Esthesiometery

The test was repeated twice and the longest filament length resulting in a positive response was recorded. The filament length was progressed in steps of 1.0 cm until a negative result was obtained, and the length was then shortened in steps of 0.5 cm until a positive result was obtained again as described previously[1].

Tear Collection and Neuromediator Analysis

At each visit, tear samples were collected from both eyes for neuromediator analysis: Schirmer strips were placed in the inferior conjunctival fornix of each eye without anesthesia, removed after five minutes, and immediately placed in Eppendorf® tubes (Eppendorf, Hamburg, Germany). Samples were stored at -80°C until analysis on the day of use.

Enzyme-linked immunosorbent assay (ELISA) was performed to analyze the neuromediators. Schirmer strips with tear samples were cut into thin sections and immersed in 200 µL elution buffer containing 0.55M NaCl, 0.33% Tween-20 (SigmaAldrich) as described previously[2]. Ultrasonication was carried out at 450 rpm and 4°C for 1 hour, followed by centrifugation and collection of the clear supernatant. Neuromediator concentration was quantitatively determined using ELISA kits (NGF and substance P kits: R&D Systems; CGRP kit: Novus Biologicals) according to the manufacturer's instructions. The optical density of samples at 450 nm was read using a microplate reader. The concentration of neuromediators was calculated according to the standard curve. Background optical density reading was set to 540 nm.

1. Cavalcanti BM, Cruzat A, Sahin A, Pavan-Langston D, Samayoa E and Hamrah P. In vivo confocal microscopy detects bilateral changes of corneal immune cells and nerves in unilateral herpes zoster ophthalmicus. Ocul Surf 2018;16:101-11.
2. Chin JY, Lin MT, Lee IXY, Mehta JS and Liu YC. Tear Neuromediator and Corneal Denervation Following SMILE. J Refract Surg 2021;37:516-23.