Tissue diagnosis of intestinal microsporidiosis using a fluorescent stain with Uvitex 2B

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Abstract

Aims—To detect intestinal microsporidiosis in paraffin wax embedded biopsy specimens using a fluorescence technique incorporating optical brighteners.

Methods—Eight HIV infected patients with confirmed intestinal microsporidiosis (six with *Enterocytozoon bienersi*, one with *Encephalitozoon intestinalis* and one with *Encephalitozoon cuniculi* infection) and 10 without infection were studied. Tissue sections of paraffin wax embedded duodenal biopsy specimens were stained with 1% Uvitex 2B, coded and analysed independently by two investigators.

Results—In all eight cases with confirmed intestinal microsporidian infection, spores could be detected easily in tissue sections using the fluorescence technique. Spores or other elements consistent with microsporidiosis were not found in the 10 patients without infection.

Conclusion—Staining of tissue sections from paraffin wax embedded intestinal biopsy specimens with stains incorporating Uvitex 2B is a rapid and easy technique for the diagnosis of intestinal microsporidiosis.

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Keywords: Uvitex 2B, microsporidiosis, HIV.

Chronic diarrhoea is a common symptom in patients infected with HIV and is often caused by infection with microsporidia.^{1,2} Three different microsporidian species, *Enterocytozoon bienersi*, *Encephalitozoon intestinalis*, and recently *Encephalitozoon cuniculi*, have been recognised in intestinal biopsy specimens of HIV infected patients.¹⁻³

Histological diagnosis of intestinal microsporidiosis in paraffin wax embedded material using routine stains is difficult because of the small size and variable staining characteristics of the parasites.^{1,4,5} Intestinal microsporidiosis can be diagnosed in paraffin wax embedded material using special stains, but most of these stains are time consuming to apply and are not suitable for routine use.^{4,6} As a result, tissue diagnosis depends heavily on electron and light microscopy of plastic or resin embedded tissue; these techniques, however, are not widely available.^{1,7-9}

Microsporidian spores can be detected in stool samples and touch preparations of intestinal biopsy specimens using fluorescent

stains with optical brightening agents such as Uvitex 2B.⁹⁻¹² Here, we report the use of a fluorescent stain incorporating Uvitex 2B for the diagnosis of intestinal microsporidiosis in tissue sections of paraffin wax embedded intestinal biopsy specimens from patients with AIDS.

Methods

Eighteen patients infected with HIV (all CDC C3) were included in the study. Eight patients had intestinal microsporidiosis, caused by *Enterocytozoon bienersi* in six, *Encephalitozoon intestinalis* in one and *Encephalitozoon cuniculi* in one, diagnosed by stool examination using a fluorescence technique incorporating Uvitex 2B^{9,10} and confirmed by transmission electron microscopy of intestinal biopsy specimens using standard procedures.^{3,7} The other 10 patients had no microsporidian spores on examination of their stool samples and no parasites in intestinal biopsy specimens examined using transmission electron microscopy.

Two biopsy specimens from each patient were obtained from the distal duodenum using a flexible fibreglass endoscope. One biopsy specimen was fixed in 10% neutral buffered formalin and embedded in paraffin wax for light microscopy without prior orientation. Tissue sections, 3 to 4 µm thick, were stained with a fluorescent stain as described by van Gool *et al* for staining microsporidia in stool samples.¹⁰ Tissue sections were deparaffinised using xylene and rehydrated through graded ethanol. Slides were fixed in methanol for two minutes and stained with 1% Uvitex 2B in phosphate buffered saline (PBS) for 30 minutes, washed in PBS for a few seconds, counterstained with 0.5% Evan's blue in PBS for 60 seconds, dehydrated through graded ethanol, and embedded in eukitt. Slides were coded and analysed independently by two investigators under a Zeiss fluorescence microscope with a 50 W mercury high pressure lamp and an excitation filter with a transmission range from 355 to 425 nm and a suppression filter of 460 nm. The other biopsy specimen was fixed in 3% glutaraldehyde and processed for transmission electron microscopy using a Zeiss EM 10 as described previously.⁷

Results

In all eight cases with a confirmed intestinal microsporidian infection, spores could be easily detected by both investigators in tissue sections

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Tissue section of a paraffin wax embedded intestinal biopsy specimen with spores of *Enterocytozoon bienewisi* in duodenal enterocytes. Fluorescent stain with Uvitex 2B, original magnification $\times 1000$.

of paraffin wax embedded biopsy specimens using the fluorescence technique (figure). Spores or other elements consistent with microsporidiosis were not found in any of the 10 patients without infection.

Spores could be detected at $\times 400$ magnification, but the morphological characteristics of the spores were only visible at $\times 1000$ magnification under an oil immersion lens. Spores were oval or round, about 1.0×1.5 μm (*E. bienewisi*) or 2.0×3.0 μm (*E. intestinalis* and *E. cuniculi*). Most spores had a brighter wall with less intense fluorescence staining of the internal structures. Some of the spores had a concave inner stripe or shadow, as described previously in stool samples by van Gool *et al.*⁹ Spores were present in clusters in the apical part of intestinal enterocytes and in the two cases with *E. intestinalis* and *E. cuniculi* infection, were also present in lamina propria cells.

The size of the spores enabled us to differentiate between *E. bienewisi* and *Encephalitozoon* spores but not between *E. intestinalis* and *E. cuniculi*.

Discussion

Diagnosis of intestinal microsporidiosis is difficult because of the small size and variable staining characteristics of the parasites.⁵ Highly experienced pathologists have reliably and consistently identified microsporidia in tissue sections using routine staining techniques such as haematoxylin and eosin or Giemsa, but sensitivity and specificity is low, especially in the case of low parasite load.¹⁵ Different stains such as a modified Gomori trichrome stain,⁴ modified Gram stains (Brown-Benn, Brown-Hopps),⁵ the Warthin-Starry stain,⁶ or immunofluorescent stains² can be used to diagnose microsporidiosis in paraffin wax embedded material on light microscopy. The

sensitivity and specificity of most of these stains are unknown and some of them are time consuming to apply and are unsuitable for routine use. Fluorescein tagged anti-*Encephalitozoon* antibodies are useful, especially for differentiating between species, but are not widely available; anti-*Enterocytozoon* antibodies have yet to be developed.² As a result, tissue diagnosis of intestinal microsporidiosis depends heavily on electron microscopy of plastic or resin embedded tissue.^{17,8}

Fluorescent stains incorporating optical brightening agents such as Uvitex 2B have been described for staining microsporidian spores in stool samples, body fluids and touch preparations of biopsy specimens, the latter requiring fresh material.⁹⁻¹² The application of these stains to paraffin wax embedded material would allow intestinal microsporidiosis to be diagnosed routinely without recourse to special staining techniques and would also be useful for epidemiological research.

Some studies found that examination of stool samples is more sensitive compared with histological examination of intestinal biopsy specimens¹⁰ but we found no such difference in sensitivity in this study.

In conclusion, staining of tissue sections of paraffin wax embedded intestinal biopsy specimens with fluorescent stains incorporating Uvitex 2B is a rapid and easy technique for the diagnosis of intestinal microsporidiosis.

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