Clinical Ophthalmology

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Better detection of *Demodex* mites by Löffler's alkaline methylene blue staining in patients with blepharitis

This article was published in the following Dove Press journal:
Clinical Ophthalmology

Katsuji Kiuchi

Kiuchi Eye Clinic, Joto-ku, Osaka, Japan

Purpose: To determine whether the Löffler's alkaline methylene blue staining method is better than no staining in detecting *Demodex* mites in the eyelashes of patients with blepharitis.

Materials and methods: Eyelashes were collected from 22 patients with blepharitis. The mean age of the patients was 82.5±6.2 years (± SD) with a range from 71 to 93 years. Eyelashes were epilated by forceps and placed individually on microscope slides. The number of *Demodex* mites was determined by conventional optical microscopy before and immediately after the addition of the methylene blue staining solution.

Results: The mean *Demodex* count before the addition of the methylene blue solution was 2.9±2.9, and it was 4.4±3.9 after the addition of the methylene blue solution ($P<0.01$, Wilcoxon test).

Conclusion: The methylene blue staining method is a simple and useful method in detecting the presence and quantifying the number of *Demodex* mites. We recommend the methylene blue staining method not only for the diagnosis of the presence of *Demodex* mites but also to evaluate the therapeutic effects of medications to eliminate the mite infestation.

Keywords: *Demodex* mites, Löffler's alkaline methylene blue, blepharitis

Introduction

Blepharitis is one of the most common diseases encountered by ophthalmologists in their daily practice. One of the causes of severe marginal blepharitis is the presence of *Demodex* mites, which are the most common ectoparasites in humans. Among the many species of *Demodex, Demodex folliculorum* and *Demodex brevis* have been detected in humans. They can be found on the eyelids, eyelashes, meibomian glands, face, and external ear tract. The incidence of *D. folliculorum* is higher in patients with blepharitis than in normal individuals,[1] and the number of *Demodex* mites is highly correlated with the age of the individual.[2] For diagnosis, it is necessary to prove the existence of *Demodex* mites from an examination of epilated eyelashes of the patient.

Recently, Kojima et al[3] and Randon et al[4] reported on a method of counting the number of *Demodex* mites in situ by confocal microscopy without removing the eyelashes. However, there are many researchers who are still using the previous method of microscopic examination of epilated eye lashes. For this, the epilated eyelashes are placed on glass microscope slides and covered with a coverslip without adding any staining solution.[5–7] The slides are then examined by conventional microscopy. In another technique, Kheirkhah et al[8] reported adding fluorescein solution to detect and count the number of *Demodex* mites in the epilated eyelashes. This method has

Correspondence: Katsuji Kiuchi
Kiuchi Eye Clinic, 6-8-16,
Higashi-Nakahama Joto-ku,
Osaka 536-0023, Japan
Tel +81 6 6962 3736
Fax +81 6 6968 4984
Email kkiuchi1@yahoo.co.jp

http://dx.doi.org/10.2147/OPTH.S157910

increased the proficiency of detecting and counting the *Demodex* mites in epilated eyelashes compared to previous methods.[8]

We reported earlier on the use of the Löffler's alkaline methylene blue supravital staining method for diagnosing conjunctivitis.[9] It is possible to deduce the cause of the infection by the differential distribution of neutrophils and lymphocytes. However, this has not been done for *Demodex* infections.

Thus, the purpose of this study was to determine the applicability of the Löffler's alkaline methylene blue staining method for detecting and counting the number of *Demodex* mites in epilated eyelashes from patients with blepharitis.

Materials and methods

The Löffler's alkaline methylene blue (Muto Pure Chemicals Co, Ltd, Tokyo, Japan) solution is made by a 10-fold dilution of the original solution with saline, and it was filtered daily through Millex AP Filter, pore size 2.0 µm (Millipore: SLAP 02550, Merck KGaA, Darmstadt, Germany) before use.

To compare the effectiveness of Löffler's alkaline methylene blue solution staining to fluorescein staining, fluorescein solution was made by moistening a fluorescein strip (FUL-GLO; Akron, Buffalo Grove, IL, USA) with 1 drop of 0.9% NaCl solution.

Eyelashes were collected from 22 patients whose mean age was 82.5±6.2 years (± SD) with a range from 71 to 93 years (Table 1).

The procedures used in this study were approved by the Institutional Review Board of the Kiuchi Eye Clinic. The procedures used in this study conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients. All patients had blepharitis. Under 12× magnification of slit-lamp biomicroscopy, a total of 8 lashes were epilated randomly from the superior eyelids of both eyes by forceps and placed separately on the center of a glass microscope slide. Each eyelash was covered with a coverslip without adding any solution and examined by a conventional optical microscope. Later, one drop of the Löffler's alkaline methylene blue solution was placed at the edge of the coverslip, and the preparation examined by a conventional optical microscope immediately. The number of *Demodex* mites was counted by the same examiner first without and then with the methylene blue solution.

Wilcoxon tests were used to determine the significance of the differences of the number of *Demodex* mites before and after adding the Löffler's alkaline methylene blue solution.

Table 1 Characteristics of patients, diagnosis, and the *Demodex* count before and after the addition of the Löffler's alkaline methylene blue solution

Case	Age (years)	Sex	Diagnosis	Methylene blue (–)	Methylene blue (+)
1	85	Female	Blepharitis	0	0
2	89	Female	Blepharitis	1	1
3	83	Female	Blepharitis	1	1
4	84	Female	Blepharitis	1	1
5	85	Female	Blepharitis	1	1
6	87	Female	Blepharitis	1	1
7	83	Female	Blepharitis	3	3
8	77	Male	Blepharitis	1	1
9	82	Female	Blepharitis	1	2
10	93	Female	Blepharitis	7	13
11	89	Female	Blepharitis	2	4
12	88	Female	Blepharitis	3	5
13	82	Female	Blepharitis	2	6
14	77	Female	Blepharitis	2	3
15	81	Female	Blepharitis	0	3
16	71	Female	Blepharitis	11	13
17	84	Female	Blepharitis	3	6
18	69	Male	Blepharitis	3	4
19	75	Male	Blepharitis	9	11
20	77	Male	Blepharitis	5	8
21	83	Male	Blepharitis	4	6
22	91	Male	Blepharitis	2	3

Results

Photographs of *Demodex* mites attached to the eyelashes of a patient with blepharitis are shown in Figure 1. The photographs of the eyelashes in Figure 1A and C were taken without any staining, and the photographs in Figure 1B and D were taken after staining with the Löffler's alkaline methylene blue solution. The *Demodex* mites are easier to detect with the blue background.

The *Demodex* mites were also more easily detected in heavy opaque dandruff (▼; Figure 2) after the methylene blue staining. The *Demodex* count before the addition of the methylene blue solution was 2.9±2.9, and it was 4.4±3.9 (± SD) after the methylene blue staining ($P<0.01$; Figure 3).

The results of our study showed that adding the Löffler's alkaline methylene blue solution and fluorescein solutions significantly improved the contrast of *Demodex* mites in the epilated eyelashes. The better detection was due to the blue background provided by the Löffler's alkaline methylene blue solution and yellow background provided by fluorescein solution (Figure 4).

In 1 of the 22 cases of blepharitis, *Demodex* mites could not be detected (Case 1), and in 7 cases (Cases 2–8), the number of *Demodex* mites detected was the same with and

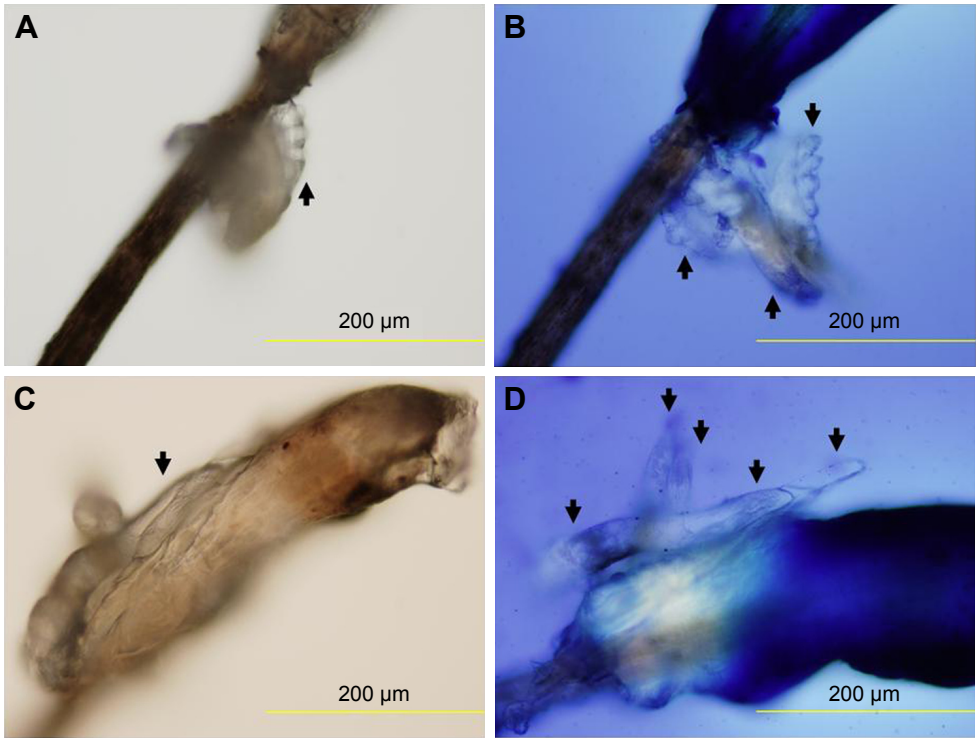

Figure 1 Photographs of eyelashes before and after the addition of the Löffler's alkaline methylene blue solution.
Notes: Before the addition of the methylene blue solution, only 1 *Demodex* mite can be seen on the eyelash (↑; **A** and **C**, respectively). After addition of the Löffler's alkaline methylene blue solution, 3 and 5 *Demodex* mites can be seen on the eyelash (↓; **B** and **D**, respectively). The *Demodex* mites are easily detected on the blue background of the methylene blue dye. Photographs taken by conventional optical microscope magnification at 200×.

without the methylene blue staining. However, in 14 cases (Cases 9–22), the *Demodex* mites could be detected better after methylene blue staining than without any staining (Table 1).

Discussion

Blepharitis generally has a good prognosis and responds well to antibacterial eye ointments or adrenal steroid-containing eye ointments in many cases. However, cases with severe blepharitis are resistant to these ophthalmic ointments, and it is possible that one of the causes of the infections is *Demodex* mites.

There are many reports on the treatments of *Demodex* infections. For example, the application of 4% pilocarpine HCL gel,[1,10] tea tree oil,[2,4,6,11] cleansing with baby shampoo,[10] antibiotic ointment,[5] and oral ivermectin in combination with topical permethrin have been reported to eliminate the *Demodex* mites.[7] In many cases, the number of *Demodex* mites count is evaluated by direct microscopic examination of the epilated eyelashes without adding any solution.

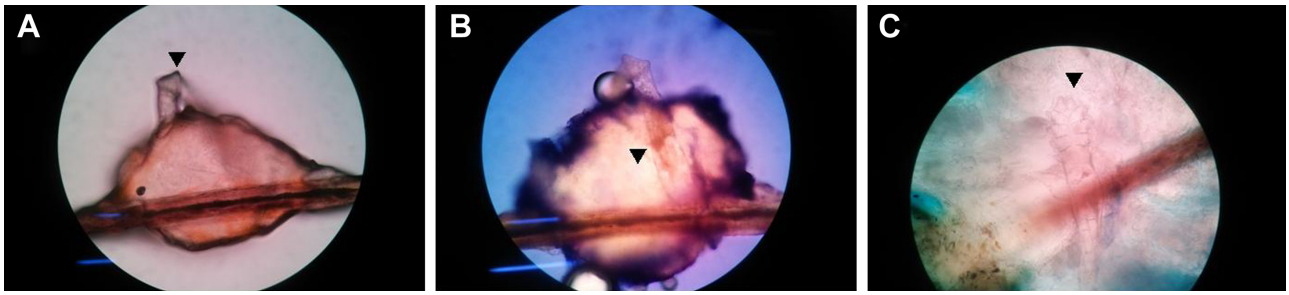

Figure 2 Images of *Demodex* mites (▼) in heavy opaque dandruff in a patient with blepharitis taken with a smartphone.
Notes: Photograph taken before (**A**) and after (**B**) the addition of the Löffler's alkaline methylene blue solution. The *Demodex* mite is more easily detected in the heavy opaque dandruff after the methylene blue staining (▼). In another case, the *Demodex* mite is more easily detected in heavy opaque dandruff (▼; **C**). The conventional optical microscope magnification is 400×, and the photograph has been magnified severalfold.

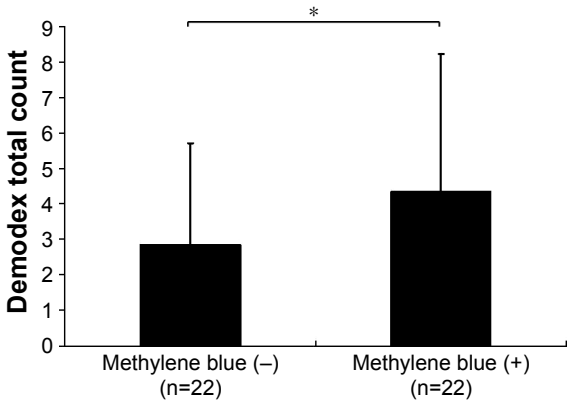

Figure 3 *Demodex* mites count before and after addition of Löffler's alkaline methylene blue solution.
Notes: The count of before the Löffler's alkaline methylene blue solution was 2.9±2.9, while the *Demodex* count of after Löffler's alkaline methylene blue is 4.4±3.9 (± SD; *$P<0.01$ Wilcoxon test).

Kheirkhah et al[8] reported that a more accurate count of the number of *Demodex* mites was obtained after the addition of fluorescein solution than without adding any solution. This was probably because of the yellow background provided by fluorescein solution improved the contrast.

The results of our study showed that adding the Löffler's alkaline methylene blue solution significantly increased the number of *Demodex* mites counted in the epilated eyelashes. This capability coupled with the blue background provided by the Löffler's alkaline methylene blue solution allowed a better visibility of the *Demodex* mites. Because eyelashes, *Demodex* mites, epithelial tissues, and eye discharges can be distinguished by their different colors, using the Löffler's alkaline methylene blue solution was probably the basis for detecting the *Demodex* mites more clearly than with the fluorescein solution (Figure 4).

One of the drawbacks of this procedure was that it was not possible to compare the results of the different types of stains, for example, fluorescein, methylene blue, and oil drops. In addition, the slides could not be preserved because the eyelashes were not fixed. However, as shown in Figure 2, digital images can be made with smartphones which will allow long-term preservation of the images.[12] These images can also be shown to a patient to obtain informed consent for treatments.[13] Further studies are required to compare the results of *Demodex* counting using the Löffler's alkaline methylene blue solution to those with other methods with the highest yield for *Demodex* detection in eyelashes of the patients with blepharitis.

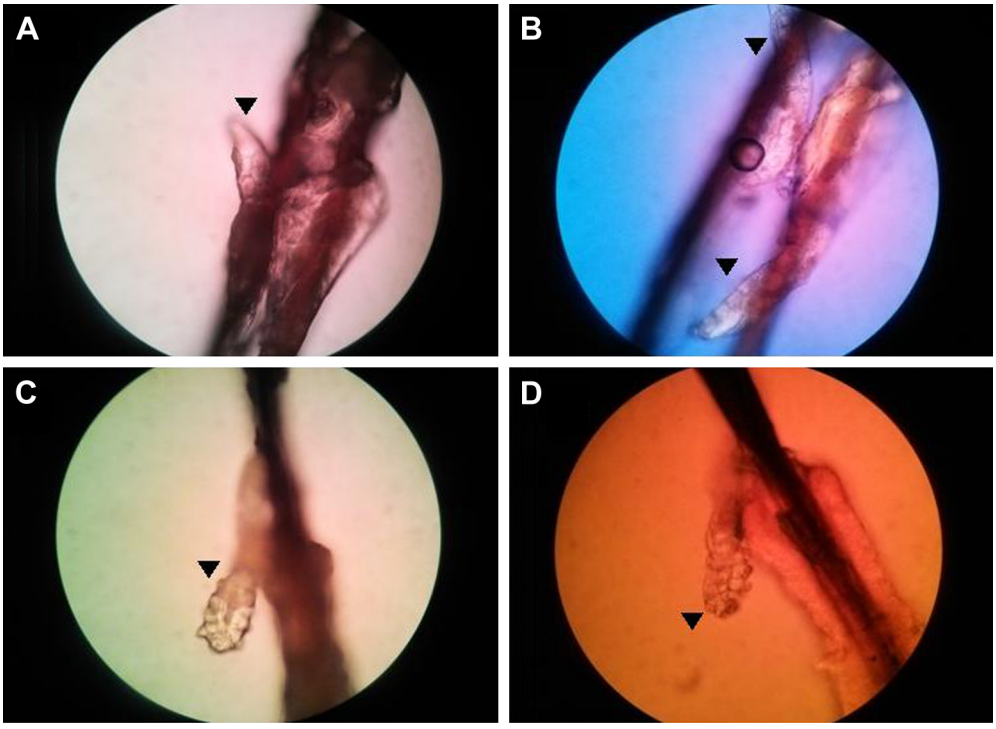

Figure 4 Comparison of the Löffler's alkaline methylene blue solution staining with fluorescein solution staining.
Notes: Photographs showing how not the addition or with the addition of the Löffler's alkaline methylene blue solution (**A** and **B**, respectively) and fluorescein solution affects the detection of the *Demodex* mites (**C** and **D**, respectively). Photographs of (**A**) and (**B**) are same eyelash, and (**C**) and (**D**) are same eyelash, respectively. Although eyelashes and *Demodex* mites (▼) were distinguished by their different colors provided by the Löffler's alkaline methylene blue solution, eyelash, *Demodex* mites, and background are stained yellow by the fluorescein solution. The conventional optical microscope magnification is 400× and image was taken with a smartphone, and the photograph has been magnified severalfold.

Conclusion

In conclusion, the use of the Löffler's alkaline methylene blue solution is simple and can be used for an easier detection of *Demodex* mites. It can be used not only for the diagnosis but also for the evaluation of the therapeutic effects of the treatment.

Disclosure

The author reports no conflicts of interest in this work.

References

1. Türk M, Öztürk I, Şener AG, Küçükbay S, Afşar İ, Maden A. Comparison of incidence of *Demodex folliculorum* on the eyelash follicule in normal people and blepharitis patients. *Türkiye Parazitol Derg*. 2007;31(4):296–297.
2. Koo H, Kim TH, Kim KW, Wee SW, Chun YS, Kim JC. Ocular surface discomfort and *Demodex*: effect of tea tree oil eyelid scrub in *Demodex* blepharitis. *J Korean Med Sci*. 2012;27(12):1574–1579.
3. Kojima T, Ishida R, Sato EA, et al. In vivo evaluation of ocular demodicosis using laser scanning confocal microscopy. *Invest Ophthalmol Vis Sci*. 2011;52(1):565–569.
4. Randon M, Liang H, El Hamdaoui M, et al. In vivo confocal microscopy as a novel and reliable tool for the diagnosis of *Demodex* eyelid infestation. *Br J Ophthalmol*. 2015;99(3):336–341.
5. Kawakita M, Kawashima M, Ibrahim O, Murato D, Tsubota K. [*Demodex*-related marginal blepharitis in Japan]. *Nippon Ganka Gakkai Zasshi*. 2010;114(12):1025–1029. Japanese.
6. Nicholls SG, Oakley CL, Tan A, Vote BJ. Demodex treatment in external ocular disease: the outcomes of a Tasmanian case series. *Int Ophthalmol*. 2016;36:691–696.
7. Damian D, Rogers M. *Demodex* infestation in a child with leukaemia: treatment with ivermectin and permethrin. *Int J Dermatol*. 2003;42(9):724–726.
8. Kheirkhah A, Blanco G, Casas V, Tseng SC. Fluorescein dye improves microscopic evaluation and counting of *Demodex* in blepharitis with cylindrical dandruff. *Cornea*. 2007;26(6):697–700.
9. Kiuchi K. Rapid alkaline methylene blue supravital staining for assessment of anterior segment infections. *Clin Ophthalmol*. 2016;10:1971–1975.
10. Inceboz T, Yaman A, Over L, Ozturk AT, Akisu Ç. Diagnosis and treatment of demodectic blepharitis. *Türkiye Parazitol Derg*. 2009;33(1):32–36.
11. Gao YY, Di Pascuale MA, Li W, et al. In vitro and in vivo killing of ocular *Demodex* by tea tree oil. *Br J Ophthalmol*. 2005;89(11):1468–1473.
12. Roy S, Pantanowitz L, Amin M, et al. Smartphone adapters for digital photomicrography. *J Pathol Inform*. 2014;5(1):24.
13. Kimura M, Enoki E, Maenishi O, Ito A, Chikugo T. [Capture of histopathological images by medical students using the digital cameras of cell phones and smart phones during histopathology classes]. *Igakukyouiku*. 2013;44(2):85–87. Japanese.