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A pilot study on corneal Langerhans cells in keratoconus☆

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ABSTRACT

Purpose: To report the density and morphology of cells that are analogous to corneal Langerhans cells and their associations in keratoconus.

Materials and methods: This prospective cross-sectional study included a convenience sample of keratoconus subjects aged between 18-65 years. Corneal topography, assessment of ocular symptoms, tear variables, corneal sensitivity, *in-vivo* confocal microscopy were performed. The number of Langerhans cells were manually counted and averaged across three central corneal images. Cell morphology was graded on a 0-3 scale, where grade 3 indicates cells with long visible dendrites. Associations of Langerhans cells with other variables were evaluated using Spearman's correlation.

Results: Twenty-one keratoconus subjects with a mean age of 43 ± 11 years were included. Eighty-one percent of them were males, 48% had mild keratoconus and 52% were contact lens wearers. Langerhans cells were present in the central cornea in 91% of subjects. Median cell density was 15 cells/mm^2(IQR: 3-21). Cell morphology of grades 2 or 3 (with short or long dendrites) was seen in 71% of subjects. There was a significant association between Langerhans cell frequency and density with male gender (rho and p-values: -0.669, 0.001 and -0.441,0.045) and between Langerhans cell density and nerve fibre tortuosity (0.479,0.028). No significant association observed with age, contact lens wear or ocular symptoms.

Conclusion: Langerhans cells were present in a significant number of subjects suggesting the possibility of inflammation in keratoconus. Based on the association of Langerhans cells with nerve parameters, we propose inflammation as the underlying cause for corneal nerve changes in keratoconus.

1. Introduction

Being an avascular tissue, the normal cornea has been assumed to be immunologically privileged and devoid of antigen presenting cells. However dendritic cells, that are equivalent to the antigen presenting Langerhans cells in the epidermis, have been identified in the ocular surface epithelium by histochemistry, immunofluorescence, and electron microscopy [1–3]. Langerhans cells have a significant role in corneal immune homeostasis by taking part in innate immunity, immune surveillance and induction of antigen-specific immune reactivity and tolerance [4].

Zhivov et al. were the first to evaluate the density, morphology and distribution of Langerhans cells in the normal human cornea using *in vivo* confocal microscopy [5]. Recent studies have reported an increase in Langerhans cell density in the corneal epithelium in various ocular surface conditions including dry eye disease, contact lens wear, corneal graft rejection and infectious keratitis [6–14].

There is an increasing consensus on the role of inflammation in the pathophysiology of keratoconus, which is evident from the reports on inflammatory molecules in the cornea and tear-film of keratoconus subjects [15–17]. Having immune-regulatory functions in the ocular surface, Langerhans cells might have a role in the pathogenesis of keratoconus. However, there is a paucity of knowledge on their involvement in keratoconus. The purpose of this study is to report the density and morphology of cells that conform to the morphology of Langerhans cells in the central cornea and their association with clinical parameters in keratoconus.

2. Materials and methods

Keratoconus subjects aged between 18 and 65 years were recruited from a single clinic for this cross-sectional study. Those subjects with associated vernal keratoconjunctivitis and/or who had prior ocular surgery and any systemic condition that may affect corneal sensitivity

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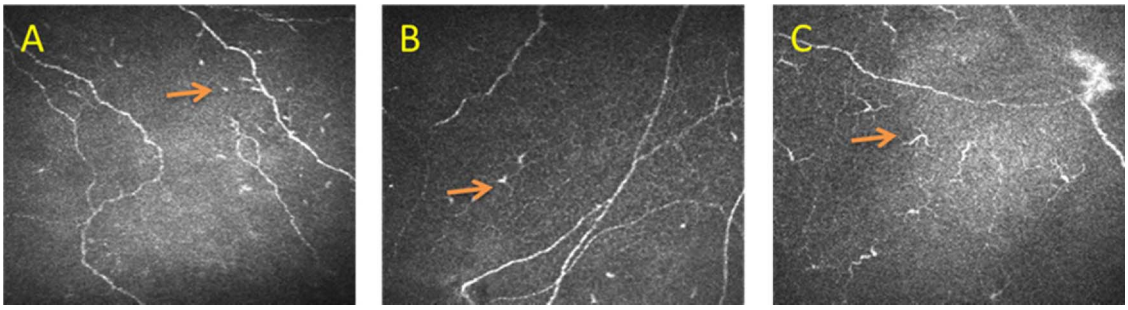

Fig. 1. Grading the morphology of Langerhans cells.
(A) *grade 1– cells without dendrites, (B) grade 2–cells with short dendrites, (C) grade 3–cells with long dendrites.*

or immunology (apart from allergy which has been associated with keratoconus) were excluded from the study. Approval to run the study was obtained from the institutional ethics committee. Written informed consent was obtained from the participants prior to recruitment and all study procedures were conducted in accordance with the Declaration of Helsinki.

The demographic data of the subjects, history of systemic and ocular allergy, ocular rubbing and contact lens wear were recorded. Subjects who could not wear contact lenses more than 4 h continuously because of ocular discomfort were considered as contact lens intolerants. The variables assessed (in the following order) included ocular symptoms using the Ocular Surface Disease Index (OSDI) questionnaire, corneal topography using the Medmont E300 corneal topographer (Medmont Pty. Ltd., Melbourne, Australia), tear variables including tear osmolarity using a TearLab Osmolarity System (TearLab Corp San Diego, CA, USA), tear volume using the phenol red thread test (Showa Yakuhin Kako Co. Ltd, Tokyo, Japan), tear meniscus height using a slit lamp biomicroscope, ocular surface staining with sodium fluorescein (Fluorets; Contacare Ophthalmics and Diagnostics, Gujarat, India) and Lissamine green dye (Akriti Oculoplasty Logistics, Hyderabad, India) in Oxford scale, central corneal sensitivity using 0.08 mm nylon filament on Cochet-Bonnet aesthesiometer (Luneau Ophthalmogie, France), and corneal sub-basal nerve parameters and evaluation of corneal dendritic cells using laser in vivo confocal microscopy. Based on the steepest keratometry reading (Steep K) from corneal topography, subjects were graded as having mild keratoconus (Steep K ≤ 52.00D) or severe keratoconus (Steep K > 52.00D). The eye with more severe keratoconus of each subject, based on the steepest keratometry reading, was included in the analysis.

For corneal sensitivity measurements, the Cochet-Bonnet aesthesiometer (Luneau Ophthalmogie, France) was mounted on a slit-lamp biomicroscope and the subjects were instructed to view a distant object while the filament slowly approached the centre of the cornea until the tip of the filament contacted the corneal surface perpendicularly and a slight bend of the filament was observed. The length of the filament was adjusted to 60 mm initially and subsequently decreased in 5 mm intervals. At each length, the procedure was repeated 4 times and the response of the subject was noted. The corneal sensitivity threshold was measured as the longest length of the filament where the subject reported 3 positive responses. The threshold was converted into pressure in gram/mm^2.

Laser scanning *in vivo* confocal microscopy of the central corneal sub-basal nerve plexus was performed using the Heidelberg Retina Tomography II (HRT) with a Rostock corneal module (RCM) (Heidelberg Engineering GmbH, Heidelberg, Germany) after anaesthetizing the cornea using 0.4% oxybuprocaine hydrochloride (Minim, Chauvin Pharmaceuticals Ltd, London, UK). During image capture, the subjects were instructed to follow a moving target on a liquid crystal display screen that was positioned at 70 cm. The HRT was set on "sequence mode" and the instrument was aligned visually to the corneal apex of the subject and 100 high-quality images of the sub-basal nerve plexus were captured while the white spot target moved on a black background. It took approximately 40 s to capture the images.

Captured images were reconstructed into montages using Photoshop Elements (Adobe Systems Inc., San Jose, CA, USA). The contrast of the montages was reversed to increase the visibility of the nerve fibres. The montages were cropped into an area of 1 square millimetre wherever possible or the maximum possible area was selected. The nerve fibres were traced using Neuron J, a plugin of ImageJ software (National Institute of Health, Bethesda, Maryland, USA) and the nerve fibre density (NFD) was calculated by measuring the total length of nerve fibres per square millimetre (mm/mm^2). The nerve fibre tortuosity was graded on a 0–4 scale as described earlier by Oliveira-Soto and Efron [18].

For Langerhans cell density, three frames with no or minimal overlap were selected for each eye and were coded by a masked observer. The number of Langerhans cells were manually counted and then decoded and averaged across the three images. The Langerhans cell density was reported per square millimetre. Langerhans cell morphology was graded on a 0–3 scale (Fig. 1), where grade 0 indicates absence of dendritic cells in the frame, grade 1 indicates cells without dendrites, grade 2 indicates Langerhans cells with short visible dendrites and grade 3 indicates Langerhans cells with long visible dendrites [19]. If more than one cell morphology was present, the predominant cells were considered for grading.

Descriptive statistics were used to describe the Langerhans cell density and morphology. Associations between Langerhans cells and other clinical variables were evaluated using Spearman's correlation and statistical significance was set at p ≤ 0.05 and moderate correlations (> 0.3) were reported as trends for this pilot study.

3. Results

This pilot study included a convenience sample of 21 keratoconus subjects with a mean age of 43 ± 11 years. Eighty six percent of subjects (18 out of 21) were Caucasians, 2 were Asians and one was Pacific Islander. Eighty one percent of total subjects were males, 48% had mild keratoconus and 52% were contact lens wearers. The demographic data of the subjects are shown in Table 1 and the study variables are shown in Table 2. Langerhans cells were present in the central cornea in 91% of keratoconus subjects. The median Langerhans cell density was 15 cells per mm^2 (IQR: 3–21 cells per mm^2). Langerhans cell morphology of grade 1 was seen in 19% of subjects and grades 2 or 3 (with short or long dendrites) were seen in 71% of subjects. There was a significant association between Langerhans cell frequency and density with male gender (rho = −0.669, p = 0.001 and −0.441, p = 0.045 respectively) and between Langerhans cell density and nerve fibre tortuosity (rho = 0.479, p = 0.028) (Table 3). Other moderate associations observed between frequency of Langerhans cells and keratoconus severity (rho = 0.340), Langerhans cell density and nerve fibre density (rho = −0.360) and tear meniscus height (rho = −0.336), Langerhans cell morphology and duration of the disease (rho = 0.326),