

## **Materials and Methods**

### *Subjects*

Thirty patients with moderate to severe CHE treated at the Department of Dermatology, Peking University First Hospital (Beijing, China), from November 2011 to March 2012 and 30 healthy volunteers were included in this study. The severity of CHE was assessed using the hand eczema severity index (HECSI) [13]. The inclusion criteria were: (1) age between 35 and 65 years; (2) hand back involvement with skin hyperkeratosis and fissures as major manifestations; (3) disease duration >3 months; (4) HECSI score  $\geq 10$  (moderate to severe), and (5) being willing to participate in the study and sign the informed consent. The exclusion criteria were: (1) severe systemic diseases or diseases that affect cognition and/or perception; (2) use of topical glucocorticoids or other immunomodulators within 1 week; use of systemic glucocorticoids, other immunomodulators, isotretinoin, or phototherapy within 1 month; (4) other hand diseases that may affect the evaluation and monitoring of CHE lesions, and (5) being unable to complete the study. All subjects provided informed consent, and the study protocol was approved by the ethics committee of the Peking University First Hospital.

### *Measurements*

All subjects underwent measurements of pH value, water content, TEWL, and integrity of the stratum corneum in both hands. The measurements were performed at the site of lesions (lesional skin) and a site approximately 3 cm away from lesions (nonlesional skin) for CHE patients. Prior to measurements, the sites to be measured were cleaned and disinfected. The measurements were performed at a temperature of  $22 \pm 2^\circ\text{C}$  and humidity of  $40 \pm 5\%$ .

pH values were measured using a pH meter (PH900, Courage + Khazaka Electronic GmbH, Germany) according to the manufacturer's instructions. Each site was measured twice, and the mean of the 2 values was calculated and used for analysis.

The water content in the stratum corneum was measured using a corneometer (CM825, Courage + Khazaka Electronic GmbH, Germany) according to the manufacturer's instructions. Each site was measured 3 times, and the mean of the 3 values was calculated and used for analysis.

TEWL was measured using a vapometer (Delfin Technologies Ltd., Finland) according to the manufacturer's instructions. Each site was measured 5 times, and the mean of the 5 values was calculated and used for analysis.

For measuring the integrity of the stratum corneum, stratum corneum stripping was performed with Scotch tape. TEWL was recorded 5 times after every 5 strips, and the mean of the 5 values was calculated and used for analysis. The change in TEWL ( $\Delta$ TEWL) was used to assess the integrity of the stratum corneum.

### *Immunohistochemistry*

Tissue samples were collected from 6 CHE cases at the site of lesions and the site approximately 3 cm away from lesions, and from 5 age- and gender-matched patients with noninflammatory skin disorders at the edge of the surgical incision. Frozen sections at 5  $\mu$ m thickness were then prepared and fixed in 4% paraformaldehyde for 10–15 min. After incubation with hydrogen peroxide at room temperature for 10 min to block endogenous peroxidase activity, the sections were incubated with mouse anti-human caspase-14 monoclonal antibody (1:100 dilution; Abcam, Cambridge, UK) overnight at 4°C. This was followed by successive incubation with polymer helper (Zhongshan Golden Bridge Biotechnology, Beijing, China) and polyperoxidase anti-mouse IgG (Zhongshan Golden Bridge Biotechnology) at room temperature for 20 min each. The sections were then visualized with diaminobenzidine (Zhongshan Golden Bridge Biotechnology), counterstained with hematoxylin, and observed under an optical microscope (Leica DM 5000B, Germany) at a magnification of  $\times 200$ . Three high-power fields were selected for each slide to calculate the mean optical density (OD) of immunohistochemical staining for caspase-14 with Image-Pro Plus 6.0.

### *Statistical Analysis*

All statistical analyses were performed using SPSS16.0 software. Numerical data are expressed as means  $\pm$  standard deviation, and categorical data are expressed as numbers (percentages). The pH value, water content, and TEWL of the stratum corneum among different groups were compared using one-way analysis of variance (ANOVA), followed by post hoc pairwise comparisons. The integrity of the stratum corneum was compared using repeated-measures ANOVA. Mean OD values between groups were compared using a paired-sample *t* test. *p* values  $< 0.05$  were considered statistically significant.