The Cornea in Classic Type Ehlers-Danlos Syndrome: Macro- and Microstructural Changes

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Purpose. To analyze in vivo corneal morphology and ultrastructural features in patients with classic Ehlers-Danlos syndrome (EDS).

Methods. Fifty patients with classic EDS and 50 age- and sex-matched control subjects were studied. A clinical evaluation was made with the Ocular Surface Disease Index (OSDI) questionnaire and a complete ophthalmic examination, including assessment of the best-corrected visual acuity and refraction, slit-lamp biomicroscopy, tear break-up time, intraocular pressure, Schirmer test without topical anesthesia, and corneal diameter. Scheimpflug camera topography and in vivo confocal microscopy (IVCM) were used to investigate corneal morphology and corneal ultrastructural features respectively.

Results. Classic EDS patients, compared to controls, had thinner and steeper corneas (P < 0.001 and P < 0.05, respectively; independent samples t-test). IVCM showed thinner stromas, lower keratocyte densities (P < 0.001), increased applanation-related stromal folds (P < 0.001; Mann-Whitney U test), and increased endothelial hyperreflective dots (P < 0.05) in these patients. The study group also had increased symptoms (OSDI score: P < 0.01, independent samples t-test) and signs (tear break-up time and Schirmer test: P < 0.001 and P < 0.05, respectively) of tear film dysfunction.

Conclusions. Patients with classic EDS had macro- and microstructural changes of the cornea, which is a target tissue of the disease. These findings should be considered to optimize clinical management of these patients and to evaluate the opportunity of adding ocular findings to the classic EDS diagnostic criteria.

Keywords: Ehlers-Danlos syndrome, connective tissue, collagen V, cornea, confocal microscopy

The Ehlers-Danlos syndromes (EDSs) comprise a wide and heterogeneous group of monogenic conditions with multisystemic and variable clinical manifestations primarily affecting the skin, ligaments and joints, blood vessels, and internal organs. The current Villefranche classification recognizes six subtypes, most of which are linked to mutations in genes encoding fibrillar collagens or enzymes involved in posttranslational modification of these proteins.

The literature reports several potential ocular complications of different types of EDS, including eyelid and conjunctival abnormalities, keratoglobus, corneal thinning and keratoconus, dry eye, pathologic myopia, angioid streaks and abnormal retinal vessels, retinal detachment, scleral atrophy, and globe perforation. Classic EDS is a heritable connective tissue disorder characterized mainly by skin hyperextensibility, abnormal wound healing, and joint hypermobility. It causes an important reduction of quality of life, and there is no etiologic therapy. The syndrome is caused in most cases by mutations of either COL5A1 or COL5A2, the genes that code for collagen chain $\alpha(V)$ and $\alpha(V)$, respectively. The current working hypothesis states that these mutations result in COL5A1 haploinsufficiency and lead to the production of approximately half the normal amount of type V collagen. A minority of mutations consist of splice-site or missense mutations in either COL5A1 or COL5A2 that lead to the production of an abnormal polypeptide chain. The polypeptide chain is incorporated into the collagen molecule and results in the production of structurally abnormal type V collagen.

Physiologically, collagen V forms heterotypic fibrils with collagen I, adjusting the diameter in an inhibitory way due to the retention of the N-terminal domain. In mice heterozygous for COL5A1, half of the fibers are normal, while the remaining ones are composed only of collagen 1 molecules and are assembled in disorderly fibrillar clusters of irregular shape and larger diameter than the normal counterpart.

Collagen V is present in small amounts (2%-5%) in the most type I collagen-containing connective tissues, but it accounts for 10% to 20% of the total collagen in cornea. The purpose of this study was to investigate corneal changes in a large
group of classic EDS patients, compared with healthy control subjects.

METHODS

Subjects

Fifty patients (32 women and 18 men; average age 36.8 ± 12.1 years, range, 16–66 years) with classic EDS were recruited consecutively at the Ehlers-Danlos Syndrome Regional Referral Center (UO Medicina del Lavoro, Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milan, Italy), where the diagnosis of classic EDS was made according to the criteria proposed by the Ehlers-Danlos Foundation (USA) and Ehlers-Danlos Support Group (UK). The control group included 50 age- and sex-matched healthy people (33 women and 17 men; age 33.9 ± 12.9 years, range, 19–62 years) attending our general clinic.

For both classic EDS patients and control subjects, exclusion criteria were topical or systemic therapy with known corneal toxicity, ocular or systemic diseases that can modify corneal homeostasis, use of contact lenses, history of ocular trauma, or previous ophthalmic surgery. All examinations were performed by the same expert operator, and all variables were examined by a single masked investigator.

The study followed the tenets of the Declaration of Helsinki, and all the participants provided written informed consent.

Clinical Evaluation

A careful case history was compiled for each participant in the study, and all the subjects completed a questionnaire for a standardized evaluation of ocular surface symptoms (Ocular Surface Disease Index [OSDI]). This is a 12-item questionnaire designed to provide a rapid assessment of ocular irritation symptoms and their impact on vision-related functioning. The OSDI score provides a valid and reliable measure of dry eye symptom severity. The participants underwent a complete ophthalmic examination including evaluation of refraction, assessment of the best corrected visual acuity (BCVA), biomicroscopic examination, measurement of the tear film break-up time (BUT), fluorescein corneal staining evaluation, intraocular pressure (IOP) measurement with Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland), and Schirmer test without topical anesthesia. The corneal diameter was obtained through an autorefractor (Canon RK-F1 Full Auto Refractor Keratometer; Canon, Tokyo, Japan).

Computerized Tomography With Scheimpflug Imaging

Image Acquisition. Examination by computerized tomography with a Scheimpflug camera (Oculus Pentacam HR; Optikgeräte GmbH, Wetzlar, Germany) was performed first to avoid image distortions caused by sodium fluorescein instillation and transitory modification of corneal curvature due to contact by confocal microscopy (see next section).

Image Analysis. We analyzed the Pentacam printout, collecting the following parameters: corneal apex pachymetry, peripheral pachymetry (mean value of 26 measurements performed at 2, 4, and 6 mm from the corneal apex), anterior chamber depth, angle width, anterior and posterior average keratometric value (Km), astigmatism, the index of surface variance (ISV), keratoconus index (KI), and the minimum radius of curvature (Rmin).

Confocal Microscopy

Image Acquisition. All subjects underwent in vivo confocal microscopy (IVCM) examination by the HRT II with the Corneal Rostock Module (Heidelberg Engineering, Dossenheim, Germany) using a scanning wavelength of 670 nm. The
Clinical Data of Classic EDS and Healthy Control Subjects

Table 1. Main Characteristics of Classic EDS Patients

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>No. of Patients</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Joint hypermobility</td>
<td>48</td>
<td>96</td>
</tr>
<tr>
<td>Skin hyperextensibility</td>
<td>47</td>
<td>94</td>
</tr>
<tr>
<td>Widened atrophic scars</td>
<td>47</td>
<td>94</td>
</tr>
<tr>
<td>Smooth, velvety skin</td>
<td>43</td>
<td>86</td>
</tr>
<tr>
<td>Complications of joint hypermobility</td>
<td>40</td>
<td>80</td>
</tr>
<tr>
<td>Easy bruising</td>
<td>38</td>
<td>76</td>
</tr>
<tr>
<td>Surgical complications</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Positive family history</td>
<td>25</td>
<td>50</td>
</tr>
<tr>
<td>Manifestations of tissue extensibility and fragility</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td>Muscle hypotonia, delayed gross motor development</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Molluscoid pseudotumors</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Subcutaneous spheroids</td>
<td>0</td>
<td>0</td>
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</tbody>
</table>

Table 2. Clinical Data of Classic EDS and Healthy Control Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Classic EDS</th>
<th>Control Group</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snellen BCVA</td>
<td>0.98 ± 0.93</td>
<td>1.00 ± 0.00</td>
<td>ns</td>
</tr>
<tr>
<td>Spherical equivalent, D</td>
<td>−0.71 ± 2.46</td>
<td>−0.82 ± 1.66</td>
<td>ns</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>12.79 ± 1.18</td>
<td>13.08 ± 1.54</td>
<td>ns</td>
</tr>
<tr>
<td>Corrected IOP, mm Hg†</td>
<td>15.75 ± 1.22</td>
<td>13.01 ± 1.51</td>
<td>ns</td>
</tr>
<tr>
<td>Corneal diameter, mm</td>
<td>11.97 ± 0.44</td>
<td>12.11 ± 0.20</td>
<td>ns</td>
</tr>
<tr>
<td>Ocular score</td>
<td>35.53 ± 25.37</td>
<td>9.95 ± 11.44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Tear BUT, s</td>
<td>6.13 ± 2.29</td>
<td>10.22 ± 2.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Schirmer test, mm/5 min</td>
<td>15.60 ± 11.79</td>
<td>20.90 ± 9.82</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

D, diopter; ns, not significant.
* t-test for independent samples.
† Based on pachymetry.21

Image Analysis. The basal corneal epithelium, subbasal plexus, anterior and posterior stroma, and endothelium images were studied. Cell densities in the different layers were measured. In all cases cell density was determined through the manual cell counting procedure present in the software, taking into consideration the whole area marked as available for the cell count. The cells partially contained in the area analyzed were counted only along the right and lower margins. Results were expressed in cells per square millimeter.

In the subbasal plexus, three parameters were taken into consideration: the number of nerves, the tortuosity, and the total length of nerves in each photogram. Total length of nerves was calculated using ImageJ software (National Institutes of Health, Bethesda, MD),21 and the tortuosity was evaluated according to grading performed by comparison with the reference images.22

The stromal thickness was obtained using the first stromal image clearly recognizable after the subbasal plexus and the last stromal image clearly recognizable before the endothelium. Features of the stromal folds were studied in the most anterior and most posterior 50 μm of the stroma. One image for every 10 μm in both cases was scored according to a grading reference scale of 0 to 3 (Fig. 1).

Stromal dots were assessed in the three most anterior and most posterior clearly recognizable stromal images, and the densities were scored according to a grading reference scale of 0 to 3 (Fig. 2). Endothelial dots were evaluated by a similar procedure (Fig. 3).

Statistical Analysis

Data derived from right eyes were used for statistical analysis, unless that eye exceeded the exclusion criteria. In those cases, the left eye was used. All data were expressed as the average ± standard deviation. For parametric measures, significant differences between the EDS and control groups were determined by the t-test for independent samples. For nonparametric measures, such as nerve tortuosity, stromal folds, and stromal and endothelial dot scores, significant differences were tested by the Mann-Whitney U test. Statistical significance was accepted at P < 0.05. Statistical analysis was performed with commercial software (SPSS for Windows v.19.0; SPSS Sciences, Chicago, IL).

RESULTS

Table 1 reports general characteristics of the enrolled patients with classical EDS.
The Cornea in Classic Ehlers-Danlos Syndrome

TABLE 3. Topographic Data of Classic EDS and Healthy Control Subjects

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Classic EDS</th>
<th>Control Group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apex pachymetry, μm</td>
<td>503.43 ± 64.87</td>
<td>544.34 ± 25.50</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peripheral pachymetry, μm</td>
<td>574.90 ± 96.98</td>
<td>594.90 ± 27.58</td>
<td>ns</td>
</tr>
<tr>
<td>Anterior chamber depth, mm</td>
<td>2.93 ± 0.46</td>
<td>2.99 ± 0.33</td>
<td>ns</td>
</tr>
<tr>
<td>Cameralar angle, grades</td>
<td>36.32 ± 6.89</td>
<td>34.17 ± 8.72</td>
<td>ns</td>
</tr>
<tr>
<td>Anterior Km, D</td>
<td>43.70 ± 1.28</td>
<td>43.10 ± 1.20</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Posterior Km, D</td>
<td>−6.27 ± 0.25</td>
<td>−6.18 ± 0.19</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Astigmatism, D</td>
<td>1.08 ± 0.86</td>
<td>0.93 ± 0.67</td>
<td>ns</td>
</tr>
<tr>
<td>ISV</td>
<td>19.86 ± 12.15</td>
<td>15.04 ± 6.17</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>KI</td>
<td>1.03 ± 0.033</td>
<td>0.99 ± 0.14</td>
<td>ns</td>
</tr>
<tr>
<td>Rmin</td>
<td>7.51 ± 0.22</td>
<td>7.61 ± 0.26</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* Mann-Whitney U test for independent samples.

Clinical Data

Clinical data for Snellen BVCA, spherical equivalent, IOP, corneal diameter, OSDI score, BUT, and Schirmer test for the classic EDS and control groups are reported in Table 2.

Compared with control subjects, EDS patients showed the following statistically significant differences: higher OSDI score (P < 0.01, t-test), decreased BUT (P < 0.001), and lower Shirmer test (P < 0.05). There were no significant differences between classic EDS and control groups for epithelial basal cell density, dendritic cell density, activated keratocyte density, stromal dots, or endothelial cell density.

Topographic Data

Table 3 reports the data relative to pachymetry and topography in the classic EDS and control groups.

Compared with control subjects, EDS patients showed the following statistically significant differences: thiner apex pachymetry (P < 0.001, t-test), higher anterior Km and ISV (P < 0.001), lower posterior Km (P < 0.05), and decreased Rmin (P < 0.05). There were no significant differences between the classic EDS and control groups in peripheral pachymetry, anterior chamber depth, cameralar angle, astigmatism, or KI.

Confocal Microscopy Data

The data relative to confocal microscopy are reported in Table 4.

Compared with the control group, confocal data of the EDS patients (Mann-Whitney U test) showed the presence of thinner stromal pachymetry (P < 0.001), higher number of nerve fibers (P < 0.01), greater fiber lengths and tortuosity (P < 0.05), lower anterior and posterior keratocyte density (P < 0.001), higher anterior and posterior stromal folds score (P < 0.001), and a higher endothelial dot score (P < 0.05). There were no significant differences between the classic EDS and control groups for epithelial basal cell density, dendritic cell density, activated keratocyte density, stromal dots, or endothelial cell density.

DISCUSSION

The clinical description of classic EDS in the revised Villefranche classification does not mention any ocular involve-ment.17 However, Col5a1 haploinsufficient mouse models of classic EDS showed several ultrastructural corneal changes that caused decreased corneal thickness.2,24 In these murine models, the pathologic stroma was composed of larger collagen fibrils with reduced density and interfibrillar space.

At present, little is known about corneal macro- and microstructural changes in human patients with classic EDS. To the best of our knowledge, the only previously described corneal findings in classic EDS are thinning and steepness found in 16 eyes of eight subjects from three unrelated families.2 In our study, we examined a large number of consecutive patients with a wide age range. Comparing classic EDS patients to controls, our data confirmed thinner and steeper corneas in the study group (Figs. 4A–D), which also had increased irregularity of the corneal surface. The changes in corneal curvature, however, did not cause increased refractive...
Corneal morphology and ultrastructure in classic EDS compared to healthy subject. The EDS cornea (A, C), compared to a normal cornea (B, D), was thinner (A, B) and steeper (C, D). In EDS (E, G), compared to a normal cornea (F, H), IVCM showed decreased keratocyte density (E, F), increased stromal folds (E, F), and increased endothelial dots (G, H).
errors (including astigmatism) nor increased prevalence of keratoconus.

We used IVC M to investigate microstructural changes in the cornea of EDS patients. This technology enables a quick, minimally invasive analysis of the in vivo ocular surface at the cellular level. Thus, this approach can provide fresh insight into corneal microstructure, inflammatory processes, and glenular changes. Interestingly, IVC M was applied by Sun et al. on corneas of Col5a1-null mouse models and showed decreased stromal thickness along with conserved epithelial and endothelial thicknesses and cell density. In our patients, we found comparable findings and several additional changes in each layer from the anterior to the posterior surfaces of the cornea.

Stromal pachymetry in the EDS group was reduced by approximately 57 µm, and the entire corneal thickness was reduced by approximately 41 µm, suggesting the importance of EDS-induced changes in the stroma. Stromal IVC M examination in the study group also showed decreased density of anterior and posterior keratocytes and increased folds (Figs. 4E, 4F). The small number of keratocytes indicates a perturbation of their survival, as suggested by Chanut-Delalande et al. for dermal fibroblasts of mutant mice. In fact, the morphological signs of apoptosis in mutant fibroblasts and the reduced number in culture could be the result of extracellular matrix alterations. These changes can cause a loss of cell–matrix interactions and may affect the storage and activation of growth factors.

Stromal folds appear as thin, interconnected lines in the extracellular matrix that were bright in the anterior and dark in the posterior stroma. These findings are frequent in images captured by laser IVC M because they are related to the corneal application of the protective cap covering the front lens. In our study, all of the confocal exams, both in EDS and control subjects, were performed by the same trained investigator, attempting in each case to minimize the microscope lens pressure on the cornea. The significantly greater number of folds and thickness in the EDS patients suggested an increased corneal laxity due to the abnormal collagen fibrils organization.

EDS corneal thinning and lower rigidity could impact IOP evaluation with Goldmann applanation tonometer (Haag-Streit). However, even after making corrections based on pachymetry, we found no IOP differences between patients and controls. We were not able to correct IOP values on the basis of corneal laxity, so there is a potential risk that pressure was underestimated. Further studies should analyze the hypothesis of an increased risk of IOP underestimation in these patients.

EDS corneas, compared to controls, also showed an increased amount of endothelial dots (Figs. 4G, 4H). These particles have already been described in Marfan syndrome, but it was hard to determine their nature. The correlation of these dots with age led us to interpret them as age-related signs of endothelial response to the poor stromal support.

Our classic EDS patients, despite having no complaints of any ocular surface symptoms, showed abnormal OSDI and BUT scores and reduced tear secretion, compared to control subjects. The high prevalence of dry eye in patients with EDS has so far been reported only in patients with hypermobility type EDS and is not easy to interpret. Gharbiya et al. proposed a role for primary alteration of the extracellular matrix of the lacrimal glands. However, this is not supported by our data that show an impairment of the stability of the tear film that was not explained by Schirmer test values. It seems more reasonable to hypothesize that the increased OSDI and decreased BUT scores are related to the ocular surface changes associated with the effects of altered fibroblasts and collagen matrix on the proliferation and differentiation of ocular surface epithelial cells. It is also likely that the nervous system has an important role as suggested by the possible autonomic dysfunction and by the increased tortuosity of subbasal fibers found in our patients.

In conclusion, patients with classic EDS show numerous morphologic and ultrastructural corneal changes that should be considered in optimizing their clinical management. Currently, classic EDS diagnostic criteria do not include ocular manifestations. However, the cornea, which is rich in collagen V, seems to be a target tissue of the disease. Our data suggest the necessity of further studies to assess the possible role of corneal changes in the diagnostic criteria for classic EDS.

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