Epstein-Barr Virus-Associated Langerhans Cell Histiocytosis of the Thyroid Gland

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Received: 30 July 2020 / Accepted: 26 October 2020 / Published online: 2 November 2020
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Abstract

Thyroid gland involvement of Langerhans cell histiocytosis (LCH) is extremely rare in both systemic and isolated disease. The role of viral infection in LCH development is not yet fully understood. Although several viruses are proposed as etiologic factors, such as Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6), they seem to play a bystander role in LCH. A 29-year old female patient with a prior history of multisystemic LCH (pituitary gland and skull bone), presented with a thyroid nodule. The patient underwent a total thyroidectomy and the histological examination revealed nodular lesions composed of sheets and clusters of histiocytes in the inflammatory background. The histiocytes stained positive for S-100 and CD1a and were negative for HHV-8, cytomegalovirus, and VE1 (anti-*BRAF*^{V600E}) on immunohistochemistry. The EBER in situ hybridization for EBV showed frequent positive-stained cells. The conventional PCR analysis for EBV was positive and qPCR analysis confirmed a significant DNA copy number difference ($p=0.02$) between the tumor and adjacent non-neoplastic thyroid tissue. PCR analysis for HHV-6, HPV, HSV was negative in both tumor and benign samples. In conclusion, the presented case showed a rare thyroid involvement by LCH associated with EBV infection, which has not been reported before. Further studies are required to investigate a possible etiologic link between EBV infection and LCH.

Keywords Langerhans cell histiocytosis · Thyroid · Epstein-Barr virus

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Introduction

Langerhans cell histiocytosis (LCH) is a rare, predominantly multisystemic disease characterized by clonal proliferation of CD1a+/Langerin+ Langerhans (dendritic) cells [1]. LCH can arise in virtually any organ system; the most common sites being bone, skin, lung, and the pituitary gland. The clinical presentation of the disease is widely variable, ranging from single indolent lesions to an aggressive multisystemic disease, and most cases are encountered in children [2]. Thyroid gland involvement, either as a single lesion or a part of the multisystemic disease is extremely rare [3].

Previous studies actively debated the role of viral infection in LCH [1, 2] and several viruses were proposed as etiological factors, including Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6) [2, 3]. According to Jeziorski et al., EBV seems to play a bystander role in pediatric LCH [4]. Herein, we report a rare case of adult LCH with thyroid gland involvement, showing EBV infection on in situ hybridization (ISH) and polymerase-chain reaction (PCR), which was not found in the neighboring non-neoplastic thyroid.

Case Presentation

A 29-year old female patient presented with thyroid gland enlargement. The patient had a biopsy diagnosis of LCH in the pituitary gland and left temporal bone, 3 years and 1 year prior to the presentation, respectively. Considering

the underlying disease, a total thyroidectomy was performed. Grossly, the thyroid gland had a multinodular appearance with an index nodule presenting as a 3.5 cm sized irregular mass in the right lobe. The histological examination showed thyroid gland architecture effacement by sheets and clusters of histiocytes in a background of reactive lymphoid follicles, eosinophils, and germinal

Fig. 1 Langerhans cell histiocytosis of the thyroid. Panoramic histology shows a nodular lesion (**a**) with classic LCH cells (**a**, inset) on hematoxylin and eosin, which is negative for thyroglobulin (**b**) and highlighted by S-100 (**c**) immunostain. On high magnification, the normal thyroid follicles, highlighted by thyroglobulin (**d**) were infiltrated by lymphocytes and CD1a (**e**) and S-100 protein (**f**) positive histiocytes. EBER in situ hybridization revealed frequent positive cells within the lesion (**g**). EBV DNA copy number on qPCR showed a significant difference ($p=0.022$) between the tumor and benign thyroid tissue (**h**)

centers (Fig. 1a). On a high power view, Langerhans cells had abundant pale eosinophilic cytoplasm and irregular shaped nuclei with prominent nuclear grooves (Fig. 1a, inset). Nodules of the lesion were negative for the thyroid tissue-specific markers thyroglobulin (Fig. 1b) and TTF-1, and positive for S-100 (Fig. 1c). On high magnification, normal thyroid follicles, highlighted by thyroglobulin (Fig. 1d) were infiltrated by neoplastic cells, immunoreactive for CD1a (Fig. 1e) and S-100 (Fig. 1f), consistent with the diagnosis of LCH. Additional workup with VE1 antibody detected no *BRAF*^{V600E} mutation.

We further investigated the possible viral etiology. Immunostaining for HHV-8 and cytomegalovirus (CMV) was negative. The EBER ISH for EBV showed frequent positive-stained cells (Fig. 1g), up to 20 cells per one high-power field. To confirm EBV status of the lesion, molecular analysis was undertaken at an accredited microbiology laboratory. The conventional PCR for EBV was positive, which prompted quantitative study of the viral load in the neoplastic tissue vs. the microdissected adjacent benign thyroid. Quantitative real-time PCR (qPCR) confirmed a significant copy number difference ($p=0.022$) between the tumor and the benign thyroid gland (Fig. 1h). PCR analysis for HHV-6, human papillomavirus (HPV) and herpes simplex virus (HSV) were negative in both tumor and benign thyroid samples.

2 years after thyroid surgery, the patient developed an LCH lesion in neck soft tissue (confirmed as recurrence on biopsy) and received several courses of chemotherapy, as per the existing protocols for LCH. Currently, 10 years after thyroidectomy, the patient is under follow-up and has been disease-free for 7 years with no new lesions on whole-body imaging.

Discussion

The first case of LCH was reported by Alfred Hand in 1893. Formerly referred to as histiocytosis X, it was renamed in 1987 as LCH [5]. LCH is the most common histiocytic disorder, characterized by the proliferation of dendritic cells with immunophenotype of Langerhans cells. The incidence rate is approximately five children per million and one adult per million, annually. The median age of presentation is 30 months [6].

LCH involvement present as a single site lesion, multiple sites in a single organ system, or multiple organ systems. While pediatric LCH patients commonly show bone, skin, and lymph nodes involvement [7], the common sites in adults are bone, lung, skin, and pituitary gland [8]. Thyroid gland involvement is particularly rare, described in about 65 cases in English literature [9] and it is more frequently reported in adults, mostly as a part of multisystemic disease.

Due to its rarity and variable clinical symptoms, thyroid LCH might be misdiagnosed as granulomatous thyroiditis, benign tumors, undifferentiated carcinoma, or lymphoma without histological examination [8]. Given that our case had a prior history of LCH, there were grounds to suspect involvement of the thyroid gland by LCH, which was confirmed by histopathological findings and immunohistochemical stains. A few cases of isolated thyroid LCH [9, 10] and co-existing LCH with papillary thyroid carcinoma (PTC) have been reported [11, 12].

The pathogenesis of LCH is not fully understood and there is still an open debate about an inflammatory reactive or neoplastic origin of the disease. LCH was believed to arise from skin Langerhans cells but gene expression analyses indicated that LCH cells were more similar to dendritic cell precursors derived from bone marrow [5]. The identification of the oncogenic *BRAF*^{V600E} mutation in about 60% of cases of LCH has provided additional evidence for its neoplastic origin [12]. Based on these findings, LCH is redefined as a myeloid neoplasm driven by misguided myeloid differentiation [5–7]. The present case showed negative staining on VE1 immunohistochemistry, indicating an absence of the *BRAF*^{V600E} mutation. This approach as a substitute to genotyping was previously validated in our laboratory [13, 14].

EBV is one of the most prevalent viruses in human, as it is estimated that about 90–95% of adults get the infection sometime in life and become lifelong carriers. EBV infection is associated with the development of various autoimmune diseases and some lymphoid and epithelial malignancies [15]. In the thyroid gland, autoimmune thyroid diseases, such as Graves' disease and Hashimoto's thyroiditis have been found to be associated with EBV infection [15, 16]. Involvement of EBV in thyroid carcinogenesis is rather controversial [17].

LCH lesions have a rich inflammatory cell infiltrate background with a median of only 8% pathological Langerhans cells. This inflammatory character of LCH lesions raises the possibility of infection and immune dysregulation as mechanisms of pathogenesis [6]. Several viruses were studied as a candidate for etiological factors of LCH. A few studies reported high EBV positivity rate in LCH tissues using ISH and PCR analysis [2, 18] and development of LCH in chronic active EBV infection has also been reported [19]. Jeziorski et al. suggested the possibility of EBV reactivation due to an immunosuppressive microenvironment in LCH [4]. They detected EBV in B lymphocytes of LCH granuloma in three out of 19 LCH cases using double staining with ISH and immunohistochemistry (IHC), which indicated that EBV was likely a bystander rather than a cause of LCH. Although a recent series focused on head-neck lesions showed an absence of EBV by ISH in all 19 studied cases [20], Shimakage et al. pointed that pre-analytical variables and ISH probe-type can greatly affect the test sensitivity [2].

All the above EBV-focused reports did not include a single case of thyroid LCH. Therefore, we performed a virology workup in our case, which eventually showed positivity on both EBER ISH and EBV PCR analysis. In our previous experience with detection of EBV by EBER in thyroid diseases, such as Hashimoto's thyroiditis, Graves's disease, and papillary thyroid carcinoma [17], we have never seen such a profound accumulation of EBER-positive cells in a lesion. Furthermore, qPCR found a significant copy number difference ($p=0.022$) between LCH and surrounding benign thyroid tissue. Since the EBER ISH double staining with IHC was not available in our setting, we can neither support nor question the above-discussed hypotheses on the exact role of EBV infection in our case. Further studies employing double staining to identify the type of infected cells in LCH would be beneficial. With respect to other viruses, which were studied in LCH but revealed no significant association with the disease, such as HHV-6 [3], CMV [1], and HSV [21], our case also showed negative results.

Identifying an oncogenic *BRAF* mutation in more than half of all LCH cases served as a major advance in the understanding of LCH pathogenesis in 2010 [5]. Our case showed that EBV and *BRAF*^{V600E} were mutually exclusive, consistently with another report on pituitary-hypothalamic LCH [22]. However, other case reports produced conflicting results on the association between EBV and *BRAF* in LCH, such as *BRAF*+/EBV+ [23] or *BRAF*-/EBV- [24]. Therefore, at the moment, there is not enough evidence to either prove or disprove a mutually exclusive *BRAF* and EBV in LCH. This should be addressed in future studies including a sufficient number of cases.

LCH treatment is determined by the extent and location of lesions. Clinical behavior is extremely variable. Patients with liver, bone marrow, and/or spleen involvement are at the highest risk for mortality. In the most recent Histiocyte Society trial, LCHIII, 5-year overall survival for patients with high-risk organ disease was 84%, compared to 99% for patients without high-risk organ disease [6]. Our patient remains disease-free after last course of chemotherapy 7 years ago.

In conclusion, thyroid involvement of LCH is unusual and may pose diagnostic challenges. EBV infection associated with LCH has been previously reported but never in the thyroid gland. The presented case showed thyroid involvement of LCH with EBV infection, proved by EBER and qPCR. Further studies are needed to investigate a possible etiologic link between EBV infection and LCH.

Author Contributions AB conceived the study design. PV and CP performed molecular analysis. SK performed immunohistochemical study and provided expert opinion. JM and AB performed slides review, literature search and drafted the manuscript. All authors approved the final version of the manuscript.

Funding The authors received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical Approval Ethical approval was obtained from the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB No. 512/63).

Informed Consent Appropriate written informed consent was obtained from the patient for publication of this case report and accompanying images.

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