Abnormal oral mucosal light reflectance: A new clinical sign of Ehlers-Danlos syndrome

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Ehlers-Danlos syndrome (EDS) is a major inherited connective tissue disorder leading to an impaired extracellular matrix structure. Although several odontostomatologic signs have been reported, their diagnostic accuracy remains to be ascertained. We tested the hypothesis that EDS is associated with an abnormal reflectance of the oral mucosa. Twelve patients with EDS-II or EDS-III and 12 age- and gender-matched controls were examined. Reflectance of the lower gingival and vestibular oral mucosa in the optical spectrum was measured using an imaging spectrophotometer. EDS patients showed significantly higher reflectance values in the 400-590 nm wavelengths ($P \leq .0002$) and significantly lower reflectance values in the red wavelengths (610-700 nm range, $P \leq .025$) than did controls. A reflectance cutoff value of $>10.51\%$ at the 400 nm wavelength identified the EDS patients with 100% sensitivity and 100% specificity. These findings indicate that an abnormal oral mucosal reflectance is a previously unrecognized clinical marker of EDS.


Ehlers-Danlos syndrome (EDS) is a heterogeneous group of inheritable connective tissue disorders with a reported prevalence of 1 in 5000 individuals. The diagnosis of EDS is made clinically, and several diagnostic signs have been previously reported. However, the absence of the oral frenula is the only congenital clinical marker of EDS known to date. Color is an essential parameter in clinical medicine, and the radiation reflected by an object is known to be dependent on its physical state and chemical composition. We hypothesized that an impaired structure of the extracellular matrix (ECM) in EDS may affect its optical properties. In particular, in the present study we tested the hypothesis that the EDS-related ECM changes are associated with an abnormal reflectance of the oral mucosa.

METHODS

Subjects

Twelve consecutive patients (5 male, 7 female, mean age 29.7 years, range 15-45 years) belonging to 7 unrelated families and fulfilling the clinical criteria for classic EDS (Online Mendelian Inheritance in Man, OMIM #130010, autosomal dominant inheritance; n = 4) or hypermobility EDS (OMIM #130020, autosomal dominant inheritance; n = 8) were recruited for the study. All patients had velvety skin with severe hyperelasticity, which was tested at a neutral site, and joint hypermobility, which was determined by a score of 5/9 or higher by use of Beighton’s 9-point scale (mean ± SD, 7.4 ± 1.2), based on the following maneuvers: (1) passive apposition of thumbs to the flexor aspect of the forearms; (2) passive hyperextension
of the fingers, parallel to the extensor aspect of the forearms; (3) active hyperextension of the elbows >10° beyond 180°; (4) active hyperextension of the knees >10° beyond 180°; (5) ability to place palms on the floor with knees extended. Joint hypermobility is defined as the presence of at least 3 positive criteria in children15,17,18 and a mobility score >4 in adults.18,19 All patients with classical EDS had atrophic scars, and 2 out of 4 had subcutaneous nodules and pseudotumors, whereas none of the hypermobility patients had any of these characteristics. We randomly selected 12 unrelated, gender- and age-matched controls (5 male, 7 female, mean age 28.5 years, range 15-45), who were free of known congenital malformations and chromosomal abnormalities and had no history of either inheritable connective tissue disorders or infantile hypertrophic pyloric stenosis. None of the controls had any features of EDS, and their hypermobility score was (mean ± SD) 2.0 ± 1.0. None of the participants had any known gingival pathology. Mean blood concentrations of major chromophores, such as hemoglobin and bilirubin, were comparable between the groups (P ≥ .95, data not shown). The study was approved by the institutional review board, and informed consent for examination and photographs was obtained.

Photography

The lower gingival and vestibular oral mucosa was chosen as the study area, owing to its easy accessibility. The attached gingiva, as well as the other periodontal tissue areas were excluded from the study. To avoid direct mucosal contact, photographs of the selected areas were acquired using a Yashica Dental Eye photocamera with an automated on-axis flashbulb, emitting standard photographic white light, and a 55-mm f 1.4 Yashica lens (Yashica-Kyocera Co, Kyoto, Japan). Kodak Elite Chrome 100 ISO/21 DIN films (Eastman Kodak Co, Rochester, NY) were used and developed according to the standard E-6 procedure. Incident illumination was about 10°, with a viewing pass nearly normal to the observed surface, and 8× magnified printed images were converted to digital spectral data by using a standard 45°-0° viewing geometry.

Reflectance analysis measurements

Oral mucosal reflectance was measured using an imaging spectrophotometer,20 after calibration against standard white before each measurement series (maximal deviation from calibration standard ±0.4%). Spatially averaged spectra were used in order to estimate the oral mucosal color in the 400-700 nm wavelength electromagnetic spectral range (violet region 400-450 nm, blue 450-475 nm, blue-green 475-500 nm, green 500-570 nm, yellow 570-590 nm, orange 590-610 nm, red 610-700 nm). Reflectance values from 21 different artifact-free and vessel-free areas were measured, and mean values were used for data analysis. Derived spectral data were reproducible (intra- and interobserver coefficients of variation (mean ± SD) 1.46% ± 0.89% and 3.72% ± 1.75%, respectively), and qualitatively comparable (±2% shift) to those obtained by direct measurements using a CM-2600d/2500d Minolta reflectance spectrophotometer (Minolta Co, Osaka, Japan), with an absolute spectral error (ΔE) of ~0.4 (i.e., average difference measure gauged over the visible spectral range). The operators performing the reflectance measurements were unaware of the subjects’ clinical findings (EDS vs. controls).

Data analysis

Differences among group means were analyzed by the paired t-test. The t-values from different regions of the optical spectrum between EDS patients and controls were analyzed by one-way ANOVA, and post-hoc pairwise differences were tested using Student-Newman-Keuls. Model discrimination was tested using receiver-operating characteristic (ROC) curve analysis. A 2-sided P < .05 was considered to be statistically significant, and the Bonferroni corrected significance levels were used. The MedCalc ver. 7.0 statistical software (MedCalc Software, Mariakerke, Belgium) was used.

RESULTS

The oral mucosa of the EDS patients showed significantly higher reflectance values in the 400-590 nm wavelengths of the optical spectrum (t-value range 4.19-13.76, df = 22, P ≤ .0002) and significantly lower reflectance values in the red wavelengths (wavelength range 610-700 nm, t-value range 2.40-5.72, df = 22, P ≤ .025) than that of controls (Fig 1). Maximum distance between group means was observed at 400 nm wavelength (t-value: 13.76, df = 22). Intergroup differences showed a high degree of certainty, with mean absolute values 18.48 ± 5.04 times larger than the corresponding experimental errors. The degree of significance for differences between groups was strongly dependent on the spectrum region, being significantly larger for the violet region (F6.24 = 18.93, P < .0001). From the ROC curve analysis results, a reflectance cutoff value of >10.51% at the 400 nm wavelength was found to identify EDS patients with 100% sensitivity and 100% specificity.

DISCUSSION

Several odontostomatologic manifestations of EDS have been previously reported, including recurrent temporo-mandibular joint subluxation,21 oral mucosa fragility with delay in oral healing, roots deformity, enamel
hypoplasia with irregularities of the amelodentinal junctions, and multiple odontogenic keratocysts. However, their diagnostic accuracy remains to be determined. The findings of the present study indicate the presence of a previously unrecognized mucosal reflectance abnormality of the oral mucosa of EDS II/III patients, leading to the largest difference in the violet wavelengths of the optical spectrum.

Three types of cones, each sensitive to different bands of the visible spectrum ("blue," maximum sensitivity 450 nm; "green," maximum sensitivity 530 nm; and "red," maximum sensitivity 560 nm), are present in humans and *Macaca fascicularis*. However, they are not evenly distributed, with a majority of red cones (64%) but very few blue cones (4%), thus leading to a relative insensitivity to shorter wavelengths (cyan to deep-blue) and relatively high sensitivity to the yellow wavelengths. In contrast, the findings of the present study indicate a maximum difference between EDS and controls for the violet region of the visible spectrum, with a maximum distance at 400 nm wavelength. Given the difficulty in assessing such a difference on a subjective basis, a top-to-bottom approach, that is, from theory to observation, was crucial in generating our hypothesis. Therefore, we reasoned that a physicochemical change in the ECM should have been associated with a change in the overall color of the mucosa, as detectable by light reflectance.

The spectrophotometric methodology used in this study is unusual in that a potential color distortion may derive from the use of photographic films prior to the spectral data acquisition. On the other hand, the main objective of the study was to detect possible color differences between EDS patients and controls. The main reasons for deciding to perform an indirect reflectance methodology included the following points: (1) to avoid artifacts potentially leading to significantly incorrect spectral estimates (e.g., blood vessels overlap, glaring interference from saliva, etc.); (2) a high technical reproducibility; (3) to minimize the lack of color homogeneity of the observed surface potentially deriving from the color nuance of the underlying venous plexus; and (4) to avoid any direct mucosal contacts, to minimize measurement-associated risks of infection. The methodology we used in this study fulfills all these criteria. Moreover, a comparison between spectral measurements performed with a standard spectrophotometer directly on the oral mucosa and the experimental results obtained by photography-mediated spectrometry indicated the ability of standard photographs to record even the smaller color differences in the imaged scene.

Nevertheless, the described color reflectance application is still to be considered a first step toward a more comprehensive quantitative color evaluation. An ideal technology would require the use of hyperspectral images, associated with the unavoidable side effects of
using complex procedures and still experimental equipment, with a lengthening of reading times, leading to increased measurement instability.

Abnormal matrix assembly abnormalities in various EDS types are well established, including both abnormal collagen fibrils diameter and elastic fibrils fragmentation. More recently, tenascin-X deficiency has been reported to mimic EDS in mice through alteration of collagen deposition, leading to a clinically distinct, recessive form of EDS in humans. In addition, gene disruption of several other matrix molecules (i.e., thrombospondin, SPARC, small leucine-rich proteoglycans) in experimental models led to phenotypes mimicking EDS. These observations support the hypothesis that alterations in the connective tissue matrix may ultimately modify the overall organization and the mechanical properties of the tissue, as well as its average optical properties. Either an increased light scattering or tissue transparency may account for the higher reflectance values observed at shorter wavelengths in the EDS population. Our findings, while stressing the importance of oral mucosal changes in the diagnosis of ECM-related disorders, offer to the odontostomatologist an additional and accurate phenotypical marker of EDS. Given its general applicability, our methodology may not be limited to EDS, but it may generate a novel non-invasive diagnostic tool, as well as a new diagnostic concept in ECM-related diseases.

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REFERENCES


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