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# Evaluation of scrotal pigmentation in infants with spectroscopic method and its correlation with 17-hydroxyprogesterone blood levels

## Yenidoğanlarda skrotal pigmentasyonun spektrometre ile değerlendirilmesi ve 17-hidroksiprogesteron kan düzeyi ile korelasyonunun incelenmesi

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## ABSTRACT

**Aim:** To evaluate the scrotal melanin density in infants using spectrometry and to determine the correlation between spectrometric evaluations, physical examinations and blood 17-hydroxyprogesterone levels.
**Material and methods:** A total of 40 infants were enrolled to the study, 22 of whom were diagnosed by a physician as having scrotal hyperpigmentation and 18 with normal scrotal pigmentation, who were admitted for the evaluation of prolonged jaundice. Age, gestational week, birth weight and scrotal pigmentation noted by the physician were recorded. Spectral data were acquired from scrotum and thigh. A correlation between the spectral measurements and the blood 17-hydroxyprogesterone level was determined by comparing spectral value in the wavelength range of 620-800 nm and 17-hydroxyprogesterone levels.
**Results:** No statistically significant difference was observed between the groups who were categorized by the physician as having "hyperpigmented" or "normal" scrotal color in terms of the infant's age, gestational week, birth weight, 17-hydroxyprogesterone level or spectrometric values. We observed a strong correlation between 17-hydroxyprogesterone levels and spectrometric values in all groups.
**Conclusion:** This preliminary study is the first one in the literature which evaluates scrotal pigmentation with an objective spectrometric method and determines its relationship with 17-hydroxyprogesterone levels. Further studies are needed to employ this method as a non-invasive, indirect screening test for the screening of congenital adrenal hyperplasia in male infants.

Key words: Congenital adrenal hyperplasia, spectrometry, scrotal pigmentation, 17-hydroxyprogesterone, spectroscopic method

## ÖZ

**Amaç:** Spektrometre ile bebeklerin skrotal melanin dansitesini değerlendirmek ve spektrometrik değerlendirme ile fizik muayene ve kan 17-hidroksiprogesteron düzeyleri arasındaki ilişkiyi belirlemektir.
**Gereç ve Yöntemler:** Çalışmaya skrotal hiperpigmentasyonu olduğu düşünülen 22 olgu ile uzamış sarılık nedeniyle başvuran normal skrotal pigmentasyona sahip 18 olgu olmak üzere toplam 40 bebek dahil edildi. Olguların yaşı, gestasyon haftası, doğum ağırlığı, anne yaşı, skrotum rengi kayıt altına alındı. Spektral veriler skrotumdan ve uyluktan elde edildi. Spektral ölçümler ve kan 17- hidroksiprogesteron seviyesi arasındaki korelasyon, 620-800 nm dalga boyu aralığındaki spektral değer ve 17- hidroksiprogesteron seviyelerinin karşılaştırılmasıyla incelendi.
**Bulgular:** Skrotal hiperpigmentasyonu saptanan ve saptanmayan grup arasında anne-olgu yaşı, gestasyon haftası, doğum ağırlığı, 17-hidroksiprogesteron düzeyi ve spektrometri değeri arasında istatistiksel olarak anlamlı farklılık saptanmadı. Tüm gruplarda 17-hidroksiprogesteron düzeyleri ile spektrometrik değerler arasında güçlü bir korelasyon gözlemledik.
**Sonuç:** Çalışmamız, literatürde spektrometre değerleriyle skrotal pigmentasyonun nesnel bir ölçüt hale getirildiği, 17-hidroksiprogesteron ile ilişkisinin incelendiği, gelecekte erkek bebeklerde konjenital adrenal hiperplazi taramasına dolaylı yoldan yardımcı olabilecek bir çalışmadır.

Anahtar kelimeler: Konjenital adrenal hiperplazi, spektrometre, skrotal pigmentasyon, 17-hidroksiprogesteron, spektroskopik yöntem

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## INTRODUCTION

Congenital adrenal hyperplasia (CAH) is an autosomal-recessive inherited disease, resulting from deficiency of any of the 7 enzymes required for cortisol biosynthesis from cholesterol [1]. 21-hydroxylase enzyme deficiency accounts for more than about 95% of all CAH cases [2]. Impaired production of cortisol, aldosterone and increased androgen synthesis are the causes of many clinical manifestations.

While female infants with CAH are identified early due to their ambiguous genitalia, male infants do not have this sign to alert the physician. This results in diagnostic delays and diagnosis can only be made when serious adrenal insufficiency develops. Therefore, attention by the physician, particularly in the case of male infants, is very important for early diagnosis of the disease. Genital hyperpigmentation developing as a result of the disease in male infants is recognized as a warning sign [3]. In clinical practice, 17-OHP blood level is required in infants with suspected genital hyperpigmentation. However, this subjective criterion can lead to missing the cases or unnecessary 17-OHP requests.

Spectroscopic methods have begun to be widely used in recent years for the diagnosis of diseases. The objective of these studies is to diagnose the disease in a non-invasive and less pain-inflicting manner at an early stage. In addition, another aim of the researches made with optical methods is to develop practical systems that will enable the real time evaluation of the tissues [4, 5]. Spectroscopy is a non-invasive technique that can determine optical properties in vivo. In this technique, light is sent to the tissue and collected after interacting with it, then analyzed via the spectrometer. The spectrum data contains information about the biochemical composition and the physical structure of the tissue, thus providing information about the tissue physiology [6, 7].

The aim of this study was to develop an objective and non-invasive spectroscopic method to measure the density of melanin pigment in neonatal scrotal tissue and investigate the compliance of the method with physician's opinion and its correlation with 17-OHP blood level.

## MATERIAL AND METHODS

### Patients

This prospective study was conducted at the well-child clinic of Social Pediatrics Department at Akdeniz University. Twenty-two male patients older than two days of age thought to have scrotal hyperpigmentation on physical examination and 18 male patients aged 15-60 days with normal scrotal pigmentation on physical examination, who were assessed due to prolonged jaundice, were included in the study. Female infants and male infants with a nevus in the genital area and infants with low birth weight were excluded from the study. The age, gestational week and birth weight of the study infants were recorded. The physical examination of all cases was performed by the same physician and the scrotum color was recorded as either hyperpigmented or normally pigmented.

### Spectroscopy System

The spectroscopy system consisted of a miniature spectrometer (Ocean Optics USB200, FL), an optical fiber probe to deliver the light to and from the scrotum (R400-7-VIS-NIR, Ocean Optics, FL), a white light source (HL-2000, Ocean Optics, FL) and a laptop computer. The optical fiber probe was made up of seven optical fibers with a core diameter of 400 µm; one of the fibers was placed at the center and the other six surrounded it. The six surrounding fibers delivered the light to the tissue and the central fiber detected reflected diffuse light from the tissue. A schematic illustration of the system is given in Figure 1.

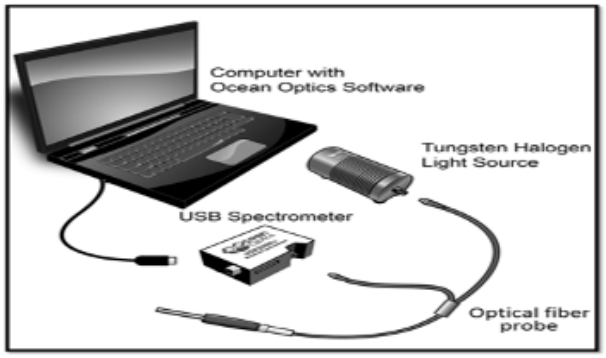


Figure 1. Schematic illustration of the spectroscopic system used in the study.

Measurements:

The necessary institutional and ethical approvals were obtained from Akdeniz University Ethics Committee (11.06.2013-115) at the beginning of the study in accordance with the Helsinki declaration. All parents gave written informed consent before participation.

The tip of the optical fiber probe was gently placed over the scrotum for the spectroscopic measurements. Before acquiring data from the scrotum, the system was calibrated as previously described [8]. Control measurements were made from the inside of the thigh to remove the effect of any components other than melanin, which are absorptive of light, located in the scrotum tissue.

The blood level of 17-OHP was determined via the RIA (DIAsource 17OH-RIA-CT Kit, DIAsource ImmunoAssays S.A., Belgium) method in all infants who had scrotal hyperpigmentation or normal pigmentation, according to the physician's evaluation. To avoid unnecessary blood sampling, infants with normal scrotal pigmentation were chosen among those who were scheduled for blood tests due to prolonged jaundice.

Data Analysis:

All the spectra were acquired in the wavelength range of 400-800 nm. The first spectrum was the background spectrum ($I_b(\square)$), acquired from the region of interest while the light source was off. The second spectrum ($I_s(\square)$) was taken to define the spectral distribution of the light source, with the probe placed nearly 1 cm above a white reflectance standard (Spectralon; Labsphere Inc, North Sutton, NH). Then, the spectra ($I_p(\square)$)were acquired from the patient's scrotum and medial side of the thigh. The spectra acquired from the patients were corrected as

$$_c(\lambda) = \frac{I_p(\lambda) - I_b(\lambda)}{I_s(\lambda) - I_b(\lambda)}$$

As seen in Figure 2, normalized spectra of scrotum and thigh are different from each other. Hemoglobin absorption is stronger on the thigh than the scrotum in the wavelength range of 500-600 nm. In all probability, the probe was located on a vein during the acquisition of the spectra from the thigh. Absorption of hemoglobin decrease above 600 nm. In the wavelength range of 620-800 nm, absorption of water is very weak, absorption of hemoglobin is lower than the absorption of melanin. Therefore, in that wavelength the range spectral shape is dominated by the absorption of melanin [9]. Basically, spectral shape of the transmitted light through the tissue depends on the tissue optical parameters defined by the absorption and scattering of the light within the tissue. Scattering depend on the physical structure of the tissue. Absorption depends on the concentration of each tissue chromophores. The difference between the thigh and the scrotum tissues in terms of the spectroscopy is the variation of melanin concentration. Scrotum tissue has more melanin than thigh tissue. Here, we assume that all other optical parameters of both tissues are similar except for the absorption of melanin. Therefore, having the ratio of the spectra provides the variation of melanin absorption in the wavelength range of 620-800 nm.

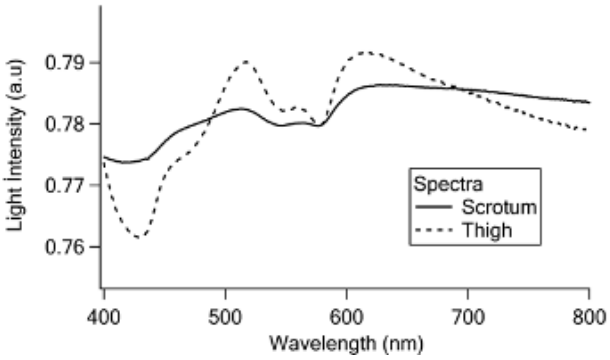


Figure 2. The spectrum measured from the scrotum and thigh: scrotum (solid line) and thigh (dashed line).

In order to obtain net absorption of the scrotum pigmentation, the scrotum spectrum is divided by the spectrum of normal tissue (the spectrum acquired from the thigh). As seen in Fig 3, the ratio is increasing with the wavelength. Slope of the ratio in the wavelength range of 620-800 nm has been chosen as a diagnostic parameter and calculated for all the patients. From this point on, we identify the "slope" as the "spectroscopic value". The correlation between the spectroscopic value and 17-OHP blood level was examined.

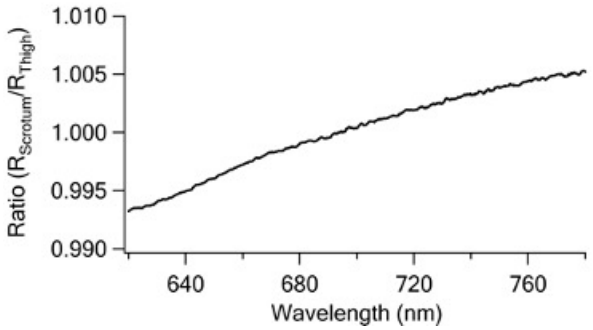


Figure 3. Ratio of the spectrum acquired from the scrotum to the spectrum acquired from thigh.

Statistical Analysis:

Data results were analyzed using the SPSS 18.0 (Chicago) software package. Descriptive statistics such as frequency distribution, mean and standard deviations were used to describe the sample. The Shapiro-Wilk normality test was used to analyze the continuous distributions of the two groups, Student-t test was performed in the cases with normal distribution, and Mann-Whitney U test was performed in the cases where non-conformity was observed. The Spearmann correlation analysis was used for the relationship analysis of the measurement variables according to the test assumptions. A 95% significance level (or α = 0.05 error margin) was used to determine the differences in the analyses. The following assumptions were made: a Spearman's correlation coefficient (r) between 0.90-1.00 indicates a very strong correlation, 0.70-0.89 a strong correlation, 0.50-0.69 a moderate correlation, 0.30-0.49 a weak correlation, 0-0.29 no correlation and a negative correlation indicates an inversely proportional correlation.

## RESULTS

Physician-diagnosed scrotal hyperpigmentation was determined in 22 of the 40 infants studied. The median age of the infants was 29 (3-60) days, the mean birth weight was 3348 ± 387 grams, the mean gestational week was 38.1 ± 1.2 weeks, the mean spectroscopic value was 66.1x10-6 ± 29.5x10-6 and the mean 17-OHP level was 6.98 ± 3.8 ng /dl. The 17-OHP level of two cases was in the range of 10-20 ng/dl. The 17-OHP level of one case was above 20 ng/dl. Nine cases (22.5%) were born at 37 weeks' gestation or less.

When infants were grouped according to the physician's evaluation, as having normal or hyperpigmented scrotum, no statistically significant difference was observed between the two groups in terms of age, birth weight, gestational age, spectroscopic value or 17-OHP levels (Table 1).

Table 1. Demographic data, spectrometric measurement values and 17-OHP levels of infants with normal or hyperpigmented scrotum according to the physician's evaluation

| | All Patients | Hyperpigmented (n:22) | Normal (n:18) | p value |
|---|---|---|---|---|
| Age (day) | 20.8±11.97 | 22.4±14,1 | 18.9±8.8 | .436 |
| Birth weight (gram) | 3348±387 | 3355±396 | 3340±386 | .849 |
| Gestational age (week) | 38.1±1.2 | 38.3±1.0 | 37.9±1.3 | .518 |
| Spectroscopic value (x10-6) | 66.1±29.5 | 69.8±31.9 | 61.6±26.6 | .377 |
| 17 OH Progesterone level (ng/dl) | 6.98±3.8 | 7.19±4.3 | 6.72±3.1 | .807 |

Data are presented as mean ± SD.

In infants with physician-diagnosed scrotal hyperpigmentation, the gestational week had a weak positive correlation with birth weight (r=0.488, p=0.021). There was a weak negative correlation between the 17-OHP level and the age of the infant (r = -0.434, p = 0.044). A strong positive correlation was found between the 17-OHP level and the spectroscopic value (r=0.869, p=0.000).

In infants with scrotal pigmentation determined to be normal according to the physician's evaluation, the age and gestational week were not correlated with any of the parameters. The birth weight was negatively correlated with the 17-OHP level (r=-0.609, p=0.007). A strong positive correlation was found between the 17-OHP level and the spectroscopic value (r=0.837, p=0.000).

In all infants, the gestational week was found to have a weak positive correlation with the birth weight only (r=0.325 p=0.041). The birth weight had a weak negative correlation with the 17-OHP level (r=-0.332, p=0.036), whereas age of the infant was not correlated with any of the parameters. A strong positive correlation was found between the 17-OHP level and the spectroscopic value (r=0.857, p=0.000) (Figure 4).

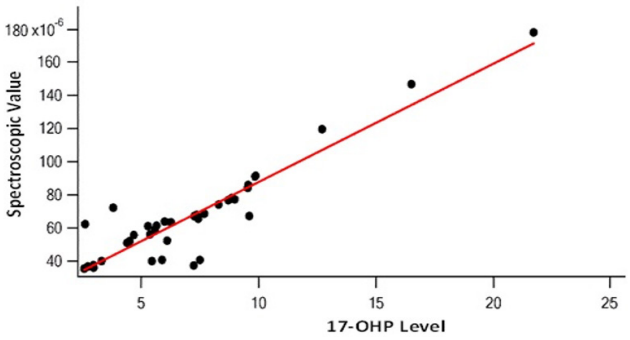


Figure 4. The relationship between 17-OHP level and spectroscopic value.

## DISCUSSION

CAH is a common and fatal childhood disease, the morbidity and mortality of which can be prevented with early diagnosis and treatment [10]. The cost of screening for 21-hydroxylase deficiency in newborns is high. However, when the results obtained from the screenings were evaluated, at least 50% of the patients identified during the screening were not clinically diagnosed [11]. Therefore, attention on the part of the physician, particularly in the case of male infants, is very important for early diagnosis of the disease, in particular in countries where there exists no screening program for CAH. Genital hyperpigmentation developing as a result of the disease in male infants is recognized as a warning sign, however the subjective evaluation of physician may lead to false-positive suspicion which may in turn result in unwarranted anxiety for the parents and higher health care costs for the system. On the other hand, false-negative evaluations on physical examinations may lead to more devastating results such as late diagnosis, severe dehydration and even death. Thus, in our study, we tried to develop an objective method to measure the density of melanin pigment in neonatal scrotal tissue. We identified a strong positive correlation between the spectrometer value and the 17-OHP level in all infants, though there was no difference in the 17-OHP values when physician was the source of information about the pigmentation of scrotum. Hence, we believe that the spectrometric method may allow the determination of CAH in a more objective manner.

According to our research, this study is the first in the literature in which scrotal pigmentation was evaluated objectively with a non-invasive spectroscopic method and its correlation with the 17-OHP was investigated. We have shown that the spectrum of the light reflecting from scrotal tissue was strongly correlated with the 17OHP levels of infants (r = 0.857). We think this method can provide an initial, non-invasive, indirect step for the screening of CAH in the future for countries which do not a have a well-established CAH screening program. Consequently, we identified several major advantages: First of all, this spectroscopic method is portable and allows the probe to be easily positioned on the scrotum due to its flexibility. Secondly, patients are examined in a non-invasive manner by the optical fiber probe. Thirdly, this method is not motion sensitive during data acquisition. Fourthly, the system is completely safe for the patients because only visible light is used in the measurements. Lastly, acquiring a spectrum from scrotum takes less than 2 minutes and therefore allows the physician to diagnose scrotal hyperpigmentation in a short time.

The most important problem with screening tests is false positives at the 17-OHP level. The latter is high during birth and rapidly decreases in the next few days. In this respect, blood samples should be taken 48-72 hours after birth, making it difficult to diagnose during this period. Therefore, in order to eliminate the effect of blood sampling on the 17-OHP level, we exclusively included infants aged 2 and more days in the study.

Preterm, sick or stressed infants have higher 17-OHP levels than healthy children. Especially in preterm infants, physiological delay in 11 β-hydroxylase enzyme activity can cause temporary increases in 17-OHP levels [12]. Van der Kamp et al. [13] reported that 17-OHP levels are correlated more with gestational week than birth weight. In our study, we found a weak negative correlation between the 17-OHP level and the birth weight (r=-0.332) but we did not find any significant correlation between the gestational week and the 17-OHP level (p=0.456). This may be ascribed to the exclusion of very young preterm infants and infants with low birth weight from the study.

In our study, we did not find any significant

difference between 17-OHP levels of patients with scrotal hyperpigmentation and those without, as revealed by the physical examination (p=0.807). We think this is important because it shows that the physician may fail to correctly diagnose CAH. However, we found a strong positive correlation between the 17-OHP level and the spectroscopic value of all patients (r=0.857, p=0.000).

As a matter of fact, Gökdemir et al. [14] found scrotal hyperpigmentation in 90 (28.12%) of 320 male infants they included in their study. This percentage indicates that the incidence of scrotal hyperpigmentation in male infants should not be underestimated and that the evaluation of scrotal hyperpigmentation through physical examination is a subjective practice that is not very helpful in diagnosis of CAH, frequently giving false positive results. This, in turn, leads to additional unnecessary interventional procedures.

The limitations of our study were that we did not examine the correlation of spectroscopic value with the 17-OHP in patients with low birth weight, and that there were no diagnosis of CAH among our patients. Therefore, the lack of a control group is a limitation of the study and this is the reason we could not provide a cut-off value for spectroscopic density in this preliminary study.

In this pilot study we observed that the 17-OHP blood levels correlated with the spectroscopic values, but not with the scrotal hyperpigmentation identified by physical examination. In order for spectrometry to be used as an indirect and non-invasive method in diagnosis of CAH, further studies are required to determine the sensitivity and specificity of the test, by comparing the spectrometric cut-off value corresponding to 10 ng/ml of the 17-OHP blood level in two large patient groups, with and without CAH diagnosis.

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**Conflict of Interest:** The authors have no conflicts of interest relevant for this article.

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