tr>
<td>E.I.</td>
<td>0.04</td>
<td>-0.02</td>
</tr>
</tbody>
</table>
cell, red blood cell, monocyte and lymphocyte cell. However, these results are inappropriate for utilizing the diagnostic method due to the visual skin color deformation index. The blood composition conditions need to compare the quantitative color index such as $L^*$, $a^*$ and E.I. After establishing the relation, it is possible to indirectly estimate the blood composition conditions at acupoints.

Previous studies reported that negative pressure caused by a suction cup induced a reduction in the volumetric fraction of melanosomes with the reduction of epidermal thickness. One of reported studies revealed that 2.4 kPa generated the 23% reduction in epidermal thickness\(^ \text{(30)} \). Moreover, the result of in vivo experimentation reported that the reduction in thickness of both the epidermis and papillary dermis was observed to be 7% at 20 kPa and 17% at 35 kPa, respectively\(^ \text{(30)} \). These studies aimed to verify the relationship between bulk tissue changes and skin surface stretching induced by applying negative pressure. It was reported that skin displacement by suction cup had a linear relationship of up to about 34 kPa. At negative pressures above 34 kPa, a linear relationship between negative pressure and skin displacement was not observed. Moreover, the skin surface closest to the suction cup generated the most stretching\(^ \text{(30)} \).

Later, before conducting the experiment and elucidating the relationship between internal organ dysfunction and skin color changes, it is necessary to confirm the relationship between melanosome reduction and reduction in epidermal thickness at 80 kPa with a certain degree of skin color variance parameter. Furthermore, the relationship between bulk tissue changes and degree of skin color deformation needs to be objectively identified.

**Conclusion**

To confirm the accuracy of color measurements, we conducted a simple linear regression between measurement values and reference values from the 24 color-checker chart. As a result, we confirmed high linearity at RGB and $L^*a^*b^*$ space over R-square 0.998. Since the $L^*$ represents light intensity within the range from 0(black) to 100(white), $L^*$ was found to decrease due to black discoloration after cupping. The $a^*$ represents color saturation within the range from $-60$(green) to 60(red). We observed that $a^*$ increased toward 60 after cupping. We observed that the various distribution of the $L^*$, $a^*$ and E.I. at every acupoint were observed by all participants. Given that each participant has a different skin surface with variable viable melanocytes within basal keratinocytes, the $L^*$, $a^*$ and E.I. differed from individual to individual. Therefore, we confirmed significant differences in skin color changes at each acupoint between identical participants\(p<0.05\). After conducting the contrast test of the one-way repeated measures analysis of variance(ANOVA), we observed that the $L^*$, $a^*$ and E.I. were not significantly different after 40 minutes\(p>0.05\). The distribution of blood lactate levels at each part was observed differently. However, Pearson correlation indicated that blood lactate was not associated with $L^*$, $a^*$ and E.I. One-way ANOVA and post hoc analysis demonstrated no significant differences at each part\(p<0.05\). Various studies have reported that negative pressure by suction cup induces a reduction in the volumetric fraction of melanosomes and subsequent reduction in epidermal thickness. Therefore, it is necessary to confirm the relationship between the reduction in melanosomes and epidermal thickness at 80 kPa and the degree of skin color deformation that occur. This must be investigated prior to considering the relationship between internal organ dysfunction and skin color deformation.

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**References**

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목적: 부항요법은 국소적 부위를 진공상태로 유지시키는 과정 중 유두층 안의 표피혈관을 파괴함에 따라 발생하는 색소, 응결, 자반, 수포, 압통 반응 등을 살펴서 오장육부의 기능상상을 진단하고 있다. 시각에 의존하는 주관적인 혈색소 판별로 인한 진단방법에서 벗어나 정량적으로 측정 및 분석이 가능하기 위하여 교차편광 촬영술을 접목하였으며, 새로운 혈색소 평가 가능성을 확인하고자 하였다.

방법: 족태양방광경의 좌/우 폐수(BL13), 심수(BL15), 간수(BL18), 신수(BL23) 총 10개에 80 kPa의 음압으로 1분 동안 자극하였다. 교차편광 촬영술을 이용하여 부항 자극 직전과 60분 이후까지 2분마다 이미지를 획득하였다. 획득한 이미지를 RGB space에서 확인하였으며, L*, a* 그리고 E.I.erythema index를 계산하여 분석하였다. 또한 부항 자극 전 각 경혈에서 석면 농도를 측정하여 색 지표들의 관계성을 통한, 근육의 상태와 부항 자극으로 유도된 피부 색 변화와의 관계를 확인하고자 하였다. 결과: 교차편광 촬영을 이용하여 획득한 이미지에서의 L*, a* 그리고 E.I 모두 부항 자극에 따른 피부 색 변화를 정량적으로 나타낼 수 있는 유의한 지표임을 확인하였다. 부항 자극 40분 후에 피부 색 변화가 더 이상 관찰되지 않았다. 또한 각 경혈에서의 석면 농도와 피부 색 변화 정도와 유의한 차이가 없음을 확인하였다. 결론: 실험을 포함하여 한의학적 색 진단을 하기 위한 방법으로 교차편광 촬영을 활용 가능성을 확인하였다. 또한 L*, a* 그리고 E.I 모두 색 지표로써 유의성을 확인하였다. 향후, 장부 기능 이상과 혈색소 반응간의 상관성을 확보하기 위한 연구가 진행하기 전에, 음압 도중 발생하는 조직 내 다양한 반응과 혈색소 반응간의 관계를 확보하고자 하는 노력이 우선적으로 진행되어야 한다.