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Association Between HTLV-1 Infection and Nasopharyngeal Carcinoma: A Case-Control Study

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Abstract

Background: The northeast of Iran is one of the endemic regions of human T-cell lymphotropic virus type-1 (HTLV-1). This study aimed to evaluate the relationship between nasopharyngeal carcinoma and HTLV-1 infection in northeast Iran, an endemic area for HTLV-1.

Methods: In this case-control study, paraffin-embedded nasopharyngeal tissue samples of patients with definitive nasopharyngeal carcinoma were evaluated for the presence of the HTLV-1 genome by polymerase chain reaction retrospectively.

Results: Thirty patients with nasopharyngeal carcinoma and 30 healthy people were evaluated. All participants were matched in terms of age and gender, and all were living in Mashhad. The HTLV-1 genome was detected in only one of the people in the healthy group.

Conclusions: The HTLV-1 infection and nasopharyngeal carcinoma did not correlate significantly.

Keywords: HTLV-1, Nasopharyngeal Carcinoma, Polymerase Chain Reaction

1. Background

Human T-cell lymphotropic virus type-1 (HTLV-1) is a retrovirus first described in 1979 as a causative agent of cutaneous T-cell lymphoma. Epidemiologic data since then have shown the association between HTLV-1 and a variety of hematologic malignancies, including adult T-cell leukemia or lymphoma (ATL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), and acute lymphoblastic leukemia (ALL) (1). This worldwide infection affects around 20 million people and often occurs in endemic areas.

Mashhad, the second largest city of Iran in the northeast of the country, has been regarded as one of the endemic regions for HTLV-1 infection. The prevalence of HTLV-1 among blood donors in Mashhad was 0.18% in 2013 (2). Also, HTLV-1 has increased the risk of nonhematologic malignancies, including liver and cervical cancer. Nasopharyngeal carcinoma, another virus associated with cancer, is related to EBV infection. Infection with EBV and HTLV-1 activates the immune system response, mostly regulatory T cells (3). The resulting inflammatory condition can lead to cancer development.

2. Objectives

This study aimed to evaluate the relationship between nasopharyngeal carcinoma and HTLV-1 infection in northeast Iran, an endemic area for HTLV-1.

3. Methods

3.1. Study Population

In this case-control study, paraffin-embedded nasopharyngeal tissue samples of patients with definitive nasopharyngeal carcinoma (NPC) were evaluated for the presence of the human T-cell lymphotropic virus type 1 (HTLV-1) genome retrospectively. The study was conducted at the Pathology Department of Qaem Educational Hospital, affiliated with the Mashhad University of Medical Sciences,

Mashhad, Razavi Khorasan, Iran. According to the pathology records during 2000 - 2013, all cases with proven nasopharyngeal carcinoma were entered into this cross-sectional study. The exact number of patients undergoing a nasopharynx biopsy without malignancy was regarded as the control group. Razavi Khorasan has been suggested as an endemic region of HTLV-1 infection worldwide, and Qaem Educational Hospital is one of the city's leading tertiary referral hospitals specialized in treating HTLV-1 and also a head and neck cancer treatment center. Nasopharyngeal tissue samples of people biopsied for reasons other than malignancies were selected randomly and evaluated as the control group. A pathologist reevaluated all slides to confirm the diagnosis.

3.2. PCR Method

3.2.1. DNA Extraction

After deparaffinization, DNA extraction was performed using DNA Isolation Kit (GENET BIO, KOREA). First, 20 μL of proteinase K enzyme was poured into sterile microtubes, and the tissue was added along with 200 μL of lysis buffer. The component was mixed and heated up to 56°C until the tissue was completely dissolved. Then, 200 μL of buffer bonding was added to the microtubes and placed again at 56°C for 10 min. Next, 200 μL of absolute ethanol was added to the microtubes, and after mixing the contents, they were transferred to extraction filter columns and centrifuged for one minute at 10,000 Revolutions per Minute (RPM). After this step, the filtered column was transferred to a collection tube, and 500 μL of washing solution 1 was added and centrifuged again at 10,000 RPM. Then, the column was transferred to another clean collection tube to add washing solution 2, and centrifugation was repeated for 14 minutes at 14,800 RPM. The solution was then placed at room temperature for 5 min and centrifuged for another 5 min at 10,000 RPM. The DNA genome was purified on 1% agarose gel and electrophoresed. Then, after staining with ethidium bromide, the transmissometer was used to detect the genome. If DNA was not seen, the extraction steps were repeated using a new sample.

3.2.2. PCR

The HTLV-1 genome amplification was carried out using two different pairs of primers specifically designed for tax and LTR regions of the viral genome (Table 1).

The method has been addressed previously by Mirsadraee et al. (4).

3.3. Ethical Consideration

The Ethics Committee of Mashhad University of Medical Sciences approved this study, and because of the retrospective nature of the study, additional ethical clearance and written consent were waived by the ethical board.

3.4. Statistical Analysis

Data were analyzed by SPSS-21 using an independent t-test and chi-square test at a significance level of 0.05.

4. Results

Thirty patients with nasopharyngeal carcinoma and 30 healthy people were evaluated. All participants were matched in terms of age and gender, and all were living in Mashhad (Table 2).

The HTLV-1 genome was detected in only one of the people in the healthy group.

5. Discussion

This study aimed to evaluate the prevalence of HTLV-1 infection in nasopharyngeal tissue samples of patients with nasopharyngeal carcinoma using PCR. Our results showed no HTLV-1 presence in the tissues. There is limited research on the association between HTLV-1 and the presence of carcinomas. In one of the early studies, Mirsadraee et al. assessed the association between HTLV-I infection and esophageal squamous cell carcinoma showing that the virus's genome was present in two patients with esophageal squamous cell carcinoma out of 85 samples. Also, the study showed no significant association between HTLV-I infection and esophageal squamous cell carcinoma (4). In another study, Nomori et al. evaluated the potential role of HTLV-I infection in the risk of bronchioloalveolar carcinoma by measuring the serologic antibodies against the virus. Out of 212 lung cancer patients, anti-HTLV-1 was positive in only eight. Despite the low number of positive patients, the authors concluded that the risk of bronchioloalveolar carcinoma is higher in HTLV-1-positive patients (5). A recent case report by Dahy et al. reported a newly diagnosed small-cell lung cancer patient with a history of HTLV-1-associated myelopathy (6). Hirata et al. evaluated the clinicopathologic features of HTLV-1-positive patients with breast cancer and found that these patients were older; however, their survival was unrelated to the virus infection (7). Other studies in this context are case reports on the association of HTLV-1 with other carcinomas, and there are no considerable studies in this context.

The study harbors several limitations, including the small sample size and the low number of patients because of the rarity of nasopharyngeal carcinoma. Multi-center studies are needed to overcome this limitation. Besides,

Table 1. Primers Used for HTLV-1 Genome Amplification

Name	Sequence (5'-3')	Amplicon (Base Pair)	Location
TaxF (forward)	AGGGTTTGGACAGAGTCTT	256 Bp	7335 - 7590
TaxR (reverse)	AAGGACCTTGAGGGTCTTA		
LTRF	CATAAGCTCAGACCTCCGGG	224 Bp	8107 - 8330
LTRR	GGATGGCGGCCTCAGGTAGG		

Table 2. Patients' Characteristics

Variable	NPC	HP	P-Value
Age	45.42 ± 20.27	46.46 ± 18.32	0.845
Gender			
Male	21 (70)	20 (667)	0.781
Female	9 (30)	10 (33.3)	
History of smoking	20 (0.66)	17 (0.56)	0.63
History of alcohol	3 (0.1)	1 (0.03)	0.54

Abbreviations: HP: healthy participants, NPC: nasopharyngeal carcinoma group
[a] Values are expressed as mean ± SD or No. (%).

the assessment of the HTLV-1 genome by PCR in the nasopharyngeal tissue samples and the absence of a serologic evaluation of antibodies against it are the other limiting factor resulting in false negative results.

5.1. Conclusions

The HTLV-1 infection and nasopharyngeal carcinoma did not appear to correlate significantly.

Footnotes

Authors' Contribution: Study concept and design: Mahmoudreza Kalantari; Acquisition of data: Shakiba Kalantari; Analysis and interpretation of data: Mohammad Reza Majidi; Drafting of the manuscript: Seyed Alireza Javadinia; Critical revision of the manuscript for important intellectual content: Sare Hosseini; Statistical analysis: Not Applicable ; Administrative, technical, and material support: Mohammad Reza Majidi and Houshangh Rafatpanah; Study supervision: Mahmoudreza Kalantari.

Conflict of Interests: Sare Hosseini is a member of the Editorial Board of this journal. Other authors have no conflict of interest.

Data Reproducibility: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: The Ethics Committee of Mashhad University of Medical Sciences approved this study, and because of the retrospective nature of the study, additional ethical clearance and written consent were waived by the ethical board.

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