Arch Pediatr Infect Dis. 2016 October; 4(4):e36299. doi: 10.5812/pedinfect.36299.

Published online 2016 August 6. **Research Article**

Human Herpesvirus-6 DNA and Langerhans Cell Histiocytosis

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Received 2016 January 17; **Revised** 2016 May 15; **Accepted** 2016 June 20.

Abstract

Background: Langerhans cell histiocytosis (LCH) is a rare histiocytic proliferative disorder of unknown etiology that mainly affects young children. The histological feature is the granuloma-like proliferation of Langerhans-type dendritic cells. The possible role of viruses such as Epstein-Barr virus (EBV, HHV-4), human herpesvirus-6 (HHV-6), herpes simplex virus (HSV) types 1 and 2, and cytomegalovirus (CMV, HHV-5) in the pathogenesis of LCH has been suggested in some studies; however, this still remains under debate.
Objectives: HHV-6 infections are reported to be associated with LCH, but no such reports could be found on Iranian children in the English-language medical literature. This study investigated the presence of HHV-6 in Iranian children with LCH.
Methods: In this retrospective study, we investigated the presence of HHV-6 DNA in 48 patients with LCH, using paraffin-embedded tissue samples, and in 48 controls (age- and tissue-matched) using the nested polymerase chain reaction (nested PCR) method. The patients had been treated at the Department of Pediatric Pathology from 2002 - 2013 and had undergone operations for reasons other than infectious disease. Only the pathology reports were retrospectively reviewed, and the patients were anonymous.
Results: There was no significant difference in the prevalence of HHV-6 detection between the LCH patients and the control subjects. HHV-6 was found by nested PCR in one (2.1%) of the 48 LCH patients and in six (12.5%) of the 48 control cases. P = 0.11 was calculated using Fisher's exact test (OR: 0.15; 95%CI: 0.02 - 1.29).
Conclusions: Our study is the first to investigate patients with LCH and its possible association with HHV-6 in Iran. Considering the P level of 0.11, which is statistically insignificant, our findings fail to support the hypothesis of a possible role for HHV-6 in the pathogenesis of LCH. These results are in concordance with previous investigations showing negative results.

Keywords: Cell Proliferation, Dendritic Cells, Histiocytosis, Langerhans Cell, Human Herpesvirus-6, Polymerase Chain Reaction

1. Background

Langerhans cells are a type of non-lymphoid mononuclear cell involved in inflammatory responses, and Langerhans cell histiocytosis (LCH) is their neoplastic proliferation, the clonality of which was first reported by in 1994 (1-3). These immature dendritic cells express lysosomal enzymes, CD1a, cytoplasmic S-100 protein, and langerin (CD207), and contain racket-shaped organelles of Birbeck granules on electron microscopy (4-6).

LCH is an enigmatic histiocytic proliferative disease of unknown etiology; however, a possible etiologic link between viruses or vaccinations and LCH has been proposed, among other environmental agents (7, 8). Epstein-Barr virus (EBV, HHV-4) is known as the etiologic agent of several malignancies, and herpes viruses are reported to cause persistent infections (9, 10). In addition, hemophagocytic syndromes in humans with several inherited immunodeficiencies are proposed to be induced by EBV and cytomegalovirus (CMV, HHV-5) (11-13). Controversial results are reported in the literature regarding the etiologic role of HHV-6 (14-19), but this is yet to be determined. Accordingly, in this study, we investigated the possible association between HHV-6 and LCH in Iranian children.

2. Objective

Considering the fact that there have been proven cases of cancers with viral etiologies and vaccinations, and the early diagnosis and treatment of viral infections could be of importance in the management of these patients, we investigated the presence of HHV-6 in Iranian children with LCH.

3. Methods

3.1. Patients and Controls

Formalin-fixed, paraffin-embedded (FFPE) tissue samples from 48 patients with a pathologic diagnosis of LCH were extracted from the archive of the pathology department of Mofid children's hospital in Tehran, Iran (a national referral center) from an 11-year period (2002 - 2013).

The diagnosis of LCH was made by a pediatric pathologist, using the histological criteria mentioned in pathology textbooks, i.e. granulomas composed of Langerhans cells with typical grooved nuclei mixed with eosinophils and other inflammatory cells. The diagnoses were confirmed using immunohistochemical techniques for CD1a, S-100 protein, and CD68 when available. After examination of the slides by light microscopy, the tissues with adequate amounts of tumor tissue were used in the study, while the tissues with tumors that were too small were excluded. All patients were Iranian, with an age range of 2 months to 10 years. Forty-eight tissue samples from non-LCH patients who were operated on for reasons other than infectious disease were also selected from the files of the Pathology Department for the same years, as controls (age- and tissue-matched to the LCH cases). These patients' procedures had been performed for conditions such as hemangioma, cystic hygroma, osteochondroma, dermatitis, emphysema, pilonidal disease, soft tissue cysts, enlarged reactive nodes, and anal fissures. The criterion for inclusion of tissue in the control group was the absence of clinical and microscopic evidence for LCH or any other malignant tumor.

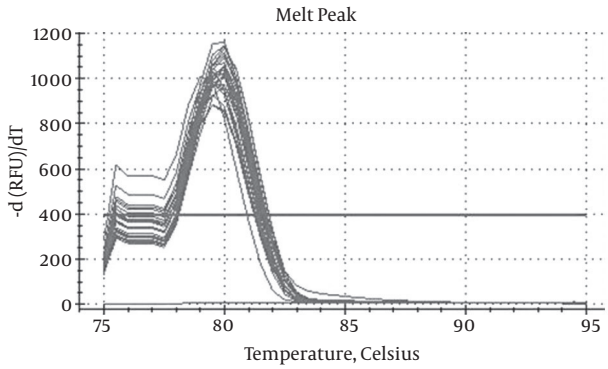
3.2. Paraffin-Embedded Tissue Section Preparation and DNA Extraction

The 5-μm-thick tissue sections were cut from paraffin-embedded blocks on a microtome and placed in sterile screw-cap tubes. It is necessary to completely remove the embedding material before DNA extraction. Xylene and alcohol solutions were used to deparaffinize and rehydrate the tissue sections. The sections were then lysed with a tissue lysis buffer and proteinase K. The samples were subjected to DNA extraction after the tissues were dissolved. The DNA was extracted from lysed-tissue samples according to the manufacturer's instructions (RTP® DNA/ RNA Virus Mini Kit; Stratec Molecular GmbH, Berlin, Germany). The extracted nucleic acids were stored at -20°C until PCR testing.

3.3. Polymerase Chain Reaction

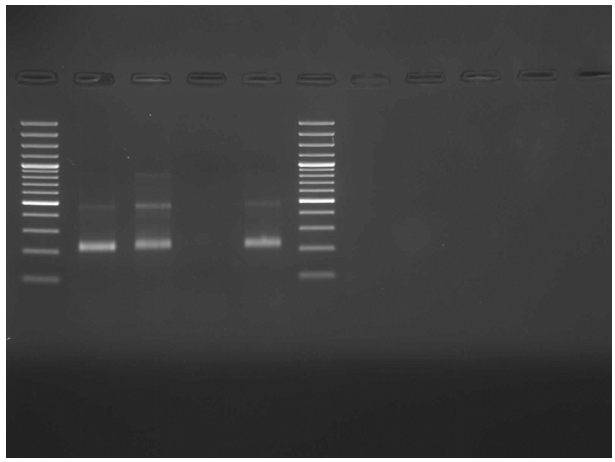
After quality control of the extracted DNA with the SYBR green real-time PCR (RT-PCR) melting curve for the beta-globin gene (Figure 1), nested PCR was applied to detect the HHV-6 genome in the samples as previously described (20). A 214-bp gene region of the main viral capsid protein was amplified using the nested primer sets (Figure 2). Primers for nested PCR were HHV-1 (outer): 5'-CAATGCTTTTCTAGCCGCCTCTTC-3' and HHV-2 (outer): 5'-ACATCTATAATTTTAGACGATCCC-3' and HHV-3 (inner): 5'-TTGTGCGGGTCCGTTCCCATCATA-3 and HHV-4 (inner): 5'-TCGGGATAGAAAAACCTAATCCCT. The limit detection of 50 genome copies of HHV-6 per reaction was determined by the nested PCR assays, using the serial dilutions of AmpliRun® HHV-6 DNA CONTROL (Vircell, S.L., Granada, Spain).

Figure 1. Beta-Globin Gene SYBR Green Real-Time Polymerase Chain Reaction Melting Curve



The graph shows that the melting temperature of the beta-globin PCR product is approximately 80.0 °C.

Figure 2. Electrophoresis of PCR Products of HHV-6 Assay



The 214-bp band is the positive signal.

3.4. Statistical and Ethical Considerations

The obtained data were compared using Fisher's exact test. $P < 0.05$ was considered to indicate statistical significance.

No ethical issues were involved in this study. Only the pathology reports were retrospectively reviewed, and the patients were anonymous. The articles used as references are valid and the data taken are reported unchanged.

4. Results

Forty-eight patients with a pathologic diagnosis of LCH were included in this study. All patients were Iranian (24 males and 24 females), with an age range of 2 months to 10 years. Age, sex, and biopsy sites for all patients are shown in Table 1. Forty-eight tissue samples from patients with non-LCH diagnoses were also selected as controls (in the same age range and tissue-matched to the LCH cases). The HHV-6 DNA was detected by nested PCR in one of the 48 LCH patients (positive results in 2.1% and negative results in 97.9%). In the control group, we detected HHV-6 in six of the 48 samples (positive results in 12.5% and negative results in 87.5%). This results in OR: 0.15; 95%CI: 0.02 - 1.29, and P = 0.11, which shows no significant difference in HHV-6 prevalence results between the LCH patients and the controls. HHV-6-DNA was found in the lymph node of a two-year-old girl with LCH (Table 1).

Table 1. Age, Sex, and Biopsy Sites of Patients

Age	Sex	Biopsy Site	HHV-6
2 y	Female	Lymph node	+
2 y	Male	Mediastinal and neck mass	-
10 mo	Male	Skin	-
1 y	Male	Soft tissue, scalp mass	-
2 y	Male	Frontal bone	-
11 mo	Male	Mastoid bone	-
1 y	Female	Skin, chest wall	-
3 y	Male	Iliac bone	-
6 y	Female	Proximal tibia	-
10 y	Male	Skull and soft tissue	-
2 y	Female	Parietal bone and soft tissue	-
2 y	Male	Skin	-
8 y	Male	Soft tissue, scalp	-
2 y	female	Soft tissue, submandibular	-
2 y	Male	Vertebral bone	-
7 y	Male	Clavicle and soft tissue	-
15 mo	Male	Abdominal mass	-
4 y	Female	Base of skull	-
2.5 y	Female	Rt lobe of lung	-
8 y	Female	Skin, abdomen	-
2 mo	Female	Soft tissue	-
2 y	Male	Soft tissue, scalp	-
3 y	Female	Frontal bone and soft tissue	-
3 y	Female	Soft tissue, scalp	-
2 y	Female	Metatarsal bone	-
16 mo	Male	Skin	-
4 mo	Female	Skin	-
1 y	Female	Skin	-
1.5 y	Male	Soft tissue, scalp	-
7 y	Male	Soft tissue, perianal	-
5 y	Female	Skin	-
6 y	Male	Skin	-
1 y	Female	Bone	-
2 y	Female	Soft tissue	-
17 mo	Male	Skin	-
4 y	Female	Lymph node	-
1 y	Female	Skin	-
2 y	Female	Skin	-
22 mo	Male	Bone	-
2 y	Female	Skin	-
1 y	Male	Soft tissue	-
18 mo	Male	Skin	-
2 y	Female	Skin	-
1 y	Male	Skin	-
1 y	Male	Soft tissue	-
2 y	Female	Skin	-
5 y	Male	Skin	-
3 y	Female	Skin	-

5. Discussion

LCH (histiocytosis X) is an uncommon disease with three overlapping clinical syndromes, including multifocal multisystemic LCH (Letterer-Siwe disease), multifocal unisystemic LCH (Hand-Schüller-Christian disease), and unifocal LCH (solitary eosinophilic granuloma) (2). Hematoxylin-eosin staining of biopsy slides shows granulomas composed of a mixture of Langerhans cells, macrophages, eosinophils, multinucleated giant cells, and lymphocytes (3, 4). The involved organs and the patient's age determine the prognosis of LCH. Children usually need treatment, whereas most adult patients with lung involvement have an indolent course of regression (5, 6, 21, 22).

Some investigators have tried to identify a link between viruses and LCH. The etiologic role of HHV-6 in LCH is debated, with conflicting results in the literature (14-19). Jeziorski et al. (17) demonstrated no significant association between EBV, CMV, or HHV-6 in the pathogenesis of LCH. McClain et al. (18) also failed to find evidence of genomes for adenovirus, CMV, EBV, herpes simplex virus (HSV), HHV-6, human immunodeficiency virus (HIV), human T-cell leukemia virus types I and II, and parvovirus in 56 cases of LCH, employing in situ hybridization (ISH) and PCR techniques.

In contrast, Leahy et al. (16) detected HHV-6 in 47% of their patients with LCH, using the PCR technique. Glotzbecker et al. (15) used the immunohistochemistry (IHC) method and ISH for detection of HHV-6, and reported a high rate of 71.4% by both methods. However, in another investigation, they reported no significant difference between 13 LCH patients compared to a control group, using qualitative and quantitative real-time PCR (23). Csire and colleagues (14) had an LCH patient with persistently de-

tected HHV-6 through 17 years of follow-up, and suggested that HHV-6 infection may be associated with development or progression of LCH.

In our study, HHV-6-DNA was detected in only one patient (2.1%) out of 48 cases of LCH. The positive result in six (12.5%) of the control samples could be attributed to coincidence. P = 0.11, which shows no significant difference in HHV-6 detection results between the LCH patients and the controls, does not support the hypothesis of a possible role for HHV-6 in the pathogenesis of LCH disease, which is in keeping with some other reports in the literature (17-19).

In previous studies (14-19), authors have declared no limitations, and their methods included serology, IHC, ISH, and PCR. However, the limitations of our study were that IHC and ISH for HHV-6 were not available for use and the patients could not be checked for the evidence of HHV-6 infections serologically.

Our study is of importance because it is the first performed on this subject in Iran. We failed to find any statistically significant differences between patients with LCH and the control group with regard to the presence of HHV-6 DNA. This is in concordance with previous negative results reported in the literature (17-19).

Acknowledgments

We thank Dr. Ahmad Reza Shamshiri for statistical analysis, Ms. Leila Poos-Ashkan and Pooneh Tavakoli for performing the PCR, and Mojgan Shohadai for technical assistance.

Footnotes

Authors' Contribution: Maliheh Khoddami: concept and design, critical revision of the manuscript for important intellectual content, supervision, and drafting and revision of the manuscript; Seyed Alireza Nadji: supervising and performing PCR tests and drafting and revision of the manuscript; Mahsa Vahdatinia: corresponding author, acquisition of data, and drafting of the manuscript.

Funding/Support: This study was financially supported by the pediatric infections research center, Mofid children's hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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