

Epstein - Barr virus Infection Documented by Lymphocyte Study after Nasal Scraping: Case Report

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Abstract: The authors describe a case of Epstein Barr virus infection (EBV) in a five-year-old girl. In the field of otorhinolaryngology diagnostics, and in the strong suspicion of a viral disease, the study of the nasal mucosa has highlighted the presence of atypical lymphocyte cells. This observation led the authors to anti-EBV antibody research and a blood count. These investigations confirmed the diagnostic suspicion. In conclusion, the optical microscope study of the nasal mucosa, can be considered a valid tool not only for the early diagnosis of viral diseases in general, but also a means to monitor over time the course of the disease and the possible consequences.

Key words: Epstein Barr virus, Pappenheim method, nasal cytology, mononucleosis

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I. Introduction

The Epstein-Barr virus (EBV) is a Herpesvirus belonging to the subfamily Gammaherpesvirinae and is the etiological agent of infectious mononucleosis and a variety of neoplastic syndromes. EBV is ubiquitous in the world population and, like other Herpesviruses, its infection remains latent for the rest of life. The main EBV receptor is also receptor for the C3d component of the complement system. The effects of the virus, caused at the nasal level, are documented by diffuse epithelial suffering. The cytological study of a nasal scraping sample reveals numerous groups containing columnar cells with cytomegaly, prominent multinucleation, shortly marked, shortened cilia; the cytoplasm, in these cells, contains an acidophilic area and a small discolored round area; the cells have a large acidophilic intracytoplasmic vacuole and Periodic Acid-Schiff stain positive (PAS+)¹. Furthermore, the pathophysiological behavior of this virus at the lymphocyte level is characterized by its entry into B lymphocytes, where the virus binds to the major histocompatibility complex of class II. Once infected, lymphocytes have peculiar characteristics².

II. Case report

A five-year child with a low-grade fever and pharyngodynia from a few days. The objective examination conducted by rhinoscopy with a pediatric rheofibroscope has documented a pale and slightly hypertrophic nasal mucosa. The adenoid vegetations were evaluated by 3rd degree. Oropharyngoscopy revealed oral hyperemia and exudative tonsillitis. Otoscopy presented a medium catarrhal otitis, bilaterally. Palpation of the lymph nodes of the neck has revealed marked lymphadenopathy. The objective picture was compatible with a disease of viral origin. To better understand the inflammatory nature of the nasal mucosa, a cytological study was performed, to highlight the presence of any cells indicating a viral pathology^{3, 4, 5}. The scraping was carried out, by crawling 2-3 times a rhinosonde (Nasal-scraping) ® on the mucosal surface of the central area of the inferior turbinate of both nostrils.

III. Method

The nasal mucosal cells were placed on an electrostatically charged cytology slide (Super Frost Plus Menzel – Gläser, Thermo Scientific, Milan, Italy). The cells were then stained according to the panoptic Pappenheim method (3 min in pure May-Grunwald dye [Carlo Erba, Milan, Italy], 6 min. in 50% May-Grunwald dye; 1 min. in bidistilled water [Carlo Erba, Milan, Italy]; and 30 min. in Giemsa solution [Carlo Erba, Milan,

Italy] diluted 1:10 v/v). The slide was then covered with a # 1 cover glass with dimensions of 24 x 50 mm and observed under an optical microscope (Nikon Eclipse 50i) at 100 x enlargement in oil-immersion.

The observation under the microscope has documented, in addition to the cytological characteristics related to viral infections, the presence of atypical lymphocytes (Downey cells), compatible with EBV infection. They were characterized by a more basophilic and vacuolated cytoplasm than normal lymphocytes and the oval, reniform and lobulated nucleus. Figs (1,2,3)

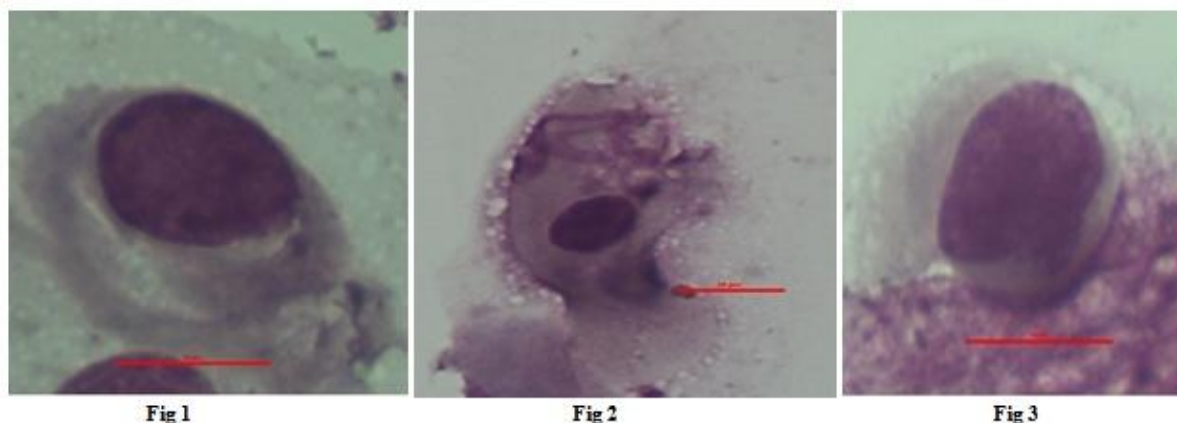


Fig 1

Fig 2

Fig 3

Fig 1 Atypical lymphocyte in an apoptotic form

Fig 2 Pleomorphic cells with abundant cytoplasm. Larger cells present a moderately basophilic vacuolated cytoplasm and lobulated nucleus with slightly scalloped edges containing a nucleolus

Fig 3 Atypical lymphocyte with a cytoplasm strongly basophilic and vacuolated; the nucleus appears oval and the scalloped edge

Subsequently, a search was performed for EBV antibodies that have documented the presence of VCA Ab-IgM equal to 10.6 (pos> 1.2) and VCA Ab-Ig G 1.1 (pos> 1.2). The leukocyte formula showed the presence of neutrophils at 29.9% (vn 43.0-65.0), Lymphocytes at 56.8% (vn20.45-45.5) and monocytes at 11.3% (vn 0, 0-12). These investigations confirmed EBV infection, hypothesized under an optical microscope, observing the lymphocytes.

IV. Discussion

EBV, whose incubation period can last up to two months, has adapted to human lymphocytes and is able to manipulate and use the different phases of B cells to establish an infection. EBV diseases derive from an overactive immune response (infectious mononucleosis), or from the lack of an effective immune control (lymphoproliferative pathologies and hairy cells leukoplakia). The respiratory system is the main pathway of access to the pathogen by these viral agents. Many begin to replicate in the nasal mucosa, sometimes without causing significant clinical manifestations, but tend to produce systemic symptoms. Most viruses (rhinovirus, coronavirus, syncytial respiratory virus [RSV], adenovirus, parainfluenza virus) often cause benign respiratory disease, while others such as EBV, coxsackie and cytomegalovirus produce much more serious diseases.

An important agent among the latter is EBV that causes infectious mononucleosis, which generally affects adolescents and young adults and leads to serious pathological syndromes such as lymphoproliferative syndrome, B-cell lymphoma, Burkitt's lymphoma and nasopharyngeal carcinoma (NPC)^{6,7,8,9}. Although NPC is relatively rare in Europe (1 case per 100,000 population), the disease remains a diagnostic challenge because it is diagnosed late in the course of the disease, when the primary tumor has already occurred on sites (laterocervical or retroangulomandibular metastases) and/or loco-regional pathologies (recurrent tubotympanitis, chronic catarrhal otitis, etc.)^{10, 11,12}

V. Conclusion

The use of an optical microscope can be a valid tool for the early detection of infectious diseases, such as the EBV one. Since it is known that the virus can be responsible for lymphoproliferative diseases and nasopharyngeal carcinomas, this method, in addition to allowing a precision analysis, allows to constantly monitor the health status of young people through targeted screening. Some rare diseases can be diagnosed faster by a critical revision of lymphocytes, in particular the recognition of abnormal lymphocytes should not be underestimated. Their morphology is complex and depends on the cell size, the quantity and color of the cytoplasm, the shape and the structure of the nucleus chromatin, characteristics that must be carefully observed to achieve a good characterization of the lymphocyte¹³. It is very difficult to identify the abnormal lymphocytes

accurately, because there are different classifications¹⁴, but we consider it important that any difference found in normal lymphocytes should make suspicious the presence of infectious or lymphoproliferative diseases. Therefore a comparison with systemic haematological studies is always necessary.

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