Research article/Raziskovalni prispevek

EPSTEIN-BARR VIRUS INFECTIONS – AVIDITY TEST FOR IgG ANTIBODIES

OKUŽBE Z VIRUSOM EPSTEIN-BARR – DOLOČANJE AVIDNOSTI PROTITELES IgG

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Key words: *Epstein-Barr virus; diagnosis; avidity; avidity index; IgG antibodies*

Abstract – Background. *We wish to introduce specific IgG avidity test as a supplementary assay in serological screening for Epstein-Barr virus infection if the status of patient cannot be resolved from a single serum sample with routine testing.*

Methods. *Avidity of IgG antibodies was determined in sera of 57 patients with different stage of Epstein-Barr virus infection. Enzyme-immuno assay was used with a short incubation of 6-molar urea included in the procedure. Urea should remove low avidity antibodies. Avidity was expressed as the avidity index. Avidity testing with commercial kit was done as well.*

Results. *Low avidity index was found for IgG antibodies of acute phase sera and high for those of past infection, recent infection and reactivation of endogenic virus.*

Conclusions. *Avidity test for IgG antibodies might be supplementary assay to prove acute infection but also to resolve some other clinical states related to Epstein-Barr virus.*

Ključne besede: *virus Epstein-Barr; diagnostika; avidnost; indeks avidnosti; protitelesa IgG*

Izvleček – Izhodišča. *S testom avidnosti za določanje protiteles IgG proti različnim antigenom virusa Epstein-Barr želimo dopolniti diagnosticiranje okužb tedaj, ko z ustaljenimi preiskavnimi postopki ne moremo točno določiti stopnje okužbe v enem serumu.*

Metode. *V 57 serumih bolnikov z različnimi stopnjami okužbe z virusom Epstein-Barr smo določali avidnost protiteles IgG. V encimsko-imunski test smo po inkubaciji serumov z antigeni vključili kratko inkubacijo s 6-molarno ureo, ki odstrani nizko avidna protitelesa. Avidnost smo izrazili z indeksom avidnosti. Improvizirano metodo smo primerjali s standardiziranim komercialnim kompletom.*

Rezultati. *Nizek indeks avidnosti protiteles IgG smo določili v serumih bolnikov z akutno okužbo, visokega pa v serumih bolnikov z okužbo v preteklosti, z nedavno okužbo in z reaktivacijo endogenega virusa.*

Zaključki. *Test avidnosti protiteles IgG lahko predstavlja dopolnilno diagnostično metodo ne le pri potrditvi akutne okužbe, marveč tudi pri razjasnitvi drugih kliničnih oblik okužbe z virusom Epstein-Barr.*

Introduction

Epstein-Barr virus (EBV) is a causative agent of infectious mononucleosis (IM); it is also related to Burkitt lymphoma (BL), nasopharyngeal carcinoma (NPC), Hodgin disease (HD), to B-cell lymphoma of immunodeficiency, T-cell lymphoma and to many other diseases (1). The outcome of the infection is strongly influenced by the virus and its host; in some cases also by environmental conditions (2).

Virus infects the cells of nasopharynx. It is replicating in these cells and released. The next cells which are infected are B-cells; EBV replicating cycle is continuing. In B-cells EBV establishes a latency; for numerous exogenic and endogenic reasons it might be reactivated oftenly (3). EBV is becoming more and more actual because of its possible oncogenicity.

The host organism responds to EBV infection by different antibodies, specific to several kinds of virus antigens. After primary infection antibodies to early antigens (EA) and viral capsid antigen (VCA) are first present subsequently followed by antibodies to nuclear antigen (EBNA) which appear only after several weeks and they always correlate to the establishement of EBV latency. In latency antibodies to EA dissapear but they reappear during EBV reactivation, while antibodies to VCA and EBNA persist lifelong (2, 4).

The diagnosis of EBV infection is ussually serological; the methods of molecular biology, like hybridization and PCR also might be a useful key in solving some clinically problematic cases. For regular diagnosis it is very important to have relatively fast, specific and sensitive test. Indirect immunofluorescence had been the most important serological method for some decades. ELISA based on the demonstration of specific antibodies to native and recombinant antigens, bount to polystirene plates now seems to be the method of choice.

Yet there are some cases which remain very difficult from the diagnostic point of view; it happens that it is not possible to define the stage of EBV infection when examined only one patient's serum. In these cases we should do some supplementary diagnostic tests which help to define acute infections

or other stages of infection. One of these tests could be the avidity test of specific IgG antibodies or Western blot.
Avidity tells about the strength of antibodies' binding to multivalent antigen. Avidity can be measured after short incubation of the antigen-antibody complex with 6 M urea (5). High avidity antibodies persist in binding to the antigen, while low avidity antibodies are destroyed after the addition of urea.
In this study we've measured the avidity of IgG antibodies (anti-EA, anti-VCA, anti-EBNA) in sera of patients previously examined by ELISA to define their immune status to EBV.
The results of »in house« avidity test were compared to the results obtained by commercially available test kit, oriented to measure avidity of IgG anti-EBV.

Material and methods

Sera of patients. In this study 74 sera of patients previously examined for EBV immune status (4, 7) were included for measuring IgG antibodies' avidity. According to their anti-EBV status sera were divided into four groups: patients' sera with acute EBV infection (n = 22), recent infection (n = 18), past infection (n = 11) and with virus reactivation (n = 23).
»In house« avidity test. All sera were repetedly tested by ELISA. After the incubation of sera with specific antigens, the plates were washed; in each well 100 µl of 6 M urea was added for 3 mins at room temperature. Urea was removed, the plates were washed again and ELISA was continued as described elsewhere (5, 7).
Avidity test with commercial kit – Enzygnost Anti-EBV IgG; Avidity Reagent for Enzygnost (Dade Behring, Marburg, Germany). This test was performed following instructions of the manufacturer (8).
Measurement of avidity. Avidity of IgG antibodies is expressed by the avidity index (AI). In »in house« avidity test AI was obtained when optical density (OD) of the sample, treated with urea, was devided by OD of the same sample which was not treated with urea. When AI is 0.50–1.00, it means high avidity of IgG antibodies and this is characteristic for past infection. When AI is < 0.25, it means low avidity of IgG antibodies and this reflects acute infection. AI values of 0.25–0.50 are equivocal (5).
By the use of commercial test kit, AI was determined in the same way as in »in house« avidity test; the only difference was that the quocient was multiplied by 100. So AI was expressed in percentages. Cut off value was 40% (8).
Statistics. The results were statistically evaluated in different EBV infections by χ^2 test.

Results

»In house« avidity test

Avidity of IgG anti-EA. IgG anti-EA were present in 31 sera tested: 18 sera belonged to patients with acute infection, 13 to patients with recent infection. In 6 sera of patients with acute infection OD fell under the cut off value after the treatment with urea. In 2 sera of patients with recent infection antibodies were destroyed after the treatment with urea. Average values of AI in acute and recent infection are presented in Figure 1. The differences are statistically significant (p = 0.05).
Avidity of IgG anti-EBNA. IgG anti-EBNA were determined in sera of 25 patients: in 17 of them with virus reactivation, in 8 with recent infection. After the treatment with urea in 8 sera of patients with reactivated virus a minor fall of OD was found. The same results were observed in the group of patients with recent infection. Average AI values in both groups were high (Fig. 2). The differences were not statistically significant.

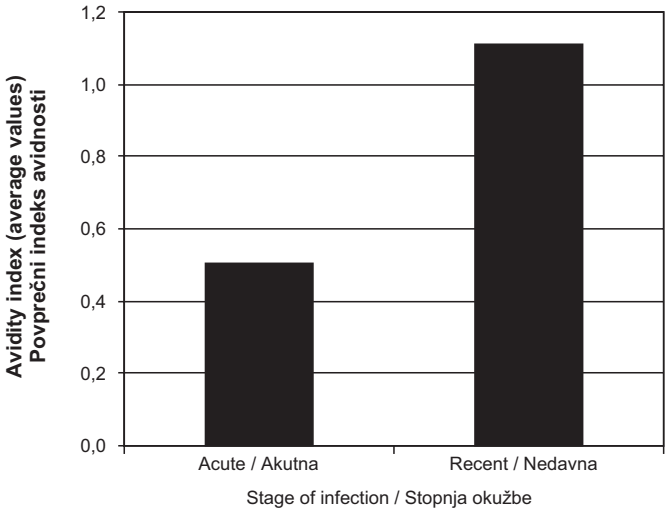

Fig. 1. *Graphical presentation of avidity index for IgG anti-EA.*

Sl. 1. *Grafični prikaz indeksa avidnosti IgG anti-EA.*

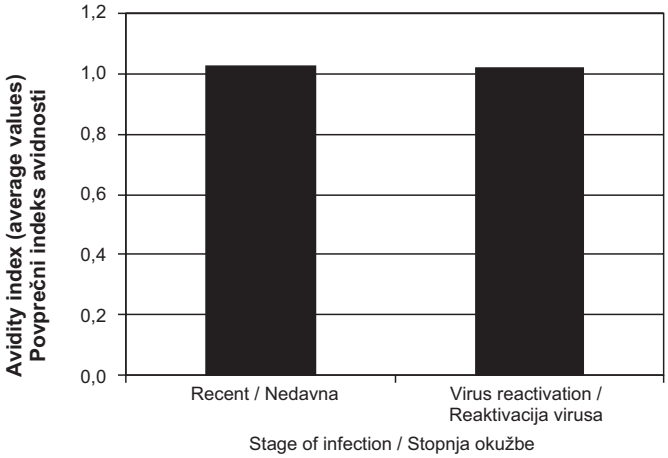

Fig. 2. *Graphical presentation of avidity index for IgG anti-EBNA.*

Sl. 2. *Grafični prikaz indeksa avidnosti IgG anti-EBNA.*

Avidity of IgG anti-VCA. IgG anti-VCA were determined in sera of 18 patients; 4 of them with acute infection, 4 with the past infection, 5 with recent infection and 5 of them experienced the virus reactivation. In all sera the fall of OD values were found after the treatment with urea. Average AI values were borderline or higher (Fig. 3). The differences were statistically significant only between patients with acute and recent infection (p = 0.05).

Commercial test kit

Avidity of IgG anti-EBV. IgG anti-EBV were found in sera of 17 patients: 7 of them had past infection, 4 patients had reactivated virus, in 3 patients acute infection was determinated and in 3 patients recent infection. In sera of patients with acute infection the evident fall of OD values were found after the treatment with »avidity reagent«. Average AI values were low in this group as well. In other groups of sera AI was high (Fig. 4). Statistically significant differences were found between sera of patients with acute and past infection and also between acute infection and reactivated virus (p = 0.05). The differences in AI between sera with acute and recent infection were not significant.

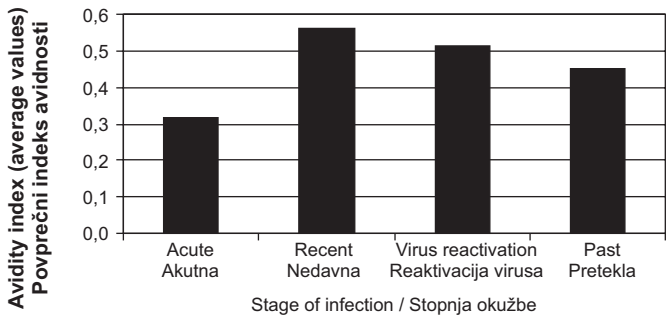

Fig. 3. *Graphical presentation of avidity index of IgG anti-VCA.*

Sl. 3. *Grafični prikaz indeksa avidnosti IgG anti-VCA.*

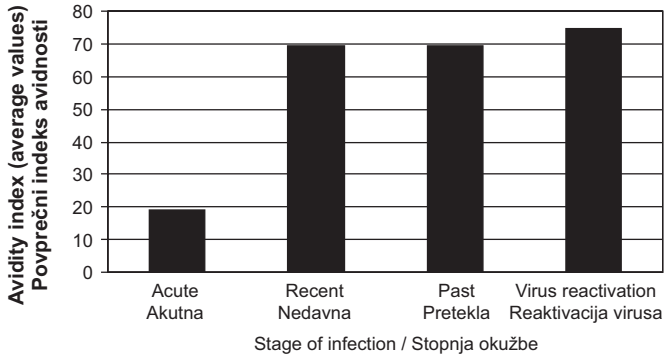

Fig. 4. *Graphical presentation of avidity index of IgG anti-EBV.*

Sl. 4. *Grafični prikaz indeksa avidnosti IgG anti-EBV.*

Specificity and sensitivity of the avidity test

The specificity of our test was 59.38% and the sensitivity 91.67%.

Discussion

The measurement of IgG anti-EBV avidity might be a supplementary diagnostic procedure in the situation of problematic clinical cases, when we are not able to define precisely the stage of EBV infection (8, 9). Normally, we do diagnosis of EBV infection by ELISA; sometimes the result is not in good correlation to the clinical state of the patient – in most cases these are adult patients with laboratory determined recent infection. This is, according to the known EBV epidemiological situation in our population, quite unusual. We suppose that these patients in fact do not experience recent infection, but they might have just gone through EBV reactivation: IgM anti-EA have dissapeared, the patients still have IgG anti-EA and of course they have permanently present IgG anti-EBNA. This patients were a perfect challange for us to introduce »in house« avidity test, which has found that both patients with »recent« infection and patients with EBV reactivation had IgG antibodies with high avidity; but as a rule, in recent infection IgG antibodies should have low avidity. In this study only adult patients were included and so it remains to compare IgG avidity of children with laboratory diagnosed recent infection. Preliminary we presume that the avidity of IgG (anti-EA, anti-VCA and eventually anti-EBNA) should be low.

Avidity test we have used in the study was not standardized. This is also the reason why the differences in optical density of »normal« sera and of sera treated with urea were not as clear as we had expected. The same problem was mentioned by Weissbrich (6). This author advises to use immunofluorescence when avidity is to be measured. So we here tested a minor number of sera with anticomplement immunofluorescence. To measure avidity, the treatment with urea was included in the test as well. The results incouraged us and we agree with Weissbrich, that the results of avidity in immunofluorescence are more easy to interpret than with ELISA.

At the end of the study the commercial avidity test was offered to us and we were able to test some sera. We believe that this test kit will be really very helpful in EBV diagnosis. By the use of this kit the avidity of all IgG antibodies are determined; the results our study point to the avidity of specific IgG and this is our scientific contribution to general knowledge of avidity.

The main privilege of the commercial test kit is that the difference between acute and all the other stages of EBV infections is clearly evident – far more clear than with the »in house« avidity test.

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