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Survival and complications in a cohort of patients with anti-Delta positive liver disease presenting in a tertiary referral clinic

Robert A. de Man[1], Renate P. Sprey[1], Hubert G. M. Niesters[2], Ruud A. Heijtink[2], Pieter E. Zondervan[3], Wim Hop[4] and Solko W. Schalm[1]

Department of [1]Internal Medicine II (Hepatology Section), [2]Virology, [3]Pathology and [4]Biostatistics, University Hospital Dijkzigt and Erasmus University Rotterdam, The Netherlands

Background/Aims: **Our aim was to evaluate the clinical outcome and survival of patients with anti-Delta positive liver disease in The Netherlands.**
Methods: **We evaluated those patients visiting our hospital between 1978 and 1993 with respect to clinical, virological and histological parameters. During the follow-up period the occurrence of complications of the liver disease and survival was determined. Thirty patients with a median age of 34 years (range 21–52) were included.**
Results: **During an average follow up of 4.8 years, nine patients died. The overall 5-year survival as estimated by Kaplan-Meyer analysis was 71%, which was comparable to hepatitis B cirrhosis patients. However, in the group without active hepatitis B replication (HBeAg-negative) a clear trend towards a worse survival was identified in Delta cirrhosis patients. Complications and deaths occurred exclusively in the patient group with cirrhotic liver disease. The complications (ascites, elevated bilirubin >34 μmol/l), variceal bleeding and spontaneous bacterial peritonitis) occurred in 52% of the patients with a follow up of more than 6 months (n=27). Fifty-seven percent of those patients died. In our population anti-Delta positive liver disease affects predominantly young patients and is related to advanced liver disease.**
Conclusions: **In view of the high death rate, liver transplantation should be considered when signs or symptoms of decompensated liver disease occur.**

Key words: **Delta hepatitis; Epidemiology; Survival.**

THE DELTA agent was first detected by Risotto et al. in Italy (1). It has been characterized as a defective RNA-virus dependent for infection on the helper functions provided by the hepatitis B virus (HBV) (2). The hepatitis Delta virus (HDV) is thought to be highly pathogenic: inoculation in a chimpanzee model induced liver damage in all recipients (3). HDV can cause acute and chronic liver disease. Two modes of infection have been identified: simultaneous infection with HBV or superinfection of persons already infected by HBV. The outcome of co-infections is most often clearance of both HBV and HDV, whereas superinfections often result in chronic HDV infection, usually progressing to cirrhosis (2,4–7). The diagnosis chronic HDV-related liver disease is usually made by detecting a high titer of antibodies against HDV in serum, but recently the polymerase chain reaction (PCR) for HDV-RNA detection in serum has become available. At present the golden standard for the diagnosis is the expression of Delta antigen in liver tissue which can be shown by immunoperoxidase staining. RNA detection in liver tissue is possible by polymerase chain reaction techniques as well as by *in situ* hybridisation (8–10).

Epidemiological studies show the existence of endemic areas in Southern Europe, the Middle East, parts of Africa and South America (2,7,11). Western Europe and North America are non-endemic areas and almost all Delta infections have occurred in intravenous drug abusers, polytransfused subjects and hemophiliacs (12–16).

The clinical course and survival in Delta patients compare unfavourably with the natural history of chronic HBV infection (17,18). Although antiviral

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Correspondence: R. A. de Man, M.D., Erasmus University Hospital, Department of Internal Medicine II, CA 326, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

therapy can decrease transaminase activity and decrease the markers of HDV in serum and liver, the overall results are still disappointing (19,20). However, the results of liver transplantation in Delta patients compare favourably with the results in chronic HBV patients. Even after hepatic allograft re-infection with the Delta virus, the survival curve is comparable to the overall survival for non-viral liver diseases (21).

Few clinical studies of Delta hepatitis have been performed in The Netherlands. The aim of our study was to determine the occurrence of complications and survival of a cohort of HBsAg positive, anti-Delta positive patients in a university-based hepatology unit. For comparison, we used a cohort of HBsAg-positive HDV-negative cirrhosis patients concurrently followed in our unit.

Materials and Methods

Study population

The Dijkzigt University Hospital serves as a tertiary referral hospital for hepatology and liver transplantation. All patients were referred to our department for evaluation, antiviral therapy and sclerotherapy. Since 1978, thirty patients with anti-HDV positive liver disease have been diagnosed in our department. The diagnosis of HDV-related liver disease was made by demonstrating HBsAg and antibodies to the Delta virus. Every patient who had antibodies to Delta on two successive occasions was entered in the study. The clinical situation at presentation was evaluated retrospectively by describing: age, sex, country of origin, ethanol intake and subjective complaints. In addition, signs and symptoms of decompensated liver disease were described: spleen size, presence of ascites, encephalopathy or esophageal varices. Ascites and splenomegaly were diagnosed by physical examination and confirmed by ultrasonography. Esophageal varices were examined by endoscopy or barium swallow, and graded I to IV according to size (22). Hepatic encephalopathy was confirmed by spectral analysis of the electro-encephalogram. Decompensated cirrhosis was defined as a history of variceal bleeding, the presence of ascites, encephalopathy or elevation of bilirubin above 34 μmol/l (23). Alcohol abuse was defined as a daily alcohol consumption of at least 50 g/day. Laboratory parameters included: aspartate aminotransferase, bilirubin, albumin, platelets, coagulation factors and the presence of HBsAg, HBeAg (E.I.A., Abbott laboratories, North Chicago, IL, USA) and antibodies to HCV, HAV, HIV, HBsAg, HBeAg and HDV (E.I.A., Abbott laboratories). The presence of antibodies to HCV was confirmed by recombinant immunoblot assay (Ortho Diagnostics, Raritan, NJ, USA). Aspartate aminotransferase, albumin and bilirubin were measured by the sequential multiple autoanalyzer (12 SMA, Technicon Instruments Corp, Tarrytown, NY, USA). Normotest and thrombotest (Wyegaard and Co., Oslo, Norway) were used as coagulation parameters.

HDV RNA

RNA was extracted essentially as described by Young et al. (24), with some minor modifications. Briefly, to 100 μl EDTA-plasma, 400 μl lysis buffer was added [4M Guanidinium-iso-thiocyanate, 25 mM sodium-citrate pH 7, 0.5% sarcosyl, 100 mM beta-mercapto-ethanol, 20 μg poly A per ml]. After briefly mixing the solutions, the samples were incubated for 10 min at 65°C. The samples were cooled for 1 min at 0°C and 500 μl isopropanol was added. The solution was mixed and centrifuged directly for 20 min at 12 000 g at room temperature. The pellet was washed once with 80% ethanol and air dried. After adding 30 μl DEPC treated bidest, 10 μl was used for the cDNA reaction.

Total RNA was denatured for 3 min at 95°C and subsequently chilled on ice. First strand synthesis was performed in a volume of 25 μl containing 50 mM Tris-HCl pH 8.3, 3 mM $MgCl_2$, 10 mM DTT, 0.5 mM of each of the four dNTPs, 150 U RNasin, 0.25 μg hexanucleotides [Promega] and 200 U MMLV reverse transcriptase [Gibco-BRL]. After incubation for 30 min at 42°C, the cDNA was incubated for 5 min at 95°C. For the reaction, 75 μl mix was added containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM of each of the four dNTPs and 1 U Taq DNA polymerase [Promega] and 50 pmol of primer 1 and 2 [primer 1, position 851–871: CGG.ATG.CCC.AGG.-TCG.GAC.CGC; primer 2, position 1275-1298: GGA.TCA.CCG.ACG.AAG.GAA.GGC.CCT. After 4 min incubation at 94°C, forty cycles were performed in a Biomed thermocycler 60 [Tecnolab, The Netherlands] with 1 min at 94°C, 2 min at 48°C and 3 min at 72°C respectively. The PCR product was analyzed on a 2% agarose gel and electroblotted onto a Hybond N+ membrane [Amersham]. The membrane was hybridized with a ^{32}P end-labelled oligonucleotide [GCG.AG-G.AGG.TGG.AGA.TGC.CA] probe (position 870–889) (25). Positive and negative control samples were included both during sample preparation and during the PCR procedure. The sensitivity of the assay is 1000 genome equivalents/ml.

Histology

In 27 patients, a liver biopsy was performed for staging of the liver disease. This was tested by immunoperoxidase staining on paraffin-embedded material for HBcAg and HBsAg in 22 cases and for the presence

of HDAg in 24 patients. The polyclonal anti-Delta serum was kindly provided by Organon Tecnika (Boxtel, The Netherlands). Positive control slides were prepared from liver tissue kindly provided by Prof. F. Negro (Molinette Hospital, Turin, Italy). The negative control slides were prepared from a liver explant specimen of an HBsAg-negative patient. The histologic diagnosis was made by two independent histopathologists according to international criteria (26).

Analysis

The characteristics of the patients were determined at the time of presentation at our unit. The clinical evaluation was repeated every 3–4 months. The follow up started at the time antibodies to the Delta virus were detected, which was at admission to our hospital in all but one case. The follow up closed at 01-01-1993. Survival analysis was done according to the Kaplan-Meier method. To determine the prognosis of patients with anti-Delta positive liver cirrhosis, their survival was compared to a cohort of HBsAg-positive HBeAg-negative patients with cirrhosis concurrent followed in our unit and described previously (23). Patients who had a follow up of more than 6 months were evaluated for their complications and clinical outcome.

TABLE 1

Initial clinical characteristics of 30 anti-Delta positive patients

	Number of patients (%)
Sex	
Male/female	25/5 (83/17)
Country of origin	
Mediterranean	17 (57)
Western Europe/Eastern Europe	12/1 (40/3)
Alcohol intake	
>5 drinks/day	11 (37)
<5 drinks/day	19 (63)
Icteric presentation	
Present	9 (30)
Absent	21 (70)
Ascites	
Present	3 (10)
Absent	27 (90)
Encephalopathy	
Present	2 (7)
Absent	28 (93)
Esophageal varices	
Grade I–II	10 (33)
Grade III–IV	5 (17)
Absent	8 (27)
Not investigated	7 (23)
Spleen size (cm)	
≤12	5 (25)
>12	15 (75)

Results

Entry characteristics

The characteristics at entry to the study are shown in Tables 1 and 2. Symptomatic disease was present in 27 patients: subjective complaints included nausea, fatigue and upper abdominal pain in the liver region.

The median age at presentation was 34 years (range 21–52 years). Symptoms suggestive of portal hypertension were found in 75% (15 out of 20 had splenomegaly >12 cm). Esophageal varices grade I–II were detected in ten, and grade III-IV in five patients, respectively. The absence of varices was documented in eight patients.

Risk factors for contracting HDV infection were: stay in an endemic area (Mediterranean) in 60% (18 out of 30), and (ex) intravenous drug abuse in 30% (9 out of 30). In three Dutch patients (10%) no risk factors were found. Seven patients had more than one risk factor. HBeAg was found in 10%, and 61% tested anti-HBe positive (tested 29 and 28 patients, respectively). Antibodies to HCV were positive in 23% (7 out of 30) and 15% (2 out of 13) had antibodies to HIV.

Survival analysis

Thirty patients had a mean follow-up period of 4.8 years (range 1 day–20 years). One patient was lost to follow up. Eight out of nine patients who died during follow up, died due to liver-related causes: liver failure (3), variceal bleeding (2), liver failure after liver trans-

TABLE 2

Initial virological and biochemical characteristics of 30 anti-Delta positive patients

	Number of patients (%)	Median	Range
Bilirubin (μmol/l)		12	4–339
≤35	24 (80)		
>35	6 (20)		
Albumin (g/l)		42	24–49
>35	22 (73)		
28–34	6 (20)		
<28	2 (7)		
AST (IU/l)		56	23–300
≤30	2 (7)		
>30	28 (93)		
Thrombotest (%)			
>35	22 (88)		
<30	3 (12)		
Virology			
HBsAg positive (tested n=30)	29 (97)		
HBeAg positive (tested n=29)	3 (10)		
Anti-HBeAg positive (tested n=28)	17 (61)		
Anti-HBcAg positive (tested n=30)	30 (100)		
Anti-HCV positive (tested n=30)	7 (23)		
Anti-HIV positive (tested n=13)	2 (15)		

TABLE 3

Complications and deaths in 27 anti-Delta positive patients with follow up more than 6 months, according to their initial histological diagnosis

	n		Died	
	Total (%)	cirr/nc[1]	Total (%)	cirr/nc
Bilirubin >34 μmol/l	14 (52)	*12/2	#8 (57)	*7/1
Encephalopathy	7 (26)	*6/1	5 (71)	*5/0
Ascites	14 (52)	*12/2	#7 (50)	*6/1
Esophageal bleeding	6 (22)	*6/0	#4 (67)	*4/0
Spontaneous bacterial peritonitis	1 (4)	1/0	1 (100)	1/0

[1]Cirrhosis/non-cirrhosis. * One patient never had a biopsy, he had clinical signs of a decompensated cirrhosis. # One patient with cirrhosis has died since the end of the follow up (not included in death rates in the table).

TABLE 4

Correlation between the presence of HDV-RNA in serum as measured by the polymerase chain reaction and immunohistochemistry for Delta-antigen on liver biopsy material

HDV-RNA PCR	Liver immunohistochemistry		
	Positive	Negative	No material
Positive	17	4	5
Negative	1	2	1

plantation (hepatic artery thrombosis in one and primary non-function in one) and hepatocellular carcinoma (1). Only one patient died of non-liver-related causes (stomach carcinoma) (Table 3).

The survival of all patients with anti-HDV positive cirrhosis was calculated and compared to a cohort of HBsAg positive cirrhosis patients. The 5-year survival was 71% (95% confidence interval 53%–89%).

The 5-year survival of all HBeAg-negative anti-Delta positive patients with cirrhosis ($n=18$) is compared to HBcAg-negative cirrhosis patients in Fig. 2. Although there is a clear trend towards a decreased survival in the Delta patients, statistical significance was not reached, possibly due to the relatively small sample of anti-Delta positive patients.

Histology

A liver biopsy was performed in 27 patients: cirrhosis was present in 66% (18 out of 27), all with chronic active hepatitis (CAH) as a sign of active disease. According to the Child-Pugh classification, 15 patients were graded as Child A, two patients as Child B and one patient as Child C. Chronic active hepatitis was present in six patients, and chronic persistent hepatitis in two. One patient presented with acute hepatitis in transition to chronic hepatitis.

Immunohistochemistry was done in 24 patients: Delta antigen in liver tissue was positive in 75%. HBsAg and HBcAg in the liver were positive in 95% and 14%, respectively. One patient was positive for all markers. The polymerase chain reaction additionally detected HDV-RNA in serum in four patients who were negative for HDAg in the liver by immunoperoxidase staining (Table 4). One patient was negative in the HDV PCR in serum, but positive in the immunoperoxidase staining in the liver biopsy.

In the group of nine patients with non-cirrhotic liver disease at presentation, the biopsy was repeated in five: four developed cirrhosis during follow up, one patient still had chronic active hepatitis. At the end of the follow-up period, 81% showed cirrhosis. The patients who

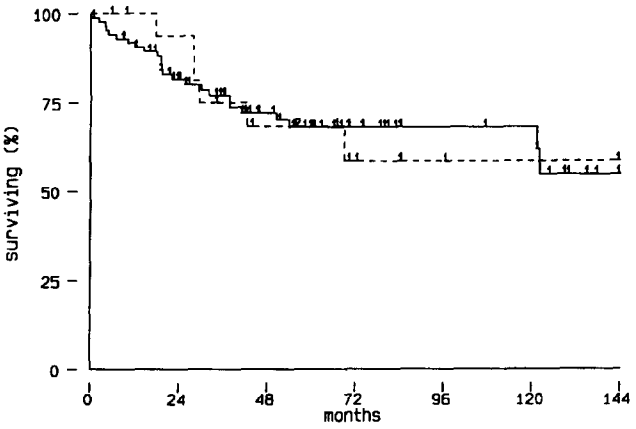

Fig. 1. Survival of all HBV cirrhosis patients (solid line; n=86) versus survival of all HDV cirrhosis patients (dotted line; n=18). The two survival curves do not significantly differ from each other. Tick-marks along curves represent follow up of patients still alive.

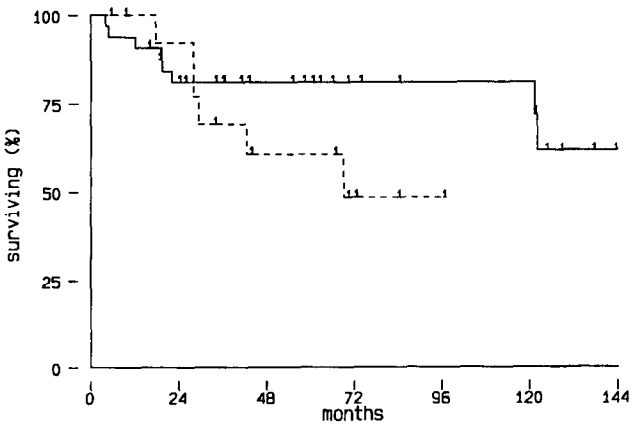

Fig. 2. Survival of patients without active HBV replication (HBeAg-negative) for HBV cirrhosis (solid line; n=33) versus HDV cirrhosis (dotted line; n=15).

showed progression to cirrhosis had chronic persistent hepatitis ($n=2$), acute hepatitis in transition to chronic hepatitis ($n=1$) and moderate chronic active hepatitis ($n=1$) at presentation.

Complications

Of patients with a follow up of more than 6 months, 52% (14 out of 27) developed complications, most frequently icteric episodes (bilirubin >34 μmol/l) and the development of ascites (Table 3).

Of five patients with esophageal varices grade III–IV at presentation, three (60%) died and two patients with liver transplantation survived. Of ten patients with varices grade I–II, two (20%) died. Five patients without varices at presentation ($n=8$) developed varices (grade I–II) during follow up. Three (38%) patients died, two had no further investigation and one had no detectable varices.

Discussion

In The Netherlands, we found anti-Delta positive liver disease in young people who are (ex)intravenous drug abusers and people who have immigrated from the Mediterranean area. This is in accordance with the expected low incidence of the Delta virus in Western Europe.

The results of this study show a 5-year survival of 71% of anti-Delta positive patients with chronic liver disease after the first presentation in our hepatology unit. This is comparable to a 5-year survival of 80% in chronic HBV patients. Furthermore, there was no difference in the main causes of death in anti-Delta positive patients and patients with chronic HBV infection (23,27,28). However, if corrected for the presence of HBeAg, a clear trend towards a worse survival in chronic HDV patients was found. This is even more remarkable if the median age of 34 years in taken into account, which is 10 years less than in our HBV cohort. Whether this is due only to the HDV infection or related to confounding factors related to intravenous drug abuse (HCV co-infection (23%), alcohol abuse (37%)) cannot be established because of the small size of the cohort.

Ninety-five percent of patients had elevated AST levels (>30 IU/l) independent of the expression of Delta antigen in the liver. Patients negative for the antigen had higher levels than patients positive for the antigen. Immunological activity while viral replication with expression of the Delta antigen has already ceased might be responsible for the hepatitis in patients negative for the expression of Delta antigen in the liver. This is supported by the detection of Delta RNA by PCR in these patients. In one patient no Delta RNA could be detected by PCR while liver immunohistochemistry was positive. This unexpected result could be explained in several ways: the PCR was performed on stored serum, RNA could have deteriorated during storage or the amount could have been below the detection limit of the PCR assay.

The complications of anti-Delta positive liver disease in this cohort were the development of ascites, an increase in bilirubin (>34 μmol/l), encephalopathy and esophageal variceal bleeding. Of the patients who had a follow up of more than 6 months, 52% developed complications; all patients had cirrhosis at that time. Of these patients, 57% died during the follow-up period.

We conclude that patients with anti-Delta positive liver disease as they presented in our hospital have a progressive liver disease leading to decompensated liver cirrhosis approximately 10 years earlier than our HBV patients. When decompensated liver disease has developed, liver transplantation is the option of choice, especially since re-infection with Delta hepatitis of the liver allograft does not affect patient or graft survival.

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