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Research Article

Evaluation of Epstein-Barr Virus Indirect Immunofluorescence Assay Results

Gorgun S[1]*, Havuz SG[2], Mehel D[3]

[1]Department of Microbiology and Clinical Microbiology Laboratory, Health Sciences University, Training and Research Hospital, Ilkadım, Samsun, Turkey

[2]Department of Microbiology and Clinical Microbiology Laboratory, Bafra State Hospital, Bafra, Samsun, Turkey

[3]Department of Otorhinolaryngology, Health Sciences University, Training and Research Hospital, Ilkadım, Samsun, Turkey

***Corresponding author:** Selim Gorgun, Department of Microbiology and Clinical Microbiology Laboratory, Health Sciences University, Training and Research Hospital, Ilkadım, Samsun, Turkey

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Abstract

Aim: Epstein-Barr Virüs (EBV) causes Infectious Mononucleosis (IM) and chronic active EBV infections, malignant diseases such as Burkitt's lymphoma, nasopharyngeal carcinoma, and posttransplant lymphoproliferative disease. More than 90% of children are infected with EBV until the age of six. The aim of this study was to diagnose EBV infection using Indirect Immuno Fluorescence Assay (IIFA) method and to evaluate these patients clinically.

Methods: The tests were studied by using EBV Indirect Immuno Fluorescence Assay (IIFA) method. A total of 247 patients, 186 adults, and 61 children, were included in the study.

Results: Five (2.7%) of the adults were EBV-Capsid antigen (EBV-CA) IgM positive, 175 (94.1%) were IgG positive and 6 (3.2%) were seronegative. 10 (16.4%) of the child patients were IgM positive, which is considered as an acute IM infection marker, whereas the child patients had a significantly higher IgM rate than adults ($p < 0.001$). 39 (63.9%) of the child patients were IgG positive and 12 (19.7%) of them were seronegative. The rate of IgG positivity in children was significantly lower than in adults ($p<0.001$). There was no significant difference between the genders in terms of IgM and IgG positivity rates in both adults and children ($p > 0.05$ for each).

Conclusion: These results suggest that the presence of an acute EBV infection should be considered when the patient has viral diseases with similar clinical picture especially for children.

Keywords: Epstein-Barr virus; Infectious mononucleosis; Viral capsid antigen

Abbreviations

EBV: Epstein-Barr virus; IIFA: Indirect immunofluorescence assay; EBV-CA: Epstein-Barr virüs capsid antigen; IM: Infectious mononucleosis; LAP: Lymphadenopathy; LPM1 and LPM2: Latent membrane protein 1 and 2; CMV: Cytomegalovirus; EIA: Enzyme immunoassay; ELFA: Enzym-linked fluorescence assay; MPBI: Microparticle based immunoassay; IB: Immunoblot; EBNA: Epstein-Barr virus nuclear antigen; EBEA: Epstein-Barr virus early antigen.

Introduction

Epstein-Barr Virus (EBV) is a double-stranded DNA virus belonging to the sub-family of gammaherpesvirinae, a type of the herpes viruses. EBV is a virus that causes Infectious Mononucleosis (IM) and chronic active EBV infections, malignant diseases such as Burkitt's lymphoma and nasopharyngeal carcinoma, and posttransplant lymphoproliferative disease [1-3]. The American Joint Committee on Cancer reported that when EBV positivity is detected in metastatic lymphadenopathies with unclear primary lesions in the neck region, it should be considered as nasopharyngeal cancer [2-6].

Although infectious mononucleosis may be asymptomatic in childhood, in some children, adolescents and adults, Lymphadenopathy (LAP) is a disease characterized by sore throat, fatigue, pharyngitis, fever, and splenomegaly. EBV is transmitted through close contact with common objects [1,4,5]. EBV settles into lymphoepithelial cells and B lymphocytes in the oral cavity, causing persistent infection. The virus first attaches to the tonsils and settles there. Once the virus enters the cell, EBV Nuclear Antigens (EBNA) in the cell nucleus become detectable, then latent membrane proteins 1 and 2 (LPM-1 and LMP-2) formed by these antigens are synthesized. Copy cells containing EBV genes are then formed and EBV virus becomes latent in B lymphocytes. In these patients, the EBV virus becomes active in cases of weakening of the immune system [1,3,6,7].

EBV's clinical picture is similar to the clinical picture of Cytomegalovirus (CMV), rubella and Toxoplasma gondii infections. Therefore, it requires a differential diagnosis. Additionally, serious complications such as intra-spleen bleeding, respiratory obstruction, myocarditis, and anemia may develop in EBV infection. Therefore, early diagnosis of EBV is crucial for patients suffering from these symptoms [3,4,7].

More than 90% of children are infected with EBV until the age of six. 90% of adults are found to be EBV seropositive. In socioeconomically low populations, infections can be seen at earlier ages [8-10].

Serologic tests such as IIFA, Enzyme Immunoassay (EIA), enzyme-linked fluorescence assay (ELFA), microparticle based immunoassay (MPBI), complement fixation test, and Immunoblot

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(IB) are used for the diagnosis of EBV infection. There is not a gold standard method for these tests. Subjective evaluation of these methods bares sensitivity and specificity problems. Therefore, problems may occur in the diagnosis of EBV [1,2,9,10]. There are advantages of working with different parameters of the tests due to suspicious results obtained in ELISA test results. Although IIFA is more sensitive than ELISA, it has disadvantages as well, since it requires experienced personnel and it can be interpreted subjectively depending on the individual.

The aim of this study was to diagnose EBV infection using IIFA method and to examine these patients clinically.

Methods

A total of 247 patients (186 adults and 61 children) with suspected infectious mononucleosis admitted to the tertiary hospital between January 2018 and December 2018 were included in the study. The patients included in the study had complaints such as sore throat, fever, and lymphadenopathy. Blood samples collected from patients were centrifuged and pellets were sent to laboratory in accordance with cold chain rules. Serum samples were included in the study on the same day or the following day. The samples included the following day were stored at +4^{0}C. Samples were examined in accordance with the manufacturer's recommendations using the IIFA kit for EBV (Europlus: Biochip Sequence EBV plus gp125/p19, Euroimmun Medizinische Labordiagnostika AG. Luebeck, Germany).Biochips coatedwith different EBV antigenswere incubated with diluent patient samples. If the reaction was positive, spesific antibodies of classes IgG and IgM attached to the antigens. In the second step, fields A, C and D were incubated with PBS, field B with urea solution and field E (EBNA) with a human complement solution. In the third step, the attached antibodies were stained with FITC- labelled anti-human globulins. Results were evaluated with a fluorescence microscope (Euroimmun, Eurostar II, Germany) in terms of EBV Capsid antigen (EBV-CA) IgG, EBV-CA IgM, EBNA, EBV-Early antigen (EBEA) and avidity.

All statistical analyses in the study were performed using SPSS 25.0 software (IBM SPSS, Chicago, IL, USA). Descriptive data were given as figures and percentages. Comparisons between the groups in terms of categorical variables were made by Pearson's Chi-square test and Fisher's Exact Test. Results were evaluated in a 95% confidence interval and $p<0.05$ level was considered significant.

This study received approval by local ethics committee decision (TUEK 23-2019 BADK/5-42). Patient consent was obtained from all participants prior to the study.

Results

The overall mean age was 36.64±22.73 years. 104 (42.1%) of the 247 patients with suspected IM, were male and 143 (57.9%) were female. 61 (24.7%) of the patients were children and 186 were adults (Table 1).

Five (2.7%) of the adult patients were EBV-CA IgM positive, 175 (94.1%) IgG positive and six (3.2%) were seronegative. 10 (16.4%) of the pediatric patients were IgM positive presenting acute EBV infection, the IgM positivity rate of the child patients was significantly higher than of adult patients ($p<0.001$).39 (63.9%) of the pediatric patients were IgG positive and 12 (19.7%) of them were seronegative. The rate of IgG positivity in children was significantly lower than in adults ($p <0.001$). There was no significant difference between the genders in terms of IgM and IgG positivity rates for both adults and children ($p> 0.05$ for each) (Table 1).

Table 1: Distribution of theseropositivityrates of patients.

Groups	EBV IgM (+) n(%)	EBV IgG (+) n(%)	EBV (-) n(%)	Total n(%)
Adult	5 (2.7)	175 (94.1)	6 (3.2)	186 (100)
Child	10 (16.4)	39 (63.9)	12 (19.7)	61 (100)
Total	15 (6.1)	214 (86.6)	18 (7.3)	247 (100)
p	<0.001	<0.001	<0.001	

EBV: Epstein-Barrvirus

Table 2: Distribution of patients according to clinics they have admitted to

Clinical	Number
Hematology	155
Pediatrichematology	24
Pediatrics	24
Infection Diseases	11
Otolaryngology Surgery	11
Internal Diseases	6
Pediatric Infection	4
Dermatology	3
Neurology	2
Oncology	2
Others	5
Total	247

155 (62.8%) patients were admitted to the hematology clinic, 24 (9.7%) were admitted to pediatrics and 24 (9.7%) were admitted to pediatric hematology clinics (Table 2).

Eight (53.3%) of 15 EBV IgM positive patients had LAP, three had anemia and one had splenomegaly. All of pediatric patients who found EBV IgM positive had cervical LAP.

Discussion

The clinical picture of EBV infections is similar to some viral infections. Accurate and early diagnosis of EBV infections, which may cause serious complications, is crucial. EBV seropositivity is very high in the normal population. Therefore, the use of methods with high sensitivity and specificity values may provide more accurate results in the diagnosis of EBV infection [4,5]. In this context, we used IIFA method for the EBV diagnosis in our study. For each parameter studied with the IIFA method, a separate patient sample is required. Despite the need for a separate reagent for each parameter, it has been reported to be highly reliable [10,11]. In addition, the IIFA assessment is subjective and can be highly reliable when performed by experienced personel [11].

In this method, IgG and IgM antibodies against EBV-CA were investigated. Acute EBV-CA infection and IgM presence were accepted as the marker of past or recent infection [9,12]. High avidity and EBNA positivity were considered to be a highly possible chronic EBV infection marker and the low avidity was considered to be a

highly possible recent EBV infection.

In the study conducted with 100 patients withsuspected IM, Feyzioglu et al. [12] found that only 2% of the patients had acute EBV infection using IIFA method and reported that 81% of the patients had a past or recent infection. In the study, IgG and IgM presence was not found in 17% of the patients. In the study conducted with minors with suspected IM and with LAP diagnosis Gumuser [9] detected that EBV-CA IgG rate was 83%. In the study conducted with nine thousand patients Sirekbasan et al. [13] have found out that %58.3 of the patients were EBV-CA IgG seropositive, IgM rate was 4.3%, and 38.4% were seronegative. Altuglu et al. [14] reported that 82.2% of the patients were IgG positive, 16.3% of them were IgM positive, and 17.8% were seronegative. In a different study Altuglu et al. [10] found that IgM positivity rate was 14.8%, IgG positivity rate was 91.3%. Fidan et al. [15] detected that 91.4% of the patients were IgG seropositive. Soylu et al. [16] reported that the rate of IgG seropositive patients was 81%. The data of our study suggests that acute infection rate was 6.1% and IgG seropositivity rate was 86.6%, seronegativity rate was 7.3%. All these data supports 80-90% seropositivity rate of developing countries [13].

In the studies conducted in other countries also reveals high seropositivity rates. Beader et al. [17] reported a EBV-CA IgG rate of 91.4% in the study they carried out in Croatia. Xiong et al. [18] found that seropositivity rate was 80% in the study they made in China. In France, Fourcade et al. [19] reported a seropositivity rate of 88% in the study included almost 50 thousand patients. Farid et al. [20] found this rate to be 86% in their study conducted with 10 thousand patients in Bahrain. Tuon et al. [21] found the EBV seropositivity rate as 98% in stissue donors in Brazil. Suntornlohanakul et al. [22] reported an adult seropositivity rate of around 95% in Thailand. Franci et al. [23] reported this rate as 65% in their study in Italy. In our study, IgG seropositivity rate was found to be 94.1%. These data show that although EBV seropositivity rate varies regionally, it is generally very high.

Gumuser [9] found that the IgG seropositivity rate in children under two years of age was 63.6%, and in the 6-12 age range it was 93.1%, and 75% in 12 years of age. Chan et al. [24] found the EBV-CA IgG rate in children under two years of age was 60% in Hong Kong. Pereira et al. [25] found a 50% IgG seropositivity rate in children under the age of four in their study in England and found this rate to be 81% in the 15-24 age group. Ferres et al. [26] reported that the IgG seropositivity rate was 76.7% in children under two years of age and 90% in patients around 20 years of age in their study conducted in Spain. In our study, the EBV-CA IgG seropositivity rate was 63.9% in pediatric patients. These data support the estimation that EBV seropositivity rates are lower in the first childhood period compared to the general population and the rate of exposure to the virus rapidly increases after the first six years of age.

Primary EBV infection was reported to occur in early childhood [24-26]. In our study, IgM presence is detected in 16.4% in child patients, which suggests an acute IM infection, whereas the rate of IgM positivity in adults was only 2.7%. The IgM positivity rate in pediatric patients was significantly higher than in adults ($p<0.001$). This data supports the idea that primary infection is more common in young age.

Sirekbasan et al. [13] have not found a significant difference between the genders in terms of seropositivity. In our study, no significant difference was found between the genders in terms of seropositivity in both adults and children. This data supports the information that the frequency of IM infection does not vary according to gender.

In our study, 155 (62.8%) patients were admitted to the hematology clinic, 24 (9.7%) were admitted to pediatrics and 24 (9.7%) were admitted to pediatric hematology clinics. According to this data, it can be thought that patients with blood-related disorders, namely anemia, are more frequently examined with a preliminary diagnosis of EBV infection. In our study, LAP, anemia, and splenomegaly were detected in 8 (53.3%) of 15 EBV IgM positive patients. This data shows that the classical findings of IM are more common in these patients.

There were some limitations in our study. Since, our study was retrospective, different methods could not be used for the patients. Therefore, positive tests could not be confirmed by the EBV Blot test and atypical findings could not be examined. However, positive test results were found to be consistent with the clinical results of patients. Patients with a diagnosis of lymphadenopathy who were asked for an EBV test and had a negative EBV IgM test were followed up and were given emprical treatment considering the development of lymphadenopathy due to nonspecific upper respiratory tract infection.

In this study, we evaluated the results of patients with suspected IM who underwent EBV-CA IgM and IgG tests conducted by using the IIFA method. The rate of primary EBV infection in childhood was higher than in adults. In addition, the seropositivity rate of past or current EBV infection was found to be similar to the rates reported in our country. These data indicate that acute EBV infection should be considered separately from other infections showing a similar clinical picture.

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Authors' Contributions

Selim Gorgun and Seda Havuz Gudul have approved the design of the work, analysis, interpretation of data, preparation of the manuscript, language edit and its submission to the journal. Dursun M. Mehel approved the design of the work, preparation of the manuscript and its submission to the journal.

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