The effect of Epstein – Barr Virus Viremia on the Progression to Severe COVID-19

Jae Hyung Im

Inha University College of Medicine: Inha University School of Medicine

Chung Hyun Nahm

Inha University College of Medicine: Inha University School of Medicine

Young Soo Je

Seoul clinical laboratories

Jin-Soo Lee

Inha University College of Medicine: Inha University School of Medicine

Hea Yoon Kwon

Inha University College of Medicine: Inha University School of Medicine

Moon-Hyun Chung

Seogwipo Medical Center

Ji-Hun Jang

Inha University College of Medicine: Inha University School of Medicine

Jeong Soo Kim

Inha University College of Medicine: Inha University School of Medicine

Jun Hyeok Lim

Inha University College of Medicine: Inha University School of Medicine

Mi Hwa Park (✉ bami.park@gmail.com)

Inha University Hospital <https://orcid.org/0000-0003-2013-0972>

Ji Hyeon Baek

Inha University College of Medicine: Inha University School of Medicine

Research

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Abstract

Background

Coronavirus disease 2019 (COVID-19) can reactivate several latent viruses. Epstein–Barr virus (EBV) is a latent virus that is frequently reactivated in patients with critical illnesses. Recently, a high incidence of viremia has been reported in patients with severe COVID-19. However, it is unclear whether EBV viremia is the result of the severity of COVID-19 or if it affects the severity of COVID-19. Therefore, we conducted a cohort study to evaluate the effects of EBV on the progression of COVID-19.

Methods

We compared the incidence of EBV viremia between the COVID-19 and non-COVID-19 groups. Simplified Acute Physiology Score (SAPS) II and lymphocyte subsets were analyzed in patients with COVID-19. We also observed that patients with COVID-19 with EBV viremia progressed to severe pneumonia more often than those without EBV viremia.

Results

Two hundred and nine patients with COVID-19 were compared with the control (non-COVID-19) group. The incidence of EBV viremia was lower in the COVID-19 group than in the non-COVID-19 group (17.1% vs. 26.8%, $P=0.034$). In the subgroup analysis of the COVID-19 group, the EBV-positive group patients had more severe COVID-19 infection than the EBV-negative group (SAPS II, 22.3 vs. 17.4%; $P=0.002$). However, progression to moderate or severe pneumonia in patients with mild COVID-19 was rather high in the EBV-negative group (not statistically significant), contrary to our expectations.

Conclusions

Although the severity of COVID-19 may affect EBV viremia, there is no evidence that EBV viremia is a factor that exacerbates pneumonia in patients with early COVID-19. The effect of EBV viremia on prolonged organizing pneumonia should be further studied.

Background

In December 2019, a novel pneumonia was first reported in Wuhan, China [1], and the causative pathogen was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [2]. Subsequently, the Coronavirus disease 2019 (COVID-19) spread worldwide and was detected in almost all countries by March 2020 [3]. COVID-19 is not only rapidly transmitted but also fatal in patients with advanced and underlying diseases, causing a serious disease burden [4].

The effect of SARS-CoV-2 on immune dysfunction and the resultant reactivation of latent viruses are still under investigation. In an Italian study, Epstein–Barr virus (EBV) viremia was observed in 40 of 42 patients with severe COVID-19 and 51 of 61 patients with moderately severe COVID-19 [5]. It was also reported that the patients with severe COVID-19 had higher levels of EBV viremia than those with mild COVID-19. However, the study was cross-sectional and did not reveal a causative relationship between EBV viremia and the severity of

COVID-19. Because of the lack of information regarding the relationship between EBV and COVID-19, we proposed the following two hypotheses and investigated them using case–control and cohort studies: i) in COVID-19, EBV viremia can occur more frequently than other infectious diseases; and ii) EBV viremia affects the disease progression of COVID-19 pneumonia.

Methods

Study population

We conducted real-time polymerase chain reaction (PCR) to detect EBV in adult patients with COVID-19 who were admitted to the Inha University Hospital from February 1, 2020, to January 11, 2021. Inpatients with infectious diseases other than COVID-19 were used as age-matched controls. EBV PCR was routinely performed during hospitalization, and if the test was not performed within 5 days, the patient was excluded from the study. Children under 15 years of age were excluded from the study. Patients with human immunodeficiency virus infection, hematologic malignancies, and immunosuppressants were excluded from the study. The Simplified Acute Physiology Score (SAPS) II was used to compare the severity between the COVID-19 and non-COVID-19 groups.

Subgroup Analysis

COVID-19 severity and lymphocyte subpopulations were investigated to analyze the factors involved in EBV reactivation. The rating of COVID-19 was divided into seven levels, as follows: Grade 1, no pneumonia; Grade 2, pneumonia but no oxygen therapy required; Grade 3, low-flow nasal cannula; Grade 4, high-flow nasal cannula/non-invasive ventilation; Grade 5, mechanical ventilation; Grade 6, extracorporeal membrane oxygenation (ECMO); and Grade 7, death.

To examine whether EBV viremia affects the progression to severe COVID-19, patients with mild COVID-19 at the time of hospitalization (Grades 1–2) were subdivided into groups with and without EBV viremia. The rates of progression of these two groups to severe COVID-19 (Grade 3–7: oxygen therapy group, Grade 4–7: acute respiratory distress syndrome (ARDS) group) were compared.

Covid-19, Ebv Pcr Test, And Lymphocyte Subpopulation Analysis

For diagnosis of COVID-19, the Allplex™ 2019-nCoV Assay kit (Seegene Inc., Seoul, Republic of Korea) was used for PCR of upper or lower respiratory tract secretions. The Real-Q EBV Quantification Kit (BioSewoom, Inc., Seoul, Republic of Korea) was used to detect EBV. The cutoff for EBV viremia was defined as 72 copies/mL, which is the reference value given in the manufacturer's insert.

The lymphocyte subpopulation was analyzed using multicolor flow cytometry (BD FACSCanto™ II, Becton Dickinson, San Jose, USA). Whole blood samples were stained with BD Multitest CD3 FITC/CD8 PE/CD45 PerCP/CD4 APC and BD Multitest CD3 FITC/CD16 + CD56 PE/CD45 PerCP/CD19 (Becton Dickinson, San

Jose, USA) and analyzed according to the manufacturer's instructions. Each lymphocyte subpopulation was presented as an absolute count (cells/ μ L).

Statistical analysis

Student's *t*-test and the chi-square test were used to compare the COVID-19 and control groups. In the subgroup analysis of COVID-19 patients, Fisher's exact test and Mann–Whitney U test were used for comparison. All tests were two-tailed, and a P-value of 0.05, was considered significant.

Ethics statement

This study was approved by the Institutional Review Board of Inha University Hospital, Incheon, Republic of Korea. All patient records were anonymized.

Results

General characteristics of the COVID-19 group

During the study period, 322 adult patients diagnosed with COVID-19 were admitted to our hospital. Fifty-two children were excluded from the study. Fifty-three patients, mainly those admitted to medical departments other than the Division of Infectious Diseases, were not prescribed EBV PCR testing. Eight patients were excluded from the study because EBV PCR was performed 5 days after hospitalization. A total of 209 patients were included in the COVID-19 group. The median duration from hospitalization to examination was 2.3 days. EBV viremia was found in 17.7% of patients with COVID-19, and the highest incidence (31.4%) was found in patients aged 70–79 years (Table 1). The incidence of EBV viremia according to the grade at hospitalization was as follows: 5/54 (9.3%), Grade 1; 21/109 (19.3%), Grade 2; 7/35 (20.0%), Grade 3; 3/10 (30.0%), Grade 4; and 1/1 (100.0%), Grade 6.

Table 1
General characteristics and EBV viremia in CIVOD-19

Age groups (years)	No.	Sex, F (%)	Severity at admission							EBV viremia (%)
			Gr1(%)	Gr2(%)	Gr3(%)	Gr4(%)	Gr5(%)	Gr6(%)	Gr7(%)	
15–29	16	10 (62.5)	15 (93.8)	1 (6.3)						3 (18.8)
30–39	26	21 (80.8)	16 (61.5)	9 (34.6)	1 (3.8)					0 (0.0)
40–49	20	12 (60.0)	8 (40.0)	11 (55.0)		1 (5.0)				1 (5.0)
50–59	30	14 (46.7)	4 (13.3)	18 (60.0)	6 (20.0)	1 (3.3)		1 (3.3)		7 (23.3)
60–69	40	17 (42.5)	4 (10.0)	27 (67.5)	6 (15.0)	3 (7.5)				5 (12.5)
70–79	35	19 (54.3)	2 (5.7)	19 (54.3)	11 (31.4)	3 (8.6)				11 (31.4)
≥ 80	42	30 (71.4)	5 (11.9)	24 (57.1)	11 (26.2)	2 (4.8)				10 (23.8)
Total	209	124 (59.3)	54 (25.8)	109 (52.2)	35 (16.7)	10 (4.8)	0 (0.0)	1 (0.5)	0 (0.0)	37 (17.7)
EBV Epstein–Barr virus, Gr1 Grade 1 = no pneumonia, Gr2 Grade 2 = pneumonia but no oxygen therapy required, Gr3 Grade 3 = low-flow nasal cannula, Gr4 Grade 4 = high-flow nasal cannula/non-invasive ventilation, Gr5 Grade 5 = mechanical ventilation, Gr6 Grade 6 = extracorporeal membrane oxygenation, Gr7 Grade 7 = death.										

Comparison of EBV viremia between the COVID-19 and non-COVID-19 groups

The COVID-19 and non-COVID-19 groups were compared. There were no statistically significant differences in age, sex, or SAPS II between the two groups. The incidence of EBV viremia (17.7%) in the COVID-19 group was lower than that in the non-COVID-19 group (26.8%), and the difference was statistically significant (Table 2).

Table 2
Comparison of EBV viremia between the COVID-19 and control groups

	COVID-19 group	Non-COVID-19 group	P value
No.	209	209	
Age [SD]	60.6 [20.3]	60.1 [SD 19.4]	0.795
Sex, female (%)	124 (59.3%)	116 (55.0%)	0.842
SAPS II [SD]	18.3 [7.8]	19.3 [SD 8.6]	0.191
EBV viremia (%)	37 (17.7%)	56 (26.8%)	0.034*
EBV Epstein–Barr virus, SD standard deviation, SAPS Simplified Acute Physiology Score			

Comparison of severity and lymphocyte subsets between EBV-positive and -negative groups

At the time of hospitalization, 37 patients had ARDS (Grades 4–7), which was 10.8% of the total number of patients in the EBV-positive group, and the EBV-negative group had 4.1%. The average SAPS II score was 22.3 in the EBV-positive group and 17.4 in the EBV-negative group, and the difference was statistically significant. There was no statistically significant difference between the lymphocyte subsets in the EBV-positive and -negative groups (Table 3).

Table 3
Comparison of severity and lymphocyte subsets between EBV-positive and –negative groups

	COVID-19 patients with EBV viremia (N = 37)	COVID-19 patients without EBV viremia (N = 172)	P value
Grade			
1	5 (13.5%)	49 (28.5%)	
2	21 (56.8%)	88 (51.2%)	
3	7 (18.9%)	28 (16.3%)	
4	3 (8.1%)	7 (4.1%)	
5	0 (0.0%)	0 (0.0%)	
6	1 (2.7%)	0 (0.0%)	
SAPS II	22.3 ± 7.5	17.4 ± 7.6	0.002*
Lymphocyte subset, 10 ⁹ /L			
CD3 [IQR]	0.84 [0.57–1.09]	0.67 [0.46–1.04]	0.121
CD4 [IQR]	0.47 [0.35–0.74]	0.41 [0.27–0.61]	0.069
CD8 [IQR]	0.33 [0.16–0.44]	0.28 [0.16–0.42]	0.466
CD19 [IQR]	0.13 [0.08–0.25]	0.11 [0.08–0.18]	0.232
CD56 [IQR]	0.21 [0.11–0.38]	0.18 [0.12–0.28]	0.319
EBV Epstein–Barr virus, IQR interquartile range, SAPS Simplified Acute Physiology Score			

Table 4
Progression during observation period in mild COVID-19 infection (Grade 1–2)

	Number	Oxygen therapy (Grade 3–7)	P-value	ARDS (Grade 4–7)	P-value
EBV-negative group, N (%)	137	17 (12.4%)		5 (3.6%)	
EBV-positive group, N (%)	26	3 (11.5%)	1.000	0 (0.0 %)	1.000
EBV Epstein–Barr virus					

Cohort study for COVID-19 pneumonia progression according to EBV viremia

At the time of hospitalization, 163 people with mild COVID-19 were divided into two groups based on the presence or absence of EBV viremia, and the prognosis of the groups was observed until 2 months or discharge/death. In the EBV-positive group (n = 26), progression to oxygen therapy was seen in 11.5% and ARDS in 0% of cases. In contrast, in the EBV-negative group (n = 137), progression to oxygen therapy was

observed in 12.4%, and ARDS in 3.7% of cases. There was no statistically significant difference in the incidence of oxygen therapy and ARDS between the two groups (P value 1.00 and 1.00, respectively).

Discussion

In patients with severe COVID-19, reactivation of viruses, such as herpes simplex, cytomegalovirus, and EBV occurs, and functional exhaustion of cytotoxic lymphocytes has been suggested as the cause [6, 7]. Since COVID-19 can cause cellular immune dysfunction [8], it can induce reactivation of latent viruses. Several studies have reported a high incidence of reactivated EBV in patients with COVID-19 [5, 9]. In addition, these studies reported that COVID-19 was more severe in patients with EBV viremia. However, these studies were cross-sectional studies; therefore, it is not known whether EBV viremia affects the progression of COVID-19. Because severely ill patients were the main test targets, and no time span over which the test was conducted was provided, the effect of EBV viremia on progression to severe COVID-19 might have been overestimated. However, we performed the PCR test within 2.3 days on average and conducted a cohort study only in patients with mild COVID-19; therefore, there is less possibility of bias for critical illness.

EBV viremia is common, even in severely ill patients with diseases other than COVID-19. Reactivation of EBV is significantly related to the dysfunction of cellular immunity [10]. EBV is latent in nearly 90% of people, which is the highest rate among herpes viruses [11]. SARS-CoV-2 viral infection can cause functional exhaustion of antiviral lymphocytes and their cytopathic effect [12]. Therefore, we expected EBV viremia to be an indicator of immune burnout. However, in this study, there was no difference in lymphocyte subset or severe expression incidence between the EBV-positive and -negative groups.

The possibility that EBV can cause reduced immunity, rather than simply being an indicator, cannot be ruled out. During EBV reactivation, EBV interferes with the activity of NK cells and helper T cells. EBV causes B-cell transformation [13] and produces proteins that primarily impair interferon production during the lytic phase [14]. With this mechanism, EBV infection or reactivation can reduce the defense against infection by other pathogens. Moreover, the pathogenicity of reactivated EBV is still being discussed. One study reported that EBV DNA was detected in the lower respiratory tract of patients with severe respiratory tract infections in which no other pathogen has been detected [15]. However, it is difficult to conclude that EBV has a cytopathic effect in all cases where other pathogens have not been identified, because it is not easy to identify the causative pathogen of pneumonia in many cases. However, some researchers claim that the pathogenicity of reactivated EBV might be the result of a compromised immune system, whereas others claim that it is just an indicator of severe illness [10].

To observe the effects of EBV viremia, we conducted a cohort study to compare the ARDS progression of COVID-19 in the EBV viremia and non-viremia groups at the time of hospital admission. Although the incidence of EBV viremia differed according to COVID-19 severity at admission, there was no higher probability of disease progression in the group with EBV viremia. Further, although the total number of ARDS cases was small, all ARDS cases occurred in the EBV-negative group. The rate of progression to pneumonia requiring oxygen therapy was not different between the EBV viremia group and the other group. Early EBV viremia does not seem to affect COVID-19 prognosis. However, for severe COVID-19, the setting may differ from that in the present study. In severe COVID-19 cases, steroid use is often prolonged. In addition, the

immunity of the host may decrease because of critical illness. In these cases, EBV viremia can persist at high levels. This persistent viremia can reduce immunity for the reasons mentioned above, and this immunocompromised status can become part of a vicious cycle that worsens EBV viremia. Therefore, in severely ill patients, including those undergoing long-term steroid treatment, further studies on EBV viremia may be needed.

This study has several limitations. First, there was no follow-up test for EBV viremia; therefore, it could not be confirmed whether EBV viremia persisted in severely ill patients. Second, the study was conducted with a relatively small number of patients. Additional studies with more patients are needed, and the mechanism of EBV viremia must be determined. Third, a comparison with the non-COVID-19 control group was challenging. In particular, patients with COVID-19 pneumonia are admitted to university hospitals earlier than those with other diseases in South Korea. However, even after accounting for this, there is no evidence that EBV viremia occurs more frequently in patients with COVID-19 than in those with other infectious diseases of similar severity, or that early EBV viremia causes severe COVID-19.

Conclusion

There is no evidence that EBV reactivation occurs more frequently in patients with COVID-19 than in those with other infectious diseases. Moreover, there was no difference in prognoses between the EBV viremia and non-viremia groups. Our results suggest that EBV viremia was more strongly related to the severity of COVID-19 or immunocompromised conditions than to the effect of EBV viremia. Further evaluation is required to determine the effect of prolonged EBV viremia in patients with severe disease or immunocompromised patients on steroids.

Abbreviations

EBV: Epstein–Barr virus; PCR: polymerase chain reaction; ECMO: extracorporeal membrane oxygenation; SAPS: Simplified Acute Physiology Score

Declarations

Ethical approval

This study was approved by the Inha University Hospital Institutional Review Board (Incheon, Republic of Korea), which waived the need for informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used during the current study are available from the corresponding authors upon reasonable request.

Competing interests

All authors declare no conflicts of interest related to this study.

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This study received no external funding.

Author contributions

JHI conceptualized the project and drafted and edited the manuscript; JHJ, JSK, YSJ, JHL, and JHN analyzed and interpreted the data; JSL, JHB, MHC, HYK, and MHP reviewed the writing and editing. The MHP and JHB supervised the project. All authors read and approved the final manuscript.

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