Contact Lens and Anterior Eye xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Contact Lens and Anterior Eye

journal homepage: www.elsevier.com/locate/clae

Conjunctival impression cytology evaluation of patients with dry eye disease using scleral contact lenses

Sarah La Porta Weber*, Rossen Mihaylov Hazarbassanov, Alex Nasaré, José Álvaro Pereira Gomes, Ana Luisa Hofling-Lima

Department of Ophthalmology, Ocular Surface Advanced Center, Federal University of Sao Paulo, 821 Botucatu St—Vila Clementino, Sao Paulo, SP, 04023-062, Brazil

ARTICLE INFO

Article history:
Received 27 June 2016
Received in revised form 1 December 2016
Accepted 21 December 2016

Keywords:
Dry eye disease
Scleral contact lens
Impression cytology
HLA-DR
Ocular surface inflammation

ABSTRACT

Purpose: To evaluate conjunctival impression cytology and HLADR expression changes after wearing scleral contact lenses (ScCLs) for moderate to severe dry eye disease (DED).
Design: Prospective interventional case series.
Methods: Forty-one eyes from 25 patients with moderate to severe DED were evaluated for Esclera ScCL treatment. Best-corrected visual acuity (BCVA) and slit-lamp findings were assessed. Impression cytology specimens were obtained from DED patients at the baseline and after wearing ScCLs for 12 months. The impression cytology specimens were analyzed using morphological results score, and HLA-DR positive cells were detected and quantified. The values were compared to assess the IC changes after wearing ScCLs.
Results: Forty-one eyes from 25 patients were fitted with ScCLs to manage DED. The underlying diseases were Stevens-Johnson syndrome (22 eyes), Sjogren's syndrome (11 eyes), graft-versus-host disease (2 eyes), dry eye after keratomileusis (2 eyes) and undifferentiated ocular surface disease (4 eyes). The HE-PAS impression cytology score did not differ significantly before and after wearing ScCLs for 12 months in DED patients ($p > 0.05$). The percentage of eyes expressing the HLA-DR antigen in the temporal conjunctiva after wearing ScCL for 12 months significantly increased in patients with Sjogren's syndrome (11.11% to 66.66%; $p = 0.0498$). In groups with Stevens Johnson syndrome and other ocular surface disorders, we did not observe statistically significant differences ($p > 0.05$).
Conclusions: The ScCLs did not change the parameters used to evaluate inflammatory processes, which were measured using conjunctival impression cytology and HLA-DR expression, except in Sjogren syndrome, in which there was an unexpected increase in HLA expression.

1. Introduction

Dry eye disease (DED) is a common tear film disorder that is caused by a tear deficiency or excessive tear evaporation, which can lead to severe disease in the cornea and conjunctiva. Affected individuals may experience many symptoms, such as foreign-body sensation, ocular fatigue and eye redness [1,2].

Artificial tear drops, tear retention treatment, stimulation of tear secretion, or anti-inflammatory drugs may be used to treat dry eyes based on the disease severity. Scleral contact lenses (ScCLs) have been increasingly used to treat moderate to severe DED [3–9].

The ScCLs are large-diameter devices that are completely supported by the sclera, vault the cornea and limbus, and maintain a fluid reservoir in the space between the lens and cornea. The unique ScCL fit characteristics protect the ocular surface from shear forces generated by eyelid movement over the cornea and continuously hydrate the ocular surface [4,5].

Conjunctival impression cytology (IC) was introduced into the ophthalmic practice in 1977 by Egbert et al. [10] and is a commonly used technique to collect cells from the ocular surface and evaluate cytological changes caused by DED. Cells obtained using IC can be graded or subjected to morphological and morphometric analyses [11–17]. Several studies with clinical and experimental evidence show that ocular surface inflammation is a key component of DED [3,18,19].

* Correspondence to: 1385 Avenida das Ubaranas, bl 14, apto 303, Aquiraz, CE, 61170-000, Brazil.
E-mail address: sarahlpweber@gmail.com (S.L.P. Weber).

http://dx.doi.org/10.1016/j.clae.2016.12.008
1367-0484/

Further, HLA-DR is a proinflammatory marker that is specifically overexpressed in patients with advanced forms of DED [20,21] and is present in the dry eyes of Sjogren syndrome patients [22].

The purpose of this study was to evaluate conjunctival impression cytology and HLA-DR expression changes after patients wear ScCLs for moderate to severe dry eye disease.

2. Methods

This is a prospective, interventional case series approved by the Ethics Committee of Federal University of Sao Paulo (Sao Paulo, Brazil). Informed consent was obtained from participants after the nature and possible consequences of the study were explained; the research was performed in accordance with the tenets of the Declaration of Helsinki.

In this study, we evaluated 41 eyes from 25 patients who were fitted with Esclera ScCLs to treat moderate to severe DED. When a patient exhibited indications for ScCLs in both eyes, we analyzed the values for each eye as independent variables because each eye presents independent anatomical and physiological features. These patients were referred to the Contact Lens Department of Federal University of Sao Paulo (Sao Paulo, SP, Brazil) from February 2013 to March 2015.

This study included patients with grades II, III and IV DED based on the DEWS report, which is also known as moderate to severe DED. Dry eye graduation as well as all follow-up assessments were performed by SLPW author. DED presents occasionally annoying or constant visual symptoms, changes in conjunctival staining and injection, changes in corneal staining and tear signs, changes in the meibomian glands, a tear film breakup time (TBUT) $\leq$10 s and a Schirmer score $\leq$10 [2]. The evaluated patients exhibited symptoms that could not be controlled using conventional treatments.

Patients with the following conditions were excluded from the study: glaucoma, disorders that affect sensitivity (e.g., herpetic disease and diabetes mellitus), corneal decompensation, active ocular infection, anatomical variations of the eyelid and conjunctiva that impair proper ScCL fitting, pregnancy and an inability to correctly handle and care for the ScCLs.

The Esclera (Mediphacos, Belo Horizonte, MG, Brazil) device uses a non-fenestrated scleral design with a diameter ranging from 16 to 18.2 mm, available sagittal vaults from 4.12 to 6.27 mm, DK/T: 141 (ISO/Fatt), and available powers from −20 to +20 diopter.

The ideal ScCL Esclera fit presented a size at least 2 mm greater than each side of the limbus and a 100 μm minimum apical clearance. The ScCL should not touch the cornea, and the edges of the ScCL should not exhibit vascular impingement, conjunctival blanching or scleral indentation. Patients with ideal fits were allowed to wear the lenses for one hour; next, apical clearance was reassessed, and overefraction was performed. All lens adjustments were made by the author SLPW following these criteria.

Follow-up visits occurred at 0, 1, 3, 6 and 12 months. The IC specimens were collected by the same researcher (SLPW) before the fitting and 12 months after the ScCL treatment. The follow-up period was 12 months for all patients. All ScCLs were following the same fitting criteria mentioned by SLPW author.

The clinical examinations included best-corrected visual acuity (BCVA) using *Early Treatment of Diabetic Retinopathy Study* (ETDRS) charts (CC-100, Topcon Corp, Tokyo, Japan), and this value was recorded as the Snellen equivalent. The corresponding logMAR was then derived from the Snellen equivalent.

A slit-lamp examination at ×10–×16 magnifications was used to detect active inflammation or structural changes, which were evidenced by scarring in the eyelid and conjunctiva, neovascularization, opacity or thinning of the cornea.

All patients were instructed to take out the ScCLs one day before the dry eye tests were performed.

2.1. Specimen collection

Impression cytology specimens were obtained for each patient using the technique previously described by Nelson [12].

Conjunctival impression cytology samples (ICSs) were obtained from the upper lid-covered and temporal regions of the bulbar conjunctiva. The same area covered and supported by the ScCLs. After the ocular surface was anesthetized with 0.5% proxymetacaine hydrochloride (Allergan, Sao Paulo, SP, Brazil), a pure nitrocellulose filter membrane disc (Millipore, Barueri, SP, Brazil) was halved and applied to the bulbar conjunctiva approximately 1 mm from the limbus using forceps. The specimen was gently pressed for 15 s and then peeled from the conjunctival surface [17]. Further, ICSs were obtained from each eye from the superior and temporal bulbar quadrants.

Using the technique described by Krenzer [17], the ICSs were fixed with using Spray-cyte™ cytological fixative (Clay-Adams™ Brand, Becton, Dickinson and Company, USA), dried and transported to the laboratory. Each ICS was halved, and each half was placed specimen-side down on a separate poly-L-lysine-coated glass slide [23]. Thereafter, each slide was placed in acetone for 1 h with continuous agitation to dissolve the bulk of the filter membrane. After a 5-min wash in tap water, the ICSs were subjected to digestion with cellulase (Sigma, St. Louis, MO, USA) for 2 h at 37 °C (10 U/ml in 0.1 M acetate buffer, pH 5) to remove residual membrane material. The specimens were washed in tap water for Hematoxylin-Periodic Acid Schiff (H-PAS) staining[13] or immunocytochemistry for HLA-DR.

2.2. Immunocytochemistry

Endogenous peroxidase activity was quenched with equal volumes of hydrogen peroxide and methanol. After blocking nonspecific binding sites with a 4% low-fat milk solution for 20 min [24], the specimens were treated using a standard avidin-biotin protocol. We performed an overnight incubation in a humid chamber at 4 °C with the primary antibody HLA-DR (1:100; Abcam, MA, USA) and thorough phosphate-buffered saline (PBS)-0.05% Tween-20 washes between each incubation. Next, the specimens were incubated with the LSAB kit amplification solutions (Labelled Streptavidin-Biotin, DAKO, CA, USA) for 25 min. The reactions were revealed using a diaminobenzidine chromogen solution (DAB). The specimen was counterstained in Mayer's hematoxylin (Sigma), dehydrated, and mounted with Erv-mount® (Erviegas, Sao Paulo, Brazil) [25]. For each run, ethanol-fixed, paraffin-embedded tissues with known reactivity for each antibody were included as positive controls [26], and one ICS served as a negative control (primary antibody exclusion).

The ICSs were examined using an Axioplan photomicroscope (Zeiss, Jena, Germany) and imaged using Axiovision version 4.3 imaging software (Zeiss, Jena, Germany).

2.3. Impression cytology evaluation

The H-PAS specimens were evaluated using a scoring system based on the sum of the scores for each morphological change, such as cellularity, cohesivity, nuclear/cytoplasm ratio, snake-like chromatin, goblet cell density and inflammation, as described by Murube (Table 1) [11].

The ICSs were evaluated for the presence or absence of HLA-DR staining. An ICS was considered positive if unequivocal cytoplasmic staining was detected in two or more cells and if staining

Please cite this article in press as: S.L.P. Weber, et al., Conjunctival impression cytology evaluation of patients with dry eye disease using scleral contact lenses, Contact Lens & Anterior Eye (2017), http://dx.doi.org/10.1016/j.clae.2016.12.008