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 into 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. Several groups have concluded that Demodex infestation leads to blepharitis 5-9. 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. 1,2 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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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. If 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).

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

<table>
<thead>
<tr>
<th>Group (Sample Size)</th>
<th>Age (y)</th>
<th>Total Epilated Lashes</th>
<th>Retain CD</th>
<th>Demodex Count per Lash</th>
<th>Did Not Retain CD</th>
<th>Did Not Retain CD but Had Demodex Count</th>
<th>Demodex Count per Lash</th>
<th>Prevalence of Demodex in Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 20)</td>
<td>59.9 ± 12.9</td>
<td>160</td>
<td>80</td>
<td>72/80 (90)</td>
<td>80</td>
<td>7/80 (8.7)</td>
<td>4.1 ± 1.0</td>
<td>20/20 (100)</td>
</tr>
<tr>
<td>B (n = 12)</td>
<td>41.1 ± 10.6</td>
<td>96</td>
<td>28</td>
<td>25/28 (89)</td>
<td>68</td>
<td>6/68 (8.8)</td>
<td>2.0 ± 1.2</td>
<td>12/12 (100)</td>
</tr>
<tr>
<td>A + B (n = 32)</td>
<td>51.2 ± 11.1</td>
<td>256</td>
<td>108</td>
<td>97/108 (90)</td>
<td>148</td>
<td>13/148 (8.8)</td>
<td>3.3 ± 3.3</td>
<td>32/32 (100)</td>
</tr>
<tr>
<td>C1 (n = 15)</td>
<td>40.8 ± 8.5</td>
<td>120</td>
<td>0</td>
<td>0 (90)</td>
<td>120</td>
<td>1/120 (0.8)</td>
<td>0.01 ± 0.09</td>
<td>1/15 (6.7)</td>
</tr>
<tr>
<td>C2 (n = 8)</td>
<td>58.5 ± 15.7</td>
<td>64</td>
<td>0</td>
<td>0 (90)</td>
<td>64</td>
<td>6/64 (9.4)</td>
<td>0.12 ± 0.41</td>
<td>4/8 (50)</td>
</tr>
<tr>
<td>C1 + C2 (n = 23)</td>
<td>47.6 ± 9.2</td>
<td>184</td>
<td>0</td>
<td>0 (90)</td>
<td>184</td>
<td>7/184 (4.7)</td>
<td>0.05 ± 0.23</td>
<td>5/25 (22)</td>
</tr>
</tbody>
</table>

FIGURE 3. Modified counting method with added alcohol. In some epilated lashes with CD fragment, no Demodex was discerned (A, C, E). Demodex head was noted near the edge of the CD fragment (C). Nevertheless, 20 minutes after adding 100% alcohol, a group of most epilated lashes (B) was preserved in each epilated lash. Indeed, after 100% alcohol was added to soften CD and stimulate migration, CD fragments in groups A and B were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash (Fig. 3C). Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1). Even if fragments of CD were retained in epilated lashes, they were completely preserved in each epilated lash. Indeed, after epilation, some epilated lashes without CD fragments were retained in epilated lashes (Table 1).
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. 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-

![Image](https://via.placeholder.com/150)

**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).

**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 52 (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.

![Image](https://via.placeholder.com/150)

**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 discarding 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 = 25) 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 Norn10 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 controversy12 resulted from potential errors in sampling and counting Demodex. Furthermore, they also explain why English11 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 reports4,10,11 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 English11 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 I) 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 eradicating 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.

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