Corneal Epithelial Thickness Measured Using Anterior Segment Optical Coherence Tomography as a Diagnostic Parameter for Limbal Stem Cell Deficiency

QINGFENG LIANG, QIHUA LE, DANIEL W. CORDOVA, CHI-HONG TSENG, AND SOPHIE X. DENG

• OBJECTIVE: Using anterior segment optical coherence tomography (AS-OCT), we investigated the epithelial thickness (ET) of the central cornea and limbal regions in patients with limbal stem cell deficiency (LSCD) as a diagnostic and staging parameter.

• DESIGN: Prospective, cross-sectional study.

• METHODS: The central corneal epithelium thickness (CET) and maximum limbal epithelium thickness (mLET) were measured in the superior, inferior, nasal, and temporal limbus on AS-OCT images of the normal and eyes with LSCD. CET was obtained by 1-point (OCT-CET1) and 3-point measurement (OCT-CET3). The values of OCT-CET1 and OCT-CET3 were compared to the CET obtained with in vivo confocal microscopy (IVCM-CET).

• RESULTS: Sixty-eight eyes of 50 patients with LSCD and 52 eyes of 34 normal subjects were included. The mean (±standard deviation) OCT-CET3 was 55.0 ± 3.0 μm (range, 50.6-62.0 μm) in the control group and 41.6 ± 10.8 μm (range, 0-56.3 μm) in the LSCD group ($P < .001$). OCT-CET3 had a better correlation with IVCM-CET ($r = 0.91$) than did OCT-CET1 ($r = 0.87$, $P = .001$). The degree of reduction in OCT-CET3 increased in more advanced clinical stages of LSCD (all $P < .001$). The OCT-CET3 cutoff value that suggests LSCD was 46.6 μm. Compared with the control group, the LSCD group had decreases in mLET in all 4 limbal regions (all $P < .001$). The sensitivity and specificity of OCT-CET3 is the highest among all mLET in detecting LSCD.

• CONCLUSIONS: Both CET and mLET were thinner in patients with LSCD than in normal subjects. OCT-CET3 appears to be a reliable parameter to confirm LSCD when there is clinical suspicion. (Am J Ophthalmol 2020;216:132–139. © 2020 Elsevier Inc. All rights reserved.)

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From the Stein Eye Institute (Q.Liang, Q.Le. D.W.C., S.X.D.) and Department of Medicine, Statistic Core-General Internal Medicine and Health Service Research (C.H.T.), David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA; and Beijing Institute of Ophthalmology, Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Key Laboratory of Ophthalmology and Visual Sciences, Beijing, China (Q.Liang).

Inquiries to Sophie X. Deng, Stein Eye Institute, David Geffen School of Medicine at UCLA, 100 Stein Plaza, Los Angeles, CA 90095, USA; e-mail: deng@jsei.ucla.edu

Corneal epithelial maintenance and regeneration depend on a sufficient amount of functional limbal stem cells (LSCs) located in the corneoscleral limbus.[1] The reduction in the population of LSCs and their dysfunction result in abnormal corneal epithelialization and invasion of the corneal surface by the conjunctival epithelium with or without corneal neovascularization—that is, limbal stem cell deficiency (LSCD).[2] Because of the limitation of clinical signs in the diagnosis of LSCD in the past,[3,4] the global consensus on the diagnosis of LSCD suggests additional laboratory tests, such as impression cytology to detect goblet cells, the use of conjunctival markers to identify conjunctival epithelial cells,[2,5–7] and/or in vivo imaging to confirm the diagnosis of LSCD.[8–13]

The epithelial thickness (ET) of the cornea and limbus is a potentially important parameter for use in evaluating and monitoring the function of LSCs.[11] The repair and renewal of the corneal epithelium are related to the amount of functional LSCs. In patients with severe LSCD, persistent epithelial defects, epithelial thinning, irregularity, and opacity are often present. Findings from others and from our group show that epithelial thinning is observed in eyes with LSCD.[9,11,13,14] An association of the degree of epithelial thinning with the severity of LSCD was found by in vivo confocal microscopy (IVCM).[11] Anterior segment optical coherence tomography (AS-OCT) is more readily available and easier to perform than IVCM. Therefore, AS-OCT potentially is a better approach to measure ET and may be effective as an initial diagnostic tool to confirm LSCD when there is clinical suspicion. Using AS-OCT, Banayan and associates[13] found that the difference between the minimal and the maximal ET and the variation of ET were significantly higher in those with LSCD than in those without LSCD. However, a precise grading and detection of the LSCD severity are still lacking. Recently, a more precise clinical staging system was established by the International LSCD Working group.[2] In the current study, we investigated different methods to

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determine ET by using AS-OCT and IVCM, and we correlated the degree of epithelial thinning in the central cornea and limbus in eyes with different clinical stages of LSCD to establish a cutoff value of ET that signifies LSC dysfunction.

METHODS

• **SUBJECTS:** This prospective, cross-sectional, comparative, single-center study was conducted at the Stein Eye Institute with the approval of the Institutional Review Board at the University of California, Los Angeles (UCLA Institutional Review Board #10-001601). All subjects were informed of the aims of the study, and the study was adherent to the Declaration of Helsinki. Appropriate consent was obtained prior to the study.

Patients with LSCD were consecutively recruited from 2009 to 2018, and the diagnosis of LSCD was first based on results from clinical presentation and then confirmed by IVCM (HRT III; Heidelberg Engineering GmBH, Dossenheim, Germany) and/or impression cytology according to the criteria set by the International LSCD Working Group.[2] All subjects underwent AS-OCT, and all patients with LSCD and 17 control subjects underwent IVCM. Impression cytology was performed for 13 patients who consented to this test. The control group consisted of subjects without a history of eye disease and free of any ocular surface abnormality that could have been detected by slit-lamp examination. The control subjects with a contact lens–wearing history (continuous use over 2 weeks) and/or a history of any ocular surface surgery were excluded.

Using a previously established clinical scoring system based on the extent of limbus and corneal surface involvement (range of total score: 2-10 points),[15] we classified the stages of LSCD as mild (range, 2-4 points), moderate (range, 5-7 points), and severe (range, 8-10 points). The mild, moderate, and severe stages correlate with the stages I, II, and III (established by the International LSCD Working Group), respectively.

• **ANTERIOR SEGMENT OPTICAL COHERENCE TOMOGRAPHY:** A Fourier-domain OCT system (RTVue-100; Optovue, Inc, Fremont, California, USA) with a wide-angle (long) corneal anterior module lens was used. The central corneal epithelium thickness (CET) and maximum limbal epithelium thickness (mLET) were measured. The cross-line scan mode was used to obtain the images of the central cornea and the single-line scan mode was used to obtain images of each limbus quadrant (superior, inferior, nasal, and temporal) with the scleral spur visible. The images of limbus without a clear scleral spur or iris root structure were taken again or excluded from the analysis. A minimum of 3 scans was acquired at each location.

CET was defined as the straight distance between the tear film (first hyperreflective layer) and the basement membrane (second hyperreflective layer).The value of CET was measured with 2 manual methods: 1-point measurement (OCT-CET1) at the center of the cornea and 3-point measurement (OCT-CET3, Figure 1, top row) at 1 mm outside the center of the cornea. In cases of severe LSCD with subepithelial fibrosis at presentation, the epithelial layer could not be detected in AS-OCT images, and the CET measurement of 0 μm was given. Eyes with epithelial defect were excluded from the study.

The mLET was measured manually. First, the limbal epithelium area (LEA) was defined as the area between the lines crossing the scleral spur and the end of the Bowman layer. Second, the thickest part with the LEA was measured, and this measurement was recorded as the mLET (Figure 1, bottom row). Lastly, the mLET was determined in 3 independent OCT scans at each limbal quadrant, and the mean value of these 3 results was considered to be the mLET in this limbal quadrant. All measurements were performed by 2 independent, masked observers.

• **IN VIVO CONFOCAL MICROSCOPY:** CET was determined with the image recognition method from "volume" scan mode of in vivo confocal microscopy.[10] Series confocal images of the central cornea were taken by using the "volume" scan mode on an HRT III. A minimum of 3 high-quality Z scans were acquired. Only those scans with the least amount of motion artifacts underwent analysis. Two independent, masked observers then measured the CET, which was defined as the scan depth difference between the most superficial layer of epithelium and the basal layer. The mean value of 3 measurements served as the IVCM-CET.

• **STATISTICAL ANALYSIS:** Statistical analysis was performed with R software (www.r-project.org). CET and mLET values (mean ± standard deviation [SD]) were summarized and compared among the control group and the group of patients with LSCD at different stages using analysis of variance methods. The relationship of CET detected by using AS-OCT and IVCM was characterized by scatterplots and Pearson correlation coefficients with all subjects. All of the above measurements (OCT-CET1, OCT-CET3, IVCM-CET, and mLET) were performed by 2 independent, masked observers. Interobserver differences were calculated to assess the interoperator variation between 2 independent observers. The receiver operating characteristic (ROC) curves (area under the curve; AUC) were generated to examine the sensitivity and specificity of CET and mLET values to detect LSCD. All tests were 2-sided, and any *P* value ≤ .05 indicated statistical significance.

RESULTS

• **PATIENT CHARACTERISTICS:** A total of 120 eyes were included: 68 eyes of 50 patients with LSCD and 52 eyes

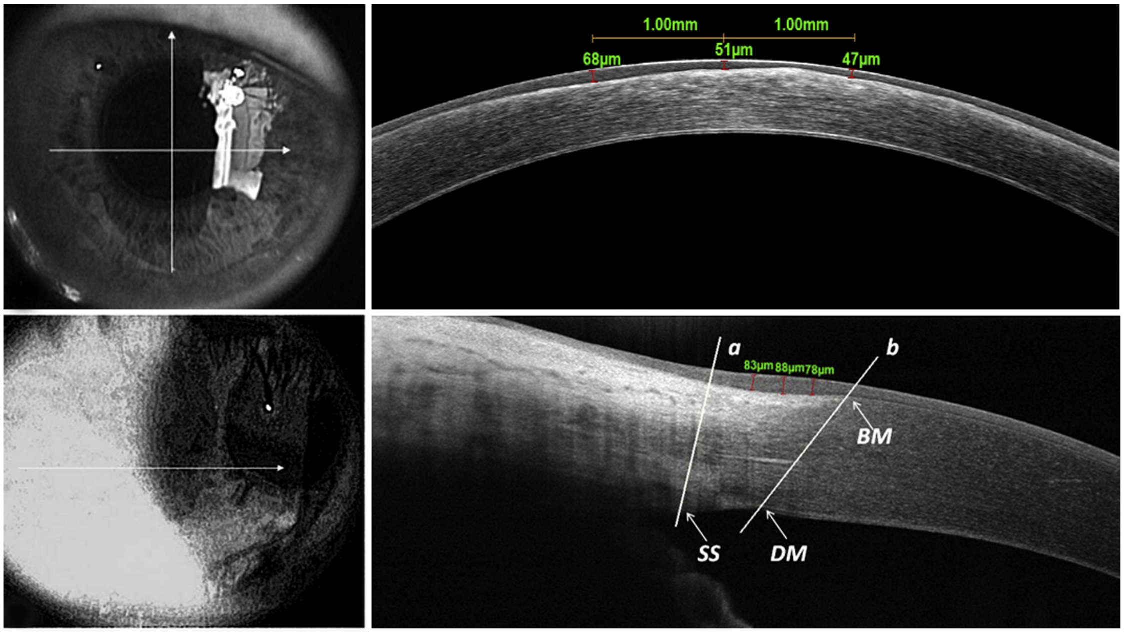

FIGURE 1. Measurement of corneal epithelial thickness (CET, Top row) and maximal limbal epithelial thickness (mLET, Bottom row) using anterior segment optical coherence tomography (OCT). The center of the cornea and 2 points at 1 mm away from the center of the cornea were located (Top left). CET was measured at these 3 locations from a point beneath the tear film (first hyperreflective layer) to the basal membrane (second hyperreflective layer). The value of CET in the center of the cornea was OCT-CET1, and the mean of these 3 points was OCT-CET3. "Line a" is perpendicular to the tangent of the limbal surface, which crosses the scleral spur (SS); "line b" is a connecting line between the ending points of the Bowman layer (BM) and Descemet membrane (DM). The limbal epithelium area (LEA) is defined by 2 white solid lines ("line a" and "line b"). The thickest part of the LEA was located, and the mLET was measured.

of 34 normal control subjects. The mean age (±SD) was 67.3 ± 17.6 years (range, 19-90 years) in the LSCD group and 50.4 ± 18.2 years (range, 19-89 years) in the control group ($P < .001$). There was no significant difference in sex between the control and LSCD groups ($P = .9$). All subjects were white. The leading etiologies of LSCD were multiple ocular surgeries (34 eyes; 50.0%), contact lens wear (11 eyes, 16.2%), drug toxicity (7 eyes, 10.3%), and mucous membrane pemphigoid (6 eyes, 8.8%).

The mild stage (stage I) of LSCD was observed in 27 eyes (39.7%); the moderate stage (stage II), in 27 eyes (39.7%); and the severe stage (stage III), in 14 eyes (20.6%). The mean (±SD) visual acuity in logMAR was 0.0 ± 0.0 in the control group and 1.1 ± 0.7 in the LSCD group ($P <$ .001). As the severity of the LSCD increased, the visual acuity decreased (Table). The clinical manifestations included stippling fluorescein staining in a vortex pattern of the cornea, minimal peripheral superficial neovascularization, and, in some patients, a persistent epithelial defect. The slit-lamp images of different stages are shown in Figure 2. The average clinical score (±SD)was 2.4 ± 1.1 points (range, 1-4 points) in stage I LSCD, 6.3 ± 1.1 points (range, 5-7 points) in stage II LSCD, and 9.0 ± 1.0 points (range, 8-10 points) in stage III LSCD. For the 13 patients who consented to impression cytology, goblet cells were detected on the cytology samples in all cases.

• CENTRAL CORNEAL EPITHELIUM THICKNESS: The method of measuring CET is described in Figure 1. Measurements were highly consistent (<5% variation) between the 2 independent observers (Supplemental Table 1; Supplemental Material available at AJO.com). The mean (±SD) OCT-CET1 was 54.0 ± 3.5 µm in normal controls and 41.4 ± 10.9 µm in the LSCD group. The mean (±SD) OCT-CET3 was 55.0 ± 3.0 µm in normal controls and 41.6 ± 10.8 µm in the LSCD group. There were no significant differences between the 2 measurements ($P > .05$). However, the OCT-CET3 had a better correlation with the IVCM-CET (r = 0.91) than did the OCT-CET1 (r = 0.87, Supplemental Figure 1; Supplemental Material available at AJO.com). Therefore, OCT-CET3 was used to evaluate CET in the rest of the study.

Most patients (36 cases, 72.0%) were older than 60 years in the LSCD group, whereas 13 subjects (38.2%) in the control group were older than 60 years. To control for the age variation, the subjects were categorized as <60 years of age and ≥60 years of age. There was no difference in the mean (±SD) OCT-CET3 values between the older age subgroup (54.9 ± 2.7 µm) and the younger age subgroup (55.8 ± 2.8 µm) of normal subjects ($P = .278$). The mean OCT-CET3 was also found to be similar between the younger and older subgroups of patients with

TABLE 1. Clinical Characteristics of Patients With Limbal Stem Cell Deficiency and Normal Controls

	Control Group	LSCD Group All	LSCD Group Stage I	LSCD Group Stage II	LSCD Group Stage III	P^a	Subgroup Comparison $P < .05$
Eyes, n (%)	52	69	27 (39.7)	27 (39.7)	14 (20.6)		
BCVA logMAR	0.0 ± 0.0	1.1 ± 0.7	0.6 ± 0.9	1.4 ± 1.3	1.6 ± 1.0	<.001	1, 2, 3, 4, 5
Clinical score	0 ± 0	5.35 ± 2.76	2.4 ± 1.1	6.3 ± 1.1	9.0 ± 1.0	<.001	1, 2, 3, 4, 5, 6
OCT-CET3 (μm)	55.0 ± 3.0	41.6 ± 10.8	49.5 ± 2.9	38.9 ± 9.6	32.8 ± 12.2	<.001	1,2, 3, 4, 5, 6
mLET (μm)							
Superior	108.4 ± 23.8	68.0 ± 21.7	72.8 ± 19.9	64.3 ± 24.3	65.8 ± 19.5	<.001	1, 2, 3
Inferior	102.0 ± 27.0	71.8 ± 21.4	71.3 ± 16.5	75.3 ± 26.3	66.0 ± 19.0	<.001	1, 2, 3
Nasal	86.7 ± 14.6	69.0 ± 17.5	72.9 ± 19.3	70.6 ± 16.5	58.4 ± 11.4	<.001	1, 2, 3, 5, 6
Temporal	84.6 ± 14.1	70.3 ± 17.4	78.0 ± 15.9	66.3 ± 16.7	63.1 ± 16.9	<.001	2, 3, 4, 5

BCVA = best-corrected visual acuity; LSCD = limbal stem cell deficiency; mLET = maximum limbal epithelium thickness; OCT-CET3 = optical coherence tomography corneal epithelium thickness 3-point measurement.

[a]P values of ANOVA performed among the control group and LSCD groups; In subgroup comparison, 1. Control vs Stage I; 2. Control vs Stage II; 3. Control vs Stage III; 4. Stage I vs Stage II; 5. Stage I vs Stage III; 6. Stage II vs Stage III.

LSCD in any of the 3 stages (all $P > .05$, Supplemental Figure 2; Supplemental Material available at AJO.com). Therefore, the age effect on CET is negligible. The remaining analyses were performed on the entire LSCD group and control group.

The epithelial layer in eyes with LSCD exhibited greater reflectivity than did the same layer, which was hyporeflective, in normal eyes. Reflectivity in the anterior stroma of eyes with LSCD was also greater than that in the eyes of control subjects (Figure 2). Compared to the mean OCT-CET3 of the control group (55.0 ± 3.0 μm), the mean OCT-CET3 of the LSCD group was significantly less (41.6 ± 10.8 μm, $P < .001$). The OCT-CET3 decreased with increasing severity of the disease (Figure 3). The OCT-CET3 was reduced by 10.3% in the group with stage I LSCD (49.5 ± 2.9 μm), by 29.6% in the group with stage II LSCD (38.9 ± 9.6 μm), and by 42.9% in the group with stage III LSCD (31.4 ± 11.8 μm, $P < .001$, Table). The cutoff value for OCT-CET3 that distinguished LSCD from a normal limbus was 46.6 μm; the sensitivity was 61.7% and the specificity was 100%. The AUC of the OCT-CET3 cutoff value was 0.973.

The OCT-CET3 in the affected (34.8 ± 11.3 μm) and unaffected (48.3 ± 3.8 μm) visual axis area differed significantly ($P < .001$). In patients with LSCD without visual axis involvement, the OCT-CET3 (48.3 ± 3.8 μm) was less than that of the control group (55.2 ± 2.8 μm; $P < .001$). Furthermore, the degree of OCT-CET3 reduction increased with the extent of the ocular surface involvement. The OCT-CET3 was 47.9 ± 5.6 μm for patients with 1 quadrant of the corneal surface affected, 41.5 ± 7.4 μm with 2 quadrants of the corneal surface affected, 37.2 ± 11.3 μm with 3 quadrants of the corneal surface affected, and 36.5 ± 10.5 μm with all 4 quadrants of the corneal surface affected ($P < .001$).

• **LIMBAL EPITHELIUM THICKNESS:** There was no difference in the mLET of the superior, inferior, nasal, and temporal quadrant between the older and younger subgroups of normal subjects ($P > .05$, Supplemental Table 2; Supplemental Material available at AJO.com). This finding suggested that age did not influence the mLET significantly. Therefore, the mLET values of the entire cohort were used in the rest of the analyses.

Compared with the mLET in the control group, the mLET in the LSCD group was significantly less in all 4 limbal quadrants (all $P < .001$, Table). The superior limbus was the most commonly affected area (51 eyes, 75.0%), followed by the inferior area (36 eyes, 52.9%), nasal area (32 eyes, 47.1%), and temporal area (27 eyes, 39.7%).

The degree of mLET reduction varied among the 4 quadrants of the limbus (Table); the greatest reduction was observed in the superior and inferior limbus. In patients with LSCD, the mLET in the clinically affected region was significantly less than that in the corresponding region of control subjects (Figure 4, all $P < .01$). Meanwhile, the unaffected regions also had a significantly thinner mLET than the corresponding limbal regions in the controls (all $P < .01$). However, there was no significant difference in mLET between the affected and unaffected limbal regions in LSCD group (all $P > .05$, Supplemental Table 3; Supplemental Material available at AJO.com). ROC curve analysis of the mLET in the control subjects and patients with LSCD revealed that the AUC of mLET cutoff value was 0.898 in the superior limbus, 0.827 in the inferior region, 0.790 in the nasal region, and 0.730 in the temporal region (Supplemental Figure 3; Supplemental Material available at AJO.com). The AUCs in all limbal regions were lower than that of OCT-CET3.

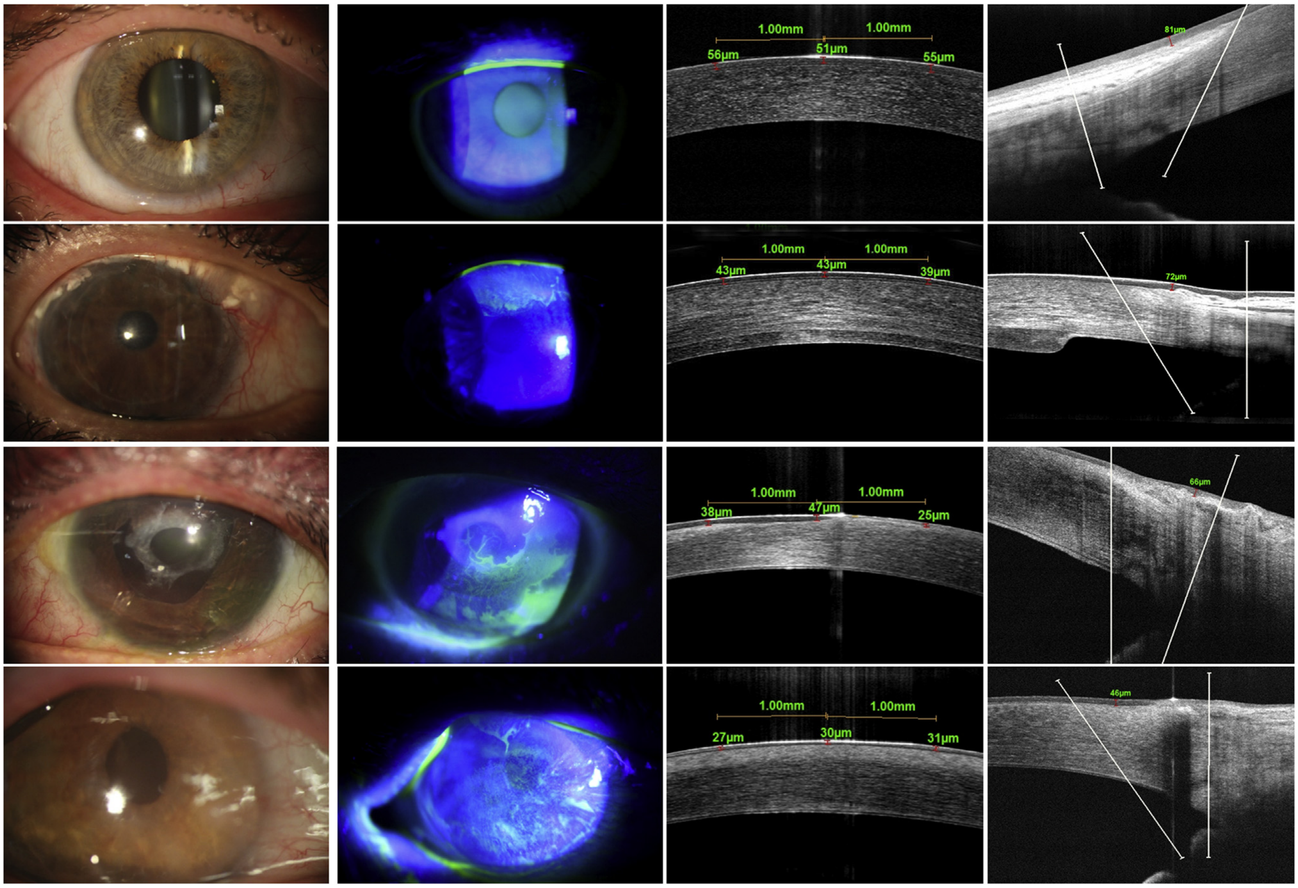

FIGURE 2. Representative examples of corneal epithelial thickness and maximal limbal epithelial thickness (mLET) using anterior segment optical coherence tomography (AS-OCT) in the control subjects and patients with limbal stem cell deficiency (LSCD) in different stages of severity. Slit-lamp photographs (left column), fluorescein staining patterns (second left column), AS-OCT images of the central (third column), and mLET (right column) in the control subjects and patients with LSCD are shown. The epithelium in the normal eye is transparent and devoid of fluorescein staining (top left). In LSCD, affected area exhibited stippled fluorescein staining in a whorl-like pattern or pooling (second column, bottom 3 images). There was epithelial thinning as outlined by the fluorescein staining in the cornea and affected limbal regions (second column bottom 3 images).

DISCUSSION

CONSISTENT WITH PREVIOUS FINDINGS BASED ON IVCM RESULTS, the ET of the cornea and limbus measured by AS-OCT decreased with the increasing severity of LSCD in the LSCD group. Our results suggest that the OCT-CET3 had the highest diagnostic value for LSCD and the mLET in the superior and the inferior limbal region had the second-highest diagnostic value.

Previous studies revealed that CET and LET varies among patients of different ethnic backgrounds[16] and age.[17] In our study, only white subjects were included in the control group. We also analyzed the variance of OCT-CET3 and mLET in different age groups, but the difference between the older and younger subjects of the control group was not significant. Yang and associates[17] measured CET and LET in 180 healthy eyes (age range, 7-83 years) and found that the CET of the central 2-mm-diameter zone had no significant change with aging. The difference among mean mLETs in different age groups was very small (about 3 µm), which is less than the 5-µm axis resolution of the AS-OCT system. The CET cutoff value between the patients with suspected LSCD and normal control subjects was similar in the older and younger subgroups. Therefore, age was not a confounding factor in the analysis of ET in LSCD measured by AS-OCT.

Although a characteristic of some corneal diseases such as keratoconus, post refractive surgery, and dry eye could present with a thinner ET, the epithelial remodeling is the primary mechanism of those diseases and the degree of epithelium thinning becomes limited in these diseases. For example, the CET reduction in patients with keratoconus is reported to be 9.2% of that of normal controls.[18] The reduction of ET in dry eye is 1.6%.[19] In LSCD, the average CET reduction was more than 20%, as epithelial renewal

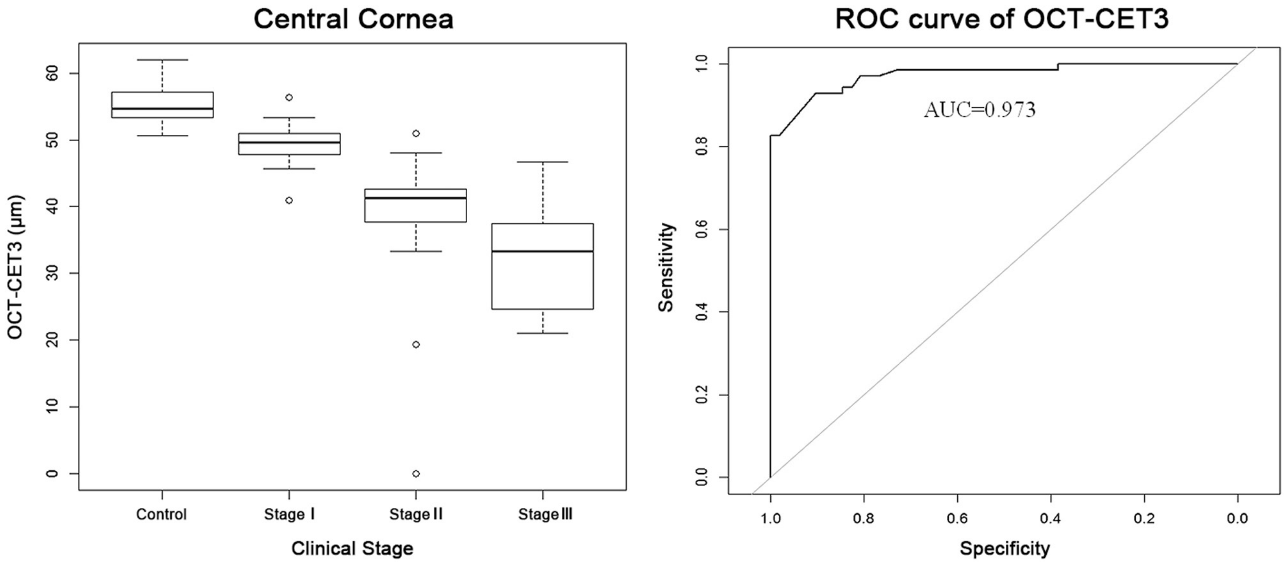

FIGURE 3. The box-and-whisker plots of the central corneal epithelial thickness obtained by 3-point measurement (OCT-CET3) in the control subjects and in patients with limbal stem cell deficiency (LSCD). The right figure is the receiver operating curve (ROC) of OCT-CET3 for detecting LSCD; the method provides (area under the curve [AUC] = 0.973) 61.7% sensitivity and 100% specificity in detecting LSCD.

was negatively influenced by the insufficient number of functional LSCs. Mehtani and associates[20] reported the CET reduction in LSCD cases was 32.6%, and Chan and associates[11] reported a 20.2% reduction in patients with LSCD. In addition, the epithelial layer in ocular surface squamous neoplasia is detected as hyperreflective above the Bowman layer using OCT.[21] However, this is often accompanied by thickening of the ET. Superficial keratectomy is necessary to obtain a diagnosis by histologic study in case of uncertainty of the underlying pathology.

In the present study, the OCT-CET3 cutoff suggests that LSCD was 46.6 μm, which is similar to the 45.5-μm cutoff obtained with IVCM. The OCT-CET reduction may be related to the decrease of basal cell density (BCD) and subbasal nerve density in the central cornea, which affect epithelial cell growth, proliferation, and regeneration. Chan and associates reported that BCD reduction was detected in mild sectoral LSCD; BCD in the central cornea decreased by 31.0% and by 23.6% in the limbus.[10] Thus, the central cornea was identified as an area representative of the global function of LSCs. Although the visual axis of the cornea appeared to be unaffected as seen by clinical examination in some patients with stage I LSCD, the OCT-CET was less than that of the normal controls. This result indicates that the central cornea homeostasis is already affected. CET could serve as a parameter to evaluate the LSC function, especially in the early stages of the disease. Moreover, OCT-CET decreases as the number of affected limbal and corneal regions increases.

It is important to note that subepithelial fibrosis, which is detected as a hyperreflective layer, was often present in eyes with chronic moderate or severe LSCD. The hyperreflectivity could interfere with the identification of the precise location of the epithelial layer and Bowman layer. Banayan and associates reported that limbal subepithelial fibrosis was detected in 76% of the eyes with LSCD and in none of the normal eyes.[13] The absence of the palisades of Vogt and limbal crypts is often observed in older individuals and is not necessarily an indication of LSCD.[22] In sectoral LSCD, a clear transition of the corneal epithelial layer (hyporeflective layer) to a thin hyperreflective layer is often observed (Figure 2, third image of the third row). Therefore, CET appears to be a more specific parameter than the absence of the palisades of Vogt in identifying LSCD.

There are limitations of our study. As mentioned above regarding the hyperreflective subepithelial fibrosis in severe LSCD, the accurate measurement of the CET and LET might be difficult. IVCM would be necessary as the next imaging test to further evaluate the phenotype and thickness of the epithelium in such cases. The current study has validated the use of AS-OCT in the measurement of ET in LSCD. CET reflects the global LSC function and could serve as the initial diagnostic test when there is clinical suspicion of LSCD. However, additional studies are necessary to further determine the role as a specific parameter in the diagnosis of LSCD.

In summary, the current study has validated the use of AS-OCT in the measurement of ET in LSCD. CET reflects the global LSC function and could serve as the initial diagnostic test when there is clinical suspicion of LSCD. Further confirmation and staging of LSCD could be achieved with IVCM.

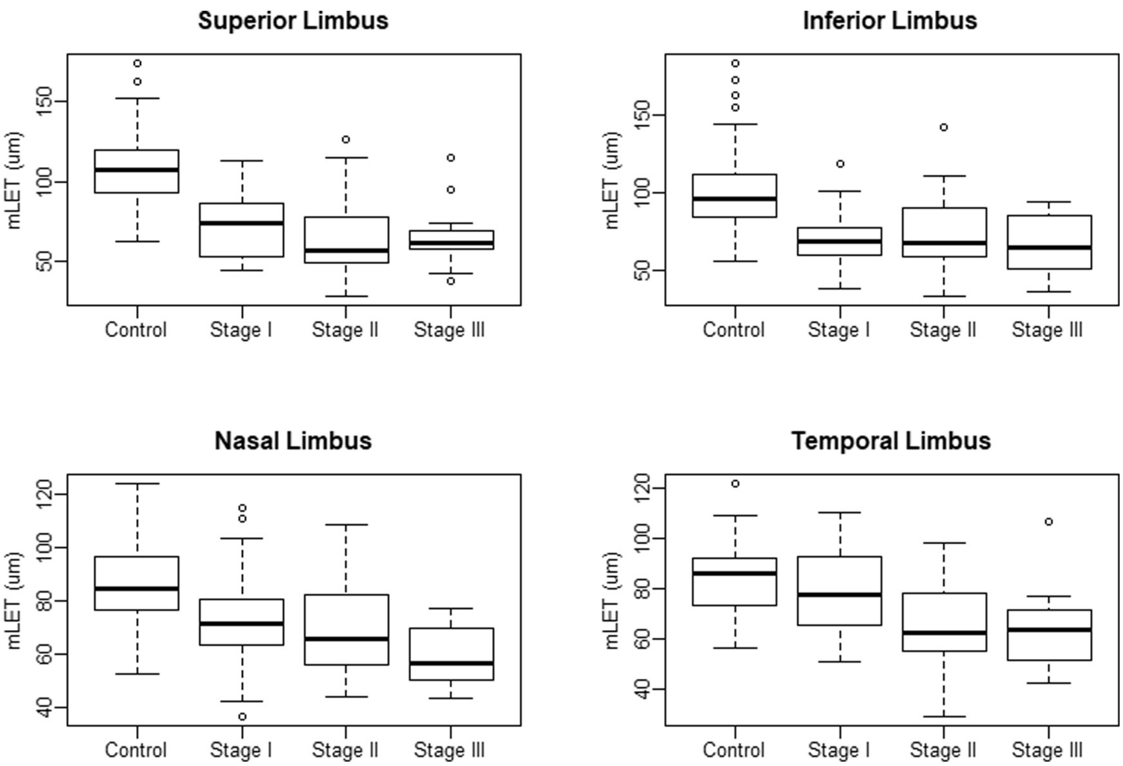

FIGURE 4. Box-and-whisker plots of maximal limbal epithelial thickness in the control subjects and in the patients with limbal stem cell deficiency at different stages. mLET = maximum limbal epithelium thickness.

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