วารสารวิชาการสาธารณสุข
ปีที่ 3 ฉบับที่ 1 ม.ค.-มี.ค. 2537

Journal of Health Science
Vol. 3 No.1 Jan.-Mar. 1994

นิพนธ์ต้นฉบับ Original Article

Survey of the Antibodies to HTLV-1/2 Among Intravenous Drug Users in Thailand

การตรวจหา แอนติบอดีย์ ต่อเชื้อ HTLV-1/2 ในกลุ่มผู้ติดยาเสพติดชนิดฉีดเข้าเส้น ในประเทศไทย

สุขใจ ผลอำไพสถิตย์ วท.บ., วท.ม*
อาชวินทร์ โรจนวิวัฒน์ วท.บ.*
สุธน วงษ์ชีรี วท.บ., วท.ม.*
สุรางค์ สงวนวงศ์ วท.บ., Dip in Bact (Major in Virology)*
ไพจิตร์ วราชิต พ.บ., ส.ม., ว.ว. (กุมารเวชศาสตร์)**
* *สถาบันวิจัยไวรัส กรมวิทยาศาสตร์การแพทย์*
** *กรมวิทยาศาสตร์การแพทย์*

Sukjai Pholampaisathit B.Sc., M.Sc.*
Archawin Rotjanawiwat B.Sc.*
Suthon Wongcheree B.Sc., M.Sc.*
Suranga Saguanwongse B.Sc., Dip in Bact (Major in Virology)*
Paijit Warachit M.D., M.P.H., Board of Pediatrics**
* *Virus Research Institute, Department of Medical Sciences*
** *Department of Medical Sciences*

Abstract

The human-T-cell leukemia-lymphoma virus type 1 (HTLV-1) causes adult T-cell leukemia-lymphoma (ATLL) and HTLV-1 associated Myelopathy. It appears to be spread worldwide. On the other hand, the prevalence of a second type of HTLV (HTLV-2) is high in intravenous drug users (IVDU) or other high risk group such as sexual transmitted disease patients. In this preliminary study, attempt was made to determine the antibody to HTLV-1/2 in a total of 300 HIV-positive plasma specimens from IVDU group from Thanyarak hospital which were screened by an Enzyme-Linked Immunosorbent Assay (ELISA). Repeatedly reactive samples were further tested by western blot assay (WB). Of the 300 specimens, 10 plasma specimens were positive for HTLV antibodies by ELISA as a screening test, while another 7 were found with absorbance to be near the cut-off value of 0.35. Seventeen specimens were confirmed by western blot and interpreted according to manufacturer criteria. Of these plasma specimens, only 5 were confirmed negative and the others 12 specimens were indeterminate. Thus, From this results, there was no indication that the antibody to HTLV-1/2 was found among the HIV-infected Thai IVDU.It is therefore advisable that plasma specimens with indeterminate be supplementary tested by polymerase chain reaction(PCR) for HTLV-1/2.

บทคัดย่อ

เชื้อ Human T-cell leukemia-lymphoma virus type 1 (HTLV-1) เป็น human retrovirus ที่เป็นสาเหตุในการเกิดโรคมะเร็งในเม็ดเลือดขาว (Adult T-cell leukemia-lymphoma) และโรคที่มีอาการของระบบประสาท (HTLV-1 associated myelopathy) สำหรับ HTLV-2 นั้นยังไม่พบว่ามีความสัมพันธ์กับโรคใดอย่างชัดเจน แต่ HTLV-2 มักจะพบมากในกลุ่มผู้ติดยาเสพติดชนิดฉีดเข้าเส้น และกลุ่มเสี่ยงอื่นๆ เช่น กลุ่มคนไข้ sexually transmitted diseases การศึกษาครั้งนี้เป็นการศึกษาเบื้องต้นเพื่อตรวจหา antibody ต่อ HTLV-1/2 ในกลุ่มผู้ติดยาเสพติด ซึ่งมีผลบวกต่อเชื้อ HIV จำนวน 300 ราย จากโรงพยาบาลธัญญารักษ์ จังหวัดปทุมธานี โดยใช้วิธี ELISA ผลบวกที่ได้จะนำไปตรวจหาซ้ำด้วยวิธีเดิม และนำไปตรวจยืนยันด้วยวิธี western blot จากการศึกษาพบว่า ใน 300 ตัวอย่าง ได้ผลบวกของ antibody ต่อ HTLV-1/2 โดยวิธี ELISA 10 ราย นำตัวอย่างทั้ง 17 ราย ซึ่งรวมตัวอย่างที่มีค่า O.D ต่ำกว่าค่า cut-off (0.35) เล็กน้อย อีก 7 ราย ไปตรวจยืนยัน พบว่า มีผลลบเพียง 5 ราย และ 12 ราย มีผล Indeterminate ดังนั้น จากผลการศึกษา ยังไม่พบ antibody ต่อเชื้อ HTLV-1/2 ในคนไทย กลุ่มผู้ติดยาเสพติด ชนิดฉีดเข้าเส้นที่ติดเชื้อ HIV-1 อย่างไรก็ตาม ตัวอย่าง plasma ที่ให้ผล Indeterminate ด้วยวิธี western blot ควรที่จะศึกษาเพื่อตรวจยืนยันด้วยวิธี PCR ต่อไป.

Introduction

Human T-cell leukemia-lymphoma virus type 1 (HTLV-1) is the etiological agent for adult T-cell leukemia-lymphoma (ATLL) and HTLV-1 associated myelopathy (HAM)[1-2]. The second type of HTLV (HTLV-2) was first identified from a patient diagnosed with hairy-cell leukemia[3]. Since that time several other patients have been identified as harboring HTLV-2.[4]

The study on the natural history of HTLV-1 infection in ATLL and other associated diseases indicated that the development of disease take a long latent period (20-30 years in the case of ATLL).[5] The virus is transmitted from mother-to-child mainly by breast feeding. In adult hood, the virus is transmitted by sexual intercourse or exposure to contaminated blood (ie. blood transfusion or intravenous drug abuse).[5]

Epidemiological studies indicated that HTLV-1 carriers are mainly distributed among the Japanese in the southern Japan, the Caribbian, parts of Central and South America and regions of western and central Africa.[1,6]

Survey for incidence of HTLV-1 infection has already been performed in mainland China[7,8], Korea and Indonesia[7]. Three and one HTLV-1 carriers were found in Taiwan province of China and mainland China, respectively, using Indirect Immunofluorescence (IF) test for the presence of anti-HTLV-1. Another study in Taiwan could detect anti-HTLV-1 by different methods like IF, ELISA, and particle agglutination (PA) test[9]. Kimura et al. had reported that 2 seropositives were detected by gelatin particle agglutination test, one was Malaysian of Indian origin, and the other was female Thai.[11] According to Bryan Page et al's study, they suggested that HTLV-1 carriers who were infected with HIV-1 have an accelerated progression to AIDS[11]. In addition, *Tax* protein, the regulatory protein of HTLV-1, plays an important role in the trancriptional activation of the HIV-1

enhancer[12].

HTLV-2 infection appears to be more prevalent in certain high risk groups including intravenous drug users, female prostitutes, patients attending sexually transmitted diseases clinic and recipients of multiple blood transfusions[13]. It is interesting to find whether the prevalence of HTLV-1/2 infection among people in Thailand neighbouring Japan or others in Asia is high or not. The objective of this study was to determine the prevalence of the antibodies to HTLV-1/2 in HIV-infected IVDU group.

Materials and Methods

Specimen

Plasma from 300 intravenous drug users (IVDU) who were HIV-seropositive by the method of ELISA, Particle agglutination (PA) or Polymerase chain reaction (PCR) were obtained from Thanyarak Hospital, Pathumthani province. The patients ranged in age from 18 to 52 years. These specimens were stored frozen at -20°C until being tested.

ELISA

Specimens were added into the wells which were coated with HTLV-1 viral lysate and were enriched with both recombinant HTLV-1 and HTLV-2 envelope (rgp46) antigens (the Diagnostic Biotechnology, Singapore). The specimens were mixed thoroughly by tapping and incubated 30 minutes at 37°C. The contents of all wells were aspirated then washed 6 times in a wash buffer, followed by the addition of goat anti-human IgG labelled with horseradish peroxidase. After another 30-minutes incubation, the solution in the wells were again aspirated and washed as above. Then, the substrate solution containing hydrogen peroxide and o-phenylenediamine was added and incubated for 15 minutes in the dark at room temperature. The stop solution, sulphuric acid was finally added to each well. The absorbance was then read at 492 nm. The specimens whose absorbances were greater than or equal to the cut-off value of 0.35 were considered initially reactive and retested in duplicate before interpretation. The specimens which were repeatedly reactive were further confirmed by western blot.

Western blot assay (WB)

Two hundred-fold-diluted plasma was incubated with a strip of western blot (The Diagnostic Biotechnology, Singapore) for 1 hour at room temperature on a Rocker plateform. After the membrane was washed 3 times, goat anti-human IgG conjugated with alkaline phosphatase was used to detect human antibodies against HTLV-1/2 and incubated for 1 hour at room temperature. The membrane was again washed 3 times and followed by adding solution of 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) and nitroblue tetrazolium (NBT). After incubation for 10-15 minutes, the substrate was aspirated and the strips were rinsed several times with water to stop the reaction. The seropositivity was determined according to the following criteria: the reactivity to *gag* (p^{19} or p^{24}) and *env* (gp^{46} or $rgp^{46\text{-}1}$) and rgp^{21} bands were considered to be HTLV-1 positive. HTLV-2 positive was defined by reactivity to *gag* (p^{24}) and *env* ($rgp^{46\text{-}2}$) and rgp^{21} bands. A specimen with immuno-reactivity to HTLV gene product but not satisfying the above criteria was called indeterminate.

Results

In estimating the prevalence of antibody to HTLV-1 and HTLV-2 in 300 IV drug users whose plasma specimens were positive for HIV, we employed commercial ELISA kit as a screening test. The positive results were being retested in duplicate by ELISA and further confirmed by commercial western blot kit. The positive results by ELISA were summarized in Table 1. There were 10 seropositive specimens whose obtical density value was greater than the cut-off value of 0.35 in 300 specimens (3.3%). The another 7 sero-negatives whose absorbance was near the cut off value of 0.35 were also included in western blot assay. None of plasma specimens was found positive by western blot. Of 12 in 17 cases had indeterminate western blot antibody reactivity with single, two or faint bands of any of rgp^{46-1}, rgp^{21} and p^{19}. No band of p^{24} can be seen. The other 5 plasma specimens were negative.

Table 1 Positive results of plasma specimens by screening ELISA confirmed by WB for HTLV-1 or HTLV-2.

No.	Patient code no.	ELISA	WB	Band of HTLV gene product
1	1	0.870 (+)	Ind	rgp^{46-1}
2	5	0.514 (+)	Ind	rgp^{21}
3	45	0.438 (+)	Ind	rgp^{21}
4	77	0.897 (+)	Neg	-
5	110	1.030 (+)	Ind	p^{19}
6	122	1.019 (+)	Ind	rgp^{46-1}
7	152	0.539 (+)	Ind	rgp^{46-1}
8	202	0.414 (+)	Ind	rgp^{46-1}
9	204	0.412 (+)	Neg	-
10	263	0.514 (+)	Ind	rgp^{21}
11	141	0.281 (-)	Ind	p^{19}
12	164	0.280 (-)	Neg	-
13	166	0.295 (-)	Ind	rgp^{21},rgp^{46-1}
14	236	0.305 (-)	Neg	-
15	257	0.291 (-)	Neg	-
16	258	0.317 (-)	Ind	rgp^{21}
17	276	0.343 (-)	Ind	rgp^{46-1}

(+): positive result
(-): negative result, these negative specimens whose O.D is below the cut-off value of 0.35 were considered to include in WB assay.
Ind : Indeterminate

Discussion

The prevalence of HTLV-1/2 in Asia has been studies in many countries in Malaysia[14], Australia and the western Pacific[15], Egypt[16], Philippines[17]. Only a low prevalence of infection has been detected. In our study, we determined the seropositivity of Thai IVDU for HTLV-1/2. Plasma specimens were screened by ELISA and repeatedly reactive samples were further confirmed by western blot. Specimens were interpreted as HTLV-1 positive when antibody to *gag* (p^{19} or p^{24}) and *env* (gp^{46} or rgp^{46}) and rgp^{21} were detected or antibody to *gag* (p^{19}) and *env* (rgp^{46-2}) and rgp^{21} band as HTLV-2.

As a result, 10/300 (3.3%) of specimens was found to be repeatedly reactive by ELISA whereas Yap et al.[14] using the first genera-

tion ELISA kit containing only HTLV-1 viral lysate and western blot kit (The Diagnostic Biotechnology) could detect only 1.6% seropositive for HTLV-1 by ELISA. This was found to be less than what we had observed. It might be the ELISA kit that we used had utilized HTLV-1 viral lysate which was enriched with both recombinant HTLV-1 and HTLV-2. However, in western blot assay, we could not detect any positive from reactive samples, whereas they could detect 2 seropositives showing reactivity to p^{19} or p^{24} and gp^{46}. In this study, our western blot kit contained recombinant glycoprotein such as rgp^{46} and rgp^{21} which is slightly different from the one used by them. In addition, Hayes et al[17] also used the same manufacturer of ELISA and WB kit as in Yap et al's study. They reported that 6 sera from adult female prostitutes were found to be strongly reactive with all HTLV-1 protein including p^{19}, p^{24} and gp^{46}. Nevertheless,they found no immunoreactive by WB in 4 ELISA seropositives (2.0%) from people who were living in another area. Nichoson et al[15] reported that no evidence of HTLV-1 infection was found in IVDU by ELISA, whereas IVDU with HIV-positive in Italy could be described as the HTLV-1 infection.[18] Nevertheless, The most of repeatedly reactive samples in this study were indeterminate western blot. In many studies, indeterminate western blot was found to be common as described by Agius et al[19] on a high percentage (25.5%) of sera gave indeterminate interpretation on western blot. In contrast to our study shows no positive. Only the majority of indeterminate could be seen. Since the results of our study were somehow different from those in many studies, this may be the sample size was not big enough to detect antibody to HTLV in our unendemic area or there was no case in Thailand. Also perhaps the serological tests were missing specimens with low antibody titers of HTLV-1/2. Though IVDU group had been reported to be a high prevalence of HTLV-2 in USA.[4,5], it is not clear if there is any difference of behaviour between the IV drug abusers in Thailand and those in USA. As there are several possible explanation for occuring indeterminate: Presence of autoantibody, circulating immune complex and high immunoglobulin level during parasitic disease.[19,20] However, indeterminate western blot samples should be determined by PCR whether a specimen is positive or negative. With the fast changing world, the communication and migration of people might occur any moment, even the behavior of people in the society. Thus, the transmission of virus could happen easier and faster than in the old days. Although there is no evidence of HTLV-1/2 infection among Thai IVDU, we certainly can not overlook to keep watchful eye on it.

Acknowledgement

We thank Thanyarak Hospital for supporting blood specimens.

Reference

1. Hausen HZ. Viruses in Human Cancers. Science 1991;254:1167-73.
2. Cartier L, Araya F, Castillo JL. Southernmost carriers of HTLV-1/2 in the world. Jpn J Cancer Res 1993;84:1-3.

3. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M. A new subtype of human T-cell leukemia virus (HTLV-2) associated with a T-cell variant of hairy cell leukemia. Science 1982;218:571-3.
4. Rosenblatt JD, Golde DW, Wachsman W. A second HTLV-2 isolate associated with atypical hairy-cell leukemia. N Engl J Med 1986;315:372-5.
5. Cann AJ, Chen ISY. Human T-Cell Leukemia Virus type 1 and 2. In: Fields BN, Knipe DM, eds. Virology Volume 2. New York: Raven press, 1990:1517.
6. Hunsmann G, Schneider J, Schmiitt J, Yamamoto N. Detection of serum antibodies to adult T-cell leukemia virus in non-human primates and in people from Africa. Int J Cancer 1983; 15:329-32.
7. Hinuma Y, Chosa T, Komoda H. Sporadic retrovirus (ATLV)- seropositive individual outside Japan. Lancet I 1983;824-5.
8. Zeng Y, Lan XY, Fang J. HTLV antibody in China. Lancet I 1984;799-800.
9. Fan FS, Lin DL, Yeh HM. Screening of antibodies to human T-cell leukemia virus type 1 (HTLV-1) in sera of blood donors and comparison of different methods. Chung Hua 1 Hsueh Tsa Chih (abstract) 1989; 44(1):71-6.
10. Kurimura T, Tsuchie H, Kobayashi S. Sporadic cases of carriers of human T-lymphotropic virus type 1 in southeast Asia. Jpn J Med Sci Biol 1986;39:25-8.
11. Bryan Page J, Lai S, Chitwood DD, Klimas NG, Smith PC, Fletcher MA.HTLV-1/2 seropositivity and death from AIDS among HIV-1 seropositive intravenous drug users. Lancet 1990;335:1439-41.
12. Bohnlein E, Siekevitz M, Ballard DW. Stimulation of the human immuno-deficiency virus type 1 enhanced by the human T- cell leukemia virus type 1 tax gene product involves the action of inducible cellular protein. J Virology 1989;63:1578-86.
13. Manns A, Blattner WA. The epidemiology of the human T-cell lymphotropic virus type 1 and type 2: etiologic role in human disease. Transfusion 1990;31:67-75.
14. Yap SF, Peh SC, Chan L, Wong HC, How VJ, Looi LM. HTLV-1 antibody study in normal individual and unselected hospital patients in Malaysia. South East Asian J Trop Med Public Health 1992;23(1):26-29.
15. Nichoson SR, Efandis T, Dimitrakakis M, Karapoulos A, Lee H, Gust ID. HTLV-1 infection in selected populations in Australia and the western Pacific region. Med J Aust 1992;156:878-80.
16. Constantine NT, Fathi Sheba M, Corwin AL, Danahy RS, Callahan JD, Watts DM. A serosurvey for HTLV-1 among high risk populations and normal adults in Egypt. Epidemiol Infect 1991;107:429-33.
17. Hayes CG, Buran JP, Oberst RB. Antibodies to human T lymphotropic virus type 1 in a population from the Philippines: evidence for cross-reactivity with Plasmodium falciparum. J Infect Dis 1991;163:257-62.
18. Antinori A, Tamburrini E, Ventura G, Nervo P, Ortona L. Prevalence and prognostic significance of anti-HTLV-1 and anti-HTLV-2 antibodies in patients with HIV infection in the Roman region. Bull 1st Sieroter Milan (abstract) 1988;67:345-50.
19. Agius G, Biggar RJ, Alexander SS. Human T lymphotropic Virus type 1 antibody patterns: Evidence of Difference byage and risk group. J Infect Dis 1988;158:1235-44.
20. Biggar RJ, Gigase PL, Melbye M. ELISA HTLV retrovirus antibody reactivity associated with malaria and immune complex in healthy Africans. Lancet 1985; 2:520-3.