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Rapid Immunochromatographic Test for Hantavirus Andes Contrasted With Capture-IgM ELISA for Detection of Andes-Specific IgM Antibodies

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Hantavirus is associated with hemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). The clinical diagnosis of hantavirus infections has been confirmed routinely by the immunofluorescence antibody assay (IFA) or enzyme-linked immunosorbent assay (ELISA). A rapid and easy diagnostic test for hantavirus infection is required. A new immunochromatographic assay for hantavirus, POC-PUU, useful for the diagnosis of epidemic nephrophaty associated with hantavirus Puumala in Europe, was evaluated in Chile. This test is based on recombinant N-protein of hantavirus Puumala, and cross-reacts with other hantaviruses. Eighty human sera were selected at random from patients from Southern Chile who were suspected with HPS. The hantavirus capture-IgM ELISA was compared with a commercially available POC-PUU test (POC PUUMALA, Reagena Ltd., Toivala, Finland). The test sensitivity and specificity of the POC-PUU test were 97 and 90%, respectively. It is important to note that although the test is not specific for Andes virus the sensitivity and specificity were above 90%, which indicates good reactivity to the Puumala nucleoprotein antigen. As this test is cost-effective, with a high negative value, rapid and easy to carry out, specialized personnel are not necessary, nor does it require specialized equipment. Its usefulness for diagnosis is important in hospitals far from reference centers and areas with a high incidence of HPS cases. ***J. Med. Virol. 79:41–44, 2007.*** © 2006 Wiley-Liss, Inc.

KEY WORDS: Hanta; POC PUUMALA; Andes virus

INTRODUCTION

Hantaviruses are rodent-borne RNA viruses that belong to the family *Bunyaviridae*. Their genome is negative stranded and tripartite encoding an RNA-dependent RNA polymerase, two envelope glycoproteins (G1 and G2), and a nucleocapsid protein (N) [Schmaljohn et al., 1985]. In humans, hantaviruses cause two distinct diseases, a hemorrhagic fever with renal syndrome (HFRS) and a hantavirus pulmonary syndrome (HPS) [Smadel, 1953; McCaughey and Hart, 2000]. In Europe, two hantaviruses cause HFRS, Puumala virus (PUUV) and Dobrava virus (DOBV) [Hujakka et al., 2003]. HPS has been reported in virtually the entire American Continent [CDC, 1993; Childs et al., 1994; Hjelle et al., 1994; Khan et al., 1995; Parisi et al., 1996]. At present, most of the reported HPS cases are from South America, where the Andes strain of hantavirus (ANDV) is responsible for most cases [Nichol et al., 1993; Padula et al., 2000]. ANDV is the only hantavirus associated with all reported cases of HPS disease in Chile. In Argentina, this virus has been identified in blood samples from patients by reverse transcription-polymerase chain reaction (RT-PCR); and in Chile, it was isolated from a human blood sample [López et al., 1997; Galeno et al., 2000].

In Chile, the first case of HPS was described in 1995, nevertheless cases have been identified retrospectively as far back as 1975 [Toro et al., 1998; Baró et al., 1999]. At present, 485 cases have been reported (37% mortality, MINSAL, 2006) in different zones of Chile, V–XI Regions [Toro et al., 1998], which confirms the prediction that the disease may appear throughout the geographic distribution of the rodent reservoir, III–XI Regions [Murua, 1998].

The clinical diagnosis of hantavirus infections has been confirmed routinarily by the immunofluorescence antibody assay (IFA) or enzyme-linked immunosorbent assay (ELISA) with native viral antigens. Due to the

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hazardous nature of hantaviruses, their slow replication, and low and variable expression in cell culture, recombinant hantavirus proteins have been produced for use as antigens for serological assays [Feldmann et al., 1993; Padula et al., 2000].

Currently, the available serological tests require specialized laboratories with trained personnel and a delay in the results of at least 4–6 hr. A rapid and easy diagnosis test of hantavirus infection is necessary. A new immunochromatographic assay for hantavirus diagnosis, POC-PUU, useful for the diagnosis of epidemic nephrophaty associated with hantavirus Puumala in Europe, was tested in Chile. This test is based on the recombinant N-protein of hantavirus Pumala, and has cross-reactivity with other hantaviruses [Hujakka et al., 2003]. In the present study, the hantavirus capture-IgM ELISA reference technique was compared with the POC PUUMALA test (Reagena Ltd.). The samples tested were from HPS suspect patients from Chile.

MATERIALS AND METHODS

Samples Tested

Eighty human serums taken during 2000–2002 were selected at random from patients from IX and X Regions (Southern Chile) who were suspected with HPS. The samples were transported under universal bio-safety standards to the Virology Laboratory, Department of Clinical Microbiology, Universidad Austral de Chile. The samples were examined by the hantavirus capture immunoglobulin M (IgM) and immunoglobulin G (IgG) ELISA, 49 sera were negative for hantavirus and 31 were IgM or IgG/IgM positive. Afterwards, serum samples were aliquoted and stored at −70°C.

Reference Method

IgG was detected in blood samples by using an ANDV specific ELISA. The IgM was detected by using capture IgM ELISA as described previously [Padula et al., 2000]. Briefly, the blood samples (including samples from the controls) were diluted 1:100 and fourfold up to 1:6,400. Recombinant ANDV nucleoprotein (N-ANDV) provided by INEI-ANLIS "Dr. Carlos Malbrán," Argentina, was used as a specific antigen [Feldmann et al., 1993; Lazaro et al., 2000; Padula et al., 2000]; and Laguna Negra antigen for IgM (was provided by Centers for Disease Control and Prevention (CDC Atlanta, EEUU).

Immunoglobulins were detected by using peroxidase-labeled affinity purified antibody to human IgG and IgM (heavy plus light chains) as conjugate. All commercial reagents were from Kirkegaard and Perry laboratory (KPL), USA. ABTS (2.2_-azino-di[3-ethyl-benzthiazoline sulfonate]) was used as the substrate for peroxidase, and absorbance was measured at 405 nm. The optical density (OD) of each test sample was subtracted from the OD of the corresponding dilution of the unspecific antigen run on the same microtiter plate. Samples with titers of less than 0.2 and 0.1 units were considered negative for IgG and IgM, respectively.

The POC PUUMALA Test

The POC PUUMALA test (Reagena Ltd.) uses a lateral-flow membrane-based assay technology, in which the baculovirus-expressed highly purified PUUV-N antigen is immobilized on the nitrocellulose membrane. The PUUV-N antigen is purified by urea extraction from Sf9 insect cells (ATCC CRL 1711) as described previously [Vapalahti et al., 1996]. The test result is visualized by using gold-conjugated rabbit anti-human IgM antibodies dried in the conjugate pad.

The POC PUUMALA test is a rapid test that uses a sample well and result window in every test device. After adding the sample to the sample well, two drops of running buffer are added. The result is detected within 5 min after addition of the running buffer by the presence (positive samples) or absence (negative samples) of the red line in the test window marked by a T. The red line is formed by precipitation of the PUUV-N-specific IgM and the gold-conjugated anti-human IgM antibodies with the PUUV-N antigen in the membrane. The control line is formed into the test window marked by a C as a consequence of the reaction between the goat anti-rabbit antibody in the control line and the gold-conjugated rabbit anti-human antibody.

Interpretation of Results

Samples were examined by a single observer, without knowledge of the cited serology results, interpreting the results as negative (−), positive (+), weak positive (++/−), or very weak reactivity (+/−−).

RESULTS

A total of 80 samples from patients suspected with HPS were examined; 31 were positive by the capture IgM ELISA reference method. These positive samples were tested by POC-PUU test. Twenty-five of these 31 samples were interpreted as positive (80.6%), 5 were interpreted as weak positives (16.1%), and 1 sample was interpreted as very weak positive (3.2%). None of these samples was interpreted as completely negative.

On the other hand, of the 49 samples negative by the capture IgM ELISA method tested by POC-PUU test, 5 were found to be weak positives (10%), 2 showed very weak reactivity (4.1%), and the remaining 42 were clearly negative (85.7%).

On the other hand, from the 49 samples tested negative for capture IgM ELISA analyzed by IgG ELISA, 5 were positive (Table I).

Specificity and sensitivity for this random sampling POC-PUU test were calculated. Weak positive results for POC-PUU were included as positive and very weak reactivity for POC-PUU were included as negative (Table II). The POC-PUU percentages calculated were 97% sensitivity and 90% specificity, with 3% false negatives and 10% false positives.

The POC-PUU test presented a moderate (9.7) likelihood ratio (LR) for a (+) test and broad changes (0.033) for a (−) LR.

TABLE I. Distribution of Results as Capture-IgM ELISA/ POC-PUU Test

Serum panel		POC-PUU test			
Reference method (ELISA)	N°	+	++/−	+/−−	−
IgM positive/IgG negative	21	15	5	1	0
IgM positive/IgG positive	10	10	0	0	0
IgM negative/IgG negative	44	0	5	2	37
IgM negative/IgG positive	05	0	0	0	5

Test, POC-PUU; +, clear positive result; ++/−, weak positive result; +/−−, very weak reactivity (ghost line); −, clear negative, no ghost line.

DISCUSSION

Considering that the risk of hantavirus infection is higher in rural areas, there is a need to use a simple test, easy to carry out, without special equipment requirement, to be applied in rural hospitals for rapid diagnosis. Hence, in this study the hantavirus capture-IgM ELISA reference technique was compared with the commercially available POC PUUMALA test. The samples tested were from HPS suspect patients from Southern Chile.

The IgM-specific POC-PUU test has important diagnostic value because it allows differentiation between acute infections (IgM-positive) and past infection (IgG-positive). Seroprevalence studies in South America have shown that some populations have had frequent exposure to hantaviruses in the absence of known clinical symptoms, as seen in some native populations from Paraguay and Northern Argentina (40 and 17%, respectively, Ferrer et al., 1998). Seroprevalence studies in Southern Chile indicated 2 and 13.1% in urban and rural areas, respectively, of Aysén (Region XI) [Valderrama et al., 1998]; global seroprevalence of 0.7% (ranging from 0 to 6%, depending on the community) in the general adult population in Region IX [Täger et al., 2003]; and 6.3% in the general population of Bahia San Pedro (Region X) [Zamorano et al., 1999].

The percentages of POC-PUU test sensitivity and specificity were 97 and 90%, respectively. It is important to note that although the test is not Andes virus specific, the sensitivity and specificity were above 90%, indicating reactivity to the Puumala nucleoprotein antigen.

Weak positive results from the POC-PUU test were interpreted as positive results and very weak reactivity was interpreted as negative. This distribution of results was made considering the clinical implications in mistaken diagnostic of negative results instead of positive. On the other hand, the high sensitivity (97%) and only 10% of false positives of POC-PUU test are appropriate in the context of the clinical epidemiological situation. The application of this test is suggested as diagnosis instrument in rural areas far from references centers, where most of the suspected cases of hantavirus infection are concentrated. This would allow an earlier prediction to send HSP suspected cases to larger reference centers for application of the capture-IgM ELISA, to confirm or exclude a false positive POC-PUU test results.

TABLE II. Contingency Table: POC-PUU/ Capture-IgM ELISA

	IgM ELISA positive	IgM ELISA negative	Total
POC-PUU (+) POC-PUU (++/−)	30	5	35
POC-PUU (+/−−) POC-PUU (−)	01	44	45
Total	31	49	80

Test, POC-PUU; +, clear positive result; ++/−, weak positive result; +/−, very weak reactivity (ghost line); −, clear negative, no ghost line.

Upon assessment of the LR of the POC-PUU test, it is significantly more likely to be related to a negative result LR (−) of the POC-PUU test. In other words, when the result is negative, there is an increased assurance of a negative case for hantavirus infection.

As with studies published on the POC-PUU test [Hujakka et al., 2001], frozen samples show a weak reaction line that may be interpreted as false positive. However, preliminary (unpublished) results of non-frozen sera from HPS suspected patients test by POC-PUU [Hujakka et al., 2003] permitted the diagnosis of HPS cases with a 100% correlation with the reference technique, the capture-IgM ELISA.

The POC-PUU is a cost-effective test with a high negative value, rapid and easy to carry out, as trained personnel is not necessary nor does it require specialized equipment.

These tests can be especially important in hospitals far from reference centers and areas with a high incidence of cases of HPS without disregarding the universal norms of caution in the handling of clinical samples.

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