International Scholars Journals

International Journal of Diseases and Disorders ISSN 2329-9835 Vol. 4 (1), pp. 001-005, January, 2016. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Extremely low prevalence of intestinal cryptosporidiosis and hygienic practices among hospitalized children with malignancies in Malaysia: A preliminary observation

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Accepted 08 November, 2015

Intestinal cryptosporidiosis has been reported to be associated with high morbidity and mortality among cancer patients. The objectives of the study were to determine the prevalence of cryptosporidiosis and to elucidate contributing factors that might influence its transmission in our children with cancer. A prospective cross-sectional study among 110 patients hospitalized for chemotherapy and 100 healthy children (used as controls) between November 2009 and January 2011 at Institute of Paediatrics, Malaysia. Stools were screened for *Cryptosporidium* by using RIDA-quick *Cryptosporidium* (R-Biopharm, Germany) and underwent formalin-ether concentration method and stained with modified Ziehl-Neelsen stain as a gold standard. Questionnaires on personal hygiene practices and risk factors, and medical records from 105 children were analyzed. All stool samples were negative for *Cryptosporidium*. Washing hands before and after taking meals and after using the toilet were practised in 85/105 (80.9%) and 79/105 (75.2%), respectively. History of previous hospitalization was observed in 35/105 (33.3%), contact with animals 31/105 (29.5%), swimming in the pools 26/105 (24.8%) and admission to day care centres in 17/105 (16.2%). History of drinking unfiltered tap water and recent travel were seen in 3/105 (2.9%) and 1/105 (0.9%), respectively. Intestinal cryptosporidiosis is very rare among children with cancer and good personal hygiene practices remain the best preventive approach.

Key words: *Cryptosporidium*, Malaysia, children, cancer, personal hygiene practices.

INTRODUCTION

Cryptosporidium, a coccidial protozoon is now increasingly being recognized as an emerging oppor-tunistic pathogen among children with cancer (Hunter and Nichols, 2002). In these children, the disease can be life threatening and even fatal (Foot et al., 1990; Tumwine et al., 2003). Recent studies documented that the prevalence of cryptosporidiosis among this immuno-compromised group varies from 0 to 22% (Pettoello-Mantovani et al., 1995; Burgner et al., 1999; Menon et al., 1999; Aksoy et al., 2003; Berenji et al., 2007). The differences in the prevalence of cryptosporidiosis are believed due to differences in study methodology, geographical location, and type of study population, sensitivity and

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specificity of laboratory methods or stage of the disease (Hunter and Nichols, 2002). Historically, heighten attention on the importance of the disease derived from several reported cases of severe infections with significant morbidity and mortality in AIDS epidemics in 1980's (Casemore et al., 1985) and later from a massive outbreak in Milwaukee, Wisconsin in 1993 where 403,000 people were involved (MacKenzie et al., 1995). Now, epidemiological data on the prevalence of cryptosporidiosis and its transmission in HIV patients has been rapidly accumulating and several risk factors have also been identified as an important transmission route for cryptosporidiosis.

Despite the general consensus on the opinion regarding the devastating consequences of *Cryptosporidium* infection in patients with HIV/AIDS and the importance of preventing them from infection, it does not seem to be a shared understanding of the risks to other groups of immunocompromised patients, especially children with malignancy.

There are relatively few studies which investigate on the prevalence of *Cryptosporidium* in children with cancer in developing countries. Moreover, the epidemiology and clinical characteristics of cryptosporidiosis in these children in those countries are still scarce and probably under-reported. For instance, the only available data from Malaysia was published in 1999 which reported only 2% of children with cancer had *Cryptosporidium* oocysts (Menon et al., 1999). In India, the prevalence of cryptosporidiosis in children with malignancies was 0.3 and 1.3%, respectively (Sreedharan et al., 1996; Rudrapantna et al., 1997). Nonetheless, higher pre-valence of cryptosporidiosis (22%) was reported in Iran among the similar immunocompromised group (Berenji et al., 2007). Interestingly, *Cryptosporidium* oocysts can be detected in asymptomatic children with cancer. This was reported in 22% of those children with different types of cancers in the United States (Pettoello-Mantovani et al., 1995). In addition, the parasite was also detected in 6.4% of children who were immunocompetent. Thus, this can be a potential reservoir for disease transmission among the patients. Previously, nosocomially acquired cryptosporidiosis has been reported in hospital staff and spread from a patient to another patient has also been documented (Ravn et al., 1991; Casemore et al., 1994). However, this does not attract much attention from the researchers. Thus, considering the characteristics of this parasite such as its robustness towards chlorine and acid (Carpenter et al., 1999; Dillingham et al., 2002), and low infective dose that can probably be as low as 10 oocysts required for inducing infection (Okhuysen et al., 1999), one could imagine how such high numbers of immunocompromised children in a close setting such as in hospitals would be affected during nosocomial outbreaks. This is further complicated by the absence of effective treatment regimen for the disease in immunocompromised children with cancer.

Therefore, the aims of this study were to investigate the current prevalence rate and elucidate factors that might influence the prevalence of *Cryptosporidium* in our children with different type of cancers.

MATERIALS AND METHODS

Patients and study protocols

This cross-sectional study was conducted in an Institute of Paediatric Hospital, Kuala Lumpur over 15 months from November 2009 to January 2011. A single fresh stool specimen was collected in wide mouth screw cap containers from children who were admitted to oncology wards for chemotherapy and other oncologic assessments. In addition, stools from healthy children (used as controls) were also collected within the same period. Children who used anti-parasitic or antibiotic drugs 2 weeks prior to the enrolment of the study were excluded.

The study protocol was approved by the Universiti Putra Malaysia Ethical Committee. Data on the clinical information were traced from medical records. Exposure to specific risk factors such as source of drinking water, history of swimming or contact with animals, history of admission to day care centres or previous hospitalization, history of recent travel as well as personal hygiene practices were obtained by a questionnaire filled by the parents or guardians. All stool samples were immediately transported to the laboratory for the identification of parasites. RIDA-quick *Cryptosporidium* (R-Biopharm, Germany) was used to screen for *Cryptosporidium* oocysts according to manufacturer's guideline. Modified Ziehl-Neelsen stain was used as a gold standard for the identification of *Cryptosporidium*. Specimens were also concentrated by formalin-ether concentration method (Khalil et al., 1991) and examined for ova and cysts examination.

Febrile neutropenia was defined as a temperature of 38°C on two occasions 4 h apart or more than 38°C on one occasion with absolute neutrophil count (ANC) between 500 and 1,000/ml. Diarrhoea was defined as the passage of 3 or more loose stools per day.

RESULTS

In this study, 210 samples obtained from 100 healthy and 110 immunocompromised children were negative for *Cryptosporidium* oocysts. In addition, routine stool examination for ova and cysts was also negative. Among children with cancers and haematological disorders, 105 had completed the questionnaire on the socio-demographic characteristics and clinical backgrounds. The age of the respondents ranged from 3 months to 17 years old (mean age of 2 years ± 0.99 SD). There were 56 boys (53.3%) and 49 girls (46.7%). Malays represented the majority of the respondents, which were 79 (75.2%) followed by the Chinese (15; 11.4%), Indians (6; 8.6%) and others (5; 4.8%). The other ethnics were from Kadazan Dusun (3; 2.9%) and Bengali (2; 1.9%). Meanwhile, 63 (60%) and 42 (40%) of the respondents came from small and large families, respectively. Table 1 showed clinical characteristics and type of cancers among the respondents. Percentage of exposure to risk factors was shown in Table 2.

Table 1. Socio-demographic and clinical characteristics of children with cancers.

Socio-demography	n (%)
Age (year)	
<1	11(10.5)
1-6	61(58.1)
7-10	20(19.1)
11-14	12(11.4)
>14	1(0.9)
Gender	
Male	56(53.3)
Female	49(46.7)
Ethnicity	
Malays	79(75.5)
Chinese	12(11.4)
Indians	9(8.6)
Others	5(4.8)
Number of siblings	
1-3	63 (60)
>3	42 (40)
Clinical characteristics	
Febrile neutropenia	7(6.7)
Diarrhoea	57(54.3)
Neutropenia	42(46.7)
Leucopenia	30(33.3)
Undergoing chemotherapy	75 (71.4)
Use of steroids	48(45.7)
Type of cancers	
Acute lymphoblastic leukaemia (ALL)	40(38.1)
Acute myeloid leukaemia (AML)	9(8.6)
Lymphoma	8(7.6)
Chronic myeloid leukaemia (CML)	2(1.9)
Suspected leukaemia	9(8.6)
Brain tumour	12(11.4)
Retinoblastoma	6(5.7)
Hepatoblastoma	4(3.8)
Wilm's tumor	3 (2.9)
Osteosarcoma	3(2.9)
Pleuropulmonary blastoma	2(1.9)
Adrenal cortical tumour	1(0.9)
Haemophagocyctic lymphohistiocytosis	3(2.9)
Aplastic anemia	2(1.9)
Pure red cell aplasia	1(0.9)

Table 2. Risk exposures of cryptosporidiosis and hygienic practices.

Factors	n (%)
History of previous hospitalization	35(33.3)
History of contact with animals	31(29.5)
History of swimming in the pools	26(24.8)
History of admission to day-care centers	17(16.2)
History of drinking unfiltered tap water	3(2.9)
History of recent travel	1(0.9)
Washing hands before and after taking meals	85(80.9)
Washing hands after using the toilet	79(75.2)

DISCUSSION AND CONCLUSION

Our study showed that none of the children with cancer was positive for *Cryptosporidium*. Likewise, our finding seems to be paralleled with others (Kern et al., 1987; Rudrapatna et al., 1997; Burgner et al., 1999). In addition, low prevalence rates have been reported elsewhere. In Turkey, 2 of 50 children (4%) with different types of cancers had *Cryptosporidium* (Aksoy et al., 2003). Recently, 3 of 72 children (4.2%) with cancers had positive stools for *Cryptosporidium* in Iran (Hazrati et al., 2011). It seems that the trend of prevalence is constantly low globally. The precise explanation for this remains unclear. Although higher transmission rate of *Cryptosporidium* has been reported during rainy season (Tzipori, 1987; Tumwine et al., 2003), seasonal variations are unlikely to influence the result of our study as it is spanned over 15 months. Moreover, failure of detection could not be related to methodological inconsistency as samples in both groups were similarly analysed, and the use of modified Ziehl-Neelsen stain as a gold standard has been widely accepted (Current and Garcia, 1991; Caccio and Pozio, 2006). In addition, RIDA-quick *Cryptosporidium* (R-Biopharm, Germany) has compara-ble sensitivity (91.6 to 98.8%) and specificity (100%) with other methods (Regnath et al., 2006; Abdel et al., 2008) and can be employed for the rapid and cost-effective screening of large numbers of faecal samples (Garcia et al., 2003; Weitzel et al., 2006). Another possible explana-tion that might influence the recovery of *Cryptosporidium* in the stools is the level of immune status of the children. In general, patients with lympho-hematopoeitic cancers are more prone to have devastating clinical outcomes compared to other type of cancers (Gentile et al., 1991). These clinical outcomes are believed to be directly related to the CD4+ lymphocyte count, and patients with CD4 counts of less than 50 are at greatest risk for both severity of disease and prolonged carriage (Hunter and Nichols, 2002; Abubakar et al., 2007). In our study, we could not comment on the level of immune status of children with malignancies as the CD4 count was not measured.

In our study, 33.3 and 46.7% of children had leucopenia and neutropenia, respectively, and only 6.7% of them had febrile neutropenia. In addition, 71.4 and 45.7% of them had received chemotherapy and steroids, respectively as well. However, they seem to be at low risk

of acquiring the disease. This might be related to the different degrees of immunosuppression in patients at the time of exposure or infection and during its course in our study, or the immunodeficiency state that might be transient and eventually returned to near normal immune function as proposed by Burgner et al. (1999). Similar findings have been documented in a study conducted by Rudrapatna et al. (1997). In their study, stools from 1,029 cancer patients who received or not received chemotherapy were all negative for *Cryptosporidium*. Undoubtedly, basic hygienic practices such as washing hands before or after taking meals (80.9%), and after using the toilet (75.2%) would have preventive benefits against infection and transmission of cryptosporidiosis in our study, albeit limited data on this. Person to person spread is common in cryptosporidiosis (Current and Garcia, 1991). In addition, asymptomatic carriers seemed to be rare in our patients. Conversely, Aksoy et al. (2003) reported higher asymptomatic carriage in children with cancers than healthy ones in USA (22 versus 6.4%). It is believed that asymptomatic carriers can be a potential source of reservoir for outbreak (Zar et al., 1985; Thomas and Kuhls, 2000). Nonetheless, oocysts can be intermittently shed with variable frequency regardless of symptoms (Dowd et al., 1999). Thus, we believe that higher chances of detection could be obtained if more than one sample is used or more symptomatic children are involved. However, association of the presence of parasite with the number of stool sample or symptomatic cases had been contradictory; some reports have recommended more than one sample (Clavel et al., 1995; Orlandi and Lampel, 2000) while others have not, particularly if acid-fast stain is employed as a diagnostic staining method (Weitzel et al., 2006). Although good personal hygiene is important in reducing the transmission, minimizing risk of exposure to socio-environmental sources, such as drinking contaminated water, animal contacts, overcrowded places and others is crucial too (Casemore et al., 1994; Kavanagh et al., 2005).

In our study, only 0.9, 2.9, 24.8, 29.5 and 16.2% of children had history of recent travelling, drinking unfiltered tap water, swimming, animals contact and day-care centres, respectively. Thus, the risk of exposure is relatively low. Our study has several limitations. T cell population study was not done to assess deficiency of T-cell, a significant risk factor for cryptosporidiosis (Baxby et al., 1984). However, the deficiency state could be transient and may eventually return to normal, and also, this could be masked by the use of immunosuppressive agents which further complicate the laboratory evaluation of immunosuppressed condition. Lastly, PCR should have been used for the definitive and confirmatory detection of *Cryptosporidium* (Orlandi and Lampel, 2000). However, it is labour-intensive and resource-demanding to be used as a screening tool in most developing countries. Results of the present study could form a basis for a larger multicentric trial to stress on the traditional approach of basic personal hygiene practices, which shall include diverse cohorts such as communities engaged in livestock industry, animal handlers and veterinary personnel, pet-shop operators, hospital staff, patients undergoing immunosuppressive therapy and organ transplant surgery.

ACKNOWLEDGEMENTS

This study was supported and funded by The People's Bureau of the Great Socialist People Libyan Arab Jamahiriya and the Research University Grant Scheme (RUGS) No. 04-02-07-0340RU. We wish to thank Ministry of Health Malaysia for giving the permission during the study period and to all staff involved in this study.

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