In vivo confocal microscopy of meibomian glands in primary blepharospasm
A prospective case–control study in a Chinese population

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Abstract
The aim of the study was to evaluate the morphological changes of meibomian glands (MGs) in primary blepharospasm (PBS) by in vivo laser scanning confocal microscopy (LSCM) and to investigate the correlations between clinical data of PBS and LSCM parameters of MGs. This prospective and case–control study recruited 30 consecutive PBS patients and 30 age- and gender-matched healthy controls. After questionnaire assessments of ocular surface disease index (OSDI), Jankovic rating scale, and blepharospasm disability index, all subjects underwent blink rate evaluation, tear film break-up time (TBUT), corneal fluorescein staining (CFS), Schirmer test, MG expressibility, meibum quality, MG dropout, and LSCM examination of the MGs. The main LSCM outcomes included the mean MG acinar area and density, orifice diameter, meibum secretion reflectivity, acinar irregularity, and inhomogeneity of interstice and acinar wall. The PBS patients had significantly higher blink rate, higher OSDI and CFS scores, lower TBUT and Schirmer test value, and worse MG expressibility than the controls (All $P < 0.05$), whereas meibum quality showed no difference ($P > 0.05$). The PBS patients showed lower values of MG acinar area, orifice diameter and meibum secretion reflectivity, and higher scores of acinar irregularity and inhomogeneity of interstices than the controls (All $P < 0.05$). For the PBS patients, the severity of blepharospasm evaluated by JCR scale was strongly correlated with MG acinar area ($P < 0.001$), orifice diameter ($P = 0.002$), meibum secretion reflectivity ($P = 0.002$), and MG acinar irregularity ($P = 0.013$). The MG expressibility was significantly correlated to MG acinar area ($P = 0.039$), orifice diameter ($P < 0.001$), and MG acinar irregularity ($P = 0.014$). The OSDI score was moderately correlated with MG acinar irregularity ($P = 0.016$), whereas the TBUT value was positively correlated with MG acinar area ($P = 0.049$) and negatively correlated to MG acinar irregularity ($P = 0.016$). The CFS score was negatively correlated to MG orifice diameter ($P = 0.008$). The LSCM provided a noninvasive tool for in vivo histopathologic studies of MGs in PBS patients. The excessive constriction of lid muscles closely related to MG morphological alterations of PBS, which offered a new research approach to interpret the interactional mechanism between dry eye and PBS.

Abbreviations: BSDI=blepharospasm disability index, CFS=corneal fluorescein staining, DE=dry eye, JRS=Jankovic rating scale, LSCM=in vivo laser scanning confocal microscopy, MAA=MG acinar area, MAD=MG acinar density, MAI=MG acinar irregularity, MGs=meibomian glands, MOD=MG orifice diameter, MSR=meibum secretion reflectivity, OO=orbicularis oculi, OSDI=ocular surface disease index, PBS=primary blepharospasm, TBUT=tear film break-up time.

Keywords: blepharospasm, confocal microscopy, dry eye, meibomian glands

1. Introduction
Primary blepharospasm (PBS) is a focal dystonia that characterized by involuntary spasmodic contractions of the orbicularis oculi (OO) muscles. PBS often begins insidiously, progressively worsens, and frequently results in increased blinking and eyelid closure.[1] This physically and mentally debilitating condition has a disabling effect on quality of life by compromising activities of daily living such as driving, reading, and shopping, and even lead to functional blindness.[2–4] In addition to the symptoms of blepharospasm, observational data showed that almost 50% of PBS patients also suffered from dry eye (DE) symptoms in varying degrees with reduced Schirmer test values and tear break-up time (TBUT).[5–8] Furthermore, several studies have indicated that DE symptoms in PBS patients could be improved by botulinum toxin injections.[9–11] These evidences made many researchers to concern about the interactional mechanism between PBS and DE. The study from Lu et al.[11] showed several key inflammation cytokines of tear fluid increased more significantly in the patients with a diagnosis of both PBS and DE than in those with DE alone and meanwhile the DE symptoms of PBS+DE patients were more severe, thus the authors suggested that blepharospasm might participate in the progress of inflammation of the ocular surface in PBS+DE patients. Fayers et al.[12] found that PBS patients were characterized with lower corneal mechanosensitivity and reduced number of nerves in the subbasal plexus which could be another possible causative of DE in PBS patients. However, few studies...
have been designed to investigate the morphology and function of meibomian glands in PBS patients. It is well known that the meibum forming the outer layer of the tear film is mainly secreted by the meibomian glands (MGs) and play a vital role in preventing tear evaporation. Meibomian gland dysfunction could cause the alterations of meibum and make the tear film unstable.\textsuperscript{13} The mechanical muscular action by the OO muscles which locate on the outside of the tarsus was supposed to act as the driving forces that result in the delivery of meibum onto the lid margin and tear film.\textsuperscript{14} Consecutive reports from 3 individual researches all suggested that the weakness of the OO muscle caused by facial nerve palsy had impact on meibomian gland morphology and had a component of obstructive meibomian gland disease.\textsuperscript{15–17} Based on the anatomic relationship between MGs and the OO muscles, we hypothesized that repeated forceful spasmodic contractions of the OO muscles might also compromise the MGs to morphologic and functional changes, which could be a possible cause of DE in PBS patients.

In vivo laser scanning confocal microscopy (LSCM) has been used in the examination of MGs in the conditions such as meibomian gland dysfunction,\textsuperscript{18,19} atopickeratoconjunctivitis,\textsuperscript{20} primary chronic dacryocystitis,\textsuperscript{21} Sjogren’s Syndrome,\textsuperscript{22} contact lens wearers,\textsuperscript{23} providing a new noninvasive tool with which to study morphologic changes in MGs. And it allows description and testing of the microstructure of MGs including MG acinar area and density, orifice diameter, meibum secretion reflectivity, and the inhomogeneity of interstices and walls of acinar units, which is difficult to be detected by infrared meibography system.

In this study, we evaluated meibomian gland morphology in PBS patients both by in vivo LSCM and infrared meibography system compared with age- and gender-matched controls. We also investigated the correlations between clinical data of PBS and MG morphologic findings in PBS patients.

2. Methods

2.1. Subjects

This study was conducted in compliance with the Declaration of Helsinki for research involving human participants and was approved by the Ethics Committee of the Eye, Ear, Nose, and Throat (EENT) Hospital of Fudan University. Written informed consent was obtained from all of the participants before the examination.

For the purposes of this prospective case-control study, 30 consecutive patients with a diagnosis of active PBS (12 male and 18 female; mean age±SD, 60.20±6.28 years) were recruited from the EENT Hospital of Fudan University, Shanghai, China. All the PBS patients should experience at least more than 6 months of blepharospasm symptom. Severity of blepharospasm was assessed using the validated Jankovic rating scale (JRS).\textsuperscript{24} Patients had to score a minimum of 3 points on the JRS for inclusion. Thirty age- and gender-matched healthy volunteers (14 male and 16 female, 59.33±6.01 years) were recruited as a control group through the same order for all participants so that any effect of order of testing would be the same for all the subjects.

2.2. Blepharospasm symptom assessment

Both severity and frequency of blepharospasm were graded by JRS questionnaire on a 5-point scale, with a score of 0 indicating least severe and a score of 4 indicating the most severe (total score range, 0–8).\textsuperscript{24} The BSDI questionnaire was used to complement the JRS as a patient reported assessment scale to evaluate the degree of impact on quality of life in the PBS patients, including driving, reading, watching TV, shopping, walking, and doing everyday activities. Each subscale was scored on a 5-point scale, with a score of 0 indicating least severe and a score of 4 indicating the most severe (total score range, 0–24).\textsuperscript{24}

2.3. Dry eye symptom assessment

The questionnaire of OSDI was used to assess the subjective symptoms of DE. The questionnaire consisted of 3 subscales including the bothersome symptoms, visual function, and environmental trigger. Each answer was scored on a 5-point scale from 0 (indicating least severe) to 4 (indicating the most severe). The total scores range from 0 to 100, with higher scores indicating more severe symptoms.\textsuperscript{26}

2.4. Video-recording assessment

The blink rate was calculated with subjects at rest and with eyes open during a video recording lasting 150 seconds and was expressed as blinks per minute. Blink was defined as a transient, bilateral and synchronous brief (<1 second) eyelid drop not associated with lowering of the eyebrows beneath the superior orbital margin.\textsuperscript{25}

2.5. Tear film stability

Tear film stability was evaluated by TBUT. TBUT was measured by instilling fluorescein into the lower conjunctival sac with a fluorescein strip (Jingming, Tianjing, China) that was moistened with nonpreservative saline solution. Then the patient was required to blink several times to ensure adequate coating of the dye on the cornea. Using the cobalt blue filter and slit lamp biomicroscopy, the interval time between the last complete blink and the appearance of the first black spot in the stained tear film was recorded as the TBUT. The test was repeated 3 times, and the average TBUT was calculated.

2.6. Corneal fluorescence staining

The CFS was measured with the same fluorescein impregnated strip as used for TBUT. The grading system recommended by NEI divides the cornea into 5 zones (central, superior, temporal, nasal,
and inferior) and for each zone, the severity of CFS is graded on a scale from 0 to 3. Therefore, the maximum score is 15. \(^{27}\)

2.7. Schirmer I test

A Schirmer test without topical anesthesia was used to assess tear production, by inserting a sterile dry strip (Jingming, Tianjing, China) in the lateral canthus of the lower eyelid, away from the cornea, for 5 minutes. Then the length of the strip that was wetted by the absorbed tears was measured to evaluate tear secretion function. Potential scores of Schirmer I test ranged from 0 to 30 mm.

2.8. MG expressibility

Assessment of MG expressibility was conducted by applying digital pressure on the upper tarsus, after which the degree of expressibility was assessed on a scale of 0 to 3 in 5 glands in the middle part, according to the number of glands expressible: 0, all glands; 1, 3 to 4 glands; 2, 1 to 2 glands; and 3, no glands. \(^{28}\)

2.9. Meibum quality

To evaluate meibum quality, eight glands of the central part of the upper lid is assessed on a scale of 0–3 for each gland: 0, clear; 1, cloudy; 2 cloudy with debris (granular); and 3, thick, like toothpaste (total score range, 0–24). \(^{28}\)

2.10. MG dropout

After the eyelids were everted, the morphological pictures of meibomian glands of both lower and upper lids were taken by non-contact infrared meibography system with the Oculus Keratograph (Oculus GmbH, Wetzlar, Germany). MG dropout of both the upper and lower eyelids was evaluated based on a 4-point scale as previously described \(^{29}\). Grade 0, no glands loss; Grade 1, glands loss was less than one-third of total gland area; Grade 2, glands loss was between one-third and two-thirds; Grade 3, glands loss was more than two-thirds of the total gland area. Scores of upper and lower lid summed scale range from 0 to 6.

2.11. Confocal microscopy

2.11.1. Image acquisition. All participants were examined using LSCM (HRT II Corneal Rostock Module; Heidelberg Engineering GmbH, Heidelberg, Germany), as described previously. \(^{22,23}\) Before each examination, a drop of 0.4% oxybuprocaine (Santen, Osaka, Japan) was instilled into the conjunctival fornix. After the upper eyelid was everted, the center of the Tomo-cap was applied onto the palpebral conjunctiva of the eyelid margin center, and then adjusted the controller manually to visualize the glandular structures in a satisfactory resolution. The applanate lens was then moved from the lid margins toward the fornix with minute vertical movements. Ten nonoverlapping images were taken during the movement. This procedure was repeated for the nasal and temporal eyelid margins. Each LSCM examination acquired 30 images in total and commonly lasted 10 minutes. Two-dimensional image sizes measured 384 x 384 pixels, with a 400 x 400 \(\mu\)m field of view. This examination was conducted by the same observer (TL) during the whole process of the study.

2.11.2. Image analysis. Three randomized, nonoverlapping, high-quality digital images of the nasal, middle, and temporal upper eyelids (total of 9 images per eyelid) were used for calculation of the LSCM parameters. We quantified the following variables: (1) MG acinar area (MAA) (manually drawn a line around the inner lumen of the acinar unit and calculated the area automatically by Image J, an open source software program from http://rsb.info.nih.gov/ij/), (2) MG acinar density (MAD) (manually marked inside each 400 x 400 \(\mu\)m frame and calculated the density automatically using Image J), (3) MG orifice diameter (MOD) (manually marked along the longest axis of orifice and calculated the diameter automatically by Image J), (4) meibum secretion reflectivity (MSR), (5) MG acinar irregularity (MAI), (6) inhomogeneous appearance of periglandular interstices, and (7) walls of acinar units. The MSR was evaluated based on a 4-point scale reported in a previous study from Villani et al. \(^{22}\) as follows: 0=black color of secretion, 1=dark gray color of secretion, 2=light gray color of secretion, and 3=white color of secretion. The inhomogeneity of interstices or walls of acinar units was quantified on a grading scale of 0 to 3 as follows: 0=absence of punctate reflecting elements, 1=slight presence of punctate reflecting elements, 2=greater presence of punctate reflecting elements, and 3=higher presence of punctate reflecting elements. \(^{22}\) The MAI was quantified on a grading scale of 0–3 as follows: 0=almost round or elliptical shape, 1=slight presence of lobulated shaped acinar units, 2=greater presence of lobulated shaped acinar units, and 3=higher presence of lobulated shaped acinar units (shown as Fig. 1).

2.12. Statistical analysis

For statistical analysis, the right eyes of all subjects were selected. Analysis was performed using SPSS V.19.0 Software (SPSS Inc.; Chicago, IL). Kruskal–Wallis test and Pearson \(x^2\) test were used to evaluate age and sex differences, respectively, between healthy controls and patients with BPS. The Mann–Whitney \(U\) test was used to determine differences of total OSDI score, TBUT, SIT, corneal staining, MG dropout, MG expressibility, and LSCM parameters between the 2 groups of subjects. Spearman or Person’s correlation analysis was used to investigate the relations between LSCM parameters and clinical data of PBS in patients with BPS. The \(P\) values less than 0.05 were considered statistically significant.

3. Result

3.1. Clinical data of PBS and dry eye

Patients with PBS scored a mean JRS value of 5.60±1.22 and a mean BSI value of 11.90±3.90. The PBS patients had experienced blepharospasm symptom for a mean duration of 2.88±1.60 years. Patients with BPS had significantly higher blink rate than the controls (\(P<0.001\), Fig. 2).

The PBS patients had significantly higher OSDI total score and CFS score than the controls (\(P<0.001\) and \(P=0.004\), respectively, Table 1). Meanwhile, the PBS patients showed significantly lower TBUT value and Schirmer test value than the controls (\(P=0.024\) and 0.003, respectively, Table 1).

3.2. MG dropout, MG expressibility, and meibum quality

There was no significant difference of MG dropout between the PBS patients and the controls (\(P=0.484\), Table 1). Patients with BPS showed worse MG expressibility than the controls (\(P=0.002\), Table 1); however, there was no significant difference of meibum quality between the 2 groups (\(P=0.297\), Table 1).
3.3. MG confocal data

The PBS patients showed significantly lower mean value of MAA ($P<0.001$, Table 2), MOD ($P=0.001$, Table 2 and Fig. 3), and MSR ($P=0.003$, Table 2) than the controls. However, there was no significant difference of MAD between the 2 groups ($P=0.399$, Table 2). And the BPS patients had significantly higher degree of MAI than the controls ($P<0.001$; Table 2 and Fig. 4). In BPS patients, inhomogeneous appearance of periglandular interstices was significantly greater than in the controls ($P<0.001$, Table 2), but there was no significant difference of inhomogeneity of the acinar wall between the 2 groups ($P=0.722$, Table 2).

3.4. Correlation between clinical data of PBS and MG LSCM parameters

The correlations between clinical data of PBS and MG LSCM parameters were shown in Table 3. The JCR score was strong negatively correlated with MAA ($P<0.001$), MOD ($P=0.002$), and MSR ($P=0.002$), and was moderate positively correlated with the degree of MAI ($P=0.013$). The BSDI score was significantly negatively related to MAA ($P=0.007$). The duration of PBS was significantly correlated with the MAA ($P=0.001$) and the degree of MAI ($P=0.017$). The blink rate was strong negatively correlated to MAA ($P<0.001$), and MSR ($P=0.005$). The OSDI score was moderate positively correlated with the degree of MAI ($P=0.016$), while the TBUT value was moderate correlated with MAA ($P=0.045$) and the degree of MAI ($P=0.016$). The CFS score was significantly negatively correlated to MOD ($P=0.008$). The MG expressibility was negatively correlated to MAA ($P=0.039$) and MOD ($P<0.001$), and was positively correlated with the degree of MAI ($P=0.014$).

4. Discussion

The current study presented the micromorphologic changes of MGs in the PBS patients by LSCM, including decreased MAA, MOD, and MSR, higher degree of MAI and inhomogeneity of the periglandular interstices compared with the age- and gender-matched controls. Most of these evidences suggested a minor role...
of the obstructive pathogenetic mechanism for the morphologic changes of MGs in PBS patients. Patients with obstructive meibomian gland dysfunction were reported to show the inverse morphologic changes of MG including increased MAA, high MSR, and increased MOD in previous studies. However, the PBS patients completely performed as the opposite condition that almost 4 times of blink rate was observed in these patients than in the controls (shown as Fig. 2). Speculatively, the alterations of MGs including the lower MEI expressibility and MG ori
cel diameter may control the ori
cel of the periglandular interstices in PBS patients than in the controls. The previous studies have indicated that the incomplete blinking for the weakness of OO muscles induced by CN VII palsy could lead to the development of meibomian gland dysfunction which was characterized with accumulative lipid secretion, glandular dilatation and meibomian gland drop-

Table 1
Clinical summary data.

|                  | PBS (n = 30) | Control (n = 30) | P     |
|------------------|-------------|-----------------|-------|
| Sex              |             |                 |       |
| Male             | 12          | 14              | 0.795 |
| Female           | 18          | 16              |       |
| JCR              | 5.60 (1.22) | —               |       |
| BSDI             | 11.90 (3.90)| —               |       |
| Duration of PBS (y) | 2.88 (1.60) | —               |       |
| OSDI             | 37.82 (20.34)| 15.82 (11.06)   | <0.001|
| TBUT (s)         | 5.53 (2.33) | 7.40 (3.30)     | 0.024 |
| CFS              | 1.20 (1.00) | 0.53 (0.90)     | 0.004 |
| SIT (mm/min)     | 4.73 (3.07) | 8.13 (4.53)     | 0.003 |
| MG dropout       | 2.33 (1.52) | 2.03 (1.10)     | 0.484 |
| MG expressibility| 0.90 (0.61) | 0.40 (0.50)     | 0.002 |
| Meibum quality   | 1.46 (1.25) | 1.13 (1.14)     | 0.297 |

BSDI, Blepharospasm disability index; CFS, corneal fluorescein staining; JCR, Jankovic Rating Scale; MG, meibomian gland; OSDI, Ocular Surface Disease Index; PBS, primary blepharospasm; SIT, Schirmer I test; TBUT, tear film break-up time.

Table 2
The LSCM parameters of meibomian glands in PBS and control groups.

|                  | PBS (n = 30) | Control (n = 30) | P     |
|------------------|-------------|-----------------|-------|
| MAA (μm²)        | 995.56 (383.22) | 1841.85 (479.52) | <0.001 |
| MAD (glands/mm²) | 97.90 (13.46)  | 102.63 (14.10)  | 0.399 |
| MOD (μm)         | 28.57 (4.68)  | 34.07 (6.44)    | 0.001 |
| MSR              | 1.50 (0.74)   | 1.50 (0.51)     | 0.005 |
| MAN              | 1.70 (0.73)   | 1.00 (0.45)     | <0.001 |
| Inhomogeneity of periglandular Intercalce | 1.73 (0.69) | 1.06 (0.59) |<0.001 |
| Inhomogeneity of acinar wall | 1.40 (0.52) | 1.33 (0.48) | 0.722 |

LSCM, In vivo laser scanning confocal microscopy; MAA, MG acinar area; MAD, MG acinar density; MAN, MG acinar irregularity; MG, meibomian gland; MOD, MG orifice diameter; MSR, meibum secretion reflectivity; PBS, primary blepharospasm; P obtained by Mann–Whitney U test.

* Presented the significant differences (P < 0.05).
controls. As suggested by Villani et al., for others conditions, the increased inhomogeneity may be seen as a sign of inflammation in tarsus and MGs. But in PBS patients we did not find obvious inflammation evidence of inflammatory dendritic cell in the periglandular interstices (data not shown). In Osama et al’s study, they found confocal sign of fibrosis in MGs of patients with atopic-keratoconjunctivitis as the possible reason for the increased inhomogeneous appearance of the periglandular interstices, but the fibrosis of MGs was absent in the PBS patients. Thus we interpreted the higher inhomogeneous appearance of the periglandular interstices in PBS patients as a confocal sign of long-term extrusion effect caused by repeated forceful spasmodic contractions of the OO muscles.

These morphologic changes of MG in PBS patients might have an evident clinical impact since the TBUT was significantly correlated with MAA and MAI, the OSDI score was associated with MAI and the CFS score was correlated with MOD (shown in Table 3). In addition, we found the PBS patients to be characterized by worse tear film stability, tear deficiency, ocular surface epithelial damage, and severer DE symptom compared with the age- and sex-matched controls, which were partly consistent with Fayers et al’s study. Fayers T and his colleagues similarly showed the PBS patients had higher OSDI total score, worse CFS, lower TBUT value compared with the controls. But they did not found the decreased Schirmer test value for the PBS patients compared with the controls. However another study from Price et al. found that the PBS patients had a significantly lower tear secretion than the control group, which was consistent with our current study.

This study is limited by its small sample size. As an exploratory study, the determination of patient numbers was not powered. Although repeated forceful spasmodic contractions of the OO muscle offered attractive explanation for the alterations of MGs in PBS patients, the current cross-sectional study still could not provide the conclusive evidence to draw the conclusion. Further powered studies will be required to verify the current findings. And longitudinal observation studies should be designed to determine the exact impact of the long-term spasmodic contractions of the OO muscle on the structure of MGs. Furthermore, we did not evaluate the lipid layer thickness which may help to support the hypothesis that the morphological alterations in MG of PBS patients may lead to the dysfunction of the MGs and alterations of the lipid layer, and then result in tear film instability.
In spite of these limitations for the current study, we still could conclude that LSCM offered the opportunity for in vivo noninvasive histopathologic studies of subtle changes of MGs in PBS patients. The excessive mechanical constriction of lid muscles might play a role in inducing MG morphological alterations of PBS patients. And the findings provided a new research approach to interpret the interactional pathologic mechanism between DE and PBS.

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Table 3

|                      | MG acinar area | MG orifice diameter | Meibum secretion reflectivity | MG acinar irregularity | Inhomogeneity of periglandular interstice |
|----------------------|----------------|---------------------|------------------------------|-----------------------|------------------------------------------|
| Age                  |                |                     |                              |                       |                                          |
| Age                  | −0.070         | 0.147               | −0.066                       | −0.002                | −0.094                                   |
| J1/2                 | 0.712          | 0.437               | 0.730                        | 0.900                 | 0.621                                    |
| BSDI                 | −0.620*        | −0.538*             | −0.550*                      | 0.440*                | 0.348                                    |
| OSDI                 | 0.000          | 0.002               | 0.002                        | 0.013                 | 0.060                                    |
| TMBT                 | −0.485*        | −0.310              | −0.300                       | 0.134                 | 0.245                                    |
| Duration of PBS      | 0.007          | 0.096               | 0.107                        | 0.480                 | 0.191                                    |
| Blink rate           | −0.566*        | −0.152              | −0.253                       | 0.432*                | 0.008                                    |
| CFS                  | 0.001          | 0.421               | 0.177                        | 0.017                 | 0.968                                    |
| OSDI                 | −0.606*        | −0.117              | −0.501*                      | 0.031                 | 0.154                                    |
| SIT                  | 0.000          | 0.557               | 0.005                        | 0.871                 | 0.418                                    |
| TBUT                 | −0.302         | −0.071              | −0.051                       | 0.436*                | 0.108                                    |
| Duration of PBS      | 0.105          | 0.710               | 0.780                        | 0.016                 | 0.568                                    |
| Blink rate           | 0.368*         | 0.164               | −0.152                       | −0.435*               | −0.155                                   |
| CFS                  | 0.045          | 0.386               | 0.422                        | 0.016                 | 0.415                                    |
| OSDI                 | −0.079         | −0.476*             | −0.086                       | 0.350                 | 0.131                                    |
| SIT                  | 0.677          | 0.008               | 0.651                        | 0.058                 | 0.400                                    |
| MG expressibility    | 0.196          | 0.136               | −0.144                       | −0.209                | 0.003                                    |
| MG expressibility    | 0.298          | 0.475               | 0.440                        | 0.269                 | 0.625                                    |
| Meibum quality       | −0.379*        | −0.010*             | −0.169                       | 0.445*                | 0.219                                    |
| Meibum quality       | 0.039          | 0.000               | 0.373                        | 0.014                 | 0.245                                    |
| Meibum quality       | 0.379          | 0.659               | 0.146                        | 0.348                 | 0.952                                    |

BDSI, Blepharospasm disability index; CFS, corneal fluorescein staining; J1/2, Jankovic Rating Scale; MG, meibomian gland; OSDI, Ocular Surface Disease Index; PBS, primary blepharospasm; SIT, Schirmer I test; TBUT, tear film break-up time.

Spearman or Person’s correlation analysis was used to investigate the relations between LSCM parameters and clinical data of PBS.

*Presented the significant correlation (P < 0.05).
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