



## Peer Review File

Article information: <http://dx.doi.org/10.21037/atm-20-7715>

### Reviewer comments

#### Reviewer A

**Comment 1:** This is a prospective study comparing two groups of patients with Demodex infestation depending on age. In the title it should be mentioned that both *Demodex folliculorum* and *Demodex brevis* are investigated in this study.

**Reply:** Thank you for your comment. As mentioned in Introduction, *Demodex folliculorum* and *Demodex brevis* are the only two demodex species affecting human. In the title “demodex profile” means the different distribution of *Demodex folliculorum* and *Demodex brevis*.

**Changes in the text:** N/A

**Comment 2:** The abstract is not clear, it does not reflect all the results described.

**Reply:** We revised the abstract to make it clearer and to include all important results.

**Changes in the text:** We added the result as advised (see Page 3, line 26-Page 4, line 56).

**Comment 3:** What do you mean by SPK in describing corneal changes in the Methods section (line 122)? In the abstract only *D. brevis* is mentioned.

**Reply:** SPK is the abbreviation for “superficial punctate keratopathy”, a mild form of corneal change. We added this full name when it first appeared in the text. *Demodex folliculorum* have been mentioned in the revision.

**Changes in the text:** We added the full name of SPK and the results of *Demodex folliculorum* as advised (see Page 9, line 136)

**Comment 4:** The results of this study are controversial: The percentage of corneal changes is very high - 73.7%. The prevalence of *D. brevis* is surprisingly low in older patients-only 4%.

**Reply:** Corneal changes were detected in 73.7% patients in the young group, which is significantly higher than that in elderly group. The prevalence of *D. brevis* is 2 (1,3) in young patients, which is higher than that in older patients as 0(0, 2) (Page 9, line 148, table1). Multivariate ordinal logistic regression revealed that higher *D. brevis* % was positive associated with corneal changes severity in the young group.

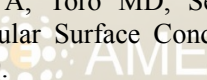
**Changes in the text:** N/A

**Comment 5:** Later it is written “Both *D. folliculorum* and *D. brevis* were detected in 48.9% (45/92) and 78.0% 152 (71/91) of elder and younger groups respectively.” But in the demographic data it is written “Among 183 patients (73 males, 110 females) with ocular demodicosis..”. What was the basis for diagnosis of demodicosis? The prevalence should be 100%.

**Reply:** We meant mix infestation of two types of demodex mites, i.e., both *D. folliculorum* and *D. brevis* were *simultaneously* detected in 48.9% elder patients and 78.0% younger patients. The other patients with ocular demodicosis had either *D. folliculorum* or *D. brevis*. Those symptomatic patients with positive *demodex* count were diagnosed as ocular demodicosis. (see reference 20, 21)

**Changes in the text:** N/A

**Comment 6:** Please cite the study:

Nowomiejska K, Lukasik P, Brzozowska A, Toro MD, Sedzikowska A, Bartosik K, Rejdak R. Prevalence of Ocular Demodicosis and Ocular Surface Conditions in Patients Selected for Cataract Surgery. J Clin Med. 2020 Sep 23;9(10):3069. 

**Reply:** Thank you and we did so in the revision.

**Changes in the text:** We have cited this paper as ref 13 in the text. (Page 5, line 67, Page 13, line 233)

## Reviewer B

**Comment 1:** Abstract section: this section is too long and too descriptive. The study aims are presented but rationale for the study is lacking. The only most important findings should be included here.

**Reply:** Thank you and we added the rationale in the revised abstract as “*Demodex* infestation is highly age-dependent. Intriguingly, our previous studies that focused on children and young adult patients suggested that the clinical features of young patients were different from those published studies enrolling mainly elderly patients. Whether age plays a role between young and elderly patients with ocular demodicosis remains unclear”. We also modified the abstract to make it clearer and more straight forward.

**Changes in the text:** We have modified our text as advised (Page 3, line 28-32).

**Comment 2:** Section: Material and methods. My major concerns are related to the methodology. I am not very convinced by your division into age groups; rather, patients should be divided into more age groups, because the difference in healthy status between people aged 35 and 45 is smaller than between 45 and 81. Why did you choose that groups? What about the group of patients aged between 35 and 45? Do you really think that person aged 45 is old and can be compared with someone who is 60 or 80?

**Reply:** Thank you and we agree that more age groups may provide more detailed findings. However, much larger sample size would be needed for more age subgroups. To mitigate the concern that the demodex infestation is age-related and much more prevalent in the elders, our previous studies focussed on patients younger than 35 years (Liang L, et al. Significant correlation between meibomian gland dysfunction and keratitis in young patients with *Demodex brevis* infestation. *BJO*, 2018. Liang L, et al. High prevalence of demodex brevis infestation in chalazia. *AJO*, 2014. Liang L, et al. Ocular demodicosis as a potential cause of pediatric blepharoconjunctivitis. *Cornea*, 2010.) That was why we arbitrarily choose 35 years. We did agree that the difference between 35 years and 45 years might not be that significant, therefore, those aged 35-45 years were not included in present study. This limitation of our study is now mentioned in the revision.

**Changes in the text:** The age groups were chosen relatively arbitrary in present study. (Page 16, line 278-279)

**Comment 3:** Line 93: exclusion criteria should be better specified: what kind of ocular and systemic disease did you mean, please clarify

**Reply:** Patient with ocular diseases other than ocular demodicosis and age-related cataract were excluded in the elderly group. Any systemic disease was also excluded.

**Changes in the text:** We added it in method. (Page 7, line 101-104)

**Comment 4:** Line 101: how demodex mites were counted? Did you count eggs and larves also? Please clarify.

**Reply:** *Demodex* was counted as reported before (Page 8, line 109- Page 7 line 116, see reference 8, 16,17). Two lashes, one from each half of each lid, were removed by fine forceps and placed separately on each end of glass slides. Lashes with CD (scales that form clear cuffs collaring the lash root) were explicitly selected, if available because random epilation of lashes might result in lower counts than those lashes with CD.

We did count eggs and larva. However, because we cannot differentiate *D. brevis* from *D. folliculorum* in eggs and larva, and in fact eggs and larva were only a minority, so we did not include eggs and larves in analyses.

If eggs and larva were included, the total counts were [7 (5, 10) in young group vs. 8 (5, 14) in elderly group,  $P=0.280$ ]. The result remains the same after excluding eggs and larva as [6 (4, 8) in young group vs. 7 (4, 13) in elderly group,  $P=0.176$ ] as shown in the manuscript (see Page 10, line 159-160).

**Changes in the text:** N/A

**Comment 5:** Lines 103-105: the authors included patients with diagnosed ocular demodicosis or asymptomatic individuals also? Patients were diagnosed as positive when they had more than three mites. What about patients who were symptomatic, were diagnosed with the disease and had two mites in microscopic examination, were they excluded? How exactly the patients were selected for the study, were they randomized?

**Reply:** Those symptomatic patients with positive *Demodex* count were diagnosed as ocular demodicosis. *Demodex* species and counts were recorded and whose total counts greater than or equal to 3 in 8 lashes were considered as *Demodex*-positive. Since *Demodex* can also be found in asymptomatic population, we did not include asymptomatic individuals. If a patient only had two mites, we'll further look for the exact hiding causes of symptoms or repeat the lash sampling. But in our study, we ruled out such ambiguous cases. We enrolled patients seen in our clinic and divided them into two groups according to their age, not randomized.

**Changes in the text:** N/A

**Comment 6:** Line 108- reference is lacking

**Reply:** Thank you, we have added the reference in the revision.

**Changes in the text:** We added the reference as advised. (Page 8, line 121)

**Comment 7:** Section: Result. Lines 144-152: This part of the text should be reformulated, it is unclear. Please explain what the numbers in brackets mean? What did you mean: “The *Demodex* count...and then *D. brevis* count.

**Reply:** We reformulated the sentences as “The total *Demodex* count was comparable between young and elder patients [6 (4, 8) vs. 7 (4, 13),  $P=0.176$ ]. However, the *D. brevis* counts and the percentage of *D. brevis* count to total *Demodex* count (*D. brevis* %) of the young group was significantly higher than that of the elderly group [2 (1, 3) vs. 0 (0, 2), 43% (25%, 100%) vs. 4% (0, 21%), both  $P<0.001$ , Table 1]. In contrast, *D. folliculorum* was more dominant in the elder group [6 (3, 10) vs. 3 (2, 6), 96% (73%, 100%) vs. 57% (40%, 82%), both  $P<0.001$ , Table 1].”

**Changes in the text:** We reformulated the sentences. (Page 10, line 159-165)

**Comment 8:** Lines 147- 148: the authors stated... “percentage of *D. brevis* to total *Demodex* (*D. brevis* %) of the young group was significantly higher than that of the elderly group [43% (25%, 100%) vs. 4 (0, 21%)...” Total *Demodex* – did you mean *D. folliculorum* and *D. brevis* or did you mean total counts? The 43% vs. 4 %? Please clarify

**Reply:** As mentioned in Introduction, *D. folliculorum* and *D. brevis* are the only two types of demodex mites affecting human. Total *Demodex* count=*D. folliculorum* count + *D. brevis* count. The *D. brevis*% meant the *D. brevis* count/total *Demodex* count in each person. The median *D. brevis*% in young patient was 43%, and the median *D. brevis*% in elderly patient was 4%.

**Changes in the text:** N/A

**Comment 9:** How many patients were with mixed infestation, with *D. folliculorum* and *D. brevis* as in your presented case. Were they excluded from the study? They were included in the group with *D. brevis* infestation?

**Reply:** Mixed infestation in our study was 48.9% (45/92) in the elder group and 78.0% (71/91) in younger groups (Page 10 line 165-167). They were NOT excluded. They were counted when we analyzed *D. brevis* or *D. folliculorum* infestation.

**Changes in the text:** We modified the sentence as “Mixed infestation, i.e. both *D. folliculorum* and *D. brevis* were simultaneously detected in 48.9% (45/92) and 78.0% (71/91) of elder and younger groups, respectively.” (Page 10 line 165-167)

**Comment 10:** Discussion. The findings of the present study deserve to be discussed more deeply, emphasizing the differences from other studies/populations. In particular, how the authors explain the differences in the frequency of *D. folliculorum* and *D. brevis* in other studies, local phenomenon? In my opinion, due to the choice of methods, the results of the study may not be representative and conclusions should be formulated with caution.

**Reply:** Thank you for highlighting this limitation of our study. The participants enrolled in our study are patients seen in cornea department they might have more severe symptoms or signs than those went to general ophthalmologists or family doctors. This selection bias was now mentioned in discussion (page, line). The difference in *Demodex* counts among studies may be explained by the different numbers of epilated lashes in those studies.

**Changes in the text:** We added the limitation in text. (Page 16, line 279-281)

**Comment 10:** References: should be updated, the only six (20%!) cited studies were published in the last five years.

**Reply:** We updated the references and included more recent studies in the revision.

**Changes in the text:** We updated the references in the text. (Page 17 to 18)

### Response to Reviewer #3:

**Comment 1:** Please specify the inclusion criteria in clinical settings. For example, we would like to know more about the clinical signs and symptoms that would prompt the investigators to perform a lash sampling and microscopic counting of *Demodex* mite.

**Reply:** The inclusion criteria were the patient diagnosed as ocular demodicosis. (page 7, line 106- Page 8, line 117) There is no highly specific symptoms of demodicosis. However, common symptoms include ocular discomfort such as dryness, foreign body sensation, itching, light sensitivity, eye fatigue, and redness. The most significant signs are cylindrical dandruff at eyelash roots. Patients presented with above mentioned clinical features would be subjected to further lash sampling and microscopic counting

of *Demodex*.

**Changes in the text:** We added the suggestive clinical signs and symptoms in method. (Page 8, line 106-108)

**Comment 2:** P. 6, Line 96 and P. 7, Line 101: demodex should be “D”emodex.

**Reply:** Thank you and we corrected those in the revision.

**Changes in the text:** We have modified the word as advised. (Page 8, line 113)

**Comment 3:** P. 7, Line 102-104: The purpose of this sentence should be clarified. Please specify if the sentence should read: Demodex species and counts for each patient were recorded and whose total counts greater than or equal to 3 in 8 lashes were recorded as Demodex-positive.

**Reply:** Thank you and we revised the sentence.

**Changes in the text:** We have modified this sentence as advised. (Page 8, line 114-116)

**Comment 4:** Please give the definition of eyelash disorder in the Methods section.

**Reply:** Lash disorders were defined as any of the following signs including trichiasis, madarosis, lash malposition, and cylindrical dandruff at the root of lashes.

**Changes in the text:** We have added this definition of eyelash disorder in Method section. (Page 8, line 127-128)

**Comment 5:** P. 6, Line 90-92. There seems to be a duplication of the text describing equal severity in both eyes.

**Reply:** Thank you and we have deleted the duplication text.

**Changes in the text:** We have deleted the duplication text as advised (Page 7, line 101-102 )