PREVALENCE OF INTESTINAL MICROSPORIDIOSIS IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED PATIENTS WITH DIARRHEA IN MAJOR UNITED STATES CITIES

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SUMMARY

To determine the prevalence of intestinal microsporidiosis in HIV-infected patients, we performed a prospective study of HIV-infected patients with diarrheal illnesses in three US hospitals and examined an observational database of HIV-infected patients in 10 US cities. Among 737 specimens from the three hospitals, results were positive for 11 (prevalence 1.5%); seven (64%) acquired HIV through male-to-male sexual contact, two (18%) through male-to-male sexual contact and injection drug use, and one (9%) through heterosexual contact; one (9%) had an undetermined mode of transmission. Median CD4 count within six months of diagnosis of microsporidiosis was 33 cells/μL (range 3 to 319 cells/μL). For the national observational database (n = 24,098), the overall prevalence of microsporidiosis was 0.16%. Prevalence of microsporidiosis among HIV-infected patients with diarrheal disease is low, and microsporidiosis is most often diagnosed in patients with very low CD4+ cell counts. Testing for microsporidia appears to be indicated, especially for patients with very low CD4+ cell counts.

KEYWORDS: Intestinal microsporidiosis; Prevalence; HIV-infected patients; Diarrhea.

Microsporidia, have been known for over a decade to cause opportunistic infections in patients with human immunodeficiency virus (HIV) infection. *Enterocytozoon bieneusi* is the most commonly identified microsporidia in patients with HIV/AIDS. Microsporidiosis usually produces diarrheal illness but can produce disseminated illness in some persons depending upon the species involved[5,13]. Few reports in the medical literature describe the prevalence of intestinal microsporidiosis; those that do are not considered reliable and generalizable[2]. One study, conducted before the widespread use of highly active antiretroviral therapy (HAART), reported that microsporidia accounted for 14.1% and 34.8% of cases of acute and chronic diarrhea, respectively, in HIV-infected patients[12]. However, since use of HAART has become widespread, very few data are available on the prevalence of microsporidiosis in HIV-infected persons[6].

One study suggests that the prevalence of infectious diarrhea (along with AIDS opportunistic illnesses) has declined in recent years[4]. It is not known whether the prevalence of microsporidiosis has declined as well. Because clinical laboratory testing for microsporidia is not part of the routine testing for ova and parasites, infection with microsporidia is likely underrecognized. Therefore, active surveillance for infection with microsporidia is necessary to estimate the true burden of this infectious disease in patients with HIV and AIDS. In addition, the sensitivity of testing for this infection could vary according to type of test used[15].

To determine the prevalence of intestinal microsporidiosis, we performed a prospective study of HIV-infected patients with diarrheal illnesses in three US hospitals that care for a high proportion of HIV-infected persons in their cities. We also examined an observational database involving medical record abstraction of HIV-infected patients at participating healthcare facilities in 10 US cities (the Adult and Adolescent Spectrum of HIV Disease Project).

METHODS

The study at three sites: CDC contracted with three sites (Denver, New Orleans, and Seattle) participating in the Centers for Disease Control and Prevention's (CDC's) Adult and Adolescent Spectrum of HIV Disease project (ASD). The study was performed at three public hospitals: Medical Center of Louisiana (formerly Charity Hospital of New Orleans), New Orleans, Louisiana (1 January 1998 through 30 September 1999); a hospital in Seattle, Washington (1 February 1998 through 30 June 2000); and Denver Health Medical Center (formerly Denver General Hospital), Denver, Colorado (30 March 1998 through 21 September 1999). These three hospitals performed supplemental medical record abstraction and tested for microsporidia in HIV-infected patients being evaluated for diarrheal illness. The study was approved by the institutional review boards of Medical Center of Louisiana (formerly Charity Hospital of New Orleans), New Orleans, Louisiana, a hospital in Seattle, Washington, Denver Health Medical Center

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At each participating hospital, consecutive diarrheal (loose) stool specimens from HIV-infected patients sent to the hospital microbiology laboratory were tested for microsporidia. One specimen was evaluated for each patient (if multiple specimens were sent, the first specimen was evaluated). The tests were performed on specimens that had been sent to the hospital laboratory as part of the patient's medical evaluation for infectious diseases. Therefore, the physician's decision to send a stool specimen for evaluation of routine diarrheal pathogens was not influenced by this study. Before this study, microsporidia testing was not routinely performed on such specimens at the participating hospitals. The Denver Health Medical Center and the Seattle hospital used Moura's quick-hot Gram chromotrope technique[11]; the Medical Center of Louisiana used the modified trichrome blue stain[14].

Stool specimens with positive test results for microsporidia were sent to the CDC Division of Parasitic Diseases laboratory for confirmation. Duplicate smears were made from each of the stool specimens; one smear was stained with chromotrope 2R and one with the quick-hot Gram chromotrope techniques. The stained smears were examined under the oil immersion lens of an Olympus BX-60 microscope. Pinkish (in chromotrope 2R stained smear) or dark violet (in quick-hot Gram chromotrope stained smear) spores confirmed infection with microsporidia. After submitting for confirmation a specimen with positive results, the participating hospital submitted the next specimen with negative results to the CDC laboratory to confirm that it was negative.

Patients whose specimens were negative for microsporidia were assigned to a control group, but they could be reassigned to the microsporidia group if a subsequent stool specimen revealed microsporidia. If a patient's specimen was positive for microsporidia, then any further stool specimens from that patient were excluded from the study for the remainder of that year.

The medical records of patients with positive test results were reviewed using the appropriate (initial or follow-up) ASD form and a supplemental medical record questionnaire that collected occupational history, duration of diarrheal illness, recent antimicrobial prescriptions, total lymphocyte count (if no CD4+ lymphocyte count was available within six months of stool testing), and other pathogens identified from the stool specimens with microsporidia.

At the end of the study, a quality assurance study was performed. Duplicate smears were made from each of the stool specimens; one smear was stained with chromotrope 2R and the other with the quick-hot Gram chromotrope techniques. The stained smears were examined under the oil immersion lens of an Olympus BX-60 microscope and scored as positive if pinkish (in chromotrope 2R-stained smears) spores with a vacuole, a median belt-like stripe, or both, were seen or if dark violet (in quick-hot Gram chromotrope-stained smears) spores with a vacuole, a median belt-like stripe, or both, were identified. To evaluate the duration of diarrheal illness and to learn whether any patients had died, the medical records of infected patients were reviewed six months after the stool was tested.

Adult and Adolescent Spectrum of HIV Disease Project: ASD is a longitudinal observational cohort study of HIV-infected persons at participating health care facilities. Its methods have been previously reported[7]. Briefly, for the initial medical record abstraction, records for the entire year before patient enrollment in the study are reviewed; information is then abstracted every six months until the patient dies or is lost to follow-up. The initial medical record abstraction collects patient information on demographics, mode of HIV exposure, any previous occurrences of conditions listed in the AIDS surveillance case definition[3] and other conditions, medications prescribed, and CD4+ cell counts during the year before study inclusion. Cities participating in ASD during the study periods were Atlanta, Georgia; Dallas, Houston, and San Antonio, Texas; Denver, Colorado; Detroit, Michigan; Los Angeles, California; New Orleans, Louisiana; New York City, New York; Bayamon, Puerto Rico; and Seattle, Washington. Puerto Rico was excluded from ASD analyses because physicians at the participating site infrequently ordered stool testing for pathogens.

The prevalence of microsporidia among patients with infectious diarrhea was also examined in the ASD database; however, microsporidia were added as organism codes for data entry in 1997. Cases recognized from the 3-city study (all confirmed at CDC) were also included in the ASD database. However, the routine practice of the ASD study includes the abstraction of a diagnosis (such as infectious diarrhea), and the organism which was documented (by physician note and laboratory test report) as causing the diagnosis without confirmation by CDC.

RESULTS

The study at three ASD sites: Among 737 specimens screened, results were positive for 11 (prevalence 1.5%). Of those with negative results, 10 were selected to be controls. By city, the results were as follows: New Orleans, eight cases among 512 specimens (prevalence 1.56%); Seattle, three cases among 159 specimens (prevalence 1.89%); and Denver, zero cases among 66 specimens (prevalence 0%). Of these 11 patients with positive results, 64% were white, 27% were black, 9% were Asian/Pacific Islander, and 100% were male. Regarding the mode of HIV transmission, 64% acquired HIV through male-to-male sexual contact, 18% through male-to-male sexual contact and injection drug use, and 9% through heterosexual contact; 9% had an undetermined mode of transmission. The median age at diagnosis of microsporidiosis was 34 years (range 29 to 46 years). Case and control patients were not statistically (p > 0.05) different with regard to median age, gender, race (white), and percentage with male-to-male sexual contact as mode of HIV transmission. The median duration of diarrheal illness for case- and control-patients overall at time of stool testing was four weeks (range 0.5 to approximately 136 weeks). The median CD4 count within six months of diagnosis was 33 cells/μL (range 3 to 319 cells/μL) for case patients and 348 cells/μL for the 10 control patients. Nine of the case patients but only one of the control patients had a CD4+ cell count below 100 cells/μL. During the six months before diagnosis, 10 of the 11 case patients (91%) had been prescribed a medication thought to have antimicrosporidial properties (seven azithromycin, six fluconazole, four albendazole, two metronidazole, and one octreotide; none received itraconazole, atovaquone, or fumagillin. Recent occupational history was not available for six of the case patients. Occupational history for the other five was as follows: one was a farmer and woodcrafter, two were health professionals, one

was an unemployed business professional, and one was an unemployed computer consultant. One patient died within six months of diagnosis.

Adult and Adolescent Spectrum of HIV Disease Project: According to the ASD database (including the three participating sites), 39 cases of microsporidia-associated disease were found during 1998 through 2002 among 24,098 HIV-infected patients followed up during these years [16 cases in 1998, 10 cases in 1999 (one of these was also a 1998 case), four cases in 2000, six cases in 2001, and four cases in 2002]. For nearly all of the cases, the species of microsporidia was not specified. Infectious diarrhea was reported for 34 of these case patients; other illness was reported for five. Twenty-seven (69%) of the case patients were men who have sex with men (including two who also had histories of injection drug use).

The overall prevalence of microsporidiosis in ASD was 0.16%; 0.23% among patients with a CD4 count < 200 cells/µL, 0.33% among patients with a CD4 count < 100 cells/µL, 0.26% among patients with male-to-male sexual contact as mode of HIV transmission (not including men who have sex with men and inject drugs), 0.20% among patients enrolled at public hospitals (92.3% of all microsporidiosis case patients were enrolled in ASD at public hospitals), and 0.11% among patients enrolled in private practices or health care maintenance organizations.

The prevalence of microsporidiosis was highest in New Orleans (0.31%). No cases were found in Los Angeles, Atlanta, Houston, Dallas, San Antonio, Detroit, and New York City. The prevalence of microsporidiosis among patients having a diagnosis of infectious diarrhea from 1998 through 2002 was 0.88% and ranged from a high of 1.15% in 1998 (1,392 patients with at least one diagnosis of infectious diarrhea) to 0.53% in 2000 (759 patients with at least one diagnosis of infectious diarrhea) (Fig. 1).
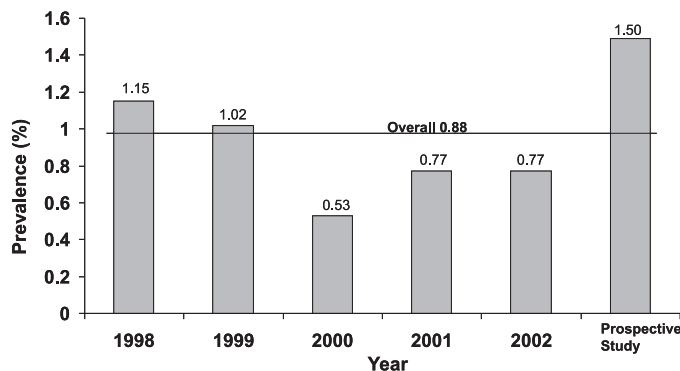

Fig. 1 - Prevalence of diagnosis of microsporidiosis in HIV-infected patients who had infectious diarrhea and were enrolled in the Adult and Adolescent Spectrum of Disease Project from 1998 through 2002 (n = 4427), and for the prospective study (Denver, Seattle, New Orleans) (n = 737).

DISCUSSION

This study demonstrates that the prevalence of microsporidiosis is low (1.5%) among HIV-infected patients for whom stool testing is performed to evaluate diarrheal disease and that microsporidiosis is most often diagnosed in patients with very low CD4+ cell counts. These data reflect the prevalence of microsporidiosis in the United States in a cohort of patients from three public health hospitals. The prevalence may be lower in patients in other settings and could be higher in other countries, especially developing countries where use of HAART may be less common. Generalizations about exposure (such as may be inferred from occupational history) are not available from our data because of the low number of cases identified in our study.

Before we performed this study, anecdotal information had suggested that microsporidiosis might be more common in New Orleans than elsewhere. In addition, the prevalence in the overall 1998 through 2002 ASD database was higher at the New Orleans site. However, our data do not support such a theory. Using a standard diagnostic approach during this study time frame, we found that the prospective prevalence in Seattle was similar to that found in New Orleans.

Our finding that most cases of microsporidiosis were diagnosed in patients with a low CD4+ cell count <100 cells/µL is consistent with the literature[1,8-10]. The difference between the median CD4+ cell count in our case patients (33 cells/µL) and that in control patients (348 cells/µL) was substantial. The finding of very low CD4+ cell count in case patients is also consistent with similar findings from two other published studies (KYAW *et al.*, mean 37 cells/µL; LEDER *et al.*, median 20 cells/µL)[9,10]. One of these studies also found that microsporidiosis was associated with a history of swimming pool exposure[10]. If confirmed in other studies, this finding could be incorporated into opportunistic illness education programs for AIDS patients.

Our study has several limitations. First, all three hospitals did not use the same test method. According to the experience of one of the authors, the prevalence rate might have been higher had the quick-hot Gram chromotrope technique been used in all hospitals because with this technique, the spores stain a dark violet color, making them easier to recognize, especially when present in small numbers. Second, some locations in the ASD database may have different practices that make the likelihood of microsporidia testing less likely, especially since it is not a routinely performed test in microbiology laboratories. Therefore, microsporidia prevalence in the ASD database may underestimate the true prevalence. Third, the diagnosis of microsporidia in stool was confirmed only for the cases identified in the 3-city study, so it is possible that misdiagnosis could have occurred, which would overestimate the prevalence. We do not have information on the laboratory practices at participating sites, which could have helped to interpret why some ASD sites had no cases diagnosed in the 5-year period examined. In addition, we did not identify large numbers of cases, we did not have exposure histories, and we had minimal occupational histories. Therefore, our study does not provide definitive findings that would be helpful in identifying risk factors for infection. Finally, the data may not be generalizable to all HIV-infected patients with diarrheal illness because the data were derived only from patients for whom stool testing was ordered by the physician.

We recommend consideration of microsporidiosis in any HIV-infected patient with diarrheal illness, especially for patients with CD4+ cell counts < 50 cells/µL. Future studies could examine whether men who have sex with men are at increased risk for microsporidiosis and how microsporidia infections are acquired. This information could be useful for creating prevention messages.

RESUMO

Prevalência de microsporidiose intestinal em pacientes infectados pelo HIV com diarréia nas principais cidades dos Estados Unidos da América do Norte

Para determinar a prevalência de microsporidiose intestinal em pacientes infectados pelo HIV foi realizado um estudo prospectivo em três hospitais dos Estados Unidos da América do Norte (EUA) e analisada uma base de dados nacional composta de dados coletados de pacientes infectados pelo HIV em 10 cidades dos EUA. De um total de 737 amostras de fezes de pacientes infectados pelo HIV que apresentavam diarréia, amostras de 11 pacientes (prevalência de 1,5%) foram positivas para microsporídios. Todos os positivos eram do sexo masculino e, entre eles, sete (64%) pacientes adquiriram a infecção pelo HIV através de relação homossexual, dois (18%) através de relação sexual e drogas injetáveis e um (9%) através de contato heterosexual, enquanto que em um paciente o modo de transmissão do HIV não foi determinado. A contagem média de linfócitos CD4 realizada até seis meses do diagnóstico de microsporidiose foi de 33 células/microlitro (3 a 319 células/microlitro). A análise da base de dados nacional (n = 24.098) mostrou uma prevalência de microsporidiose de 0,16%. A prevalência de microsporidiose em pacientes HIV-positivos com diarréia é baixa. Entretando, como a microsporidiose é mais frequentemente diagnosticada em pacientes com contagens de CD4 muito baixas, a indicação de pesquisa de microsporídios é justificada, especialmente para estes pacientes.

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