Science, Engineering & Education,8, (1), 2023, 29-35

The Influence of SARS-COV-2 Viral Infection on the Serological Level of IgM Antibodies in Patients with Infectious Mononucleosis

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Received 20 September 2023, Accepted 16 October 2023

ABSTRACT

Infectious mononucleosis (IM) is a disease caused by the Epstein-Barr virus (EBV), a member of the herpes virus family, which usually occurs in older children, adolescents, and young adults between the ages of 15 and 25. In young adults, infectious mononucleosis often causes fever, cough, pharyngitis and malaise, and lymphadenopathy. The similar symptoms of COVID-19 and EBV-induced IM suggest a possible association. The aim of this study is to investigate the possibility of coinfection with Epstein-Barr virus in COVID-19 patients. 80 serum samples from IM patients from January 2020 to December 2022 are included and analyzed by enzyme-linked immunosorbent assay (ELISA) for diagnosis and determination of EBV-VCA IgM stage. Patients were classified into an EBV/SARS-CoV-2 coinfection group based on serologic results. A high frequency of reinfection and coinfection with EBV was found in patients with COVID-19 (23.42 % - 34.58 %). C-reactive protein (CRP) and aspartate aminotransferase (AST) results were higher in EBV SARS-CoV-2 coinfection during disease. EBV coinfection may be associated with disease severity at COVID -19.

Keywords: Infectious mononucleosis, COVID-19, EBV IgM stage.

INTRODUCTION

Infectious mononucleosis is an acute viral disease caused by Epstein-Barr virus (HHV4) and cytomegalovirus (CMV, HHV5), which belong to the group of herpes viruses (Herpesviridae) and are transmitted by infectious droplets. The infection usually occurs in older children as well as in adolescents and young adults, but most often in young people between 15 and 25 years of age. It persists in the body throughout life in association with fibromyalgia syndrome and nasopharyngeal syndrome.

The main symptoms of chronic EBV infection are fever, reactive adenopathy, and polyneuropathy. After an incubation period of 10 - 14 days to several weeks, similar symptoms with SARS–COV-2 appear: fever, sore throat, cervical lymphadenitis, exudative pharyngotonsillitis and hepatosplenomegaly, monocytosis with atypical lymphocytosis and jaundice or eczema, and correct diagnosis of IM is very important. EBV produces persistent infection

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with periodic reactivation. The disease may manifest with clinically imperceptible symptoms to severe forms such as malignant tumors [1]. Mononucleosis syndrome is primarily defined cytohematologically and combines two white line disorders in the peripheral blood: a constant increase of mononucleocytes by 50 - 80 % or more than 800 mononucleocytes in 1 mm^3 and the appearance of atypical large mononuclear cells (15 - 25 μm) with hyperbasophilic cytoplasm, and oval and bulky nucleus. They activated T lymphocytes that have come into contact with an antigen, virus, bacteria, parasites and drugs. They are also called hyperbasophilic mononucleocytes or more commonly atypical lymphocytes, which are not normally present in circulating blood. Their number is variable and depends on the pathogen. The erythrocyte and platelet lines are unchanged [2]. According to the literature, IM occurs sporadically, in rare cases epidemically in spring and autumn. After infection, whether manifest or inconspicuous, the virus remains in the oropharynx for a long time and is isolated as a virus carrier via saliva [3, 4]. Solid immunity is acquired after recovery. However, Sacco found that a certain number of B lymphocytes persist throughout life, which means that the latent infection persists [5]. In addition, Sallman and colleagues concluded that EBV/CAEBV (chronic active Epstein-Barr virus) is a rare syndrome characterized by permanent symptoms as well as mononucleosis symptoms and increased peripheral blood load EBV DNA in immunocompetent individuals [6]. Sacco and coworkers concluded that the virus can also spread via the blood, through blood transfusions and organ transplants. Infected epithelial cells have also been found in the cervix or semen, suggesting the possibility of EBV spread through sexual contact. Kissing, sharing personal items such as toothbrushes, eating and drinking with an infected person can lead to the spread of EBV [7]. Based on individual studies and the resulting estimates at the national level in the United States, significant socioeconomic differences in EBV seroprevalence have been found in all age groups [8, 9].

Recently, some studies found that COVID-19 patients were positive for EBV-VCA IgM antibodies and EBV reactivation might be related to the severity of COVID-19 [10, 11]. However, high levels of inflammation were not observed. EBV and HHV6 reactivation may contribute to some features of acute disease as a predisposition to post-acute sequelae [12].

COVID-19 is a multistage disease that begins with viral replication and leads to disruption of the immune system response, organ failure, and recovery or death. The similar symptoms of COVID-19 and EBV induced IM suggest a possible relationship, so we hypothesized that EBV co-infection may be present in COVID-19 patients.

The primary diagnosis of EBV virus is important to eliminate other infectious diseases with similar symptoms. The choice of diagnostic methods depends on the evolution of patient demographic characteristics in combination with the status of laboratory analysis.

A practical way to provide laboratory evidence is the heterophilic antibody test, used as a standard procedure for clinical diagnosis [13]. The tests are used to detect IgM antibodies that rise during generalized immune regulation associated with acute primary EBV infection. The sensitivity of the test is 85 % and the specificity is 100 %. The results of the antibody test may be negative at the beginning of the infection, but positivity increases during the first 6 weeks of infection [14].

Serologic tests rely on the detection of EBV antibodies in a serum sample from an infected patient. Although serological tests have a high degree of variability in EBV diagnosis, they are still preferred and most commonly used for comparison with other tests providing reasonable criteria for determining the patient's level of infection [7, 15].

The most useful tests for detecting antibodies are VCA IgG, VCA IgM, and EBNA-1 IgG, which are usually measured using the enzyme immunoassay method. VCA IgM antibodies were present in 75 % of patients in the acute phase of the disease. All patients with infectious mononucleosis develop VCA-IgG antibodies, making this the best test to detect prior EBV infection. Antibodies to EBNA-1 develop slowly and usually cannot be detected until 90 days or more after disease began. For this reason, the presence of EBNA-1 antibodies during the acute phase of disease indicates acute primary EBV infection. Worldwide, EBV infection can be classified into multiple stages by measuring VCA IgG, VCA IgM, and EBNA-1 IgG antibodies in serum samples (Table 1) [16].

In this work, the possibility of coinfection with Epstein-Barr virus in patients with confirmed SARS-Cov-2 coronavirus was investigated by determining EBV antibodies in serum samples. Analysis of IgM in serum as the first immunoglobulin class produced in a primary response to viruses was important for monitoring viral activity over time.

EXPERIMENTAL

Study design

Between January 2020 and December 2022, EBV IgM analysis was performed in four groups of patients with COVID-19, men > 25 years, men < 25 years, women > 25 years, women < 25 years. High liver enzyme levels (AST, ALT), white blood cell monocytes and C-reactive protein (CRP) were characteristic of the patients included in this study. Exclusion criteria included: patients with COVID-19 vaccination, positive history of allergic reaction, clinically significant immunodeficiency states, significant liver or kidney disease, patients with active malignant disease, pregnancy or lactation in female patients.

The study was conducted in a private medical laboratory Synlab.

EBV antibodies determination

Detecting and diagnosing EBV antibodies in a serum sample from patients, and also determination of the statistical level of infection rate was performed using the enzyme-linked immunosorbent assay (ELISA MR – 96A / MINDRAY), based on the principle of antibody-antigen binding. The reference value for the diagnosis of infectious mononucleosis by this method is in the following ranges: < 20 U mL^{-1} negative; 20 - 40 U mL^{-1} borderline; > 40 U mL^{-1} positive (Table 2).

Procedure

Human serum from the patient's venous blood is used for the application of the ELISA method. The method is applicable for 5 days after sample collection if the serum is stored in

Table 1. Stages of EBV infection with antibody results by enzyme immunoassay ELISA method [15].

Stages of infection	Time of disease onset	VCA IgM	VCA IgG	EBNA 1 IgG
EBV naive	-	negative	negative	negative
Acute primary infection	0 - 3 weeks	positive	positive or negative	negative
Subcutaneous infection	4 weeks - 3 months	positive	positive	negative
Convalescent infection	4 - 6 months	negative or positive	positive	negative or positive
Past infection	> 6 months	negative	positive	positive

Table 2. Epstein-Barr virus, reference values and working methods.

		Sample	Reference value:	Unit	Method
EBVME	EBV-VCA-IgM abs>> Addition virus capsid IgM antigen(VCA)	Serum 1 mL	<20 negative 20-4 border line >40 positive	U mL^{-1}	LIA

the refrigerator at a temperature of 2 - 8 °C. On the other hand, it can be stored in the freezer at a temperature of (-70°C – -20°C) if the material is kept longer for preparation. Once the stored material is thawed, it should not be refrozen. It must be thawed and stirred well before use. Heat inactivation of the sample is not recommended. The ELISA method involves several sequential procedures to prepare the material. In the first step, the patient's serum sample is diluted in an already prepared diluent in a ratio (3:1) and incubated in a thermostat at a temperature of 37°C for about 1 hour. Then diluent is added in the second step and the color of the reaction with the antibodies present in the wells becomes blue again. After the third washing step in the wells, 3,3',5,5'-trimethylbenzene (TMB) is added. The sample is incubated with this solution in the wells for an additional 15 minutes in the dark and dried at room temperature. In the final step, the wells are not washed, and the reagent STOP is added, which turns the solution yellow and stops the reaction. The plate with the wells is placed in an ELISA reader, which measures the absorbance at 450/620 nm and gives the final result of the sample.

RESULTS AND DISCUSSION

The clinical presentation of infectious mononucleosis can vary, but in the young population EBV disease is often subclinical [18] and may begin suddenly with high fever, diarrhea, sore throat, and tonsillitis [5]. According to Mohsheni and coworkers, EBV can cause atypical clinical features affecting different organ systems [19]. In the studies by Kimura et al., infection with EBV (symptomatic or asymptomatic) was associated with many neoplastic autoimmune diseases. After symptomatic EBV infection, the disease history of infectious mononucleosis is a strong risk factor for the occurrence of Hodgkin's lymphoma, nasopharyngeal carcinoma, Burkett's lymphoma, and multiple sclerosis, but according to Balfour and Dunmire, the reason for the association between these diseases and primary symptomatic EBV infection is not well understood [13]. In another study by Henry et al., the association of Hodgkin`s lymphoma with EBV is explained by immunohistopathological examination of a patient diagnosed with multiple sclerosis [19]. In countries such as Equatorial Africa and New Guinea with holoendemic malaria, 100 % of those tested were EBV positive, which was classified as endemic Burkitt's lymphoma, an abdominal tumor in children. Gero-Ramos and coworkers simulated chronic immunity to pathogens and an increase in the incidence of Burkitt's lymphoma [20]. Nasopharyngeal carcinoma, a malignant disease of the epithelial cells of the nasopharynx that is common in China, probably plays an important role in the development of mononucleosis along with genetic factors. According to Shygoshi et al., EBV/DNA, EBVNA1, and LMP1 were detected in the isolated tumor cells, and high levels of EBV antibodies were also detected [21].

According to literature data [20], the association of infectious glandular fever with other diseases is less likely in European countries. To obtain valuable information about the association of EBV with patients infected with SARS-CoV-2, and to determine the source of infection and find more truth about the unexplained pneumonia, reliable and rapid serological diagnostic method

was used. Serological tests and seroconversion reactions for EBV IgM antibodies were performed in this study to screen Epstein-Barr virus in COVID-19 patients for coinfection.

From January 2020 to December 2022, 330 patients infected with SARS-Cov-2 were enrolled in this study, including 107 patients in 2020, 112 patients in 2021, and 111 patients in 2022 with infectious mononucleosis history or symptoms. There was small difference between the EBV seropositive patients of the two sexes during the study period, and the ratio of males to females was 15.45 % and 12.73 %, respectively. The development rate of EBV antibody detection in patients by age and gender was 2 - 5 years: 4.2 %; 5 - 10 years: 1.5 %; 10 - 15 years: 2.7 %; 16 - 25 years: 3.0 % and > 25 years 3.3 % in males, and female: 2 - 5 years: 2.7 %; 5 - 10 years: 1.5 %; 10 - 15 years: 1.5 %; 16 - 25 years: 4.2 % and > 25 years: 2.7 %. According to the statistical data presented in Table 2, the number of EBV-infected patients decreases slightly in the third year after the onset of the pandemic at COVID-19, mainly due to the lower number of EBV-positive patients over 16 years of age. In addition, the percentage of total seropositive EBV patients in the age range 16 - 25 years is higher, in which a decrease in acquired immunity or the influence of certain viruses could influence reinfection and cause seropositivity to increase again. Trends in total and individual EBV seropositive infections are shown in Table 3 and Fig. 1, with patients grouped by age and gender for each year from 2020 to 2022.

Fig. 1. Detection and presentation of EBV antibodies by age and sex in COVID-19 patients from January 2020 to December 2022.

	Total (n = 330)	2020 (n = 107)	2021 (n = 112)	2022 (n = 111)
	Characteristic n (%)			
Age				
2-5 years	23 (6.97 %)	8 (7.48 %)	8 (7.14 %)	7 (6.30 %)
5-10 years	10 (3.03 %)	1 (0.93 %)	3 (2.68 %)	6 (5.41 %)
10-15 years	14 (4.24 %)	7 (6.54 %)	1 (0.89 %)	6 (5.41 %)
16-25 years	26 (7.88 %)	12 (11.21 %)	8 (7.14 %)	6 (5.41 %)
>25 years	20 (6.06 %)	9 (8.41 %)	10 (8.93 %)	1 (0.90 %)
Total	93 (28.18 %)	37 (34.58 %)	30 (26.78 %)	26 (23.42 %)
Gender				
Male	51 (15.45 %)	21 (19.6 %)	15 (13.39 %)	15 (13.51 %)
Female	42 (12.73 %)	16 (14.95 %)	15 (13.39 %)	11 (9.91 %)

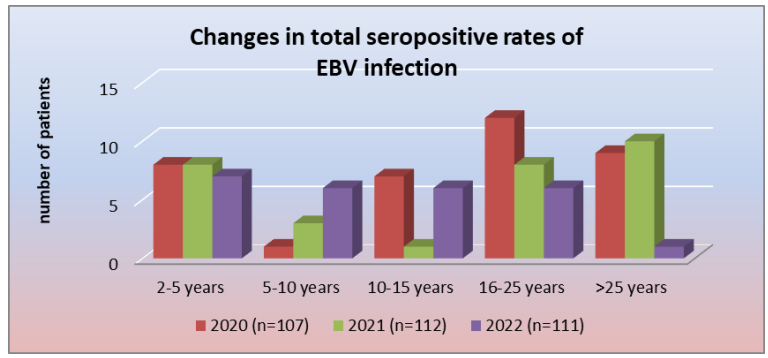

Table 3. Changes in the overall rate of EBV seropositive infections.

The low incidence of acute EBV infection in COVID-19 can be attributed to some factors, such as reduced immunity during the illness. Some studies found that positive rates of various respiratory viruses, atypical pathogens and enteroviruses decreased during COVID-19.

CONCLUSIONS

Patients were classified into the EBV/ SARS-COV-2 coinfection group based on EBV IgM serological results. We found a high frequency of coinfection with EBV in patients with COVID-19. C-reactive protein (CRP) and aspartate aminotransferase (AST) results were higher in EBV SARS-Cov-2 coinfections during illness. EBV coinfection may be associated with COVID -19, which is still under investigation.

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