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High prevalence of HTLV-I infection in Mashhad, Northeast Iran: A population-based seroepidemiology survey

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ABSTRACT

Background: Mashhad, in the northeast of Iran has been suggested as an endemic area for human T cell lymphotropic virus type I (HTLV-I) infection since 1996.
Objectives: We performed a community-based seroepidemiology study to examine the prevalence and risk factors for HTLV-I infection in the city of Mashhad.
Study design: Between May and September 2009, overall 1678 subjects from all the 12 geographical area of Mashhad were selected randomly by multistage cluster sampling for HTLV antibody. The study population included 763 males and 915 females, with the mean age of 29.1 ± 18.5 years. 1654 serum samples were assessed for HTLV antibody using ELISA and reactive samples were confirmed by Western blot and PCR.
Results: The overall prevalence of HTLV-I infection in whole population was 2.12% (95% CI, 1.48–2.93) with no significant difference between males and females ($p = 0.093$) and the prevalence of HTLV-II seropositivity was 0.12% (95% CI, 0.02–0.44).

The HTLV-I Infection was associated with age ($p < 0.001$), marital status ($p < 0.001$), education ($p = 0.047$), and history of blood transfusion ($p = 0.009$), surgery ($p < 0.001$), traditional cupping ($p = 0.002$), and hospitalization ($p = 0.004$). In logistic regression analysis, age was the only variable that had a significant relation with the infection ($p = 0.006$, OR = 4.33).
Conclusions: Our results demonstrated that Mashhad still remains an endemic area for HTLV-I infection despite routine blood screening. Thus, further strategies are needed for prevention of the virus transmission in whole population.

1. Background

Human T cell lymphotropic virus type I (HTLV-I) is a member of Retroviridae family which has been associated with two main diseases; HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and adult T cell leukemia (ATL).[1,2] The prevalence of HTLV-I infection worldwide is estimated to be 15–20 millions.[3] The main endemic regions of HTLV-I are southwestern Japan, parts of Africa, South America and the Caribbean basin.[4]

More recently the northeast of Iran, Mashhad, has been suggested as a new endemic region of HTLV-I. In 1996, The prevalence of HTLV-I infection in Mashhad was estimated to be ~2% in blood donors.[5] Similarly, Safai et al., reported the prevalence of 3% in healthy individuals who referred to general medical laboratories or blood banks.[6] Mashhad, the capital of Razavi Khorasan province, is the second largest city in Iran with ~2.5 million population in the last census in 2006.[7] Moreover, it is thought that more than 20 million persons a year make the pilgrimage to Mashhad, as one of the holiest cities in the world.HTLV-I seroprevalence rates are sex and age dependent, increasing with age and higher in females than males.[8–10] Discrepancies in sexual behavior and breast-feeding

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practices could contribute to the heterogeneity in prevalence rates in different countries. Studies have shown a difference in the absolute prevalence rates in Japan, Jamaica and the USA; however, these countries demonstrate the same pattern of age and sex-specific prevalence.[9–11]

Lower socioeconomic status such as education is shown to be associated with HTLV-I infection in both endemic and non-endemic areas.[9,12–15] Furthermore, it has been suggested that social and environmental factors may influence the HTLV-I transmission not only in endemic countries but also across the world.[16,17]

In the previous study, we demonstrated the decline tendency in the seropositivity of HTLV-I among blood donors in Mashhad to 0.45% in the years of 2004–2006.[18] However, there is no informative data regarding whether the prevalence rates of HTLV-I infection in the population has changed since 1996. Furthermore, the influence of factors such as age, sex and socioeconomic status, which could contribute to HTLV-I infection, has not been studied in this endemic city so far.

2. Objectives

In the present cross-sectional study we investigated the prevalence rates of HTLV-I infection and some related factors in the general population of Mashhad.

3. Study design

3.1. Study population

From all 12 municipality areas of the city with an estimated 2.5 million residents, 1678 individuals were selected by multistage cluster sampling between May and September 2009. The sampling method was described in details elsewhere.[19] Briefly, each area included some districts and many subdivisions. Some blocks were chosen from one subdivision in every district. About 20 households in each block and only one person in each household were selected randomly. The pilgrims and travelers to Mashhad were not included in this survey. Information regarding demographic characteristics such as sex, age, socioeconomic status and data on medical history including blood transfusion, dentistry procedure, surgery, hospitalization, and traditional cupping (*Hijamat*) were collected. History of behavioral risk factors such as unsafe sex and intravenous drug injection was not investigated due to cultural limitations.

The study was approved by Research Deputyship of Iranian Academic Center for Education, Culture & Research (ACECR) regarding scientific and ethical issues. Informed consent was obtained from all participants and in case of children the written consent was signed by their parents.

3.2. Serological assay and confirmation tests

Five ml of blood samples were obtained from each individual. Serum was separated through centrifugation, DNA was extracted from whole blood cells, and both samples were stored at −20 °C. Serum samples were screened for the presence of anti-HTLV-I antibodies with the MP Diagnostics HTLV I/II enzyme linked immunosorbent assay (ELISA) 4.0 (MP Biomedicals Asia Pacific Pte Ltd, Singapore) according to the manufacturer's instructions. All reactive samples on serologic screening were tested further by Western blot (WB) analysis according to the manufacturer's instructions (MP Diagnostics HTLV Blot 2.4, MP Biomedicals Asia Pacific Pte Ltd, Singapore). Polymerase chain reaction (PCR) was also carried out on all positive ELISA samples for further confirmation of HTLV-I infection. Briefly, Genomic DNA was extracted from peripheral blood mononuclear cells (PBMC) using an available commercial kit (Blood mini kit, Qiagen, Germany) and PCR amplification was performed using specific primers for *tax* (5′-AGGGTTTGGACAGAGTCTT-3′ and 5′-AAGGACCTTGAGGGTCTTA-3′) and *LTR* regions (5′-CATAAGCTCAGACCTCCGGG-3′ and 5′-GGATGGCGGCCTCAGGTAGG-3′).

3.3. Statistical analysis

Descriptive data were summarized as mean, standard deviation and/or percents and were analyzed by SPSS 16.0 using *Chi square* and *t* tests. Binary logistic regression analysis was used to estimate the potential risk factors for HTLV-I infection. Variables in the equation included: age (35 and above vs. <35), marital status (widowed vs. others), Literacy (illiterate vs. literate), and history of transfusion, surgery, traditional cupping, and hospitalization (yes vs. no). A p value <0.05 was considered statistically significant.

4. Results

The study population consisted of 1678 individuals ranged from 1 to 90 years. Seven hundred sixty three of them were males (45.5%) and 915 (54.5%) were females. The mean age of males was 27.9 ± 19.0 and for females was 30.0 ± 18.0 and the male to female ratio was 1.19. Twenty-four persons refused blood withdrawal and were excluded from subsequent analyses. One thousand six hundred and fifty-four serum samples were analyzed for anti-HTLV antibodies.

In the primary screening of the samples by ELISA, 56 (3.39%) were positive for HTLV antibodies. The WB results demonstrated that 35 (62.5%) out of 56 ELISA positive specimens were HTLV-I positive (including two reactive samples for both HTLV-I and HTLV-II), four samples (7.1%) were indeterminate and HTLV positivity was not confirmed in 17 cases (30.4%). According to the WB results, the overall prevalence of the HTLV-I infection in the population study was 2.12% (35/1654) (95% CI, 1.48–2.93), while HTLV-II positivity was 0.12% (2/1654) (95% CI, 0.02–0.44).

In order to confirm the infection further, all the 56 positive ELISA samples were reexamined by PCR using specific primers for HTLV-I. All of the WB positive samples, including two HTLV-I/II dual infection serums were confirmed to be HTLV-I, while the presence of virus was not confirmed in negative and indeterminate samples.

The HTLV-I infection rate for females was 2.66% (24/903) and for males was 1.46% (11/751). No significant differences in the seroprevalence were observed between males and females, although the infection rates were 10 times higher in females older than 65 years compared to males of the same age group (23.08% vs. 2.38%, $p = 0.005$) (Fig. 1A and B). Both two cases with HTLV-I/II dual infection were females; one was 29 and other 58 years old.

Seroprevalence was associated with age, marital status and literacy, increasing significantly among those older than 35 years ($p < 0.001$), illiterates ($p < 0.001$) and widowed individuals ($p < 0.001$) (Table 1). On the other hand, income, ethnic background, employment status, and place of birth had no significant impact on HTLV-I infection. In addition HTLV-I infection was associated with the history of blood transfusion ($p = 0.009$), surgery ($p < 0.001$), traditional cupping ($p = 0.002$), and hospitalization ($p = 0.004$) (Table 2).

In logistic regression analysis, age ($p = 0.006$, OR = 4.33; 95% CI, 1.52–12.29) was the only factor that could predict significantly the risk of infection.

5. Discussion

The preliminary seroepidemiology of HTLV-I infection in Mashhad, Iran was reported in 1996.[5,6] However, the risk factors associated with HTLV-I infection have not been assessed so far.