High Prevalence of *Demodex* in Eyelashes with Cylindrical Dandruff

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Purpose. To determine the prevalence of *Demodex* in eyelashes with cylindrical dandruff (CD).

Methods. A modified sampling and counting method was applied to 55 clinical cases. Patients were divided in to group A ($n = 20$) with diffuse CD, group B ($n = 12$) with sporadic CD, and group C ($n = 23$) with clean lashes or greasy scales, of which the latter was divided into subgroup C1 ($n = 15$) without lid hygiene and subgroup C2 ($n = 8$) using daily lid hygiene for the past year. Each patient underwent a routine complete eye examination and modified counts of *Demodex*.

Results. *Demodex* was found in all group A and B patients ($n = 32$) with CD, which was significantly higher than the 22% of group C patients ($n = 23$) without CD ($P < 0.001$). The *Demodex* counts were 4.1 ± 1.0 and 2.0 ± 1.2 per epilated lash with retained CD, significantly higher than the 0.2 ± 0.5 and 0.2 ± 0.4 per lash without retained CD in groups A and B, respectively (each $P < 0.001$) and than the 0.01 ± 0.09 and 0.12 ± 0.41 per lash in subgroups C1 and C2, respectively (each $P < 0.001$). *Demodex* was still found in CD fragments left on the lid skin after epilation. Five *Demodex brevis* mites were found among the 422 *Demodex* specimens.

Conclusions. The modified sampling and counting method showed that the prior controversy regarding *Demodex* has resulted from miscounting and confirmed that lashes with CD are pathognomonic for ocular *Demodex* infestation. Lid hygiene with shampoo reduces *Demodex* counts but does not eradicate the mites. (*Invest Ophthalmol Vis Sci.* 2005;46: 3089–3094) DOI:10.1167/iovs.05-0275

The *Demodex* mite (class Arachnid and order Acarina) is an elongated ectoparasite with an obvious head–neck part and a body–tail part, of which the former has four pairs of stumpy legs. Among a wide range of reported species, only two, *Demodex folliculorum* and *Demodex brevis*, are found on the human body surface. The adult *D. folliculorum* is 0.35 to 0.4 mm long and is commonly found in small hair follicles. *D. brevis* is 0.15 to 0.2 mm long and lives deep in the sebaceous glands. Both *Demodex* species often coexist in the same skin area and tend to gather in the face, cheeks, forehead, nose, and external ear tract, where active sebum excretion provide a favorable habitat for breeding.

In the eye, *D. folliculorum* is found in the lash follicle, whereas *D. brevis* burrows deep into the lash's sebaceous gland and the meibomian gland.[1] Several groups have concluded that *Demodex* infestation leads to blepharitis.[2–9] However, the pathogenic potential of these mites remains unclear, because a low number of *Demodex* can be found in the skin and lashes of asymptomatic individuals. No research has convincingly demonstrated whether a minimal number of mites must be present to produce symptoms. The central requirements in addressing this question are accurate sampling and counting of mites on removed lashes.

Cylindrical dandruff (CD) in eyelashes, a common finding in some patients with blepharitis, has been regarded as pathognomonic of *Demodex* infestation,[4,10,11] although controversial results have also been presented.[12] We speculated that such a controversy is generated in part by the method and accuracy of *Demodex* sampling and counting. As a first step toward understanding the pathogenic role of ocular *Demodex* infestation, we sought to determine whether the conventional method of counting *Demodex* carries the potential for error. Using a modified sampling and counting method, we report herein that eyelashes with CD indeed had significantly higher *Demodex* infestation. The pathogenic significance of our findings is further discussed.

Materials and Methods

Patients and Subgroups

This study was in compliance with the tenets of the Declaration of Helsinki for the study of 55 patients seen at the Ocular Surface Center (Miami, FL). All underwent a routine, complete eye examination and external photography. A modified method of lash sampling and *Demodex* counting was used.

Cylindrical dandruff (CD), also known as cylindrical casts, are scales that form clear cuffs collaring the lash root (Figs. 1A, 1B). Characteristic CD could be distinguished from greasy scales, which did not rest on and were not connected with the root of the lash (Fig. 1C). Based on the presence and the extent of CD, patients were divided into three groups: group A ($n = 20$), with diffuse CD in more than 10 lashes on the upper lid (Fig. 1A); group B ($n = 12$), with sporadic CD in less than 10 lashes on the upper lid (Fig. 1B); and group C ($n = 23$), with lashes with greasy scales (Fig. 1C) or clean lashes without greasy scale or CD (Fig. 1D). Group C was further divided into two subgroups: C1 ($n = 15$), persons who had never used lid hygiene and C2 ($n = 8$), those who had used a daily lid scrub with shampoo for the past year. Group A with diffuse CD could easily be identified under the lower magnification of slit lamp examination. In contrast, group B was not readily identified by the same method, but with the lid under high

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Supported in part by an unrestricted grant from the Ocular Surface Research and Education Foundation, Miami, Florida.

Submitted for publication March 3, 2005; revised April 6, 2005; accepted April 12, 2005.

Disclosure: **Y.-Y. Gao**, None; **M.A. Di Pascuale**, None; **W. Li**, None; **D.T.-S. Liu**, None; **A. Baradaran-Rafii**, None; **A. Elizondo**, None; **T. Kawakita**, None; **V.K. Raju**, None; **S.C.G. Tseng**, None

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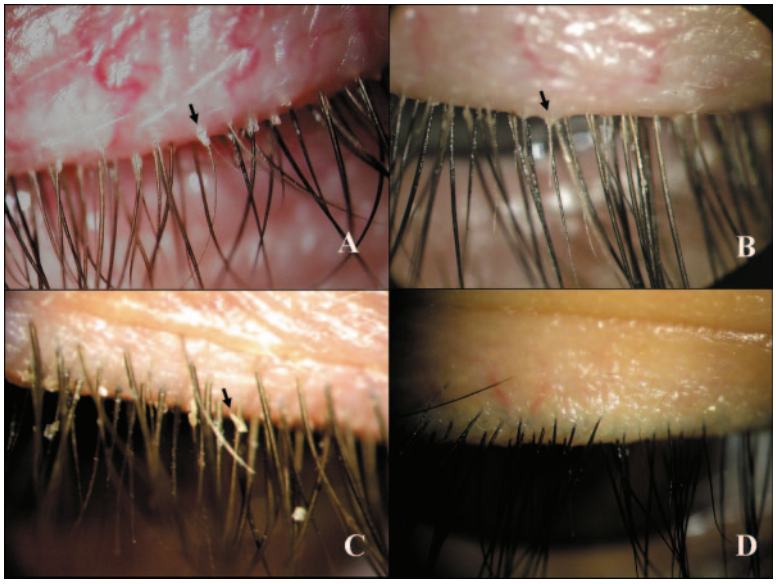

FIGURE 1. Representative photographs of eyelashes with diffuse CD (**A**), sporadic CD (**B**), noncharacteristic greasy scales (**C**), and clean lashes (**D**). CD (**A**, **B**, *arrows*) is contiguous at the base of the lash from the surface of the skin. In contrast, greasy scale is not, and can be randomly located on different parts of the lash trunk (**C**, *arrow*).

magnification, we could see less than 10 pieces of CD located on the upper lid.

Modified Method of Sampling and Counting *Demodex*

Under a slit lamp microscope (SL-2ED; Topcon, Tokyo, Japan) at a magnification of ×25, two lashes, one from each half of each lid, were removed by fine forceps and placed separately on each end of glass slides. Thus, eight lashes were prepared on four slides. For groups A and B, lashes with CD were intentionally selected. In group C, we chose lashes that were of a different color and brittle, as is characteristic of lashes that have a higher tendency to harbor *Demodex.*[11] In the conventional method, a drop of oil was added to the lash before a coverslip was mounted. Our preliminary study indicated such a maneuver frequently caused the free *Demodex* to float away from the lash, resulting in miscounting. Thus, we mounted a coverslip onto each lash before slowly pipetting 20 μL of saline to the edge of the coverslip to surround the lash (Fig. 2A), and we watched the procedure under the microscope. This maneuver resulted in preservation of the *Demodex* that had a loose contact with the lash at the tip (Figs. 2B–2D). Under the microscope, the status of the lash including the location of CD in relation to the follicle was recorded and photographed, and the number of *Demodex* was counted in a conventional manner. The results showed that the conventional counting method also caused miscounting if a compacted CD was preserved. Under the latter scenario, we pipetted 20 μL of 100% alcohol (Sigma-Aldrich, St. Louis, MO) into the edge of the coverslip, and prolonged the counting time up to 20 minutes to allow the embedded *Demodex* to migrate from the CD.

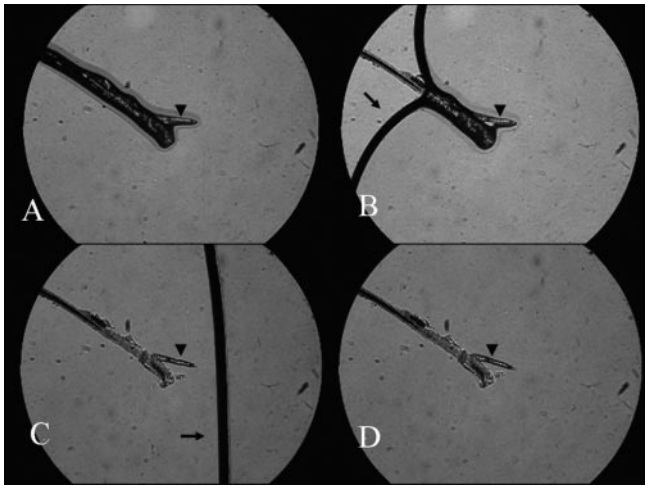

FIGURE 2. Modified mounting method. A coverslip was laid on a dry epilated lash (**A**). A *D. folliculorum* was loosely attached at the tip of the lash follicle (*arrowhead*). An aliquot of 20 μL of saline was slowly pipetted onto the left side of the coverslip (**B**, **C**, *arrow*), and the saline was allowed to spread to the field. This maneuver is important to avoid detaching this *Demodex* from the lash (**D**).

Statistical Analysis

Summary data are reported as the mean ± SD, compiled and analyzed on computer (Excel; Microsoft, Redmond, WA). The data between groups were evaluated by *t*-test. Test results were reported as two-tailed probabilities, with $P < 0.05$ considered statistically significant. The differences in incidence were evaluated by the Fisher exact test, with $P < 0.05$ again regarded as statistically significant.

RESULTS

The results of key demographic information and *Demodex* count are summarized in Table 1. The average age of group A patients was 59.9 ± 12.9 years, which was significantly older than group B patients (41.1 ± 10.6 years; $P < 0.001$). The average age of subgroup C1 patients was 40.8 ± 8.5 years, which was significantly younger than those in subgroup C2 (58.5 ± 13.7 years; $P = 0.006$). There was no difference in age between group C1+C2 and group A+B ($P = 0.09$). In group C2 ($n = 8$), lid hygiene with shampoo was prescribed by ophthalmologists for two patients, to treat meibomian gland dysfunction, and for three patients, for lash dandruff and sticky eyelids noted in the morning on awakening. Three patients acquired knowledge of lid hygiene from the Internet after experiencing stickiness and dryness of the eye. Symptoms recurred in three patients when lid hygiene was stopped for 2 to 3 days.

Sources of Counting Errors

Despite the fact that CD was identified in group A and B by slit lamp examination (Figs. 1A, 1B) and epilation was performed on those lashes with CD by our modified method, CD was not

TABLE 1. Summary of *Demodex* Counts in the Study Groups

Group (Sample Size)	Age (y)	Lash Count					*Demodex* Count per Lash			*Demodex* Count per Patient	Prevalence of *Demodex* in Patients (%)
		Total Epilated Lashes	Retain CD	Retain CD with *Demodex* (%)	Did Not Retain CD	Did Not Retain CD but Had *Demodex* (%)	CD In Lashes	No CD In Lashes	Total		
A ($n = 20$)	59.9 ± 12.9	160	80	72/80 (90)	80	7/80 (8.7)	4.1 ± 1.0	0.2 ± 0.5	2.2 ± 2.7	17.3 ± 4.2	20/20 (100)
B ($n = 12$)	41.1 ± 10.6	96	28	25/28 (89)	68	6/68 (8.8)	2.0 ± 1.2	0.2 ± 0.4	0.70 ± 2.1	5.6 ± 2.8	12/12 (100)
A + B ($n = 32$)	51.2 ± 11.1	256	108	97/108 (90)	148	13/148 (8.8)	3.3 ± 3.3	0.2 ± 0.4	1.6 ± 2.9	12.9 ± 3.3	32/32 (100)
C1 ($n = 15$)	40.8 ± 8.5	120	0	0	120	1/120 (0.8)	0	0.01 ± 0.09	0.01 ± 0.09	0.07 ± 0.26	1/15 (6.7)
C2 ($n = 8$)	58.5 ± 13.7	64	0	0	64	6/64 (9.4)	0	0.12 ± 0.41	0.12 ± 0.41	0.9 ± 0.8	4/8 (50)
C1 + C2 ($n = 23$)	47.6 ± 9.2	184	0	0	184	7/184 (4.7)	0	0.05 ± 0.23	0.05 ± 0.23	0.35 ± 0.65	5/23 (22)

FIGURE 3. Different amounts of CD fragments were retained in epilated lashes. In contrast to a normal clean lash without CD (**A**), fragments of CD were found at locations other than the follicle (**B–D**) after epilation. In some epilated lashes, no CD fragment was retained but free *D. folliculorum* (*arrow*) was preserved near the follicle (**E**), using our modified method (also see Fig. 2).

completely preserved in each epilated lash. Indeed, after epilation, 50% ($n = 160$) and 29% ($n = 96$) of the lashes retained CD fragments in groups A and B, respectively (Table 1). Even if fragments of CD were retained in epilated lashes, they were adherent at different locations in the follicle (Fig. 3). Free *Demodex* could occasionally be detected in the lashes without CD after epilation (Fig. 3E). Some lashes with fragments of CD appeared devoid of *Demodex* when observed under the microscope (Figs. 4A, 4C). However, after 100% alcohol was added to soften compacted CD and to stimulate *Demodex*, we observed more *Demodex* migrate from the CD fragment during the 20-minute period of observation (Figs. 4B, 4D, respectively). In some lashes with more intact CD, this modified method disclosed a family of an egg, larvae that had three to four pairs of poorly developed legs and a slender body, and an adult with four pairs of well-developed legs and a stumpy body, that had been hidden in the compacted CD (Fig. 4E). Furthermore, epilation may not have removed CD with the lash (Fig. 5). We frequently found the remaining CD still attached to the lid skin (Fig. 5A). In such a situation, if we removed the remaining CD from the lid skin and treated with 100% alcohol,

FIGURE 4. Modified counting method with added alcohol. In some epilated lashes with CD fragment, no *Demodex* was discerned (**A**, **C**, *insets:* images taken at lower magnification), although a suspected *Demodex* head was noted near the edge of the CD fragment (**C**, *arrow*). Nevertheless, 20 minutes after adding 100% alcohol, a group of *Demodex* moved out of the compacted CD on both of these two lashes (**B**, **D**). In another example, after the CD was completely dissolved by 100% alcohol, a family of *D. folliculorum*, including an egg (*arrowhead*), two larvae which had three to four pairs of poorly developed legs and a slender body (*arrows*), and an adult with four pairs of well-developed legs (*) and a stumpy body were revealed adjacent to the lash follicle (**E**).

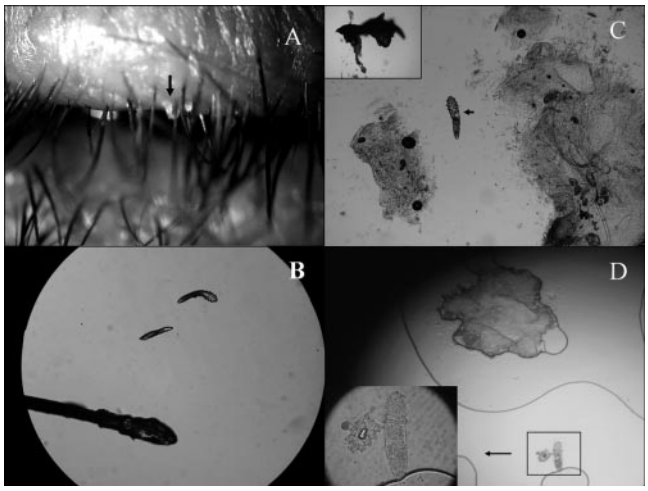

FIGURE 5. *Demodex* in the remaining CD after epilation. Most CD after epilation was left on the skin surface (**A**, *arrow*). The epilated lash was later found to be free of CD and there were two free *Demodex* nearby (**B**). Nevertheless, after the remaining CD was removed and treated with 100% alcohol, a free *D. folliculorum* (*arrow*) was found in the midst of the cellular debris (**C**, *inset*: lower magnification of remaining CD taken from the skin). In another example, fragments of a *Demodex* body and a dead *Demodex* were found in the remaining CD (**D**, *inset*: higher magnification).

we were able to detect both intact (Fig. 5C) and fragmented (Fig. 5D) *D. folliculorum*.

Correlation of *Demodex* Counts and Lashes with CD

Therefore, we adopted the aforementioned modified method to sample and count *Demodex* in 55 patients. When we grossly divided them into those with clinically evident CD (groups A+B) and those without (group C), we noted that *Demodex* was detected in all 32 (100%) patients in the former groups and in 5 (22%) of 23 patients in the latter. The difference was statistically significant ($P < 0.001$), indicating that *Demodex* infestation was more prevalent in patients with clinically evident CD. The actual count of *Demodex* per patient, on average, was 12.9 ± 3.3 *Demodex* mites in groups A+B, which was more than 30-fold higher than in group C (0.35 ± 0.65; $P < 0.001$). When we calculated the number of *Demodex* per lash, we noted that the average was 1.6 ± 2.9 *Demodex* in groups A+B, also significantly higher than in group C (0.05 ±0.23; $P < 0.001$). These data collectively indicated that there was a significant quantitative difference in *Demodex* infestation between those with clinically evident CD and those without.

Among the 422 specimens studied, we found 5 *D. brevis* scattered in five different patients, three in group A and two in group B (Fig. 6). These *D. brevis* mites had an evenly distributed head–body ratio which was significantly different from that of *D. folliculorum* (Fig. 4E), and *D. brevis* were found singly, seldom trapped inside CD.

Because clinical examination showed that group A had diffuse and group B sporadic CD, we then compared the *Demodex* count separately between these two groups. Although *Demodex* was detected in 20 (100%) of 20 and 12 100%) of 12 in groups A and group B, respectively, the average *Demodex* count per patient was 17.3 ± 4.2 and 5.6 ±2.8, and the average *Demodex* count per lash was 2.2 ± 2.7 and 0.70 ±2.1, in group A and group B, respectively. Such differences were significant ($P < 0.001$), indicating that the extent of *Demodex* infestation correlated with the clinical severity of CD.

Because CD was not completely retained in the epilated lash, even if the lash with clinically evident CD was intentionally removed (Figs. 3, 5), we thus compared the *Demodex* count between lashes retaining CD and those not retaining CD in groups A+B. *Demodex* was detect in 97 (90%) of 108 of the former lashes, but in 13 (8.8%) of 148 of the latter lashes—a 10 fold difference ($P < 0.001$). The *Demodex* counts were 4.1 ± 1.0 and 2.0 ± 1.2 per epilated lash with retained CD, significantly higher than the 0.2 ± 0.5 and 0.2 ± 0.4 per lash without retained CD in groups A and B, respectively (each $P < 0.001$), and than 0.01 ± 0.09 and 0.12 ± 0.41 per lash in subgroups C1 and C2, respectively (each $P < 0.001$). These results further indicated that *Demodex* was highly prevalent in lashes with CD, and the prevalence correlated with the clinical severity of CD.

Although all patients in group C had clean lashes without CD by clinical examination, subgroup C1 did not practice lid hygiene, whereas subgroup C2 did. Therefore, we wanted to compare the *Demodex* count between these two subgroups. *Demodex* was detected in 6.7% ($n = 15$) of the patients in subgroup C1 and in 50% ($n = 8$) in subgroup C2 ($P = 0.03$). The *Demodex* count per patient was 0.07 ± 0.26 in subgroup C1, significantly less than the 0.9± 0.8 in those in subgroup C2 ($P = 0.03$). The *Demodex* count per lash was 0.01 ± 0.09 in subgroup C1, which was also significantly less than the 0.12 ± 0.41 in subgroup C2 ($P = 0.03$). Collectively, these data indicate that even if the lashes did not show clinically evident CD, patients who routinely practiced lid hygiene had a higher prevalence of *Demodex* infestation than those without lid hygiene. This counterintuitive result will be discussed further.

DISCUSSION

The conventional method of counting *Demodex* involves random epilation of four nonadjacent lashes per lid and addition of a drop of oil (peanut oil is preferred) before mounting with a coverslip.[4] This method carries the potential for the following five errors. First, because the chance of detecting *Demodex* was much higher by sampling those with CD when compared with those without CD (Table 1), random epilation of lashes may result in a lower count if lashes without CD are epilated. Second, addition of oil before mounting the coverslip may induce undercounting, by allowing nonadherent *Demodex* to float away, especially in those lashes without retained CD fragments (Fig. 3E). Third, even if lashes with CD were intentionally epilated, different amounts of CD fragments were ac-

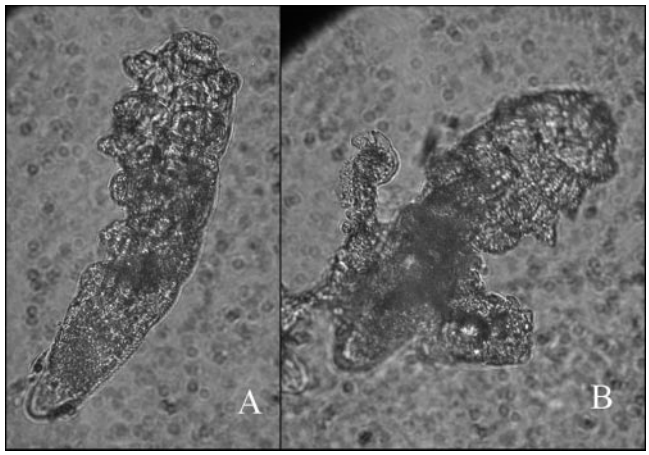

FIGURE 6. *D. brevis* found during lash sampling. The *D. brevis* mite has an evenly distributed head–body ratio that is significantly different from *D. folliculorum* (Fig. 4E). *D. brevis* was found singly, seldom trapped inside CD. (**A**) Dorsal and (**B**) ventral views.

tually retained (Fig. 3). Fourth, *Demodex* embedded in compact CD fragments could not be counted with accuracy without adding alcohol (Fig. 4). Fifth, even if only those lashes with clinically evident CD were epilated, some CD fragments that harbored *Demodex* still adhered to the lid skin (Fig. 5). These potential errors collectively explain why use of the conventional method could lead to miscounting of *Demodex*.

Accordingly, we modified the sampling and counting method, as described herein. In brief, we intentionally epilated those lashes with CD, put coverslips over them, and observed them by microscope. If no CD was discerned or CD was loose and *Demodex* could be easily discerned, saline was pipetted at the edge of the coverslip, and the counting was performed in a conventional way. If there was compacted CD, 100% alcohol was added, and the observation time was prolonged for up to 20 minutes to allow alcohol to dissolve the CD and stimulate live *Demodex* to migrate. Using this modified method, we found *Demodex* in all 32 patients (100%) with clinically evident CD. This prevalence was significantly higher than the 22% ($n = 23$) found in those in group C without clinically evident CD ($P < 0.001$). *Demodex* was 10 times higher in epilated lashes with CD fragments than in those without. This prevalence is notably higher than that reported by Norn[10] who observed "mites having been four times more often in the follicles of such lashes than in those of cylinder free lashes." Taken together, these results lead us to conclude that the prior controversy[12] resulted from potential errors in sampling and counting *Demodex*. Furthermore, they also explain why English[11] found that "the incidence of the mites often depends on the number of lashes epilated and the experience of the observer in the technique of examination." Using the modified method, we believe that selection of two, rather than four, lashes per lid is sufficient to achieve a meaningful sampling for *Demodex* counting.

Because the *Demodex* count per lash and per patient in group A, which had diffuse CD, was significantly higher than that in group B, which had sporadic CD, we also conclude that patients with more clinically evident CD tend to have more severe *Demodex* infestation. Recognizing that CD was not completely retained in epilated lashes even though only lashes with clinically evident CD were selectively sampled (Figs. 3 5), we found that the *Demodex* count per lash in group A was still significantly higher than that in group B for lashes retaining CD. Taken together, these results disclose that the clinical severity judged by lashes with CD correlates well with higher *Demodex* infestation. We thus concur with previous reports[4,10,11] that clinical manifestation of CD is pathognomonic for *Demodex* infestation.

Our studies also showed that *Demodex* is abundantly embedded in compacted CD, and CD is not always completely removed with the lash during epilation. These observations are consistent with Coston's observation that "[although] those (*Demodex*) which happen to hold so tightly as to come out with the lash are seen, many more may be left in the follicle."[4] Furthermore, our observations are consistent with the histologic findings of English[11] that CD consists mostly of keratins and lipids and that the infested follicles show distension and epithelial hyperplasia with an increase in keratinization adjacent to the claws of the mite.[1] This is why it is important to add an immersion solution such as alcohol to dissolve the CD/*Demodex* complex formed by keratins and lipids to achieve more accurate *Demodex* counting. Further improvement of the counting method should be directed to removing all of the CD during epilation.

D. folliculorum is frequently found in the lash follicle. Although *D. brevis* was also found in the lash sampling,[3] it was not mentioned in studies of *Demodex*-related blepharitis.[1,2,4–7,9,11] In this study, we found that *D. brevis* was present singly and not trapped in CD. Future studies are needed to determine the pathologic role of *D. brevis*.

There was a decrease in *Demodex* infestation between group A and group B (Table 1) that correlated with age. This finding resembled prior observations made by Norn,[10] who noted that *Demodex* prevalence increases with age. Thus, we speculate that if left untreated, *Demodex* infestation deteriorates with age, presumably through progressive propagation and spread of its population despite a short life cycle of 2 to 3 weeks. Judging from the sites of *Demodex* infestation—that is, the lash root and the meibomian gland—it is plausible that *Demodex* infestation causes blepharitis and contributes to ocular surface irritation. This speculation is also inferred by the comparison of subgroup C1 with subgroup C2. Patients in subgroup C2 had been treated with lid hygiene for at least 1 year because of the clinical diagnosis of either blepharitis or meibomian gland dysfunction or because of ocular discomfort. Although lashes were free of CD by slit lamp examination, the *Demodex* count and prevalence in subgroup C2 were still significantly higher than in subgroup C1. Because *Demodex* infestation could still be detected when clinically evident CD was absent in subgroup C2, we also believe that our modified sampling and counting method is valuable for detecting "subclinical" *Demodex* infestation.

Because the average age of patients in subgroup C2 was significantly older than that of those in subgroup C1 ($P = 0.006$), we believe that subgroup C2 may have had CD with much higher *Demodex* infestation before lid hygiene. The reason that lashes without clinically evident CD were still infested with *Demodex* in subgroup C2 could be in part that CD formed in the area close to the follicle and was buried under the skin (Fig. 3). Because lid hygiene was beneficial in reducing patients' symptoms and its discontinuation led to relapse of symptoms in some patients in subgroup C2, we strongly suspect that *Demodex* infestation is pathogenic and that its pathogenicity is dictated in part by the amount of infestation. The finding that *Demodex* could still be detected in 50% of subgroup C2 patients suggests that the technique of lid hygiene was inconsistently practiced among different patients. In addition, lid hygiene using shampoo cleans only CD extending outside the skin, but does not eradicate *Demodex* buried deep under the skin. If the latter is the cause of the problem, it would be desirable to develop a more effective therapy, not solely by cleansing but rather by killing *Demodex* buried deep in the follicle. Furthermore, a prospective and long-term study in a larger population may be needed to determine whether *Demodex* infestation should be controlled, if not eradicated, at an earlier age when there is still no irreversible damage to the lashes and meibomian glands.

Acknowledgments

The authors thank Hua He, Ching-Liang Kuo, and Armand Hornia for oversight of the project and comments on the manuscript.

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