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Correspondence

Performance of microscopy for detection of microsporidial spores from stool samples of HIV infected individuals with diarrhoea

Sir,

Intestinal microsporidiosis is the most common cause of chronic diarrhoea in treatment-naïve HIV infected patients[1]. Intestinal microsporidiosis due to *Encephalitozoon (Septata) intestinalis* and *Enterocytozoon bieneusi* are also most frequently reported among immunocompromised patients such as transplant recipients[2-4]. Laboratory diagnosis of microsporidiosis was initially based on the detection of microsporidial spores in stool specimens by light microscopy using special stains[5]. Electron microscopy, wherever possible, was also used for detection of microsporidia[6-8].

Diagnosis of intestinal microsporidiosis has been markedly improved by the use of Weber's chromotrope stain and its modified procedure[1,5,9], and fluorescence staining with fluorochromes such as Uvitex 2B or calcoflour white which binds to the chitin in the endospore layer of the microsporidial spore[1,8,10,11]. Molecular technique such as polymerase chain reaction (PCR) became available later for confirmation of micropsoridia. Additionally, species specific PCR can be used to identify different species of microsporidia[12-16]. However, due to resource constraints, most laboratories rely on the use of microscopy of stool samples for diagnosis of intestinal microsporidiosis. Limited studies have been carried out in India on detection of microsporidia[8,17,18]. We compared light microscopy with modified trichome stain, fluorescence microscopy using Uvitex 2B and PCR to detect microsporidia in HIV infected individuals with diarrhoea during January 2009 to May 2010 on consecutive HIV infected patients with diarrhoea admitted at Naidu Municipal Corporation Hospital, Pune. The study protocol was approved by the institutional ethics committee & Naidu Municipal Corporation Hospital.

The study included 331 patients [65 (51 males, 14 females)] HIV infected adults >18 yr and 266 HIV (uninfected individuals) with diarrhoea. This study was carried out only on HIV infected individuals with diarrhoea. Study patients were interviewed using the structured questionnaire. Diarrhoea was defined as two or more liquid or three or more soft stools per day All the patients had history of diarrhoea of <14 days and were treatment-naïve HIV-infected individuals. Stool samples were collected in wide mouth, leak proof, clean sterile containers and then transported to National AIDS Research Institute (NARI) within 4 h of collection. If there was a delay in the transportation, the samples were preserved at 4°C. The samples were immediately processed after receiving at NARI for microscopy by the conventional method[8]. Stool samples were stained by modified trichome and Uvitex 2B by the method described earlier[4,6,8], and were subjected to light microscopy and fluorescence microscopy, respectively. A portion of the stool was stored at -70°C for further molecular analysis. DNA was extracted from frozen samples using the QIA amplication DNA tissue kit (Qiagen, Inc, Germany). PCR was performed using specific primers as described by Najla *et al*[19].

The median duration of diarrhoea was found to be 3 days (IQR 2.75 - 5) and median number of episodes of diarrhoea was noted as 6 in HIV-infected individuals (n=65). The mean age was found to be 35.1 yr ranging from 18-60 yr. Male (n=51) outnumbered the females (n=14). The CD4 counts were available only for 35 patients [CD4 count 200-499 cells/µl (n= 29) and CD4 count ≥500 cells /µl (n=6)] Examination of 65 stool samples from 331 patients by light microscopy with modified trichome stain showed microsporidial spores (oval shaped, measuring 1-2 µm in diameter under oil immersion) in four samples. Fluorescence microscopy using Uvitex 2B also showed microsporidial spores in the same four (6.2%) HIV-infected individuals. PCR detected microsporidial spores in stool samples of five (7.7%) individuals including four found positive by light and fluorescent microscopy (Fig.). Of these

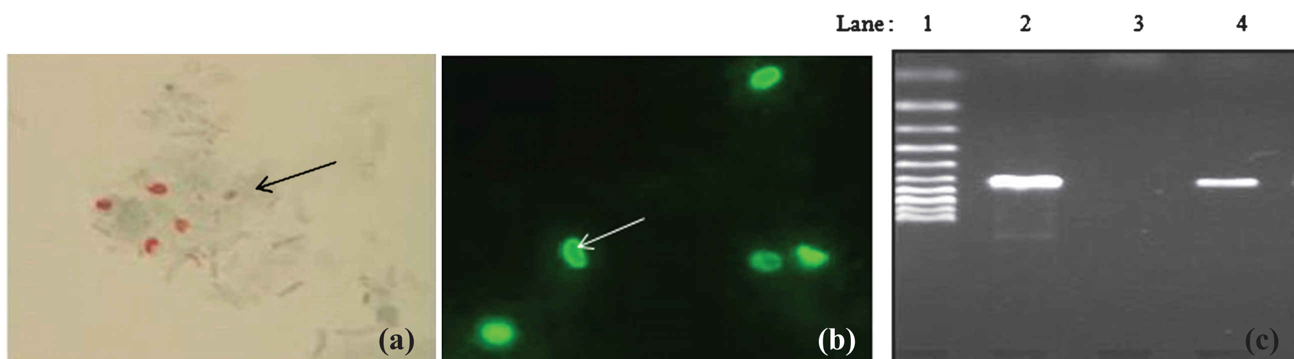

Fig. (a) Stool smear stained with modified trichome stain showing microsporidial spores (arrow); **(b)** Stool smear stained by Uvitex 2B & examined with UV light. Spores of microsporidia show typical elongated shape (arrow); **(c)** Agaraose gel electrophoresis of PCR product agarose gel electrophoresis of polymerase chain reaction (PCR product. Lane1: Marker 100 bp, Lane 2: positive sample, Lane 3: negative control and Lane 4: positive control).

five samples positive by PCR, three were identified as *Enterocytozoon bieneusi* and two were identified as *Encephalitozoon intestinalis*. A repeat light microscopy and fluorescent microscopy on one sample positive only by PCR did not yield positive results. Thus, the results, though limited by a small number of positives, indicate that trichome and Uvitex 2B stains work well for diagnosis of intestinal microsporidiosis. PCR as expected was more sensitive and yielded one additional positive sample.

In conclusion, with well trained laboratory staff both the Uvitex 2B and modified trichome stain can be used for microsporidia detection. PCR can increase sensitivity and species identification. The study was limited by small sample size and small number of positives and hence warrants additional testing on a large number of samples.

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