Early Diagnosis of Human Immunodeficiency Virus Infection by p24 Antigen Detection

Col AK Praharaj*, Dr (Mrs) K Angadi⁺, Lt Col AT Kalghatgi[#], Lt Col S Tripathy^{**}, Lt Col MPS Sawhney⁺⁺, Col A Nagendra^{###}

Abstract

p24 antigen was estimated in human immunodeficiency virus (HIV) sero-negative individuals attending various sexually transmitted diseases (STD) clinics and also in sero-negative voluntary blood donors. A total of 300 STD cases and 500 voluntary blood donors, who also acted as controls, were included in this study. Antibody to HIV was detected by ELISA and was confirmed by western blot. In sero-negative individuals, p24 antigen detection was carried out by standard assay and immune-complex dissociation assay (ICD assay) using ELISA method and confirmation was done by neutralisation assay. In voluntary blood donors, 4 (0.8%) individuals were found to be HIV positive and no sero-negative individual was positive for p24 antigen. 41 out of 300 patients attending STD clinics were found to be positive for HIV and in 259 sero-negative patients, p24 antigen was detected in 6 (2.3%) cases by ICD assay whereas only 4 cases were detected by standard assay. By estimating p24 antigen an additional 2.3% HIV positive cases that were in window period were detected. Further, an ICD assay improves the detection of p24 positive individuals.

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Key Words : ICD assay; p24 antigen ; STD; Window period

Introduction

HIV infection is predominantly a STD world wide. History of STD is associated with an increased risk of HIV infection, genital ulcer being a co-factor. Recent data suggests that in the presence of other STDs, individuals are 3-8 times more likely to acquire HIV if exposed to the virus through sexual contact [1].

Though the detection of antibody to HIV is the main modality of diagnosis of HIV infection in adults, antibodies are not detectable before 4-6 weeks of infection. During this window period, HIV-PCR for detection of nucleic acid, HIV culture and p24 antigen assay can be used to detect HIV infection [2]. We carried out p24 antigen assay in patients attending STD clinics and in voluntary blood donors who were sero-negative for HIV to diagnose infection during window period. As p24 antigen (Ag) remains bound to antibody forming immune complex, immune complex dissociation was also carried out and compared with standard assay.

Material and Methods

500 voluntary blood donors and 300 patients attending various STD clinics were included in the study. Informed consent was obtained from each subject and pre and post-test counselling was carried out. History of sexual exposure and physical findings were noted.

5 ml of blood was collected from each subject; serum was separated and stored at 70°C till further tests. Antibody to

HIV was detected by ELISA using commercial kit (HIV detect, USA) and manufacturer's instruction was followed. Sera found reactive were retested by rapid test (Immunocomb, Organics, Israel) and another ELISA (Lab system, Finland). Estimation of p24 antigen was carried out by standard as well as ICD-HIV-p24 Ag7 assay using a commercial kit (Inno genetics USA). Manufacturer's instructions were followed. Acid treatment procedure was done on all specimens immediately prior to p24 antigen ELISA as described by Nishanian et al [3]. In brief, serum was thawed at the time of assay, and 100µl of the specimen was mixed with 50µl of 0.5N HCl (pH2.5-3.0), incubated for 1 hour at 37°C and neutralised with 50µl of 0.5N NaOH to pH 6.8-7.2 as determined by pH indicator paper. Detection of p24 Ag was carried out by ELISA following manufacturer's instructions. Positive tests were confirmed after an anti-p24 Ag neutralisation assay using commercial kit (Innogenetics, Belgium). A positive p24 Ag result required demonstration of a greater than 50% reduction in optical density with specific p24 Ag neutralisation.

Result

Out of 500 voluntary blood donors in the control group, 4 individuals (0.8%) were found to have antibody (Ab) to HIV. None of the sero-negative blood donors tested positive for p24 Ag. Out of 300 patients with STD, 41 (13.66%) were found to be sero positive for HIV, and out of 259 sero-negative patients with STD, 6 (2.3%) were found to be positive for p24 Ag (Table 1) by ICD assay whereas only 4 cases were detected by standard assay. Genital ulcers were detected in 194 STD cases including the six p24 Ag positive cases.

*Senior Advisor (Pathology and Microbiology), 151 Base Hospital, C/o 99 APO, ⁺Post Graduate Student in Microbiology, [#] Associate Professor, Department of Microbiology, ^{###}Professor and Head, Department of Microbiology, Armed Forces Medical College, Pune - 411 040, ^{**}Classified Specialist (Pathology), Command Hospital (Northern Command), C/o 56 APO, ⁺⁺Classified Specialist (Dermatology), Base Hospital, Barrackpore.

Table 1

HIV positivity in blood donor and STD cases

Category	Total No tested for HIV Ab	Total No positive for HIV Ab	Total No tested for p24 Ag (sero-negative)	Positive for 24 Ag
Voluntary blood donors	500	4 (0.8%)	496	Nil
STD cases	300	41 (13.66%)	259	6 (2.3%)

(4 by standard assay)

Discussion

The current world-wide expansion of the AIDS epidemic is primarily driven by the sexual transmission of HIV and in future will be determined largely by the degree to which sexual transmission could be reduced. STDs have been associated with risk of HIV infection in a number of studies [4,5,6]. STDs increase susceptibility to infection by causing genital lesions that facilitate viral entry or by increasing the number of target cells for HIV (activated CD4⁺ and monocytes). Genital ulcers in particular have been identified as an independent risk factor [1].

Routine screening of blood for antibodies (Ab) to HIV has been in practice to detect infection. But Ab screening cannot detect infection during the window period. Various tests like HIV-PCR, culture and p24 Ag detection are available for detection of HIV infection during window period. Facilities for PCR and HIV culture are available only in a few selected laboratories, are costly and labour intensive. Detection of p24 Ag by ELISA is a simple method, though the sensitivity is lower as compared to other viral diagnostic tests. In neonates, the sensitivity of simple p24 Ag test is 0-20% till one month of age and 55-75% by 3-6 months of age. In asymptomatic adults, it is 4% and during acute HIV infection (just before sero conversion) 55-75% [2]. In general, p24 Ag is detectable early in primary HIV infection, but after 6-8 weeks, a rise of anti-p24 Ab leads to the formation of immune complex that greatly inhibits the sensitivity of standard assay for p24 Ag. Acid treatment in ICD-HIV-p24 Ag assay increases the sensitivity by 3-8 folds as compared to standard assay by dissociating the immune complex and destroying the Ab and freeing the p24 Ag for detection [3].

p24 Ag detection as a screening procedure has been recommended and is the practice in blood banks in USA along with screening of Ab to HIV. It has been seen that the p24 Ag can be detected only in 4% of individuals in asymptomatic stage, but 50-75% can be detected during acute HIV infection [2]. In our study, among the voluntary blood donors, sero-positivity was found in 4 individuals (0.8%), but none of the sero-negative

individuals tested positive for p24 Ag. Out of 496 seronegative cases, not a single blood donor was found to be positive for p24 Ag. This is due to the fact that individuals during acute HIV infection, when p24 Ag positivity is highest, do not report for blood donation due to illness. In USA, where routine screening of blood donors for p24 Ag is mandatory, only 7 units were positive for p24 Ag out of 11,40,000 sero-negative blood donors [1]. In India, routine screening by p24 Ag assay is not recommended in low risk population such as voluntary blood donors.

Out of 300 patients attending various STD clinics, 41 (13.66%) were found to be positive for Ab to HIV. The over all sero-positivity of STD cases in India ranges from 1.5% to 49%. In Maharashtra, highest incidence of 49% was seen in Sangli whereas, in Pune it ranged from 2.5% to 25% in different studies [8]. Out of 259 patients, who were sero negative for HIV, p24 Ag detection was carried out and 6 cases (2.3%) were found to be positive by ICD assay whereas only 4 cases were detected by standard assay. Thus, additional 2 cases could be detected after immune complex dissociation. Though Ab to HIV is not detected during window period, the low titre of Ab at the earliest stage of formation gets complexed to the declining level of p24 Ag. Immune-complex dissociation releases the free p24 Ag which is detected by ELISA. This was the reason why we were able to detect additional 2 cases by ICD assay. These patients were in the window period and by carrying out p24 Ag detection, we were able to detect additional 2.3% of HIV infected cases. Three of the 6 patients were found to have Ab to HIV when retested after three months. The other 3 cases were lost to follow-up. Bollinger et al in a study in Pune detected an additional 1.5% of sero-negative STD patients having HIV infection by p24 Ag estimation [9]. The lower rate of positivity by them as compared to our study may be due to the use of standard assay by them, which has a lower sensitivity than the ICD-HIV-p24 Ag assay [3]. Manfredi et al compared ICD p24 Ag assay with HIV-1 RNA levels on 3129 samples and found ICD p24 assay to be less sensitive compared with viral load assessment [10]. Presence of genital ulcers has been attributed as an independent risk factor for acquiring HIV infection [1]. In our study, all the 6 additional cases that had p24 Ag positivity were found to have genital ulcers. Over all, genital ulcers were found to be present in 34 of the 41 sero-positive individuals (82.93%). Bollinger et al in their study in Pune had similar findings i.e. 79% sero-positive cases with ulcers [9].

Patients with history of STD reporting to STD clinics for treatment may not have Ab to HIV as they may be in the window period. Hence, in these patients, diagnosis

of HIV can be missed if additional tests like HIV-PCR, culture or p24 Ag detection are not performed. Though the sensitivity of p24 Ag is lower than PCR and culture, it can detect additional cases during acute HIV infection. The sensitivity of DNA PCR is 95% after two weeks of infection and 99.9% after 6 months of infection [2]. The estimation of p24 Ag is simpler to carry out and cheaper as compared to PCR/culture. Though plasma RNA RT-PCR is equally sensitive as compared to DNA PCR, it is mostly used for viral load assay in patients under treatment for monitoring purpose [2]. As ICD-HIV-p24 Ag assay increases the sensitivity 3-8 fold [3], it can be used as a routine screening procedure in high-risk population such as STD patients who are seronegative. In low-risk population such as blood donors, estimation of p24 Ag may not be cost effective in third world countries like India. Voluntary blood donors should be questioned about sexual contact with commercial sex workers, and if necessary p24 Ag detection may be carried out.

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