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Comparison of several ELISA tests for detecting the presence of IgG and IgM against herpes simplex viruses

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Abstract

Four enzyme-linked immunosorbent assays designated test 1 (ETI-HSVK-G 1/2); test 2 (ETI-HSVK-M 1/2); test 3 (ETI-HSVK-G 2), and test 4 (BioElisa HSV2 IgG) were studied to evaluate different stages of herpes simplex virus (HSV) infection. Samples (50 sera and 14 cerebrospinal fluid) were included in four groups. Group 1 consisted of samples from patients with primary HSV infections; group 2 comprised samples from patients with recurrent HSV infections; group 3 were samples nonreactive to HSV; and group 4 were samples from patients with infections by other herpes viruses (4a, chickenpox; 4b, herpes zoster; and 4c, infectious mononucleosis by Epstein-Barr virus). The percentages of agreement between tests 1 and 2 were 100 and 72.1%, respectively. The total diagnostic values of tests 1 and 2 were: 100 and 50% sensitivity, respectively; and 100 and 89% specificity, respectively. Few positive results for HSV-2 infection were found, and so, tests 3 and 4 were not evaluated. The results of tests 3 and 4 for a chickenpox patient, and a herpes zoster patient were not in agreement.

Introduction

Herpes simplex virus (HSV) infections have a high prevalence in our environment (Essers *et al.*, 1991; Kavallierou *et al.*, 1991). The diagnosis of these infections is usually carried out by detecting specific antibodies against HSV. The presence of specific antibody has a definite predictive value in childhood, but its significance decreases with age. Due to the elevated prevalence in children, the absence of antibodies in serum at every age has only negative predictive value, and antibody detection in cerebrospinal fluid (CSF) is necessary to confirm herpetic encephalitis (Guerrero *et al.*, 1996).

The investigation of antibodies against herpes viruses was performed by enzyme-linked immunosorbent assay (ELISA) which detected immunoglobulin G (IgG) and/or immunoglobulin M (IgM) using infected cell or synthetic antigens. When cell-expressed antigens are employed, a greater sensitivity is guaranteed because multiple epitopes are used. When only a few of the recombinant antigens are utilized, the specificity is increased, but sensitivity probably decreases.

The two HSV types (HSV-1 and 2) share many antigens, and only a few of those (*e.g.* envelope glycoproteins, gC and gG) are antigenically distinct. Recently, three new commercial recombinant ELISA tests to detect IgG or IgM against both HSV types, together or separately, have become available. In the present report, we have analysed the diagnostic value of these new techniques to distinguish different HSV states of infection.

Material and methods

Samples

Fifty serum and fourteen CSF samples were processed at the Microbiology Department, University Hospital San Cecilio during 1997. They were investigated using IgG (test 1) and IgM (test 2) against HSV 1 + 2 (ETI-HSVK-G 1/2; DiaSorin, Italy). In test 3 IgG was against HSV-2 using ETI-HSVK-G 2 (DiaSorin); and in test 4 HSV-2 was against IgG (Biokit, BioElisa, Spain).

The 64 samples were divided into four groups. Group 1 consisted of two samples from primary HSV-infected individuals defined by the presence of compatible clinical signs, low avidity IgG, and specific IgM (Enzygnost HSV, DadeBehring, Germany) according to Gutiérrez *et al.* (1997). Group 2 comprised six samples from recurrent HSV-infected individuals, defined by the presence of compatible clinical signs, high avidity IgG increasing between two serum samples, and without specific IgM (Gutiérrez *et al.*, 1997). Group 3 contained 27 samples (14 sera and 13 CSF) from subjects without antibodies against HSV. Finally, group 4 had 29 samples from other herpes viral infections (group 4a, 14 chickenpox; group 4b, four herpes zoster; group 4c, eleven mononucleosis infections caused by Epstein-Barr virus (EBV). The different diseases were defined by the presence of compatible clinical signs, low avidity IgG, and specific IgM, except in the case of herpes zoster (Enzygnost, DadeBehring) according to Gutiérrez *et al.* (1997). In group 4a, seven samples had IgG against HSV. In group 4b, one sample had IgG against HSV. In group 4c, one sample had IgG against HSV. No sample had IgM against HSV.

Techniques

Enzygnost (DadeBehring, Germany) is an indirect ELISA, described previously by us (Gutiérrez *et al.*, 1994; Gutiérrez *et al.*, 1999), which uses peroxidase-conjugated goat antihuman IgG or IgM. The antigens are an extract from infected cells expressing multiple viral antigens. The sample dilutions employed were 1:231 for IgG, and 1:42 for IgM (containing anti-IgG). The chromogenic substrate was tetra-

methylbenzidine. In order to evaluate the IgG titre the α-Method system was utilized (Dopatka and Schuy, 1996). The IgM is reported in qualitative form.

Tests 1 and 2 (ETI-HSVK 1+2) used an indirect ELISA for IgG, and a capture ELISA for IgM, involving peroxidase-conjugated murine anti-human IgG (test 1) or IgM (test 2). The antigen is the viral recombinant glycoprotein B. The sample dilutions employed were 1:1 for IgG, and IgM. The assays were carried out on 96-well plates coated with the recombinant antigen, and were processed by an automated delimiter (Tecan Megaflex, Austria) and a Behring ELISA processor III system (BEP III; DadeBehring), for a total running time of 3 h. The chromogenic substrate was tetramethylbenzidine. The results were expressed in qualitative form. A positive result was registered when the sample absorbance at 450/630 nm ($A_{450/630}$) was higher than the $A_{450/630}$ of cut-off positive samples. Values around a cut-off 90–110% were retested, and only the second result was recorded.

Test 3 (ETI-HSVK-G 2) was carried out like test 1, but its antigen was the recombinant HSV-2 glycoprotein G2. In test 4 (BioElisa HSV-2G) a similar method was used to that previously described, except that its total running time was 2.5 h. The sensitivity and specificity, for tests 1 and 2, were determined when primary or past infection groups were compared. The IgM value was used to diagnose the current disease.

Table 1 Results for anti-HSV-1 + 2 immunoglobulin G (IgG) and immunoglobulin M (IgM), and anti-HSV-2 IgG in the serum groups

Serum groups	Samples	Positive results (agreement %): Test 1	Test 2	Test 3	Test 4
1	2	2 (100)	1 (50)	0	0
2	6	6 (100)	3 (50)	1	1
3	27	0 (100)	0 (100)	0	0
4a	14	7 (100)	2 (72.2)	0	1
4b	4	1 (100)	0 (100)	0	1
4c	11	1 (100)	4 (60)	0	0

Group 1, HSV primary infection; group 2, HSV recurrent infection; group 3, nonreactive sera and CSF; group 4a, samples from chickenpox patients (seven samples have anti-HSV IgG); group 4b, samples from herpes zoster patients (one sample has anti-HSV IgG); group 4c, EBV primary infection (one sample has anti-HSV IgG). Test 1, ETI-HSVK-G 1/2, DiaSorin, Italy; test 2, ETI-HSVK-M 1/2, DiaSorin; test 3 ETI-HSVK-G 2, DiaSorin; test 4, BioELISA HSV-2 IgG, Biokit, Spain.

Table 2 Results of agreement between tests 3 and 4

Test	Test 3 (positive)	Test 3 (negative)
4 (positive)	1	2
4 (negative)	0	61

Results

The results obtained are given in Table 1. The mean percentage agreement for test 1 was 100%, and for test 2 was 72.1%. This poor result was due to the presence of anti-HSV IgM during the course of chickenpox and EBV infectious mononucleosis, as well as the lack of anti-HSV IgM during primary or recurrent HSV. The diagnostic reliability for tests 1 and 2 were: sensitivity, 100 and 50%, respectively, and specificity, 100 and 89%, respectively. In tests 3 and 4 we found that positive results for HSV-2 infection were infrequent. Discrepancies in the results between tests 3 and 4 (one for a chickenpox patient, and another for a herpes zoster patient) were discovered. The results of conformity for tests 3 and 4 are given in Table 2.

Discussion

The enzyme-linked immunosorbent assay is one of the laboratory methods most extensively employed to detect HSV-specific IgM or IgG, because of its easy performance, and its acceptable sensitivity. Until recent times, reliable commercial ELISA kits, which facilitate distinguishing between HSV-1 and HSV-2, were not available; only Western-blot tests offered greater guarantees. However, these assays are expensive, there is a lack of automation, and they are difficult to interpret (Eis-Hübinger *et al.*, 1999; Cowan *et al.*, 1996; Ashley and Wald, 1999; Enders *et al.*, 1998).

We employed a commercial recombinant antigen ELISA to detect specific HSV IgG (test 1) and IgM (test 2). However, its efficiency was reduced to detect specific IgM, because test 2 had lower sensitivity and specificity. Perhaps this was due to undetectable IgM levels.

Moreover, patients who were IgM positive showed clonal lymphocyte B stimulation in the course of the other infection. We conclude that test 2 was less useful in detecting primary or recurrent HSV infections, and test 1 was useful in detecting IgG.

In previous reports a reliable HSV serous diagnosis has been achieved with few variations in sensitivity and specificity evident among the enzyme immune assays. This has facilitated differentiating between HSV-1 and HSV-2 (Eis-Hübinger *et al.*, 1999; Ashley *et al.*, 1998). Indeed, tests 3 and 4 (ELISA) with antigen gG2 were used to detect and compare specific HSV-2 IgG, and two negative results in test 3 disagreed with results from test 4. This was due to its reduced sensitivity to detect specific antibodies against HSV-2 in subjects infected by other herpes viruses and antibodies to HSV. Test 4, employed in our study, was able to detect IgG against HSV-2 in subjects with infections by other herpes viruses (chickenpox, herpes zoster). Therefore, we conclude that test 4 may be more useful in detecting specific IgG against HSV-2.

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