Quantitative skin color measurements in acanthosis nigricans patients: colorimetry and diffuse reflectance spectroscopy

Bensachee Pattamadilok[1], Suneetha Devpura[2], Zain U. Syed[1], Oma N. Agbai[1], Pranita Vemulapalli[1], Marsha Henderson[1], Steven J. Rehse[3], Bassel H. Mahmoud[1], Henry W. Lim[1], Ratna Naik[2] & Iltefat H. Hamzavi[1]

[1]Department of Dermatology, Henry Ford Hospital, Detroit, MI, USA, [2]Department of Physics and Astronomy, Wayne State University, Detroit, MI, USA, and [3]Department of Physics, University of Windsor, Windsor, ON, Canada

Key words:
acanthosis nigricans; colorimetry; diffuse reflectance spectroscopy

Correspondence:
Dr Iltefat H. Hamzavi, M.D., Department of Dermatology, Henry Ford Hospital, New Center One, Rm 8441, Detroit, MI 48202, USA.
Tel: +1 313 916 2972.
Fax: +1 313 916 5334.
e-mail: ihamzav1@hfhs.org

Accepted for publication:
8 March 2012

Conflicts of interest:
Dr. Hamzavi has served as an investigator for Dow Pharmaceuticals, Abbott, Pfizer, Kythera, Estee Lauder, Johnson and Johnson and Cipher. Dr. Lim has served as a consultant for La Roche-Posay, Clinuvel, and Procter and Gamble and an investigator for Clinuvel and Estee Lauder.

Summary

Tristimulus colorimetry and diffuse reflectance spectroscopy (DRS) are white-light skin reflectance techniques used to measure the intensity of skin pigmentation. The tristimulus colorimeter is an instrument that measures a perceived color and the DRS instrument measures biological chromophores of the skin, including oxy- and deoxyhemoglobin, melanin and scattering. Data gathered from these tools can be used to understand morphological changes induced in skin chromophores due to conditions of the skin or their treatments. The purpose of this study was to evaluate the use of these two instruments in color measurements of acanthosis nigricans (AN) lesions. Eight patients with hyperinsulinemia and clinically diagnosable AN were seen monthly. Skin pigmentation was measured at three sites: the inner forearm, the medial aspect of the posterior neck, and anterior neck unaffected by AN. Of the three, measured tristimulus L*a*b* color parameters, the luminosity parameter L* was found to most reliably distinguish lesion from normally pigmented skin. The DRS instrument was able to characterize a lesion on the basis of the calculated melanin concentration, though melanin is a weak indicator of skin change and not a reliable measure to be used independently. Calculated oxyhemoglobin and deoxyhemoglobin concentrations were not found to be reliable indicators of AN. Tristimulus colorimetry may provide reliable methods for respectively quantifying and characterizing the objective color change in AN, while DRS may be useful in characterizing changes in skin melanin content associated with this skin condition.

Acanthosis nigricans (AN) is a dermatologic condition consisting of hyperpigmented, velvety plaques that display hyperplasia of the epidermis and papillary dermis on histopathology. AN was shown to have a direct association with hyperinsulinemia (1) and is considered a cutaneous marker for insulin-resistant states including type 2 diabetes mellitus and polycystic ovarian syndrome (2).

Dermatologists have traditionally depended on their eyes for the task of measuring skin color, but there is an inherent subjectivity to this type of measurement that can be overcome through the use of optical diagnostic instruments. In addition, it is difficult to reproducibly quantify small changes in pigmentation that are derived from a clinician's assessment. Two methods frequently used to objectively quantify and characterize skin color measurements are tristimulus colorimetry (3) and diffuse reflectance spectroscopy (DRS) (4). In DRS, skin chromophores such as melanin, oxyhemoglobin and deoxyhemoglobin are quantified.

Neither colorimetry nor DRS has been fully explored as a way to study the hyperpigmentation of AN. In a previous work, we investigated the use of chemometric data analysis techniques to correlate colorimetery and DRS measurements with visually diagnosed AN lesions (5). The current study aimed to quantify the efficacy of colorimetry in quantifying color change and DRS in characterizing the chromophore change associated with AN.

Materials and methods

Data used in this study were obtained from eight female patients, aged 12 years and older, who suffered from AN as diagnosed by a dermatologist prior to enrolling in this study. All patients required elevated fasting insulin levels with normal fasting glucose levels to suggest they were in an insulin-resistant condition. In addition, all patients were sent for consultation with an

endocrinologist for treatment of their hyperinsulinemia with diet control, weight reduction and/or oral metformin during the time studied.

Measurements of pigmentation were acquired from locations within the AN neck lesion on the posterior neck and the lateral neck. Control measurements were made on the inner surface of the forearm and on the skin over the clavicle. During measurement, the photo receivers of both devices were placed perpendicularly on the skin with light pressure to avoid the skin blanching effect The colorimeter obtained 3 measurements at 1 spot, and the DRS obtained 10 measurements at 10 different spots as the DRS probe area is 10 times smaller than the colorimeter probe area. The averages of the measured values were calculated. Photographs of the measurement areas of AN and normal skin were marked so that subsequent measurements could be taken at approximately the same location and to observe and document clinical changes. All measurements were taken in an exam room at a dermatology outpatient clinic at Henry Ford Hospital. Follow-up measurements were conducted for eight consecutive months.

The chromameter (Minolta CM-2600d, Osaka, Japan) possessed an 8 mm measurement/illumination aperture diameter. A pulsed xenon lamp emitted an intense white light covering the entire visible spectrum and illuminating the specimen uniformly. The chromameter measured L^*, a^*, and b^* values as defined by the standard CIE system. The L^* parameter expresses color brightness or luminance between a value of 100 for total white and 0 for total black. The a^* parameter represents changes from a positive value for red to a negative value for green. The b^* parameter represents changes from a positive value for yellow to a negative value for blue.

The DRS light source (LS-1 Series Tungsten Halogen Light Source HL-2000, Mikropack and Ocean Optics, Inc., Dunedin, Florida, USA) emitted light in the visible to near-infrared range (360 nm–2000 nm). This source was connected to one end of a bifurcated fiber optic probe (measurement/illumination diameter of 2.5 mm), which also collected reflected light into a spectrophotometer (BWTEK, Inc., Newark, Delaware, USA). The spectrophotometer measured diffuse reflectance spectra in the range of 350 nm–850 nm. These spectra were used to calculate melanin, deoxyhemoglobin and oxyhemoglobin chromophore concentrations. Prior to every patient measurement, both instruments were corrected for detector dark current and were calibrated using manufacturer-provided white and black tile standards.

Results

The percent difference measured between AN lesion and control measurements for the first 4 months of the trial are shown in Table 1. We found that L^*, b^* and melanin values measured in AN lesions were different from normal skin with statistical significance, as shown by their very low P-value ($P < 0.001$). This P-value analysis implied that L^*, b^* and melanin may be good parameters to objectively quantify skin color in AN lesions, as L^* and b^* values tended to be lower while melanin values were higher in lesion tissues. Data in subsequent months were similar.

Table 1. Percent difference in measured parameters between lesion skin and normal skin

Variable	N	Mean	SD	P value
L^* 1st month	8	−27.56	7.86	< 0.001
L^* 2nd month	8	−26.83	7.63	< 0.001
L^* 3rd month	8	−27.65	7.56	< 0.001
L^* 4th month	8	−29.48	7.77	< 0.001
a^* 1st month	8	−29.26	15.16	< 0.001
a^* 2nd month	8	−19.49	25.88	0.071
a^* 3rd month	8	−17.84	27.74	0.112
a^* 4th month	8	−16.31	30.60	0.176
b^* 1st month	8	−47.85	12.98	< 0.001
b^* 2nd month	8	−42.26	17.24	< 0.001
b^* 3rd month	8	−42.17	18.46	< 0.001
b^* 4th month	8	−43.92	18.53	< 0.001
Melanin 1st month	6	33.68	10.45	< 0.001
Melanin 2nd month	8	27.50	10.37	< 0.001
Melanin 3rd month	8	30.11	14.56	< 0.001
Melanin 4th month	8	31.05	12.98	< 0.001
Oxyhemoglobin 1st month	6	64.29	114.45	0.227
Oxyhemoglobin 2nd month	8	10.92	32.92	0.380
Oxyhemoglobin 3rd month	8	−3.43	73.03	0.898
Oxyhemoglobin 4th month	8	10.10	61.29	0.655
Deoxyhemoglobin 1st month	6	−32.08	27.05	0.034
Deoxyhemoglobin 2nd month	8	−44.81	26.06	0.002
Deoxyhemoglobin 3rd month	8	−32.02	25.87	0.010
Deoxyhemoglobin 4th month	8	−44.91	35.59	0.009

The left three graphs of Fig. 1 show the colorimetry L^*, a^* and b^* difference between forearm (control) tissue and AN lesion skin (square markers), as well as the difference between forearm tissue and healthy clavicle or 'normal neck' (control) (star markers) for a single patient over the course of 8 months. Data are typical. The right three graphs show the difference in calculated melanin, oxyhemoglobin, and deoxyhemoglobin concentrations. It is noted that almost all of the measurements fall within one standard deviation of the average and show no discernable trend with time, a result consistent with visual diagnosis of no change over the course of 8 months of treatment. In addition, normal neck tissue is shown to have very similar values to normal forearm tissue, as evidenced by the difference of those values being relatively close to zero, indicating that the choice of forearm tissue as a control was valid.

Discussion

L^* and b^* color parameters obtained from the AN lesions were shifted by more than 3σ from correspondingly healthy neck tissue for all the patients, which corresponds to less than 0.27% chance that the measurement differed by chance alone. These parameters are therefore sensitive enough to differentiate AN from normal pigmented skin color. However, no parameters obtained from DRS possessed sufficient sensitivity to diagnose AN for all the patients. Melanin concentration in the AN lesion

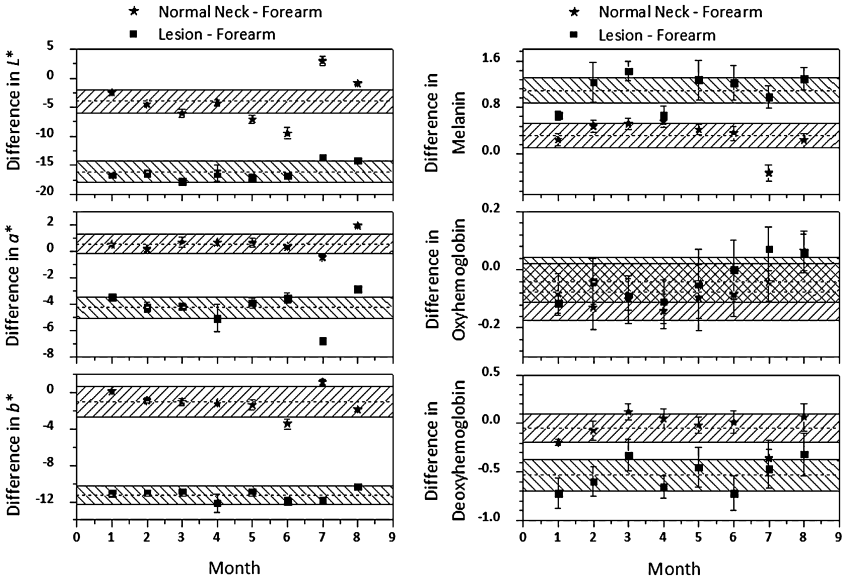

Fig. 1. Patient 1; comparison between normal neck-normal forearm and AN lesion skin-normal forearm.

showed a significant statistical difference of more than 2σ from the corresponding healthy neck tissue in all the patients except one.

By performing a Pearson correlation analysis on the data measured by both instruments, we found a strong negative correlation between L^* and melanin values. We also found that L^* and b^* decreased while melanin increased in AN compared to normal skin. Taken together, this suggests that the hyperpigmentation of AN may have a higher melanin content than the normal pigmented skin. This study supports the conclusion that the brown color of AN is caused by hyperkeratosis of the epidermis as well as increased melanin content.

Colorimetery L^* and b^* seem to be consistent measures of objective skin color in AN lesions, as the measurements were different statistically compared to measurements obtained from the control sites. Although a^* and melanin values are indeed indicative of a change in AN patients (relative to healthy controls), they do not appear to be reliable. Further experiments need to be carried out to draw any conclusions. The oxyhemoglobin and deoxyhemoglobin values did not show a significant difference between patient and control subject data, therefore we would conclude that these values are unreliable for use as an objective color measurement of AN skin lesions. Because these tools measure different aspects of objective skin color (skin chromophore content in DRS and objective color in colorimetry), and because there were no appreciable changes in the clinical appearance of AN lesions to correlate with colorimetry and DRS measurements during the course of the study, we cannot conclude that one tool is more clinically relevant than the other in evaluation of AN. Further studies are indicated to evaluate the correlation of these instruments with dynamic color change associated with skin disease.

At present, a significant limitation of the DRS instrument seems to be that the size of the measurement probe is small. Therefore DRS is ideal for skin lesions which are homogeneous or smaller in size than the integrating sphere aperture of the colorimeter. Another limitation to this study was the fact that AN lesions, as well as other skin conditions, possess a velvety, rough texture, which causes significant light scattering at the skin surface. A larger DRS probe would be able to capture the light that is reflected from these relatively large surface features. In the future, studies will be conducted to determine if the amount of scattered light from the lesion can be correlated with visual diagnosis of AN and/or colorimetery measurements.

Acknowledgements

The study was funded in part by a grant from the Rodzik fund, the Shahani Fund and the C.S. Livingood Lectureship and Educational Fund of the Department of Dermatology, Henry Ford Hospital, Detroit, MI.

References

1. Kahn CR, Flier JS, Bar RS *et al*. The syndromes of insulin resistance and acanthosis nigricans. Insulin-receptor disorders in man. *N Engl J Med* 1976; **294**: 739–745.
2. Guran T, Turan S, Akcay T, Bereket A. Significance of acanthosis nigricans in childhood obesity. *J Paediatr Child Health* 2008; **44**: 338–341.
3. Andreassi L, Flori L. Practical applications of cutaneous colorimetry. *Clin Dermatol* 1995; **13**: 369–373.
4. Stamatas GN, Zmudzka BZ, Kollias N, Beer JZ. In vivo measurement of skin erythema and pigmentation: new means of implementation of diffuse reflectance spectroscopy with a commercial instrument. *Br J Dermatol* 2008; **159**: 683–690.
5. Devpura S, Pattamadilok B, Syed ZU *et al*. Critical comparison of diffuse reflectance spectroscopy and colorimetry as dermatological diagnostic tools for acanthosis nigricans: a chemometric approach. *Biomed Opt Express* 2011; **2**: 1664–1673.