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Microsporidia in Stools from Cancer Patients

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RAPID COMMUNICATION

Microsporidia are widespread obligate intracellular parasites which have been recently more akin to degenerate fungi than to protozoa (Gill and Fast, 2006). They are found widely widespread in invertebrates and vertebrates but was rarely seen in humans until the AIDS epidemic (Schwartz *et al*., 1996). *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most common species associated in opportunistic infections seen in immunosuppressed individuals such as transplant-recipients (Kelkar *et al*., 1997; Orenstein *et al*., 2005), AIDS patients and occasionally in immunocompetent individuals in the form of travellers' diarrhea (Field, 2002).

There have been studies associating parasitic infections in cancer patients such as cryptosporidium (Noureldin *et al*., 1999; Baqai *et al*., 2005; Bialek, 2005), visceral leishmaniasis (Sah *et al*., 2002; Bialek, 2005), liver flukes (Hughes *et al*., 2006), *Blastocystis hominis* (Noureldin *et al*., 1999; Tasova *et al*., 2000);, *Strongyloides stercoralis* (Reddy *et al*., 1983; Dini *et al*., 1987; Bialek, 2005) and *Toxoplasma gondii* (Klastersky, 1985; Bialek, 2005). In Malaysia there were reports of extra-hepatic cholangiocarcinoma co-existing with biliary ascariasis which were seen in 2 cases involving rural Malay women (Lim and Selliah, 1994) and pediatric cancer patients receiving chemotherapy with parasitic infections (Menon *et al*., 1999). Besides scanty reports of microsporidia in cancer patients (Yazar *et al*., 2003, Botero *et al*., 2003) there has been no report of microsporidia seen in cancer patients in Malaysia despite its occurrence in HIV population. The present study is pivotal in establishing the occurrence of microsporidia in cancer patients and to assess if the organism is opportunistic when compared to the prevalence of microsporida in normal population.

There hundered and eleven and 144 fresh stool specimens were collected from the oncology clinics of three hospitals and from a normal healthy population respectively over a period of 2 years. All specimens were randomly obtained from confirmed cancer patients undergoing chemo-and/or radiotherapy, s who visited the oncology clinic. The normal health population were renadomly selected from a community during a local medical camp organized by volunteers. From the cancer patient population, 36.0% (112/311) were males and 64.0% (199/311) were females._____health population? Specimens were concentrated with a water-ether sedimentation method as described by Van Gool *et al*. (1994). A thin smear was made before being stained with modified trichrome stain as described by Weber *et al*. (1992). The result was examined by light microscopy under oil immersion. At least 100 fields were examined, in duplicate, before a specimen was declared negative, as recommended by Bendall and Chiodini (1993).

21.9% (68/311) were found to be positive for microsporidia. 69.2% (47/68) were from females, while 30.88% (21/68) were from males. The difference in infection between genders was not statistically significant (female vs. male = 47/199 vs. 21/112, p-value = 1). 7.36% (5/68) of the positive specimens also had co- or multiple infections with other gastrointestinal parasites such as *Blastocystis hominis*, Giardia sp. *Dientamoeba fragilis*, *Ascaris lumbricoides* and *Trichuris trichura*, while 92.64% (63/68) were single infections. 29.41% (20/68) of these patients had been newly diagnosed and/or had not undergone any treatment, while 70.59% (48/68) had already undergone chemo-and/or radiotherapy at the time the specimens were collected.

The organisms were visible against a clear background as ovoid or ellipsoid spores, measuring 1.0-1.5 μm, that were brightly outlined in dark pink, with the polar zones staining slightly darker than the centres. While a light pink central vacuolar zone was seen in most of the spores that were detected, an extremely faint, belt-like stripe was observed in other spores, girdling the equatorial region (Fig. 1 and 2). This is an identifying characteristic of Microsporidia sp. the polar filament by which the parasite uses to infect other cells with its sporoplasm.

Only 4.41% (3/68) of the positive specimens were diarrheic, suggesting that most microsporidial infections are asymptomatic. One patient had undergone 11 cycles of therapy for colorectal cancer, while the other had

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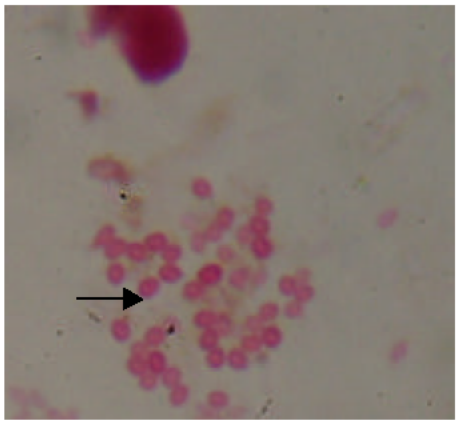

Fig. 1: Fresh stool specimen from a cancer patient with microsporidiosis. Spores are numerous and widely scattered throughout the specimen. A faint polar filament is visible in one of the spores (arrow). X scale

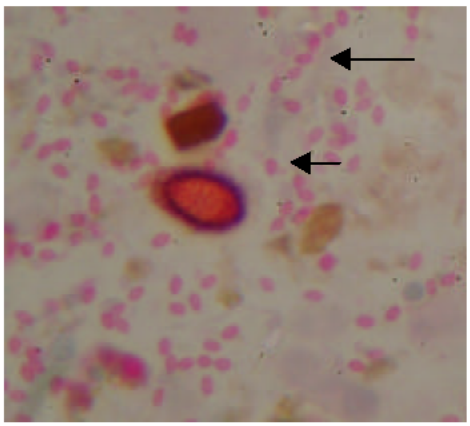

Fig. 2: Positive stool specimen showing ellipsoid-shaped spores with dark pink outlines and polar filament (arrow)

undergone 2 cycles of treatment for a cancer that was not specified in the patient data. The remaining patient had just been diagnosed with breast cancer and had not begun treatment.

While the majority of positive specimens were from individuals who had undergone therapy, the difference between the pre-and post-treatment groups was not statistically significant (infected pre-treatment vs. infected post-treatment = 20/103 vs. 48/208; p-value = 1). However, when the findings were compared with the results of screening in a normal healthy population (a control group, results not shown), the difference was statistically significant (normal vs. cancer = 5/144 vs. 68/217; p-value = 0.001). It has been reported that immunosuppressive treatment increases the probability of acquiring parasitic infections, generally with a high degree of severity (Smith *et al*., 1988; Manheimer and Soave, 1994; Rotterdam and Tsang, 1994). This is because the immunocompromised cellular or humoral responses that have undergone qualitative and/or quantitative alterations (due to immunosuppressive therapy) that impede them from acting efficiently against the infections, which is manifested in the deterioration of their overall condition (Botero *et al*., 2003). This finding concurs with previous studies that microsporidia are opportunistic as evidenced by a larger proportion of patients after chemotherapy having a higher numbers of spores (Connolly *et al*., 1988; Smith *et al*., 1988; Souza-Dias *et al*., 1988; Manheimer and Soave, 1994).

CD4+ T-lymphocyte counts were not established in this study, but previous studies showed that cancer patients infected with microsporidia had CD4+T-lymphocyte levels of less than 50 cells mm^{-3} (Botero *et al*., 2003). As the specimens were collected in random fashion, a more controlled study involving the collection of stool specimens from a patient from the moment of diagnosis, before and after each cycle of treatment, right up to the end of the treatment, as well as from a follow-up visit to the clinic would yield more thorough data on such opportunistic infections.

To the best of our knowledge, this is the first report of microsporidial infection in Malaysian cancer patients and the study confirms that it is imperative to include microsporidia in the screening of opportunistic pathogens in stools especially from cancer and other immunosuppressed patients as the spores could contribute to complications.

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