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HANTAVIRUS PULMONARY SYNDROME. REPORT OF THE FIRST THREE CASES IN SÃO PAULO, BRAZIL

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SUMMARY

The hantavirus pulmonary syndrome was first recognized in cases that occurred in the U.S. in 1993, which served as an alert not only for American physicians but also for physicians in other countries for the identification of the disease. In the city of São Paulo, Brazil, 3 cases of the syndrome were recorded in 1993. The patients were young brothers residing in the Mata Atlântica (Atlantic Forest) region submitted to recent deforestation. Two of the patients died of acute respiratory insufficiency and the third recovered without sequelae. In the surviving patient the diagnosis was established by a laboratory criterion based on the detection of specific IgM and IgG class antibodies by indirect immunofluorescence. In the two patients who died, the diagnosis was confirmed by laboratory tests using immunoperoxidase technique for hantavirus in tissue, in histological lung and heart sections in one case, and by clinical and epidemiological data in the other.

KEYWORDS: Hantavirus pulmonary syndrome; Hantavirus; Emergent disease.

INTRODUCTION

Hantavirus disease, considered to be an emergent disease[1], started to attract again the attention of investigators after the occurrence of cases with predominantly pulmonary involvement in the U.S. in 1993[2,8]. The disease attacked previously healthy individuals who first presented symptoms similar to those of the flu, with a febrile prodrome that rapidly evolved to non-cardiogenic pulmonary edema, respiratory insufficiency and shock[23]. The investigations carried out in the U.S. showed that this syndrome was caused by hantavirus, with the isolation and identification of three viruses: Sin Nombre in the Southwestern region[5], Black Creek Canal virus (BCCV) in Florida[10,18], and Bayou virus in Louisiana[12,15]. Further investigation revealed the epidemiological chain as well as the natural reservoirs at those sites: the small wild rodents *Peromyscus maniculatus*, *Sigmodon hispidus*, *Oryzomys palustris* and others[16,19].

Studies showing hantavirus infection have been carried out in Brazil. LeDUC et al.[13,14] carried out a serologic survey on urban rats (*Rattus norvegicus*) in different towns in South America in the 1980's. These investigators detected positivity of serum samples from rats captured in the towns of Belém, São Paulo, and Recife-Olinda in Brazil, and in Buenos Aires, Argentina. A virus isolated from one of the rats captured in Belém was found to be antigenically similar to the *Seoul* virus. The results of these surveys showed that hantavirus was disseminated throughout South America.

In a serologic survey carried out in the Amazon Region, PINHEIRO et al.[17] detected anti-*Hantaan* virus antibodies in 7% of the human blood samples studied. VASCONCELOS et al.[22], in a serologic survey carried out in 1991 after the occurrence of several fatal human cases of hemorrhagic fever in Manaus, Brazil, found *Hantaan* positivity by immunofluorescence in 45.2% of the serum samples from 84 persons who resided close to the fatal cases. These investigators detected 54.3% positivity in sera from 48 blood donors at the Manaus blood bank submitted to the same test, and in 10.4% of 48 serum samples from individuals living in Tucuruí, Pará.

In the 1980's, IVERSSON et al.[9] carried out a serologic survey among persons living in the states of São Paulo and Paraná and detected 3% hantavirus positivity by ELISA, 15.6% of which were also positive by immunofluorescence and by the neutralization technique with plate reduction.

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The objective of the present study was to report the occurrence of human hantavirus disease in Brazil with predominantly respiratory clinical manifestations.

CASE REPORTS

We report the occurrence of human hantavirus infection in the State of São Paulo, Brazil, in November and December 1993. The patients were 3 white brothers who were previously healthy and had no history of disease. They were born in São Paulo city and had been living for 2 months in the rural zone of the city of Juquitiba, Ribeira Valley region, São Paulo.

Case 1 was an 18-year-old patient who started to show clinical symptoms on November 21, with fever and frontoparietal headache of medium intensity that subsided with analgesics. One day later he felt rotary dizziness, adynamia, anorexia, generalized myalgia that prevented him from walking, and a dry cough. These symptoms persisted for 4 days, when the patient started to improve gradually, with spontaneous resolution of the clinical picture, except for the persistence of a cough without expectoration. The patient came to the First Aid Unit of the Emílio Ribas Infectology Institute 15 days later when he was feeling well, with no symptoms or clinical alterations upon physical examination. The visit was motivated by the death of two brothers with the same symptoms during the same period. The patient was followed up for 1 month and no symptoms were observed. The laboratory tests performed (blood glucose levels, urea, creatinine, sodium, potassium, pyruvic and oxaloacetic glutamic transaminase and blood counts) were normal. Serologic tests for syphilis (VDRL) and leptospirosis (microscopic serum agglutination) were negative. Serologic tests for hantavirus were performed at the Evandro Chagas Institute (Belém, Pará) by indirect immunofluorescence on slides prepared with VERO cell lines infected with the *Hantan hantaan* virus (A549P15, VEROP3). The serum sample collected on December 7, 1993 showed the presence of IgM class antibodies with a 1:128 titer, and IgG antibodies with a 1:32 titer. A sample collected on December 20, 1993 also showed the presence of IgM antibodies at 1:16 titer and of IgG antibodies at 1:32 titer. On January 5, 1994, no IgM class antibodies were detected and IgG antibodies were detected at a titer of 1:128.

Case 2 was a 20 year-old patient whose symptoms had started on December 3, when he presented with general malaise, fever that was not measured, nausea, vomiting, a dry cough, and a lumbar pain. On that occasion he went to the Municipal Fisrt Aid Unit of Juquitiba where he received medical care and was submitted to a chest X-ray which showed no alterations. Symptomatic medication was administered and the patient was discharged. Two days later he was seen at the First Aid Unit of the Emílio Ribas Infectology Institute, where he reported persistent high fever (39°C), accompanied by sweating, dizziness, fainting spells and decreased urine volume. Upon admission to the hospital he was in good general condition, walking, oriented, with good color, febrile (39°C), hydrated, tachypneic (RF = 30 respiratory movements per minute) and with good peripheral perfusion. Pulmonary examination revealed crepitant stertors in the middle and upper thirds of the right hemithorax.

Three hours after admission he presented progressive hemodynamic worsening progressing to shock and respiratory insufficiency, with no clinical signs of bleeding. He was transferred to the intensive care unit where treament with volemic replacement (intravenous fluids), vasoactive drugs and antibiotics was started.

The patient was intubated and placed on mechanical ventilation due to progressive respiratory failure ($PaO_2 < 60$ mmHg with a face mask). At first he was submitted to mechanical ventilation in order to maintain an arterial oxygen saturation (SaO_2) of 0.9 or more. He developed oliguria and metabolic acidosis and was treated with diuretics and vasoactive drugs. Shock still persisted 4 hours later, hemodynamic worsening occurred and the patient died 15 hours after admission. Laboratory tests showed 53% hematocrit (normal values, 42 to 46%), 17.7 g% hemoglobin (normal values, 13 to 16 g%), and a platelet number reduced to 58,000/mm^3 (normal values, 200,000 to 400,000/mm^3).

The patient suddenly developed radiologic alterations with a bilateral pulmonary infiltrate with heterogeneous condensation in the middle and lower thirds of the right hemithorax.

Anatomopathological examination showed pulmonary changes compatible with pulmonary edema and bilateral hydrothorax (400 ml on the left and 700 ml on the right) and cardiac dilatation. Microscopic lung examination revealed interstitial pneumonia with focal points of acute broncho-pneumonia and focal areas with macrophages containing hemosiderin. The liver also presented congestion and hemosiderin.

Immunoperoxidase technique for leptospirosis were performed in the pathology division of the Adolfo Lutz Institute on lung, spleen and kidney sections, with no detection of antigens. Using an immunoperoxidase technique for hantavirus carried out on lung sections revealed structures that were suggestive of hantavirus particles. Immunoperoxidase technique carried out in the pathology service of the Emílio Ribas Infectology Institute on heart sections showed structures that were suggestive of hantavirus particles (Figure 1).

A frozen lung section was submitted to the PCR-RT test in the division of viral disease and ricketts of the National Center of Infectious Diseases, Center of Disease Control and Prevention, Atlanta, GA, U.S., but no viral bands were detected.

Case 3 was a 16-year-old patient with a history of headache, fever (39°C), adynamia, asthenia, myalgia and cough without expectoration starting on November 24. Worsening of the respiratory symptoms led him to seek medical care at the Municipal First Aid Unit of Juquitiba 2 days later, when he was

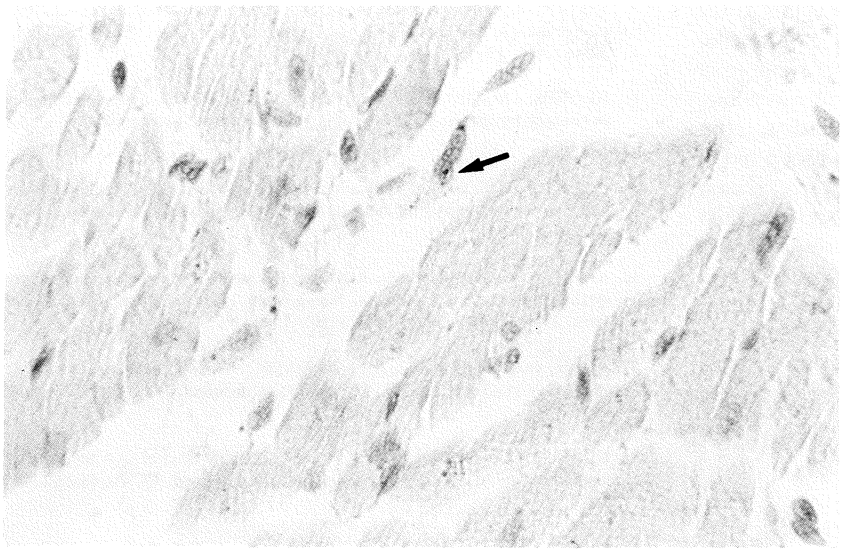

Fig. 1 – Immunolocalization of hantavirus antigens in a histological section of the heart of a patient who died of hantavirus pulmonary syndrome. × 800 (Case 2).

admitted with fever and skin pallor. Signs and symptoms worsened during hospitalization, with the development of dyspnea, chest and abdominal pains, acute respiratory insufficiency, pulmonary edema and shock, followed by death 36 hours after admission. Samples of biological material for virologic diagnosis were not obtained from this patient. The only examination performed on this patient was a chest X-ray which was altered.

DISCUSSION

The patients with hantavirus disease were residing in a region of the Atlantic Forest which had been recently submitted to deforestation. Living conditions were precarious, without appropriate storage space for human food or for animal feed. The dwelling was constantly invaded by wild rodents looking for food.

The virus is transmitted horizontally among rodents, and infect rodents shed virus in saliva, urine and feces for many weeks following infection, but duration of shedding and the period of maximum infectivity are not known. Transmission to humans is thought to occur predominately after infect rodent excreta are inhaled, althougt infections after rodent bites have been reported[11].

The major symptoms observed in the cases that occurred in São Paulo were fever and respiratory involvement, as also recorded in the U.S.[11]. However, in the 2 cases with laboratory confirmation reported here, the patients complained of dizziness as an important symptom, a fact that was not properly elucidated and that should be better investigated if other cases should arise.

The laboratory tests for the detection of hantavirus antibodies showed that the first patient presented specific IgM and IgG titers by indirect immunofluorescence on a slide in serial serum samples, which established the etiologic diagnosis. This patient showed a good clinical course and was cured. This suggests that human pulmonary hantavirus disease may occur with symptoms of different intensity, requiring greater attention even in cases of mild respiratory manifestations.

Hemoconcentration and platelet depletion were observed in the second patient and the diagnosis was confirmed by immunoperoxidase technique in histological lung and heart sections, revealing structures that were suggestive of hantavirus particles. However, the frozen lung tissue fragment used for the RT-PCR tests did not show viral bands, probably because Brazilian viruses differ from those detected in the U.S. and require different primers for sequencing. This fact was later confirmed by NICHOL et al., cited by KHAN et al.[10], who established the viral sequence of a Brazilian case of hantavirus pulmonary syndrome.

The third patient, who was not submitted to specific laboratory tests, was considered to be a case of hantavirus disease on the basis of clinical and epidemiological criteria.

The appropriate clinical history, in combination with a fever, unexplained acute respiratory distress syndrome, hemoconcentration and thrombocytopenia, should help to identify new patients.

The information obtained in the present cases demonstrates the urgent need to isolate and identify the Brazilian hantavirus(es) in order to permit the development of serologic, immunohistochemical and PCR tests appropriate for our strain(s).

After the occurrence of hantavirus pulmonary syndrome was established in Brazil, it became clear that a better understanding of the epidemiological chain is needed, with the determination of the animal reservoir involved. Another need that arose after these cases was the setting up of an epidemiological surveillance system with proper attention paid to this public health problem, which was probably present earlier in our midst but remained undiagnosed.

RESUMO

Síndrome pulmonar por hantavírus. Relato dos três primeiros casos em São Paulo, Brasil

O síndrome pulmonar por hantavírus foi reconhecido a partir dos casos ocorridos nos EUA em 1993. Esse alerta serviu

tanto para os médicos americanos como para os de outros países na identificação de casos dessa doença. Na cidade de São Paulo, Brasil, em 1993, foram registrados 3 casos com essa manifestação da hantavirose; eram irmãos, do sexo masculino, jovens, residentes na região da Mata Atlântica, com desmatamento recente. Dois dos casos evoluíram para a morte causada por insuficiência respiratória aguda e o terceiro com recuperação sem seqüelas. No paciente sobrevivente o diagnóstico foi estabelecido por critério laboratorial pela pesquisa de anticorpos específicos das classes IgM e IgG pela técnica de imunofluorescência indireta. Dos casos que evoluíram para o óbito, um foi confirmado laboratorialmente pela técnica de imuno-histoquímica para hantavírus em tecido, em cortes histológicos de pulmão e coração; e no outro o diagnóstico foi clínico e epidemiológico.

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