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Herpes Simplex Virus and Langerhans Cell Histiocytosis

Maliheh Khoddami[1], Seyed Alireza Nadji [2], Paria Dehghanian[3*]

1. Pediatric Pathology Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

2. Virology Research Center (VRC), National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

3. Pediatric Infections Research Center, Research Institute for Children Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background and objective: Langerhans cell histiocytosis (LCH) is a rare histiocytic proliferative disorder of unknown etiology and mainly affects young children. The histological feature is granuloma-like proliferation of langerhans-type dendritic cells. Although the possible role of viruses such as *Epstein-Barr virus* (*EBV*, *Human Herpes virus -4*), *Human Herpes virus-6* (*HHV-6*), *Herpes Simplex virus* (*HSV*) types 1 and 2 and *Cytomegalovirus* (CMV, *Human Herpes virus-5*) is suggested in the pathogenesis of LCH by some investigators, its exact pathophysiology has not been cleared yet. In this study, we investigated the presence of *HSV* types 1 and 2 in Iranian children with LCH

Methods: In this retrospective study, we investigated the prevalence of presence of *HSV* types 1 and 2 (in 30 patients with LCH), using paraffin-embedded tissue samples and 30 age and tissue-matched controls (operated for reasons other than infectious diseases) from the Department of Pediatric Pathology, Tehran, Iran, by nested Polymerase Chain reaction method. No ethical issues arose in the study, because only the pathology reports were reviewed and patients were anonymous.

Results: We failed to find *HSV* types 1 and 2 DNA in any of the 30 patients with LCH or the control group.

Conclusion: According to our findings, *HSV* types 1 and 2 do not appear to have any etiologic role in the pathogenesis of LCH in Iranian children. These results are in accordance with previous investigations with negative findings.

Corresponding information: Paria Dehghanian, MD, Pediatric Infections Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. E. mail: Paria_email@yahoo.com.

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Introduction

Langerhans cells are a type of non-lymphoid mononuclear cells involved in inflammatory responses; LCH is the neoplastic proliferation of these cells, the clonality of which was first reported by in 1994 (1-3). These immature dendritic cells express lysosomal enzymes CD1a, cytoplasmic S-100 protein, langerin (CD207) and contain the 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 vaccination and LCH has been proposed among other environmental agents (7, 8). *EBV* 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 human with several inherited immunodeficiency types are proposed to be induced by *CMV* (11-13). Controversial results are reported in the literature regarding the etiologic role of *HHV-6* (14-19). Although no positive result is reported regarding

HSV (14, 17, 18), the etiologic role of *HSV* is yet to be determined. Accordingly, in this study, we investigated the possible association of *HSV* types 1 and 2 and LCH in Iranian children.

Materials and Methods

Patients and Controls

Formalin-fixed, paraffin-embedded (FFPE) tissue samples of 30 patients with pathologic diagnosis of LCH were extracted from the archive of Pathology Department of Mofid Children's Hospital in Tehran, Iran (one of the national referral centers) for a 10-year period (2002-2012). Diagnosis of LCH was made by a pediatric pathologist, using the histological criteria mentioned in the 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 technique for CD1a, S-100 protein and CD68 when available. After examination of slides by light microscopy, tissues with adequate amount of tumoral tissue were used in the study and those with too small tumors were excluded. All patients were Iranian and age ranged between 2 months and 10 years. Thirty tissue samples with non-LCH diagnoses, who were operated for reasons other than infectious diseases, were also selected from the files of the Pathology Department (between the years 2002 and 2012), as controls (age and tissue-matched to LCH cases). These patients were operated for reasons such as hemangioma, cystic hygroma, osteochondroma, dermatitis, emphysema, pilonidal disease, soft tissue cysts, enlarged reactive nodes, anal fissure, etc. The inclusion criterion of tissue in control group was absence of clinical and microscopic evidence for LCH or any other tumor.

Paraffin-Embedded Tissue Section Preparation and DNA Extraction

The 5 µm-thick- tissue sections were cut from paraffin-embedded blocks on a microtome and put into sterile screw–cap tubes. It is necessary to completely remove the embedding material before DNA extraction. The xylene and alcohol solutions were used to deparaffinization and rehydration of the tissue sections. For this purpose, the section-contained tubes were heated in dry oven at 55-60°C for 5 minutes, positioned so as to allow drainage of melting paraffin. Then Xylene (1 mL) was added to the tubes and incubated at room temperature for two minutes. The tubes were centrifuged at high speed and Xylene was discarded. This was repeated once more in fresh xylene for one minute. For washing and rehydration, 1 mL of 100%, 95% 70% ethanol timely were added to the tubes for two minutes. Then the sections were immersed in 1X PBS for two minutes. The PBS was discarded from tube by centrifugation and pipetting.

Then the sections were lysed by a tissue lysis buffer (containing EDTA 0.05 mM –Tris 0.01mM-SDS 0.2 %; pH 8.0) and Proteinase K (5 mg /ml). The samples were subjected to DNA extraction when the tissues were dissolved. DNA was extracted from the lysed-tissue sample according to the company's instructions (RTP® DNA/ RNA Virus Mini Kit Procedure; Stratec Molecular, Berlin, Germany). The extracted nucleic acids were stored at -20°C before performing PCR.

Polymerase Chain Reaction

The quality control of the extracted DNA was performed using SYBR Green Real-time PCR-Melting curve for beta globin gene using GH20 primer; GAAGAGCCAAGGACAGGTAC and PCO4 primer; CAACTTCATCCACGTTCACC (Figure 1).

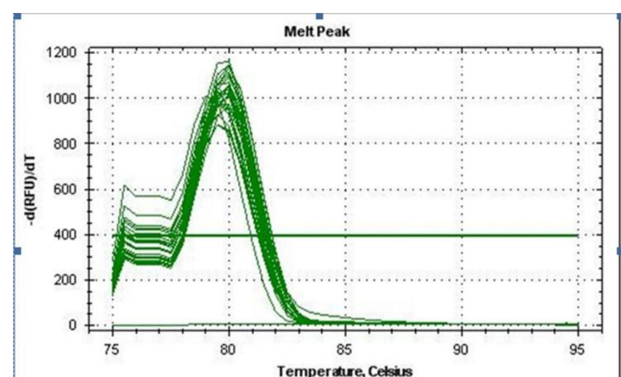

Figure 1. Beta-globin gene SYBR Green Real-time PCR–Melting curve. The melting temperature of Beta-globin PCR product is around 82.5 °C.

The Thermo Scientific™ Maxima™ SYBR™ Green 2X qPCR Master Mix and 10 pmol per reaction of the GH20/PCO4 primers were used to conduct the quality control.

To detect *HSV*-1/2, two sets of nested-PCR were applied to detect *HSV*-1/2 genomes in the samples as described before (20). The primer sequences are as below: HSV1F1 ATCRCGGTAGCCCGGCCGTGTGACA; HSV1R1 CATACCGGAAGCCACCACACAA; HSV2F1 TCAGCCCATCCTCCTTCGGCAGTA; HSV2R1 GATCTGGTACTCGAATGTCTCCG; HSV1F2 CATAYCGACCACACCGACGA; HSV1R2 GGTAGTTGGTCGTTCGCGCTGAA; HSV2F2 AGACGTGCGGGTCGTACACG; HSV2R2 CGCGGTCCCAGATCGGCA. By using the gpD HSV-1/2 sequence primer sets, the 140 and 100 bp fragments came positive for *HSV*-1 and *HSV*-2, respectively (Figure 2).

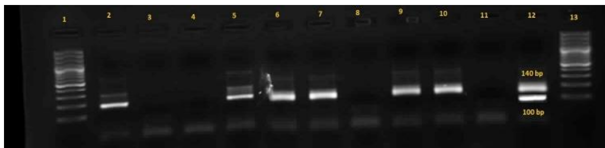

Figure 2. The gel electrophoresis picture of HSV1/2 nested PCR assay. The 140 and 100 bp bands are related to HSV1 and HSV2 positive cases, respectively. Lanes 1 and 13: 100bp DNA size marker; lanes 2 to 10: clinical samples; lane 11: negative control of PCR and lane 12: positive control of HSV detection kit.

The limit detection of 50 genome copies of *HSV*-1/2 per reaction was determined by the nested PCR assays using serial dilutions of *AmpliRun® HSV1, HSV2* DNA *CONTROL* (Vircell, Spain). The positive clinical samples and the Vircella DNA control were used as positive controls in our assay.

Statistical and Ethical Considerations

Data was analyzed using Chi-Square or Fisher's exact test. P ≤0.05 was considered to indicate statistical significance.

In this study, no ethical issues were involved. Only the pathology reports were reviewed retrospectively and patients were anonymous.

Results

Thirty patients with a pathologic diagnosis of LCH were included in this study. All patients were Iranian (16 male and 14 female) and the age ranged between 2 months and 10 years. Age, sex and biopsy site of all patients are shown in Table 1. Thirty tissue samples with non-LCH diagnoses were also selected as controls (age and tissue-matched to LCH cases). *HSV* DNA was not detected in any of 30 LCH patients or in the control group (Table 1).

Table1. Age, Sex and Biopsy Site of Patients

Age (years)	2.96±2.55
Gender	
Female	14 (46.67%)
Male	16 (53.33%)
Biopsy site	
Soft tissue	10 (33.33%)
Bone	7 (23.33%)
Soft tissue and Bone	4 (13.33%)
Skin	7 (23.33%)
Lymph node	1 (3.33%)
Lung	1 (3.33%)

Discussion

Considering the fact that LCH could have an etiologic association with viruses, as suggested by previous studies (7,8,14,15,19), this study was conducted due to the lack of information about such a possible link in Iranian children in English medical literature.

It is proposed that the direct oncogenic mechanism of HSV-1 and 2 is by induction of unscheduled DNA synthesis. Both viruses have an anti-apoptotic activity in lytically infected cells, particularly HSV-2 protein ICP10PK, which inhibits apoptosis through activation of Ras/Raf-1/MEK/ERK Pathway. HSV1 was associated with prostate cancer, melanoma and cervical cancer and was detected in benign and malignant thyroid tumors (21).

LCH (histiocytosis X) is an uncommon disease with three overlapping clinical syndromes which includes multifocal multisystemic LCH (Letterer-Siwe disease), multifocal unisystemic LCH (Hand-Schüller –Christian disease) and unifocal LCH (solitary eosinophilic granuloma) (2). Hematoxylin-eosin stain of biopsy slide shows granulomas composed of a mixture of Langerhans cells, macrophages, eosinophils, multinucleated giant

cells and lymphocytes (3, 4). The involved organs and 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, 22, 23).

Although some investigators have tried to seek a link between viruses and LCH, they have failed to report any positive results for many viruses including *HSV* (16-18).

Jeziorski et al. (16) demonstrated no significant association between *EBV*, *CMV* or *HHV-6* in the pathogenesis of LCH. On the contrary, Leahy et al. (14) detected *HHV-6* in 47% of 30 patients with LCH, but their cases had negative results for *HSV* using PCR technique. Glotzbecker et al. (15) used the immunohistochemistry method (IHC) and in situ hybridization (ISH) for detection of *HHV-6* and reported a high rate of 71.4% by both methods. However, they reported no significant difference between patients with LCH and the control group in their study on 13 patients with LCH, using qualitative and quantitative real-time PCR (24). Csire and colleagues (19) had a LCH patient with persistent *HHV-6* detection through 17 years of follow up and suggested that *HHV-6* infection may be associated with development or progression of LCH.

Concerning *HSV*, McClain et al. (17) used sensitive ISH and PCR techniques in 56 cases of LCH and found no positive results. In their review of the literature, McClain et al. (18) did not find any positive reports regarding *HSV* in LCH. We also failed to find the *HSV* type 1 and 2 DNA in either of the LCH patients, which is in concordance with some other results reported in the literature (14, 17, 18).

In previous studies (14-19) authors declared no limitations and the methods used included serology, IHC, ISH and PCR. However, IHC and ISH for *HSV* were not available for us, which is considered as the main limitation of our study.

Conclusion

Our investigation was the first study performed in Iran on this subject. Our negative result for *HSV*-1/ 2 is similar to previous studies in the literature (14, 17, 18). Performing such studies could be of importance, because vaccination or an early diagnosis and treatment of viral infections could prevent development of LCH in the future.

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Conflicts of interest

Authors had no conflicts of interest to be reported.

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