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Serological markers of EBV primary infection

Infectious mononucleosis (IM) was the first disease to be attributed to Epstein-Barr Virus (EBV) infection but seroepidemiological, immunohistochemical and molecular studies suggest that EBV plays an important role in the pathogenesis of several malignancies, e.g., nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease, some sarcomas, breast, gastric and cervical cancers (2, 3, 5, 7).

Clinical manifestations similar to IM can also be induced by a number of other pathogenic infectious agents including cytomegalovirus, Toxoplasma gondi, hepatitis viruses, human immunodeficiency virus (HIV) and others (1). The term "mononucleosis syndrome" is often applied until specific etiologic agent is identified. Confirmation of an acute diagnosis of EBV IM is generally sought by a positive heterophile antibody test – agglutination by patient's serum with horse or ship red blood cells (1, 6). However, difficulties in diagnosis arise when the heterophile test is negative or when clinical manifestations are atypical (6). Heterophile antibody negative IM has been demonstrated in 10 to 20% of adults with an even greater percentage in children with acute IM infections (6). For these individuals, IM diagnosis may be confirmed by identification of antibodies to specific EBV protein antigens.

The aim of the work was to analyse serological markers of EBV infection: VCA IgM, VCA IgG, EBNA IgG in acute symptomatic phase IM.

MATERIAL AND METHODS

We examined 24 patients, 11 women at the age of 18–26 and 13 men at the age 16–24 in acute symptomatic phase of mononucleosis (IM), who were hospitalised at the Department of Infectious Diseases, Medical University of Lublin during the period 2003–2004. The control group included 18 healthy person, 13 women at the age of 16–28 and 5 men at the age of 17–25. One time in all the examined people we assessed the level of serum markers of EBV assessed infection: EBV VCA IgM, EBV VCA IgG, EBV EBNA IgG immunoenzymatic method by Vironostica ® Bio - Merieux. The obtained data were analysed with the use of U Mann - Whitney statistical test.

RESULTS

The clinical characteristics of the patients are shown in Table 1. All IM patients presented fever, sore throat, cervical lymphadenopathy and fatigue. Exudative tonsilitis was present at diagnosis in 20 (83.3%) patients and 2 (8.3%) patients were clinically jaundiced. The serum level EBV VCA IgM, EBV VCA IgG and EBV EBNA IgG in IM patients and control group are shown in Table 2. Serum samples in control group presence low, less that 20 IU/ml, titers of EBV VCA IgM. EBV VCA IgG was high, more that 20 AU/ml in all samples of control group. We observed low (<20 AU/ml) EBV EBNA IgG serum level in all healthy individuals. In 21 (87.5%) IM patients EBV VCA IgM was high (>20 AU/ml), in 2 (8.3%) EBV VCA IgG was low (<20 AU/ml) with high level of EBV VCA IgM. All IM patient presented low (<20 AU/ml) level of EBV EBNA IgG.

Table 1. Symptoms and clinical signs of the infectious mononucleosis patients

Patient No.	Fever	Exudative tonsilitis	Sore throat	Cervical lymphadenopathy	Jaundice	Fatigue
1	+	+	+	+	-	+
2	+	+	+	+	-	+
3	+	+	+	+	+	+
4	+	-	+	+	-	+
5	+	+	+	+	-	+
6	+	+	+	+	-	+
7	+	+	+	+	-	+
8	+	+	+	+	-	+
9	+	+	+	+	-	+
10	+	-	+	+	-	+
11	+	+	+	+	-	+
12	+	+	+	+	-	+
13	+	+	+	+	-	+
14	+	+	+	+	-	+
15	+	+	+	+	-	+
16	+	+	+	+	-	+
17	+	+	+	+	-	+
18	+	+	+	+	-	+
19	+	-	+	+	-	+
20	+	+	+	+	+	+
21	+	+	+	+	-	+
22	+	+	+	+	-	+
23	+	-	+	+	-	+
24	+	+	+	+	-	+

Table 2. Comparison of cytokine and serological markers in infectious mononucleosis (IM) and control group (C)

		EBV VCA IgM (pg/ml)	EBV VCA IgG (pg/ml)	EBV EBNA IgG (pg/ml)
Minimum	IM	8.68	9.31	0.01
	C	0.00	20.85	0.13
Maximum	IM	198.00	213.62	2.51
	C	7.35	213.62	19.27
Median	IM	145.83	112.24	0.08
	C	1.04	196.23	1.18
SD	IM	66.38	80.70	0.60
	C	2.21	65.74	6.28

We observed statistically important difference between IM patients and control group in levels: EBV VCA IgM ($p<0.001$), EBNA IgG (<0.001). We did not observe the statistically important difference in the level of VCA IgG ($p=0.142$).

DISCUSSION

EBV infection is ubiquitous, but is usually asymptomatic except in teenagers and young adults, some 50 percent of whom experience the syndrome of acute IM at the time of primary infection (3, 5). Although the virus established a permissive infection in a number of cell types in the mouth and pharynx, including the ductal cells of the salivary glands, it is the proliferation of latently infected B lymphocytes in lymphoid tissue and the resultant cellular immune reaction against these EBV-infected cells which give rise to the syndrome of IM (2, 5). EBV gains access to B lymphocytes via the surface receptor for the complement component C3d (CR2), also known as CD21, and transforms them into lymphoblasts capable of indefinite proliferation *in vitro*. This capacity is conferred on the cell via a small set of EBV genes which are expressed in the absence of viral replication. In addition to "immortalizing" the cell, however, some of these latent genes induce an immune response against the EBV-infected cells, which leads to their destruction. Destruction is accomplished initially by NK cells and nonspecifically reactive T cells which probably respond to EBV-induced activation antigens, and later by specifically reactive cytotoxic T lymphocytes which recognize virally coded proteins on the cell surface in the context of HLA class I antigens. The clinical syndrome of IM, if it occurs at all, is thus limited in duration and rarely fatal (5).

Rapidly fatal IM and a variety of EBV-associated lymphoproliferative syndromes do, in fact, occur in immunosupressed individuals. In the case of acquired immunosuppression developing some time after primary EBV infection, whether due to a virus infection or to the administration of immunosuppressive drugs, any subsequent EBV-associated lymphoproliferative syndrome can only result from a "reactivation" of EBV infection. In practice, this means that a higher level of EBV replication occurs at sites of productive infection, potentially giving rise to a higher rate of B-cell infection and transformation and a poorer ability to destroy EBV-infected B cells. It is still uncertain whether a pool of latently infected B lymphocytes, regulated in size by T cells, persists after primary EBV infection, or whether such cells are repeatedly infected during passage through tissues harboring and releasing EBV, only to be destroyed by cytotoxic T lymphocytes. By whatever means, a balance is struck so that a small number of virus-infected B cells is always detectable in the blood-stream of EBV-seropositive adults, the level of which is regulated by HLA-restricted, EBV-specific cytotoxic and/or suppressor T cells (2, 3, 5). In immunocompetent conditions EBV infections remain under control of some mechanisms of host defense, and this immunological response has an impact both on clinical source and consequences of the disease (3, 7).

Diagnosis of IM is based upon clinical manifestations which generally include sore throat, fever, lymphadenopathy, and malaise in conjunction with hematological evidence for lymphocytosis and serological evidence for the presence hetorophile antibody and/or antibodies to EBV specific proteins (3, 4, 6). In our study we observed fever, sore throat and cervical lymphadenopathy in all IM patients. EBV are included in hepatotrophic viruses and two our patients had the clinical symptoms of *hepatitis viralis*. IM diagnosis may be confirmed by identification of antibodies to specific EBV protein antigens which include viral capsid antigen (VCA) and early antigen diffuse [EA(D)]. The presence of VCA IgM antibody usually sufficious for diagnosis of IM. However, verification should be sought clinically with additional relevant information (8).

In our study we used to assess the level of serum markers of EBV infection: EBV VCA IgM, EBV VCA IgG, EBV EBNA IgG immunoenzymatic method (ELISA). VCA p 18 peptide, a VCA-specific marker protein unique to EBV, is utilized in microelisa system EBV VCA IgM together with a VCA p 18-specific monoclonal antibody as conjugate. This well characterized peptide consists of 56 amino acids encoded by open reading frame $BFRF_3$. It contains immunodominant epitopes of the p 18 protein that is a major component of the viral capsid antigen complex (8, 9, 10).

We used as controls serum samples obtained from healthy volunteers. We observed lower than 20 AU/ml, titers of EBV VCA IgM. In 89.29% IM patients EBV VCA IgM was a reactive result (>20 AU/ml). Our study showed statistically important difference between IM patients and control group in levels EBV VCA IgM ($p<0.001$). Results of some studies have proved finding of

class IgM antibodies against EBV VCA IgM to be one of the best diagnostic methods for confirmation of IM. These antibodies might be present in serum up to 3 months after acute symptomatic IM. VCA p 18 peptide, a recently defined VCA-specific marker protein unique for EBV, is utilized in microelisa system EBV VCA IgG together with goat anti-human IgG as conjugate (8, 9, 10). EBV VCA IgG was high, more that 20 AU/ml in all samples of the control group. In 92.86% sample serum from IM patients there was a high level of EBV VCA IgG, only in two (7.14%) patients EBV VCA IgG was low (<20 AU/ml) with a high level of EBV VCA IgM. We did not observe statistically important difference in the level of VCA IgG (p=0.142). Most symptomatic IM patients (>80%) show near-peak antibody levels of VCA IgG and IgM when first examined. VCA IgM antibodies usually disappear in 2 to 3 months whereas VCA IgG antibodies persist indefinitely.

Microelisa system EBV EBNA IgG utilizes a synthetic peptide of Epstein Barr Nuclear Antigen (EBNA)-1-protein. It comprises 59 amino acids containing several domains of the EBNA-1-protein known to be immunodominant. The peptide detects antibodies to EBNA with high degree of sensitivity and specifity. The Gly–Ala repeat has been excluded due to its known cross-reactivity with autoantigens (6). In our study all serum samples in both groups were negative.

Reactive results (≥20 AU/ml) indicated exposure to EBV. In this case, the presence of IgM to VCA and IgG to EBNA should be determined, in order to assess the phase of infection. Our study indicated that positive EBV VCA IgM, positive EBV VCA IgG and negative EBV EBNA IgG is a typical serologic profile in IM patients.

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SUMMARY

Infectious mononucleosis (IM) was the first disease to be attributed to Epstein-Barr Virus (EBV) infection but seroepidemiological, immunohistochemical and molecular studies suggest that EBV plays an important role in the pathogenesis of several malignancies. Clinical manifestations similar to IM can also be induced by a number of other pathogenic infectious agents including cytomegalovirus, *Toxoplasma gondi*, hepatitis viruses, human immunodeficiency virus (HIV) and others. IM diagnosis may be confirmed by identification of antibodies to specific EBV protein

antigens. The aim of the work was to analyse serological markers of EBV infection: VCA IgM, VCA IgG, EBNA IgG in acute symptomatic IM. We examined 28 patients in acute symptomatic phase of mononucleosis (IM), who were hospitalised at the Department of Infectious Diseases, Medical University of Lublin in 2003–2004. The control group included 18 healthy persons. One time in all the examined people there was assessed the level of serum markers of EBV infection: EBV VCA IgM, EBV VCA IgG, EBV EBNA IgG by the immunoenzymatic method. The obtained data were analysed with the use of U Mann-Whitney statistical test. Our study indicated that positive EBV VCA IgM, positive EBV VCA IgG and negative EBV EBNA IgG is a typical serologic profile in IM patients.

Serologiczne markery pierwotnej infekcji EBV

Mononukleoza zakaźna (MZ) była pierwszą chorobą etiologicznie związaną z zakażeniem wirusem Epstein-Barr (EBV), ale badania seroepidemiologiczne, immunohistochemiczne i z zakresu biologii molekularnej sugerują, że EBV odgrywa istotną rolę także w różnych procesach rozrostowych. Obraz kliniczny MZ może przypominać infekcję wywołaną cytomegalowirusem, pierwotniakiem *Toxoplasma gondi*, innymi wirusami hepatotropowymi czy wirusem HIV. Potwierdzeniem rozpoznania MZ jest wykazanie obecności przeciwciał przeciwko białkom wirusa w surowicy krwi chorych. Celem pracy była ocena poziomu przeciwciał anty VCA IgM, anty VCA IgM, anty EBNA IgG w surowicy krwi w ostrej objawowej MZ. Badaniami objęto 28 chorych z MZ, hospitalizowanych w Klinice Chorób Zakaźnych AM w Lublinie w latach 2003–2004. Grupę kontrolną stanowiło 18 osób zdrowych. U wszystkich badanych poziom anty VCA IgM, anty VCA IgM, anty EBNA IgG w surowicy krwi oznaczano jednorazowo metodą immunoenzymatyczną ELISA. Uzyskane dane liczbowe poddano analizie statystycznej z wykorzystaniem testu U Mann-Whitneya. Nasze badania wykazują, że dodatni EBV VCA IgM, dodatni EBV VCA IgG i negatywny EBV EBNA IgG to typowy profil serologiczny chorych w ostrej objawowej fazie MZ.