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Abstract

The incidence of hepatitis A (HA), hepatitis B (HB), and non-A, non-B hepatitis (NANBH) was 27%, 30% and 43% among 73 patients with sporadic hepatitis. Epidemiological data (geographical distribution, seasonal variation, age, sex, and occupation) were not distinguishing of the type of hepatitis. Neither intrafamilial infection nor previous contact with viral hepatitis patients could be demonstrated in the NANBH cases. Fever and jaundice were less frequent in NANBH than in HA. Maximum levels of SGPT, serum bilirubin, ZTT, and gamma-globulin were significantly lower in NANBH than in HA and HB. Ten of 29 NANBH patients (35%) presented abnormal SGPT activities for more than 6 months, and four (14%) more than 12 months. In the ten patients with prolonged courses, jaundice was more frequent and maximum levels of SGPT were higher than in patients with transient courses. Histopathologic findings were not markedly different from those of HA and HB. Bile duct damage, fatty deposition, and giant multi-nucleated cells were recognized in 6, 12, and 2 NANBH patients, respectively. There were no characteristic ultrastructural changes in NANBH.

KEYWORDS: acute hepatitis, sporadic non-A, non-B hepatitis, liver histopathology, liver ultrastructure

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CLINICAL AND HISTOLOGICAL FEATURES OF SPORADIC NON-A, NON-B HEPATITIS

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Abstract. The incidence of hepatitis A (HA), hepatitis B (HB), and non-A, non-B hepatitis (NANBH) was 27 %, 30 % and 43 % among 73 patients with sporadic hepatitis. Epidemiological data (geographical distribution, seasonal variation, age, sex, and occupation) were not distinguishing of the type of hepatitis. Neither intrafamilial infection nor previous contact with viral hepatitis patients could be demonstrated in the NANBH cases. Fever and jaundice were less frequent in NANBH than in HA. Maximum levels of SGPT, serum bilirubin, ZTT, and γ-globulin were significantly lower in NANBH than in HA and HB. Ten of 29 NANBH patients (35 %) presented abnormal SGPT activities for more than 6 months, and four (14 %) more than 12 months. In the ten patients with prolonged courses, jaundice was more frequent and maximum levels of SGPT were higher than in patients with transient courses. Histopathologic findings were not markedly different from those of HA and HB. Bile duct damage, fatty deposition, and giant multi-nucleated cells were recognized in 6, 12, and 2 NANBH patients, respectively. There were no characteristic ultrastructural changes in NANBH.

Key words : acute hepatitis, sporadic non-A, non-B hepatitis, liver histopathology, liver ultrastructure.

Since the development and wide spread availability of sensitive and specific serological tests for the diagnosis of hepatitis A (HA) and hepatitis B (HB), it has become apparent that there are other hepatitis viruses, namely non-A, non-B hepatitis (NANBH) viruses. However, as there are no reliable markers for NANBH, the diagnosis is by exclusion of HA, HB, Epstein-Barr virus and cytomegalovirus infection. In 1975, the first reported occurence of non-transfusion-related endemic NANBH was detected in an area of Costa Rica (1). Such cases have been reported since in the United States (2), Europe (3, 4), and many other locations (5, 6). In reports from the United States and Europe, the incidence of sporadic NANBH was 12-25 % (2-4), and that of non-A, non-B post-transfusion hepatitis was about 90 % (7-10). In Japan, NANBH occurs in approximately 45-50 % of the sporadic hepatitis cases (11, 12), and about 70-90 % of the post-transfusion hepatitis cases (12).

Non-A, non-B post-transfusion hepatitis is a major cause of chronic liver

disease (10, 12-16). Sporadic NANBH also plays an important role in chronic sequelae of liver disease (17, 18). Bamber *et al.* (17) described fatty deposition, sinusoidal cell activation and bile duct damage as histopathological features of sporadic NANBH. Electron microscopic analysis has revealed characteristic hepatocyte nuclei and cytoplasmic changes in experimentally infected chimpanzees (19). Moreover, virus-like intranuclear particles have been observed in a case of sporadic NANBH (20).

Because of the lack of specific markers, we decided to compare the epidemiological, clinical, histological and ultrastructural features of NANBH, HA and HB in an attempt to determine distinguishing characteristics of sporadic NANBH.

PATIENTS AND METHODS

Seventy three acute viral hepatitis patients were admitted to Okayama University Hospital between 1978 and 1982, excluding those with drug— or alcohol-induced liver injury, obstructive jaundice or previous chronic liver disease. None had received blood transfusion. The patients were classified into three groups : HA, HB, and NANBH. HA was defined by the presence of IgM antibody to hepatitis A virus (IgM anti-HAV) (21). HB was defined by the presence of hepatitis B virus surface antigen (HBsAg) and the absence of IgM anti-HAV. NANBH was defined by the absence of IgM anti-HAV, HBsAg and antibody to hepatitis B virus core antigen (anti-HBc). Epstein-Barr virus (EBV) or cytomegalovirus (CMV) infection was excluded clinically and serologically. IgM anti-HAV was detected with a commercially available radioimmunoassay (HAVAB-M, Abbott Laboratories), as was anti-HBc (CORAB, Abbott Laboratories). A reversed passive hemagglutination test and radioimmunoassay (AUSRIA II, Abbott Laboratories) were used to detect HBsAg. EBV and CMV infection was ascertained by measurement of anti-viral antibodies with complement fixation tests.

In 20 of 31 patients with NANBH, 6 of 20 patients with HA and 8 of 22 patients with HB, liver biopsies were performed in the acute or convalescent phase of the illness. All biopsy specimens for light microscopy were fixed and sectioned by routine methods. The stains employed were : hematoxylin and eosin, Gordon and Sweets' reticulin, orcein, Azan-Mallory, periodic acid-Schiff-diastase after amylase digestion, and Perls' iron. Biopsy specimens from 4 NANBH patients, who presented SGPT abnormalilies for more than 6 months, were investigated by electron microscopy. The specimens were fixed in 2.5 % glutaraldehyde and 1.0 % osmium tetroxide, dehydrated through a graded ethanol series and embedded in Epon. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed under a Hitachi H-700H transmission electron microscope operated at 75kV.

RESULTS

Epidemiology. The geographical distribution of the NANBH patients in the Okayama region was not distinctive from that of the HA and HB patients. The patients were scattered throughout the region and were not clustered in specific areas.

NANBH represented 43 % of the cases of sporadic viral hepatitis, with HA and HB evenly split for the rest (Table 1). Half the cases of NANBH occurred during winter and spring (from December to May). This seasonal distribution

TABLE 1. THE OCCURRENCE OF SPORADIC HEPATITIS

	Number of patients			
	HA	HB	NANBH	Total
1978	4	4	8	16
1979	3	3	5	11
1980	6	4	2	12
1981	5	6	10	21
1982	2	5	6	13
Total	20	22	31	73
(%)	(27)	(30)	(43)	

HA : hepatitis A, HB : hepatitis B, NANBH : non-A, non-B hepatitis.

TABLE 2. EPIDEMIOLOGICAL AND CLINICAL COMPARISON OF THE THREE TYPES OF SPORADIC HEPATITIS.

	HA	HB	NANBH
Sex male/female	14/6	16/6	21/10
Age mean	39	40	40
(range)	(17-59)	(10-72)	(20-74)
Jaundice	19/20 (95%)	17/22 (77%)	15/29 (52%)
Fever	18/20 (90%)	8/22 (36%)	13/29 (45%)
General fatigue	18/20 (90%)	15/22 (68%)	22/29 (76%)
Gastro-intestinal symptoms	19/20 (95%)	19/22 (86%)	20/29 (69%)

HA : hepatitis A, HB : hepatitis B, NANBH : non-A, non-B hepatitis.

is different from HA, in which 80 % of the cases occurred in this period, and from HB, in which only one-third occurred in this period.

Cases of all three types of hepatitis presented similar age and sex profiles (Table 2). Males predominated approximately 2 to 1 in all three types.

Doctors, nurses and laboratory employees comprised 25 %, 27 %, and 19 % of the patients with HA, HB, and NANBH, respectively. There was no significant difference in this respect among the three types. Among the HA patients, 6 (three families) were designated as having had intrafamilial infection. There were no intrafamilial infection cases among the HB and NANBH patients, none of whom had a history of previous contact with viral hepatitis patients. In the Okayama region, a NANBH outbreak has not been observed for the last 5 years, and evidence of transmission of NANBH via food and water was not detected.

Clinical aspects. Jaundice and fever were significantly less frequent in patients with NANBH than in patients with HA (Table 2). Only half of the patients with NANBH were icteric or febrile. The frequency of jaundice and gastro-intestinal symptoms in NANBH patients was less than in the other two types.

Laboratory comparison (Table 3). Mean peak SGPT, serum bilirubin, ZTT and

TABLE 3. LABORATORY ANALYSIS OF PATIENTS WITH SPORADIC HEPATITIS*.

Parameter	HA n=20	HB n=22	NANBH n=29
Maximum			
SGOT (IU/L)[e]	652 (176~2420)	1100 (633~1920)	410 (177~942)
SGPT (IU/L)[a,e]	1220 (443~3380)	1740 (846~3580)	599 (273~1320)
T.Bil. (mg/dl)[b,d]	7.6 (4.2~14)	6.0 (2.1~17)	2.6 (0.92~7.2)
γ-GTP (IU/L)	169 (92~309)	98 (31~307)	121 (47~308)
TTT (MU)[b]	7.8±1.1	3.4±2.0	2.8±3.9
ZTT (KU)[b,e]	10±3.9	8.4±2.4	6.3±3.2
γ-glob. (g/dl)[a,c]	1.5±0.39	1.3±0.35	1.1±0.37
IgG (mg/dl)	1540±493	1560±484	1360±268
IgA (mg/dl)	275±122	332±138	238±139
IgM (mg/dl)[b]	510±357	201±70	208±121
Prothrombin time (sec)	13.2±1.65	13.1±1.14	13.0±1.30
Minimum			
T.Cho. (mg/dl)	130±32.8	144±33.8	181±46.7

HA : hepatitis A, HB : hepatitis B, and NANBH : non-A, non-B hepatitis.

* SGOT, SGPT, T.Bil. and γ-GTP values are given as the mean (mean−SD~mean+SD) obtained by log conversion. Other values are given as the mean±SD.

between NANBH and HA

a $p < 0.01$

b $p < 0.001$

between NANBH and HB

c $p < 0.05$

d $p < 0.01$

e $p < 0.001$

TABLE 4. NUMBER OF PATIENTS WHO PRESENTED ABNORMAL SGPT VALUES* FOR EXTENDED PERIODS.

	> 6 months	> 12 months
HA	2/20 (10%)	0/20 (0%)
HB	1/22 (5%)	1/22 (5%)
NANBH	10/29 (34%)	4/29 (14%)

HA : hepatitis A, HB : hepatitis B, NANBH : non-A, non-B hepatitis.

* > 50 IU/L

γ-globulin values for patients with NANBH were significantly lower than those for patients with HA or HB. Mean peak SGOT values for NANBH patients were significantly lower than those for patients with HB. Mean peak TTT and IgM values for patients with HA were significantly higher than those for patients with HB or NANBH.

Chronicity. Two of the 31 NANBH patients were diagnosed as having fulminant hepatitis and were excluded from this part of the study. Ten of the remainder presented abnormal SGPT activities for more than 6 months, and four showed abnormalities for more than 12 months (Table 4). Only two of the 20 HA patients had abnormal SGPT activities for more than 6 months, one of whom

TABLE 5. CLINICAL COMPARISON OF NANBH SUBGROUPS.

	prolonged [a,c]	recovered [b,c]
Sex male/female	7/3	12/7
Age mean	43	37
(range)	(24-74)	(20-62)
Jaundice[a]	9/10 (90%)	6/19 (32%)
Fever	4/10 (40%)	9/19 (47%)
General fatigue	9/10 (90%)	7/19 (37%)
Anorexia	9/10 (90%)	7/19 (37%)
Nausea, vomiting	4/10 (40%)	8/19 (42%)

HA : hepatitis A, HB : hepatitis B, and NANBH : non-A, non-B hepatitis.
a-SGPT abnormalities for $>$ 6 months.
b-SGPT abnormalities for $<$ 6 months.
c-see Table 4
d- $p < 0.05$

TABLE 6. LABORATORY ANALYSIS OF THE SPORADIC NANBH SUBGROUPS*.

Parameter	Prolonged group[a] n = 10	recovered group[a] n = 19
Maximum		
SGOT (IU/L)[b]	629 (304~1300)	323 (143~733)
SGPT (IU/L)[c]	1070 (647~1780)	433 (208~903)
T.Bil. (mg/dl)	3.0 (1.2~7.5)	2.3 (0.78~7.1)
γ-GTP (IU/L)	142 (70~288)	112 (39~316)
TTT (MU)	3.8±6.2	2.3±1.3
ZTT (KU)	7.0±3.9	6.0±2.4
γ-glob. (g/dl)	1.2±0.51	1.1±0.25
IgG (mg/dl)	1270±197	1420±287
IgA (mg/dl)	337±187	181±42
IgM (mg/dl)	154±42	239±139
Prothrombin time (sec)	13.0±1.03	13.1±1.49

HA : hepatitis A, HB : hepatitis B, and NANBH : non-A, non-B hepatitis.
a-see Table 4 and 5.
* SGOT, SGPT, T.Bil. and γ-GTP values are given as the mean (mean−SD~mean+SD) obtained by log conversion. Other values are given as the mean±SD.
b-$p < 0.05$, *c*-$p < 0.001$.

had acute renal failure (22) and the other of whom had intrahepatic cholestasis (23). One of the 22 HB patients showed abnormal SGPT activities for more than 12 months; this patient also presented intrahepatic cholestasis.

The ten NANBH patients who presented abnormal SGPT activities for more than 6 months (prolonged group) were compared to the remaining 19 cases who recovered within 6 months (recovered group) (Tables 5 and 6). Age and sex did

TABLE 7. HISTOLOGICAL FEATURES OF NANBH.

	Prolonged group							Recovered group (case No.)												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Enlargement of portal tract	+++	+++	+++	+++	++	++	+	−	−	−	−	+	+	−	++	+	++	−	−	−
Cell infiltration of portal tract	+++	+	−	++	++	++	+	−	−	−	+	−	++	−	+	+++	++	++	−	−
Destruction of limiting plate	++	−	++	−	−	−	−	−	−	−	−	−	++	−	−	−	−	−	−	−
Portal-central bridging necrosis	+	−	+	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	−	−
Bile duct damage	++	−	−	−	+	+	+	−	−	−	−	+	−	−	−	−	+	−	−	−
Giant cell with multi-nuclei	−	−	−	+	−	−	−	−	++	−	−	−	−	−	−	−	−	−	−	−
Fatty deposition	+	+	−	−	+	+	++	−	−	−	−	−	++	+	++	−	+	+	+++	+++
Cell infiltration of sinusoid	+++	+	+	+	+	+++	−	++	++	+	−	+	++	+	++	+	++	++	+	+
Time from onset to liver biopsy (months)	4	6	6	6	6	3	5	1	2	2	4	1	2	2	2	2	2	4	3	2

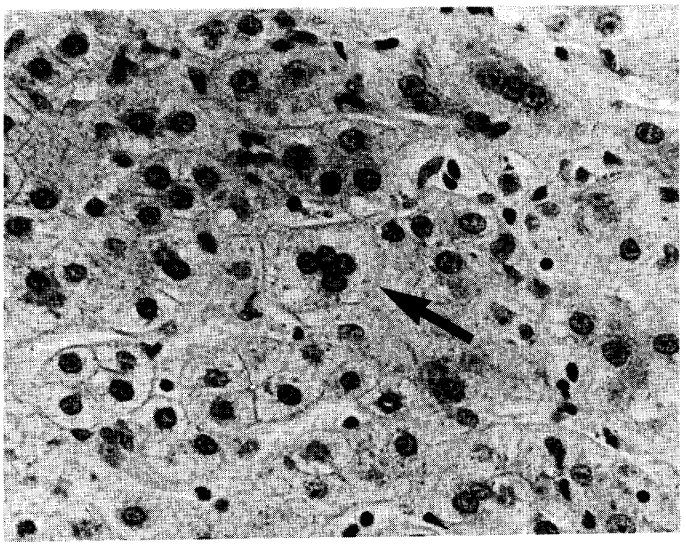

Fig. 1. Giant multi-nucleated cell (arrow). NANBH patient No. 9. Hematoxylin and eosin. × 400.

not differ significantly. Jaundice, general fatigue, and anorexia were more frequent in the prolonged group. Mean peak SGOT and SGPT levels were significantly higher in the prolonged group than in the recovered group, while the other parameters did not distinguish between the groups.

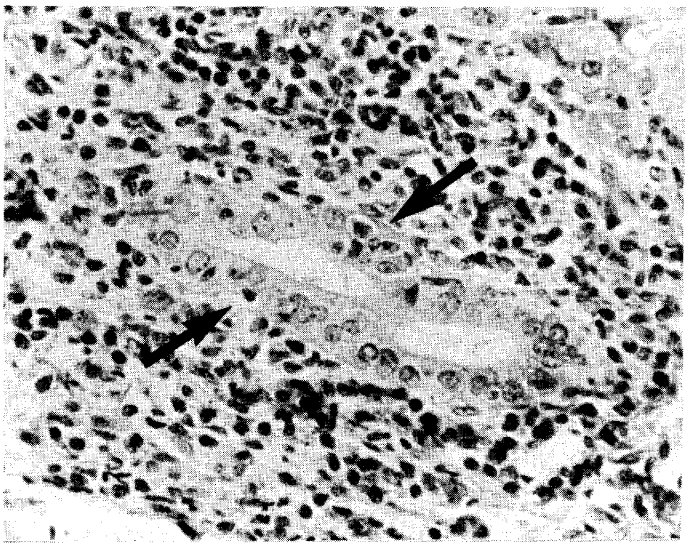

Fig 2. Interlobular bile ducts surrounded by dense inflammatory cells. The basement membrane is partly destroyed and lymphocytic infiltration (arrows) is seen in the bile duct epithelia. NANBH patient No. 1. Hematoxylin and eosin. × 400.

Light microscopic findings. In all specimens, the extent and type of injury and reparative and immunologic responses were ascertained histologically Histologically NANBH was not remarkably different from HA and HB. In the histological evaluation of NANBH, many factors were given a semiquantitative score from (−) to (+++) (Table 7). In two of the patients with NANBH, giant multi-nucleated cells were recognized without a cholestatic pattern (Fig. 1), although the appearance of giant cells in HA or HB cases was consistently accompanied by cholestasis. Bile duct damage (Fig. 2) and fatty deposition were recognized in 6 and 12 of the patients with NANBH, respectively. The bile duct epithelia presented swollen, lightly stained, and partly vacuolated cytoplasm. The ducts were infiltrated by lymphocytes, and their arrangement was distorted, but bile duct alterations were mild in all cases. The degree of fatty deposition was slight to moderate, and the cytoplasmic fat droplets varied in size. Most of the cases of the prolonged group presented portal tract enlargement with dense inflammatory cell infiltration. In two prolonged cases, moderate destruction of the limiting plate was observed. Furthermore, piecemeal necrosis, an important marker of chronicity, was seen in one of 20 NANBH patients (case No. 1).

Ultrastructural alterations. Tissue specimens of four patients with NANBH who presented abnormal SGPT activities for more than 6 months were investigated by electron microscopy. Ultrastructural changes commonly seen in acute hepatitis were observed in all these specimens. Swollen cytoplasm, dilated smooth and rough endoplasmic reticula, swollen mitochondria, and flattened cell membranes

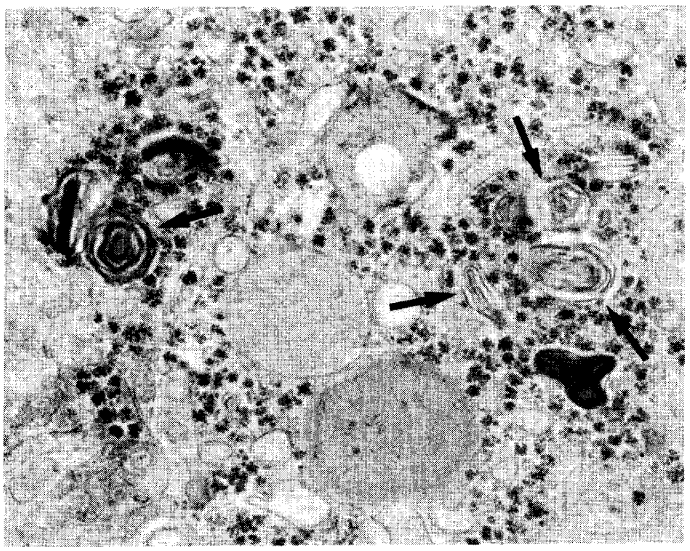

Fig. 3. Lamellar inclusions (arrows) in the hepatocytic cytoplasm. NANBH patient No. 2. × 2900.

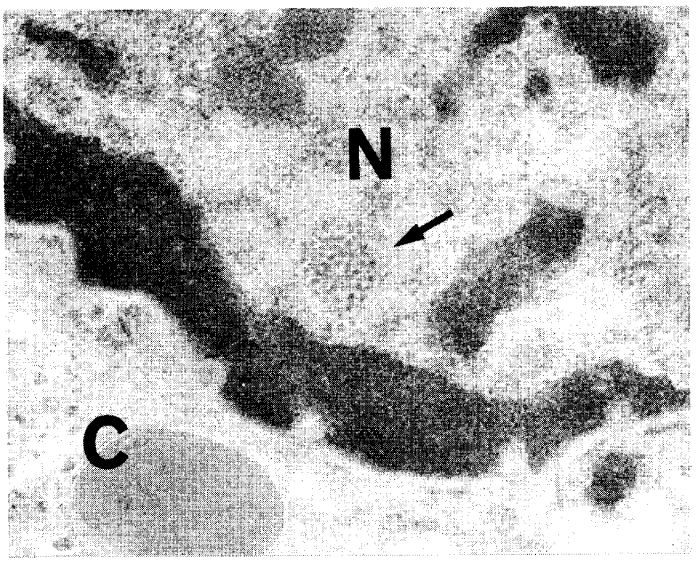

Fig. 4. Intranuclear virus-like particles (arrow) about 20 nm in diameter. The particles are located at the periphery of the hepatocytic nucleus. NANBH patient No. 1. × 39000. N : nucleus. C : cytoplasm.

(loss of microvilli) were observed. Glycogen particles were reduced numerically. Circular membranes and lamellar inclusions (24) were seen in a few specimens (Fig. 3). In one case, 20 nm diameter intranuclear particle aggregates were ob-

served at the nucleus periphery (Fig. 4). Tubular, double unit membrane changes of hepatocytes, which have been observed in experimentally infected NANBH chimpanzees (19), were not recognized.

DISCUSSION

NANBH accounted for about 43 % of the sporadic hepatitis cases of our study, which is in accordance with previous studies from Japan (11, 12). This rate is more than double that in the United States and Europe (2-4).

The male : female ratio was about 2 : 1. In the United States and Europe, male predominance is partially related to the inclusion, in studies, of intravenous drug abusers (3) and male homosexuals (4). Such cases were absent from our study. Furthermore, NANBH did not demonstrate any seasonal variation, as Alter *et al.* (18) reported.

Since Prince *et al.* (7) first reported post-transfusion hepatitis without serological evidence of exposure to hepatitis B virus, many reports of post-transfusion NANBH have followed (8, 9, 25). Evidence exists for the existence of at least two parenteral transmissible agents responsible for NANBH (19). Transmission via an enteral route was present in a waterborne epidemic in the Kashmir valley (26). Among our HA patients, 6 (three families) were designated as having had intrafamilial infections, with the transmission route considered to have been enteral, via food or close contact (27). However, in the NANBH patients, there was no evidence of either enteral or parenteral transmission of NANBH. Doctors, nurses, and medical employees, thought to have a high risk of exposure to NANBH agents, comprised 6 of the NANBH patients (19 %), but they did not have previous exposure to NANBH agents. In this study, the transmission route of NANBH remained unclear.

Clinically, NANBH appeared to be milder than HA or HB. Mean peak SGPT, serum bilirubin, ZTT and γ-globulin values were significantly lower. Similar data have been reported in many previous studies (3, 4, 28-30), and such findings seem to be common of NANBH whether sporadic or post-transfusion.

The most important clinical aspect of NANBH is its high frequency of progression to chronicity. Berman *et al.* (31) reported that 12 of 26 patients (46 %) with non-A, non-B post-transfusion hepatitis presented elevated SGPT activities for more than 1 year after disease onset. They reported histological criteria for chronic sequelae in 8 of these patients. Chronic hepatitis occurs in as many as 40 % to 60 % of non-A, non-B post-transfusion hepatitis cases (31-33). The frequency of progression is only slightly lower in sporadic cases. Villarejos (34) investigated 22 patients with sporadic NANBH, and found 5 patients (23 %) to have abnormal SGPT activities for more than 6 months. Liver biopsies revealed chronic active hepatitis in 4 individuals. In another study, 16 of 57 patients (29 %) exhibited prolonged elevated SGPT levels (35). Herein, 10 of 29 sporadic cases (34 %) possessed abnormal SGPT levels for more than 6 months, whereas SGPT

returned to normal within 6 months in almost all the HA and HB patients. The clinical symptoms of jaundice, general fatigue and anorexia were observed more frequently in the prolonged cases than in the remaining 19 patients with the transient course. The maximum levels of aminotransferase in patients with a prolonged course were higher. Initially high SGPT levels have been reported to be associated with chronicity by Berman *et al.* (31), but other investigators have not found such a relationship (10, 12, 14). Thus, in the acute phase of sporadic NANBH, a reliable biochemical marker for progression to chronic hepatitis remains elusive.

Bamber *et al.* (17) studied 12 patients with sporadic NANBH and designated characteristic histopathological features : fatty change, bile duct damage and excessive cellularity of sinusoids in relation to the degree of necrosis. In our study, fatty deposition and bile duct damage were also noted, but these findings did not distinguish NANBH. Sinusoidal cell infiltration in NANBH was not as prominent as in HA or HB. In two of 20 NANBH patients, giant multi-nucleated cells were recognized in the absence of intrahepatic cholestasis. Bamber *et al.* (17) found similar giant cells which were reminiscent of those observed in neonatal hepatitis. While Kryger *et al.* (36) could not find histological evidence of chronic active hepatitis in 5 NANBH patients biopsied one year after the acute attack, Bamber *et al.* (17) described 2 cases of chronic lobular hepatitis, 1 of chronic persistent hepatitis and 3 of chronic active hepatitis continuing for 6-28 months after disease onset in his survey of 12 cases. Although our biopsy specimens were obtained within 6 months of the onset, almost all specimens from the prolonged cases exhibited portal tract enlargement with dense inflammatory cell infiltration, suggestive of progression to chronic hepatitis.

Peculiar tubular structures, composed of two unit membranes sandwiching electron opaque material, and intranuclear aggregates of 20-27 nm virus-like particles have been detected by electron microscopy in experimentally infected NANBH chimpanzees (19). Similar, spherical intranuclear particles have been described occasionally in hepatocytes of patients with NANBH (20, 37). We also found such intranuclear particles, measuring about 20 nm in diameter, in one case of sporadic NANBH. However, the size of these particles was not as uniform as that of core particles in hepatocytic nuclei of patients with type B chronic hepatitis (38). Liver biopsy specimens from 4 patients with sporadic NANBH exhibited electron microscopic findings common to acute hepatitis. Marciano-Cabral *et al.* (24) have reported fused, circular membranes and lamellar inclusions within the cytoplasm of hepatocytes. They suggested that these cytoplasmic alterations represented hepatocellular modifications associated with human NANBH. We also found such materials, however, these changes were seen not only in NANBH but also in other liver diseases, and are considered non-specific. These virus-like particles and cytoplasmic alterations should be proven specific by immunoelectron microscopy. While epidemiological, clinical, laboratory and histopathological features may be suggestive of NANBH, no parameter is diagnostic.

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