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Full Length Research paper

Detection of human T-cell lymphotropic virus Type-1 among patients with malignant hematological diseases in Capital of Iran, Tehran

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Human T-cell lymphotropic virus type-1 (HTLV-1) is a deltaretrovirus linked causally to adult T-cell leukemia or lymphoma (ATL), and HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). The aim of this study was to detect HTLV-1 infection in patients with malignant hematological diseases and also determining the prevalence of HTLV-1 in these patient groups. Sixty patients with malignant hematological diseases were included in the study and tested by enzyme-linked immunosorbent assay (ELISA) for anti-HTLV-1, and Real time-PCR for the sequences from HTLV-1 tax gene. The mean age of patients was 33.9 ± 18.3 years. 18 of the subjects were found HTLV-1 seropositive using ELISA and the viral prevalence by Real time-PCR was 12%. HTLV-1 was found in 25% of patients with acute myelogenous leukemia (AML), 58.3% of patients with chronic myelogenous leukemia (CML), 16.7% of patients with acute lymphoblastic leukemia (ALL), and no detected in patients with lymphoma. The present study revealed that HTLV-1 is prevalent in patients with malignant hematological diseases and in our study. The major HTLV-1 associated syndromes were chronic myelogenous leukemia and acute lymphoblastic leukemia.

Key words: Human T-cell lymphotropic virus type-1, malignant hematological diseases, prevalence, Iran.

INTRODUCTION

A type C retrovirus human T-cell lymphotropic virus type 1 (HTLV-1) is the causative agent of two distinct human diseases, adult T-cell leukemia or lymphoma (ATL), and a chronic progressive demyelinating disorder known as HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Matsuoka and Jeang, 2007). HTLV-1 infection has also been associated with a variety of chronic inflammatory diseases such as uveitis (Mochizuki et al., 1996), Sjo¨gren's syndrome (Eguchi et al., 1995), chronic arthropathy (Hasunuma, 1997), infective dermatitis (Lee and Schwartz, 2011), polymyositis synovitis (Sowa, 1992), thyroiditis (Desailloud and Hober, 2009), and bronchioalveolar pneumonitis (Sugimoto et al., 1993). The role of HTLV-1 infection in these disorders is still under investigation. It is estimated that 10 to 20 million people world-wide are infected with HTLV-1 (Johnson et al., 2001). This infection is endemic in southern Japan, the caribbean basin, central Africa, central and south America, the melanesian islands in the Pacific basin, and in the aboriginal population in Australia (Proietti et al., 2005). In Iran, this virus has been found in isolated pockets that HTLV-1 infection is endemic (Khorasan, the northeastern province of Iran). The prevalence of HTLV-1 infection in Mashhad was 0.77% among blood bank donors (Tarhini et al., 2009). However, little is known on the prevalence of HTLV-1 in patients with malignant hematological diseases in Iran, including a possible HTLV-1 association with other malignancies (Table 1).

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Table 1. Prevalence of HTLV-1 among patients with malignant hematological diseases in Iran.

Hematological malignancies	Patient No	Mean age	Male / female	HTLV-I antibody positive (%)	HTLV-I PCR positive (%)
Acute myelogenous leukemia (AML)	26	29.8±12.2	19/7	8 (30.8)	3 (11.5)
Chronic myelogenous leukemia (CML)	21	51±14.6	16/5	7 (33.3)	7 (33.3)
Lymphoma	3	27.3±2.5	2/1	1 (33.3)	0 (0)
Acute lymphoblastic leukemia (ALL)	10	12.3±6.2	7/3	2 (20)	2 (20)
Total	60	33.9±18.3	44/16	18 (30)	12 (20)

The association between retroviruses and hematologic malignancies is also described. There have been few studies on the association between human T cell lymphotropic virus type 1 (HTLV-1) infection and malignancy risk (Inoue et al., 2008). It is still controversial whether or not HTLV-1 infection affects the incidence of several malignancies. Adedayo et al. (2004) found an association between HTLV-1 and lymphoid malignancies in Dominican population. There are case reports of HTLV-1 in lymphoid malignancies except ATL (Starkebaum et al., 1987). Little is known on the prevalence of HTLV1 in patients with various hematologic. The association between retroviruses and hematologic malignancies is also described. There have been few studies on the association between human T cell lymphotropic virus type 1 (HTLV-1) infectionand malignancy risk (Inoue et al., 2008). It is still controversial whether or not HTLV-1 infection affects the incidence of several malignancies.

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MATERIALS AND METHODS

Study design

In this cross-sectional study 60 patients with established malignant hematological diseases who were admitted to oncology Unit of Hazrate Rasul Hospital, Tehran, Iran, from 2009 to 2010 were enrolled. Diagnosis of malignancy was confirmed based on pathology (histology) findings.

The malignancies were as follows: acute myelogenous leukemia (AML) (26 cases), chronic myelogenous leukemia (CML) (21 cases), acute lymphoblastic leukemia (ALL) (10 cases) and lymphoma (3 cases).

Collection and preparation of samples

About 5 ml of peripheral blood were collected from each patient into EDTA-containing vacutainer tubes. Plasma was stored at -70°C until anti HTLV-1 antibody analysis. Blood buffy coat were isolated from EDTA-treated blood by centrifugation and stored at -70°C for later detection. All patients gave written consent to participate in this study, which conforms to the guidelines of the 1975 Declaration of Helsinki.

Immunoassay for anti-HTLV-1

Serum samples were examined for anti HTLV-I antibody by enzyme-linked immunosorbent assay (ELISA) method using anti HTLV-1 antibody kit (ELISA; Dia. Pro Diagnostic Bioprobes, Milan, Italy). Assay procedures and the interpretation of the results were performed in accordance with the instructions provided by the manufacturer.

Real time polymerase chain reaction

To detect HTLV-1 provirus in peripheral blood cells DNA was extracted from 200 µl blood Buffy coat using the High pure extraction kit (Roche Diagnostics GmbH, Mannheim, Germany). Quantitative determination of the amplified products was done with the Rotor Gene 6000 (Corbett Research, Australia) Real-time detection system in accordance with the instructions provided by the manufacturer and subjected to PCR with a Maxima probe qPCR Master Mix (2X) kit (Fermentas, Germany). The primer set for the HTLV- 1 tax gene was PXF (5´-CAAACCGTCAAGCACAGCTT-3´) positioned at 7163 to 7182 and PXR (5´-TCTCCAAACACGTAGACTGGGT- 3´) positioned at 7385 to 7364 and the probe for HTLV-1 tax gene was PXT (5´-TTCCCAGGGTTTGGACAGAGTCTTCT- 3´) positioned at 7331 to 7355 (Gabet et al., 2003). The thermal cycler profile is optimized and validated with heat activation (15 min at 95°C) of hot-start *Taq* polymerase was followed by 40 cycles of denaturation (30 s at 95°C), annealing (30 s at 50°C), and extension (30 s at 72°C). For positive control, DNA was extracted from a HTLV-1 producing human T-cell line (MT-2).

Statistical analysis

Data analyses were done by SPSS software version 11 (SPSS, Chicago, IL). Descriptive results were presented as frequencies, and 95% confidence intervals.

Table 2. Demographic characteristics of patients positive to HTLV-1.

Case	ELISA	PCR	Syndrome	Duration of blood transfusion in month	Age/gender
1	+	-	Lymphoma	Unknown	25/M
2	+	+	CML	2	69/M
3	+	+	AML	2	50/F
4	+	-	AML	3	31/M
5	+	+	AML	1	31/M
6	+	+	CML	2	42/F
7	+	+	CML	3	42/M
8	+	+	CML	3	36/M
9	+	+	AML	5	21/M
10	+	+	CML	2	54/M
11	+	+	CML	5	43/F
12	+	+	CML	6	65/M
13	+	-	AML	4	31/M`
14	+	+	ALL	5	21/M
15	+	+	ALL	1	4/F
16	+	-	AML	2	41/M
17	+	-	AML	4	25/M
18	+	-	AML	3	32/M

The correlation between different factors was evaluated by chi-square test (x^2), or Fisher's exact test when an expected value was less than 5.

RESULTS

Sixty patients with established malignant hematological diseases were recruited in this study. The mean age of patients was 33.9 ± 18.3 years. Out of 60 patients, 44 (73.3%) were male. According to the type of hematological malignancy, 26 (43.3%) with acute myelogenous leukemia (AML), 21 (35%) with chronic myelogenous leukemia (CML), 3 (5%) with lymphoma, and 10 (16.6%) with acute lymphoblastic leukemia (ALL) consist our study population (Table 2).

Eighteen of sixty cases of malignant hematological diseases were positive with ELISA for HTLV-1 antibody, obtaining an HTLV-1 seroprevalence of 30% (18/60). The Molecular method of Real time-PCR that amplifies sequences from the tax region provided a viral prevalence of 20% (12/60). Statistical comparisons showed that ELISA detected higher positive results ($P < 0.05$) than Real time-PCR. HTLV-1 antibody was found in 30.8% of patients with acute myelogenous leukemia, 33.3% of patients with chronic myelogenous leukemia, 20% of patients with acute lymphoblastic leukemia and 33.3% in lymphoma patients. In our study the major HTLV-1 associated syndromes were chronic myelogenous leukemia and acute lymphoblastic leukemia.

In this study, a significant difference was seen; the history of blood transfusion ($p=0.04$) between patients with positive and negative results for HTLV-1 infection.

DISCUSSION

HTLV-1 causes adult T-cell leukemia (ATL) and HTLV I-associated myelopathy (tropical spastic paraparesis), a nononcogenic neurologic disease, arthropathy, and Sjogren's syndrome, infective dermatitis of childhood, hyperinfective strongyloidiasis (Gotuzzo et al., 1999), and Norwegian scabies (Blas et al., 2005). HTLV-1 is cell associated and is spread in cells after blood

transfusion, sexual intercourse, or breastfeeding. The information and understanding of HTLV-1 prevalence in different population and patients groups is crucial because it may be useful in establishing prophylactic measures to decrease rates of viral transmission from infected individuals.

In the present study, we demonstrate that the prevalence of HTLV-1 infection in patients with malignant hematological diseases in Iran is 20% and HTLV-1 seroprevalence is 30%. Whereas the gold standard method for the diagnosis of HTLV-1 infection is the detection of HTLV-1 genome in the specimen of patients, it seems that the prevalence of HTLV-1 infection in our study population is about 20%.

There are several reports which demonstrated a comparable HTLV-1 prevalence to our study. Farias de Carvalho et al. (1997) found a seroprevalence of 28.9% among patients with T-cell lymphoid malignancies in Brazil. Adedayo and Shehu (2004) found a 38.6% of HTLV-1 seropositives in all hematological malignancies in India. Miyagi et al. (2002) found a HTLV-1 prevalence of 26.1% in 88 cases of non-Hodgkin's lymphoma in Japan. Barrientos et al. (2005) in southern Chile found an HTLV-1/2 viral prevalence in patients with malignant hematological diseases 18%, and in chronic lymphoproliferative disorders 27% (Barrientos, 2005). The overall HTLV-1 prevalence rate found in our study group is greater than that seen in some studies but is closed to the others.

On the other hand, HTLV-1 is among the infectious agents that can be transmitted via blood transfusion (Matsuoka and Jeang, 2007). In the present study, significant difference was seen between patients with and without HTLV-1 infection. These patients with HTLV-1 infection may acquire this infection from blood transfusion, despite all of the requirements for screening the blood supply. Therefore, the present study suggests that serious consideration must be given to prevent HTLV-I infection via transfusion in hematological malignanant patients. Routine serological screening for HTLV-I antibody and detection of HTLV-1 genome in blood donors is indicated to permit deferral of blood product donations by asymptomatic HTLV-1 carriers.

In conclusion, the results of this study show an association between HTLV-1 and malignant hematological diseases. Therefore, the possibility of HTLV-1 infection should be considered in patients who suffer malignant hematological diseases.

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