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Research Article

Clinical Manifestations of Treatment Naïve HIV Infected Patients in Pakistani Population

Ahmad F[1], Imran M[1], Yusuf NW[2], Atif M[1], Akram N[1], Fatima Z[3] and Waqar AB[1]*

[1]Department of Medical Lab Sciences (DMLS), FHAS, ICBS, Pakistan

[2]Department of Pathology, Allama Iqbal Medical College, Pakistan

[3]Department of Radiological Sciences and Medical Imaging (DRSMI), FHAS, ICBS, Pakistan

***Corresponding author:** Ahmed Bilal Waqar, Department of Medical Lab Sciences (DMLS), Imperial Post Graduate Medical Institute (IPGMI), FHAS, ICBS, 25 B Lower Mall, Lahore, Pakistan

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Abstract

The Human Immunodeficiency Virus (HIV) infection is a global burden with the most prevalence in sub-Saharan Africa. Although the prevalence of HIV in Pakistani population is low but it is ranked as a high risk country. The current study was designed to investigate the clinical manifestation of 600 HIV untreated patients from several major cities of Punjab, the largest province of Pakistan. These patients were tested for other co-infections like Hepatitis B virus (HBV), Hepatitis C virus (HCV) and Tuberculosis (TB). CD4+ and CD8+ T lymphocytes profile of all the subjects was also determined by fluorescence activated cell sorting (FACSCalibur) using BDTritestCD4/CD8/CD3 antibodies. The results showed that in all untreated HIV subjects, CD4+ T lymphocytes were decreasing with the disease progression, while the primary level of CD8+ T lymphocytes cells was decreased but later on it kept increasing. The ratio of CD4+ T lymphocytes /CD8+ T lymphocytes was also decreased with disease progression.

Keywords: HIV; CD4+; TB; Transmission; Infection

Introduction

Human Immunodeficiency Virus (HIV) is one of the major viral infections worldwide. HIV infection leads to a progressive failure of immune system leading to Acquired Immuno-Deficiency Syndrome (AIDS). Since its discovery in 1983, HIV infection is the subject of major focus as numbers of patients are increasing with each passing day. Now HIV infection is a global dilemma [1]. According to recent estimates, worldwide 37 million people are living with HIV infection with the addition of 3.1 million new cases each year. Thus HIV infection is becoming an alarming threat to world population. In Pakistan, approximately 83,468 individuals are living with HIV-1 infection. In total 18 AIDS control centers in Pakistan; only 7568 individuals living with HIV-1 (PLHIV-1) are registered. Out of these registered cases only 3211 adult and 70 children were on Antiretroviral Therapy (ART) [2]. As the treatment facility against HIV-1 is increasing day by day, therefore the life span of infected people is also increasing. Additionally, the recent advances in ART are also preventing the onward HIV-1 transmission as the treatment suppresses the viral on set and reservoir.

There are two types of HIV viruses depending on the origin. HIV-1 is believed to be originated from chimpanzee (SIV1), while HIV-2 is believed to be originated from monkey (SIV11). Thus both types of viruses have zoonotic origin and now successfully infecting humans. The most prevalent type of HIV infection across the world is HIV-1 infection [3].

HIV-1 is a retrovirus and deviates from the central dogma of Molecular Biology (DNA to RNA; RNA to protein). After entry into CD4+ T-cell, HIV-1 RNA is converted to DNA and integrates into the host genome. The host machinery and energy is exploited to produce viral proteins. The structure of HIV-1 shows that it comprises of envelop, outer lipid bilayer, two outer surface glycoproteins, gp41 and gp120; two layers, matrix layer of MA proteins and capsid layer of CA proteins. The capsid layer surrounds a layer of core proteins containing two single strands of RNA along with reverse transcriptase and integrase protein [3]. There are four HIV-1 accessory proteins (Nef, Vif, Vpu, and Vpr). These proteins facilitate the evasion of viral escape from both the cell mediated and the intrinsic immune system. These proteins modify the hostile environment of the host cell to ensure the viral persistence, dissemination and transmission [4].

HIV-1 infection leads to the development of symptoms within 2-6 weeks in 40-90% patients. These symptoms are flu, fever, fatigue, night sweats, lymphadenopathy, headache, nausea and diarrhea. As these symptoms are also associated with other diseases such an influenza and tuberculosis therefore, the primary care clinicians generally do not diagnose early HIV-1 infection [5,6]. Antibodies against HIV-1 are formed within 3-12 weeks [7].

HIV-1 life cycle is characterized by three phases. The first phase of HIV infection, early or acute infection is commonly diagnosed by detectable HIV-1 RNA or p24 antigen [8,9]. Soon after HIV-1 infection, antibody test is useless as HIV-1 antibodies are produced in 3-12 weeks. Therefore, the suspected patients must be screened for HIV-1 RNA. Low copy numbers of HIV-1 RNA i.e. less than 10,000 copies per/ml represent false positive result as acute HIV-1 infection causes a high copy number of HIV-1 RNA, i.e. more than 100,0000 copies per/ml [10,11]. HIV-1 treatment at acute stage lower viral set point [12-14], reduces virus reservoir [15], disease progression and viral mutation [16]. However, early treatment is also linked with prolonged exposure to ART which may play a role in the development of drug resistance, drug toxicities and serious side effects on individual's quality of life. The second phase is connected with low viral titer in the blood and may last for years. The third and the last stage are characterized by high viral count and severe drop in CD4+ T-cells leading to a weak immune system.

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According to the current treatment guidelines of adult HIV infected subjects the treatment initiation should be with 2 different nucleoside reverse transcriptase inhibitors e.g. amivudine/abacavir, emtricitabine/tenofovir disproxil fumarate and their efficiency is further boosted up by a nonnucleoside reverse transcriptase inhibitor (efavirenz or rilpivirine) or integrase strand transfer inhibitor (dolutegravir, elvitegravir, or raltegravir), or inhibitor (darunavir or atazanavir). The treatment failure is rapidly confirmed and drug resistance test may be performed before switching therapy. There are also available alternative treatment options. Treatment switching due to cost effects, adverse side effects or inconvenience should not jeopardize antiretroviral potency [17].

Current study focuses on the clinical aspects of HIV/AIDS subjects residing in the largest province of Pakistan (Punjab). HIV/AIDS patients were investigated for the presence of diseases like HBV, HCV and TB. This study gives an overall clinical association of co-infection in untreated HIV/AIDS subjects.

Material and Methods

The current study was conducted at the Department of Pathology, Allama Iqbal Medical College, Lahore Pakistan. There were included 600 HIV/AIDS treatment naïve subjects from April 2012 to Feb 2013. The department has a reference laboratory of Punjab AIDS Control Program (PACP), for their lymphocytes profiling (CD4+ count) of HIV infected subjects. All the enrolled subjects were already diagnosed with HIV infection and referred from multiple centers of PACP, Pakistan. HIV infected treatment naïve subjects of both genders; male and female were included without any age restriction. Written permission was taken from all the enrolled subjects that their clinical findings may be published in a scientific journal without disclosing their identity.

Blood samples were collected in a sterile K_3EDTA (Ethylene Diamine Tetra Acetic Acid) tube for lymphocyte profiling and in a sterile BD vacutainer rapid serum tubes for detection of HCV, HBV co infections.

CD4+ /CD8+/CD3+profiling

CD4+ /CD8+/CD3+profile were determined by BD Tritest CD4/CD8/CD3 antibodies using TruCount Tubes through BD FACSCalibur flow cytometer. The blood samples were processed immediately within 2 hours of samples collection. Lyse no wash method was performed using whole blood for acquisition of sample. BD Tritest CD4/CD8/CD3 antibodies which include fluorochrome labeled monoclonal antibodies were used to perform CD4+ /CD8+/CD3+profiling.

HBV, HCV and TB Detection

HBV diagnosis was performed by accurate diagnostic kit, USA which is based on immunochromatographic technique having approximately 95% accuracy. HCV detection was done by accurate diagnostic kit with relative sensitivity, specificity, and accuracy of >99.8%, 99.9%, and 99.9%, respectively. Later on, positive cases were further confirmed by Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). Pulmonary TB was diagnosed by clinical features along with physical examination and radiograph (Chest X-ray). Further confirmation of TB was done by three consecutive sputum smears examination for Acid Fast Bacilli (AFB) using Ziehl-Neelsen staining technique (Z-N stain)

Table 1: Possible routes of HIV transmission in accordance with the patient history.

Possible Causes	Frequency	Percent
Infected Needles	155	25.8
Heterosexual contact	120	20.0
IV Drug Abuse	105	17.5
Surgery	65	10.8
Spouse of HIV Infected Person	50	8.3
MSM	45	7.5
Blood Transfusion	40	6.7
From Infected Parents	20	3.3
Total	600	100.0

Results

Six hundred treatment naive HIV subjects were selected for the current study. The age of patients ranged between 04-68 years and the mean age of 31.80 and standard deviation of 10.903. Percentage frequency of gender wise HIV infection of male, female and transgender was; 64.1%, 33.3% and 2.5%.

The history data of HIV patients revealed that infected needles (25%) use was the major possible route of HIV transmission followed by heterosexual contact (20%), intravenous drug use (17.5%), surgery (10.8%), spouse of HIV infected person (8.3%), male sex with male (7.5%), blood transfusion (6.7%) and vertical transmission (3.3%) as shown in Table 1.

About half number of patients (50.8%) has no opportunistic infections as shown in Figure 1. The most frequent infection associated with HIV patients was HCV (21.6%), TB (9.1%) and HBV (4.1%). The frequency of triple infection; HIV, HCV and TB was 7.5%, while HIV, HBV and TB frequency was 1.6% as shown in Figure 1.

CD4+ T cells are important immune cells for clearance of HIV infection. It is established that patients possessing high frequency

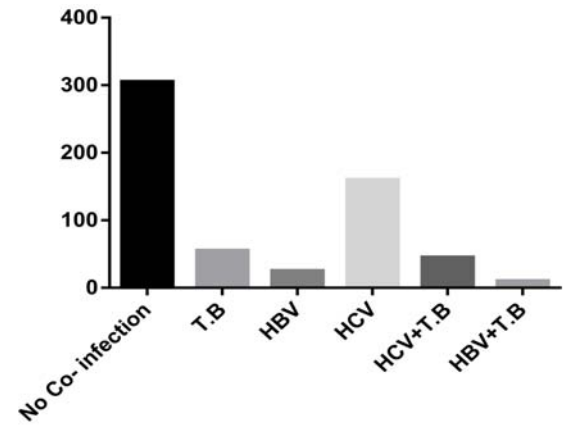

Figure 1: Frequency of co-morbidities associated with HIV infected subjects. Graph shows that 305 patients had no co-infection, 55 were co-infected with TB, 25 were co-infected with HBV and 130 were co-infected with HCV, 45 had HCV+TB and10 had HBV +TB.

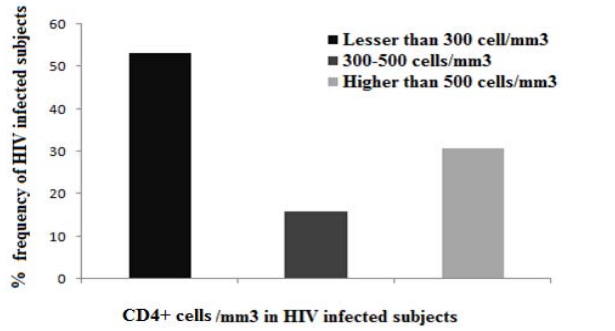

Figure 2: Frequency of CD4+ T cells in treatment naive HIV infected subjects.

CD4+ T cells prior to initiation of treatment have more chances control viral replication. Absolute CD4+ T cell count in majority of enrolled patients (53.3%) was less than 300 cells/mm^3. The frequency of CD4+ T cell in 15.8% patients was between 300-500 cells/mm^3, while in 30.8% patients it was above 500 cells/mm^3 (Figure 2).

CD4+ T cells counts of 600 untreated HIV individuals was determined by BD FACSCalibur flow cytometer; 53.3% of untreated HIV infected individuals had absolute CD4+ count less than 350 cells/mm^3, 15.8% had absolute CD4+ count between 350-500 cells/mm^3 and 30.8% had absolute CD4+ count greater than 500 cells/mm^3. Thus only 30.8% of HIV treatment naïve patients had the chances to spontaneously control viral replication without ART.

CD8+ T cell are the key players of immune system against HIV infection. These cells respond to the initial infection and control the infection for many years. It is ultimate failure of these immune cells that leads to the condition of AIDS. Absolute CD8+ T cell count in 5.8% enrolled patients (53.3%) was less than 300 cells/mm^3. The frequency of CD8+ T cell in 41.7% patients was between 300-1000 cells/mm^3, while in 52.5% patients it was above 1000 cells/mm^3 (Figure 3).

CD8+ T cells counts of 600 untreated HIV individuals was determined by BD FACSCalibur flow cytometer; 5.8% patients had absolute CD8+ count less than 300 cells/mm^3, 41.7% had absolute CD8+ count between 300-1000 cells/mm^3 and 52.5% had absolute CD8+ T count more than 1000 cells/mm^3. Thus initially, approximately half number of patients had an appropriate CD8+ T count but with the disease progression and time span, it is likely that CD8+ T cells count will get decreased.

Discussion

In this cross sectional study 600 HIV/AIDS subjects were enrolled for relevant clinical features and their T cell subsets were determined by FACSCalibur. These HIV/AIDS subjects were mostly from families with lower socioeconomic status. Majority of patients infected with HIV were male (53.3%). The most probable reason is more exposure of male to routes of HIV transmission such as sharing of needles and intravenous drug users. The results of current study demonstrated that the prevalence of HIV infection due to infected needles and intravenous drug abuse was 25% and 17.5% respectively. Worldwide studies showed that one of major reasons for the spread of HIV-1 infection (5-10%) is the practice of using shared syringes. This number rises to 80% in Central Asia and Eastern Europe

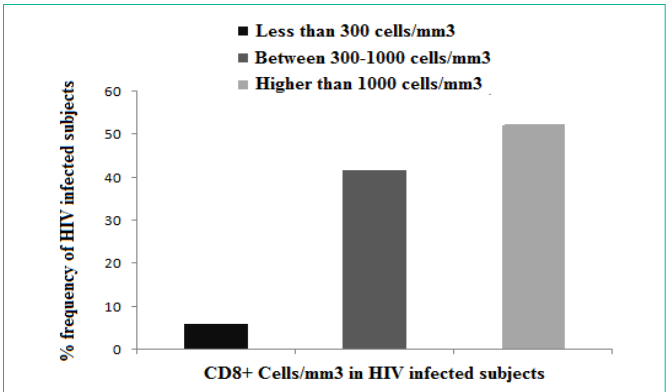

Figure 3: Frequency of CD8+ T cells in treatment naive HIV infected subjects.

[18]. In Pakistan, it is estimated that about 4.25 million people are addicted to drugs with 430,000 people who inject drugs nationwide. It is found that mostly (4 million), People Who Inject Drugs (PWID) uses cannabis as an injecting drug. In Punjab around 80% of PWID drug share syringes, 66% in Sindh and 50% in Khyber Pakhtun Khwa (KPK). The main reason for this harmful practice is lack of awareness and poverty.

It is estimated that each year there occur 448 million cases of treatable sexually transmitted infections across the world. Approximately, 80% of new HIV-1 transmission cases occur by sexual route [19]. In Pakistan, in 2011 the prevalence of HIV-1 among Hijra, or Transgendered Sex Workers (HSW), Male Sex Workers (MSW) and female sex workers was 5.2%, 1.6% and 0.6% respectively [2]. The patients' history of the current study highlighted this route of HIV transmission. HIV transmission by sexual activity was 20%.

The other major route of HIV-1 transmission is blood transfusions. However, since 1980, tremendous efforts have been made worldwide to prevent HIV-1 transmissions by blood transfusions [20]. The rate of HIV-1 seropostivity in blood donors decreased from 3.5 to 0.5 per 10,000 donations [21]. Despite all the precautionary measures, HIV-1 transmissions by blood transfusions may still occur due to window period or by escape of HIV-1 from blood screening test. HIV-1 window period is defined as the time interval, when the person is infected but has not developed antibodies yet. It was observed in the current study that HIV transmission by blood transfusion was 6.7%. The practice of safe blood transfusion is progressed in Pakistan since 1997. A survey of 23 blood transfusion centers out of 40 in Karachi (Pakistan) in year 2007-2008 showed that all of these centers were screening blood for HBV, HCV and HIV-1. Furthermore, these centers were screening blood for malaria and syphilis also. In most of these centers screening was performed by semi-automated Enzyme Immunoassay (EIA) [2].

The most common opportunistic infection associated with HIV infection was HCV. HCV infection is very common in Pakistan. Approximately 10 million people are HCV infected in Pakistan. TB was the second most prevalent infection observed in HIV subjects of the current study. Worldwide studies reports TB as the most dominant found in HIV infected individuals.

Decrease in CD4+ T counts is an important marker of HIV disease progression [22]. Low CD4+ T cells count in treatment naive

patient is an alarming sign to HIV patients as these patients have lesser chances to achieve CD4 > 200 cells/μL [23]. Long term highly active anti retroviral studies of six years have shown that HIV treatment naïve patients possessing CD4 count > 350 cells/μL recovered to normal CD4+ level. On the other hand, HIV patients with low CD4+ level before treatment initiation did not achieve normal CD4+ level [23]. In the present study CD4+ T cell count was decreased in enrolled HIV subjects, 53.3% subjects have CD4+ count 350 cells/mm^3. CD4+ T cells are the most important immune cells and target of HIV. At the last stages of HIV infection CD4+ T cell count is drastically declined and there come the stage of AIDS. Absolute CD8+ T cell count was high in most of the enrolled HIV subjects. In 52.5% subjects, its count was above 1000 cells/mm^3.

Conclusion

It was observed in the current study that the most common route of HIV transmission was infected needles and the most opportunistic infection associated with HIV infection was HCV. CD4+ cell count in majority of HIV untreated patients was less than 350 cells/mm^3, while CD8+ level was high in most HIV untreated subjects.

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