Author's response to reviews

Title: Histopathological Evaluation OF Ocular Microsporidiosis by different stains

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Version: 2 Date: 30 March 2006

Author's response to reviews: see over
March 30, 2006

Editor

BMC Clinical Pathology

Sub: MS: 6421603369057585 – Histopathological Evaluation Of Ocular Microsporidiosis by different stains

Dear Editor,

I am herewith submitting a resubmission of our manuscript after addressing the concerns of the reviewer for your kind perusal and consideration for publication in your esteemed journal.

REVIEWER 1: Comments

1. It is interesting that the authors use two stains that are not generally used in rendering the diagnosis, i.e., modified acid fast and acridine orange. Why were they chosen?

Reply: Modified acid fast stain (1% acid fast stain) have been previously used by many authors (Awadalla HN et al. J Egypt Soc Parasitol. 1998 Dec;28(3):729-38) and Though the use of acridine orange for the diagnosis of microsporidia has not been reported before we included it in our study as acridine orange has been reported to be useful in diagnosing Acanthamoeba, which is another protozoan parasite causing ocular infections. Moreover, we wanted to evaluate the entire spectrum of stains available in our laboratory that were commonly used in the diagnosis of microbial keratitis. We have also explained for the same in the introduction and discussion section of our manuscript.
2. They apparently omit the commonly used modified Brown-Brenn or Brown-Hopps Gram stains, others have gotten excellent results with these stains.

   **Reply**: The Gram stain used in our study was the same as Brown-Brenn and the same information has been changed in our manuscript.

3. Although, they address it in their Discussion section, it is not clear why the authors didn’t present the results of two common successful diagnostic preparations, i.e., scrapings and touch imprints. Otherwise the methods are generally appropriate and well defined.

   **Reply**: We have already published our results with corneal scrapings (Reference 17) and we were additionally interested in evaluating stains to help us to identify more number of clinically suspected cases in tissue sections wherein the corneal / conjunctival scrapings have failed to reveal organisms and a diagnostic biopsy may be indicated. This has been addressed in our Discussion section.

4. The biggest problem with this manuscript and the one that requires major compulsory revision is in the photomicrographs. It is difficult to correlate the descriptions of the spore appearance in the text to that in the figures. Perhaps the problem is that they are of too low magnification. Since the viewing was at 1,000x, why are most of the photographs at 500x?

   **Reply** – the photographs have been changed but the magnification essentially remains at 500x as we wanted to emphasize that the diagnosis can be easily made at that magnification and higher magnification may not be required. Additionally we
have corrected in our manuscript that the viewing was at 500x and not 1000x as written earlier.

5. It is impossible to identify the central band or the polar dot in any figures. Incidentally, it has not been proven that the band, seen by LM, corresponds to the coiled polar filament. Because the figures are problematic, the Results and Discussion sections, and the conclusions are also a problem.

Reply – The figures have been changed and the stains in which the polar dot or central band was visible, (GMS, acid fast stain and Gram’s Chromotrope) we have highlighted with arrowheads in the figures. We hope that our results and conclusions are now clear.

6. Figure 1 is much too low magnification to confirm the description.

Reply – the figure highlights with an asterix deep stromal infiltrates which is evident at that magnification

7. There is a discrepancy between the description of the spore color in GMS in the text and in Table 2, brown versus black (the correct color).

Reply – The text has been modified to reflect our observation that the spores were brown in color
8. The acid fast stain is noted in the text to stain the spores bright red on a bluish background, however in the figure (4a), they are all dark blue, and the Masson trichrome (3d), which is almost black as opposed to the red mentioned in the text.

Reply – Figure 4a has been changed to show bright red spores on a bluish background and diagonal band has been highlighted with arrowheads, however there are also unstained spores which appear blue in color but the polar dot is still visible to aid in diagnosis. We have also changed in the text that the spores appear deep red to black in Masson trichrome stain as suggested by the reviewer.

9. The Cacofluor white (3a) barely stains the spores, which is not so if done correctly. The same is true for the other fluorochrome stains. The picture of the acridine orange stain (3b) does not correspond to the text at all.

Reply - We have addressed this problem in our Discussion section previously that unlike corneal scrapings, (reference 17) we observed that Calcofluor white and acridine orange were less suitable for microsporidia detection in paraffin sections mostly due to the background noise. We have also corrected our text information on acridine orange to correspond to our photograph.

10. It is interesting that the ease of standardizing the Gram chromotrope technique is alternatively described as easy versus not being standardized in their country.

Reply – Though the technique is easy to standardize, but it has not been done so in our country due to lack of information and awareness about this method and we
hope to increase knowledge about diagnosis of this emerging clinical entity through this manuscript.

11. It would be very important to have speciated these microsporidia, which could have been done at the CDC.

Reply – The Gram’s chromptrope was done at the CDC for confirmation of our diagnosis and our ongoing molecular studies will allow speciation of these organisms and increase information on the differential staining patterns of these organisms. This suggestion is however well taken and the limitation has been mentioned in our manuscript.

Thanking you
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