Author's response to reviews

Title: Retinal Changes in Visceral Leishmaniasis by Retinal Photography

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Response to reviewers

Reviewer 1

This is an interesting report of retinal signs in visceral leishmaniasis. It advances understanding in this area by providing retinal data that is more comprehensive, and from a larger group of patients, than previously described. It highlights an interesting area for further research and deserves to be published.

Minor essential revisions

Results para 1, and Table 2: Is the sample size used to generate the retinal data in table 2 n=30, or n=14/30?

The sample size for table 2 was n=30, 6 of whom had abnormal findings, as stated on page 7, lines 5-6.

The original number of eligible consecutive patients should be given (presumably >30), followed by the number of subjects excluded for various reasons, followed by the number of patients excluded from measurements of tortuosity and width (and grading of colour images?).

The original number of eligible consecutive patients was 30, as no patients met the exclusion criteria. This has been added to page 7, line 6.
No patients were excluded from measurements of tortuosity and vessel width but it was only possible to obtain measurements using the VAMPIRE software for 14 patients, as stated on page 8, line 2.

Presumably some patients did not have the minimum of 9 overlapping photographs. If so this should be stated, with reasons why these photographs were not taken in certain subjects.

All patients had a minimum of 9 overlapping photographs. This has been added on page 7, lines 6-7.

Were any of the data in Table 2 derived from ophthalmoscopy, or are the data entirely from grading of colour fundus images?

All data in table 2 are from grading of colour fundus images. This has been added to the legend for table 2.

Methods, para 3, and Tortuosity, para 1: More detail is needed about the method of measuring vessel geometry in cases and controls.

We have added additional details, as requested, to page 6 lines 1-13.

Was blood vessel tortuosity measured from single retinal images, or images that had been stitched together using Photoshop? Some vessel segments in the images in (fig 1) appear to show notching, presumably at points where adjacent images are not aligned exactly. Could this interfere with measurements of vessel geometry? If so, and vessel geometry measurements from the healthy controls were made from single images rather than composite images, could this lead to a false impression of genuine significant differences between the study group and controls? Steps taken to address these possibilities should be described in the methods, and/or discussed as limitations.

Images were stitched using Photoshop only for presentation. Those analysed for tortuosity were not stitched using Photoshop but by the VAMPIRE software. Both patients and healthy controls underwent retinal photography using the same camera and images were processed using exactly the same methodologies. This has been added to page 6, lines 15-17.

Was the optical magnification in images from healthy controls comparable to images from patients? The use of different fundus cameras for patients and healthy controls could introduce a bias towards apparently wider or thinner vessels in one or other group. The methods for obtaining images from healthy controls should be described, and the possibility of bias in measurements of vessel width should be addressed. If images from patients and controls were taken at different magnifications one option would be to report arteriole/venule ratios.

Both patients and healthy controls underwent retinal photography using the same
camera with the same optical magnification and images were processed using exactly the same methodologies. This has been added to page 6, lines 15-17.

Methods, para 3: The description of eye examination does not include an account of measuring visual acuity, colour vision, or visual fields. Since results for visual acuity, colour vision, and visual fields are presented in the manuscript, a short description should be added to explain how these were tested.

Thank you. This has been added to page 5, lines 13-14: “, visual acuity by Snellen chart, colour vision by Ishihara plates, visual fields by confrontation”

Discretionary revisions

Methods, para 3: The term ‘saccadic vessel’ may be a typo

Apologies, we have replaced the term ‘saccadic vessel’ with the term ‘arcade vessel’.

Methods, para 3: How were disagreements between the two masked observers settled?

The phrase “and differences resolved through consensus” has been added to page 5, line 22.

Tortuosity, para 1, and Methods para 5: How was the difference in tortuosity between eyes tested?

By Wilcoxon matched pairs signed rank test, as stated on page 7, lines 1-2.

Results, para 2: The authors may want to add references to what retinal lesions might be expected in patients with a history of diseases they list, e.g. malaria (Maude et al., Trans R Soc Trop Med Hyg 2009), the association between ciprofloxacin and retinal detachment, etc.

We would prefer to omit this with the intention of minimising the length of the text as those with previous eye disease had no retinal lesions.

Reviewer 2

This is a novel and well-conducted study of retinal abnormalities in VL. It describes fundus abnormalities in VL apparently for the first time. There is a major contradiction in the results and confusion surrounding changes in the nerve fibre layer. (see below)

Major Compulsory Revisions

Pg 7 line 19 states “Vessel widths were lower for retinal venules 2 optic disc diameters from the optic disc in VL than in healthy controls... There were no differences in the widths of venules 1 or >2 optic diameters from the disc or in
arterioles” and yet in Table 2, 4 of the subjects had thickened retinal veins, venules and/or arterioles. I presume the former is VAMPIRE measurements and the latter is subjective assessment. How do the authors account for this discrepancy? Page 8 Line 7 also contains this contradiction with Pg 7 line 19.

Thank you. The results in table 2 were indeed subjective and blinded assessments of individual sets of photographs. The VAMPIRE assessments included a comparison with healthy controls in the same population. It appears that this population had relatively thicker retinal vessels and there were no differences in vessel thickness between those with and those without leishmaniasis. We have therefore removed the word “thickened” from table 2 and from page 8, line 16.

Table 2. I am not sure what “Exaggerated response” of the nerve fibre layer means or what a “mild” NFL is. Are these changes in the appearance of the NFL on colour fundus images? I suggest the changes are described. I wonder whether what is being alluded to here is swelling of the nerve fibres in the maculo-papular bundle, but then page 8 line 12 discusses thinning.

Thank you. We apologise that this was not clear. We have re-reviewed the images and the references to “exaggerated NLM response” relate to increased reflectiveness of the internal limiting membrane (ILM) as seen on colour fundus images. This can be a normal finding, particularly in younger patients. We have thus removed references to it from table 2 and the text. Additionally, we have amended the description for patient 2 in table 2 from “vascular arteritis” to “periarteriolar whitening/sheathing”.

Table 2 and Discussion. Raised intra-cranial pressure is a possible cause of the thickened retinal vessels and NFL swelling so could account for some of these abnormalities and should be discussed.

This has been added to page 8, lines 22-23. “Another possible cause of increased tortuosity is raised intracranial pressure, although this is not thought to occur in VL.”

Page 8 lines 9-12. This is confused. I am not sure how a small vessel vasculopathy could cause an exaggerated NFL response, but then what this ‘response’ is is unclear. Thinning of the NFL suggests atrophy and is a chronic change seen in glaucoma and ON. This is notoriously difficult to detect on colour images.

As above, we have removed all references to “exaggerated NFL response” as it was probably a normal variant.

Page 8 line 13/14. Perivasular whitening, superficial retinal haemorrhages and CWS are possible features of retinal vasculitis, which is very interesting but a pattern which does fit other known diseases which cause retinal vasculitis.

This been added to page 9, lines 1-2: “, including known causes of retinal
vasculitis,"

The authors took fundus photos. Why are none being included to illustrate the abnormalities, particularly the uncertain ones: perivascular whitening and NFL changes.

Figure 1C and 1D shows perivascular whitening, as stated in table 2.

Figures 1E and 1F have been added to show perivascular whitening.

Minor Essential Revisions

Pg5 Methods. Patients had ophthalmoscopy and fundus photographs. Did ophthalmoscopy contribute to the identification of the abnormalities in Table 2; or were these findings purely on examination of fundus images?

All findings were from examination of fundus images. “On retinal photography” has been added to the legend for table 2 to reflect this.