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Optical Imaging Technology: A Useful Tool to Identify Remission in Cushing Disease After Surgery

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Abstract

We recently reported the use of optical imaging technology to quantify facial plethora in endogenous Cushing syndrome (CS). In the present study, we studied a larger cohort of patients with Cushing disease (CD) and examined water content fraction as well as blood volume fraction as bio-optic markers for determining the efficacy of this methodology as a predictor of lasting remission after surgery for CS. We imaged 49 patients before and after transsphenoidal surgery (TSS) for Cushing disease (CD); 22 patients were also seen at 3–6 months, and 13 patients 12 months post-operatively. On all patients, we used multi-spectral imaging (MSI) to evaluate hemodynamic distributions as well as water content at a specific area of the face. We found a

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Conflict of Interest

The authors declare that they have no conflict of interest.

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decrease in blood volume fraction after vs. before surgical treatment in the tested facial area in 37 of the 40 patients, as determined with biochemical markers ($p < 0.001$). All patients that were followed up for up to 12 months showed the same decrease from preoperative values and they remained in remission from CD. We conclude that MSI can be used for the evaluation of remission from CD, at least in the immediate post-operative period and up to one year after surgery. The use of this technology can supplement biochemical and other testing for the evaluation of the various treatment modalities available for patients with CD.

Keywords

cushing disease; optical imaging technology; bio-optic marker; facial plethora; serum cortisol; water content volume

Introduction

The growing use of optical imaging technology in biomedical studies provides the opportunity to improve diagnostics and treatment of a variety of diseases. Exposure to endogenous glucocorticoids or Cushing syndrome (CS) is hard to diagnose; criteria for its cure are also elusive. The type of CS that depends on excess adrenocorticotrophic hormone (ACTH) secreted by a benign adenoma of the pituitary gland is known as Cushing disease (CD) and in like other forms of CS its diagnosis and cure from pituitary surgery can be difficult to assess [1–3]. CD is associated with an increased risk of cardiovascular and metabolic manifestations, coagulopathies, psychiatric complications, osteoporosis and infections leading to increased morbidity and mortality [4] and increased reoccurrence of the diseases later, even when cured [5–7]. Follow up studies for CD patients of 5 to 10 years after transphenoidal pituitary microsurgery (TSS) treatment have suggested decreased survival rates among patients with persistent or recurrence vs. with control population [8–12].

CS is infrequent with an estimated incidence of 0.2–5.0 per million people per year and a prevalence of 39–79 per million in certain populations [13–16], which makes it a rare disease [17]; CD is estimated to affect approximately 8 people per million [18]. Due to its rarity, diagnosis of CS can be problematic. TSS is the treatment of choice for CD, but the post-treatment relapse rate, reported in different studies, ranges from 0–34 % [19–25]. But it should be noted that there is a remarkable variety in the reported rate of remission and recurrence. Some factors can influence the evaluation, so in the lack of consensus on remission and recurrence criteria, their diagnoses remain challenging.

The above highlights the importance of new diagnostic method for CS and CD. In addition, innovative methods are needed to assess cure of CS and CD after TSS or other therapies. At present, cortisol levels are monitored after surgery, but fail to predict long term cure accurately [22, 23, 26].

We explored what we believe is a robust method to assist clinical evaluation to evaluate remission in patients with CD after surgery. This work is continuation of our recently published study [27], which reported blood volume fraction of the cheek that quantifies

facial plethora, which is one of the longest known and most distinguishable symptoms of patients with CS, as a biomarker of short term remission. The present study, with a larger population, examined water content fraction as well as blood volume fraction as bio-optic markers in patients with CD. We then investigated the efficacy of this methodology as a predictor of lasting remission from surgical treatment. Our data showed that quantitation of face vasculature function of individuals with CD before and after surgery can be used to predict remission. This technology can help to enhance clinical decision making in CD and provide a potential non-invasive quantitative platform to complement clinical assessment with mapping biological chromophores of tissue in other diseases.

Materials & Methods

Instrumentation and methods

We employed a modified diffuse near infrared multi-spectral imaging (MSI) system consisting of charged-coupled device (CCD) camera, light source, polarizer, cross-polarizer and mechanical filter which take 2 dimensional images of the target in 6 wavelengths of 700, 750, 800, 850, 900, and 1000 nm with spectral bandwidth of 25 nm, as demonstrated by Vogel et al. and Jana et al. [28, 29]. This imaging system takes multiple simultaneous images. Each of these images has been acquired through filters with different spectral sensitivities. The mechanical filter splits the back-scattered light in the pre-defined spectrum, and enables the capturing of images of one particular wavelength at a time until the complete multispectral images are produced after a sequence of exposure times. In the near infrared spectral region (700–1000 nm) physiological chromophores (such as oxy-hemoglobin, deoxy-hemoglobin, blood, water, and melanin) have a distinguishable light absorption coefficient spectrum which allows approximating their concentration by measuring the reflected light from the tissue at different wavelengths [30, 31]. A light integration with 2 layered-skin model (epidermis and dermis) was applied to identify the fraction of blood volume and water content. Back-scattered light intensities were analyzed within the model while considering the value of the reduced scattering coefficient of the dermis, and the known light absorption coefficients of the physiological parameters of interest. The model includes biological parameters such as blood, oxy- and deoxy-hemoglobin, water, and melanin. It is assumed that the scattering properties in the skin layers are almost the same. Melanin concentration and epidermal thickness were considered as known parameters. Melanin volume concentration was estimated based on Fitzpatrick skin typing scale [30, 31]. Based on the values extracted from literature review [32, 33], the epidermal thickness of the patient's cheek was estimated to be 80–100 μm . It should be noted that unlike oxyhemoglobin concentration of blood, blood volume and water content fraction are not very sensitive to epidermal thickness.

Clinical protocol

The study was initiated in August 2012 and completed in March 2017. Forty-nine of the 57 patients (86 %) with CD who received surgical treatment for ACTH-secreting pituitary tumors at the National Institutes of Health (NIH) during this period participated in the study recruited through clinical protocol 97-CH-0076, conducted by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD). Patients (or their

parent/legal guardian) gave written informed consent (and assent from older children). Eight out of the 57 patients were excluded: 3 had fever at time of post-surgery imaging, 2 had severe facial acne, and 3 had a variety of other issues that did not allow them to complete the study. Of the 49 patients, 34 were females; the mean age was 18.5 ± 12.3 years. Three of those patients received 2 consecutive surgeries (TSS) within 8–16 days and were imaged twice; thus, the final number of scanned cases was 52. The diagnosis and etiology of CS were confirmed as previously described [34], as well as by histology.

For post-TSS patients, the procedure for clinical assessment involves the measurement of serum cortisol and ACTH after surgery beginning on postoperative day (POD) 2, and repeated daily until POD 9 or 10. During this postoperative period, dexamethasone was administered to the patients after who required glucocorticoids replacement. Oral dexamethasone was started on POD 5 for patients with serum cortisol levels consistent with remission of CS (cortisol $< 5 \alpha$ mcg/dL). For patients with serum cortisol levels that were not consistent with remission of CS, dexamethasone replacement was withheld until POD 9 when results of the postoperative corticotropin-releasing hormone (CRH) stimulation test were available. Thirty-eight CD patients were on 0.5 mg of dexamethasone per day at the time of postoperative multispectral imaging (MSI) assessment; these patients had been on dexamethasone for an average of 3.3 ± 2.4 days at the time of imaging.

Previously we reported a clinical study conducted at NIH that considered a patient in remission in the immediate postoperative period if average 24-h urine free cortisol levels were $< 20 \mu\text{g}/\text{m}^2$, and that all patients with lasting remission had a mean morning cortisol value after TSS of $< 6.5 \mu\text{g}/\text{dl}$ ($179.3 \text{ nmol}/\text{l}$). The specificity of the mean morning cortisol value in long-term remission was 100 % (66 of 66; confidence interval, 95–100 %) [35]. Because a few patients from our old cohort reported by Batista et al. with postoperative cortisol levels between 3 and $6 \mu\text{g}/\text{dl}$ faced in the meantime recurrence, and only those with levels below $3 \mu\text{g}/\text{dl}$ achieved sustained remission (unpublished data), for the purposes of this study only patients with postoperative measurements of plasma cortisol less than $3 \mu\text{g}/\text{dl}$ were considered cured [36–38].

Data acquisition

Each CD patient had images taken prior to TSS as a baseline and after TSS. The pre-surgery imaging varied in the range of 4 days to one year before TSS date. The post-surgery imaging time occurred in the range of 1–18 days after the TSS date depending on the patient's physical condition. Also, some patients had imaging performed during follow-up sessions (6 months, one year post-TSS) in addition to the 2 previous rounds of imaging.

The region of interest (ROI) was the right cheek to assess facial plethora, but the right upper arm area was also imaged as a control. All undertaken steps including the placement of $6.2 \times 6.2 \text{ cm}^2$ marker, setting exposure time ranging 200–450 ms, room darkness, and lack of patient's motion during imaging, were well monitored and executed by 2 people. One obtained the images and the other provided observations to maximize reproducibility and standardization. The measurement was performed up to 3 times for the selected exposure time for each patient.

Data processing

Calibration and artifact curvature removal are the major pre-processing steps. Utilizing the white reflectance card, calibration facilitated removing of all spatial and spectral inhomogeneities of illumination as well as spectral sensitivity of the CCD camera. An algorithm based on curvature correction method [29] was applied to wipe out the image artifacts generated by the curvature biased intensity distribution of the patient's cheek, which mask the variations associated with the tissue optical properties.

We used a stepwise approach which consisted of 2 phases of data processing to examine the impact of each variable in the model in 2 phases and avoided divergence problems in numerical reconstruction. Considering the low light absorption of water and fat in the range of 700–850 nm wavelengths, phase I just focused on blood volume and oxygenation fractions as unknown parameters and water and fat fractions was neglected. In phase II, the output of phase I was used as input and just 2 wavelength-based images of 900 and 1000 nm were used in the computation to calculate the water content fraction. Data analysis and reconstruction process was done by software. The physiological parameters of interest were reconstructed using Levenberg-Marquardt (LM) algorithm [39] by applying nonlinear least square real value function fit on each pixel of the corrected light intensity separately. The final value is based on the mean of all pixels' quantities due to entire region imaged.

Statistical analysis

Frequency distributions and simple descriptive statistics depicting the data are reported as number (percentage) or mean $\pm$ SD. The intra-class correlation coefficients as indicative of intra-observer reliability for blood volume and water content fraction measurements before and after surgery were 0.998 and 0.997, 0.972, and 0.998, respectively. These significant numbers proved that the reconstructed biological parameters, which were computed in 3 different times for the same subjects, are indistinguishable. Also, pre- and post-surgery data blood volume and water content fractions data both fit well within a Gaussian distribution. As the data analysis method is not dependent on the observer, any operator who can comply with the instructions is able to achieve the same result; therefore, inter-observer reliability was not applicable. The paired t test was used to compare pre and postoperative A blood volume (change in blood volume) and water content fractions data between remission and non-responder subjects. One way analysis of variance (ANOVA) and Fisher (least significant difference) procedure (the t method) were applied to examine the difference between the follow up sessions. Percentage changes were defined by dividing the blood volume fraction by preoperative blood volume fraction and were used to pictorially show the distribution of change. The receiver operating characteristic (ROC) curve illustrated the performance of blood volume fraction in discriminating between remission and non-responding patients. The F1 score and Matthew's correlation coefficient identified the accuracy of cured patients' classifications. Intra-observer reliability was measured with intra-class correlation using the 2 ways ANOVA. All data were assessed for their distributions, and appropriate tests were used accordingly; all tests were 2-sided, with statistical significance defined as $p < 0.05$. Statistical analyses were performed using Minitab version 17. (Minitab Inc.).

Results

Identifying remission

Baseline patient characteristics and pre- and postoperative biochemical results are presented in ► Table 1. Forty patients were in remission, where all but 3 (92 %), showed a decrease in blood volume fraction in ROI ($p < 0.001$). A decrease in water content fraction related to region of interest ROI was observed in 35 of the 40 patients (87 %). These patients also had a significantly lower blood volume and water content fraction postoperatively than before surgery, respectively (17.4 ± 0.04 % vs. 14.7 ± 0.03 %; $\Delta -12.2 \pm 0.18$ %; $p < 0.001$; ► Fig. 1a) and (44.1 ± 0.2 % vs. 38.5 ± 0.2 %; $\Delta -11.6$ % ± 0.03 ; $p = 0.001$; ► Fig. 1b).

In contrast, in all 12 patients who had persistent CD after surgery, blood volume and water content fractions in ROI increased after surgery respectively (17.3 ± 0.04 % vs. 20.2 ± 0.06 %; $\Delta 16.5 \pm 0.11$ %; $p < 0.001$; ► Fig. 1a) and (40.3 ± 0.03 % vs. 46.1 ± 0.03 %; $\Delta 16.3 \pm 0.17$ %; $p < 0.001$; ► Fig. 1b). Thus, the change in blood volume and water content fraction in ROI was significantly different between patients that were in remission and those that were not ($p < 0.001$) as can be seen in ► Fig. 1a and b. Three patients required 2 consecutive surgeries because the initial TSS was unsuccessful. In each case, the cheek blood volume fraction of the patient remained elevated after the first surgery. However, after the second therapeutic surgery, the average blood volume fraction in the ROI dropped in all 3 patients. ► Fig. 2 illustrates the variation in facial plethora and blood volume fraction of 2 of these patients before and after both the first and second surgeries. ► Fig. 2 also shows the advantage of the quantitative measurements obtained from MSI against visual inspection. While documenting facial redness correlated well with the corresponding blood volume changes, the bottom images reflected apparently incorrect interpretations with regards to cure. Thus, evaluation of facial plethora by the naked eye is often unreliable in predicting remission from CD. ► Fig. 3 points to the strong correlation between blood volume a water content changes; the Pearson correlation coefficient is 0.79 that is quite remarkable making blood volume a great marker to follow. The ROC curve for blood volume and water content fraction had area under curve (AUC) of 0.95 and 0.91, respectively, confirming the ability of our method to correctly classify patients in remission and those not in remission state (Supplemental Fig. 1S). The calculated F1 score and Matthew's correlation coefficient for blood volume and water content fraction of the cheek with clinical assessments were 0.96, 0.93 and 0.86, 0.79, respectively.

Patients who were in remission and the method failed to identify them correctly

There were 3 patients that were in remission and were misidentified by MSI. It is likely that physiological conditions accounted for the inappropriately increased blood volume fractions in 2 of the 3 cases. One of them had a postoperative urinary tract infection at the time of post-surgery imaging. Another developed significant hypertension and was placed on amlodipine and atenolol medications that can be associated with flushing.

Follow up of all patients for up to a year

We saw patients with CD post-operatively first at 3–6 months (taper of glucocorticoid dose was initiated at 3–4 months post-op) and then again at 12 months, at which point most are

off replacement with glucocorticoids. At time of follow-up optical imaging patients were changed to daily dexamethasone replacement to allow for HPA axis testing. So far, 22 patients were seen immediately both post-operatively and at 3–6 months, and 13 were also seen at 12 months. All of them remained in remission and their data, interestingly, retain the smaller blood volume fraction of the ROI in the follow up sessions, confirming the validity of the technique in identifying lack of exposure to excess glucocorticoids. Supplemental Fig. 2S indicates the mean of first and second follow up is less than before and after surgery. Blood volume fraction, although higher at 12 months vs. 3–6 months, is lower compared to preoperative values ($p < 0.001$).

Bayesian analysis

Using the Bayesian theorem, we computed the probability of clinical (bio-chemical remission) evaluation given bio-optic results (processed MSI) based on blood volume fraction variation of the cheek. Supplemental Fig. 3S shows the values for remission and non-responder, respectively, at 100 and 78 %.

Discussion

Our prior report quantified facial plethora in CS for the first time. We extended this observation to a larger cohort and explored water content fraction of the cheek as another biomarker for assessment of remission from CD both immediately postoperatively and up to a year after TSS. The results showed a decrease in the average blood volume and water content fraction in the cheek area for patients who were identified clinically in remission. We observed the opposite change in these bio-chemical parameters for patients whose post-operation cortisol levels were judged to be in non-responder state. Their average blood volume and water content fraction of cheek area increased after TSS. We are not able to explain this increase with certainty; we may hypothesize that this relates to postoperative inflammation and/or cytokine release on top of continuing increased cortisol levels.

The goal of this study was to use the MSI system to assist in the determination of the treatment outcome for CD. In addition, this study was the first to our knowledge that used MSI to quantify facial plethora based on water content fraction. The correlation between blood volume and water content fraction of cheek data may be helpful to explore the potential biological relationship between the 2-physiological parameters in facial plethora and similar conditions. Previous reports on skin composition showed that interstitial fluid makes up a major part of water content in both the dermis and epidermis (roughly 40 % and 15–35 %, respectively) [40, 41]. Furthermore, this interstitial fluid reached the skin layers by passing through the walls of blood-carrying capillaries. Regarding the fact that capillary walls only allow interstitial fluid to cross freely, we can speculate that when blood flow through those capillaries is increased, interstitial fluid penetration into the dermis and epidermis also increases. The data from this study support this hypothesis.

Unlike in our previous study [27], we now followed our patients for up to a year. The data show that MSI is a promising tool to assess remission not only immediately post-operatively but also in follow-up, at least up to 12 months. This technique is safe, noninvasive, fast, and inexpensive, making it ideal to monitor a disease or condition over time. Also, this

technology is not dependent on a threshold to define facial plethora because we are comparing preoperative to postoperative changes in the same individual.

Although this study included a substantial sample size for CD, which is a rare disease, the findings should be considered as preliminary. Continuation of the study and follow-up imaging of more patients is required to verify these results. This is a suitable time to introduce this technology into centers which provide treatment to CD patients and to collect data that will confirm the validity of this technique. This technique has 2 limitations. First, the elements associated with the study such as rarity of the syndrome and the need for more studies to confirm results in a larger CD patients sample, may put restriction on the generalizability of the findings. The second limitation originates from methodology, which include interference of patient fever and cortisol injection on imaging day, or severe acne and facial hair with the method. Additional studies are needed to confirm variables associated with interference with optical imaging technology. Moreover, the technology is applicable just for those biological targets whose location is no more than 3 mm below skin surface. Additionally, this limitation discloses the method has potential to use in other diseases perhaps to use quantifying facial plethora change as a marker of recurrent disease, or to distinguish patients with the corresponding therapy outcomes.

In conclusion, the reconstructed bio-optic markers (blood volume and water content fraction of cheek) as quantitative indicators of facial plethora are signs of early and lasting remission in patients with CD. It is possible that this technology can be used to monitor the corresponding therapy outcomes. However, as Bayesian analysis confirmed this technique is complementary to clinical evaluation and is able to serve as a tool in enhancing clinical decision making and it is not suggested as an alternative to current bio-chemical markers. It should be noted that in the future this kind of spectroscopy can be easily implemented using a handheld mobile device, with easy access at outpatient as well as inpatient care.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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► Fig. 1.

a and **b** show boxplot of mean and median percentage change indicating change in blood volume and water content fraction after surgery, compared to before surgery, in each of the remission and non-responded Cushing's patients. The distribution shows median confidence interval box and interquartile range. • & □ represents respectively mean and median in each category of remission and non-responded CD patients.

► **Fig. 2.**

Facial plethora in the 2 patients with CD before TSS, after the first TSS (non-responded), and after the second TSS (remission), The blood volume fraction percentage change of the patients' left cheek at different imaging sessions are depicted in the right graphs.

► **Fig. 3.**

This shows correlation between water content and blood volume change percentage. Blue: True negative for both of water content and blood volume fractions. Green: False negative for both of water content and blood volume fractions. Red: True positive for both of water content and blood volume fractions. Purple: True positive for blood volume fraction but false negative for water content fraction.

► Table 1

Baseline patient characteristics.

	CD	
n	49	
Age (years)	18.5 ± 12.3 (6–55)	
Female/male, n (%)	34 (69)/15 (31)	
Race, n (%)		
Asian	2 (4)	
Black	6 (12)	
White	40 (82)	
Other/unknown	1 (2)	
Ethnicity, n (%)		
Latino or Hispanic	9 (18)	
Not Latino or Hispanic	40 (82)	
	Preoperative cortisol AM, µg/dl	Postoperative cortisol AM, µg/dl
All patients	19.7 ± 12.4 (3.8–93)	4.1 ± 5.6 (1–23.6)
Remission	19.8 ± 13.7 (3.8–93)	1.5 ± 0.8 (1–7.6)
Not responder	19.4 ± 6.8 (7.5–30.9)	12.8 ± 5.9 (4.1–23.6)
	Preoperative ACTH AM, pg/ml	Postoperative ACTH AM, pg/ml
All patients	51.3 ± 57.3 (5–393)	17.6 ± 21.7 (5–113)
Remission	52.5 ± 61.8 (6.8–393)	13 ± 17.6 (5–83.5)
Not responder	43.2 ± 42.2 (5–139)	32.8 ± 29.8 (5–113)
	Preoperative UFC, µg/24 h	Postoperative UFC, µg/24 h
All patients	230.1 ± 217.6 (10.3–1135)	7.7 ± 27 (1.3–173)
Remission	232.2 ± 217.8 (28.2–1135)	3.3 ± 6 (1.3–21.6)
Not responder	223.7 ± 229.4 (10.3–836.9)	20.2 ± 51.4 (9.8–173)

Data are expressed as mean SD (range), unless stated otherwise.

UFC: Urinary free cortisol.