Detection of HIV-1 Window Period Infection in Blood Donors Using Borderline Anti-HIV Results, HIV-1 Proviral DNA PCR, and HIV-1 Antigen Test

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Abstract

Prevention of transmission of HIV-1 *via* blood transfusion has been carried out by the National Blood Center by screening donated blood with anti-HIV and HIV antigen tests. To increase the safety measure, detection of proviral DNA by PCR has been proposed; however, it was impractical to test all samples by PCR. From August 1994 to September 1995, there were 296,169 blood donors with 0.32 per cent prevalence of anti-HIV positive. From these donors, 153 samples of which the anti-HIV enzyme immunoassay optical density (OD) between cutoff and 80 per cent of cutoff value (borderline results) were selected for PCR testing. One out of 153 borderline cases showed positive by PCR test for HIV-1 proviral DNA. However, this case was also positive by HIV antigen test. Therefore, most of the samples with borderline anti-HIV results were true negative for HIV infection. On the other hand, there were 8 HIV antigen positive samples which had anti-HIV OD below the borderline value determined in this study. This finding confirmed the necessity of using both the anti-HIV and HIV antigen tests for screening of donated blood.

Human immunodeficiency virus (HIV) is the causative agent of acquired immunodeficiency syndrome (AIDS), the fatal incurable disease. Prevention of HIV transmission *via* blood and blood product transfusions from HIV-infected persons is the most crucial task of the Blood Bank. A standard practice of screening for anti-HIV in donated blood has decreased the risk of HIV transmission from blood transfusion. Nevertheless, transmission of HIV from anti-HIV negative blood has occurred[1,2]. Therefore, the additional HIV antigen test has been included in blood donor screening at the National Blood Center, Thai Red Cross Society. However, HIV transmission was still able to occur despite this effort[2]. Screening of HIV-1 proviral DNA in blood donors using

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polymerase chain reaction (PCR) has been proposed[3]. Due to the tedious procedure and high cost, it is not practical to test every unit of donated blood by PCR; therefore, a strategy of selecting only the samples that had borderline anti-HIV results for PCR testing was explored in this study.

MATERIAL AND METHOD

A total of 296,169 blood samples of voluntary blood donors from the National Blood Center, Thai Red Cross Society from August 1994 to September 1995, and 372 voluntary blood donors from newly recruited soldiers of the Royal Thai Army were studied. All samples were tested for anti-HIV and HIV antigen.

The HIV antibody test was the third generation enzyme immunoassay (EIA) using the kit from Abbott Laboratories, Chicago, U.S.A. In addition, gel particle agglutination (GPA) test was used for anti-HIV detection in some samples. The reagents for GPA were from Fujirebio, Tokyo, Japan. The test procedures were done according to the manufactural protocols.

The HIV antigen test was performed by EIA using the kit from Abbott Laboratories, Chicago, U.S.A. The test method was also done as suggested by the manufacturer except that serum was used instead of culture supernatant. Nested PCR technique was used for detection of the HIV-1 proviral DNA by using primers for the *pol* gene as described[3].

The borderline anti-HIV result was defined as having the optical density (OD) value of the EIA ranging from the cutoff value level down to 80 per cent of the cutoff value of each batch tested. These samples were usually classified as anti-HIV negative but were hypothesized that there might be cases of HIV infection that have anti-HIV in the low level.

RESULTS

Two groups of blood donors were enrolled into this study. The first group was 296,169 samples of voluntary blood donors from the general population. This group had 956 anti-HIV positive samples giving a rate of 0.32 per cent (Fig. 1) and thus classified as the low risk group. The second group were 372 samples from newly recruited soldiers of the Royal Thai Army. This group had 18 anti-HIV positive samples giving a rate of 4.8 per cent and thus classified as the high risk group.

Of 295,213 anti-HIV negative samples in the low risk group, only 153 samples had borderline anti-HIV results by EIA. The incidence of anti-HIV borderline EIA OD during 13 months of this study at the National Blood Center was 0.05 per cent (153/296,169). These samples were further tested for HIV-1 proviral DNA by PCR (Fig. 1). Only one case was detected for HIV-1 proviral DNA by PCR technique. This case was also positive for HIV antigen test by EIA at the same time. The 25th date of follow-up sample

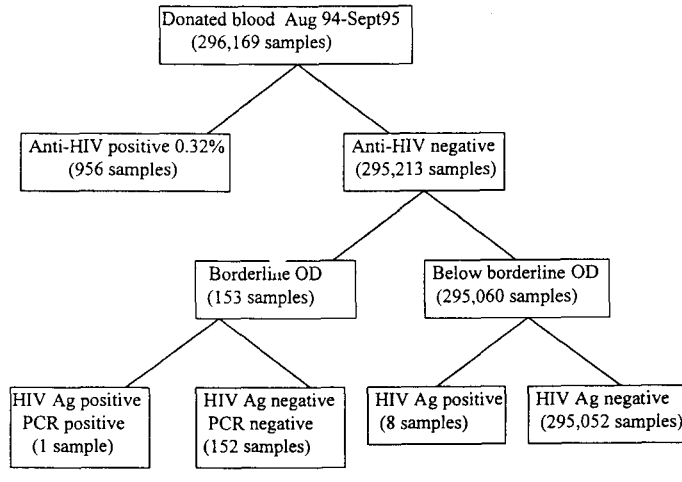

Fig. 1. HIV-1 test results of the group of general population blood donor (low risk group).

Table 1. The test results of the positive case for HIV-1 PCR.

Test	Results
Antibody test (EIA)	borderline*
Antibody (GPA)	Negative
Antigen test (EIA)	Positive
HIV-1 PCR	Positive

*Cutoff OD in duplicate=0.112 and 0.114, mean=0.113 (criteria: OD>0.113=positive, OD 0.0904=80% cutoff, OD 0.0904-0.113=borderline result, OD<0.904=negative). This sample's OD in duplicate=0.097 and 0.094. After 25th date of follow-up, this donor became to be positive for antibody test by both EIA and GPA.

became positive for anti-HIV by both EIA and GPA. The test results and anti-HIV OD of this case are summarized in Table 1.

In 354 new army recruits with anti-HIV negative by EIA, all of them showed negative HIV antigen by both PCR and EIA. In addition, all of these samples were also negative for anti-HIV by GPA.

DISCUSSION

In the group of borderline anti-HIV results, only one case was found to be positive for HIV-1 PCR. This result showed that 0.65 per cent (1 of 153) of the borderline OD cases had HIV-1 infection. Because HIV-1 antigen was also positive in this case, the combination of the antigen and antibody tests currently used by the National Blood Center were good enough for screening such a case. In Blood Banks that do not have HIV antigen screening, the blood with borderline EIA OD should be selected out as described in this study. For a Blood Bank that GPA was used for screening anti-HIV, this strategy could not be used because all borderline EIA OD cases had negative result by GPA. On the other hand, this study showed that 99.35 per cent (152/153) of the borderline cases were most likely true negative as they were negative for both HIV antigen and HIV-1 PCR tests. The primers for *pol* PCR used in this study was confirmed for its superior sensitivity over other primers for other HIV-1 genes (3-5). Blood Banks that have not screened for HIV antigen still missed 0.0027 per cent (8 in 296,169) of antigen positive cases per 13 months. These 8 samples had anti-HIV level below the borderline value (Fig. 1). This means that The National Blood Center have saved 8x3 or 8x4 patients from HIV-1 transmission by transfusion, as one blood unit was processed to yield 3 or 4 blood components. This results confirmed the necessity of antibody and antigen test combination for blood screening. For the army recruits, although its high prevalence of HIV infection was (4.8%), we could not detect the window period by either PCR or antigen tests.

This study shows similar results to other studies that the window period of HIV-1 infection could be detected by either antigen test (EIA) or proviral DNA PCR assay(6,7). In conclusion, HIV antigen test should be added to the screening of donated blood to reduce the transmission of HIV antigen through blood transfusion rather than screening by anti-HIV test alone.

ACKNOWLEDGEMENT

This study was supported by grant from the Japanese Foundation for AIDS prevention.

(Received for publication on July 11, 1997)

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การตรวจหาการติดเชื้อ HIV-1 ระยะแรกเริ่ม ในเลือดบริจาคโดยใช้ค่าก้ำกึ่งใกล้จุดตัดของ Anti-HIV, HIV-1 Proviral DNA PCR และ HIV-1 Antigen

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ศูนย์บริการโลหิตแห่งชาติมีมาตราการป้องกันการติดเชื้อ HIV จากการรับเลือดโดยการตรวจหาทั้ง anti-HIV และ HIV antigen ในเลือดที่รับบริจาค มีการเสนอการตรวจ provial DNA โดยใช้วิธี PCR เพื่อเพิ่มความปลอดภัยยิ่งขึ้น แต่วิธี PCR ไม่เหมาะสมที่จะนำไปใช้ตรวจเลือดทุกราย จากเลือดที่รับบริจาค จำนวน 296,169 ราย ในช่วงเดือน สิงหาคม 2537 ถึง กันยายน 2538 ซึ่งมีอัตราเลือด anti-HIV บวก 0.32 เลือดจำนวน 153 รายที่มีค่า optical density (OD) ของ anti-HIV โดยวิธี enzyme immunoassay อยู่ในช่วงต่ำกว่าค่าจุดตัดจนถึง 80% ของจุดตัด (ช่วงค่าก้ำกึ่ง) ถูกเลือกเพื่อนำมาตรวจโดยวิธี PCR มีเพียง 1 รายใน 153 ราย ที่ให้ผลบวกโดยวิธี PCR อย่างไรก็ตาม เลือดรายนี้ยังให้ผลบวกด้วยวิธี HIV antigen อีกด้วย ดังนั้นเลือดที่ให้ค่าในช่วงก้ำกึ่งส่วนใหญ่ จึงเป็นรายที่ไม่มีการติดเชื้อ HIV ในขณะที่มีเลือด 8 ราย ที่มีค่า OD ต่ำกว่าในช่วงค่าก้ำกึ่งทั้งสิ้น ให้ผลบวกกับการตรวจด้วย HIV antigen ผลการศึกษานี้จึงยืนยันความจำเป็นของการใช้การตรวจ anti-HIV คู่กับการตรวจ HIV antigen เพื่อตรวจกรองเลือดที่รับบริจาค

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