Acta Biomed 2021; Vol. 92, Supplement 1: e2021135 DOI: 10.23750/abm.v92iS1.10204

Case report

Pediatric cerebral stroke induced by Epstein-Barr virus infection: role of interleukin overexpression

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Abstract. *Introduction:* Ischemic stroke is an important cause of death and long-term morbidity in children. Viral respiratory infections are emerging as important risk factors for ischemic stroke in this age group of patients. Direct action of virus against cerebral vessels, autoimmune reactivity, and an increased production of cytokines have all been advocated as main factors in determining ischemic stroke. *Case Report:* We report the case of an EBV-induced ischemic stroke in a caucasian 2-year-old female. The cerebrospinal fluid samples showed positivity of polymerase chain reaction for EBV infection, also confirmed by pharyngeal swab culture. Increased levels of interleukin 6 and interleukin 1β were also detected in the cerebrospinal fluid. *Discussion:* EBV infection has been identified as an important cause of neurological involvement in children. Findings of increased levels of interleukin 6 and interleukin 1β in the cerebrospinal fluid of the child with EBV induced-ischemic stroke seems to validate the role of pro-inflammatory cytokines as crucial mediators of cerebral thrombus formation. *Conclusions:* We believe that this report can be useful to clarify some pathophysiological mechanisms related to ischemic stroke related to Epstein-Barr Virus (EBV) infection in children. (www.actabiomedica.it)

Key words: Epstein-Barr virus, children, interleukins, cerebral stroke, personalized medicine

Introduction

Ischemic stroke is among the top 10 causes of death in children and an important cause of long-term morbidity (1). Focal neurological deficits, such as hemiplegia, are the most common signs in children, but headaches, impaired speech, and seizures have also been described (1). About one half of ischemic strokes occur in children with no risk factors, albeit it can be associated with a variety of conditions, such as congenital heart diseases, hematologic and vascular disorders, and infections (1). Over the last years, several reports of viral respiratory infections have been emerging as important risk factors, such as EBV infection (2). EBV virus infection was pinned down as a cause of respiratory infections, ranging from self-limited to severe illness both in adults and children. A small percentage of children may develop more severe symptoms, such as pneumonia, hepatotoxicity and neurological involvement. Several hypotheses were planned in order to explain the particular virulence of EBV, including down-regulation of type 1 interferon expression, apoptosis, and hyper-induction of pro-inflammatory cytokines, such as previously reported in some bacterial infections due to Gram-negatives strains (3). Increased production of pro-inflammatory

cytokines has also been observed in experimental models of brain ischemia, as well as in patients with acute ischemic stroke (4). Cytokines, such as interleukin-6 (IL-6) and interleukin-1β (IL-1β), appear to be crucial mediators of this type of responses. IL-6 levels rise in both serum and cerebrospinal fluid (CSF) after ischemic stroke and elevated IL-6 expression has been associated with greater stroke severity and earlier neurological worsening. IL-1β stimulates the synthesis of tissue factor (TF) by monocytes and endothelial cells, thus activating the coagulation cascade toward thrombus formation. According to the roles of pro-inflammatory cytokines in the formation of ischemic stroke, we decided to evaluate the CSF levels of IL-6 and IL-1β in a child with EBV infection to clarify the possible correlations between these molecular markers and cerebral ischemia. Only a few cases of EBV induced-ischemic stroke and neurological involvement have been reported in literature. We also performed a review of the literature of all pediatric cases showing neurological involvement related to this condition.

Case Report

A 2 years old caucasian female was admitted to the emergency room of our Hospital because of acute onset, left hemiparesis and persistent weakness. Five days earlier she had developed an angina, dry cough associated to high fever (39.5 C°). The clinical examination showed an awake and nervous child with an increased heartbeat and respiratory frequency, weakness of left face, arm, and leg with hypertonia (left spastic hemiparesis) and impaired speech. Upon admission, blood examinations showed leukocyte count (2820/mm^3). C Reactive Protein, electrocardiogram, chest X-Ray, and computed tomography of the brain without contrast, were all normal. Electroencephalogram (EEG) revealed moderate slow anomalies in the right cerebral hemisphere. Early lumbar puncture highlighted an increased dropflow speed. The CSF samples were normal, but virological examinations showed the positivity of polymerase chain reaction (PCR) for EBV infection, along with the presence of EBV RNA copies (> 800 copies/mL). Pharyngeal swab culture confirmed an infection of EBV, which was identified through PCR. Moreover, in order to measure interleukin levels, CSF samples were centrifuged for 10 min at 5,000 rpm and the supernatants were immediately stored at −70 °C up until their analysis. As controls, we used CSF samples collected from 5 children, age related to the EBV patient, with a diagnosis of head injury (2 patients) and viral meningoencephalitis (3 patients), who had had an ischemic stroke as a complication of their condition. IL-1β and IL-6 were measured using commercial immunoenzymatic kits (Human Quantikine by R&D Systems). In the EBV patient, we found significantly ($p<0.001$) higher levels of IL-6 (135.5 pg/mL) and IL-1β (20.2 pg/mL) compared to the controls (Fig. 1-2). After the results of the lumbar puncture, a supportive treatment was started in the child with EBV. Two days after the admission, a first magnetic resonance imaging (MRI) revealed a massive ischemic area in the right parietotemporal region, along with lateral ventricular compression (Fig. 3a). Magnetic resonance angiography highlighted a focal occlusion in the origin of the right middle cerebral artery (MCA), with a lack of terminal arteries representation right after the trifurcation (Fig. 3b). Treatment with mannitol and aspirin was started. Biochemical examinations, homocysteine, coagulation tests and auto-immunity screenings (ANA, anti-ds DNA, anti-ANCA) were negative. Screenings for thrombophilia showed normal values of Protein C (73% with normal range 70-140%) and Protein S (62% with normal range 54-110%) activity. Factor VIII was 140% (normal range 70-140%). Factor V-Leiden, PT mutation G20210, Lupus Lyke Anti-Coagulant, anti-cardiolipin antibodies, anti-protrombin antibodies, and anti $\beta_2$glicoprotein antibodies were negative. Transthoracic echocardiogram and doppler sonography of cervical vessels were also negative. Microbiological serology for Varicella zoster virus (VZV), Cytomegalovirus (CMV), H1N1, Herpes simplex virus (HSV), Mycoplasma, Borrelia, Rickettsie, Coxsackie, Influenza B, Parainfluenza 1,2,3, Echovirus, Respiratory syncytial virus (RSV), and Adenovirus were either negative, or showed a previous contact. Eighteen days after, a follow-up MRI highlighted an evolution towards a sub-acute phase and showed a focal ischemic area in the cerebral peduncle that was missing

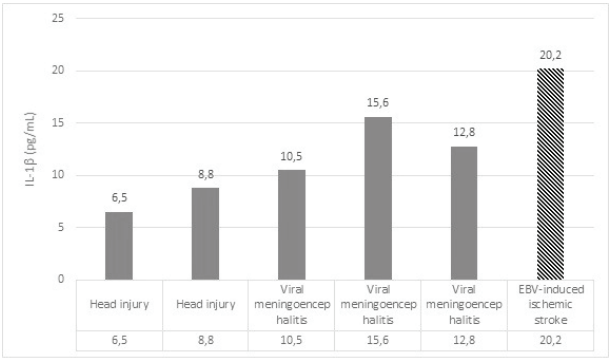

Figure 1. IL-1β CSF levels in EBV-induced ischemic stroke and in controls (CSF: cerebrospinal fluid).

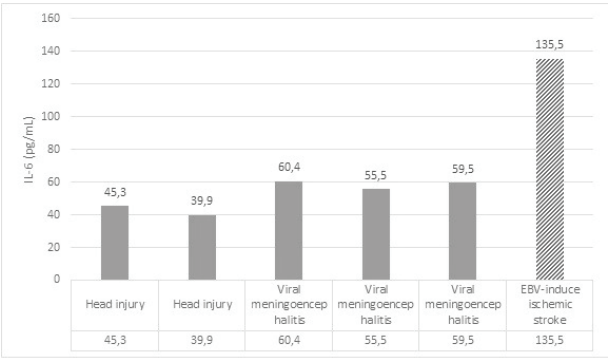

Figure 2. IL-6 CSF levels in EBV-induced ischemic stroke and in controls (CSF: cerebrospinal fluid)

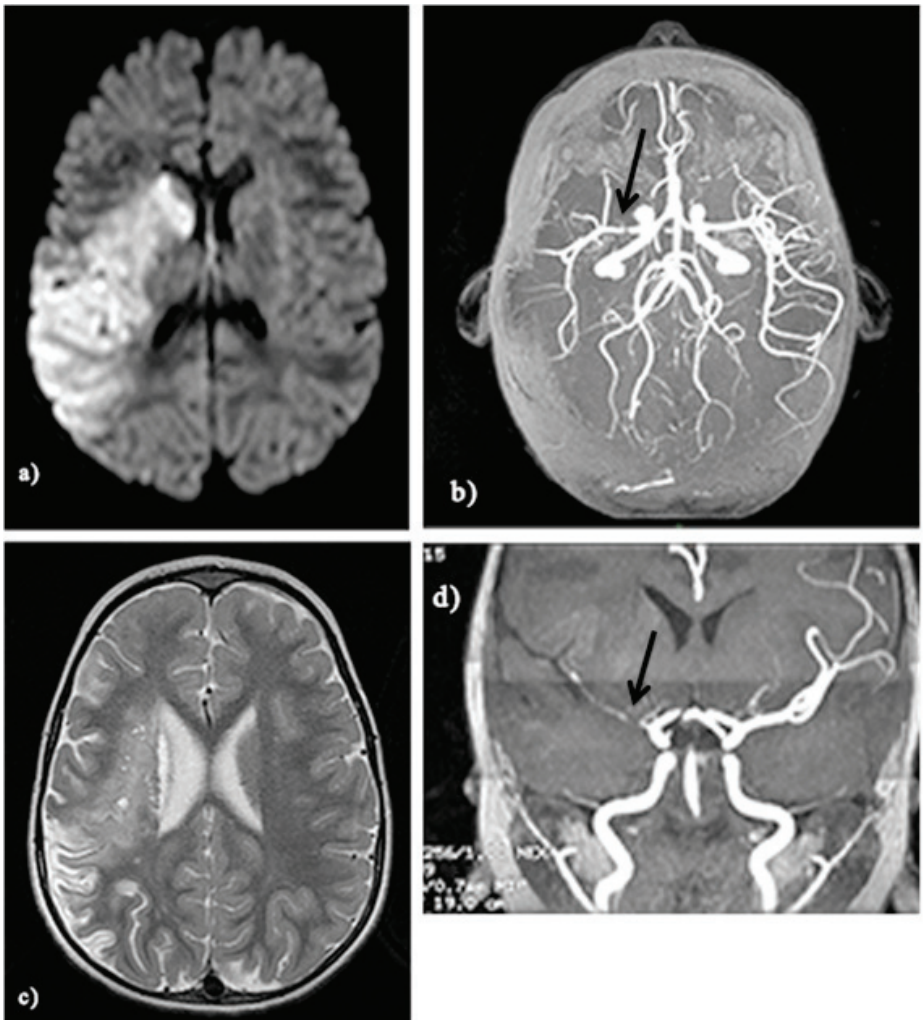

Figure 3. MRI evolution. a) Massive ischemic area in the right parietotemporal region along with lateral ventricular compression; b) Magnetic resonance angiography highlighted focal occlusion in the origin of the right middle cerebral artery, see black arrow; c) The checking MRI highlighted an evolution to a sub-acute phase; d) Angiography confirmed the focal occlusion of the right MCA origin and the lack of terminal vessels (see black arrow). MRI: magnetic resonance imaging; MCA: middle cerebral artery.

in the previous MRI (Fig. 3c). A magnetic resonance angiography confirmed the focal occlusion of the right MCA origin and the lack of terminal arteries (Fig.3d). Based on her clinical improvement, the patient was discharged after 23 days from the admission with a recommendation to continue the therapy with acetylsalicylic acid. One month later, Anticardiolipin antibodies, anti $\beta_2$glicoprotein antibodies, antinuclear antibodies, anti-ANCA antibodies, IgG and IgM vs Borrelia burgdorferi, protein C and S activities, and coagulation tests results were in the normal range. A follow up EEG showed no changes. After 4 months from discharge, the brain MRI did not highlight any lesion evolution, while the left arm movement impairment persisted, along with a slight limp. The child's speech was normal.

Discussion

To the best of our knowledge, only a few cases of EBV induced-ischemic stroke and neurological involvement have been reported in literature (Table 1). In these patients, a direct action of EBV infection on cerebral vessels, autoimmune reactivity, or increased production of cytokines were hypothesized (5-9). Different reports highlighted the role of cytokines in inflammatory injury of cerebral vessels in patients with ischemic stroke and virus infection, also demonstrated by infecting a mouse model with EBV infection (10). It is still unclear whether cytokine-related inflammation is a causal mechanism or a secondary phenomenon induced by the ischemic process. EBV infection causes the activation of the host's macrophages and lymphocytes, determining the release of pro-inflammatory cytokines (3). Cytokine upregulation may cause epithelial cell damage by increasing the production of nitric oxide synthase and cyclo-oxygenase, and by promoting the release of excitatory amino acids and neurotrophins, in both experimental and clinical models of cerebral ischemia and viral meningoencephalitis (7). In particular, IL-1β and IL-6 have been identified as specific markers of neuro-inflammation mechanisms in different models of acute brain injury (10). IL-1β is a major link between inflammation and coagulation; in fact, it is able to stimulate the synthesis of TF from monocytes and endothelial cells. On the other hand, IL-6 contributes to post-ischemic inflammation via the release of adhesins and matrix metalloproteinases, responsible

Table 1. Pediatric cases of EBV-related neurological involvement and cerebral ischemic stroke.

REF.	Year	Sex	Age	Symptoms	Coagulation tests	Familiarity	Nasal-Pharyngeal Swab Culture	MRI Findings
5	2014	M	4 y	Dry cough, rhinorrhea, fever, myalgia, chills, weakness, impaired speech	Unrevealing	Negative	PCR positive for EB Virus	Sub-acute infarct of the left middle cerebral artery. Angiography: focal partial occlusion of left middle cerebral artery origin
8	2014	M	9 y	Seizure, apathy, drowsiness, hemiplegia	Normal	Negative	PCR positive for EBV	Hyperintensities with restricted diffusion at the top of right frontal lobe. Angiography: normal
9	2010	F	12 y	Fever, seizures, impaired speech	Normal	Negative	PCR positive for EBV	Acute necrotizing encephalopathy
6	2010	F	3 y	Fever, focal convulsion, loss of consciousness	Normal	Negative	PCR positive for EBV	Bilateral perirolandic hyperintensity with restricted diffusion and hyperintense lesions in both thalami

MRI: magnetic resonance imaging; PCR: polymerase chain reaction; REF: references.

for leukocyte adhesion and contributing, along with IL-1β, to the activation of the coagulation cascade toward thrombus formation (4). Our results confirmed experimental and clinical findings, since this child with EBV-induced ischemic stroke showed an earlier and more intensive production of IL-1β and IL-6 in the CSF compared to controls, thus suggesting that this upregulation exerts a key role in the development of neuro-inflammation mechanisms responsible for ischemic stroke and pain related to this condition (11).

Conclusions

Although this study does not provide enough evidence to support a definitive statement regarding the cause of the CSF cytokine increase measured in this patient with EBV virus infection, we believe that this report could help demonstrate that cerebral damage is, at least partially, cytokine mediated, thus helping clarify some pathophysiological mechanisms related to ischemic stroke in EBV-infected patients.

Conflicts of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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Arrived: 11 July 2020
Accepted: 9 October 2020
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