

Characteristics of patients dying from acute viral hepatitis in Serbia

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Background/aims: Background/aims: Acute viral hepatitis is complicated rarely with severe liver failure due to many factors associated with the etiology, patient age, and time of development of hepatic encephalopathy, etc. The aim of this study was to identify some of the clinical and laboratory features associated with a fatal outcome in patients dying from acute viral hepatitis in Serbia. **Methods:** Clinical and laboratory data from 47 patients hospitalized from January 1989 - December 2006 were reviewed retrospectively. Serological tests for hepatitis A, B, C, D, and E viruses, herpes simplex viruses, cytomegalovirus, and Epstein-Barr virus were done. Histological features were assessed from 35 liver tissues. The electronic base, SPSS for Windows (version 11.0), was used for statistical analysis. **Results:** The majority of the patients had alanine aminotransferase (ALT) >20x the normal value, serum bilirubin >300µmol/L, prothrombin time >25 seconds (s), and white blood cell count >12 x 10⁹/L. Regression analysis revealed activity of alanine aminotransferase >20x the normal value to be associated with fulminant (p=0.015) and serum bilirubin concentration with subfulminant hepatitis (p=0.008). Hepatitis B virus was the most commonly detected virus (70%). Massive hepatocyte necrosis vs. sub-massive with bridging necrosis were found to be independent of clinical presentation. **Conclusions:** Hepatitis B virus infection, severe impairment of liver function tests, and confluent hepatocyte necrosis and infection characterize patients dying from acute viral hepatitis in Serbia. High activity of alanine aminotransferase reflects rapid and extensive acute viral liver injury, while deep jaundice is more common in a protracted course of the disease.

Key words: Liver failure, acute hepatitis, fulminant, hepatitis B virus

Sırbistan'da akut viral hepatitten ölen hastaların özellikleri

Amaç: Akut viral hepatit etyolojiye, hasta yaşına ve hepatik ensefalopatinin gelişme zamanına bağlı olarak nadiren ağır karaciğer yetmezliği ile komplike olur. Bu çalışmanın amacı Sırbistan'da akut viral hepatitli vakalarda mortalite ile ilişkili klinik ve laboratuvar verilerin tespit edilmesidir. **Yöntem:** Ocak 1989'dan Aralık 2006'ya kadar hastaneye yatırılan 47 hastanın klinik ve laboratuvar verileri retrospektif olarak incelenmiştir. Hepatit A,B,C, D ve E için, herpes simpleks, sitomegalovirus ve Epstein-Barr virüsü için serolojik testler yapılmıştır. Hastaların 35'inde karaciğer histolojisi incelenmiştir. İstatistik analiz için SPSS (v11.0) kullanılmıştır. **Bulgular:** Hastaların çoğunluğunda alanin aminotransferaz düzeylerin normalin 20 katından yüksekti, serum bilirubin düzeyleri >300 µmol/L, protrombin zamanı >25 sn ve lökosit sayısı >12x10⁹/L olarak bulundu. Regresyon analizi sonucunda alanin aminotransferaz > 20x normal fulminan seyir ile ilişkili bulunurken (p=0,015); serum bilirubin düzeyi subfulminan hepatit ile ilişkili bulundu (p=0,008). Hepatit B en sık tespit edilen (%70) virüstü. Massif hepatosit nekrozu ve sub-massif nekroza köp-rüleşme nekrozunun eşlik etmesinin klinik prezentasyondan bağımsız olduğu görüldü. **Sonuç:** Hepatit B virüsü enfeksiyonu, karaciğer testlerinde ağır bozulma ve konfluen hepatosit nekrozu Sırbistanda akut viral hepatitten ölen hastaların karakteristik özelliğidir. Yüksek alanin aminotransferaz düzeyleri hızlı ve yaygın akut viral hasarı yansıtırken, sarılık uzamış hastalık seyrinde ortaya çıkmaktadır.

Anahtar kelimeler: Karaciğer yetmezliği, akut hepatit, fulminan, hepatit B virüsü

INTRODUCTION

Acute liver failure (ALF) is defined as a rapidly developing impairment of hepatocyte function (1).

This disease is complicated with multi-organ failure (MOF), most commonly presented with hepatic

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encephalopathy and hemorrhage, which is clinically designated as fulminant hepatitis failure (FHF) (2,3). Systemic inflammatory response syndrome (SIRS), expressed by increased pro- and anti-inflammatory cytokines, has recently been identified as the essential event in the progression and determination of the outcome in ALF patients (4). Early predictive factors for the prognosis of ALF are recognized in general but are still under evaluation (5-9).

If acute/subacute viral hepatitis is complicated by encephalopathy, it is designated as fulminant (FVH) and subfulminant viral hepatitis (SFVH), respectively (1).

The aim of this study was to identify some clinical and laboratory data associated with fatal outcome in patients dying from acute viral hepatitis in Serbia.

MATERIALS AND METHODS

Forty-seven consecutive patients who died from (S)FVH were enrolled in this retrospective study. Fulminant and subfulminant hepatitis was diagnosed according to the definition by Bernuau et al. (1) (ALF with developed hepatic encephalopathy < 2 weeks, or 2 weeks - 3 months after the onset of jaundice, respectively). Patients were admitted to the Institute for Infectious and Tropical Diseases, Belgrade, Clinical Center of Serbia, from January 1989 to December 2006 with hepatic encephalopathy (HE) of at least grade 1-2 that further progressed to grade 3 and 4 (coma). Surviving patients were followed in the same institution. The investigated patients were collected from secondary medical centers all over Serbia. Patients were aged from 1-77 years (mean: 41.37 $\pm$ 19.7); 26 were males. Their medical history was obtained from family members along with available medical reports. Diseases that preceded acute hepatitis were present in 22 patients, most often expressed as chronic conditions (diabetes mellitus, arterial hypertension, duodenal ulcer, etc.). None of the patients had been treated before admission with well-known hepatotoxic or immunosuppressive drugs, nor were they alcoholics or intravenous drug abusers. They did not use herbal medicines or edible mushrooms. One patient was in the first trimester of pregnancy. Duration of hospitalization varied from 1-19 days (mean: 6 $\pm$ 5). During hospitalization, patients were under standard intensive supportive care for liver failure. No antiviral drugs (e.g., lamivudine, interferon, etc.) or corti-

costeroids were administered. Liver transplantation was not available for these patients during that period. All patients died in coma, and in a minority of them, coexisting hepatorenal syndrome (HRS) was evident.

Routine hematological and biochemical tests for liver function were performed. Values of alanine aminotransferase (ALT), total serum bilirubin, prothrombin time (PT), and white blood cell (WBC) count were evaluated in the study.

The viral diagnosis of acute hepatitis was made after serological testing for hepatitis A (HAV), B (HBV), C (HCV), D (HDV), and E (HEV) viruses, using commercial enzyme-immunoassay kits (Ortho EIA; BioRad ELISA). Serological tests for herpes simplex viruses 1 and 2, cytomegalovirus (CMV), and Epstein-Barr virus were also done. Auto-antibodies for autoimmune liver disease (anti-nuclear, anti-smooth muscle, anti-mitochondrial, and anti-liver kidney microsomal 1) and serum and urine copper concentration were determined. Specimens of blood and urine were taken from all patients for bacterial and fungal cultures.

Post-mortem percutaneous liver biopsy was done in 35 patients. Liver tissues were paraffin-embedded and routinely stained. The hepatic pathology expert assessed the degree and type of cellular necrosis, features of cholestasis and hepatocyte regeneration.

Statistical Analysis

Normally distributed data were evaluated using Student's t-test. Non-normal data were compared using non-parametric tests (Mann-Whitney test). Non-parametric variables were calculated by chi-square or Fisher's exact test. Significant variables were entered into a univariate logistic regression model. The electronic database organized in SPSS for Windows (version 11.0) statistical package was used for the analyses, and results are presented with 95% confidence interval (95% CI). A probability value of $p < 0.05$ was considered significant.

RESULTS

Clinical Presentation and Demographic Data of the Patients

There was a significantly higher frequency of patients suffering from FVH (39/47) than from SFVH (9/47) ($p = 0.000$). Bleeding (15/47) and HRS (14/47) were noticed in a minority of patients ($p = 0.013$ and $p = 0.006$, respectively). Frequencies of male

gender (26/47), age over 40 (28/47) and diseases preceding acute hepatitis (22/47) were not statistically significant among the patients. Comparison of demographic data (gender, age distribution, age over 40) and prevalence of preceding diseases between patients with FVH and SFVH also did not show any significant differences in frequency.

Values of laboratory parameters in patients dying from acute viral hepatitis are presented in Table 1.

The majority of patients had serum ALT activity >20x the normal value (82.6%), total bilirubin value >300 µmol/L (68.1%), PT >25 seconds (s)

(87.2%), and WBC >12 x 10⁹/L (75%). The frequency of patients with PT >50 s was not significant (63.8%).

A comparison of laboratory parameters between patients with FVH and SFVH is presented in Table 2.

Statistical analysis showed significantly higher values for total bilirubin and a higher frequency of patients with bilirubin >300 µmol/L in patients with SFVH than in those with FVH (p=0.002 and p=0.042, respectively). A significantly higher frequency of patients with ALT value 20x higher than the normal value was found in patients with FVH compared with those with SFVH (p=0.022). Regression analysis revealed the significance of ALT over 20x the normal value for FVH (p=0.015; Exp(B)=8.500; 1.506-47.962) and total serum bilirubin for SFVH (p=0.008; Exp(B)=1.007; 1.002-1.012).

Bacterial and fungal blood and urine cultures remained negative.

Serological investigation confirmed the viral etiology in 36/47 (76.59%) patients. The most commonly detected virus was HBV (33/47) (p=0.006). HBV was equally detected in both FVH and SFVH. HAV infection was detected in two patients with FVH. Two patients with SFVH had anti-HCV antibodies. In a one-year-old child with SFVH and positive anti-HCV who had received a blood trans-

Table 1. Laboratory data of the patients dying from acute viral hepatitis

Parameter	Value	Range
Bilirubin (µmol/L) ^a (Mean ± SD)	411.8 ± 220.5	84-828
ALT (IU/L) ^b (Mean ± SD)	2015.7 ± 1667.5	100-8520
PT (seconds [s]) ^c (Mean±SD)	59.5 ± 26.7	16.3-≥100
WBC count ^d (Mean±SD)	16.4 ± 7.4	5.3-35.6

Abbreviation and normal values for laboratory parameters:

^aTotal serum bilirubin <17.5 µmol/L

^bAlanine aminotransferase <41 IU/L

^cProthrombin time <12 s (International Normalized Ratio=1)

^dWhite blood cell count, from 4-10 x 10⁹/L

Table 2. Comparison of laboratory parameters between patients dying from fulminant (FVH) and subfulminant viral hepatitis (SFVH)

Parameter	FVH (n=38 pts)	SFVH (n=9 pts)	p ^a value
Bilirubin ^b (Mean±SD)	371.7±19.1	606.9±125.2	<0.01
>300 µmol/L (n)	24	8	<0.05
ALT ^c (Mean±SD)	2183.1±1740.4	1220.6±995.1	NS
>20x than normal (n)	34	4	<0.05
PT ^d (Mean±SD)	61.6±26.8	48.8±25.9	NS
>25 s (n)	34	7	NS
>50 s (n)	27	3	NS
WBC count ^e (Mean±SD)	16.1±7.2	17.2±8.6	NS
>12 x 10 ⁹ /L (n)	24	8	NS

^aSignificance <0.05 (t-test, Mann-Whitney test for parametric variables; chi-square or Fisher's exact test for non-parametric variables)

Abbreviation and normal values:

^bTotal serum bilirubin <17.5 µmol/L;

^cAlanine aminotransferase <41 IU/L;

^dProthrombin time <12 s (International normalized ratio=1);

^eWhite blood cell count, from 4-10 x 10⁹/L.

fusion, immunoglobulin M antibodies to CMV (IgM-anti CMV) were simultaneously detected. Six of 33 patients with FVH (18.2%) were HBV surface antigen (HBsAg)-negative at admission but were positive for immunoglobulin M antibodies to HBV core antigen (IgM anti-HBc).

Auto-antibodies were negative and serum and urine copper concentrations were normal in all patients.

Massive hepatocyte necrosis (confluent centrilobular necrosis) was found in 60% of patients. Sub-massive necrosis with bridging necrosis (mostly portal-central) was evident in the other patients. No statistically significant difference in frequency distribution of the histological findings (massive necrosis vs. sub-massive with bridging necrosis) was noted among total patients or according to clinical presentation (FVH vs. SFVH). Features of cholestasis were observed in 20% of patients equally in both groups, and hepatocyte regeneration was noticed in one patient who died from SFVH. Histological assessment did not detect features of chronic liver disease (e.g., piecemeal necrosis, etc.).

DISCUSSION

The primary idea of this study was to describe the patients dying from FVH with the intention to start a campaign for liver transplantation for these patients (never done in our country).

Focusing on this motivation, we analyzed retrospectively the available clinical and laboratory data of patients with acute viral hepatitis in the final stage of their illness.

Patients were admitted into the hospital with encephalopathy of at least grade 2 that progressed to coma and death. However, in a large series from the United States, survival of ALF patients with encephalopathy grade 1-2 without transplantation was 52% over three weeks (10). In our study, the majority of patients suffered from FVH rather than SFVH (83% vs. 17%), although an early study reported high mortality in both acute and subacute ("late onset") hepatic failure (2). Later investigations demonstrated that the patients with shorter time intervals between onset of jaundice and HE showed a better prognosis (3,11-13). In this context, the limitation of our study is the absence of data of patients with acute hepatitis with liver failure who survived, in order to evaluate the total frequency of different clinical forms

and their outcome. Certainly, including patients that survived FVH as a control group and comparing their data with those of deceased patients would be very useful to evaluate the criteria for liver transplantation. That will be a focus of our subsequent investigation.

Generally, patients with a severe form previously diagnosed as viral hepatitis are hospitalized in our institution, while patients suspected of other etiological diagnosis (autoimmune hepatitis [AIH], adrenoleukodystrophy [ALD], metabolic diseases, etc.) are referred to gastroenterology units in Belgrade. Undoubtedly, it would be very attractive to collect all patients with FHF in collaboration with our colleagues from gastroenterology units to analyze their overall data.

Analyzing the demographic data of the patients, an additional and intriguing finding was the independence of age over 40, which was well established as a "stable" risk factor for poor prognosis (5). For this finding, we have no adequate explanation.

Laboratory data demonstrated a huge hepatocyte necrosis in association with high activity of ALT (20x higher than normal) and extensive cholestasis with total bilirubin >300 $\mu\text{mol/L}$ in the majority of patients. The histological finding of massive and submassive hepatocyte necrosis with bridging necrosis also indicates widespread liver injury causing liver failure. Furthermore, the absence of features of hepatocyte regeneration with the exception of one patient suggests liver repair deficiency. Otherwise, HRS and bleeding (from the gastrointestinal or genital tract) were observed in a minority of patients (<30% in both clinical forms). However, it is reported that functional renal failure develops in approximately 55% of ALF patients and is more common in SFVH (2,14). The finding of bleeding in a minority of patients is believed to be a consequence of extensive treatment with high doses of fresh frozen plasma in those not listed for liver transplantation. The same explanation can be given for the insignificant frequency of patients with PT >50 s.

The increase in WBC count in most patients (72%) (>12 $\times 10^9/\text{L}$), as one of the criteria of SIRS, supported the current infection. Negative microbial cultures were probably the result of urgent and wide antibiotic treatment of extremely ill patients.

Concerning the etiology, HBV was the most common cause of death in acute viral hepatitis (70%).

That is related to the prevalence of HBV in our country (from 2%-7%) and also confirms our previous investigation on the etiology of FVH (15,16). As it is well known that fulminant hepatitis B has a poor prognosis, this study was also conducted towards another campaign, i.e. vaccination against this virus. Unfortunately, at present, vaccination is not completely accepted among our adult population. In spite of the efforts of public health organizations, according to national epidemiological data, the morbidity of acute hepatitis B in a 5-year period (from 2003-2007) did not decrease significantly (4.90/100,000 and 4.47/100,000, respectively). Regarding additional data, fatal outcome in 2007 was reported in 0.3% of patients with acute hepatitis B (17).

Acute hepatitis A and C were detected in only four of the investigated patients. However, it is accepted that these viruses rarely cause ALF in industrialized countries (18-20). The prevalence of HEV is not fully established in our region and is still controversial, but this virus also seldom causes liver failure except during pregnancy (21,22). Undetectable HBsAg in 18.2% of FVH patients indicated strong host-immune response producing short-lasting massive liver necrosis. Unfortunately, serum from patients with concurrent finding of antibodies to HCV and CMV is unavailable for further investigation (i.e. searching for viral geno-

mes) to verify whether HCV alone or these viruses simultaneously caused hepatitis. Concerning diagnostic procedures in this study, we used sensitive serological tests for detecting hepatitis viruses using commercial tests according to the Centers for Disease Control and Prevention (CDC) recommendations for detecting acute viral hepatitis (23). The study began in 1989, and no commercial or home-made tests for viral genomes were available for routine diagnosis in our institution. Undoubtedly, detection of viral genomes could indicate more about the etiological causes, or eventually identify co-infections with different viruses that can lead to severe hepatitis (24,25).

In conclusion, our study shows that fatal outcome in acute viral hepatitis is mostly associated with HBV infection and confluent hepatocyte necrosis producing severe damage to liver function and infection. The high activity of ALT reflects short-term extensive viral liver injury, while deep jaundice is typical for a protracted course of the disease. Thus, awareness of increased values regarding jaundice must alert the clinician to the development of a protracted disease course that requires additional medical treatment. The accessibility of liver transplantation along with accumulation of knowledge and experience is certainly of vital importance for this medically common incurable disease (26,27).

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