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INCIDENCE OF HUMAN T-LYMPHOTROPIC VIRUS TYPE 1(HTLV-1) AMONG BLOOD DONORS FROM ILAM, IRAN

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Abstract: Ilam city is the center of Ilam province, a western province of Iran. In the present study, HTLV screening were performed among 960 serum samples from blood donors by using enzyme-linked immunosorbent assay (ELISA), from March/21/2006 to May/27/2007. In primary screening, 3 (0.003125) samples were positive (two males and one female). Positivity of the samples were confirmed by Western blot (WB) analysis. The WB results indicated that, of 3 positive ELISA specimens, 2 specimens (66.6%) were HTLV-1, and 1 specimen (33.3%) could not be confirmed. For further evaluation, the HTLV-1-WB positive samples and HTLV-1-seropositive but WB-negative sample were examined by PCR. Results showed that the HTLV-1 WB-positive samples were determined as HTLV-1 and the negative sample could not be confirmed for HTLV-1 by PCR. The incidence of HTLV-1 infection in our study was 2/960 (0.00208) among blood bank donors, which confirms the city of Ilam as a non-endemic area, compared to other regions in Iran (Mashhad: 0.77% and Mazandaran: 1.6%) and in the world.

Keywords: • HTLV • incidence • blood donor • Western-Blot • PCR • Ilam

Introduction

Human T-Lymphotropic virus type 1 (HTLV-1) was first identified in humans in 1980 (11) and 1982 (5). It is the etiologic agent of two distinct human diseases, adult T-cell leukemia or lymphoma (1) and a chronic, progressive demyelinating disorder known as HTLV-1-associated myelopathy /tropical spastic paraparesis(2).HTLV-1 has worldwide distribution but it is endemic only in certain parts of the world such as southwestern Japan, the Caribbean basin, Africa, part of South America, southern Italy, Taiwan, and the United States (6). Routes of infection include transfusion, sharing of needles or syringes with infected individuals, sexual contact, and breastfeeding; transplacental transmission is also suspected (7,9). Cellular blood products are the main source of transfusion associated HTLV transmission, whereas fresh frozen plasma, cryoprecipitate, or coagulation factor concentrates appear not to cause infection (4,10). Screening of Blood product in Iran began from 1996. The study performed before 1996 showed high incidence (2%) of HTLV in Khorasan province(13), and therefore all blood product from this province undergo HTLV testing but not in other region of Iran. To avoid HTLV-1 transmission by transfusion, screening of blood donation for HTLV-1/2 infection has been mandatory in several countries: in Japan since 1986; in the United States since 1989; in Canada since 1990; in French Caribbean since 1989 and in the entire French territory since 1991; in The Netherlands since 1993; in Sweden, Denmark, and Iran since 1994; and more recently in Portugal and Greece. Such screening is still under debate in other countries.

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The incidence of HTLV-1 infection in our study was 2/960 (0.00208) among blood bank donors, which confirms the city of Ilam as a non-endemic area, compared to other regions in Iran (Mashhad = 0.77% and Mazandaran =1.6%) and in the world. The present study was carried out to estimate the incidence of HTLV among blood donor from Ilam.

Material and Methods

Subjects

A total of 960 blood donors were tested for HTLV-1/2 during March/21/2006 to May/27/2007 obtained from blood transfusion organization of Ilam. The donors were 85.6% male and 14.14% female, with a mean age of 32 years (ages ranged between 18 and 65 years). All donors fulfilled the criteria for blood donation, which included a clinical examination and an interview to record the history of previous infectious diseases, surgery, blood transfusion, heart diseases, anemia, and information on foreign travel. Those who donate are more likely to be male, white, between the ages of 20 and 40 years, and to have higher incomes and educational status than those who do not. The questionnaire used in this study was specifically developed and contained questions on risk factors which required answers in the format of 'ever/never'. Inquiries about past medical conditions and sexually-transmitted diseases were done in lay-person language to ensure understanding by the blood donors. No attempts were made to physically examine the donors for tattoos or physical signs of diseases. The red blood cells were used for ABO typing while the sera were collected asepticaly after centrifugation at 1800 g for ten minutes into sterile containers and preserved at -20C.

Medical Interview

The medical history covered information about possible risk factors for blood born infection. A tube of blood was drawn for further blood tests. After the first visit, the nurse clinician recorded information about any reported blood born infection risk factors.

Questionnaire

The initial (baseline) questionnaire asked about the period prior to notification. It included questions about self-esteem, perceived locus of control, and optimism; sexual and social functioning; coping styles; social support; health concerns; positive health behaviors; and depressive symptoms. At the 2-week follow- up visit, subjects were asked to complete another questionnaire. All HTLV-1-positive subjects were informed of the test result and were prohibited from redonation.

ELISA assays

Serum samples were screened for HTLV-1 by using enzyme-linked immunosorbent assay ELISA;(ZeptoMetrix,Buffalo, Buffalo, New York) in blood transfusion organization of Ilam.

Western blot assays

All repeatedly positive samples were confirmed by Western blotting (Problot –HTLV ;Fujirebbio,Inc, Tokyo,Japan). Our index of HTLV-1 seropositivity was reactivity to GAG (P19 with or without P24) and two ENV (GD21 and rgp46-I).

DNA purification and PCR

The peripheral blood mononuclear cells (PBMCs) DNA was extracted by a nonenzymatic method and then analyzed for HTLV-1 sequence. PCR amplification was performed with two primer sets, positions at gag, 1423-1444 sense strand and 1558-1537 antisense strand and tax, 7597-7618 sense strand and 7723-7702 antisense strand. The PCR mixture contained a 1-µlit sample, 10 pmol of each primer 200 µlit concentrations of each deoxynucleotide triphosphate, 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM MgCl2, and 1 U of *Thermos aquaticus* (*Taq*) enzyme (CinnaGen, Inc., Tehran, Iran). The reaction mixture was incubated for 5 min at 94°C and then subjected to 30 cycles consisting of 1 min at 94°C, 1 min at 53°C, and 30 s at 68°C. The final annealing step was performed for 5 min at 68°C in a DNA thermal cycler (Biotech Inc). The reaction mixtures were stored at 4°C until they were analyzed by agarose gel (1.5%) electrophoresis. To confirm the PCR fidelity, two blood samples were amplified and sequenced by using an automated sequencer (ABI model 377).

Statistical methods

Incidence rates were calculated with their 95% confidence interval. Statistical comparison of the groups was done by χ^2 analysis with or without Yates' correction, depending on whether the expected values were greater than 5 or between 3 and 5.

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Results

A total of 960 blood samples were analyzed for HTLV-1 contamination. In the primary screening (ELISA), 3(0.003) samples were positive (1 female, 38 years and 2 male, 23 and 48 years). All samples were assayed in duplicate, and positive samples were confirmed by WB analysis. The WB results indicated that, of these 3 positive ELISA specimens, 66.6% (2 specimens) (1 male and 1 female) were HTLV-1, and 0.33% (1 specimen) was not confirmed. ELISA negative results may be occurred due to false positive reaction of kit.

The incidence of the infection was 0.002 among blood bank donors. In order to confirm and determine the HTLV strains, the HTLV-1-seropositive samples, were examined by PCR. The PCR products corresponding to the *tax* and LTR regions of the HTLV-1 genome were sequenced and resulted in a complete homology with the cosmopolitan strain of HTLV-1.

A significant correlation exists between increasing age and incidence of infection (*P* value of correlation, 0.0001 for men and 0.0002 for women). It is also concluded that seroincidence rate in females is higher than males.

Discussion

There is no defined treatment for patients infected with HTLV-1, but the accurate knowledge of seroincidence rates in different population groups may be helpful in establishing prophylactic measures to reduce rates of viral transmission from infected individuals. The overall 0.00208% HTLV-1 seroincidence rate found in Ilam blood donors is lower than that seen in similar studies in Mashhad(0.77%) and Mazandaran(1.6%)(18,19), the United States (0.004%), France (0.004%), and Brazil (0.42%). Higher seroincidence in blood donors has been found in Jamaica (2.1%) (12). Such comparisons must be made cautiously because screening tests, specificity in marker levels, and medical selection of blood donors can vary from one study to another, but we have attempted to accomplish standard screening tests. The present study confirms, by using both serological and PCR detection methodologies, that Ilam is a region where HTLV-1 infection is not endemic. In a previous study, seropositivity was reported to be 3% among the patients referred to the clinic with HTLV-1-associated disease symptoms in Iran (13). ELISA kits have high sensitivity and low specificity; thus, it may not be a reliable screening tool. Therefore, positive ELISA results should be confirmed by WB or PCR. The WB seropositivity parameters used (HTLV blot 2.4 kit) a recombinant spiked WB assay, which is more stringent than those previously used with whole-virus lysate WB. The epidemiology of HTLV-1/2 has been largely defined through the use of antibody-based tests. PCR has also been a useful tool for facilitating epidemiological studies for distinguishing virus type and for quantifying viral presence (3). Age and sex relationships have been identified as contributing factors to HTLV-1 seroincidence in all areas where this virus is highly endemic (8). Female predominance could be related to a preferential sexual transmission from husband to wife. Our study also revealed a strong age-dependent rise in seroincidence rate. This pattern is also well documented in previous studies, which could be explained by a cohort effect (14) and by cumulative effect of infections occurring over the lifetime of individuals, such as by heterosexual transmission. The age-dependent rise in HTLV-1 incidence could also be explained by a birth cohort effect (12). Is our blood supply as safe as it can be? We conclude that it is as safe as state-of-the-art methods allowed to be used in our countries. However, we cannot say that a zero-risk blood supply has been achieved here or elsewhere. The current risk of transfusion-transmitted infection attributable to repeat donors is extremely low, with an estimated per-unit risk of 1 in 10 million for HIV, 1 in 3 million for HCV, 1 in 72 000 for HBV and 1 in 1.1 million for HTLV(17). Despite advances in testing, it remains critically important to maintain a rigorous donor selection process. Appropriately focused donor education regarding inclusion and exclusion criteria together with state-of-the-art testing have brought us to the current level of safety.

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