An Investigation Report of HIV Infection through Blood Transfusion in 2007

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Abstract

The main routes of HIV transmission include blood, sexual behavior, and mother-to-child vertical transmission. Since 1988, each batch of donated blood in Taiwan is supposed to be screened with HIV-1 antibody test. To ensure the safety of blood recipients, beginning in 1995 both HIV-1 and HIV-2 antibody tests are performed to prevent transmission of HIV through blood transfusions. The Taiwan Centers for Disease Control (TW-CDC) has investigated the periodic follow-up reports of blood recipients and one suspected blood transfusion-associated HIV case was reported in December of 2007. As a result a series of investigations about the blood donors and the blood recipients were done including retrieving cases, sample collecting, and diagnostic work. Through PCR and gene sequence alignment, we discovered that the blood donor was w the window period for

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HIV transmission and there were no tests performed on the donated blood, which resulted in two people becoming infected with HIV. This was the first reported case of HIV transmission that happened by way of blood products from a donor who was within the transmission window period. Patients received personal counseling and medication. In addition to improving the sensitivity of tests and shortening of the window period, we should emphasize the importance of developing standard operating procedures. This will help to preserve blood samples and enhance the consulting skills of donor care specialists in the future so as to prevent HIV infection through receiving window-period blood products and clear out the details of this incident.

Keywords: AIDS, blood transfusion

Introduction

Acquired Immunodeficiency Syndrome, also as known as AIDS, is an infectious disease caused by Human Immunodeficiency Virus (HIV), the number of people living with HIV has risen to 30 million before the end of 2007. Some 2.5 million people were newly infected with the virus in 2007 and 2.1 million people, mostly productive young adults, died of AIDS-related illnesses [1]. In addition, according to an epidemiological survey of HIV infection in Taiwan, there have been a total of 15,011 HIV infected citizens since the first reported HIV infected case in 1973 to the end of 2007, while 13,109 people are currently living with HIV.

There are three main routes of HIV infection: blood, sexual behavior, and mother-to-child vertical transmission. Currently, two different HIV screening tests are used to confirm HIV infection according to the rules and suggestions from World Health Organization (WHO) and the US Centers for Disease control and Prevention (US CDC). For instance, reported HIV-positive cases are either

defined by Enzyme-linked immunoassay (ELISA), Particle Aggregation (PA) or other methods. In addition, Western Blotting (WB) test is required to double confirm the positive results. To prevent HIV infection via blood transfusion and ensure the safety of blood recipients, beginning in 1988 and since 1995 the authorities required that donated blood products should be checked with a HIV-1 antibody test. Both HIV-1 and HIV-2 antibodies are definitely required to confirm the positive results of HIV infection.

The principle of ELISA, PA, and WB is based on detecting the HIV antibodies produced by those infected with HIV. However, no antibodies appear in the early phase of infection, also as known the window period. Therefore, to ensure the safety of citizens receiving donated blood and to screen for HIV infection during the window period, the Centers for Disease control of Taiwan (Taiwan CDC) made tremendous effort in tracking the donation records and recipients lists of newly reported cases of HIV infection. In addition, Taiwan CDC requested that the Taiwan Blood Service Foundation (The Blood Foundation) permanently keep those persons off their blood donor list.

After receiving the lists of recipients from The Blood Foundation, Taiwan CDC tracked recipients according to the tracking principles of the “Taiwan AIDS Prevention Manual” [2]. These cases were based on the final records of blood donation for those newly diagnosed HIV infection and not from the screening process done before blood donations. For the cases of HIV infection diagnosed before blood donations, the tracking date will begin with the last date of donation. Since 1988, each batch of donated blood was screened for HIV using HIV antibody tests. Therefore, we considered donation in 1988 as a dividing standard. If people donated their blood before Jan 1988 (no records after Jan 1988), those blood recipients who accepted blood 3 years prior to the last time they donated

blood should be checked for HIV infection. However, considering the progression after infection, recipients who had accepted blood before 1977 did not have to be examined for HIV infections. If HIV-positive blood donors having records after Jan 1988, those who received their blood at that time should be traced for HIV infections in the last 6 months before their last blood donation tested negative. Besides, if the blood recipients had HIV positive results during the investigation, we must trace recipients who had accepted blood for HIV infections in the last 6 months, the local public health bureau would need to accomplish the tracking tests in 14 days to the trace living recipients that have been confirmed by tracking principles. The incident of HIV infection caused by blood transfusion was discovered through the tracking principles mentioned above.

Material and Method

We used laboratory diagnostics and epidemiologic studies to figure out if the incidences of HIV infection were due to blood transfusions. In order to obtain relevant samples, we contacted The Blood Foundation to obtain the blood products from donor A, however the donated blood samples registered on July 2007 were not preserved. The only HIV-positive blood sample left was registered on November 2007. The samples of recipients B and C were sent to Taiwan CDC separately and a series of blood transfusion diagnostic tests were performed.

A. Epidemiological surveys and case report

The incident to be discussed was revealed by CDC Taiwan on November 22, 2007. A newly reported HIV-positive case (Donor A) was routinely matched with the records from The Blood Foundation while donation records and the recipient lists were provided by The Blood Foundation. We discovered that there was data of a recent HIV-negative donor A on July 3, 2007 which include

3 blood recipients. Consequently, we delivered two separate, official letters to local public health bureau about the data of living recipients B and C on 13 December, 2007 for further investigation. Taiwan CDC was notified by the department of health, Kaohsiung city government on December 21, 2007 that recipient B was positive for HIV infection, confirmed by Western blotting (WB) assay. In addition, Taiwan CDC immediately contacted the public health bureau of Pingtung County Government that was in charge of the case of recipient C who used the same blood product as recipient B. The HIV-positive case was diagnosed using an antibody test at the hospital where recipient C had been staying at the time and the blood samples were sent to Taiwan CDC by the local bureau for double confirmation. Confirmation of HIV in the blood transfusion was made by WB assay at Center for Research and Diagnostics, Taiwan CDC on December 27, 2007. Recipient D who received the same blood product died in the hospital.

Donor A was a single male in his twenties working in the service industry, his donated blood product was HIV positive and confirmed by WB assay when donating in November of 2007. In addition, the blood product was kept for serological survey and prohibited from being use. A blood transfusion center reported that the risk factor in this case was male to male sex. Donor A had donated blood 18 times and one blood product, in July of 2007, tested negative. Furthermore, the local public health bureau retrieved past medical records of donor A which revealed the HIV test was negative a week after donation which meant that donor A was not confirmed to have HIV infection during the window period when he donated the blood that caused the tragedy of transmitting HIV to 2 of the 3 recipients (recipient B and recipient C). Recipient B was an 81 year-old male who was infected while receiving platelet

concentrates because of gastrointestinal tract hemorrhage. Recipient C was a 54 year-old widow who was infected after receiving fresh frozen plasma, and recipient D was a 78 year-old widow who had received packed RBC.

This was the 17th time donor A had donated blood in this incident of HIV transmission via the transfusion of infected blood. According to blood donation records, when counseling donor A he was reported to be healthy and denied having MSM or any risk of HIV virus infection. In conclusion, the retrieving of history records to match the number of donated blood bags assured that the blood products of donor A were in fact transfused to recipient B, recipient C and recipient D, and each case was reported by local public health bureau and medical treatment organization via the law of "Human Immunodeficiency Virus Prevention and Control, and Safeguarding Rights of Infected people".

B. Confirmation tests of blood transfusion infection

a. Reverse Transcription-Polymerase Chain Reaction; RT-PCR

1. Reverse Transcription

Add viral RNA 10ul to solutions including 50 pmole 75mM KCl, 50mM Tris-HCl, 3mM $MgCl_2$, 10M DTT, dNTP mixture 0.5nM, RNasin 0.5λ38U/ul, antisense primer 35R, 1-R-RT21, and R-PR-probe (sequences are shown in Table 1 and 2), then began with 70℃ for 10 minutes, next, add 100 units MMLV-reverse transcriptase (Promega, Cat.# M1701) and incubate at 37℃ for 45 minutes.

2. Polymerase Chain Reaction (PCR)

(1) First round PCR

cDNA derived from reverse transcription reaction was served as templates to run PCR. Add cDNA in the mixture of 50 mM KCl,

10mM Tris-HCl, 3mM $MgCl_2$, 0.1% Triton-X 100, dNTP mixture 1 mM, and 50 pmole each primer pairs 44F/35R, 1-F-RT18/1-R-RT21, F-MAW26/R-PR-Probe (sequences were showed at Table 1 and 2) for amplifying Env and Pol., consequently, 5 units Taq Polymerase (Invitrogen) were added to the mixture, then elevated the temperature to 94℃ for 3 minutes for polymerase activation, and followed by 35 PCR cycles (94℃ 1minute, 48℃ 1 minute, and 72℃ 2 minutes), finally, incubate at 72℃ for 15 minutes.

(2) Nest-PCR

The product of first round PCR 5ul was conducted in a solution containing 50 mM KCl, 10mM Tris-HCl, 3mM $MgCl_2$, 0.1% Triton-X 100, dNTP mixture 1 mM, and 50 pmole each primer pairs 33F/48R, 2-F-RT19/2-R-RT20, Nest-F-PR/Nest-R-PR, moreover, 5 units Taq Polymerase (Invitrogen) were conducted with the solutions, then elevated to 94℃ for 3 minutes for polymerase activation, followed by 35 PCR cycles (94℃ 1minute, 48℃ 1 minute, and 72℃ 2 minutes) , and then incubated at 72℃ for 15 minutes.

Table 1. Primer pair sequence of RT-PCR and Nest-PCR for amplifying Protease and Reverse transcriptase

Primer	Sequence[a]	Gene	Polarity
1-F-RT18	GGAAACCAAAAATGATAGGGGGAATTGGAGG	Pol	Sense
1-R-RT21	CTGTATTTCTGCTATTAAGTCTTTTGATGGG	Pol	Anti-sense
2-F-RT19	GGACATAAAGCTATAGGTACAG	Pol	Sense
2-R-RT20	CTGCCAGTTCVAGCTCTGCTTC	Pol	Anti-sense
F-MAW26	TTGGAAATGTGGAAAGGAAGGAC	Gag	Sense
R-PR-probe	GGCAAATACTGGAGTATTGTATGG	Pol	Anti-sense
Nest-F-PR	CAACTCCCCCTCAGAAGCAGGAGCCGATAGACA	Gag	Sense
Nest-R-PR	ACATCCATTCCTGGCTTTAATTTTACTGGTACAGT	Pol	Anti-sense

Table 2. Primer pair sequences of RT-PCR and Nested PCR for amplifying HIVC2V3 gene

Primer	sequence	polarity
44F	5'-ACAGTRCARTGYACACATGG-3'	Sense
35R	5'-CACTTCTCCAATTGTCCITCA-3'	Anti-sense
33F	5'-CTGTTIAATGGCAGICTAGC-3'	Sense
48R	5'-RATGGGAGGRGYATACAT-3'	Anti-sense

b. Sequencing

ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) was used to label to nucleic acids for analysis. To ensure the quality of nucleic acids sequencing, high purity of nucleic acid ($OD_{260/280}$ >1.8) was prepared to serve as the sequencing template. The amounts of nucleic acid needed for analysis are listed as below: double-stranded DNA (e.g. plasmids): 200~500 ng, single-stranded DNA: 50~100 ng, PCR products: 30~90 ng. Mix appropriate amounts of template nucleic acids, 3 μl pre-mix (contains Tris-HCl buffer, pH9.0, $MgCl_2$, dNTP mix, labeled A-dye terminator, AmpliTaq DNA Polymerase FS with thermally stable pyrophosphatase), 3.2~5.0 pmole primer (Nest-PR-F/Nest-PR-R, 33F/48R, 2-R-RT20/2-F-RT19) and an appropriate amounts of deionized water to make a final volume of 10 μl. After mounting a layer of paraffin oil, proceed all reactants to 94℃–preheated PCR machine. Program was set as follow: 94℃ for 30 seconds, 55℃ for 15 seconds, 60 ℃ for 4 minutes and repeat 25 cycles. The reaction was terminated at 4℃.

c. Similarity % Analysis

Sequencing data was analyzed by MegAlign software in DNA Star. A 345 bp-fragment derived from C2V3 region of HIV virus coat protein was

analyzed by Jotun Hein method or Clustal Method. The percentage of sequence similarity analysis was measured by selecting Sequence Distance.

Results

HIV/RNA was extracted from serum of blood specimen from donor and recipient and specific primer was designed against C2V3, a highly variable region of HIV genome, to amplify the gene segment. Sequencing results was further uploaded to NCBI website to perform sub-type analysis. Blast results revealed that the virus isolated from the three people was B sub-type and it is highly similar to HIV-1 isolate TW-CDC-113. To compare the sequence similarity, sequence data was analyzed further by MegAlign software in DNA Star. Results showed that there is 1.4 % sequence variation in C2V3 region between Donor A and Recipient B and 1.7% between Donor A and Recipient C (Figure.1). Generally speaking, different sources of infection usually result in at least more than 5 % variation in genetic sequence. Finally, phylogenetic analysis (Figure.2) also confirmed that there is a definite correlation between Donor A and the two Recipients, Recipient B and C, and the genetic type is B sub-type.

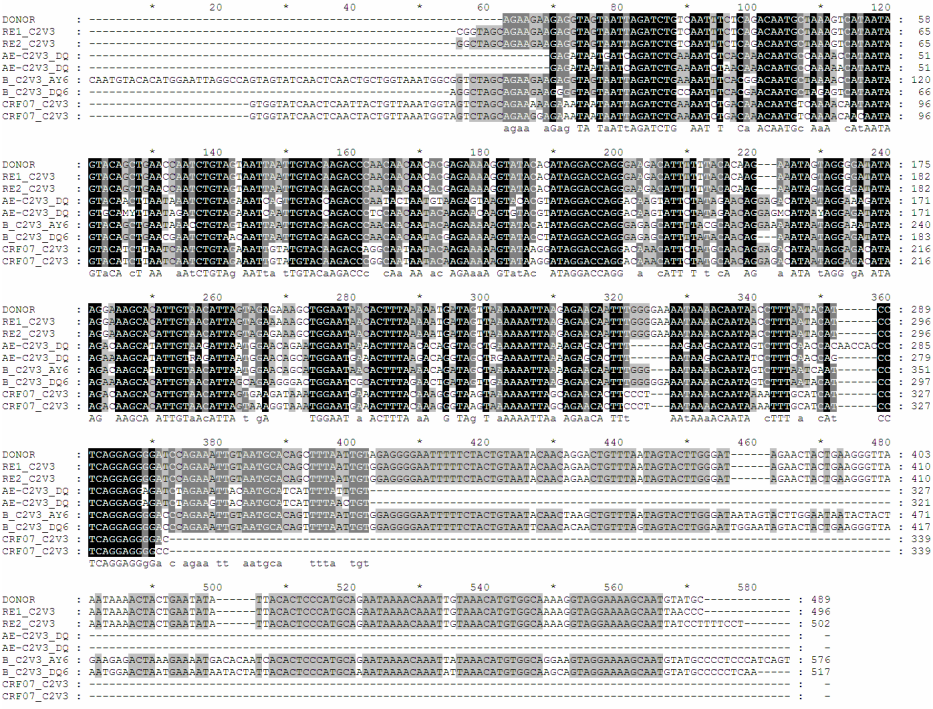

Figure 1. Sequence alignment of C2V3 region between donor and recipients

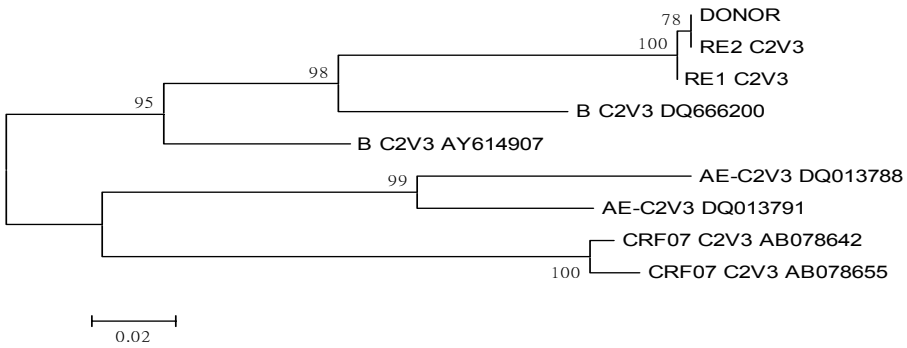

Figure 2. Analysis of phylogenetic tree

Discussion

The primary reason for this blood transfusion infection was the false negative test results due to the window period. Compared with Western blot analysis, the use of Nucleic Acid Amplification Testing (NAT) can shorten the window period. However, the false negative blood sample drawn in July 2007 was not preserved by the blood center, therefore we could not prove if the NAT method is the better solution to exclude the window period and obtain the positive results. In order to clear doubts, we strongly suggest that blood centers should establish standard operation protocols to preserve the blood sample or specimen from the donors in the future.

Although the NAT analysis can shorten the window period by about 10 to 15 days, it still cannot reduce the incidents of HIV infection caused by blood transfusion to zero. In other countries, it cases have been reported of HIV infection caused by transfusion even though the blood was checked to be negative by NAT [3]. It remains to be determined the cost benefit of using the higher-cost NAT analysis. Consequently, In addition to applying new tests in order to reduce the case number of HIV infections by shortening the window period, related actions, including random inspection of blood centers by Department of Health, will be performed to ensure the quality of execution according to the Fourth Five-year Plan for the Prevention and Cure of Acquired Immune Deficiency Syndrome [4]. Meanwhile, enhancement of monitoring and management of recipients, tracking of blood donation records of registered HIV carriers and proper health education guidelines calling upon the public not to use the donating of blood as a means of testing themselves for HIV infection will also help to reduce the number of people infected by blood transfusions. We would like to ask

Taiwan Blood Service Foundation to improve the precautionary requirements for equipment as well as donors, counseling donors not to donate blood if they are from a high risk group, and supervise blood centers to conduct the proper counseling. We would also like to ask Taiwan Blood Service Foundation to continue the work of "Conscience Call-back" and enhance the work of digital management of each registered case of high risk group screening.

After the incident, Taiwan CDC would like to ask Taiwan Blood Service Foundation once more to improve their counseling skills that are done before potential donors donate blood. Most importantly, we ask them to call upon people not to test themselves for HIV by way of donating blood, otherwise you may endanger the life of others. If people have doubts about whether they are in a high risk group or what behaviors might endanger them to becoming infected by HIV we hope people will be sure to perform their examination at the Public Health Bureau, a local medical center or nineteen qualified anonymous examination centers for further screening. Taiwan Blood Service Foundation will also remind people who might be in a high-risk group including HIV carriers, venereal disease carriers, those who have performed dangerous sexual behaviors, or who are homo-sexual or bi-sexual, not to donate their blood. The donor responsible for this incident is homosexual and the donor care specialists did not discover this at the time of counseling. The public health guidelines in blood center should emphasize the "Requirements of no blood donation", "No blood donation if one is in a high-risk group", "Penalties for inappropriate blood donation" and "Conscience call-back". Most importantly, educate people to the fact that HIV is still infectious during the widow period.

Conclusion

In Taiwan, HIV screening for all blood donations has been conducted since 1988. However, due to the limitation of current technologies, there are about 6~12 weeks during the window period where HIV cannot be detected by current detection methods. Because of this limitation, eighteen people have suffered HIV infection from blood transfusions [5]. Therefore, to ensure the safety of blood transfusion, it is important to develop more sensitive methods to reduce the rate of the false negatives during the window period. More importantly, conscience notification from blood donors of their own medical history of possible infectious diseases or discussion of whether they may belong to a high risk group for HIV infection before drawing blood is a better way to reduce HIV infection caused by blood transfusion. Therefore, the importance of consultation prior to blood donation is not secondary to the procedure of routine screening after blood drawing. In the future, the government should improve the professional training of consulting skills for donor care specialists and fulfill the work of consultation. In addition, public health education should also emphasize the importance of the safety of blood transfusion and that donors should be honest to themselves about their health conditions. In incidences of unsafe sexual behavior or venereal diseases during the HIV window period, blood donation should be absolutely prohibited. It is also forbidden to test oneself for HIV infection through the process of donating blood. If donors have doubts about the safety of their own blood, “conscience callback” is the right thing to do. It takes efforts and cooperation from everyone to reduce the risks associated with blood transfusion.

The safety of blood transfusion is an issue of major concern around the world. In western countries, great amount of funding has been spent annually, not

only on monitoring the infectious diseases caused by blood transfusion but also on including non-infectious blood transfusion monitoring into safety issues [6]. Safety policies regarding blood transfusion include blood collection, examination, sub-package and shipping before transfusion. Most importantly, it is urgent to establish a complete database for all recipients, monitor transfusion reaction, and establish feedback mechanisms and protocols for blood preservation. We expect the network of safety policies for blood transfusion will be more complete in Taiwan in the near future.

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