Opportunistic infections in relation to cd4 level among HIV seropositive patients from central Nepal

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ABSTRACT

CD4 cells status of HIV patients provides one of the benchmarks against the progression of HIV/AIDS. Regular investigation of opportunistic infection in HIV patients is one of the major components of HIV/AIDS care and support service. Between October 2007 and May 2008, a cross-sectional analytical study was carried out in Tribhuvan University Teaching Hospital with an objective to find the relationship between CD4 level and opportunistic infections. After taking informed consent pre-structured questionnaire was filled and specimens were collected to investigate major opportunistic infections (OIs) as per standard microbiological procedure. All the information were entered into SPSS 11.5 system and analyzed. Of the 150 patients, 100 (66.7%) were males and 50 (33.3%) were females. The age group 21-30 years was predominant (42.7%) followed by 31-40 years (42%). Oral candidiasis was found to be the predominant OIs (32.0%) followed by streptococcal pneumonia (28.7%), *Salmonella* infection (20.7%), cryptosporidial infection (19.3%) and tuberculosis (10.0%). Significant relationship could be established between low CD4 count (<200) and the appearance of oral candidiasis ($\div^2$=9.16, $p<0.05$) but no such relationship could be established regarding other OIs. So, it can be concluded that appearance of oral candidiasis is the strong evidence of advanced stage of HIV infection.

Keywords: Candidiasis, CD 4 count, HIV/AIDS, Nepal.

INTRODUCTION

Human immunodeficiency virus (HIV) causes progressive impairment of the body's cellular immune system leading to increased susceptibility to tumors, and the fatal conditions knows as acquired immunodeficiency syndrome (AIDS).[1] The unique feature in the pathogenesis of HIV/AIDS is that the primary target cell for HIV is immune cells bearing CD4 marker at their surface. With the infection of HIV, there will be gradual decrease of human immune cells bearing CD4 antigen receptor, the most important being T helper cells (CD4 T cells), B lymphocytes, macrophage and natural killer cells leading to development of wide varieties of OIs i.e. severe infections induced by agents that rarely cause serious diseases in immune competent individual. In this way AIDS related mortality and morbidity, which is significantly higher in number as compared to other diseases, is actually due to opportunistic infections (OIs) rather than HIV itself.[2] So, success of any HIV/AIDS care and management project relies on effective diagnosis and treatment of opportunistic infections. In the era of effective antiretroviral therapy (ART), diagnosis and treatment of OIs is an integral part of this treatment strategy because some OIs regimens (for e.g Anti tuberculosis drugs) and ART regimen should not be administrated at the same time to prevent drug induced toxicity.[3] CD4 cell count is best validated predictors of likelihood of developing OIs. Besides, it has great utility in clinical consideration of HIV disease classification and AIDS definition, assessment of prognosis and designing of clinical trials, for e.g. making decision about initiation of ART/prophylaxis.[4] Selection of appropriate CD4 cut off for initiating ART/appropriate prophylaxis can be made on a regional basis depending on OIs incidence and availability of antimicrobials. There are wide range of OIs affecting different system, for example, respiratory tract infection, gastrointestinal tract infection, urinary tract infections, sexually transmitted infection and disseminated infection.[5]

The CD4 cells co-ordinate a number of immunological functions and as these cells are decreased (due to HIV), the risk and severity of OIs increases, resulting the death of the patients. So, CD4 count is an important parameter to initiate OIs prophylaxis e.g. co-trimoxazole preventive therapy (CPT) before the agent deteriorates the body, and conversely, observation of specific OIs gives the predictive value of CD4 counts in blood suggesting the initiation of treatment for e.g. ART. Once the standard value of CD4 count and the OIs incidence rate at that stage is obtained, this can be used as a model for other resource limited settings (where CD4 count facilities are not available) to take decision about the initiation of

Table-1: Socio-demographic characteristics of studied subjects by gender

Characteristics	Male No. (%)	Female No. (%)	Total No. (%)
Age group (Yrs)			
1-10	1 (1.0)	0 (0)	1 (0.7)
11-20	3 (3.0)	0 (0)	3 (2.0)
21-30	39 (39.0)	25 (50)	64 (42.7)
31-40	43 (43.0)	20 (40)	63 (42.0)
41-50	14 (14.0)	4 (8)	18 (12.0)
51-60	0 (0)	1 (2)	1 (0.7)
Total	100 (100)	50 (100)	150 (100)
Marital status			
Married	56 (56.0)	18 (36.0)	74 (49.3)
Unmarried	31 (31.0)	2 (4.0)	33 (22.0)
Widow	9 (9.0)	30 (60.0)	39 (26.0)
Divorced	4 (4.0)	0 (0)	4 (2.7)
Total	100 (100.0)	50 (100.0)	150 (100.0)
Education			
Illiterate	31 (31.0)	31 (62.00	62 (41.3)
Primary	44 (44.0)	10 (20.0)	54 (36.1)
Lower Sec.	2 (2.0)	0 (0)	2 (1.3)
Secondary	22 (22.0)	8 (16.0%)	30 (20.0)
Higher Sec.	1 (1.0)	1 (2.0%)	2 (1.3)
Total	1 (1.0)	1 (2.0%)	2 (1.3)
Occupation			
Unemployed	28 (28.0)	15 (30.0)	43 (28.7)
Farmer	30 (30.0)	23 (46.0)	53 (35.3)
NGO/INGO	16 (16.0)	6 (12.0)	22 (14.7)
Teacher	0 (0)	1 (2.0)	1 (0.7)
Volunteer	13 (13.0)	3 (6.0)	16 (10.7)
Business	8 (8.0)	2 (4.00	10 (6.7)
Driver	2 (2.0)	0 (0)	2 (1.3)
Foreign job	1 (1.0)	0 (0)	1 (0.7)
Student	2 (2.0)	0 (0)	2 (1.3)
Total	100 (100.0)	50 (100.0)	150 (100.0)
HIV transmission			
Sexual	49 (49.0)	49 (98.0)	98 (65.3)
IDU	49 (49.0)	1 (2.0)	50 (33.3)
Mother to child	2 (2.0)	0 (0)	2 (1.3)
Total	100 (100.0)	50 (100.0)	150 (100.0)

prophylaxis/treatment. Additionally CD4 count is an important parameter for assessing the efficacy of ART.[2]

In 2007, the total number of people living with HIV/AIDS (PLHA) in the world was 33 millions (30 – 36 millions).[6] As of 17 Oct 2009, the recorded number of PLHA in Nepal as reported by National centre of AIDS and STI control (NCASC) was 14,787.[7] So far in Nepal, only gastrointestinal manifestation in HIV/AIDS patients in relation to CD4 status were done in a local setting with limited sample size but specific studies aimed at making standard profile of different OIs according to CD4 were lacking.[8] So, this study is conducted with a general objective to explore the relationship between CD4 level and different types of opportunistic infections which may be helpful for the development of guidelines regarding the initiation and monitoring of ART/prophylaxis in resource limited setting of Nepal. Furthermore, the result of the findings will be helpful in the prognosis of different OIs in HIV/AIDS patients.

MATERIALS AND METHODS

This work was carried out in Tribhuvan University Teaching Hospital between October 2007 and May 2008. This research was approved by Institute of Medicine Research Committee, Kathmandu. Altogether 150 PLHA volunteers (both symptomatic and asymptomatic) were included in the study. They were selected from TUTH VCT, Maharajgunj; Navakiran Plus HIV/AIDS Care home Budhanilakantha; Sparsha Nepal HIV/AIDS care home, Sanepa and Crisis Centre, Lalitpur. 25% of 600 HIV positive persons registered over seven months period in the above organizations were randomly selected to get a sample size of 150. The interviewer went to these organizations to take the interview and to collect the specimens of the identified HIV positive subjects. Patient selection was done by random sampling method using the patients' lists available in the respective sites.

After taking verbal informed consent, they were interviewed to fill up the pre-structured questionnaire. Then, specimens were collected which included sputum (three sputa specimen – first spot, early morning, and second spot), stool and oral swab. All the three sputa were used to make separate smear and stained by Ziehl Neelsen method, early morning sputum was inoculated into a blood agar (for culturing pneumococci) and finally it was processed by modified Petroff's method prior to inoculation into two sets of Lowenstein Jenson media. If the suspected colonies were obtained in the respective culture media they were further confirmed by the biochemical tests as per standard microbiological laboratory procedure.[9] Similarly, a part of stool specimen was used to make wet mount

Table-2: Distribution of opportunistic infections in studied subjects by age and sex

Age/ sex	Candidiasis Number (%)	Pneumonia Number (%)	Salmonella infection Number (%)	Cryptosporidial infection Number (%)	Tuberculosis Number (%)
Age group					
1-10	1(2.1)	1(2.3)	1(3.2)	0(0)	0(0)
11-20	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
21-30	18 (37.5)	23 (53.5)	13 (41.9)	9 (31.0)	5 (33.3)
31-40	23 (47.9)	12 (27.9)	12 (38.7)	15 (51.7)	7 (46.7)
41-50	6 (12.5)	7 (16.3)	5 (16.1)	5 (17.2)	3 (20.0)
51-60	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	48 (100)	43 (100)	31 (100)	29 (100)	15 (100)
Sex					
Male (n=100)	34 (34.0)	34 (34.0)	18 (18.0)	27 (27.0)	10 (10.0)
Female (n = 50)	14 (28.0)	9 (18.0)	13 (26.0)	2 (4.0)	5 (10.0)
Total (N=150)	48 (32.0)	43 (28.7)	31 (20.7)	29 (19.3)	15 (10)

preparation, another part was processed and stained by modified Ziehl Nelsen staining technique (for the detection of *Cryptosporidium parvum*) and remaining part was used to inoculate into xylulose lysine deoxycholate agar plate for the isolation of *Salmonella* spp. If the pink colonies were obtained in the culture plates, they were inoculated into biochemical tests (Indol, Methyl red, Vogues Prauskar, Citrate utilization, urease, motility) for confirmation of S*almonella* spp. For the diagnosis of candidiasis, oral swab specimen was cultured potato dextrose agar and if the desired colonies were obtained they were further confirmed by wet mount preparation as well as germ tube culture as per standard microbiological procedure.[9] Data obtained from laboratory result and field visit were entered into SPSS 11.5 (statistical package for social sciences) and analyzed.

RESULTS

Among 150 PLHA volunteers, 100 (66.7%) were males and 50 (33.3%) were females. The age group 21-30 years was predominant (42.7%) followed by 31-40 (42.0%). Most of them were illiterate (41.3 %), married (49.3%), Farmer (35.3%) and acquired HIV infection through sexual means which was as high as 98.0% in females. In contrast to the females, the males exhibited the equal chance of acquiring HIV infection by sexual as well as sharing injection as shown in Table-1.

Oral candidiasis was found to be the predominant OIs whose prevalence was found to be 32.0% followed by streptococcal pneumonia (28.7%), salmonellae infection (20.7%), cryptosporidial infection (19.3%) and tuberculosis (10.0%). Males exhibited the higher prevalence of candidiasis, pneumonia and cryptosporidial infection where as female exhibited the higher prevalence of salmonella infection. All types of OIs are predominant in productive age groups, 21- 40 years as shown in Table-2. Significant relationship was established between low CD4 count (<200) and the appearance of oral candidiasis ($\div^2$=9.16, p<0.05) but no such relationship could be established regarding other OIs ($\div^2$ values ranging from 0.08 to 3.49, p>0.05). 50.9% candidiasis was observed in those patients with CD4 count less than 200 cells per cu mm blood. Similarly, 17.0% prevalence of tuberculosis was observed as soon as the CD4 level falls below 200. However, in other OIs, no such trends were observed (Table-3).

DISCUSSION

One of the most important findings of this study is the documentation of significant relationship between the appearance of candidiasis and low CD4 count (<200). Although high prevalence of TB was obtained in patients with low CD4 count (<200), the association was statistically insignificant. Except TB and candidiasis, no regular trend was observed in the prevalence of other OIs with respect to CD4 count. This might be due to Co-trimoxazole preventive therapy (CPT) i.e as most of the patients were using Co-trimoxazole, this might have influenced the appearance of other OIs regardless of CD4 count. On the basis of this study it can be recommended that initiation of ART should be considered as soon as the appearance of oral candidiasis. Furthermore, special emphasis should be given for the screening of tuberculosis for those patients with CD4 count less than 200. A hospital based study of Far Western Nepal documented 25.9% oral candidiasis, 33.3% esophagial candidiasis in the patients with CD4 count between 101-200.[8] Similarly, 88.0% candidiasis was identified as the predominant OI in HIV patients with median CD4 count

Table-3: Association between opportunistic infections and CD4 of patients*

Opportunistic infections		CD4 <200	CD4 200 and above	x^2, 1 df
Candidiasis	Yes	27	19	9.16
	No	26	57	(Significant)
Pneumonia	Yes	18	24	0.08
	No	35	52	
Salmonella	Yes	10	21	1.31
	No	43	55	
Cryptosporidium	Yes	13	14	0.70
	No	40	62	
TB	Yes	9	5	3.49
	No	44	71	

* Finding of this table is based on the study of 129 HIV volunteers because 21 (150-129) volunteers lacked CD4 report at the time of specimen collection. So, the prevalence of individual OI in this table may also differ from Table-2.

120/cumm in Eastern India.[10] Our study documents that bacterial pneumonia (due to *Streptococcus pneumoniae*) to be the second common opportunistic infection accounting 28.7% (overall prevalence) and predominant OI in the patients with CD4 count 200 and above accounting 31.5% (24/76). In a study carried out in Italy among the HIV patients, *S. pneumoniae* was identified as the most frequent pathogen (60%).[11] Ten percent prevalence of tuberculosis documented by our study is similar to other studies conducted in different parts of Nepal. A hospital based study in Western Nepal documented 10.8 % prevalence of TB among HIV patients while another similar study in Central Nepal documented 23 % prevalence of TB.[12,13] As high as 57.0% prevalence of tuberculosis was observed in HIV seropositive patients from Eastern India.[10] Our study also documents the higher prevalence of enteric opportunistic pathogens such as *C. parvum* and *Salmonella* spp in HIV/AIDS patients. Previous study done in central Nepal documented 10.7% prevalence of *C. parvum* in HIV/AIDS patients.[14] A study carried out in HIV patients of Thailand documented 7.0% prevalence of *Salmonella* spp.[15]

Oral candidiasis was found to be the predominant OIs accounting as high as 32.0% and was significantly associated with low CD4 count (<200). Therefore, its appearance can be taken as a strong evidence of low CD4 level. Similarly, high prevalence of tuberculosis was observed in patients with low CD4 count but the association was statistically insignificant. No such trend was observed in other OIs.

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