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INFLAMMATORY DISORDERS

Quantitative evaluation of lipid layer thickness and blinking in children with allergic conjunctivitis

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Abstract

Purpose To quantitatively evaluate the lipid layer thickness (LLT) and blinking in children with or without allergic conjunctivitis (AC), and to compare those between the different types of AC.

Methods For this case–control study, 81 children with symptomatic AC with an average age of 9.62 ± 2.67 years were enrolled and subdivided according to the subtypes of AC, including seasonal/perennial allergic conjunctivitis group and vernal keratoconjunctivitis (VKC)/atopic keratoconjunctivitis (AKC) group. Another 82 age-matched healthy children were enrolled as control group. All subjects underwent routine eye examination and measurements of LLT, the number of incomplete or total blinking, partial blinking rate by the LipiView interferometer over a 10-s period. Other ocular surface assessment included fluorescein tear breakup time (TBUT), lower tear meniscus height, meibomian gland loss (MGL), meibum expressibility and quality.

Results Pediatric patients with AC had significant thinner LLT, shorter TBUT, decreased total blinking but increased partial blinking rate, especially in those with VKC/AKC (all $P < 0.05$). A significant deterioration of meibomian gland parameters was observed in AC group when compared with control subjects, demonstrated by severe upper and lower MGL, lid margin abnormalities, decreased meibum expressibility, and abnormal meibum quality, all of which were worse in the severe type of AC (all $P < 0.05$). Thinner LLT was significantly correlated with decreased TBUT ($\beta = 3.666$, $P < 0.001$) and severity of upper MGL ($\beta = -7.701$, $P = 0.002$).

Conclusion Decreased LLT and blinking disorders in pediatric patients with AC may contribute to lipid layer deficiency in the long run, which should be considered and appropriately diagnosed for a more precise treatment.

Key Message:

- Meibomian gland dysfunction is a major long-term complication of allergic conjunctivitis (AC). However, the quantitative changes of meibomian gland function, such as lipid layer thickness (LLT) in children with AC remains unclear.
- Children with AC had decreased LLT and increased partial blinking rate, especially in those with more severe type of AC.
- Thinner LLT was significantly correlated with decreased tear breakup time and severity of upper meibomian gland loss.
- Quantitative evaluation of LLT might be useful in the assessment of the AC related dry eye in pediatric patients.

Keywords Allergic conjunctivitis · Lipid layer thickness · Blinking · Meibomian gland

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Introduction

Allergic conjunctivitis (AC) is a worldwide common eye disease and frequently occurs in children. TFOS DEWS II classified AC as a probable risk factor for dry eyes disease (DED) [1]. In pediatric patients with AC, the prevalence of DED ranges from 12 to 97.5% [2, 3]. A growing number of literatures reported the instability of tear film in children with AC, evidenced by a decreased tear breakup time (TBUT) [2, 4–6].

Meibomian gland dysfunction (MGD) is a major long-term complication of AC [7]. It is known that the lipid layer derives from meibomian gland secretions and plays a significant role in stabilizing the tear film. As the outermost layer of the tear film, lipid layer retards tear evaporation from the aqueous layer, lowers the surface tension at the air interface, and promotes redistribution of the tear film over the ocular surface after blinking [8]. One of the previous studies, reported by Suzuki et al., assessed the alterations of lipid layer thickness (LLT) in adult patients with seasonal allergic conjunctivitis (SAC) [9]. The researchers noted an unexpected and a significant thickened LLT in SAC patients and reported a negative correlation between the LLT and TBUT. They inferred this as the compensate for the tear film instability. However, children and severe types of AC were excluded, and LLT was qualitatively evaluated in that research.

Blinking plays an integral role in ocular surface homeostasis by promoting the meibomian gland and meibum distribution into the tear film [7]. The number of complete blinking has been reported to be significantly associated with increased TBUT while incomplete blinking was associated with decreased TBUT [10]. Besides, the forceful blinking significantly increases LLT [11], and the deficiency of LLT was associated with incomplete blinking [12, 13]. Subjects exhibiting incomplete blinking had more severe MGL, reduced tear film stability, and thus trended to develop DED [10, 13, 14]. It is generally believed that the discomfort caused by AC increases blinking particularly in pediatric patients. However, the alterations of the total or incomplete blinking and the relationship between the incomplete blinking and LLT in children with AC have not been fully elucidated.

The aim of this study was to quantitatively evaluate LLT and blinking in children with active AC and compare that with healthy children, then observe the differences of LLT and blinking between the mild and severe types of AC, and finally analyze the association between the ocular surface parameters and LLT in AC group.

Materials and methods

Subjects

This cross-sectional, case–control study followed the tenets of the Declaration of Helsinki and was approved by the Medical Ethical Committee of Zhongshan Ophthalmic Center (2019KYPJ134), Sun Yat-sen University, Guangzhou, China. All the AC subjects with an age between 6 and 18 years were recruited from cornea clinic and the age-matched healthy control subjects were recruited among outpatients who visited for regular screening for refractive error at Zhongshan Ophthalmic Center from November 2019 to August 2020. Due to the impact of COVID-19, recruiting children from both groups were stopped between January 2020 and May 2020. Informed consent was obtained from all the subjects and their parents or guardian before the start of any study-related procedures.

Patients with AC were diagnosed according to the AAO diagnostic criteria for AC 2019 [15], and further subdivided into SAC/perennial allergic conjunctivitis (PAC) group and vernal keratoconjunctivitis (VKC)/atopic keratoconjunctivitis (AKC) group to understand the alterations of LLT in the different types of AC. We excluded the children who could not cooperate with our measurement, patients who had a history of ocular trauma or surgery, other ocular surface diseases such as giant papillary conjunctivitis induced by contact lens or ocular surface implant, infective conjunctivitis, lacrimal duct obstruction disease, eyelid disorders, intraocular diseases, who had worn contact lenses, used a punctual plug and topical ocular medications include eye drops and ointments within the prior 3 months. That is, all the active AC in our study were first onset or relapsed without being medicated for this episode yet. In the control group, subjects who had ocular surface or intraocular diseases were excluded, except the mild ametropia (spherical equivalent was from + 3.00D to − 3.00D) or mild DED.

Clinical measures

Medical history, including the duration and medication of AC history since the first episode, incidence seasons, and systemic condition such as allergic rhinitis (AR) were collected for each patient.

After examination of the best-corrected visual acuity (BCVA), refractive error (RT-5100; NIDEK, Tokyo, Japan) and intraocular pressure (Tx-200 tonometer; Canon, Japan), other ocular surface measurements were performed sequentially as follows: (1) LLT and blinking were obtained using LipiView interferometer (TearScience, Morrisville, North Carolina, USA). Considering the compliance and tolerance of the children, the examination time was adjusted to 10 s. Only the conformance factor of > 0.8 was used. The mean LLT, the number of incomplete blinking and total blinking were recorded, then the partial blinking rate was calculated as the rate between the incomplete blinking and the total blinking. (2) The

lower tear meniscus height (TMH) was measured using keratograph 5 M (K5M; Oculus, Optikgerate, Germany) in tear meniscus mode, and the mean height of three measurements was recorded. (3) Severity of AC was evaluated using slit-lamp based on the 5–5-5 exacerbation grading scale [16]. The scale consists of three graded groups and each group contains five clinical signs: 100-point-grade group (100 points for each, including active giant papillae, gelatinous infiltrates of the limbus, exfoliative epithelial keratopathy, shield ulcer, and papillary proliferation at lower palpebral conjunctiva), 10-point-grade group (10 points for each, including blepharitis, papillary proliferation with velvety appearance, Horner-Trantas spots, edema of bulbal conjunctiva and superficial punctate keratopathy), 1-point-grade group (1 point for each, including papillae at upper palpebral conjunctiva, follicular lesion at lower palpebral conjunctiva, hyperemia of palpebral conjunctiva, hyperemia of bulbal conjunctiva and lacrimal effusion). The total point is from 0 to 555. (4) TBUT measurement was performed using fluorescein through a slit-lamp with the cobalt blue filter. The sterile fluorescein strip (Tianjin Jingming New Technological Development Co., Ltd., Tianjin, China) was wetted by a drop of physiological saline and touched to the inferior fornix. Then, the children were instructed to blink three times to ensure adequate mixing of the dye and asked to refrain from blinking during the test. The interval between the last complete blink and the appearance of the first corneal black spot was measured three times consecutively with a stopwatch and the average of three measurements was calculated [5, 17]. (5) Lid margin abnormalities, meibum expressibility, and meibum quality were checked by a standard force with the sterile cotton swab. Lid margin abnormalities were scored as 0 (absent) or 1 (present) each for the following four parameters: irregularity lid margin, vascular engorgement, plugging of meibomian gland orifices, and anterior or posterior replacement of the mucocutaneous junction. The total score is from 0 to 4 [18]. The quantity and quality of meibum were evaluated and graded on the central five glands of the upper eyelid as published [19]. Specifically, the meibum expressibility was graded as follows: 0 for all glands expressible, 1 for three to four glands expressible, 2 for one to two glands expressible, and 3 for no glands expressible. The meibum quality was graded as follows: 0 for clear fluid, 1 for cloudy fluid, 2 for cloudy particulate fluid, 3 for inspissated, like toothpaste. (6) Meibomian gland loss (MGL) of the upper and lower eyelid was graded by meibography using K5M as reported [18] to generate the meiboscore, that is, 0 for no MGL, 1 for less than one-third MGL, 2 for one-third to two-third MGL, 3 for more than two-third MGL. The examination room was maintained at the temperature of 24 to 26℃ and the humidity was 40 to 50% [17, 20].

Statistical analysis

All the subjects receive examinations of both eyes and only the more severe eye was analyzed. If both eyes had the same ocular surface changes, the right eye was selected. All statistical analyses were performed with SPSS Statistical Software (version 23.0; SPSS Inc., Chicago, IL, USA). Data were presented as means ± standard deviations (SD) or n (%). The normality of variables data was assessed using the Shapiro–Wilk test. Mann–Whitney *U* nonparametric analysis was adopted for the continuous data and ordinal categorical variable. Categorical data such as sex and the history of AR were analyzed using the Pearson Chi-Square test. The univariate and multivariate linear regression analysis were used to evaluate the impact of clinical variables on LLT in AC group. In multivariate linear regression, only significant variables in the univariate were analyzed. *P* values of <0.05 were considered statistically significant.

Results

A total of 81 AC pediatric patients with a mean age of 9.62 ± 2.67 years (range 6–18 years) were included in this study. The mean duration of AC history since the first episode was 19.6 ± 23.76 months (range 0–120 months). Another 82 age-matched healthy individuals were enrolled as control group. There were 23 patients with SAC, 28 with PAC, 25 with VKC and 5 with AKC. The age of SAC/PAC group and VKC/AKC group was comparable. The male in the AC group was significantly more than that of control (81.5 vs. 43.9%, $P<0.001$; Table 1), especially in the VKC/AKC group (100 vs. 70.6%, $P=0.001$; Table 2). The number of children with AR in the AC group was significantly more than that of the control (56.8 vs. 13.4%, $P<0.001$; Table 1), primarily came from the patients in the SAC/PAC group (66.7 vs. 40.0%, $P=0.019$; Table 2).

The mean BCVA in the AC group was 0.85 ± 0.17, which is worse than that of the control (0.96 ± 0.12, $P<0.001$; Table 1). In the two subgroups of AC, a worse BCVA could be observed in patients with VKC/AKC (0.78 ± 0.19 vs. 0.89 ± 0.14, $P=0.007$), for whom had longer duration of AC and worse clinical signs (all $P<0.05$, Table 2).

LLT and TBUT was decreased in AC group

LLT was thinner (48.91 ± 21.62 vs. 72.29 ± 22.68 nm, $P<0.001$) and TBUT was shorter (3.73 ± 2.74 vs. 7.67 ± 3.08 s, $P<0.001$) in children with AC compared with control subjects (Table 1 and Fig. 1a,b). In the two subgroups of AC, a decreased LLT was observed in the VKC/AKC group when compared with the SAC/PAC group

Table 1 Clinical variables and ocular surface status in AC and control group

Parameters	AC group ($n=81$)	Control group ($n=82$)	P value
Age, y	9.62 ± 2.67	10.07 ± 2.79	0.286
Sex, n (%)			
Female	15 (18.5%)	46 (56.1%)	< 0.001*
Male	66 (81.5%)	36 (43.9%)	
Allergic rhinitis, n (%)			
No	35 (43.2%)	71 (86.6%)	< 0.001*
Yes	46 (56.8%)	11 (13.4%)	
BCVA (logMAR)	0.85 ± 0.17	0.96 ± 0.12	< 0.001*
Ocular surface parameters			
Lipid layer thickness, nm	48.91 ± 21.62	72.29 ± 22.68	< 0.001*
Incomplete blinking, n	1.93 ± 1.72	2.12 ± 1.79	0.434
Total blinking, n	2.68 ± 1.92	3.80 ± 2.60	0.004*
Partial blinking rate, %	63.65 ± 39.06	50.59 ± 37.86	0.024*
TMH, mm	0.19 ± 0.13	0.18 ± 0.07	0.438
TBUT, s	3.73 ± 2.74	7.67 ± 3.08	< 0.001*
Upper MGL (0–3)	1.00 ± 0.85	0.32 ± 0.49	< 0.001*
Lower MGL (0–3)	0.63 ± 0.72	0.13 ± 0.34	< 0.001*
Lid margin abnormalities (0–4)	0.88 ± 1.08	0.37 ± 0.73	< 0.001*
Meibum expressibility (0–3)	1.19 ± 1.28	0.23 ± 0.56	< 0.001*
Meibum quality (0–3)	1.23 ± 1.43	0.28 ± 0.81	< 0.001*

* Significant ($P<0.05$)

AC, allergic conjunctivitis; *BCVA*, best-corrected visual acuity; *logMAR*, logarithm of the minimum angle of resolution; *TMH*, tear meniscus height; *TBUT*, tear breakup time; *MGL*, meibomian gland loss

(41.20 ± 15.93 vs. 53.45 ± 23.32 nm, $P=0.014$). However, no significant difference was found in TBUT between the two subgroups of AC (Table 2 and Fig. 1c,d).

Total blinking was reduced but partial blinking rate was increased in patients with AC, especially those with severe types of AC

There were significantly decreased number of total blinking (2.68 ± 1.92 vs. 3.80 ± 2.60, $P=0.004$) and increased partial blinking rate (63.65 ± 39.06 vs. 50.59 ± 37.86%, $P=0.024$) in children with AC compared with control subjects (Table 1 and Fig. 2a,b). In the two subgroups of AC, a decreased number of total blinking (2.17 ± 1.95 vs. 2.98 ± 1.86, $P=0.032$) and an increased partial blinking rate (74.72 ± 41.67 vs. 57.14 ± 36.29%, $P=0.014$) were also observed in the VKC/AKC group compared with the SAC/PAC group (Table 2 and Fig. 2c,d). However, there was no significant difference of the number of incomplete blinking in both the groups of AC and control and between the two subgroups of AC.

Meibomian gland parameters were worse in AC group

As shown in Table 1, there were significantly higher meiboscore in the upper and lower lids and worse lid margin abnormalities, meibum expressibility and quality in the AC group than those of the control (all $P<0.001$). In the two subgroups of AC, the meiboscore of both upper and lower was significantly higher in pediatric patients with VKC/AKC (all $P<0.05$), especially in the upper lid. Other meibomian gland parameters as lid margin abnormalities, meibum expressibility and quality were significantly more severe in the VKC/AKC group (all $P<0.001$, Table 2).

Decreased TBUT and severity of upper MGL were significantly associated with decreased LLT in AC group

In AC group, decreased TBUT ($\beta=3.666$, $P<0.001$, Fig. 3a) and severity of upper MGL ($\beta=-7.701$, $P=0.002$) were significantly correlated with thinner LLT on both univariate and multivariate linear regression analysis. Partial blinking rate ($\beta=-16.079$, $P=0.009$, Fig. 4a), lid margin abnormalities ($\beta=-4.558$, $P=0.041$), meibum expressibility ($\beta=-5.396$, $P=0.004$) and meibum quality ($\beta=-5.416$, $P=0.001$) were significantly associated with LLT only in the univariate linear regression analysis (Table 3).

In the SAC/PAC group, TBUT ($\beta=3.604$, $P=0.001$, Fig. 3b), upper MGL ($\beta=-13.258$, $P=0.004$), Meibum expressibility ($\beta=-6.665$, $P=0.031$) and meibum quality ($\beta=-5.953$, $P=0.030$) were also significantly associated

Table 2 Clinical variables and ocular surface status in SAC/PAC and VKC/AKC subgroups

Parameters	SAC/PAC group ($n=51$)	VKC/AKC group ($n=30$)	P value
Age, y	9.47 ± 2.64	9.87 ± 2.75	0.443
Sex, n (%)			
Female	15 (29.4%)	0 (0.0%)	0.001*
Male	36 (70.6%)	30 (100%)	
Allergic rhinitis, n (%)			
No	17 (33.3%)	18 (60.0%)	0.019*
Yes	34 (66.7%)	12 (40.0%)	
The duration of AC, month	13.65 ± 19.21	29.70 ± 27.44	0.001*
BCVA (logMAR)	0.89 ± 0.14	0.78 ± 0.19	0.007*
Ocular surface parameters			
Lipid layer thickness, nm	53.45 ± 23.32	41.20 ± 15.93	0.014*
Incomplete blinking, n	1.92 ± 1.70	1.93 ± 1.80	0.912
Total blinking, n	2.98 ± 1.86	2.17 ± 1.95	0.032*
Partial blinking rate, %	57.14 ± 36.29	74.72 ± 41.67	0.014*
TMH, mm	0.17 ± 0.09	0.24 ± 0.18	0.132
TBUT, s	4.00 ± 2.74	3.27 ± 2.72	0.207
Upper MGL (0–3)	0.63 ± 0.63	1.63 ± 0.81	< 0.001*
Lower MGL (0–3)	0.45 ± 0.61	0.93 ± 0.79	0.003*
Lid margin abnormalities (0–4)	0.45 ± 0.76	1.60 ± 1.16	< 0.001*
Meibum expressibility (0–3)	0.75 ± 1.06	1.93 ± 1.29	< 0.001*
Meibum quality (0–3)	0.71 ± 1.19	2.13 ± 1.36	< 0.001*
5-5-5 score	14.02 ± 20.36	139.13 ± 98.70	< 0.001*

* Significant ($P<0.05$)

AC, allergic conjunctivitis; *SAC*, seasonal allergic conjunctivitis; *PAC*, perennial allergic conjunctivitis; *VKC*, vernal keratoconjunctivitis; *AKC*, atopic keratoconjunctivitis; *BCVA*, best-corrected visual acuity; *logMAR*, logarithm of the minimum angle of resolution; *TMH*, tear meniscus height; *TBUT*, tear breakup time; *MGL*, meibomian gland loss

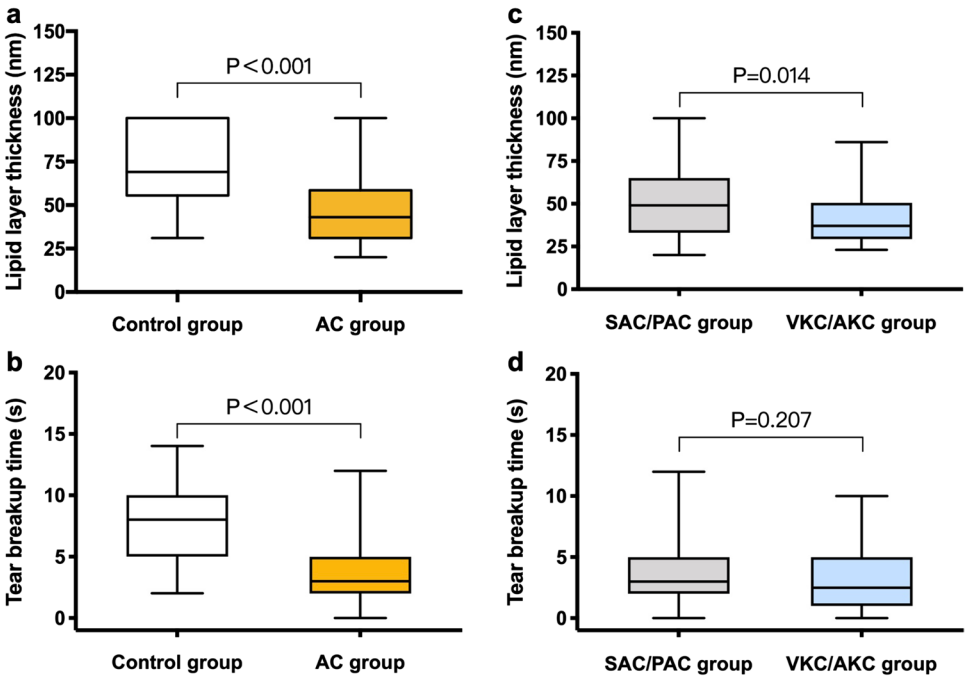

Fig. 1 Box plots showing decreased lipid layer thickness and tear breakup time in AC group vs. control group (**a**–**b**), and in VKC/AKC group vs. SAC/PAC group (**c**–**d**)

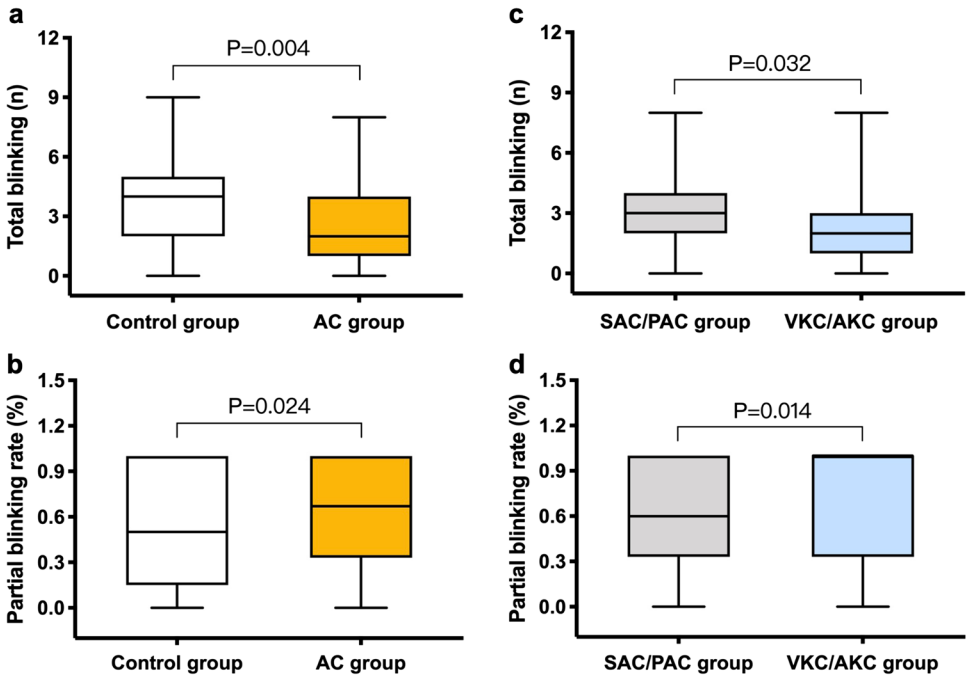

Fig. 2 Box plots showing decreased total blinking and partial blinking rate in AC group vs. control group (**a**–**b**), and in VKC/AKC group vs. SAC/PAC group (**c**–**d**)

with LLT (Table 4). However, TBUT (β = 3.392, $P = 0.001$, Fig. 3c) and partial blinking rate (β = − 15.821, $P = 0.023$, Fig. 4c) were significantly correlated with LLT in the VKC/AKC group (Table 5).

Discussion

This study quantitatively evaluated LLT and blinking pattern in pediatric patients with AC. The results showed a thinner LLT, decreased total blinking, and increased partial blinking rate in children with AC, especially in severe type of AC. The thinner LLT was associated with decreased TBUT and severity of upper MGL.

In recent years, LLT can be quantitatively measured in a noncontact way using the interferometer by analyzing the color and brightness of the lipid layer interference images [21]. In the healthy control subjects of our study, the average LLT was similar to a previous report [17], while TBUT was shorter than the data (10.04 ± 1.79 s) from children in the southwest China with a mean age of 4.76 ± 0.86 years [3]. The average TBUT in VKC/AKC group was close to a previous study (VKC: 4.5 ± 1.0 s, AKC: 3.1 ± 1.6 s) [22], while the value in the SAC/PAC group was shorter than the reported data (6.54 ± 1.48 s) from the children with a mean age of 4.75 ± 0.83 years [3].

Contrary to our conclusions, Suzuki et al. reported surprising results that SAC patients with tear film instability had thicker LLT and showed negative correlation with TBUT [9]. However, our current results indicated that the LLT was significantly thinner in the SAC/PAC group compared with the control group (data not shown). These discrepancies might

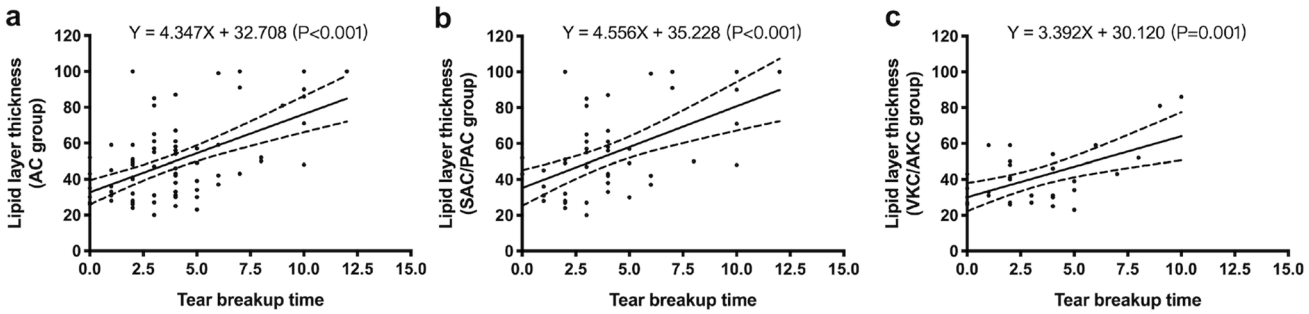

Fig. 3 Scatter plots showing positive association between lipid layer thickness and tear breakup time in AC group (**a**), SAC/PAC group (**b**), VKC/AKC group (**c**)

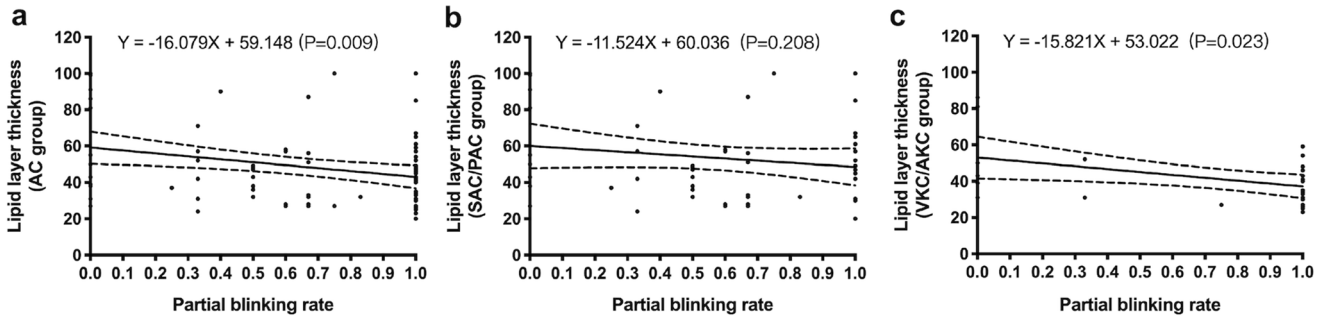

Fig. 4 Scatter plots showing negative association between lipid layer thickness and partial blinking rate in AC group (**a**) and VKC/AKC group (**c**), but not in SAC/PAC group (**b**)

be explained as follows: firstly, the device and the method of the measurement are different. In their study, LLT was qualitatively measured with DR-1 interferometry, and tear film instability was linked to a higher reading of the LLT measurement by this machine. In contrast, LLT of our study was quantitatively measured by LipiView interferometer, while tear film instability was associated with decreased LLT [23, 24]. Secondly, patient inclusion criteria were different. Children and more severe types of AC as VKC and AKC were excluded in Suzuki's study, and none of the eyes had giant conjunctival papillae, superficial punctuate keratitis, meibomian gland disease and less than 5 mm of tear secretion in Schirmer's test.

Anti-allergic eye drops used for previous episodes in some patients included olopatadine hydrochloride, azelastine hydrochloride, etc. Some studies have demonstrated that preservatives in anti-allergic eye drops impair the corneal and conjunctival epithelial cells [25–28]. That is why we excluded patients who had used topical ocular medications within 3 months prior to enrollment. However, it remains unknown whether the anti-allergic component itself has any influence on LLT; further study should be carried out to clarify this issue. Besides, we did not exclude children accompanied by AR or were using nasal sprays because AR is quite common in children with AC. In our study, no association was observed between the history of AR and LLT.

Table 3 The univariate and multivariate linear regression analysis to evaluate the factors associated with LLT in AC group

	Univariate regression		Multivariate regression	
Factors	β (95% CI)	*P* value	β (95% CI)	*P* value
Age	0.591 (−1.216 to 2.398)	0.517		
Sex	−8.042 (−20.296 to 4.211)	0.195		
Allergic rhinitis	1.206 (−8.502 to 10.914)	0.805		
The duration of AC	−0.072 (−0.276 to 0.131)	0.480		
BCVA	4.452 (−24.134 to 33.037)	0.757		
Ocular surface parameters				
Incomplete blinking	−1.396 (−4.187 to 1.396)	0.323		
Total blinking	−1.330 (−3.831 to 1.170)	0.293		
Partial blinking rate	−16.079 (−27.938 to −4.220)	0.009*		
TMH	−21.556 (−57.820 to 14.709)	0.240		
TBUT	4.347 (2.871 to 5.822)	<0.001*	3.666 (2.214 to 5.118)	<0.001*
Upper MGL	−11.052 (−16.170 to −5.933)	<0.001*	-7.701 (-12.371 to -3.031)	0.002*
Lower MGL	−2.729 (−9.473 to 4.014)	0.423		
Lid margin abnormalities	−4.558 (−8.936 to −0.180)	0.041*		
Meibum expressibility	−5.396 (−8.993 to −1.799)	0.004*		
Meibum quality	−5.416 (−8.588 to −2.244)	0.001*		
5-5-5 score	−0.020 (−0.075 to 0.036)	0.488		

* Significant ($P < 0.05$)

AC, allergic conjunctivitis; *BCVA*, best-corrected visual acuity; *TMH*, tear meniscus height; *TBUT*, tear breakup time; *MGL*, meibomian gland loss; *CI*, confidence interval

Table 4 The univariate and multivariate linear regression analysis to evaluate the factors associated with LLT in SAC/PAC subgroup

Factors	Univariate regression		Multivariate regression	
	β (95% CI)	*P* value	β (95% CI)	*P* value
Age	0.385 (−2.148 to 2.917)	0.761		
Sex	−2.856 (−17.379 to 11.668)	0.694		
Allergic rhinitis	−9.382 (−23.182 to 4.417)	0.178		
The duration of AC	0.025 (−0.323 to 0.374)	0.884		
BCVA	12.650 (−33.758 to 59.058)	0.586		
Ocular surface parameters				
Incomplete blinking	−0.273 (−4.221 to 3.675)	0.890		
Total blinking	−1.790 (−5.352 to 1.773)	0.318		
Partial blinking rate	−11.524 (−29.668 to 6.620)	0.208		
TMH	19.523 (−58.160 to 97.206)	0.616		
TBUT	4.556 (2.495 to 6.617)	<0.001*	3.604 (1.598 to 5.610)	0.001*
Upper MGL	−18.143 (−27.380 to −8.905)	<0.001*	−13.258 (−21.974 to −4.543)	0.004 *
Lower MGL	2.503 (−8.440 to 13.447)	0.648		
Lid margin abnormalities	−7.977 (−16.552 to 0.568)	0.067		
Meibum expressibility	−6.665 (−12.712 to −0.617)	0.031*		
Meibum quality	−5.953 (−11.322 to −0.585)	0.030*		
5-5-5 score	0.155 (−0.171 to 0.481)	0.344		

* Significant ($P < 0.05$)

AC, allergic conjunctivitis; *BCVA*, best-corrected visual acuity; *TMH*, tear meniscus height; *TBUT*, tear breakup time; *MGL*, meibomian gland loss; *CI*, confidence interval

Table 5 The univariate and multivariate linear regression analysis to evaluate the factors associated with LLT in VKC/AKC subgroup

Factors	Univariate regression		Multivariate regression	
	β (95% CI)	*P* value	β (95% CI)	*P* value
Age	1.343 (−0.838 to 3.524)	0.218		
Sex	-	-		
Allergic rhinitis	9.528 (−2.289 to 21.345)	0.110		
The duration of AC	−0.001 (−0.226 to 0.224)	0.992		
BCVA	−27.012 (−57.935 to 3.911)	0.084		
Ocular surface parameters				
Incomplete blinking	−3.085 (−6.299 to 0.129)	0.059		
Total blinking	−2.469 (−5.486 to 0.548)	0.105		
Partial blinking rate	−15.821 (−29.299 to −2.343)	0.023*		
TMH	−22.752 (−55.839 to 10.335)	0.170		
TBUT	3.392 (1.538 to 5.245)	0.001*	3.392 (1.538 to 5.245)	0.001*
Upper MGL	−2.467 (−10.035 to 5.100)	0.510		
Lower MGL	−2.608 (−10.402 to 5.185)	0.499		
Lid margin abnormalities	1.821 (−3.437 to 7.080)	0.484		
Meibum expressibility	−1.182 (−5.962 to 3.597)	0.616		
Meibum quality	−2.428 (−6.872 to 2.017)	0.273		
5-5-5 score	0.050 (−0.010 to 0.109)	0.098		

* Significant ($P < 0.05$)

AC, allergic conjunctivitis; *BCVA*, best-corrected visual acuity; *TMH*, tear meniscus height; *TBUT*, tear breakup time; *MGL*, meibomian gland loss; *CI*, confidence interval

The potential influence of nasal condition or the use of nasal sprays on LLT was still unclear, and further comparative study might answer these questions.

Frequent eye blinking is believed common in children with AC, especially concomitant with DED. However, there was no research to confirm this. In our report on the quantitative measurement of blinking in pediatric patients with AC, the results showed a decreased total blinking in the AC group, especially in those with VKC/AKC. Whereas, there was no statistical significance of total blinking between the SAC/PAC group and control group (data not shown). The decreased total blinking in patients with VKC/AKC might relate to the mechanical insult to the cornea caused by the giant papillae or the proliferative lesion in the upper palpebral conjunctiva. Furthermore, LLT decreased and partial blinking rate increased more significantly in the severe type of AC. According to the previously reported [12, 13], we could speculate that the decreased total blinking and the increased partial blinking rate might reduce the meibum expelling and distribution into the ocular surface, thus leading to a thinner LLT.

In addition, TMH was measured to evaluate the quantity of tear secretion. Although a watery eye is a common clinical sign of AC [15], our results showed a lack of significant difference of TMH between the AC and control group, which suggested that pediatric patients with AC have relatively normal tear secretion. Consistent with TFOS DEWS II [7], more AC children in our study have evaporative dry eye rather than the aqueous tear deficiency type of dry eye.

Next, we evaluated the meibomian gland between different groups. A significantly higher upper and lower MGL and worse lid margin abnormalities, meibum expressibility and quality were discovered in pediatric patients with AC, especially in those with VKC/AKC. MGD, lid margin abnormality, and the changes of lipid composition give rise to the hyposecretion of lipids and tear film instability [29, 30]. Previous confocal microscopy examinations in patients with VKC and AKC have disclosed that the extensive periglandular inflammatory cell infiltration accelerates meibomian gland fibrosis and atrophy, decreases in size and density of the meibomian gland acinar unit, increases lid margin vascularity and hyperkeratinization, and promotes orifice obliteration and narrowing [31–33]. Moreover, a significantly more frequent upper meibomian gland duct distortion in patients with PAC due to the inflammatory changes in conjunctival tissue and the frequently rub eyes was found, leading to a poor quality and quantity meibomian expression [34]. It can be concluded that the inflammatory mediator infiltration and the repeatedly rubbed eyes in AC children may damage the structure and function of meibomian gland, and consequently cause alterations in the meibum quality or quantity, ultimately decrease LLT and aggravate tear stability.

In the multivariate linear regression analysis, the severity of upper MGL was statistically correlated with thinner LLT in the AC group. One of the possible reasons is that the papillary hypertrophy or proliferative lesion mainly occurred in the upper eyelid, and thus the long-term and recurrent inflammation stimulation is more likely to cause the upper MGL, especially in patients with VKC and AKC. In addition, the upper meibomian gland contributes more to the lipid pool and subsequently to the LLT [29]. Compared with the lower eyelid, the upper eyelid has more pronounced movements during blinking, which promotes the squeezing of upper meibomian gland. Besides, the direction of meibum secretion in the upper eyelid is consistent with the gravity direction, making the secretion more easily and continuously. Moreover, the upper meibomian glands are larger in number and have a longer length, and hence have approximately double total volume and secretory capacity versus the lower meibomian gland [29, 35].

One weakness of the present study is the small sample size, especially in the relatively rare type of AKC. The second weakness is that the ocular surface disease index questionnaire was not performed in our study because the questionnaire is unsuitable for children. Lastly, all the patients in the AC group were in the disease active stage, and the changes of LLT after anti-allergic therapy were not evaluated. Future research with a larger sample size of each group and the comparison of LLT before and after treatment are still needed.

In conclusion, children with AC had lipid tear deficiency as decreased LLT and blinking disorders. Thinner LLT was associated with decreased tear stability and severity of upper MGL. Evaluation of LLT might shed light on the plausible mechanism of DED associated with AC in pediatric patients and provide meaningful reference for a more precise treatment. Future research with a larger sample size is still needed.

Author contribution All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Boyu Yang, Kuiyao Wen, Jing Li, Shiyao Zhang, and Zixin Fan. The first draft of the manuscript was written by Boyu Yang, Xiaoling Liang, and Lingyi Liang, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability Data are not transparent.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate The research protocol was approved by the Medical Ethical Committee of Zhongshan Ophthalmic Center (2019KYPJ134), Sun Yat-sen University, and written informed consent was obtained from each patient. The study adhered to the tenets of Declaration of Helsinki.

Consent for publication Consent was obtained from each author.

Conflict of interest The authors declare no competing interests.

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