Clinical and anatomopathological aspects of patients with hantavirus cardiopulmonary syndrome in Uberaba, Minas Gerais, Brazil

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ABSTRACT

The hantavirus cardiopulmonary syndrome is considered an emerging disease in the Americas. Since 1993, thousands of cases have been reported from different countries, but mainly from Brazil. This study aims to describe some epidemiological, clinical and anatomopathological aspects of patients with hantavirus who presented poor outcome and were autopsied in a teaching hospital in Brazil, from 2000 to 2014. Of the 10 patients included, nine were male (mean age 43.5 years) and seven reported previous contact with rodents. Fever was present in eight of ten patients, dyspnea in nine of ten and myalgia in seven of ten patients; hemoconcentration, leukocytosis, thrombocytopenia and renal involvement were evidenced in all the 10 cases. At autopsy, the main alterations were seen in the lungs: pleural effusion (8/10 cases), increased weight 2.5 to 3 times, congestion/edema (10/10), interstitial mononuclear inflammation (10/10), alveolar hemorrhage (7/10), pulmonary collapse (7/10), hyaline membranes (7/10) and alveolar neutrophilic infiltrate (2/10). Pericardial effusion (2/10), mild myocardium inflammation (4/10), right ventricle dilatation (1/10), polyploidy nuclei (3/10) and pericardial diffuse petechial (1/10) were also observed. The other organs exhibited discrete and non-specific alterations. Currently, this syndrome continues to be associated with high mortality directly linked to a late diagnosis and/or a misdiagnosis in the medical centers where these patients were seen for the first time. The anatomopathological findings at autopsy revealed the final phase of the process with pulmonary alterations, allowing a direct correlation with the severity of respiratory distress observed in these patients at admission.

KEYWORDS: Hantavirus infections. Autopsy. Hantavirus pulmonary syndrome. Hantavirus.

INTRODUCTION

Hantavirus is a tri-segmented and enveloped RNA virus belonging to Hantavirusidae family and Orthohantavirus genus¹. The virus is kept in the wild by chronic rodent infection, and human infection occurs by direct contact with contaminated animal excreta (saliva, feces, and urine) or by aerosols²,³. The first case of hantavirus cardiopulmonary syndrome (HCPS) was reported in North America in 1993; in the same year a similar case occurred in Juquitiba city, Sao Paulo State, Brazil. The isolated virus in this case was renamed with the city’s name⁴. Since then, several thousand cases have been reported in a number of Brazilian States and other countries. Different viruses have been associated with HCPS, such as Araraquara, Castelo dos Sonhos, Anajatuba, Laguna Negra and Rio Mamore⁵,⁶. From 1993 to 2018, 2,117 cases of HCPS were reported from 15 Brazilian
federal units, mainly in the South region (33.7%) followed by the Midwest region (25.7%) and Southeast region (24.2%). Most cases acquired the infection in a rural or wild environment, and half of them were involved with occupational activities related to agriculture. The lethality rate observed was 46.5% to 50%; in São Paulo State recent data pointed to 62.1% of lethality. HCPS comprises four clinical phases: prodromal, pulmonary edema and shock, diuresis and convalescence. The prodromal phase is characterized by non-specific symptoms, such as fever, myalgia, nausea, vomiting, diarrhea, dry cough and headache. After 3 to 6 days, the patient presents abrupt pulmonary edema, respiratory distress and shock. Those who survive evolve with diuresis and subsequently enter the convalescence period.

Several authors have suggested that the viral replication associated with hematological alterations and the host immune responses of specific tissue proinflammatory type constitute the most important pathophysiological mechanism in HCPS. Chronologically, this fact coincides with the evolution to severe respiratory failure due to endothelial lesion, leading to the increment of capillary permeability that ultimately explains the injuries seen in the final phase.

According to the literature review, there are few studies focused on the autopsy findings of patients with HCPS. For emerging diseases, these studies become extremely relevant and can contribute to improve the quality of clinical diagnosis and patients’ management, and to the better understanding of pathophysiological aspects. Since the first report of HCPS, sporadic cases have been seen every year in the Triângulo Mineiro Southeast region of Brazil. The present study aims to present some clinical and autopsy findings of patients with HCPS from a teaching hospital in Brazil.

**MATERIALS AND METHODS**

This descriptive and retrospective morphological study reports 10 patients with HCPS who died a few hours or days after admission, and who underwent autopsy from 2000 to 2014 at the Special Pathology Unit, Clinical Hospital of the Triângulo Mineiro Federal University, Uberaba, Brazil. The diagnosis of hantavirus infection was ascertained by enzyme-linked immunosorbsent assay (ELISA) to hantavirus specific IgM antibody in eight cases, and by immunohistochemistry (Hantavirus NC Antibody HN-1, Santa Cruz Biotechnology) in the other two cases by the analysis of lung, liver, or renal tissue fragments. The most relevant epidemiological, clinical and laboratory data of these cases were recorded through a review of the patients’ medical records and autopsy reports.

The study was approved by the Research Ethics Committee of the University under the protocol Nº 1170.

**RESULTS**

From 2000 to 2014, 867 autopsies were performed, of which 17 (1.9%) had a presumptive HCPS diagnosis, and ten had diagnostic confirmation by ELISA or immunohistochemistry methods. From these, nine cases were male (median age 43.5 years). Most of these cases occurred during the winter season in the Triângulo Mineiro region, and previous contact with rodents had been reported in seven cases. Fever, dyspnea and myalgia were the most common reported symptoms; and hemoconcentration, leukocytosis, thrombocytopenia and renal involvement were observed in all the 10 cases. Nine patients were severely ill with respiratory distress when they were admitted at the tertiary hospital and four of them presented shock. Death occurred due to respiratory failure in all the 10 cases. The time elapse between hospitalization and death ranged from 3 hours to 5 days (Table 1).

The results of the autopsies revealed that the body mass index ranged from 17.8 to 28.9 kg/m² (mean = 25.6 kg/m²). The mean weight of the hearts was 357.8 g [eRR 250-350 g], and in two cases pericardial effusion was evidenced. Discrete mononuclear inflammatory infiltrate was seen in four cases, discrete dilatation of right ventricle in one case, nuclear polyplody of the myocardial cells was seen in three cases and diffuse petechial in pericardium was evidenced in one case. Pleural effusion ranged from 120 to 1,680 mL in the eight cases in which this measurement was possible. The lungs weighed approximately 2.5 to 3 times the normal value (mean = 794.5 g) [eRR 275-400 g]. In the microscopic examination, congestion/edema was intense in five cases, moderate in two and discrete in three cases. Pulmonary hemorrhage was present in seven patients (discrete in three, moderate in three and intense in one case). Collapse was evident in seven cases, mainly at the bases of the lungs and associated with massive pleural effusion. In seven cases, hyaline membranes were revealed, focal in four and diffuse in three patients. All cases showed alveolar septa thickening with inflammatory infiltrate predominantly composed of lymphocytes (discrete in eight and moderate in two cases). Alveolar neutrophilic infiltrate was seen in two cases, probably associated with secondary bacterial infection. Table 2 summarizes the weight of the lungs and hearts of the 10 patients necropsied with hantavirus.

The average brain weight was 1440 g [eRR 1100-1400 g] and there were signs of edema in nine cases. Evidence of bleeding in the cerebellum and cerebral parenchyma was seen in one case. Half of the cases presented hepatomegaly.
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Table 1 - Main epidemiological and clinical data of 10 patients with hantavirus cardiopulmonary syndrome presenting poor outcome.

| Case | Age (years) | Gender | Occupation/profession | Previous contact with rodents | Onset of symptoms days | Time of hospital stay (days) | Severity of clinical status at admission |
|------|-------------|--------|-----------------------|-------------------------------|------------------------|-----------------------------|------------------------------------------|
| 1    | 45          | M      | Engineer              | +                             | 4                      | 2                           | Mild                                     |
| 2    | 58          | M      | Seller                | NR                            | 7                      | 3                           | Severe                                   |
| 3    | 44          | M      | Bricklayer            | +                             | 2                      | <1                          | Shock                                    |
| 4    | 44          | M      | Driver                | +                             | 7                      | 1                           | Severe                                   |
| 5    | 14          | M      | Student               | NR                            | 4                      | <1                          | Shock                                    |
| 6    | 36          | M      | Driver                | +                             | 4                      | 1                           | Shock                                    |
| 7    | 47          | M      | Driver                | +                             | 1                      | <1                          | Moderate                                 |
| 8    | 29          | M      | Farmer                | NR                            | 3                      | 5                           | Severe                                   |
| 9    | 43          | M      | Farmer                | +                             | 4                      | <1                          | Shock                                    |
| 10   | 43          | F      | Farmer                | +                             | 5                      | 2                           | Severe                                   |

NR = not reported; + = positive.

Table 2 - Weight of lungs and hearts in 10 necropsied patients with Hantavirus infections at the Clinical Hospital of UFTM.

| Case | Right lung weight | Left lung weight | Heart weight |
|------|-------------------|------------------|--------------|
| 1    | 1,105 g           | 950 g            | 440 g        |
| 2    | 1,010 g           | 930 g            | 350 g        |
| 3    | NR                | 845 g            | 365 g        |
| 4    | 1,100 g           | 1,250 g          | 375 g        |
| 5    | 600 g             | 500 g            | 245 g        |
| 6    | 970 g             | 980 g            | 353 g        |
| 7    | 592 g             | 580 g            | 525 g        |
| 8    | 580 g             | 680 g            | 354 g        |
| 9    | 662 g             | 622 g            | 276 g        |
| 10   | 640 g             | 500 g            | 295 g        |

NR: non-registered.

with a mean weight of 1725 g [eRR 1200-1600 g]; hepatic steatosis was observed in five cases, severe congestion in four, periportal lymphocytic infiltrate in four, and cholestasis in two. In eight cases there was splenomegaly with an average weight of 251 g [eRR 100-150 g]; there was red pulp hyperplasia in nine and immunoblastic-type cells in three cases. Even though the macroscopic aspect of the kidneys was normal, eight cases had congestion and two presented evidence of acute tubular necrosis. The bone marrow examination showed delayed maturation of one or more of the cell lines. Figure 1 shows the main findings of autopsy of the HCPS cases herein described.

DISCUSSION

The epidemiological and clinical profiles of the 10 cases of HCPS herein evaluated are similar to those described in other regions of the country and in the American continent. There was a predominance of males in economically productive age with activities related to the rural area. Regardless of occupation, environmental changes caused by human activity represents the main predisposing risk factor described in epidemiological surveys. Previous contact with rodents is a fact observed in most cases, which is in accordance with data already described. The chronological evolution of clinical symptoms until the onset of dyspnea occurs in a similar way in most cases, and the fast progression of disease led patients to ask for medical care. Due to the seasonality and eventual occurrence of hantaviruses, other more common clinical diagnoses with similar symptoms, such as sepsis, leptospirosis, dengue fever and Influenza often emerge in the differential diagnosis.

The first laboratory evaluation of these patients may raise the hypothesis of HCPS. Hemoconcentration, left shift leukocytosis and thrombocytopenia were present in all individuals, which has been observed in other reported case series. These findings, together with severe hypoxemia and bilateral and homogeneous pulmonary congestion on the chest X-ray—should alert the clinicians to the diagnosis of HCPS. These patients are initially managed as if they have had sepsis and a vigorous fluid infusion is performed, sometimes worsening the pulmonary congestion, contributing to the poor outcome observed in many cases.

The most relevant necropsy findings herein described are predominantly restricted to the lungs. They appeared congestive, edematous, collapsed and with marked increased weight, aside from the presence of pleural effusion in most cases. Microscopic examination revealed edema, hemorrhage, hyaline membranes and mild...
interstitial mononuclear inflammatory infiltrate, with intact alveolar wall type I pneumocytes and no activation of type II pneumocytes. In animal models, a chest plain obtained during the cardiopulmonary phase (some hours before euthanasia) showed severe interstitial infiltrate suggestive of pulmonary edema, besides pleural effusion, as well as cardiomegaly, particularly at the expense of the right ventricle\textsuperscript{13}. In addition, the lungs exhibited diffuse and coalescing areas of hyperemia, edema, fibrin, macrophages and scarce neutrophils\textsuperscript{13}. Similar findings with

Figure 1 - Photomicrographs of the lungs showing the main pulmonary alterations in patients with HCPS: A) mild inflammatory mononuclear infiltrate in the alveolar septa (HE, 400 x); B) intra-alveolar edema (HE, 200 x); C) hyaline membrane lining the alveolar wall (HE, 200 x); D) collapse focus in the left (HE, 40 x); E) intra-alveolar hemorrhage (HE, 200 x); F) hemorrhage and neutrophilic inflammatory infiltrate (HE, 400 x).
marked alveolar and interstitial pulmonary edema, minimal epithelial injury and mononuclear interstitial infiltrate were observed in most cases and have been presented and previously described in a case series of autopsies from Chile\textsuperscript{14}.

In a study carried out from 1999 to 2002, the hearts of 14 individuals who died during the acute phase of HCPS were compared with others who died due to acute pancreatitis with lung injury or chest trauma. Those who presented HCPS had mild biventricular dilation, inflammatory process with mild to moderate interstitial edema, congestion and mononuclear cell infiltrates. Diffuse reduction of myocytes, coagulative necrosis, basophilic cytoplasm appearance and pyknosis were also found\textsuperscript{19}. In contrast, the cases in the present study showed few heart alterations, limited to pericardial effusion in two cases, right ventricular dilation in one case and mild myocardial chronic inflammation in four cases.

Despite the minimal features observed in several organs, this is consistent with the systemic involvement of HPCS, which is supported by studies that detected the presence of viral RNA by reverse transcription polymerase chain reaction in several tissues, and also by hybridization techniques on endothelial cells of the lungs, liver, spleen, kidneys and heart\textsuperscript{13,19}. In vitro experimental models suggested that the endothelial damage is directly related to the viral cytopathic effect leading to endothelial dysfunction and capillary vasodilation. However, the intrinsic physiopathogenic mechanism of this lesion remains unclear\textsuperscript{13,20}.

The autopsy practice is still an important tool in teaching hospitals, allowing the clarification of a diagnosis and a clinical pathological correlation that ultimately assesses the accuracy of the clinical diagnosis. Paradoxically, in recent years, the evidence indicates a substantial decrease in this practice and/or its replacement by modern image-based methodologies, which are not yet available for this purpose in most resource-contraint places.

The anatomopathological features seen at the autopsy of cases with HCPS reveal the final phase of the process with structural pulmonary alterations, allowing a direct correlation with the severity of the respiratory distress observed at admission.

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AUTHORS’ CONTRIBUTIONS

JPVS: review of cases and preparation of the text; SJA: accomplishment of autopsies and review of the text; MLSV: review of clinical data and the manuscript text; AMRM: accomplishment of autopsies, review of cases and preparation of the text.

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