

Human Immuno-Deficiency Virus Co-Infection with Hepatitis B Virus and Baseline CD4+ T Cell Count among Patients Attending a Tertiary Care Hospital, Nepal

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ABSTRACT

Background: Since 1981, when the first AIDS case was reported, worldwide, more than 34 million people have been infected with HIV. Almost 95 percent of the people infected with HIV live in developing countries. As HBV & HIV share similar routes of transmission by sexual intercourse or drug use by parenteral injection, co-infection is common. Because of the limited access to healthcare & HIV treatment in developing countries, HIV-infected individuals are present late for care. Enumeration of CD4+ T cell count at the time of diagnosis has been useful to initiate the therapy in HIV infected individuals. The baseline CD4+ T cell count shows high immunological variability among patients.

Methods: This prospective study was done in the serology section of the Department of Microbiology over a period of one year from August 2012 to July 2013. A total of 13037 individuals subjected for HIV test were included in the study comprising of 4982 males & 8055 females. Blood sample was collected by vein puncture aseptically with standard operational procedure in clean & dry test-tube. All blood samples were screened for HIV as described by WHO algorithm by Immuno-chromatography rapid kits. Further confirmation was done by biokit ELISA method as per the manufacturer's guidelines. After informed consent, HIV positive individuals were screened for HBsAg by Immuno-chromatography rapid kits (Hepacard). Further confirmation was done by biokit ELISA method as per the manufacturer's guidelines. EDTA blood samples were collected from the HIV sero-positive individuals for baseline CD4+ T count. Then, CD4+ T cells count was determined by using FACS Calibur Flow Cytometer (BD).

Results: Among 13037 individuals screened for HIV, 104 (0.8%) were found to be infected comprising of 69(66.34%) males & 35 (33.65%) females. The study showed that the high infection was noted in active age group (30.76%), housewives (28.7%) & in heterosexual route (80.9%) of transmission. Out of total HIV infected individuals, distribution of HBV co-infection was found to be 6 (5.7%). Baseline CD4+ T cell count of HIV infected patient was found higher (mean CD4+ T cell count; 283cells/cu.mm) than HBV coinfecting patients (mean CD4+ T cell count; 91 cells/cu.mm). Majority (77.2%) of HIV infected & all co-infected individuals were presented in our center late (CD4+ T cell count; < 350/cu. mm) for diagnosis and care. Majority of co- infected 4 (80%) were late presented with advanced AIDS stage (CD4+ count; <200/cu.mm).

Conclusions: The study showed a high percentage of HIV sero-positive & co- infected individuals. Baseline CD4+ T cell count of majority of HIV infected individuals was found to be low. Hence, more sustained and vigorous awareness campaigns & counseling still need to be done in order to promote early diagnosis and management.

Keywords

HIV/AIDS, HBsAg, Co-infection, CD4+ cell.

Introduction

Human immunodeficiency virus (HIV) is a lentivirus, a member of the retrovirus family that causes acquired immunodeficiency syndrome (AIDS), a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections. HIV infects vital cells in the human immune system such as helper T cells specifically CD4+ T cells, macrophages, and dendritic cells. When CD4+ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to secondary infections. Globally, an estimated more than 35 millions people were living with HIV [1].

HIV was firstly recognized in 1980's, since then the epidemic spread out rapidly all over the world. The trend of the number of people living with HIV/AIDS is growing substantially from year to year and reached to its high level. UNAIDS/WHO estimate for December 2002 that around 42,000,000 people living with HIV/AIDS and 3,100,000 AIDS related deaths in that year alone [2]. According to the US Global Health Policy report, the number of people living with HIV in the world is estimated to be 33,400,000 and more than 2 million people have died due to this epidemic in the year 2008 [3]. The overall adult HIV prevalence in south-east Asia is 0.7%, relatively lower than the 5.7% prevalence in sub-Saharan Africa [4]. It is estimated that approximately 15,000 people are infected with HIV every day [5]. Almost 95 percent of the people infected with HIV live in developing countries and the situation is especially critical in sub-Saharan Africa where around 68 percent of all adults and 90 percent of all children infected with HIV live [6]. It is still a serious problem all over the world especially in the third world countries. Different data sources reveal that the prevalence rate of the pandemic is very high in developing countries as compared to the first world countries.

Since the first case in July 1988 [7,8], the reported number of HIV infections in Nepal has gradually increased to 10,546 HIV cases and 1,610 AIDS cases of December 2007 [9]. Until the late 1990s, Nepal was classified as having a low-level epidemic. However, after 1997, Nepal has been experiencing a concentrated epidemic with rapid spread amongst high-risk groups [8,10]. The HIV prevalence among the general population has been estimated to be approximately 0.5% [11]. Currently, Nepal is in the concentrated epidemic phase with 12,933 HIV positive and 2,151 people living with AIDS by the end of 2009 [12]. HIV is characterized as a concentrated epidemic in Nepal with HIV prevalence of 0.30 per cent among adult aged 15-49 years in 2011 [13]. In the year 2008, sero-prevalence of HIV has been determined to be 0.12% in the study conducted in Kathmandu, Nepal, among blood donors [14]. Many HIV-positive individuals have also been exposed to HBV. A person who is infected with both HIV and HBV is said to have a HIV/HBV Co-infection [15]. Infection with HIV and HBV are often found in the same individual because of shared routes of transmission [16]. Many of the countries with a high HBV disease

burden are also affected by a high HIV burden, leading to frequent HIV/HBV co-infection [17]. Worldwide, it is estimated that 10% of the 40 million HIV-infected individuals have chronic hepatitis B [18]. The prevalence of chronic HBV infection is approximately 10 times higher in people living with HIV than the general population (higher in homosexual than IV drug users, or heterosexual) [19,20]. Studies suggest that as many as 70% - 90% HIV positive people have evidence of past or current HBV infection in the United States [21]. Co-infection of HBV in HIV has been estimated to be 2.6% in the Nepalese blood donors [22]. Both the viruses are transmitted through sexual and percutaneous route in majority of circumstances. The association of these viruses to cause human infection in Nepal has not been well established.

Between 10% to 30% of HIV-infected individuals in the Western world reported to present late for care [23,24]. and higher percentage in developing countries, particularly in sub-Saharan Africa, South-East Asia and South America, because of the limited access to healthcare and HIV treatment [25]. There is the limited availability of trained personnel and laboratory facilities in many developing countries like Nepal. These resources are needed to determine when individuals should start Anti Retrovirus Therapy (ART) -the World Health Organization currently recommends that people start ART when their CD4 count drops below 350 cells/ μ l [26], and to monitor treatment responses over time so that viral resistance to ART is quickly detected. A total lymphocyte count can be used as a surrogate measure to decide when to start treatment, repeated CD4 cell counts are the only way to monitor immunologic responses to treatment, a level of monitoring that is rarely sustainable in resource-constrained settings. Enumeration of CD4+ T cell count at the time of diagnosis has been useful to initiate the therapy in HIV infected individuals. The study helps to correlate the Immunological status of HIV positive patient and co infected patients with HBV by counting CD4+ lymphocytes at the time of diagnosis.

Methodology

This prospective study was done in the serology section of the Department of Microbiology and Voluntary Counseling and Testing Centre, Institute of Medicine, Tribhuvan University Teaching Hospital (TUTH), Kathmandu, over a period of one year from august 2012 to July 2013. A total of 13037 individuals attended TUTH for HIV screening were selected for study. Written informed consent was obtained from the patient before enrollment. Ethical approval was taken from Institutional Review Board (IRB), Institute of Medicine, Tribhuvan University Teaching Hospital, and Kathmandu, Nepal.

Sampling Procedure

All individuals subjected for HIV test were included in the study. Blood samples received in the Department of Clinical Microbiology Laboratory, TUTH, were tested as described by WHO algorithm for HIV antibody. HBsAg test and CD4+ T cell count were performed for HIV infected individuals.

After informed consent, all participants included HBV co-

infected were interviewed by questionnaire on marital status, place of residence, knowledge about HIV, route of transmission and caste etc. All these collected data from each individual were recorded in clinical and serological / immunological profile form. Immunological profile (baseline CD4 count) was determined by using FACS Caliber Flow Cytometer (Becton-Dickinson).

Blood sample collection, handling and transportation

Blood sample was collected by vein puncture aseptically with standard operational procedure in clean and dry test-tube. Next blood samples were collected in a tube containing Ethylene Diamine tetra-acetic acid (EDTA) from HIV proved patients for CD4 Lymphocytes counts. Collection and transportation of specimens for CD4+ T cell count was done according manufacturer's instructions.

Laboratory Procedures

HIV testing

Blood samples were tested for HIV antibodies according to the WHO algorithm for voluntary counseling and testing by using three commercially available rapid test kits such as Determine HIV1/2 (Abbott laboratories, Japan co LTD), SD Bioline HIV 1 /2 3.0 and HIV 1 /2 TRIDOT (J. Mitra and Co. Pvt. Ltd., New Delhi, INDIA). Tests were done according to the manufacturer's instruction.

The results were interpreted as positive, negative or invalid on the basis of bands developed on the strip/well. Specimens were first tested with the Determine HIV-1/2 test kit (Abbott Laboratories, United Kingdom). If a sample was reactive, it was tested with the SD Bioline HIV-1/2 3.0 test (Standard Diagnostics, South Korea). In the event of a discordant result, HIV 1 /2 TRIDOT (J. Mitra & Co. Pvt. Ltd., New Delhi, INDIA) was used. The final result of the algorithm scored a sample as positive if two of the three rapid and simple HIV assays were reactive. If found positive, further confirmation was done by using Biokit ELISA method.

HBsAg testing

Patients with HIV positive were screened serologically for HBV infection by HEPACARD One Step Rapid Visual Test for the Qualitative Detection of HBsAg in Serum/Plasma (J. Mitra & Co. Pvt. Ltd., New Delhi, India) according to manufacturer's instruction. Patients found HBsAg positive by rapid test were further confirmed with Biokit ELISA according to the manufacturer's manual.

CD4 +T cell count

EDTA blood samples were collected from the HIV sero-positive individuals above 15 years of age for baseline CD4+ T count to find out the baseline immunological status at the time of diagnosis according to manufacturer's instructions by using FACS Caliber Flow Cytometer (Becton-Dickinson). Then CD4 count was expressed in cells/cu.mm. In this method, TruCount, test tubes that contain a known number of brightly fluorescent polystyrene beads were provided by the manufacturer. Fresh whole blood from the K2EDTA tube for each patient was accurately pipetted

into the tubes and mixed with fluorochrome-labeled monoclonal antibodies. The erythrocytes were lysed, and this mixture was analyzed on the flow cytometer. All samples were processed and analyzed within 4 hrs of blood collection.

Data Processing and Analysis

Data were analyzed using SPSS version 17.0 and interpreted according to frequency distribution, percentage. Chi-square test. Significant association of demographic data wherever applicable with P value of < 0.05 regarded as significant.

Results

Among the total 13037 HIV screened individuals, greater parts were female 8055 (61.8%) than males 4982 (38.2%). Among individuals screened for HIV, 104 (0.8%) were found to be infected (Figure 1) comprising of 69 (66.34%) males & minimum percentages were females 35 (33.65%). The sero-positivity was found significantly high in male (p=0.001) (Table 1).

Age groups (yrs)	Female (%)	Male (%)	Total (%)
0-10	4 (3.84)	6 (5.76)	10 (9.6)
11-20	0 (0.00)	6 (5.76)	6 (5.76)
21-30	12 (11.5)	15 (14.4)	27 (25.94)
31-40	12 (11.5)	20 (12.2)	32 (30.76)
41-50	6 (5.76)	14 (13.46)	20 (19.2)
51-60	1 (0.96)	6 (5.76)	7 (6.72)
61-70	0 (0.00)	2 (1.92)	2 (1.92)
Total	35 (33.65)	69 (66.34)	104 (100)

Table 1: Distribution of HIV Positive Individuals According to Age Group and Gender-wise.

■ HIV Infected patients ■ HIV non-infected patients

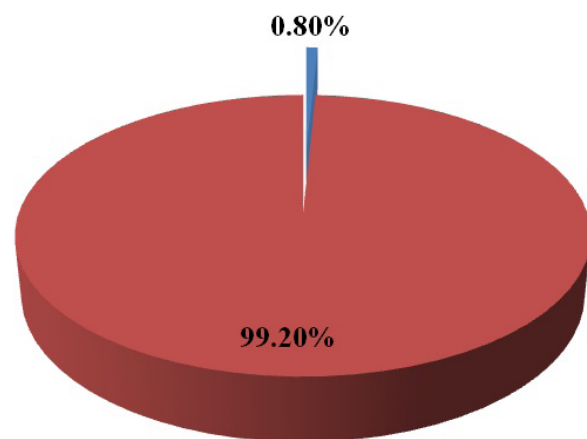


Figure 1: Distribution of HIV infected Individuals.

Age group wise distribution of HIV infected individuals indicated that greater part of infected individuals 32 (30.76%) were between 31-40 years age group and a very few of infected patients 2 (1.92%) were between 61-70 years age group. Out of total investigated, it was found that majority of individuals were housewives 25 (28.75%) followed by migrants 17 (19.54%) and

the lowest prevalence 1 (1.15%) was observed in teachers. The majority 72 (80.90%) of individuals were found to be contacted HIV through heterosexual route than vertical (13.50%) and intra venous route (5.60%).

Out of 104 HIV sero positive subjects, 6 (5.76%) were positive for HBV. (Figure 2) Among the HIV infected individuals above 15 years of age, mean baseline CD4 count was 283/cu mm. The lowest CD4 count was 20 cells /cu mm and highest was 1079 cells/cu mm. The mean baseline CD4 count among co-infected patients was found 91 cells/ cu mm. The lowest count was 14 cells/ cu mm and highest was 224 cells/cu mm (Table 2).

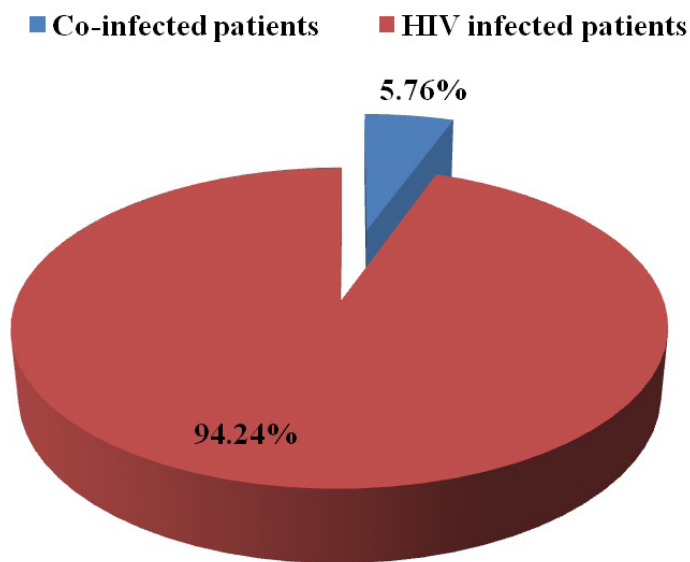


Figure 2: Distribution of HBV Co infection among HIV Infected Individuals.

Descriptions	HIV Infected Individuals (n=52)	HBV Co-infected Individuals (n=5)
Lowest base line CD4 count	20 cells/cu.mm	14 cells/cu.mm
Highest base line CD4 count	1079 cells/cu.mm	224 cells/cu.mm
Mean baseline CD4 count	283 cells/cu.mm	91 cells/cu.mm

Table 2: Status on Baseline CD4 Count among HIV Infected and HBV Co-infected Individuals above 15 Years of Age.

Majority of individuals, among the HIV mono-infected (40.40%) and co-infected (80.00%), were in advanced AIDS stage (CD4 count<200/cumm) at the time of diagnosis (Table 3).

Level of CD4 count	HIV mono-infected number (%)	HBV co-infected number (%)	HIV sero-positive number (%)
<200 cells/mm ³	21 (40.40)	4 (80.00)	25 (43.86)
200-349 cells/mm ³	18 (34.60)	1 (20.00)	19 (33.34)
≥350 cells/mm ³	13 (25.00)	0 (0.00)	13 (22.80)
Total (%)	52 (100)	5 (100)	57 (100)

Table 3: Distribution of Baseline CD4 Count among HIV Infected Individuals above 15 Years of Age.

Discussion

Among the total 13037 individuals who were enrolled for this study, 62% were females. The male to female ratio was 0.61:1. According to this study, prevalence of HIV was 0.80% in studied population. This was greater than detected prevalence (0.5%) in 2009 in the country by UNAIDS [27], 0.56% for South and South-East Asia in 2001 [28], and the also mentioned 0.2-0.5% for India in 2008 by UNAIDS/WHO [29]. This finding was also higher than blood donors reported 0.33% in 2008 by Tiwari et al. [30], 0.222% in 1993 among normal Nepalese people by Shrestha et al. [31], and 0.30% in 2012 among adult aged 15-49 years group by MOHP/NCASC [32]. This detection of higher distribution may be due to being a major referral and tertiary care centre of the country. However, the rate was found lower than 8.8% in Sub-Saharan Africa and 2.3% among Caribbean in 2001 [11] and 1% in Thailand among the adults in 2011 reported by WHO/ SEA [33]. A retrospective study among healthy Nepalese males done in 2003 showed the higher (1.6%). Prevalence of HIV than this finding reported by Joshi et al. [34]. Another hospital based study conducted in United Mission Hospital; Tansen Palpa in 2001 by Napit et al. had also shown higher (10%) prevalence among clinically suspected cases [35].

According to this study, among HIV infected individuals, majority were males (66.34%) and 33.66% were females. Similar findings were reported in Thailand by Chotiprasitsakul et al. in 2010 [36], and in central Nepal by Sharma et al. in 2010 [37], which showed 60.3% and 66.6% were males among HIV sero-positive individuals respectively. But, it was reported as contrast from Nigeria where females were higher than males. Studies conducted in 2010 by Ijioma et al. [38], and in 2012 by Iroezindu et al. [39], showed 60.0% and 66.0% of the HIV-infected individuals were females among HIV infected individuals respectively.

Our data indicates that the prevalence of co-infection of HBV in HIV infected individuals was found to be 5.76%. Other studies suggest that as many as 70% - 90% HIV positive people have evidence of past or current HBV infection in the United States [40]. Since a majority of patients spontaneously clear HBV without treatment, however the rate of active infection is much lower. The percentage of HIV/HBV co-infection prevalence reported in HIV infected individuals in Australia by Abdel et al. [41], in 2007 was 6.3%, Nigerian researchers by Ekanem et al. [42], in 2011 was 12.1%. Similarly, higher prevalence (25.5%) was also reported by French researchers Lipiroth et al. [43], in 2007, 8% by Hennessey et al. [44], in 2009 incarcerated individuals in three U.S. cities, 9% by Shanmugam et al. [45], in 2007 in India, 9.9% by Jain et al. [46], in 2009, New Delhi, India and 5-15% in Britain, 2008 by Omland et al. [33]. Low prevalence of HIV/HBV co-infection was 0.033% reported by Ghimire et al. [47], in 2002, among total volunteer blood donors and 3.8% in 2008, among sex-trafficked women and girls in Nepal by Silverman et al [48].

In our study, Among the HIV infected individuals mean baseline CD4 count was 283 cells/cumm (range: 20 to 1079 cells/cumm). The mean baseline CD4 count among co-infected individuals

was found 91 cells/ cumm (range: 14 to 224cells/cumm). Approximately 77% of HIV infected individuals were found to be baseline CD4 count less than 300 cells/cumm. This showed that majority of the individuals first presented to diagnose the disease at a time when the CD4+ T Lymphocytes cell count was already in the range requiring commencement of ART. This shows that most of the individuals still present late to the hospital. Quite a large percentage (43.86%) of individuals had a CD4+ T Lymphocytes cell count less than 200 cells/ cumm at presentation. A study done in Northern Nigeria by Nwokedi et al. [49], 2007; showed that 50-54% of HIV infected individuals had CD4+ T Lymphocytes cell count less than 200 cells/cu.mm at presentation. Another study by Ebenezer et al. in 2010, Nizeria, showed that the majority of HIV infected individuals (71.8%) had a CD4+ T- Lymphocytes cell count less than 350 cells/cumm at the time of presentation [50].

In a study in India, Gautam et al. showed that all HIV positive individuals found to be baseline CD4+ T lymphocytes cell count less than 200 cells/cumm at presentation [51]. The major reason for this high proportion of HIV individuals presenting with low CD4+ T Lymphocytes cell count is late presentation. Late presentation of HIV patients for care is definitely a major problem to our environment. About 10% to 30% of HIV-infected individuals in the Western world reported to present late for care by Sabin et al. in 2004 [52]. and higher percentage in developing countries, particularly in sub-Saharan Africa, South-East Asia and South America, because of the limited access to healthcare and HIV treatment was reported by Sepkowitz et al. in 2006 [53].

Stigma and delay in seeking health care, reluctance to utilizing voluntary testing and counseling services and health system delays in referral may be other possible explanations. Thus, priority must be given to identifying HIV-infected individuals and starting treatment earlier in the course of their illness, before they develop severe opportunistic infections. More sustained and vigorous awareness campaigns still need to be done to diagnose this disease early.

Conclusion and Recommendation

The study showed a high percentage of HIV sero-positive & co-infected individuals. Baseline CD4+ T cell count of majority of HIV infected individuals at the time of diagnosed was found to be low, i.e. preponderance of HIV infected individuals were presented in our centre late for care (CD4 count :< 350/cumm). That's why more sustained and vigorous awareness campaigns, regular general health check up & counseling still need to be promoted for early diagnosis and management. There is also a need to accelerate the integration of hepatitis B virus screening and treatment programme into HIV/AIDS programme.

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