OPPORTUNISTIC INFECTIONS AND HIV CLINICAL DISEASE STAGE AMONG PATIENTS PRESENTING FOR CARE IN PHNOM PENH, CAMBODIA

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Abstract. This prospective, cross-sectional study sought to assess the spectrum of HIV-associated complications and disease stage among individuals presenting for first-time care in Phnom Penh, Cambodia between November 2001 and September 2002. One hundred patients participated in this study. All study participants presented with advanced stages of HIV disease. Seventy-four percent of the subjects had CD4 cell counts <50 cells/mm^3. Tuberculosis was the most common AIDS-defining illness among participants, with a prevalence of 43%. A spectrum of other opportunistic infections, including cryptosporidiosis (13%), severe bacterial infections (12%), cryptococcosis (12%), and *Pneumocystis jiroveci* pneumonia (10%), was identified. These findings underscore the need for widespread HIV treatment and prevention in this setting. Increased screening for HIV and routine health maintenance for those infected are urgently needed in order to facilitate management of both opportunistic infections and the secondary prevention of HIV infection.

INTRODUCTION

Cambodia's first case of HIV was reported in 1991. Fueled by post-war political and economic instability, HIV prevalence in Cambodia rapidly increased. Although recent data indicate that HIV prevalence has begun to decrease, without access to adequate economic, professional, and material resources, Cambodia has struggled to meet the HIV treatment and prevention needs of its people as more of those infected present with advancing disease. Approximately 1.9% of Cambodians between 15 and 49 years of age are currently living with HIV, and prevalence rates in subgroups are significantly higher (NCHADS).

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While HIV prevalence and risk behaviors among sentinel groups in Cambodia are tracked through annual surveillance, data regarding the health of HIV positive individuals in the region are scarce (Inverarity *et al,* 2002; Senya *et al,* 2003; Vajpayee *et al,* 2003). Cambodia's neighboring countries have similar HIV transmission patterns, including high rates of HIV infection among sex workers; there are, however, also significant inter-country differences. In both Thailand and Vietnam, for example, a significant percentage of reported HIV infections are among injection drug users in urban centers (Anonymous, 2003a,b); whereas, in Cambodia, the population of injection drug users remains small (National Center for HIV/AIDS, 2002). Given regional differences in pathogens and routes of HIV transmission, data collected from previous studies may not be generalizable to Cambodia.

With funding from sources such as The Global Fund and the Clinton Foundation, Cam-

bodia has begun to scale-up HIV treatment programs. These programs must address the allocation of antiretrovirals and other scarce medical resources. They must also establish protocols for the management of opportunistic infections in a setting with limited diagnostic tools. A more in-depth understanding of the clinical manifestations of HIV will be important for the development of HIV treatment programs and protocols.

This prospective study sought to add to the existing data regarding clinical manifestations of HIV in Cambodia, with the aim of contributing information necessary for effective and efficient scaling-up of HIV care in Cambodia. This was accomplished both by assessing the spectrum of opportunistic infections among HIV infected individuals and by identifying patients' presenting disease stage as determined by the HIV staging system of the World Health Organization (WHO, 2006).

MATERIALS AND METHODS

Study design and setting

A prospective, cross-sectional study was conducted among 100 HIV positive patients presenting for first time care at either Sihanouk Hospital Center of Hope (SHCH) or at the hospital's HIV resource center in Phnom Penh, Cambodia between November 2001 and September 2002. This study was reviewed and approved by the institutional review boards of Brown University, The Miriam Hospital, and SHCH. Written informed consent was obtained from all study participants.

Eligibility and enrollment

All adults who tested positive for HIV and who presented for care for the first time on a Monday, Wednesday, or Friday, were eligible to participate. Alternate days were chosen to allow for consent and data collection to be completed given the high volume of potential subjects. HIV testing was performed in accordance with WHO recommendations (UNAIDS, 1997). Patients whose serum was reactive on two rapid assays (Abbott Determine and Dipstick HIV 1+2), were considered HIV infected. Test results of enrolled patients were confirmed by Western blot. Patients were referred to the study by HIV testing staff. All study subjects received standard pre- and post- HIV test counseling.

Study procedure

Each participant completed a face to face questionnaire concerning demographic information, HIV risk behaviors, HIV knowledge, clinical symptoms, and medical and family history.

Blood samples were taken for complete blood count (CBC), electrolytes, lymphocyte subsets, and serum albumin. Chest X rays, a purified protein derivative (PPD) tuberculosis test, abdominal ultrasound, and a wet mount stool examination were also performed. Additional tests were performed as clinically indicated. A stool culture, *Clostridium difficile* assay for toxin a (Oxoid Toxin Detection Kit; Sykehuset Asker og Baerum), and modified acid-fast staining were conducted for patients presenting with diarrhea. Non-induced sputum samples were obtained from patients presenting with a cough, dyspnea, or an abnormal chest X ray. This sputum was analyzed for Gram stain, bacterial culture, acid-fast bacilli (AFB) stain and culture, and *Pneumocystis jiroveci* pneumonia (PCP) immunofluorescent stain. A routine and mycobacterial blood culture and a serum cryptococcal antigen test were performed for subjects presenting with fever ≥38.5ºC. A lumbar puncture and a serum cryptoccocal antigen test were completed for patients presenting with central nervous system symptoms. A needle aspiration was performed to obtain a specimen for Gram and AFB stains for patients presenting with inflammatory peripheral lymph nodes. When pus was present upon incision or aspiration, a sample was taken for mycobacterial and routine cultures.

Based on clinical and laboratory findings, each patient was assigned a disease stage in accordance with WHO HIV disease staging guidelines. In accordance with these guidelines, only HSV cases with >1 month duration were considered in assessing HIV disease stage. As outlined in WHO guidelines, in this study, chronic herpes simplex virus (HSV) was diagnosed on clinical grounds without confirmatory laboratory testing.

One month after enrollment, subjects returned to the hospital to review their study results. The following month, subjects were asked to meet with study staff to ensure that data collection was complete.

All study subjects received standard medical care, and treatment and prevention of opportunistic infections were in accordance with hospital protocols, including cotrimoxazole for PCP prophylaxis. At the time of this survey, no antiretroviral therapy (ART) was available.

Data analysis and statistical methods

In testing for differences by gender, age, and occupation, the independent-samples *t*-test, and Pearson chi-square were used for continuous and categorical variables, respectively. Because the median age of participants was roughly 35, we considered differences among individuals <35 *vs* ≥35 years of age. Occupation was divided into the following categories: farmers, laborers, business people, and soldiers. Those involved in other, less common, occupations were considered as a group. We used an alpha probability of 0.05 as the threshold for statistical significance in two-tailed comparisons. All statistics were performed using Stata v.8 (Stata Corp, College Station, TX).

RESULTS

Study population

Of the 419 patients tested for HIV during the recruitment period, 119 (26.5%) tested positive for HIV. A total of 100 individuals, 39 women and 61 men, participated in the study. Participants were between 20-56 years of age, with a mean age of 34.1 years (SD=7.7 years). Thirty participants were from Phnom Penh, and 70 were from surrounding provinces. Seventy-six participants were married, 4 were divorced, 11 were widowed, and 9 were unmarried. Thirty-six participants were laborers, 28 were farmers, 15 were soldiers, and 21 reported other occupations.

Laboratory and clinical data

No statistically significant gender differences in laboratory data were identified. The median value of the absolute lymphocyte count (n=89) was 855 cells/mm^3 (range 170-9,300 cells/mm^3). The median hemoglobin value (n=88) was 9.25 g/dl (range 4.1-17.6 g/dl).

Among those whose CD4 cell counts were assessed (n=89), median absolute CD4 cell count was 15 (range 0-274). Seventy-four percent of subjects had absolute CD4 counts ≤50 cells/mm^3, and in nearly 30% of participants, CD4 counts were <10 cells/mm^3.

PCP stains were performed on sputum from 36 of 45 subjects presenting with signs of pneumonia. Only 1 of 10 patients believed to have PCP on clinical grounds was found to have a positive PCP stain on sputum. More than half (52.1%) of patients (n=96) had abnormal chest X rays. Abnormal abdominal ultrasound findings were recorded in 54.5% of patients (n=99). Of the patients (>50%) who reported having acute or chronic (>3 weeks) abdominal pain, 73% had abnormal abdominal ultrasound findings which included one or more of the following: lymphadenopathy, hepatomegaly, splenomegaly, and splenic abscess. While those with abdominal pain were more likely to have abnormal ultrasound findings, a substantial minority (20%) of asymptomatic patients also had abnormal findings.

Opportunistic infections and clinical conditions

Data on clinical conditions are presented in Table 1. Upon study enrollment, 15 sub-

Table 1
HIV-associated complications (n=100).

		Gender (%)			Age (yrs), mean			Residence (%)		
	n	M (n=61)	F (n=39)	p	With infection	Without infection	p	Phnom Penh (n=30)	Provinces (n=70)	p
Oral hairy leukoplakia	90	98.4	87.2	0.022	-	-	-	-	-	-
Oral candidiasis	80	73.8	69.2	0.622	-	-	-	-	-	-
Weight loss (>10%)	63	59	69.2	0.302	-	-	-	-	-	-
Esophageal candidiasis	39	36.1	43.6	0.452	-	-	-	-	-	-
Tuberculosis										
Extrapulmonary	28	31.2	23.1	0.381	35.4	33.5	0.27	24.1	29.6	0.583
Pulmonary	25	24.6	25.6	0.906	36.16	33.4	0.116	27.6	23.9	0.703
Cryptosporidiosis (with diarrhea)	13	6.6	23.1	0.017	36.6	33.7	0.201	10.3	14.1	0.614
Severe bacterial infections	12	14.8	7.7	0.289	36.7	33.7	0.213	20.7	8.5	0.087
Cryptococcosis	12	13.1	10.3	0.668	31.8	34.4	0.288	13.8	11.3	0.724
Pneumocystis pneumonia	10	8.2	12.8	0.452	33	34.2	0.649	17.2	7	0.123
Herpes simplex virus	6	6.6	5.1	0.769	31.7	34.2	0.435	3.5	7	0.492
Vulvovaginal candidiasis (women)	3	-	7.7	-	36.3	34	0.606	1.4	6.9	0.144
Isosporiasis	2	0	5.1	0.074	26	34.2	0.136	3.5	1.4	0.509
Histoplasmosis	2	1.6	2.6	0.747	21.5	34.3	0.019	3.5	1.4	0.509
Non-typhoid *Salmonella* septicemia	2	1.6	2.6	0.747	32	34.1	0.705	3.5	1.4	0.509
Mycobacterium avium bacteremia	2	3.3	0	0.253	29.5	34.2	0.401	0	2.8	0.361
Mean # of complications[a]	1.6	1.5	1.7	0.271	-	-	-	1.7	1.5	0.275

[a]Excluding weight loss, oral candidiasis, and oral hairy leukoplakia

jects (35% of TB-infected subjects) presented with pulmonary tuberculosis (PTB), 18 subjects (42%) presented with extrapulmonary tuberculosis (EPTB), and 10 subjects (23%) presented with both PTB and EPTB. (Ninety-five patients underwent PPD testing. During the 48-72 hour period following the PPD skin test, no patient developed any induration). Overall, TB of some form was the single most common AIDS-defining illness, diagnosed in a total of 43 patients.

There were two cases of histoplasmosis and no cases of penicilliosis. Two individuals reported mucocutaneous herpes simplex virus (HSV) infection for a duration of >1 month, and 4 additional subjects reported mucocutaneous HSV infections with a duration of <1 month. Weight loss >10% of body weight, oral candidiasis, and oral hairy leukoplakia were the most common conditions identified among the study population; each of these clinical conditions affected more than 50% of all participants. Excluding these most common, but not AIDS-defining, HIV-associated complications, the majority (68%) of study participants had 1 or 2 concurrent opportunistic infections, and 20% of participants presented with 3 or 4.

Upon analysis of the type of HIV-associated complications among study participants, few statistically significant differences were

found by gender, age, or residence. Histoplasmosis appeared to affect younger patients and hairy leukoplakia was seen more frequently in males compared to females. The mean number of HIV-associated complications was roughly the same among persons of various occupations; the groups of farmers, laborers, soldiers, and participants with other employment had a mean of approximately 1.5 HIV-associated complications each.

Fifty-nine subjects reported having persistent or intermittent diarrhea for more than 1 month preceding the interview. Sixty-eight subjects reported having a prolonged fever during the same time period.

Disease staging

According to WHO staging system for HIV infection, all 100 study participants presented with advanced stages of disease. Ten percent of participants had stage III disease, and 90% had stage IV disease. The percentage of stage III and stage IV disease was similar among both women and men.

While all subjects presented with opportunistic infections which reflected stage III or stage IV disease, some subjects also presented with additional conditions associated with stage II disease. Weight loss <10% was identified in 11% of subjects and minor mucocutaneous findings were identified in 6% of subjects. Twenty-nine percent of participants reported having herpes zoster within the preceding five years.

DISCUSSION

With respect to clinical manifestations of HIV infection, few statistically significant differences were found among the demographic groups examined. One explanation may be that the sample size was not adequate to detect subtle differences between groups. Distribution of HIV-associated complications was similar among women and men. Anthropologic data are scarce, but given the traditional role of women as caregivers in society (Ministry of Women's and Veteran's Affairs, 2003), we might have expected that men would present earlier in their disease, whereas women might defer their own care while caring for others. This hypothesis was not borne out. We also did not find significant differences in the profile of complications among women and men. However, gynecologic issues were not a focus of this study, and more research is necessary to examine this aspect of HIV disease. Further, we suspected that older persons may have been infected with HIV for longer, and therefore would present at later stages of disease. However, this hypothesis could not be supported. Older patients may have been infected later in life and, therefore, have the same duration of infection as younger patients. Alternatively, those patients who have survived until older age may be those with more robust immune response. We also suspected that subjects' exposure to various complications may have varied by occupation or by residence in rural or urban areas. However, given that persons may have one primary occupation while supplementing their income with additional activities, data regarding occupation may be unreliable. The same may be said for residence, with many Cambodians moving between urban and rural areas for work.

All patients presenting for care for the first time had advanced stages of HIV infection. Seventy-eight of 89 (88%) patients had absolute CD4 counts < 50 cells/mm^3 rendering them highly susceptible to a broad spectrum of HIV-associated complications. Based on WHO guidelines for the treatment of HIV in resource-limited settings (WHO, 2004), all patients in this cohort were appropriate candidates for ART; however, no patients were receiving ART. There is an urgent need for improved access to appropriate antiretroviral therapy in Cambodia.

These findings indicate that clinical examination is an efficient and cost effective

means of determining ART eligibility. Given that all patients presented at advanced stages of HIV disease, measuring CD4 cell counts may not be necessary to ascertain eligibility for ART at this stage of the Cambodian epidemic. There were similar findings in a retrospective study conducted among another cohort at SHCH; 94% of patients eligible for ART were identified based on clinical criteria alone (Chel *et al,* 2004). As therapy becomes more widely available, however, people may begin to present while asymptomatic or at earlier stages of disease.

Despite the high prevalence of tuberculosis among subjects, the results of all PPD skin tests were negative. This inconsistency between clinically diagnosed cases of tuberculosis and PPD skin test results may be attributed to several factors. In severely immunocompromised individuals with latent tuberculosis, a PPD test may not produce sufficient induration (Markowitz *et al,* 1993). In addition, recent evidence suggests that there may be a link between ethnicity and responsiveness to tuberculin skin tests among patients infected with pulmonary tuberculosis (Delgado *et al,* 2004). It is unlikely that inaccurate interpretation of skin tests resulted in the under-representation of positive results, since all PPDs were thought to be completely without induration and were verified by more than one clinician. As the inconsistency between tuberculosis prevalence and PPD skin test results indicates, in this setting, PPD tests have little value in the diagnosis of tuberculosis in symptomatic HIV positive patients. Thorough clinical examination and screening should be conducted to identify tuberculosis among patients with HIV. In addition, universal screening for HIV should be conducted among patients diagnosed with TB.

In this study, PCP staining on sputum appeared to have a low sensitivity. While this test is highly dependent on collection technique, it may be more cost-effective to diagnose PCP empirically in Cambodia, keeping in mind that alternative diagnoses, such as fungal or mycobacterial pneumonias, are also common among these patients. A more reliable technique for PCP diagnosis would be the staining of induced sputum or bronchoalveolar lavage (BAL) (Golden *et al,* 1986). However, induced sputum carries a high risk of contamination and contagion, and patients are often too hypoxic to perform a BAL safely.

The high prevalence of concurrent complications also has important implications for health care providers. While patients with HIV may present with symptoms of one opportunistic infection, providers must be aware of the potential for concurrent infections. This is a major challenge in settings such as Cambodia where diagnostic capabilities are limited and many diagnoses are made on clinical grounds. A patient's failure to respond to therapy may represent an inaccurate diagnosis or the presence of multiple, concurrent infections.

One potential limitation of the study is that only symptomatic patients suspected of having HIV were tested. This may have biased the study to enroll only those with advanced HIV disease. Unfortunately, this reflects the reality in Cambodia; HIV is commonly diagnosed only when patients present for care due to symptomatic disease. It is hoped that increasing availability of antibody testing and effective treatment will facilitate diagnosis of asymptomatic patients who would also benefit from prevention and care interventions.

Participants in this study presented at advanced stages of HIV disease. New interventions should encourage individuals to seek HIV testing earlier. Earlier diagnosis will both facilitate prevention and treatment of opportunistic infections among HIV-positive persons and help to prevent secondary transmission. Development of cost-effective and clinically viable protocols will play a significant role in the scaling-up of HIV prevention and care in Cambodia.

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