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Investigation of Epstein-Barr Virus antibodies by ELISA and IFA methods

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Abstract

Epstein-Barr Virus (EBV), play role in etiology of malignancies as Burkitt Lymphoma and Nasopharyngeal Carcinoma alongside very common situation like Infectious Mononucleosis. Also in patients groups, like transplant and oncologic patients whose immune system especially depressed detection of EBV reactivation is important. In this study, investigation of results defined by Immunofluorescent Antibody (IFA) and Enzyme-Linked ImmunoSorbent Assay (ELISA) methods aimed. Between 2017 July and 2020 July in our laboratory, With 7455 samples Anti-VCA IgM results detected by the ELISA method were 3.9% positive, 94.1% negative, and 3% borderline. With 5510 samples Anti-VCA IgG results detected by the ELISA method were 82,3% positive, 16.1% negative, and 1.6% borderline. With 449 samples, 32.9% of Anti-VCA IgM, 96.8% of Anti-VCA IgG,55% of Anti-EA IgG, and 93.5% of Anti-EBNA antibody results detected by the IFA method were positive. Positive Anti-VCA IgM results were 3% by ELISA and 25% by the IFA, positive Anti-VCA IgG results were 96.3% by ELISA and 98% by IFA. ELISA Anti-VCA IgG sensitivity was found to be 96.3% and ELISA Anti-VCA IgM sensitivity was found to be 12.9% in statistical analysis, considering IFA as the gold standard. For serological diagnosis of acute EBV infection or reactivation of latent infection EBV Anti-VCA IgM, Anti-VCA IgG, Anti-EBNA IgG, Anti-EA IgG, and Anti-VCA IgG avidity antibodies should be evaluated together.

Keywords: epstein-barr virus, immunoflourescent antibody, enzyme-linked immunosorbent assay, anti-epstein-barr nuclear antigen

1. Introduction

Epstein-Barr virus (EBV) is a virus from the Herpesviridae family, which can be seen quite frequently in the world, can be transmitted through oropharynx secretions through close contacts such as kissing, blood, and common items. It contains DNA as genetic material. Infectious mononucleosis (IM) is a clinical condition that can occur with symptoms such as lymphadenopathy (LAP), pharyngitis, fever, and splenomegaly in young and adult patients, while pediatric patients often pass without symptoms (1, 2). The virus can cause malignant transformation in B and T lymphocytes, epithelial cells, and smooth muscle cells. It has been shown to be associated with various cancers such as Burkitt's Lymphoma (BL), nasopharyngeal carcinoma (NFC), post-transplant lymphoproliferative disease (PTLD), gastric carcinoma, Hodgkin, and non-Hodgkin lymphoma, and leiomyosarcoma (3). In immunocompromised individuals, EBV reactivation occurs when cytotoxic T lymphocytes, B lymphocytes, as well as latent antigens are affected and cause malignant changes. This system is quite balanced to normal conditions in a healthy individual and causes almost no specific symptoms and signs. In cases where the immune system is weakened, T cell activity is reduced, such as in a solid organ or stem cell transplants, or HIV infection, virus reactivation can cause serious complications (4).

The fact that EBV infections have become an important problem in immunocompromised patients, whose number is increasing, has increased the importance of EBV specific tests (5). It is important to detect and demonstrate the reactivation of latent EBV, especially in immunocompromised patients such as organ and bone marrow recipients or cancer patients (6). In this study, it was aimed to examine the EBV antibody results determined by Enzyme-Linked ImmunoSorbent Assay (ELISA) and Immune Fluorescent Antibody (IFA) test.

2. Materials and Methods

EBV viral capsid antibody Anti- (VCA) IgM in 7455 serum samples sent to our laboratory from various clinics of our university's hospital between July 2017 and July 2020 and Anti-VCA IgG antibodies in 5510 serum samples was investigated by ELISA method (Architect, Abbot, Wiesbaden-Germany). EBV Anti-VCA IgM, Anti-VCA IgG, Anti-EarlyAntigen (EA) IgG, Anti-Epstein-Barr Nuclear Antigen (EBNA) IgG antibodies and Anti-VCA IgG avidity in 449 serum samples with IFA method (Euroimmun, Luebeck-Germany) status has been investigated. In addition, in this study, Anti-VCA IgG and Anti-VCA IgM antibody results determined by IFA and ELISA were compared in 164 samples sent simultaneously from the same patients. The IFA method was accepted as the gold standard and the sensitivity and specificity of the ELISA test were calculated.

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3. Results

Hematology 3534 (47.4%), pediatric hemato-oncology 756 (10.2%), infectious diseases 448 (6.1%), internal diseases 324 (4.3%), pediatric nephrology 289 (3.8%) and 2104 (28.2%) from other clinics among the 7455 anti-VCA IgM antibodies investigated by ELISA, 298 (4.1%) were positive, 7018 (94.1%) were negative, 139 (1.8%) were determined as intermediate values (Table 1). The average age of these patients, whose age range is 1-88, is 46, the gender distribution is 3986 (53.5%) male and 3469 (46.5%) female.

Table 1. EBV Anti-VCA IgM and Anti-VCA IgG antibody results determined by ELISA

	Anti-VCA IgM n (%)	Anti-VCA IgG n (%)
Positive	298 (4.1%)	4539 (82.3%)
Negative	7018 (94.1%)	886(16.1%)
Intermediate	139 (1.8%)	85 (1.6%)
Total	7455 (100%)	5510 (100%)

Hematology 1924 (34.9%), pediatric hemato-oncology 692 (12.6%), internal diseases 536 (9.8%), infectious diseases 327 (5.9%), pediatrics 298 (5.4%) and 1733 (31.4%) samples from other clinics. Of the 5510 samples investigated for anti-VCA IgG antibody, 4539 (82.3%) were positive, 886 (16.1%) were negative, and 85 (1.6%) were determined as intermediate values (Table 1). The average age of these patients, whose age range is 1-79, is 34, the gender distribution is 3101 (56.2%) male and 2409 (43.8%) female.

Hematology 398 (88.6%), pediatrics 29 (6.5%), and 22 (4.9%) from other clinics, 148 (32.9%) of 449 samples investigated by IFA method had Anti-VCA IgM, 435 (96.8%) had Anti -VCA IgG was found to be positive in 247 (55%) Anti-EA IgG, 420 (93.5%) with anti-EBNA antibodies, low avidity in 33 (7.3%) of the samples studied with the IFA test, 416 (92.7%), high avidity was detected (Table 2). The average age of these patients, whose age range is 1-67, is 41, the gender distribution is 237 male (52.7%) and 212 female (47.3%).

Table 2. EBV profile results determined by IFA

	Positive	Negative	Total n (%)
Anti-VCA IgM n(%)	148 (32.9%)	301 (67.1%)	449 (100%)
Anti-VCA IgG n(%)	435 (96.8%)	14 (3.2%)	449 (100%)
Anti-EA IGG	247 (55%)	202 (45%)	449 (100%)
Anti-EBNA IgG	420 (93.5%)	29 (6.5%)	449 (100%)
	High n(%)	**Low n(%)**	**Total n(%)**
Anti-VCA IgG Avidity	416 (92.6%)	33 (7.3%)	449 (100%)

Anti-VCA IgM and Anti-VCA IgG antibodies were studied simultaneously with IFA and ELISA tests in a total of 164 samples, 146 of whom were from the Hematology clinic, in 3% of the patients with the Anti-VCA IgM antibody ELISA, in 25% with IFA, Anti-VCA IgG antibody was detected as positive in 96.3% by ELISA and 98.1% by IFA (Table 3). While ELISA and IFA were consistent, differences were found in Anti-VCA IgM results. ELISA Anti-VCA IgG sensitivity was found to be 96.3%, while ELISA Anti-VCA IgM sensitivity was 12.9% in the statistical analysis performed by accepting IFA as the gold standard. Anti-VCA IgM antibody results, the p-value was <0.00001 ($p < 0.05$), Anti-VCA IgG results were statistically insignificant; the p-value is .310579 ($p < 0.05$) when two methods were compared with Pearson Chi-Square test. EBV other antibody results of these patients are given in Table 4. The average age of these patients, whose age range is 3-77, is 45, the gender distribution is 94 (57.3%) male and 70 (42.7%) female.

Table 3. Comparison of Anti-VCA IgM and Anti-VCA IgG antibody results in patient samples studied with ELISA and IFA method

	Anti-VCA IgM n (%)		Anti-VCA IgG n (%)	
	ELISA	IFA	ELISA	IFA
Positive	5 (3%)	41 (25%)	158 (96.3%)	161 (98.1%)
Negative	159 (97%)	123 (75%)	6 (3.7%)	3 (1.9%)
Total	164 (100%)	164 (100%)	164 (%100)	164 (100%)

Table 4. IFA EBV Profile results of patients compared with ELISA and IFA antibody results

	Positive n (%)	Negative n (%)	Total n (%)
Anti-EA IgG	99 (% 57)	65 (%43)	164 (%100)
Anti-EBNA IgG	134 (%81.4)	30 (%18.6)	164 (%100)
	High avidity n(%)	**Low avidity n(%)**	**Total n (%)**
Anti-VCA IgG Avidity	157 (%94.7)	7 (%5.3)	164 (%100)

4. Discussion

By detecting antibodies produced against four different antigens of EBV, the infection is diagnosed serologically and the infection period is determined. These antigens; VCA is the diffuse component of EA (EA / D), the restrictive component of EA (EA / R), and the nuclear antibody (EBNA). In acute infection, EBV VCA IgG, IgM, and EA antibodies are positive, and EBNA antibodies are negative. Four weeks after the onset of the acute period, VCA IgM disappears, while VCA IgG is detected positive in serum for life. Anti VCA IgG and EBNA are persistent for life and are an indicator of chronic virus carriers (7). Specific serological tests for EBV antigens are used to identify EBV infection and to distinguish between other mononucleosis-causing infections. The diagnosis of primary and past EBV infection can often be made by looking at only 3 parameters: anti-VCA IgM, anti-VCA IgG and anti-EBNA IgG antibodies. Most likely, anti-VCA IgM and anti-VCA IgG positivity as well as anti-EBNA IgG negativity favor acute infection, presence of

anti-VCA IgG and anti-EBNA IgG, absence of anti-VCA IgM past infection (8). In cases where there is only anti-VCA IgG in the absence of anti-VCA IgM and anti-EBNA IgG, or in cases where all three parameters are present, it may be difficult to diagnose infections such as acute, past or reactivation serologically. The presence of isolated anti-EBNA IgG may also raise suspicion. To interpret such profiles, detection of anti-IgM and anti-IgG antibodies by IFA, immunoblot test, detection of anti-VCA IgG avidity and anti-EA / D antibodies, and viral genome determination by molecular methods can be used. These tests can be useful to identify possible infection status and to resolve problems that may arise in routine laboratory practice (6, 8, 9–11).

Table 5. Serological profiles and interpretations in EBV infection

Anti-EBV Antibodies			Evaluation
VCA IgM	**VCA IgG**	**EBNA IgG**	
Negative	**Negative**	**Negative**	Not exposed to EBV infection
Positive	**Negative**	**Negative**	Acute infection early or nonspecific*
Positive	**Positive**	**Negative**	Acute infection
Negative	**Positive**	**Positive**	Past infection
Negative	**Positive**	**Negative**	Acute or past infection *
Positive	**Positive**	**Positive**	Late primary infection or reactivation *
Negative	**Negative**	**Positive**	Past infection or nonspecific *

*: Atypical serological profile

Table 6. Possible causes of atypical ebv serological profiles and further review suggestions

Atypical Profile	Possible Causes	Further Review
Isolated VCA IgG positivity	EBV VCA IgM may not have been produced, can be found in low concentration (false negativity), can occur 1-2 weeks after VCA IgG. In 5% of past infections, EBNA IgG may not be produced or may be produced below the detection limit (False negativity), present in immunocompromised patients may disappear over time.	-Immunoblot -VCA IgG Avidity -EBV DNA Research -Heterophil Antibody Tests -Repetition of tests after 30 days -Anti EA-IgG research
Combination positivity of EBNA IgG, VCA IgM and VCA IgG	VCA IgM may remain positive for several more months after acute infection, may occur in EBV reactivation, may persist from primary infection. Late period of primary infection where EBNA IgG is newly formed. False positivity can be found in VCA IgM during CMV, Parvovirus B19, Toxoplasma gondii, HAV, HIV infections.	-Immunoblot -VCA IgG Avidity -EBV DNA Research -Heterophil Antibody Tests -Repetition of tests after 30 days -Anti EA-IgG research - Parvovirus IgM and CMV IgM analysis
Isolated EBNA IgG positivity	VCA IgG Loss in previous infection	-Immunoblot -Anti EA-IgG research -Heterophil Antibody Tests

After primary infection, EBV can enter the latent phase and then reactivation can be observed depending on the immunological status of the host. In reactivation, virus replication and excretion usually occur asymptomatically. In rare cases, reactivation is associated with clinical manifestations such as EBV-associated lymphoproliferative disorders, mostly in individuals with compromised T-cell immune systems, such as in AIDS patients and transplant recipients. In addition, EBV is also associated with Burkitt's Lymphoma and Nasopharyngeal Carcinoma in individuals with strong immune systems (12). Due to such reasons and its importance in the differential diagnosis, early and correct diagnosis of EBV is very important. Conventionally, antibodies against EBV are measured by IFA. IFA is considered the 'gold standard' in the serological diagnosis of EBV infection (1, 13). The use of IFA in EBV infected cells is the reference method for determining specific EBV antibodies (14). However, the disadvantages of the IFA method are nonspecific immunofluorescent staining, difficulties in standardization, requiring experienced personnel, and subjective interpretation of the results. It is therefore important to determine the sensitivity and specificity of the ELISA method, in which many sera can be evaluated more practically, compared to IFA (15, 16). EBV infections are acquired at different ages in different socioeconomic groups, and this may affect clinical presentation (17). The positivity rates in various age groups in a variety of low seroprevalence studies reported from Turkey at 70%, was reported to be the highest at 99.4% (18-21). In these studies, seroprevalence was investigated using the ELISA method and it is consistent with the results of our study.

Haque et al. (15) found 97% compatibility between IFA and ELISA in a study, while ELISA was found to be less sensitive than IFA. Farber et al. (23), EBV VCA IgG was measured by ELISA and IFA, and the compatibility between the two methods was examined, and ELISA was shown to be 95% compatible with IFA for VCA IgG. According to IFA, the sensitivity of ELISA for EBV VCA IgG was determined as 94% and specificity was 97.8% (23). Michalek et al. (22) emphasized that serology and DNA analysis should be evaluated together in the diagnosis of EBV infections in pediatric oncology patients, and serological tests alone are not sufficient in the diagnosis. Serological profiles that can be obtained by the ELISA method and their interpretations are given in Table 5, and some atypical situations that may be encountered in interpreting the profiles are given in Table 6. In the interpretation of serological profiles, acute or previous infection or reactivation comments cannot always be made clear.

Kaşifoğlu et al. (24), ELISA and IFA compliance rates were found to be 100% for seronegativity, 100% for acute primary infection, 22.2% for late primary infection, and 92.1% for the previous infection. In our study, while ELISA and IFA Anti-VCA IgG results were consistent, differences were found in Anti-VCA IgM results. In the statistical analysis performed by accepting the IFA as the gold standard, ELISA Anti-VCA IgG sensitivity was found 96.3%, while ELISA Anti-VCA IgM sensitivity was found 12.9%.

In our study, when the VCA IgM IFA and ELISA results were compared, a difference was found in the positivity rates. When we examine this result, it is known that the evaluation of IFA test requires experienced personnel. Experienced personnel are employed in our laboratory as well. At the same time, it was found that the VCA IgM antibody positivity evaluation of the kit used in this study was a little more difficult, besides easily detecting other antibodies. We think it is important for the manufacturing company to consider this assessment.

VCA IgM antibodies can persist for months after acute infection (25) and reappear in reactivation situations (26). In some cases, VCA IgM may not be produced or appear in VCA IgG after 1-2 weeks, or they are produced in concentrations too low to be detected by standard methods (8). It may be useful to consult the EA IgG and Anti-VCA IgG results to interpret the VCA IgM antibodies investigated by both IFA and ELISA methods in acute or past infection or reactivation situations.

Especially for Anti-VCA IgM, there is a need to compare IFA and ELISA results in larger patient groups. In the serological diagnosis of acute EBV infection, late primary infection, or reactivation, anti-VCA IgM, Anti-VCA IgG, Anti-EBNA IgG, Anti-EA IgG and Anti-VCA IgG avidity antibodies of EBV antibodies should be evaluated together.

Conflict of interest

None to declare.

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None to declare.

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