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Original Article

Belgrade Hantavirus Infection is Associated With the most Severe Clinical Form of Hemorrhagic Fever with Renal Syndrome

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Abstract

Background. Hemorrhagic fever with renal syndrome (HFRS) is an acute viral disease characterized by fever, hemorrhage and acute renal failure and is caused by closely related zoonotic viruses of genus Hantavirus of the family Bunyaviridae. Hantaviruses produce a spectrum of illnesses with specific manifestations depending on the particular virus involved. Hantaan and Belgrade/Dobrava viruses cause the most severe disease, Puumala the least severe, and Seoul produces disease of intermediate severity. While Hantaan, Puumala and Seoul may cause a wide disparity in disease manifestations even with the same serotype, Belgrade virus, which is found in the former Yugoslavia, is associated only with severe form of illness.

Methods. 128 patients with HFRS have been treated in our unit during the period from 1989 to 2004. We retrospectively analyzed 108 of them and 20 followed prospectively.

Results. The disease is serologically confirmed in all 128 patients: Hantaan in 45%, Belgrade in 35% and Puumala in 20%. Clinical course of the disease followed up by six phases: prodromal phase in 45%, febrile in all patients, hypotensive in 30,5%, oliguric phase in 90,6%, diuretic and convalescent phase in all patients. All patients had acute renal failure (ARF), which was anuric at 30% of them, and treatment with dialysis was necessary in 55%. We observed favorable effect of "an early dialysis" to the course of the disease and its outcome. More than half of those treated with dialysis were infected with Belgrade virus-62%. Acute respiratory distress syndrome (ARDS) was presented in 11%. The cause of the disease in 8 of them was Belgrade, and in 6 Hantaan virus. 3 patients (2,3%) died, in 2 infected with Belgrade the cause of death was cerebral hemorrhage, and 1 infected with Hantaan died of shock. After the convalescent phase we noted a chronic renal failure in 8 patients (6,25%) who had a severe form of the disease. All of them had hypovolemic shock, severe ARF, ARDS, visceral haemorrhage and disseminated intravscular coagulation (DIK). The cause of the disease in 5 patients was Belgrade, and in 3 Hantaan virus. In 4 of them infected with Belgrade we noted a progression to terminal renal failure. Other chronic sequeale were hypertension in 3 patients, hypothyreosis in 2, hypocorticism in 1, sterility in 2, and acute myocardial infarction in 1.

Conclusions. According to clinical characteristic and syndromes that follow HFRS, most of our patients had severe form of illness. Belgrade virus infection is associated with a more severe disease type and development of chronic renal failure as a sequela of the disease. The long term follow up of renal function in these patients is recomended.

Keywords: Acute renal failure, Belgrade/Dobrava virus, hantaviruses, hemorrhagic fever with renal syndrome

Introduction

Hemorrhagic fever with renal syndrome is an acute viral disease characterized by fever, hemorrhage and acute renal failure and is caused by closely related zoonotic viruses of genus Hantavirus of the family Bunyaviridae [1]. Hantaviruses have single-stranded, negative-sense RNA genomes that are divided into three segments: small (S), medium (M) and large (L) segment, which encode the nucleocapsid protein, the glycoprotein precursor and the putative RNA polymerase, respectively [2]. Each hantavirus is maintained in nature by infecting a single rodent species, which serves as its primary natural reservoir. Transimission to humans occur predominately trough inhalation of aerosols of contaminated rodent excreta [3]. More than 30 different hantaviruses have been distinguished so far, at least half are related to disease in humans. One of the main features of hantaviruses is the close association between the virus type and the host species. This results in the circulation of distinct hantaviruses in the Old and New World and in geographical clusters of hantavirus genetic variants [4]. These viruses cause two types of disease in humans: hemorrhagic fever with renal syndrome (HFRS) in Asia and Europe and hantavirus pulmonary syndrome (HPS) in North and South America. HFRS is caused by Hantaan, Belgrade/Dobrava, Seoul and Puumala viruses, while HPS is caused by Sin Nombre and related viruses (New World hantaviruses). Hantaviruses produce a spectrum of illnesses with specific manifestations depending on the particular virus involved. Hantaan and Belgrade/Dobrava virus causes the most severe disease, Puumala the least severe- called nephropathia epidemica, and Seoul produces disease of intermediate severity [5]. While Hantaan, Puumala and may cause a wide disparity in disease manifestations even with the same serotype, Belgrade virus, which is found in the former Yugoslavia and the Balkan Peninsula, is associated predominately with severe form of illness [6]. It has been estimated that hantaviruses cause over 200 000 cases of clinically manifested disease world-wide, with contribution of China and Korea with more than 100 000 cases. The lethality of hantavirus infection is known to be 0,1-1% for

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Puumala virus associated disease, 5-15% for Hantaan and Belgrade virus associated disease, and 25-50% for hantaviruses causing HPS [7].
The aim of this study is to present clinical manifestations and laboratory findings in patients with HFRS with particular analysis of those infected with Belgrade virus.

Patients and methods

128 patients with HFRS have been treated in our unit during the period from 1989 to 2004. There were 126 males and 2 females, aged from 18 to 62 years. We retrospectively analyzed 108 patients treated in Military Medical Academy during the period from 1989 to 2000 with all data received from available medical records. Another 20 patients hospitalized in the period from 2000 to 2004 were followed prospectively, during the acute phase of illness. Diagnosis in both groups was made from the clinical symptoms and signs, laboratory findings, and confirmed by serologic tests to detect specific anti hantavirus antibodies: enzyme linked immunosorbent assays (ELISA) IgM and indirect immuno-fluorescence assay IgG (performed in Torlak Institute of Immunology and Virology). In 24 patients renal biopsy was done and tissue samples were analysed by light microscopic, electron microscopic and immunohystochemical methods in Institute of pathology Military Medical Academy.
Tipical clinical symptoms and signs, as well as clinical syndromes that follow HFRS and the laboratory findings were analysed in all patients. We compared results obtained in our study with results of two other author groups about HFRS caused by Hantaan and Seoul viruses in Korea and by Puumala in Sweden with noted differences and similarities.

Results

Hantavirus infections occur in two main seasonal outbreaks: in the beginning of the spring (from second half of March until the end of May) and in the end of summer (August). During the largest epidemic in 1994/95, frequency of the disease was equal trough the whole year, without seasonal changing.
The patients came to our unit in different phases of the disease. Only two of them were hospitalized in the febrile phase (1,6%), 26 in hypotensive (20,3%), and most of them were in oliguric phase - 80 patients (62,5%). In polyuric phase there was 16 patients (12,5%), and 4 (3,1%) in convalescence phase.
The disease was serologically confirmed in all 128 patients: Hantaan in 45%, Belgrade in 35% and Puumala in 20%.
Clinical course of the disease followed up by six phases (Table 1). Clinical manifestations and frequency of some symptoms and signs are shown in Table 2 with comparison to clinical picture of HFRS in other part of the world. All our patients had acute renal failure (ARF), which was anuric at 30% of them.

Table 1. Comparison of clinical course of the HFRS in patients treated in Military Medical Academy and those presented by Lee JS (5)

Phase	Frequency Korea % of patinetns	Frequency MMA* % of patients	Average duration Korea (days)	Average duration MMA* (days)
Prodromal	10-20	45	3-10	3-10
Febrile	100	100	3-6	3-8
Hypotensive	37	30,5	1	few hours-1 day
Oliguric	60	90,6	3-5	3
Polyuric	95	100	7-14	10
Convalescence	100	100	4-8 weeks	3-12 weeks

* MMA- Military Medical Academy

Table 2. Comparison of clinical manifestations in HFRS in patients treated in Military Medical Academy and those presented by Lee JS (5), and Settergren B et al (18)

Symptoms and signs	Korea- HTN† (229 pts*) % of pts*	Korea-SEO‡ (40 pts*) % of pt*	Sweden-PUU§ (355 pts*) % of pts*	Serbia, Montenegro 3 serotypes (128 pts*) % of pts*
Fever	100	100	94	100
Headache	96	45	45	100
Anorexia	96	95	95	100
Myalgia	69	51	37	82
Abdominal pain	90	72	48	100
Back pain	92	28	66	95
Blurred vision	54	9	7	25
Subconjuctival hemorrhage	14	8	3	75
Melena	16	5	< 1	32
Hematemesis	3	5	< 1	30
Hypertension	60	36	10	65
Shock	24	-	-	25
Hypotension	34	10	30	30
ARDS	-	-	-	11

*Pts- patients, † HTN- Hantaan virus, ‡ SEO- Seoul virus, § PUU- Puumala virus

Belgrade virus was serologically confirmed as etiologic agent in 45 patients (35%). All of these patients had severe ARF which was anuric in more than half (20 patients of total number of 38 anuric patients). Dialysis was necessary for the treatment of ARF in 43 (95,5%) patients infected with Belgrade. Among these patients, 45% had hypotension, 33% developped shock, in 74% severe visceral hemorrhage were presented (melena, hematemesis, bleeding into central nervous system) and 28% had disseminated intravscular coagulation (DIK). Eight patients infected with Belgrade (17,7%) developed acute respiratory distress syndrome (ARDS). In another 6 with ARDS, Hantaan virus was the cause of the disease (Table 3).

Laboratory findings are similar to those in Korean HFRS (Table 4). All patients had acute renal failure (ARF) with elevation of serum creatinine from 135 to 1700 μmol/l, and serum blood urea nitrogen (BUN) from 11 to 70 mmol/l. Proteinuria was presented in all patients with average values of 7,2g per day and the largest of 22g per day.

Table 3. The most important clinical and laboratory characteristics of patients with HFRS caused by three serotypes treated in Military Medical Academy

Symptoms and signs	BGD† (45 pts*) % of pts*	HTN‡ (58 pts*) % of pts*	PUU§ (25 pts*) % of pts*
Fever	100	100	100
Headache	100	100	100
Abdominal pain	100	100	100
Back pain	100	86	80
Hypotension	45	29	4
Shock	33	29	-
Visceral hemorrhage	74	69	-
ARF‖	100	100	100
Anuric ARF‖	45	31	-
Treatment with dialysis	95	46	-
DIC	28	19	-
ARDS**	18	10	-
CRF††	11	5	-

*Pts- patient, † BGD- Belgrade virus, ‡ HTN- Hantaan virus, § PUU- Puumala virus, ‖ ARF- acute renal failure, ¶ HBI- hronična bubrežna insuficijencija, **ARDS- acute respiratory distress syndrome, ††CRF- chronic renal failure

Table 4. Comparison of laboratory findings in HFRS in patients treated in Military Medical Academy and those presented by Lee JS (5), and Settergren B et al (18)

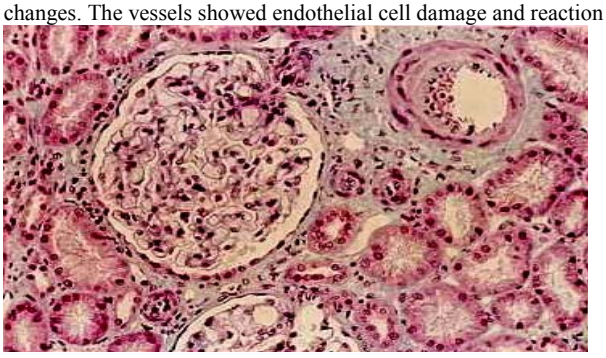
	Korea HTN‡ (229 pts†) % of pts†	Korea SEO§ (40 pts†) % of pts†	Sweden PUU‖ (355 pts†) % of pts†	MMA* HTN‡,BGD¶,PUU‖ (128 pts†) % of pts†
Leukocytosis	91	75	57	95
Thrombocyto-penia	96	80	62	91
Elevated BUN	98	90	99	100
Elevated creatinine	98	90	99	100
DIC	20	3	< 1	19
ElevatedAST, ALT	5	22	-	55
Hematuria	85	-	73	95
Proteinuria	100	100	89	100

*MMA- Military Medical Academy, †Pts- patients, ‡HTN- Hantaan virus, §SEO- Seoul virus, ‖PUU- Puumala virus, ¶BGD- Belgrade virus

Major histopathologic findings on renal biopsy tissue samples were: interstitial nephritis with hemorrhage into medullar interstitium, edema and inflammatory cell infiltrations followed by tubular epithelial and luminal alterations. There were slight glomerular mesangial changes and mild swelling of epithelial cells of Bowman's capsule. The vessels showed endothelial cell damage and reaction (Figure 1).

Treatment of HFRS consists of symptomatic and supportive care and strict management of fluid and electrolytes. Patients who developed ARDS treated with oxygenation and mechanical ventilation. Treatment with dialysis was necessary in 70 patients with ARF (55%). Indications for starting this method of treatment have been moved to lower values of serum creatinine of 400-500 μmol/l. Dialysis were performed once daily, with average 3 to 4 procedures by patient.

Fig. 1. Histopathologic findings in HFRS show interstitial nephritis with hemorrhage into medullary interstitium, edema and inflammatory cell infiltrations followed by tubular epithelial and luminal alterations. There were slight glomerular mesangial changes. The vessels showed endothelial cell damage and reaction



Three patients (2,3%) died, in 2 infected with Belgrade the death was caused by cerebral hemorrhage, and 1 infected with Hantaan died of shock. After the convalescent phase we noted chronic renal failure in 8 patients (6,25%) who had severe form of the disease. All of them had hypovolemic shock, severe ARF, ARDS, visceral haemorrhage and disseminated intravscular coagulation (DIK). Cause of the disease in 5 patients was Belgrade, and

in 3 Hantaan virus. In 4 patients infected with Belgrade we noted progression to terminal renal failure, in 2 of them kidney transplantation has been done, another two are on chronic dialysis program.
Other chronic sequeale were hypertension in 3 patients (2,34%), hypothyreosis in 2 (1,56%), hypocorticism in 1 (0,78%), sterility in 2 (1,56%), and acute myocardial infarction in 1 (0,78%).

Discussion

HFRS is endemic in the Balkan Peninsula and former Yugoslavia, where sporadic cases and outbreaks have been reported. Hantaviruses associated with disease in humans in Balkans are Belgrade/Dobrava virus carried by the yellow-necked mouse (Apodemus flavicollis), and Puumala carried by red bank vole (Clethrionomys glareolus). Dobrava was originally isolated in Slovenia where a number of severe cases of HFRS had occured [8]**,** and genetic analyses showed that Dobrava is a unique hantavirus type. Belgrade virus was isolated from blood and urine specimens collected from Yugoslavian patients (in central and south Serbia) with clinically severe HFRS [9]. Nucleotide sequencing of the G2-encoding region in the medium segment of the viral genome, reverse transcribed and amplified by polymerase chain reaction, revealed Belgrade virus to be substantially different from Hantaan and other major serotypes, but identical to Dobrava virus [10]. It has been reported that Belgrade/Dobrava virus carried by yellow-necked mouse (Apodemus flavicollis) is associated with severe clinical form of HFRS with a fatality rate up to 10 % [8,9]. Recently, another host for Belgrade/Dobrava virus was reported- striped field mice (Apodemus agrarius), causing a milder disease than that associated with A. flavicolis in Estonia, Russia, Slovakia and Balkans. [11,12]. These findings suggest that striped field mice (Apodemus agrarius), which are known to be natural host for Hantaan in Asia, also carry Dobrava in Central and Eastern Europe [13]. Puumala virus causes nephropathia epidemica, a milder form of HFRS, with a fatality rate less than 1% [14] (Table 5). It has been reported that Hantaan virus, which is endemic for Asia, causes HFRS in Balkans and the former Yugoslavia [6].

Table 5. Relationship between rodent host, hantaviruses related to human disease, and severity of clinical form of the disease

Genus	Rodent host	Clinical form
Hantaan (HTN)	Apodemus agrarius (Asia, Europe)	Severe
Seoul (SEO)	Rattus norvegicus (World wide)	Moderate
Puumala (PUU)	Clethrionomys glareolus (Scandinavia,Europe, Balkans)	Mild
Belgrade/Dobrava (BGD/DOB)	Apodemus flavicolis (Balkans)	Severe
	Apodemus agrarius (Balkans, Estonia, Russia, Slovakia)	Mild

We serologically confirmed three serotypes of hantaviruses as etiologic agents of HFRS in our patients: Hantaan in 45%, Belgrade in 35% and Puumala in 20%. The disease showed two main seasonal outbreaks: in the beginning of the spring and in the end of the summer, like other reported cases [4]. During the largest epidemic in 1994/95, frequency of the disease was equal trough the whole year, without seasonal changing, because of the war and large migrations of army forces and population. Patients were hospitalized in later phases of the disease, mostly in oliguric phase with manifested ARF. Clinical manifestations and laboratory findings of Hantaan and Puumala associated disease are similar to these in other parts of the world. There is a wide disparity in disease manifestations from asymptomatic, mild, to severe form, even with the same serotype [5]. This is reported for Hantaan and Puumala viruses, but Belgrade virus is associated mostly with more severe disease type [6,15].
Clinical course of the HFRS in our patients followed up by six phases, similar to Korean HFRS caused by Hantaan virus. We noted higher frequency of prodromal and oliguric phase (Table1), also as a more frequent anuric ARF in 30% of patients, in opposite to 10% in Korean HFRS due to Hantaan [5]. Treatment with dialysis was necessary in 70 patients with ARF (55%). Indications for starting dialysis have been moved to lower values of serum creatinine of 400-500 μmol/l. We observed favorable effect of "an early dialysis" to the course of the disease and outcome, with normalisation of BUN and creatinine in average 14 days. Dialysis was performed once daily, with average 3 to 4 procedures by patient. More then half of those treated with dialysis were infected with Belgrade virus-62%. Most of these patients infected with Belgrade developed severe clinical manifestation with high frequency of hypotension, shock, visceral hemorrhage (melena, hematemesis, bleeding into central nervous system) and DIK. Eight patients infected with Belgrade and six infected with Hantaan developed acute respiratory distress syndrome (ARDS). It is well known that ARDS is associated with HPS, but it can be part of the severe clinical form of HFRS.
We noted long duration of the convalescent phase, over 3 months in half of our patients. After the convalescent phase we noted chronic renal failure in 8 patients (6,25%) who had severe form of the disease. All of them had hypovolemic shock, severe ARF, ARDS, visceral haemorrhage and disseminated intravscular coagulation (DIK). The cause of the disease in 5 patients was Belgrade, and in 3 Hantaan virus. In 4 patients infected with Belgrade we noted progression to terminal renal failure, in 2 of them kidney transplantation has been done, another two are on chronic dialysis program. These results agree with studies that show an association between past hantaviral infection and chronic renal disease [16,17]. Other chronic sequeale were hypertension in 3 patients (2,34%), hypothyreosis in 2 (1,56%), hypocorticism in 1 (0,78%), sterility in 2 (1,56%), and acute myocardial infarction in 1 (0,78%).

Conclusion

According to clinical characteristic and syndromes that follow HFRS, most of our patients had severe form of illness. Belgrade virus infection is associated with a more severe disease type and development of chronic renal

failure as a sequela of the disease. The long term follow up of renal function in these patients is recomended.

Conflict of interest statement. None declared.

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