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Cornea Confocal Microscopy Study of Patients with Preclinical Keratoconus

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Abstract: Purpose: To report the difference of corneal confocal microscopy examination in patients with preclinical keratoconus or clinical keratoconus. Methods: 8 unilateral keratoconus patients were examined by confocal microscopy before they had cross-linking surgery. As control groups, 23 patients with myopia and 13 patients with keratoconus in both eyes were also examined by confocal microscopy. Then we used Image J to compared the density of epithelium cell, length of cornea nerve fiber, density of Langerhans cell, density of keratocyte in shallow, middle, deep stroma, and the width and grayscale value of fold near Descement membrane for each group. Result: There was differences in corneal epithelial cell count between three groups (4358.27 ± 635.14 cells/mm^2 versus 4057.81 ± 316.29 cells/mm^2 versus 3522.65 ± 978.10 cells/mm^2). The density of keratocyte in stroma showed a tendency to decrease with increasing depth. The density of keratocyte in keratoconus was less than that in normal eyes. The fold showed much wider and daker in keratoconus eyes than in normal eyes. The density of Langerhans cell was more in keratoconus group than it in normal group. Conclusion: We first report the differences in corneal epithelial cell count and fold near Descement membrane. It might provide a new way to diagnose early keratoconus. The difference of density of Langerhans cell suggested us the possibility of inflammation in keratoconus.

Keywords: Confocal, Keratoconus, Epithelial Cell, Fold

1. Introduction

It is well known that the keratoconus occurs in both eyes of patients. Topography examination provides evidence for diagnosis in patients with keratoconus. It is important but very difficult to diagnose early stage of keratoconus. For the early stage of keratoconus patients, the topography and tomography are always normal. The confocal microscopy could provide the detailed information of cornea construction with magnification around 800. It may help the early diagnosis of keratoconus. Several studies have shown that there are many structural differences in the cornea between patients with keratoconus and normal patients. [1] But do these structural differences already exist in preclinical patients and normal people? If so, can these structural differences be detected by confocal microscopy to enable early diagnosis of keratoconus? In order to answer the question of whether the confocal microscopy will help the early diagnosis of keratoconus, we studied 8 eyes with preclinical keratoconus, 13 eyes with diagnosed keratoconus and 23 eyes with only myopia for compare.

By this study, we find there are differences between keratoconus eyes and preclinical eyes in the density of keratocyte in anterior stroma and in deeper stroma. Moreover, there are differences between the normal and keratoconus eye in the fold near the Descement membrane. We demonstrated structural differences in the corneas of patients with keratoconus compared to normal group. And these differences were detectable by confocal microscopy. But in preclinical patients, these differences were less pronounced. As we known, it is the first time to investigate and compare the fold near the Descement membrane between the normal and keratoconus eye by confocal microscopy.

2. Methods

2.1. Participants

As the experimental group, newly diagnosed patients, in which one eye showed typical keratoconus and the other was completely normal on corneal topography exam, were included in this study (preclinical group). In this group, the best corrected visual acuity of normal eyes must not be less than 50/50, and the corneal topographic examination must be completely normal. Patients with diagnosed keratoconus in both eyes (keratoconus group) and patients with only myopia (normal group) were included in this study for compare. All patients came from the Myopia Laser Center, Zhongshan Ophthalmic Center.

2.2. Confocal Microscopy

All patients underwent an ophthalmic examination including refraction (uncorrected and corrected visual acuity), slit lamp evaluation, corneal topography using Pentacam HR Scheimpflug imaging system (Oculus Optikgerate gmbh, Wetzlar Germany). The normal eye of the unilateral keratoconus patients and only right eye of the control group underwent the laser-scanning, using the confocal microscopy（HRT，German). For each exam, the images with good contrast, best focusing, without fold and motion were chosen. A full 400 × 400-micron square frame was used for the analysis. The following parameters of cornea were analyzed and calculated from images obtained by confocal microscopy by Image J (version 1.53f51): density of epithelium cell, length of cornea nerve fiber, density of Langerhans cell, density of keratocyte in shallow, middle, deep stroma, and the width and grayscale value of fold near Descement membrane.

For the experiment of fold near Descement membrane, one image with highest resolution will be chosen. Then we used *straight* tool to measure the width, *color picker* tool to measure the grayscale value. Each fold will be measured 5 times to take the average. The range of grayscale value was 0~255 in which 0 means all black and 255 means all white.

For the other experiment, 3 images with highest resolution will be chosen. Each measurement will be repeated 3 times to take the average.

All images were acquired by a same researcher who was masked about the study groups. Data was analyzed and compared by another researcher.

2.3. Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM, USA) statistical software. The continuous variables were described using mean and standard deviation. The t test was used to compare the parameter values within the groups. p value < 0.05 was considered statistically significant.

3. Result

According to our inclusion criteria, in preclinical group we included 8 subjects, 13 subjects in keratoconus group, and 23 subjects in normal group. The mean age of each group was 25.25 years, 22.22 years, 25.14 years.

Table 1 shows the findings and the comparison between each group.

Table 1. *The mean of parameters with the standard deviation.*

	N group (N=23)	PC group (N=8)	KC group (N=13)	N versus PC	N versus KC	PC versus KC
Epithelium cells	4358.27 ± 635.14	4057.81 ± 316.29	3522.65 ± 978.10	0.025	0.017	<0.001
CNFL	13.56 ± 4.55	12.18 ± 2.18	10.69 ± 4.46	0.097	0.501	0.458
LC	41.17 ± 28.29	53.91 ± 39.23	54.68 ± 43.78	0.186	0.035	0.568
Width of fold	6.15 ± 2.11	8.52 ± 2.97	20.3 ± 11.94	0.277	<0.001	0.003
Grayscale value of fold	51.95 ± 17.38	49.72 ± 15.00	46.28 ± 10.25	0.365	0.016	0.253
Keratocytes in shallow stroma	788.17 ± 132.42	675.00 ± 114.07	435.57 ± 203.84	0.609	0.006	0.010
Keratocytes in middle stroma	297.10 ± 47.12	264.84 ± 58.43	216.82 ± 81.02	0.398	0.001	0.097
Keratocytes in deep stroma	318.30 ± 43.73	190.62 ± 25.66	196.15 ± 62.32	0.200	0.072	0.017

N Group means normal group, PC Group means the group of unaffected eyes of unilateral keratoconus, KC Group means keratoconus group.

The cornea epithelium cells count in normal group was 4358.27 ± 635.14 cells/mm^2, in preclinical group was 4057.81 ± 316.29 cells/mm^2, in keratoconus group was 3522.65 ± 978.10 cells/mm^2. The corneal epithelial cell count showed significant differences in the comparison of the three groups. As the disease progresses, the corneal epithelial cell count is constantly decreasing (Figure 1).

The length of cornea nerve fiber (CNFL) was 10.69 ± 4.46 mm/mm^2 in keratoconus group, 12.18 ± 2.18 mm/mm^2 in preclinical group, and 13.56 ± 4.55 mm/mm^2 in normal group. But there were no significant differences between three groups (Figure 2).

The density of Langerhans cell was 54.68 ± 43.78 cells/mm^2 in keratoconus group, 53.91 ± 39.23 cells/mm^2 in preclinical group, and 41.17 ± 28.29 cells/mm^2 in normal group. The keratoconus group has more Langerhans cells than the normal group (P=0.035). Though the preclinical group seems had more Langerhans cells than the normal group, there was no statistical difference. (Figure 3).

The density of keratocytes in shallow stroma was 435.57 ± 203.84 cells/mm^2 in keratoconus group, 675.00 ± 114.07 cells/mm^2 in preclinical group, and 788.17 ± 132.42 cells/mm^2

in normal group.

The keratocytes density in middle stroma was 216.82 ± 81.02 cells/mm^2 in keratoconus group, 264.84 ± 58.43 cells/mm^2 in preclinical group, and 297.10 ± 47.12 cells/mm^2 in normal group.

The keratocytes density in deep stroma was 196.15 ± 62.32 cells/mm^2 in keratoconus group, 190.62 ± 25.66 cells/mm^2 in preclinical group, and 318.30 ± 43.73 cells/mm^2 in normal group (Figure 4).

In the shallow stroma, compared to the other two groups, the density of keratocytes showed a significant decrease in the keratoconus group (P=0.006, P=0.010). In the middle stroma, the density was less in keratoconus group than normal group (P=0.001). In the deep stroma, the cell density of the keratoconus group is less than that of the preclinical group (P=0.017). The strange thing is that there is no difference between the two and the normal group.

The width of fold was 20.3 ± 11.94 pix in keratoconus group, 8.52 ± 2.97 pix in preclinical group, and 6.15 ± 2.11pix in normal group. The grayscale value of fold was 46.28 ± 10.25 in keratoconus group, 49.72 ± 15.00 in preclinical group, and 51.95 ± 17.38 in normal group.

The width of fold in keratoconus group was much more wide than normal group (P<0.001) and preclinical group (P=0.003). The grayscale value in keratoconus group was smaller than normal group (P=0.016), which means it was darker in keratoconus eyes (Figure 5).

4. Discussion

Previous study found there were apoptosis of epithelium in the patients with keratoconus. [2-7] Our study found the cornea epithelium cells of keratoconus eye is less than preclinical keratoconus eye and normal eye. The cornea epithelium cells of preclinical keratoconus eye were less than the normal eye. There was significant difference of epithelium cells between those results. Other study found the same result as ours. Our study indicated the cornea epithelium cells decrease in the preclinical stage of keratoconus. Cornea epithelium may be the potential parameters for differentiating preclinical keratoconus with normal eye.

Though many research [3, 5-11] have found that CNFL is reduced and morphologically altered in patients with keratoconus, we don't find the same result. The P value was very close to 0.05, we think we may find a more positive result if we have more participants.

Whether keratoconus is an inflammatory disease has been debated for a long time. Though keratoconus does not meet all the classic criteria for an inflammatory disease, the lack of inflammation has been questioned. The Langerhans cell (LC) is one of the major cells of inflammation. A study [12] showed the relationship between LC and nerve changes in patients with keratoconus, and demonstrated the density of LC was 15 cells per mm^2. Our research compares more eyes in different stage, and it is the first study to compare the density of LC between keratoconus and preclinical keratoconus. Some study shown the LC in the patients with keratoconus was more than that in the normal eye. Our study had the same result. There was not significant difference of LC density between the eyes of preclinical stage of keratoconus and keratoconus. Our study indicated the density of LC may not provide information for early diagnosis of keratoconus.

It has been reported that overall keratocyte density is lower in keratoconic corneas [2-8, 13-15]. The decrease of keratocyte in patients with keratoconus due to the apoptosis of keratocyte. The density of cornea stroma keratocytes have not studied by previous studies in the preclinical stage of keratoconus. Our research in the first-time analysis the density of keratocyte density and compared with that of patients with myopia.

Previous study demonstrated the density of anterior stroma keratocyte was 662-463 cells/mm^2 in the keratoconus patients and 786 cells/mm^2 in normal eye. The density of posterior stroma keratocyte was 236-208 cells/mm^2 in the keratoconus patients and 293 cells/mm^2 in normal eye. The density of stroma keratocyte was lower than that of normal eye.

Our study found the similar result with other study: in shallow stroma, the density of keratocyte was decreasing with the continuous progress of keratoconus. Our study indicated the density of shallow stroma keratocyte has value in distinguishing normal eye, keratoconus, and preclinical keratoconus.

We are the first to investigate the middle stroma keratocyte density, because the middle stroma is the position where the laser refractive surgery has been performed. Based on our study, we found there was a difference between normal eye and keratoconus. As in anterior stroma and posterior stroma, the density was less in keratoconus eyes.

The density of keratocyte in deep stroma had an unexpected conclusion: the density in preclinical group was less than that in clinical, though both were less than normal group. We thought it was due to the limitation of sample size. So, the credibility of these data may relatively low.

In the later stage of keratoconus bright lines located deep in the stroma adjacent to Descement membrane called Vogt's striae could be observed by slit lamp. It is very difficult to found any abnormal of cornea by slit lamp in the stage of preclinical stage of keratoconus. Some research mentioned the fold near Descement membrane [16-18]. In our study we found black fold near Descement membrane in almost all eyes, especially in patients with keratoconus. Those folds may be the preclinical Vogt's striae. As we known, it is the first time to investigate and calculated the fold near the Descement membrane. The confocal microscopy has the capability to demonstrate the detailed construction of cornea due to the magnification of around 800, allows us to have a deeper understanding of black fold and Vogt's striae. Some study demonstrated the back surface of cornea will show the earliest alteration in the patients with keratoconus in the tomography and topography examination. Because the cornea confocal microscopy will have the more magnification than the

topography instrument, theoretically the cornea confocal microscopy has the capability to demonstrate the change of back surface of cornea early than topography or tomography instrument.

Our research showed the width of fold of keratoconus is bigger than that in the preclinical stage keratoconus, also bigger than that in the normal eyes. The fold became wider and darker along with the progression of keratoconus. The analysis of fold near the Descement membrane may help distinguish the early stage of keratoconus with keratoconus and normal eye.

5. Conclusion

Early identification of keratoconus becomes more and more imperative for preventing iatrogenic corneal ectasia. There are so many examination ways, however, the research said there is no uniform screening criteria [19, 20]. We need to find more different ways to identify early stage of keratoconus. It is first time to compare and analysis the cornea confocal microscopy in patients with preclinical keratoconus eyes and normal eyes. In our study, we newly found the difference of cornea epithelium cells count between normal preclinical clinical eyes. It may be a new way for the diagnosis of early stage of keratoconus. Besides, we found the fold near Descement membrane appear deeper and darker in keratoconus eyes. As we known this is the first time to analysis the fold near Descement membrane. It may give us a better understanding of the formation of Vogt's striae.

Our study was limited by the sample size and hospital basement. Our next work will expand the sample size.

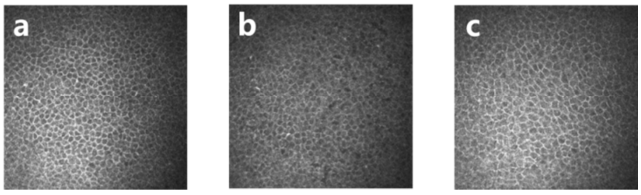

Figure 1. *Epithelium cells in different group, (a) normal group. (b) preclinical group. (c) keratoconus group.*

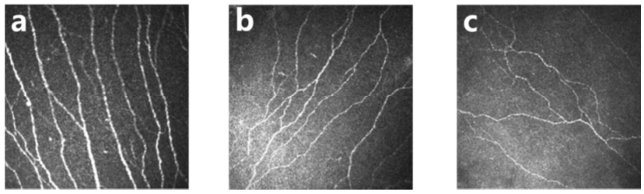

Figure 2. *CNFL in different group, (a) normal group. (b) preclinical group. (c) keratoconus group.*

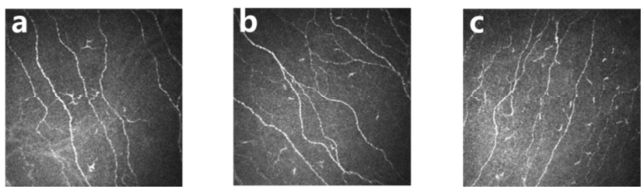

Figure 3. *Langerhans cells in different group, (a) normal group. (b) preclinical group. (c) keratoconus group.*

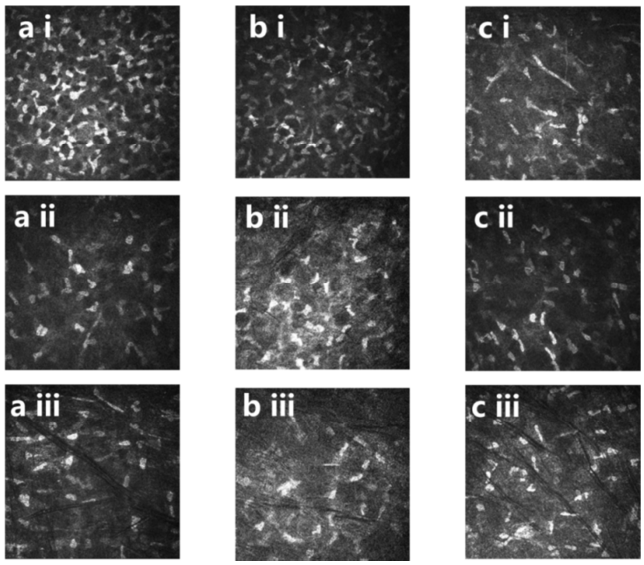

Figure 4. *Stroma keratocyte in different group and different depth, (a) normal group. (b) preclinical group. (c) keratoconus group. (i) shallow stroma. (ii) middle stroma. (iii) deep stroma.*

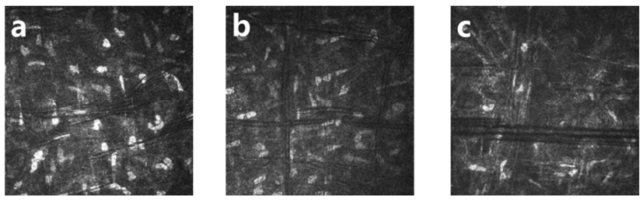

Figure 5. *Fold in different group, (a) normal group. (b) preclinical group. (c) keratoconus group.*

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no competing interests.

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