

## Frequency of HTLV-1 Antibody in Children of 6 Months to 14 Years Old Hospitalized in the Pediatric Ward of Imam Reza Hospital, Mashhad, Iran

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### Abstract

#### Background

Human T cell lymphotropic virus type I (HTLV-1) infection is endemic in specific regions of the world, including northeastern Iran. Besides mother to fetus transmission, it can be transmitted through breast feeding, sexual contact, and blood transfusion. The aim of this study was to determine the frequency of HTLV-1 in children.

#### Materials and Methods

This cross-sectional study was conducted on children from 6 months to 14 years of age hospitalized in Imam Reza Hospital between January 2016 and January 2017. HTLV-1 antibody testing was done on all patients admitted within one year, and the positive results were further confirmed by Polymerase chain reaction (PCR). After determining the frequency, the types of feeding and possible transmission ways of the virus were investigated.

#### Results

Out of the 1358 children admitted, 758 entered the study and were tested for HTLV-1 antibody. The result was positive in 58 patients (7.65%) who were further tested by PCR and 11 (1.45%) had positive antibody result. Out of the 58 positive children, 28 (48.3%) were male and 30 (51.7%) female. Most of the children were in the age range of 6 to 30 months and breastfed. In terms of location, most of them lived in Mashhad (58.62%).

#### Conclusion

Our results demonstrated that the frequency of HTLV-1 antibody among children of 6 months to 14 years was 7.65%. They were tested by PCR and 1.45% were positive. This region therefore still remains an endemic area for HTLV-1 infection.

**Key Words:** Antibody, Children, HTLV-1, Iran, PCR.

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## 1- INTRODUCTION

Human T cell lymphotropic virus type I (HTLV-1) is a member of Retroviridae family and is the causative agent for two diseases of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T cell leukemia (ATL) (1). The HTLV-1 infection was first observed in Japan, but the virus is now found all over the world (2). The exact number of people who are HTLV-1 positive in the world is unknown, but it is estimated that about 15–20 million persons are infected with HTLV-1 worldwide (3). The HTLV-1 virus is endemic in various parts of the world such as Southwestern Japan, Caribbean Basin, Central and South Africa, parts of the Middle East, and Southeastern United States (3). Also, the northeast of Iran (Khorasan Province) is known as one of the endemic regions (4).

In a study by Rafatpanah et al., the overall prevalence of HTLV-1 infection in Mashhad was reported as 2.12% (5), and Safai et al. found the prevalence of 3% in healthy individuals who referred to medical laboratories or blood banks in Mashhad (6). The reasons of HTLV-1 clustering, such as the high ubiquity in Southwestern Japan but low prevalence in neighboring regions of Korea, China and Eastern Russia are still unknown (7). The major modes of transmission are well understood, and the three modes of HTLV-1 transmission are mother to child, sexual transmission, and transmission with contaminated blood products (8).

Mother-to-child transmission can occur through placenta, perinatally or by breastfeeding (9). Nonetheless, evidence suggests that transplacental and perinatal transmissions are uncommon. Therefore, most cases of mother-to-child transmission are resulted from ingestion of breast milk (10). The vertical transmission of HTLV-1 increases with longer duration of breastfeeding and occurs in 10–25% of breastfed infants (11). However, formula

feeding can reduce mother-to-child transmission from 20.3 to 2.5% (12). Infection with HTLV-1 is diagnosed by the detection of anti-HTLV antibodies, usually by enzyme-linked immunosorbent assay (ELISA). Because of cross-reaction with HTLV-2, this test has some false-positive results, so it needs to be confirmed and typed by western blot (1, 13). The western blot analysis can differentiate between infection with HTLV-1 and HTLV-2 (13).

The polymerase chain reaction (PCR) is based on proviral DNA extraction of peripheral blood mononuclear cells and can directly detect DNA provirus; this method is used to determine the status of the infection, the serological tests validity, and the distinction between infections with HTLV-1 and HTLV-2. The PCR test is also used to diagnose the infectious virus between by exposure and serum changes (14). The aim of this study was to determine the frequency of HTLV-1 virus antibodies existence in children hospitalized in the pediatric ward of Imam Reza Hospital in Mashhad, Iran.

## 2- MATERIALS AND METHODS

### 2-1. Study design and population

This cross-sectional study was conducted on all children aged between 6 months and 14 years hospitalized in the pediatric ward of Imam Reza Hospital (Mashhad, Iran), from September 2016 to September 2017.

### 2-2. Inclusion and exclusion criteria

The inclusion criteria were any child who was hospitalized during this period of time, and children with a genetic abnormality or poor general condition, under 6 months of age, and the ones whose family did not agree to participate were excluded.

### 2-3. Methods

At first, all children who were hospitalized were assessed using a questionnaire of

demographic data and interview with the parents, then the collected information was classified based on the available variables. Sampling of children was performed by two trained nurses. After filling out the patient's demographic information forms, a 5 cc venous blood sample was collected from each individual. The blood sample was added to a tube containing the anticoagulant and stored in the refrigerator until it was transferred to the laboratory. After transferring the sample to the laboratory of microbiology and virology of Imam Reza Hospital, first 1 cc of the whole blood sample was put in a microtube and kept in freezer with a temperature of  $-20^{\circ}\text{C}$ . In the next step, the rest of the sample was centrifuged, and the plasma was separated. To identify HTLV-1-infected samples, an ELISA test was done on the isolated plasma sample. PCR was also carried out on all positive ELISA samples for further confirmation of HTLV-1 infection. For this purpose, DNA was extracted from peripheral blood mononuclear cells (PBMC) using an available commercial kit.

#### 2-4. Data Analyses

After collecting data, the data were analyzed by IBM SPSS software version

22.0. P-value less than 0.05 was statistically significant.

#### 2-5. Ethical consideration

The parents completed an informed consent form. Moreover, the study protocol was fully approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.MUMS.fm.REC.1391).

### 3- RESULT

During one year, the total number of hospitalized children in the pediatric ward of Imam Reza Hospital was 1358, of which 758 entered the study. There were 403 boys (53.2%), and 355 girls (46.8%) with the average age of  $6.35 \pm 1.76$  years. The minimum and maximum age of children was 6 months and 14 years, respectively. Out of the 758 patients tested, the frequency of positive HTLV-1 Ab (IgG) was 58 patients (7.65%), who were tested by PCR and 11 were positive (1.45%). The sex distribution of positive HTLV-1 Ab children was 28 (48.3%) males and 30 (51.7%) females. As illustrated in **Table.1**, the highest frequency was in the age range of 6 to 30 months and the lowest frequency was in the age range of 31 to 60 months.

**Table 1.** Frequency distribution of positive HTLV-1 antibody children based on age.

Feature	Age category	Data, Number (%)
Positive ELISA test	6 to 30 months	31 (53.45)
	30 to 60 months	12 (20.69)
	More than 60 months	15 (25.86)
Positive PCR test	6 to 30 months	7 (63.64)
	30 to 60 months	3 (27.27)
	More than 60 months	1 (9.09)

The distribution of feeding type among children with positive HTLV-1 Ab is provided in **Table.2**. In addition, 25 (43.11%) patients under study were born by vaginal delivery and 33 (56.89%) patients with cesarean section. The

relationship between the frequency of positive HTLV-1 Ab children and the child's place of residence was also investigated, and there were 34 patients (58.62%) living in Mashhad, 4 people (6.90%) in Sabzevar, 5 cases (8.62%) in

Neyshabur, and 15 children (25.86%) living elsewhere. In children who were positive HTLV-1 Ab, only five of the

mothers agreed with the laboratory tests, with three positive HTLV-1 Ab mothers and two negative.

**Table-2:** Frequency distribution of positive HTLV-1 antibody children based on nutrition and feeding.

Feature	Category	Data, Number (%)
Type of child feeding	Breastfed	29 (50)
	Formula-fed	12 (20.69)
	Both breast milk and formula or cow milk	17 (29.31)

#### 4- DISCUSSION

This study aimed to determine the frequency of HTLV-1 antibody in children who were admitted to the Imam Reza Hospital. In our patients, HTLV-1 antibodies were positive, using ELISA, in 58 children (7.65%), who underwent PCR and 11 were found to be real positive (11/58, around 20% of positive antibodies). The frequency of actual positive HTLV-1 Ab was therefore 1.4% in our population. Mashhad, in the Northeast of Iran, is one of the endemic regions infected with HTLV-1 virus. Epidemiological studies should be conducted there, especially on age groups that are particularly sensitive, to have up-to-date information about the prevalence of the disease, which regulates health-related plans and decisions (6). In a cross-sectional study in Neyshabur, Farid Hosseini et al. reported the prevalence of HTLV-1 infection in the general population to be 3.4% (15). In the same population in Neyshabur, another study by Hedayati-Moghaddam et al. reported the prevalence of HTLV-1 infection as 7.2% (16). However, few studies have been conducted on the prevalence of HTLV-1 infection in children of the endemic areas. The aim of this study was therefore to investigate it in children who were admitted to the Imam Reza Hospital. In our patients, HTLV-1 antibodies were

positive, using ELISA, in 58 children (7.65%), who underwent PCR and 11 were found to be real positive (11/58, around 20% of positive antibodies). Hence, the frequency of actual positive HTLV-1 Ab was 1.4% in our population. In 2003, Abbaszadegan et al. revealed that the incidence of HTLV-1 was positively correlated with age among blood donors in Mashhad (17). Similar result was also reported in the studies of Larsen et al. (18), Eshima et al. (19), Rahimzadegan et al. (20), and Hedayati-Moghaddam et al. (16). In our study, the prevalence of HTLV-1 infection was not associated with aging, and the most positive cases were seen in infants (6 to 30 months old).

Abbaszadegan et al. reported that the prevalence of HTLV-1 was higher in women than in men (17). Also, in the study of Rafatpanah et al. in Mashhad, the prevalence of HTLV-1 infection showed a significant difference between men (1.46%) and women (2.66%), and was higher in women (5). However, in the work by Farid Hosseini et al., it was reported to be higher in men than in women (15), and in the study of Hedayati-Moghaddam et al., the sex variable was not significantly associated with the prevalence of HTLV-1 (16). In our study, among the patients infected with the virus (positive HTLV-1 Ab), 48.3% were boys and 51.7% were girls, which did not differ

much in terms of sex. Vertical transmission is one of the HTLV-1 transmission routes via placenta or at delivery and through breastfeeding. A study was conducted by Hamedi et al. in Mashhad to investigate the transmission of HTLV-1 virus from mother to fetus, so that a practical solution for the transmission control can be provided (21). Out of 407 pregnant mothers, in 6 patients (1.5%) the HTLV-1 antibody was positively reported by ELISA, and the virus was found in the mother's blood by PCR. The umbilical cord sample of all six infants of these mothers was also positive, which could be a sign of infection in uterus (21). In an investigation by Hino in 2011, a significantly higher risk of short-term breast-feeding (<6 months) than bottle-feeding was reported (7.4% vs. 2.5% respectively,  $P < 0.001$ ) (22). In another study in Japan, 16283 pregnant women were studied, and seropositive prevalence of HTLV-1 in them was 5.4% (23).

The serum change in their infants who were breastfed for less than 7 months was 3.8% and in formula-fed infants it was 5.6% (23). A retrospective study by Bittencourt et al. investigated the neonates of HTLV-1 positive mothers (24). They found that the transmission rate in breastfed neonates was 41%, confirmed by PCR, and none of the formula-fed neonates was positive, while 81.5% of children were born by elective cesarean section (24). The results are similar to our study where the highest prevalence of HTLV-1 infection in relation to infant nutrition was 29 patients (50%) who were breastfed. In our study however, 25 patients (43.11%) were born by vaginal delivery and 33 (56.89%) by cesarean section.

#### 4-1. Study Limitations

The limitations of our study include the single center approach as well as the small number of children enrolled.

## 5- CONCLUSION

The frequency of HTLV-1 antibodies presence among children aged between 6 months and 14 years was 7.65%; the children were further tested for PCR and 1.45% were positive. In case of HTLV-1 seropositivity, mothers should be informed about the risk of transmission, and bottle-feeding should be strongly promoted. Since Mashhad is an endemic region in the Northeastern Iran, it is recommended that more children be tested, so that the results of this work will be more generalized.

## 6- ACKNOWLEDGEMENT

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## 7- CONFLICT OF INTEREST: None.

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