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# Molecular evolutionary analyses implicate injection treatment for schistosomiasis in the initial hepatitis C epidemics in Japan

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***Background/Aims*: The mortality due to hepatocellular carcinoma (HCC) has ranged widely in various areas of Japan since 30 years ago and the incidence was particularly high in once *Schistosoma japonicum* (*Sj*)-endemic areas. Our aim was to estimate the spread time of hepatitis C virus (HCV) infection in the past with possible relevance to a higher incidence of HCC in once *Sj*-endemic than *Sj*-nonendemic areas.**

***Methods*: During 2001, 131 strains of HCV-1b were obtained from patients in three previously *Sj*-endemic areas, as well as *Sj*-nonendemic areas in Japan and a cross-sectional study was conducted on them with molecular evolutionary analyses.**

***Results*: A phylogenetic tree reconstructed on HCV-1b sequences in the NS5B region disclosed 2 independent clusters for *Sj*-positive and -negative groups with a high bootstrap value. The estimated effective number of HCV-infections indicated a transition from quiescence to rapid exponential growth in the 1920s among patients with schistosomiasis, which is 20 years earlier than that among patients without schistosomiasis.**

***Conclusions*: The estimated spread time in previously *Sj*-endemic areas in Japan coincides with injection treatment for *Sj* since 1921. A high incidence of HCC there would be attributed to a long duration of HCV infection since 1920s.**



*Keywords*: Hepatitis C virus; *Schistosoma japonicum*; Molecular evolutionary analysis; Hepatocellular carcinoma

## 1. Introduction

Recently, the molecular clock has been successfully applied to long-term serial serum samples containing hepatitis C virus (HCV) from the US and Japan and estimated the spread time of HCV in the 1930s in Japan, which is 30 years earlier than that in the US in the 1960s [1]. Insofar as a long duration of HCV infection is the most important factor for the development of hepatocellular

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*Abbreviations* HCV, hepatitis C virus; Anti-HCV, antibody to HCV; HCC, hepatocellular carcinoma; *Sj*, *Schistosoma japonicum*.

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carcinoma (HCC), it can be predicted that the incidence of HCC will increase in the US over the next 2–3 decades. Thus, a combination of classical epidemiological approaches and molecular evolutionary analyses would be particularly useful in the study of contagious diseases, typified by HCV infection.

The way how individuals contracted HCV infection has remained unclear in Japan. Recently, a Japanese report (Ministry of Health, Labour and Welfare: Distribution of age-adjusted mortality rate from liver cancer by prefecture between 1971 and 1975, Tokyo, 2001) indicated that the mortality due to HCC has already varied widely in various areas of Japan since 30 years ago; the incidence of HCC was much higher in Saga/Fukuoka, Hiroshima and Yamanashi Prefectures, which were once endemic for schistosomiasis japonica in the long past. Hence, a high incidence of HCC in the 1970s would be related to HCV transmitted by injection treatment for *Schistosoma japonicum* (*Sj*) conducted since 1921 in these areas. In fact, shared needles and syringes for intravenous injection treatment with antimonyl potassium tartrate or sodium antimony tartrate posed a significant risk for HCV transmission in endemic areas [2]. Indeed, the prevalence of antibody to HCV (anti-HCV) is high (36.5; 95% CI=28.1–44.9%) in patients with chronic schistosomiasis [2] and therefore, HCV infection is considered responsible for the development of HCC in patients with chronic schistosomiasis.

Since, once popular intravenous injection for schistosomiasis was a risk factor for HCV transmission, the spread time of HCV in the areas once endemic for *Sj* in Japan would deserve determination. In this study, molecular evolutionary analyses using principles of both population genetics and mathematical epidemiology [3] were applied to HCV-infected patients with and without a past history of chronic schistosomiasis in once *Sj*-endemic areas.

## 2. Meterials and methods

### 2.1. Sample collection

In Japan during 2001, 181 random serum samples positive for anti-HCV were obtained from patients with chronic liver disease in widely separated areas previously endemic for *Sj*, including Kofu in Yamanashi ($n=75$), Katayama in Hiroshima ($n=50$) and Chikugo in Saga/Fukuoka Prefectures ($n=56$). Schistosomiasis was diagnosed by ultrasonographic (US) and/or computer tomographic (CT) modalities or serological examinations [4]. Two kinds of serological tests, which can detect past history of schistosomiasis, were available in this study. In brief, IgG antibodies binding to two different *schistosome antigens, Sj* adult worm antigen and *Sj* egg antigen, were detected using an enzyme-linked immunosorbent assay (ELISA). As it is now accepted that ELISA titer of egg antigen-specific IgG is reliable for case-detection rather than IgG for adult worm antigen [4–6], the results based on the egg antigen-specific IgG were accepted in this study. Samples of more than 0.25 of optical density at 415 nm were determined to be positive, as previously confirmed [4–6]. The serum samples were tested for anti-HCV by Lumipulse II Ortho HCV (Ortho-Clinical Diagnostics K.K., Tokyo, Japan). As patients with *Sj* treatments were estimated to be old, relatively older patients were selected in the *Sj*-endemic areas to match age factor that might influence duration of HCV infection or HCC incidence. For a cross-sectional study, 30 serum samples were obtained from patients infected with HCV in Aichi Prefecture where *Sj* has not been endemic. The age- and sex- matced patients were also selected from the *Sj*-nonendemic areas excluding influece of these factors on HCC incidence. The study protocol conformed to the 1975 Declaration of Helsinki and was approved by Ethic Committees of institutions. Every patient gave a written informed consent to participate in the virological research of HCV. Information of injection treatment for *Sj* was obtained by means of self-administrated questionnaires or structured interviews. None had been treated with interferon therapy for HCV infection. HCC incidence was estimated by historical information from patients themselves and/or medical records during 2001. HCC was diagnosed by liver biopsy or combination of imaging modalities such as US, enhanced CT and angiography.

### 2.2. Genotyping and sequencing

Nucleic acids were extracted using a SepaGean RV-R Nucleic acid extracting kit (Sanko Junyaku Co., Ltd., Tokyo, Japan) in accordance with the manufacturer's protocol. They were reverse-transcribed to cDNA using SuperScript II Rnase $H^-$ Reverse Transcriptase (Invirogen Corp., Carlsbad, California, USA) and random hexamer primer (Takara Shuzo Co. Ltd, Tokyo, Japan) by the method described previously [7].

A sequence spanning 339 nucleotides (nt) in the NS5B region was amplified by polymerase chain reaction (PCR) with primers described previously [1]. PCR products were directly sequenced with Prism Big Dye (Applied Biosystems, Foster City, California, USA) in an ABI 3100 DNA automated sequencer. To reduce the number of artificial substitutions arising in PCR, PLATINUM Pfx DNA Polymerase (Invirogen Corp.) with a very high fidelity was used. The sequences determined were utilized to confirm HCV genotypes and construct phylogenetic trees.

### 2.3. Test for clustering between Sj-positive and -negative groups

The phylogenetic tree was first constructed to examine the evolutionary history for *Sj*-positive and *Sj*-negative groups by the neighbor joining method [8]. Furthermore, to test whether either *Sj*-positive or *Sj*-negative group have evolved independently or not, we conducted an interior branch test for the neighbor-joining tree [9]. Thereafter, a *t*-test was conducted for the interior branch length and its standard error, which is computed using the bootstrap procedure.

### 2.4. Demographic model

A reconstructed tree was built on the NS5B sequence of 339 nt by a heuristic maximum-likelihood topology search with stepwise-addition and the nearest neighbor-interchange algorithms. Tree likelihood scores were calculated using HKY85 with the molecular clock enforced by PAUP version 4.0b8.

As estimates of the demographic history, a nonparametric function $N(t)$, known also as the skyline plot, was obtained by transforming coalescent intervals of an observed genealogy into a piecewise plot that represents an effective number of infections through time [3,10]. A parametric maximum-likelihood was estimated by several models with the computer software Genie v3.5 to build a statistical framework for inferring the demographic history of a population on phylogenies reconstructed on sampled DNA sequences [10]. This model assumes a continuous epidemic process in which the viral transmission parameters remain constant through time. Model fitting was evaluated by likelihood ratio tests of the parametric maximum-likelihood estimates [11,12].

### 2.5. Statistical method

Data for continuous variables were demonstrated as the mean$\pm$ standard deviation. The Fishers' exact test, Chi square test with Yates' correction and one-way ANOVA followed by the Scheffe's multiple comparison test were used to evaluate differences in the mean age, sex ratio

and incidence of HCC between groups, respectively. Differences with $P$ values less than 0.05 were considered significant.

## 3. Results

Of 181 anti-HCV positive samples, 113 were classified into HCV genotype 1b (HCV-1b), which is predominant in Japan. Fifty-two of 181 samples (29%) were negative for HCV RNA or incomplete for sequencing and the remaining 16 samples (9%) of genotype 2a were excluded in this study due to a minor population. Of the HCV-1b strains, 47 were recovered from patients in Yamanashi, 31 in Hiroshima and 35 in Saga/Fukuoka Prefectures. Along with 18 HCV-1b strains in Aichi Prefecture serving as controls, a cross-sectional study was conducted on them with molecular evolutionary analyses. The patients in areas previously endemic for *Sj* revealed a significantly higher prevalence of chronic schistosomiasis [24/47 (51%) in Yamanashi (Kofu area), 21/31 (68%) in Hiroshima (Katayama area) and 19/35 (54%) in Saga/Fukuoka (Chikugo area)] than that in Aichi Prefecture (0/18 [0%], $P<0.0001$). There were no significant differences in the mean age or sex ratio among patients from these four areas (Fig. 1). Although the mean age of *Sj*-positive patients was just higher than that of *Sj*-negative patients in once *Sj*-endemic areas or matched-control patients in Aichi Prefecture, there were also no significant differences between these groups (Table 1).

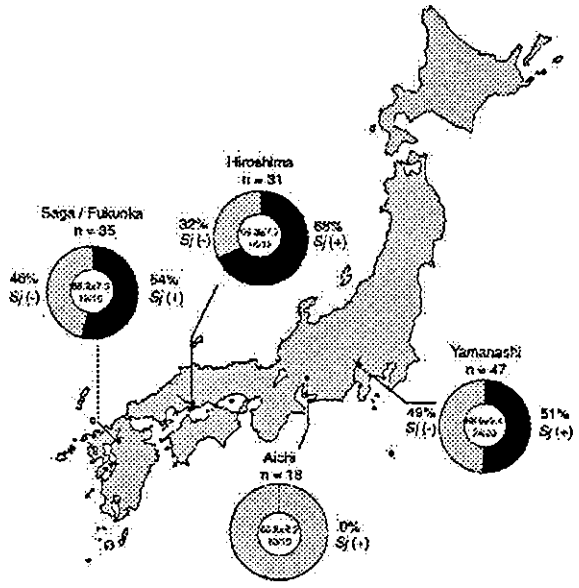


**Fig. 1. Geographic distribution of *Schistosoma japonicum* (*Sj*) and characteristics of patients infected with HCV. *Sj* (+) and *Sj* (−) denote, respectively, presence and absence of infection with *Sj* diagnosed by ultrasonographic and/or computer tomographic methods or serological examinations. Pie graphs include the age (mean ± standard deviation) and sex ratio (male/female).**

**Table 1**
**Characteristics of patients with and without schistosomiasis**

| | Schistosoma japonicum | | Controls (Aichi) |
|---|---|---|---|
| | Positive ($n=64$) | Negative ($n=49$) | ($n=18$) |
| Mean age | | | |
| Total | 69.9±7.7 | 67.4±8.7 | 66.5±9.2 |
| Yamanashi | 69.9±7.2 | 67.3±11.2 | |
| Hiroshima | 71.2±8.7 | 67.6±6.5 | |
| Saga/Fukuoka | 69.0±7.7 | 67.5±7.1 | |
| Sex (male/female) | | | |
| Total | 34/30 | 24/25 | 9/9 |
| Yamanashi | 13/11 | 11/12 | |
| Hiroshima | 10/11 | 5/5 | |
| Saga/Fukuoka | 11/8 | 8/8 | |
| Incidence of HCC | 25/55 (45%) | 11/48 (23%) | 3/18 (17%) |

The incidence of HCC in *Sj*-positive patients was significantly higher than that in *Sj*-negative patients ($P=0.0226$) or controls ($P=0.0488$).
Abbreviations: HCC, hepatocellular carcinoma.

For cross-sectional study on the viral population size between HCV-infected patients with and without a past history of schistosomiasis, a phylogenetic tree for HCV-1b strains in the *Sj*-positive and -negative patients was constructed with use of the maximum-likelihood method enforced by the molecular clock as introduced in our previous report [1] and an independent study by Pybus et al. [3]; a substitution rate of $5.3\times10^{-4}$ per site per year [1,3] was assumed for HCV. The phylogenetic tree disclosed 2 independent clusters for *Sj*-positive and -negative groups, with a high bootstrap value (81%) by the interior branch testing (Fig. 2), which is comparative with past epidemiological backgrounds in Japan. From distinct evolutionary histories in the two populations, the effective number of HCV-1b infections through time, $N(t)$, were assessed by the skyline plot. The parameters for several models in Genie v3.5 [3,10] were also examined. Time $t$ was then transformed to year using the same rate, assuming the collecting time (year 2001) as the present. Fig. 3 shows the skyline plots and population growth for *Sj*-positive and -negative patients, according to a specific demographic model in Genie v3.5 with three parameters, piecewise expansion growth model, that was evaluated by the likelihood ratio testing [11,12]. Molecular evolutionary results thus obtained supported our previous study in which the divergence time of the most recent common ancestor of HCV-1b in each area in Japan was estimated before 1850 [1]. Our estimates of the effective number of HCV-infections showed a transition from constant size to rapid exponential growth in the 1920s among patients with chronic schistosomiasis in endemic areas, which is 20 years earlier than that among patients without schistosomiasis in the 1940s. Information on HCC was available for 121 of the 131 patients with HCV-1b. Although they were relatively small in number, the incidence of HCC was significantly higher in *Sj*-positive than -negative patients ($P=0.0226$) or controls ($P=0.0488$) (Table 1).

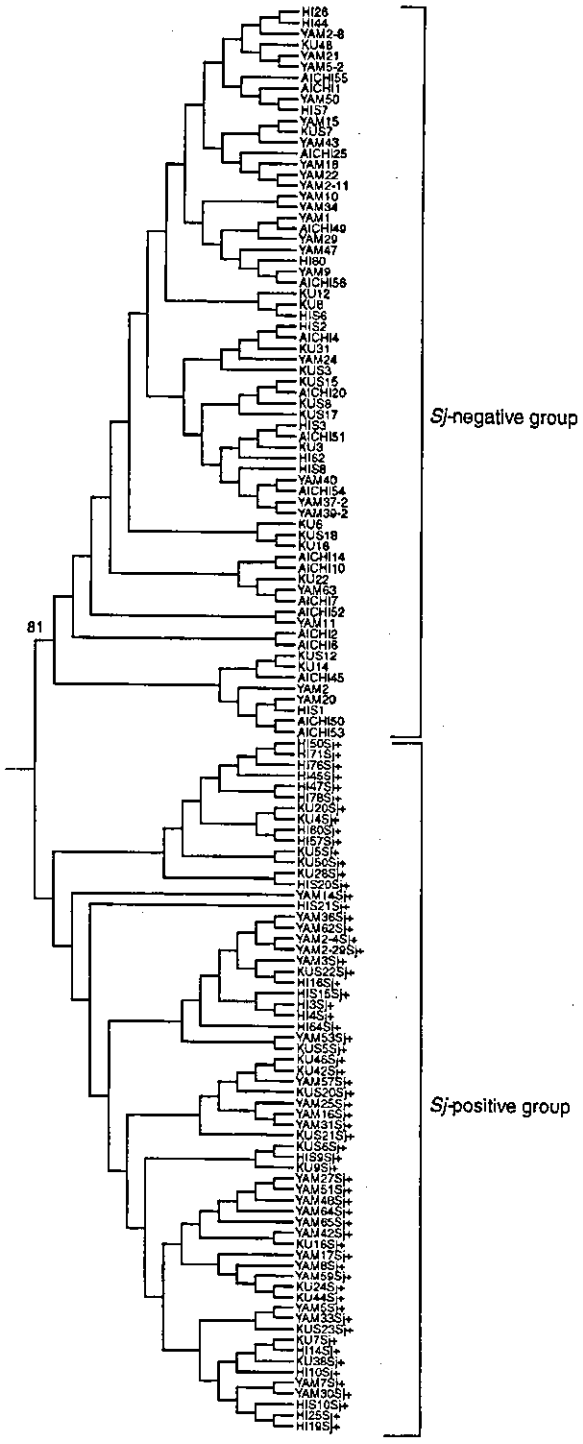


**Fig. 2. A phylogenetic tree constructed on NS5B sequences of HCV-1b strains in *Schistosoma japonicum* (*Sj*)-positive ($n=64$) and -negative ($n=67$) groups. The numbers in the tree indicate bootstrap reliability by the interior branch test. *Sj*+ indicates *Sj*-positive strains. YAM; Yamanashi, HI/HIS; Hiroshima, KU/KUS; Saga/Fukuoka, Aichi; control strains.**

## 4. Discussion

The specific demographic model based on the neutral theory [3,11,12], which has a constant size in the past and changes to exponential growth until the present, is applied to investigate the Japanese endemic of HCV. By means of the molecular evolutionary analyses, the spread time of HCV in *Sj*-positive patients was estimated 20 years earlier than that in *Sj*-negative patients from three areas in Japan where *Sj* was previously endemic (Yamanashi, Hiroshima, Saga/Fukuoka Prefectures). The spread time of HCV much earlier in *Sj*-positive than -negative patients indicates that the previous intravenous injection treatment with antimony compounds (antimonyl potassium tartarate or antimony sodium tartarate) on patients with schistosomiasis since 1921 [2] would have been a significant risk factor for HCV transmission in endemic areas through re-used needles and syringes. Indeed, it might be possible that HCV transmission from *Sj*-positive patients to *Sj*-negative patients occurs in the once *Sj*-endemic areas, but we could not find such strains in this study. One of the reasons is that residents in the village around the river, where schistosomiasis had been the most prevalent, might have been isolated from those in the other areas of the same Prefecture in the past due to the endemic disease 'schistosomiasis'. Interestingly, most Japanese strains from *Sj*-nonendemic areas in the database clustered with the *Sj*-negative group of the present study. Hence, factors other than the injection treatment for *Sj*, such as intravenous stimulants popular during and after World War II [13] and medical treatments including transfusion with blood units from paid donors in the past, would have imposed the risk for HCV transmission in most areas in Japan [14]. In addition, there would have been opportunities for HCV transmission through inadequately sterilized needles and syringes in general practices, which have contributed to a large reservoir of chronic HCV infection in Japan during the 1950s [13]. Such inadequately sterilized medical injections were still common in the less-developed world in the 20th century. WHO estimates that unsafe injections result in 2.3–4.7 million new HCV infections worldwide every year [15].

Although the spread time of HCV in *Sj*-positive group was earlier than that in *Sj*-negative group, there was no significant difference of mean age between the 2 groups. Two possibilities are considered. One is a sampling bias; as patients with *Sj* treatments were estimated to be old, relatively older patients were selected in the *Sj*-endemic areas to match age factor that might influence duration of HCV infection or HCC incidence. Second, the ages that patients had been infected with HCV were different between the 2 groups; the treatments for *Sj* in Japan were mainly conducted among relatively younger people including school children after screening of *Sj* [4,16,17], while the

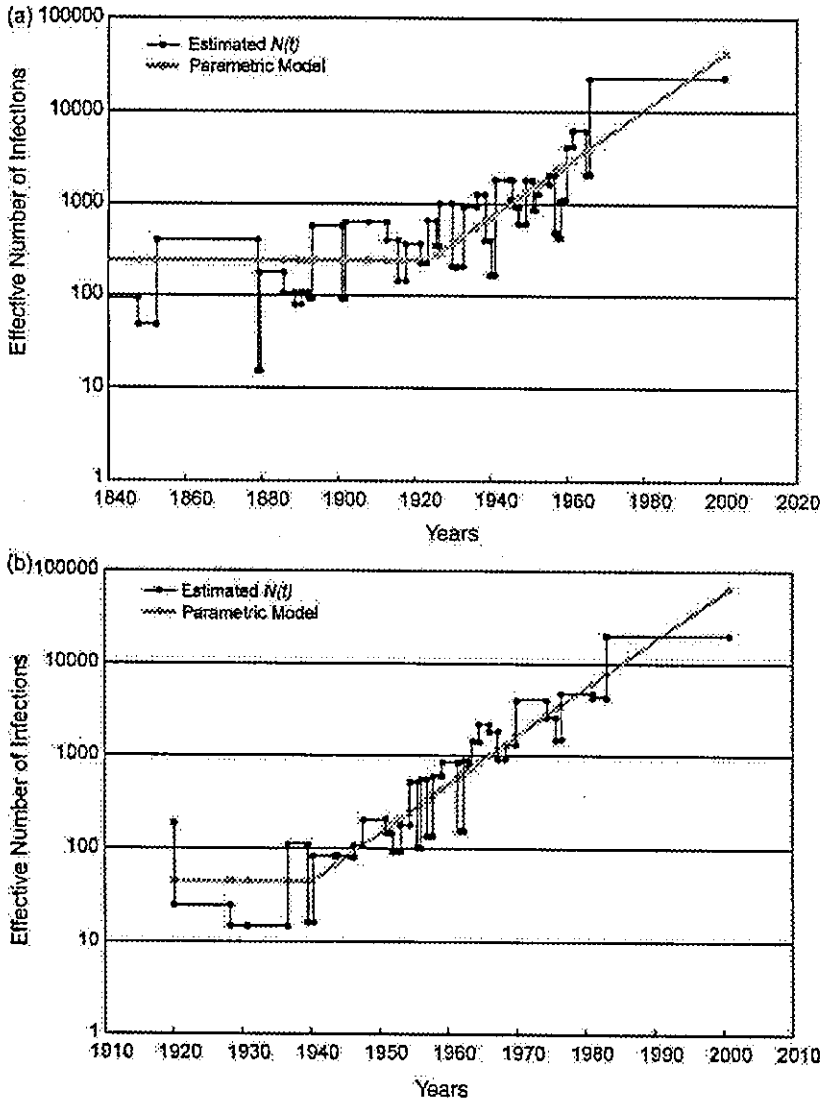


**Fig. 3. The maximum-likelihood estimates of $N(t)$ on the effective number of infections with HCV genotype 1b in Japan for *Schistosoma japonicum* (*Sj*)-positive group (a) and *Sj*-negative group (b) separated in the phylogenetic tree (Fig. 2). The parametric model is indicated by the grey line and stepwise plots by the black line that represents corresponding nonparametric estimates of $N(t)$ (number as a function of time). Genetic distances are transformed into a time scale of year using estimates of the molecular clock in the NS5B region.**

other risk factors such as blood transfusion were found in older people excluding at least children.

A disease possibly caused by schistosomal infection in Japan is documented in a book written some 300 years ago. In 1847, the clinical picture of this disease was precisely described by Yoshinao Fujii in the book 'Katayama-ki' that documented an endemic disease in Katayama area as Katayama's disease (equivalent to schistosomiasis). Water-borne epidemics of schistosomiasis prevailed in inhabitants around rivers (the tributaries of the Fuji river in Yamanashi, the Takaya river in Hiroshima and the Chikugo river in Saga/Fukuoka) in Japan, mediated by small shellfish (Miyairi-kai) serving as the natural host. More than 200,000 individuals were estimated to have been infected with *Sj* in Yamanashi Prefecture alone during 1965 through 1990 [16] and approximately 1,000,000 patients in the entire Japan since 1920s [17]. To cope with these endemics, more than 10 million intravenous injections with antimony compounds had been given in Japan since 1921 [17]. Thus, Japan would have started ahead of any other countries, in terms of HCV spread in association with schistosomiasis, wherein intravenous drugs were invented. Although acute schistosomal infection has disappeared in Japan since long ago, there are still elderly people with

chronic schistosomiasis in previously endemic areas, some of whom are developing HCC [2,14]. Substantial transmission among regions is supported by the lack of regional clustering of HCV sequences in this study.

A similar situation is reported in the Nile delta in Egypt where schistosomiasis once prevailed mediated by small shellfish [18] and the national campaigns for injection treatment with antimonyl potassium tartarate (tartar emetic) from the 1961 until 1986 are suspected to have given rise to the highest endemicity of HCV in the world ever, involving >20% of the national population there [19]. The prevalence of anti-HCV is extremely high (>70%) in patients with schistosomiasis there [18,20,21]. Highly prevalent HCV infection in the general Egyptian population accounts for most HCC cases in Egypt [22]. A question may arise whether schistosomiasis alone is responsible for the development of HCC. Patients co-infected with HCV and *Schistosoma mansoni* (*Sm*) may have a high incidence of viral persistence, accelerated fibrosis and development of HCC [23,24]. A recent population-based study between two large populations with district histories of *Sm* and hepatitis C infections, however, failed to indicate any interaction between *Sm* infection and the prevalence or severity of hepatitis C [25]. Moreover, no significant histological differences were found between anti-HCV-positive Egyptian patients with and without schistosoma [26]. Hence, the long duration of persistent HCV infection would be a more important factor for the development of HCC than the pathogeneticity of *Sm* itself.

Estimating the effective number of HCV infections has been very informative in looking back epidemic spreads of HCV infection in the Unites States [1] and Egypt [12,27]. In addition, it would also be useful in predicting the population size and extent of HCV infection. Studies to foresee future spreads of HCV would be required to cope with and prevent healthcare problems where de novo infections are increasing. The advantage of molecular evolutionary analyses, its ability to accurately estimate the dynamics of HCV based on a limited number of isolates in particular [3], will extend its application anywhere in the world where clinical sequelae of persistent HCV infection pose increasing burdens on the public health of nations.

In conclusion, the evolutionary analyses indicated that the estimated spread time in previously *Sj*-endemic areas in Japan coincides with injection treatment for *Sj* conducted since 1921. The high incidence of HCC in *Sj*-endemic areas is most likely attributed to long duration of HCV infection there transmitted through injection treatments.

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# Early Dynamics of Hepatitis C Virus in the Circulation of Chimpanzees with Experimental Infection

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**Key Words**
Chimpanzees · Chronic hepatitis · Hepatitis C virus · Nucleic acid amplification testing · Transfusion

**Abstract**
Two chimpanzees were inoculated with hepatitis C virus (HCV) and followed on a daily basis for 12 days. HCV RNA became detectable in their sera on day 5 by polymerase chain reaction with the detection limit of $10^2$ copies/ml. Based on an exponential growth observed until 8 or 9 days after inoculation in their sera, the doubling time of HCV in the circulation was estimated at 6.3–8.6 h and log time (time required to grow 10-fold) at 31.3–42.9 h. The exact doubling time of HCV determined in them would help plan an efficient strategy for screening out blood donors in the window period of infection between the exposure and the development of antibody to HCV in serum.



There still remains a residual risk of contracting hepatitis C virus (HCV) infection after transfusion with blood units without the antibody against it (anti-HCV) detectable by the second- and third-generation immunoassays [1]. Anti-HCV is not raised in the circulation of individuals during the 'window period' after the exposure to HCV that is estimated at an average of 55 days in chimpanzees with experimental infection [2] and 41 days in human beings [3]. In order to identify early HCV infection, nucleic acid amplification testing (NAT) has been introduced to transfusion services [3, 4]. NAT can detect by far the most blood units in the window period of HCV infection, but cannot identify them all on a theoretical basis [5]. Even when 200 µl of serum from a donor is tested by the individual NAT, approximately $10^2$ copies/ml of HCV RNA are required to produce a positive result. The sensitivity is reduced further in a mini-pool NAT performed on 50 donors in the current practice, in which a single donor is represented by merely 4 µl of serum. In actuality, HCV infection can occur in the recipient of platelet concentrates from a blood donor testing negative by NAT [6].

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The doubling time and log time (time required for growing 10-fold) of HCV in the circulation are prerequisite to planning a strategy for efficiently detecting HCV infection in blood donors as well as for understanding the limit of current screening methods based on polymerase chain reaction (PCR). They have not been determined accurately, however, due to the lack of in vitro systems for HCV culture. The documented doubling time of HCV varies widely from 2.4 h (0.1 day) [3] to 14.9 h [7] or 17.3 h (0.72 day) [5].

The dynamics of HCV growth in an early phase of infection were analyzed in the circulation of 2 chimpanzees for determining how soon HCV RNA increases to $10^2$ copies/ml that can be detected by the individual NAT. Further, the doubling time and log time of HCV RNA were estimated based on the exponential growth in their sera during an early phase of infection.

Two chimpanzees entered the experimental transmission study – chimp No. 224 (C224: male, 14 years old, weight 59.1 kg) and chimp No. 267 (C267: female, 7 years old, weight 49.0 kg). They both were kept in individual cages and received humane care in accordance with all relevant requirements for the use of primates in an approved facility. Neither of them had serological or molecular virological evidence for past or present HCV infection before the inoculation. They received inocula while they were under anesthesia with ketamine hydrochloride.

Fresh-frozen plasma was obtained from a donor who was in the window period of infection with HCV genotype 1b. It was separated within 6 h after blood collection, and contained HCV RNA at a titer of $8.4 \times 10^6$ copies/ml determined by Taq Man PCR (Applied Biosystems Japan, Tokyo, Japan). The plasma was kept frozen at –80° in 1-ml aliquots; the infectious activity of the plasma in chimpanzees decreased >100-fold during these procedures [8]. An aliquot (1 ml) of this plasma was thawed in a 37° bath and injected intravenously to C224. The other chimpanzee (C267) was inoculated with a passage of another fresh-frozen plasma through a chimpanzee that had been inoculated with 1 ml of it and developed viremia [8]; the plasma was donated by an individual in the window period of co-infection with HCV genotypes 1b and 2a. Serum obtained from the chimpanzee 7 weeks after inoculation was aliquoted in 1-ml volumes and snap-frozen in liquid nitrogen. An aliquot was serially diluted 10-fold with self serum of C267, and 1 ml of a $1{:}10^3$ dilution containing approximately 200 copies of HCV RNA was injected intravenously to C267; it corresponded to 10 times the minimal infectious dose of HCV in chimpanzees [8]. After the inoculation, serum samples were obtained from them daily during the same hour in the morning (9–10 a.m.) for the first 12 days. They had been kept frozen at –80°, and were tested for HCV RNA simultaneously in the same assay by Taq Man PCR.

The dynamics of HCV RNA in sera from the 2 chimpanzees during an early phase of infection are illustrated in figure 1. HCV RNA was first detected in serum taken 5 days after the inoculation from them both by Taq Man PCR. Then, HCV RNA titers increased exponentially on the log scale for 8 days after inoculation in C224, and until 9 days in C267. Thereafter, HCV RNA in them deviated from the straight line of exponential growth. Based on the linearity of an initial exponential growth and the coefficient of determination, the doubling time of HCV replication was calculated to be 6.3 and 8.6 h, and the time required to grow 10-fold (log time) to be 31.3 and 42.9 h in C224 and C267, respectively; they were in a remarkably good agreement.

The growth of HCV was closely followed in the circulation of 2 chimpanzees, 1 of whom (C267) had been inoculated with passaged and calibrated HCV from a chimp in the preacute phase of infection [8]. The growth curves of HCV in the 2 chimpanzees were strikingly similar. HCV RNA was not detected for 5 days, then increased exponentially until the 8th or 9th day, and decreased thereafter. The failure in detecting HCV RNA during the initial 4 days would be attributed, in part, to a limited sensitivity of the Taq Man PCR method ($10^2$ copies/ml). When the linear growth was extrapolated beyond the detection of HCV RNA in serum, however, it converged to day 3 in 1 chimp and a little later than day 2 in the other (fig. 1). Hence, it would be reasonably delineated that HCV would have started circulating in both chimpanzees as early as 2–3 days after infection, a few days before HCV RNA became detectable by PCR 5 days after the inoculation.

The time from HCV transmission to the first detection of viral RNA in the circulation is called the 'eclipse' phase [3], which may vary by the size of inoculated dose and the sensitivity in detecting HCV RNA. The eclipse phase of 5 days observed in the chimpanzees (C267) inoculated with 10 times the minimal infectious dose of HCV [8] was longer than that of 3 days reported for an experimentally transmitted chimpanzee inoculated with 0.5 ml of human serum containing $10^{6.5}$ chimp infectious doses per ml [9]. Since high-dose transmission by transfusions has been excluded by anti-HCV screening [1] and low-dose infection through blood units in the NAT window period is at issue nowadays [3, 4], a longer eclipse phase would be

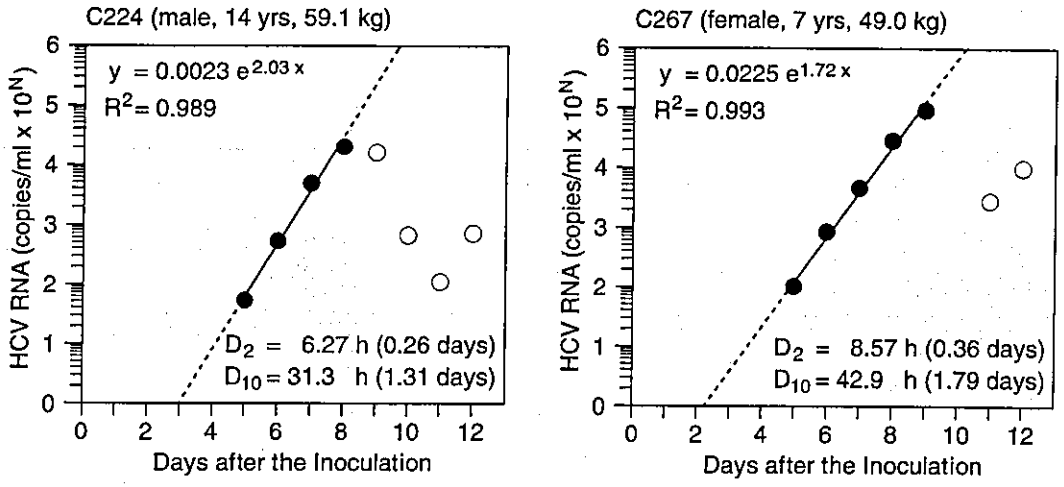


**Fig. 1.** Exponential growth of HCV during an early phase of HCV infection in 2 chimpanzees experimentally transmitted with pedigreed inocula. HCV RNA was determined by Taq Man PCR. Solid circles and the solid line represent the linear exponential growth of HCV. Dotted lines show an imaginary growth below the detection limit in initial days (to the left) as well as a putative exponential growth beyond the linearity (to the right). Open circles are HCV RNA levels that deviated from the linearity of exponential growth. $R^2$ = Coefficient of determination; $D_2$ = doubling time; $D_{10}$ = the log time required for growing 10-fold.

more informative practically. An observed interval between 'true' eclipse phase (2–3 days) and PCR eclipse phase (up to 5 days), however, indicates that NAT would not be able to close the window spanning a few days after the exposure to HCV.

The duration of exponential HCV growth is called the 'ramp-up' phase [5]. Should the ramp-up phase last until all susceptible hepatocytes are infected, the slope of exponential growth would hardly be influenced by the size of HCV dose. Doubling times of HCV in the circulation calculated on the exponential growth of viral RNA in the 2 chimpanzees were 6.23 and 8.36 h, respectively. They were half as long as 14.9 h [7] and 17.3 h [5] reported in human beings. It is not clear how such a big discrepancy has arisen. Although species differences may give an account on it, a rigorous and meticulous design for experimental transmission conducted in chimpanzees would hardly be feasible for HCV infection in the transfusion setting. This issue needs to be looked into and settled, since the doubling time of HCV is crucial in working out measures for increasing the blood safety.

The results obtained in this study would help determine the size of pool in NAT for efficiently screening HCV RNA in blood donors. Due to extremely fast replication of HCV, the merit of reducing the size of pool would have its own limit. Based on the doubling time of 6.3–8.6 h in this study, the window can be narrowed by at most 1.3–1.8 days even by performing the individual NAT, in place of a mini-pool NAT on 50 donors in the current practice. This goes along with the mathematical model of Weusten et al. [5] who calculated the risk of contracting HCV infection to decrease only to one half by performing the individual NAT in comparison with a mini-pool NAT on 50 donors. Despite the doubling time of HCV, which is much shorter than hepatitis B virus (62.4 h) or human immunodeficiency virus type 1 (20.5 h) [10], there would be a limitation in narrowing the window period by performing the individual NAT. For further increasing the safety of blood transfusion, in terms of the risk for HCV infection, the other strategies would need to be considered, such as condensing HCV RNA in more amounts of serum from individual donors before performing a mini-pool NAT.

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# わが国における E型肝炎の実態とその対策

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## はじめに

E型肝炎の発生には地域差があり、多くは赤道周囲の国々や地域（東南アジア、中南米など）でみられる[1,2]。そのため、従来は我が国ではE型肝炎の流行地域への渡航による輸入感染症の一つとして考えられてきた。しかし、最近、海外渡航歴がなく国内で感染したE型肝炎（血中HEV-RNA陽性）が少なからず認められ、しかもこれらの症例のHEV遺伝子型は我が国固有のHEV株であることから、にわかにE型肝炎が注目されてきている[3-7]。さらに、我々の検討[8]により原因不明の劇症肝炎の中にE型劇症肝炎例が存在することが初めて明らかとなり、その実態調査も求められている。一方、HEVの感染様式は、主として糞口感染と考えられてきたが、最近、人畜共通感染症（Zoonosis）の可能性[9]が指摘されており、その確証も得られつつある。

本稿では現時点（2004年1月）での我が国におけるE型急性肝炎および劇症肝炎の実態を中心に述べる。

## 原因不明の急性肝炎および劇症肝炎におけるE型肝炎の頻度

1998年から2001年末までに当科で経験した急性肝炎（重症型を含む）166例であるが、A型肝炎、B型肝炎、C型肝炎、薬剤性肝炎、自己免疫性肝炎およびアルコール性肝炎と診断された例、EBウイルス、サイトメガロウイルス、TTウイルスなどの肝炎ウイルス以外のウイルスによる急性肝炎例を除くと、残りの25例が原因不明の急性肝炎であった。これらの症例について入院時の保存血清を用いてHEV-RNAを測定したところ、4例

表1　急性肝炎および劇症肝炎の成因（岩手医科大学第一内科）

| 成　因 | 急性肝炎 | 劇症肝炎 |
|---|---|---|
| A型 | 44例（26%） | 1例（3%） |
| B型 | 30例（18%） | 9例（27%） |
| C型 | 8例（5%） | |
| 非A非B非C型 | 21例（13%） | 19例（58%） |
| **E型** | **4例（2%）*** | **2例（6%）**** |
| 薬剤性 | 33例（20%） | 1例（3%） |
| 自己免疫性 | 3例（2%） | |
| アルコール性 | 6例（4%） | |
| 肝炎ウイルス以外のウイルス（EBV、CMVetc.） | 16例（9%） | |
| TTウイルス | 1例（1%） | 1例（3%） |
| 計 | 166例（100%） | 33例（100%） |

*：原因不明の急性肝炎25例中16%に相当
**：原因不明の劇症肝炎21例中、血清の保存されていた19例中10.5%に相当

(16%) がHEV-RNA陽性を示し、E型急性肝炎と診断された[9]。一方、劇症肝炎 (1992-2001年) 33例のうち非A非B非C型の劇症肝炎と診断されたのは22例であるが、入院時の血清が保存されていた19例について血中HEV-RNAを測定したところ、2例 (10.5%) が陽性を示し、劇症肝炎全体では6%の頻度を占めた (表1)。

我が国における非A非B非C型の急性肝炎におけるE型急性肝炎あるいは劇症肝炎の頻度についての報告をみると、多施設共同研究による原因不明の急性肝炎例におけるE型急性肝炎の検討[5]では、北海道地域において急性肝炎に占めるE型の頻度が25%と高い。また、大西ら[10]も札幌地域における過去5年間に経験した急性肝炎145例の中に原因不明の急性肝炎68例を認めたが、そのうち血清が保存されていた59例について血中HEV-RNAを測定したところ16例 (27.1%) が陽性を示し、急性肝炎全体の11%がE型であったことを報告している。また、松井ら[11]は非A非B非C型急性肝炎15例中5例 (14%) に、劇症肝炎10例中1例 (10%) に、小島ら[12]は非A非B非C型急性肝炎104例中5例 (4.8%) に、劇症肝炎21例中1例 (4.8%) にE型を認めている。さらに、国立病院療養所肝疾患ネットワーク参加施設における実態調査[13]では、1990年から2002年までの過去13年間に診断した非A非B非C型急性肝炎の中からランダムに選択した311例について、初診時の血清中のHEV抗体 (IgM-HEV抗体およびIgG-HEV抗体) を測定したところ9例 (2.9%、うち8例はHEV-RNA陽性) がE型肝炎と診断され、その陽性率は2000年以降に高いと報告している。以上の成績は、国内においてE型急性肝炎の発生頻度が最近増加してきていること、その発生には地域差がみられることを示している。なお、劇症肝炎におけるE型の頻度については現在、難治性の肝疾患調査研究班においてE型劇症肝炎の全国実態調査が進められており、その結果が待たれるところである。

## E型急性肝炎の臨床像

HEV感染の多くは不顕性に経過するが、感染者の一部は2～9週 (平均6週) の潜伏期を経て急性肝炎を発症し、その大部分は予後良好である。しかし、稀に重症化し、とくに妊娠後期の妊婦がE型肝炎に罹患すると高率 (10～20%) に劇症化し、予後不良であることが報告されている[14]。

E型肝炎の確定診断は現在のところ血中HEV-RNAの測定が必要であるが、HEVのウイルス持続期間は平均16.6日 (最高39日)[15]と言われているため、入院時に既に陰性化している場合もありうる。現在、臨床応用されているIgM-HEV抗体およびIgG-HEV抗体測定法を用いる場合にはペア血清 (初診時および1～2週後) を用いて抗体価の推移を観察し、IgM-HEV抗体の低下または陰性化を条件に診断することが出来るが、未だ改良が必要であり、感度および特異性の高いIgM-HEV抗体およびIgG-HEV抗体の測定法の開発が急務である。

急性肝炎としての臨床像が他の肝炎ウイルスによる急性肝炎の臨床像と異なるかについては症例数が少なく十

表2　当科および関連施設のE型肝炎例

| 症例 | 年齢 | 性 | 年度 | 臨床病型 | 初発症状 | T-Bil (mg/dl) | AST/ALT (IU/l) | PT (%) | 遺伝子型 | 転帰 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 46 | 男 | 1998 | 急性肝炎 | 頭痛、倦怠感、関節痛 | 4.1 | 1624/2824 | 84 | III | 生存 |
| 2 | 47 | 女 | 1999 | 急性肝炎 | 発熱、倦怠感 | 0.8 | 1415/1430 | 68 | III | 生存 |
| 3 | 72 | 男 | 2001 | 急性肝炎 | 感冒様症状 | 20.0 | 1050/914 | 100 | III | 生存 |
| 4 | 56 | 男 | 2001 | 急性肝炎 | 頭重感、食欲不振、発熱 | 5.7 | 539/2966 | 100 | III | 生存 |
| 5 | 67 | 女 | 2002 | 急性肝炎 | 掻痒感、倦怠感、食欲不振 | 2.2 | 2583/2273 | 80 | III | 生存 |
| 6 | 62 | 男 | 2002 | 急性肝炎 | 倦怠感 | 18.0 | 1054/2400 | 80 | III | 生存 |
| 7 | 71 | 男 | 2002 | 急性肝炎 | 背部痛 | 6.1 | 1101/1962 | — | III | 生存 |
| 8 | 73 | 男 | 2003 | 急性肝炎 | 倦怠感、食欲不振 | 1.9 | 1099/672 | 73 | III | 生存 |
| 9 | 61 | 男 | 1993 | 劇症肝炎 | 倦怠感、食欲不振、嘔吐 | 23.5 | 184/572 | 6 | IV | 死亡 |
| 10 | 60 | 男 | 1995 | 劇症肝炎 | 倦怠感、食欲不振、嘔吐、下痢 | 35.0 | 2276/2256 | 13 | III | 死亡 |
| 11 | 65 | 女 | 2001 | 遅発性肝不全 | 倦怠感、掻痒感 | 36.0 | 1965/2153 | 9 | III | 死亡 |
| 12 | 59 | 女 | 2002 | 亜急性肝炎 | 倦怠感、食欲不振 | 28.1 | 1458/819 | 24 | III | 生存 |

分検討されていないが、経口感染による代表的なA型肝炎と比較した我々の検討[16]では、E型急性肝炎はA型肝炎に比較して発症時の年齢が有意に高く、性差では男性が有意に多い。発症時の臨床症状では発熱（>38℃）の頻度が有意に低く、全身倦怠感は有意に多い。肝機能ではピーク時の血清ZTT、IgM値がA型急性肝炎で有意に高いが他の検査値には明らかな差異を認めない（但し、黄疸のピーク値までの日数は有意に短い）。したがって、E型急性肝炎の診断はその臨床像や血液生化学検査からのみでは不可能である。しかし、重症化例を除くと肝機能は速やかに改善し正常化する例が多い。

表2に我々の施設および関連病院で経験したE型急性肝炎および重症肝炎例のまとめを示した。なお、亜急性肝炎と遅発性肝不全の症例はいずれも中年女性であり、入院当初は薬剤性肝障害の診断であったが、入院時の血清HEV-RNAが陽性を示したことより、E型肝炎と診断された例である。したがって、これら2例の重症化には薬剤とHEV感染が関与していたものと考えている。E型急性肝炎の多くは予後良好で慢性化することなく治癒すると考えられるが、重症化または劇症化の要因についてはHEVそのものに起因するのか、あるいは他の原因や合併症が関与しているのか慎重に検討する必要がある。

## E型急性肝炎および劇症肝炎の遺伝子型

HEVの遺伝子型は大別して4つに分類される。遺伝子型Iは主にアジア・アフリカなどの流行地から報告された株、遺伝子型IIはメキシコから報告された株、遺伝子型IIIは米国で発見された株、遺伝子型IVは中国より最初に報告された株であるが、遺伝子型IIIおよびIVはグループ内で多様性に富んでいる[17,18]。これまで報告された多くの分離株の分子系統樹をみると、各々の地域毎に特有の固有株が存在していることが明らかになっている。実際に、わが国で発見された固有のHEV株はIII型あるいはIV型であり、その多くは各地域毎に分かれている。また、北海道にはIV型が多く、本州地域ではIII型が多い傾向にある。急性肝炎と劇症肝炎のHEV遺伝子型の違いをみると、報告例の地域により影響を受けるが、全体として急性肝炎ではIII型、劇症肝炎ではIV型が多い傾向にある。表3にこれまでわが国で報告された劇症肝炎例での遺伝子型を示したが、7例中4例がIV型である[8,19-21]。今後、遺伝子型の違いが肝炎の重症化または劇症化に関わるか否かについても検討が必要と考えられる。

## 対策

急性肝炎例あるいは劇症肝炎例の多くは感染経路は不明である。わが国においては糞口感染、水系感染はほとんど考えられず、感染対策を立てることは困難な状況にある。当初、HEV遺伝子の分子系統樹の解析から豚が感染源として注目されたが、実際に流通市場に出回っている肉類からの感染は考えにくく、Zoonosisの確証は得られていなかったが、最近、市販されている豚レバーにHEVが存在することの報告[20]、鹿生肉や猪肉の摂取により発症したE型急性肝炎例の報告（これら獣肉と肝炎

表3　E型劇症肝炎の本邦報告例

| 報告例 | 年齢 | 性 | 年度 | 臨床病型 | 遺伝子型 | 予後 |
|---|---|---|---|---|---|---|
| 1. | 61 | 男 | 1993 | 急性型 | III | 死亡 |
| 2. | 60 | 男 | 1995 | 亜急性型 | IV | 死亡 |
| 3. | 66 | 男 | 1998 | 急性型 | IV | 死亡 |
| 4. | 64 | 男 | 2002 | 急性型 | IV | 死亡 |
| 5. | 58 | 男 | 2002 | 亜急性型 | IV | 死亡 |
| 6. | 51 | 男 | 2001 | 亜急性型 | III | 生存 |
| 7. | 34 | 女 | 2002 | 亜急性型 | IV | 死亡* |

1～3：Suzuki et al. New Engl J Med 347：1455, 2002.
4～5：Yazaki et al. JGV 84：2351, 2003.
6～7：Ohnishi et al. Hepatol Res 25：213, 2003.
※生体肝移植後に死亡

発症者のHEV遺伝子型が一致)[22,23]がなされた。したがって、これら動物の生肉あるいはレバーなどを摂取する機会には加熱などの処理を十分に行うことが大切であり、そのような啓蒙活動を行う必要がある。さらに、昨年わが国でも初めて輸血後のE型急性肝炎例の報告[24]がなされた。血液ドナーはウイルスのウインドウ期にある感染者であったという極めて稀なケースであるが、一般住民のIgG-HEV抗体価の陽性率が上昇してきている可能性が指摘されていること[25]などを考慮すると、輸血による感染もありうることを認識する必要がある。

## おわりに

E型急性肝炎および劇症肝炎についてその実態を中心に解説した。今後、E型肝炎は増加することも予想されるが、正確な血清学的な診断法の確立とともに感染経路の解明、ウイルス学的解析などをさらに進める必要がある。


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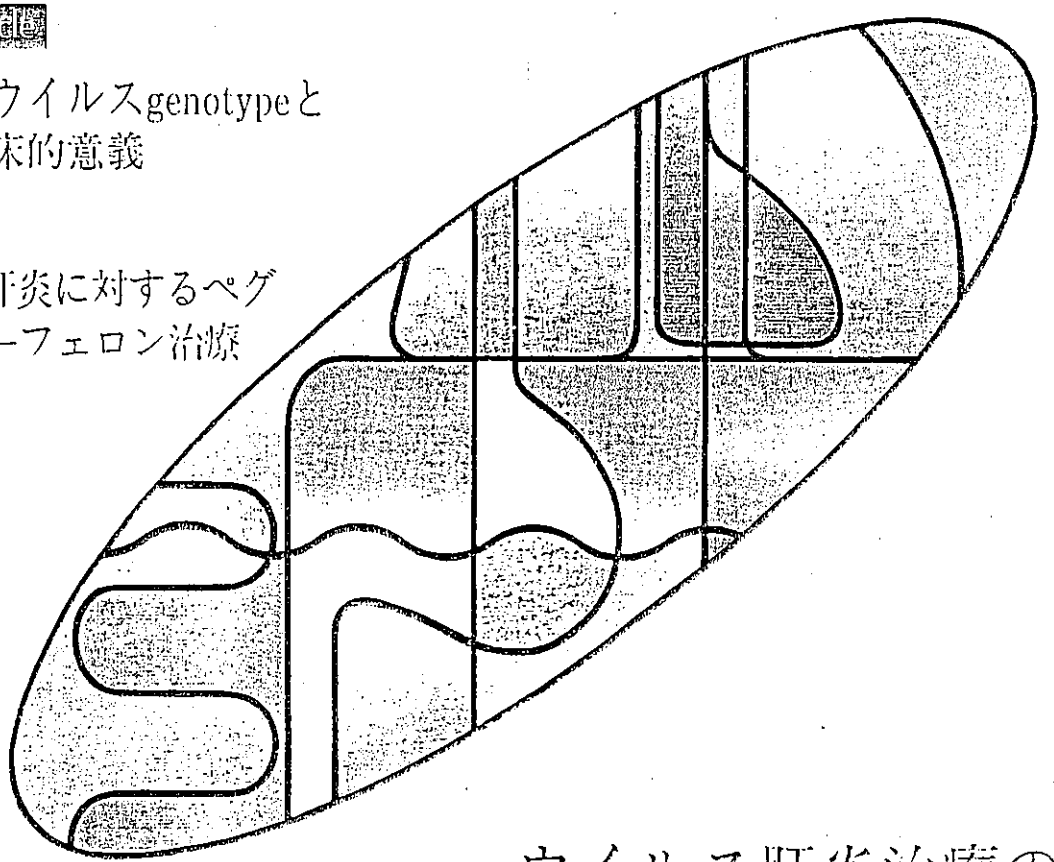
## 特集 ウイルス肝炎
## ―新たな治療法―

Special Article

B型肝炎ウイルスgenotypeとその臨床的意義
溝上雅史

C型慢性肝炎に対するペグインターフェロン治療
堺　隆弘



総説

ウイルス肝炎治療の展望
小俣政男　岡上　武　榎本信幸　加藤直也

南江堂

# ≪ウイルス肝炎の抗ウイルス療法≫
# B型肝炎重症化例の治療

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滝川康裕　鈴木一幸*

## はじめに

B型肝炎ウイルス(HBV)は，わが国における肝炎の成因ウイルスとして今なお重視されている．B型急性肝炎のほとんどは散発性の発症であり，その予後は良好であるが，まれに重症化あるいは劇症化をきたすことがある．実際に，わが国の劇症肝炎においては成因の約40%をHBVが占めており，その対策はきわめて重要である[1]．

HBVによる重症化例(劇症化例も含む)は，①HBVの初感染による重症化例と，②HBVキャリアからの急性増悪による重症化例，に分かれるが，後者には，他疾患の治療のために用いる抗癌薬や免疫抑制薬などの使用によりHBVの再増殖をきたし，肝障害の増悪をきたした例も少なからず認められる．また，劇症肝炎においてはHBVの初感染例とHBVキャリアからの発症例では，後者は亜急性型の経過をたどることが多く予後が不良であることも知られている[2]．しかし，最近，抗ウイルス療法においてはインターフェロン(IFN)だけではなく，強力な抗ウイルス作用を有する経口の抗ウイルス薬(lamivudineなど)の登場によって，HBVによる重症化例の治療の向上が期待されている[3,4]．

本稿では，B型肝炎の重症化例(プロトロンビン時間(PT)が入院時または経過中に40%以下を示した例)および劇症化例(昏睡II度以上の肝性脳症を伴う例)の治療について，最近の動向をふまえて解説する．

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## 肝炎重症化(劇症化)の頻度とその誘因

肝炎ウイルス別にみた急性肝炎の劇症化率は0.14～4.7%であり，成因によっても異なる[5～7]．過去5年間(1998～2002年)に当科で経験したHBVの初感染による急性肝炎は24例であるが，そのうち重症化例は10例(42%)であった．一方，同時期におけるHBVキャリアからの肝機能急性増悪例は18例あり，うち14例が重症化例であった．われわれの施設での重症化率はきわめて高いが，この原因としては，われわれの施設がとくに重症肝炎の治療センターとして位置付けられているためと考えられる．

肝機能の急性増悪による重症化または劇症化をきたした誘因は多岐にわたる．多くはHBVキャリアの自然発症例であるが，副腎皮質ステロイドや抗癌薬などの使用によってHBVのreactivationが生じ肝炎の急性増悪をきたした例も少なからずみられる．治療を行ううえでは，肝炎の急性増悪の原因がHBVのreactivationによるものか否かを判定することがきわめて重要であるが，肝機能増悪前のHBVのウイルス量が把握されていないことも多く，重症化した時点ではすでにウイルス量が低値であることもしばしば観察されるので，HBVのreactivationによるものかを判断することがむずかしい場合もある[8]．

## B型肝炎重症化時の治療

### 1．一般的治療

重症例に対しては，強力ネオミノファーゲンCの大量静注(80～100 ml)療法とともに，肝再生を

期待したグルカゴン-インスリン(G-I)療法，血液凝固線溶異常(DICを含む)に対する抗凝固線溶療法(gabexate mesilate，アンチトロンビンIII(AT-III)製剤)，抗炎症および過剰な免疫反応を抑えるステロイドパルス療法が行われている．さらに劇症化例に対しては，血漿交換と血液濾過透析を組み合わせた人工肝補助療法とともに，全身管理や合併症対策を含めた集学的治療が必要となる．最近ではプロスタグランジン製剤(肝細胞保護，微小循環調節，炎症性サイトカインの産生抑制を期待)や，免疫抑制薬であるcyclosporinの使用も試みられている[2,9]．

2．抗ウイルス療法

抗ウイルス療法は一般的にはHBVキャリアからの重症化例に対して適応があると考えられ，ウイルスの増殖を抑制することにより持続的な肝細胞壊死の進展を阻止することが目的である．一方，HBVの初感染による重症化例や劇症化例においては，広範な肝細胞壊死が生じた時点ではすでに血中よりHBVがクリアされており，血中ウイルス量も低下していることが多いことより，抗ウイルス療法の適応となることは少ないと考えられている．しかし，HBVの初感染による重症化例や劇症化例に対する抗ウイルス療法の適応についてのコンセンサスはいまだ得られていないので，肝機能の経過ならびに血中ウイルス量をみながら，個々の症例ごとに対応すべきと考えられる．最近，われわれも初感染による急性肝炎例でHBV DNA量が高く，黄疸および肝機能異常が持続する例に対して抗ウイルス薬であるlamivudineを使用し，良好な経過をたどった1例を経験している．

1）IFN療法：重症化例または劇症化例に対するIFNの使用法に関しては厳密な意味では統一されてはいない．通常，慢性肝炎例に対する治療方法と同様にIFN-$\alpha$あるいは$\beta$製剤1日300～600万単位を連日筋注または静注し，HBV DNA量を経時的に測定しながら減量または隔日投与する．

2）lamivudine：lamivudineはヒト免疫不全ウイルス(HIV)の治療薬として開発されたヌクレオシド誘導体の抗ウイルス薬であるが，HBVに対しても増殖抑制効果を示すことから，わが国では2000年秋よりB型慢性肝炎例に対して使用が可能となった薬剤である．B型慢性肝炎に対する臨床治験成績では血中HBV DNA量の速やかな減少が認められており，副作用も少ないことが報告されている[10]．

HBVキャリアの肝炎重症化例に対しても，早期から本剤を投与することにより劇症肝炎への移行を阻止できる可能性が，また劇症化例に対しては救命率の向上が期待されている[3,9]．さらに，副腎皮質ステロイドや抗癌薬投与によってHBVのreactivationが予想される症例においては，これらの薬剤投与初期より定期的にHBV DNA量を測定し，増加傾向を認めた場合はlamivudineを投与して肝機能の悪化を防止する試みも行われている．実際に，当科における治療成績でも重症化にいたる前にlamivudineを投与した症例については肝炎の急性増悪を認めていない．

現在，HBVによる肝炎重症化例および劇症化例に対する本剤の効果については，難治性の肝疾患調査研究班(班長：埼玉医科大学 藤原研司先生)において全国的なprospective studyが行われており，その評価が待たれるところである．

lamivudineの至適投与量については慢性肝炎例と同様に1日100 mgを経口投与するのが一般的である．しかし，本剤が効果を発揮するためにはある程度の時間が必要であるため，HBV DNA量の多い例では，初期には投与量を増量したり，あるいはIFNとの併用も考慮すべきと考えられる．lamivudineの使用期間あるいは中止時期については，慢性肝炎ではe抗原からe抗体へのseroconversionが起こり，6ヵ月以上HBV DNAが陰性であれば中止可能としているが，seroconversionがみられない例ではいつまで投与を継続するかの判断がむずかしい場合が多く，重症化例または劇症化例についても今後の検討事項となって

Table 1. Lamivudine使用例の内訳(岩手医科大学第一内科)

| No. | 性，年齢 | 発症前肝病変 | 急性増悪の誘因 | lamivudine併用の有無 | IFN併用の有無 | 転帰 |
|---|---|---|---|---|---|---|
| 1 | 女，54 | CH | 化学療法(悪性リンパ腫) | + | + | 劇症化(死亡) |
| 2 | 男，56 | ASC | 自然発症 | + | + | 劇症化(死亡) |
| 3 | 女，47 | CH | 自然発症 | + | + | 改善(生存) |
| 4 | 女，25 | CH | 自然発症 | + | + | 改善(生存) |
| 5 | 女，37 | CH | 自然発症 | + | − | 改善(生存) |
| 6 | 男，46 | CH | 自然発症 | + | − | 改善(生存) |
| 7 | 男，27 | ASC | 自然発症 | + | + | 改善(生存) |
| 8 | 男，73 | AH | (初感染) | + | − | 改善(生存) |
| 9 | 男，53 | AH | (初感染) | + | − | 劇症化(死亡) |
| 10 | 女，43 | FH | (初感染) | + | + | 改善(生存) |
| 11 | 男，64 | CH | 自然発症 | − | + | FH(死亡) |
| 12 | 女，24 | CH | 自然発症 | − | + | FH(改善) |
| 13 | 男，27 | CH | 自然発症 | − | + | LOHF(死亡) |
| 14 | 男，66 | CH | ステロイド(ネフローゼ) | − | + | 改善後死亡(脳出血) |
| 15 | 男，26 | CH | 自然発症 | − | − | 改善 |
| 16 | 男，51 | CH | 自然発症 | − | − | 改善 |
| 17 | 男，29 | ASC | 自然発症 | − | − | 改善 |
| 18 | 男，51 | ASC | ステロイド(悪性リンパ腫) | − | + | 改善 |

CH：慢性肝炎，ASC：無症候性キャリア，AH：急性肝炎，FH：劇症肝炎，
LOHF：遅発性肝不全

いる．

さらに，lamivudine投与の問題点として，投与期間が長期になればなるほど高率にYMDDモチーフの変異を生じ，肝炎の再燃を起こす可能性があることである[9,10]．変異株が出現し肝炎が再燃したときの対策としては，通常はlamivudineを継続投与しIFNを併用する方法がとられる．急性増悪による重症化例では，肝予備能の改善が得られ重症肝炎より離脱しえたと判断できれば，いったん本剤を中止して経過をみるという考え方もある．lamivudineの使用に際しては今後，どのような例が変異を起こしやすいのか，その頻度はどのくらいなのかなどについて症例を積み重ねて明らかにする必要があり，耐性株の出現時の対応についてもさらに検討が必要である．

最近，lamivudineに代わる抗ウイルス薬が開発されてきており，米国では2002年9月よりadefovir(ヘプセラ，Gilead社)の使用が可能となっている．わが国においてもlamivudineに代わる抗ウイルス薬の使用が可能になれば，B型肝炎によ

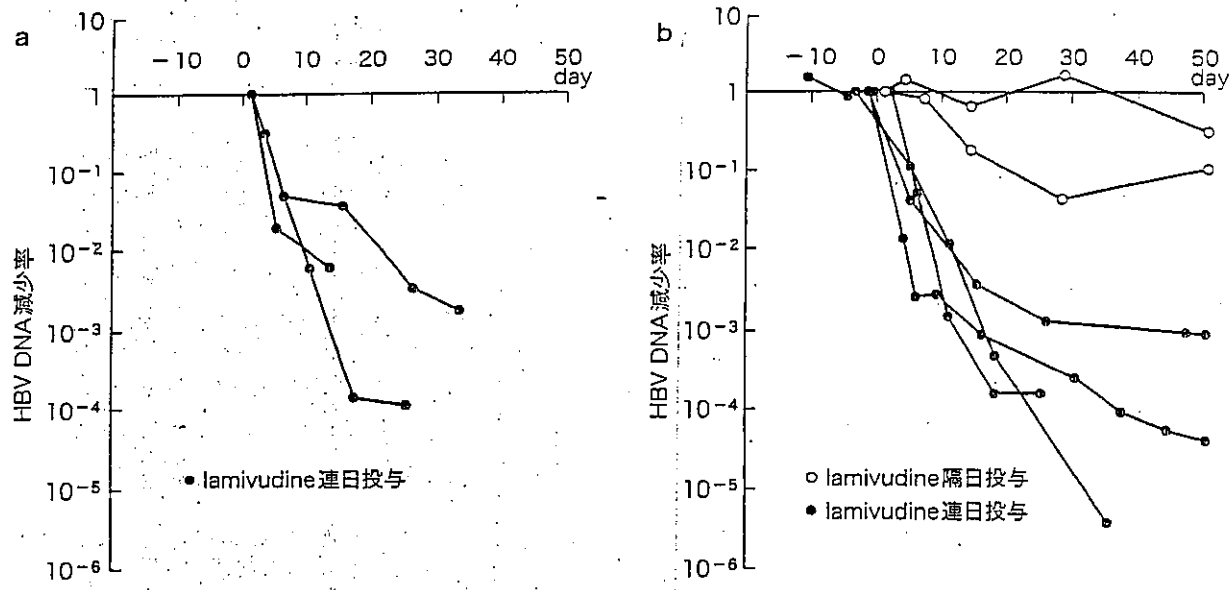


Fig. 1. 抗ウイルス療法施行例の HBV DNA 量の推移
a：lamivudine と IFN 併用療法例
b：lamivudine 単独療法例

る重症化例あるいは劇症化例に対する対処法にも進展がみられるものと期待される.

3．抗ウイルス療法の実際(自験例)

重症化例または劇症化例に対する lamivudine 治療を行った自験例での成績(Table 1)を示す. lamivudine 投与を受けた 10 例のうち初感染例は 3 例(うち 1 例は PT は 40%以上であったが, 黄疸が持続し肝機能の改善が認められなかったため使用), 他の 7 例は HBV キャリアからの急性増悪例である. IFN は 6 例に併用した. 発症から lamivudine 投与までの日数は 6〜45 日であり, 入院直後より使用した例が多いが, 重症化時には他の治療を行い, PT が改善(>60%)したのちに肝炎の再燃防止を目的に使用した例もある. 一方, lamivudine 非投与 8 例はいずれも HBV キャリアからの急性増悪例であり, lamivudine が市販される以前に経験した例である. 5 例に IFN を使用している. なお, その他の併用療法はさまざまであったが, lamivudine 投与例と非投与例のあいだには大きな差を認めていない.

lamivudine 投与例と非投与例の肝機能および血中 HBV DNA 量の推移を検討すると, lamivudine 投与例, 非投与例とも T-Bil の推移には差異を認めなかった. 血清 ALT は lamivudine 投与例で非投与例に比べてその低下が速やかな傾向が認められたが, 各種治療を併用しているためその評価は困難であった. 血中 HBV DNA 量は抗ウイルス療法施行例で減少が認められたが, lamivudine 単独療法例と IFN 併用療法例とに分けて検討すると, 両者間には明らかな差異は認められなかった(Fig. 1). したがって, 抗ウイルス効果は lamivudine によるものと推定され, 重症化例に対しては現時点では lamivudine を第一選択薬として投与するのが望ましいと考えられる.

抗ウイルス療法と予後を検討すると, lamivudine あるいは IFN 投与にかかわらず劇症化した例では 1 例を除いて死亡しており, 大部分が HBV キャリアからの発症例である. いずれにしても高度の黄疸(T-Bil>10 mg/dl)や PT の低下(<40%)をきたす前に lamivudine の投与を考慮する必要があると思われる.

代表的な例(27 歳, 男性, 症例 No. 7)の臨床経過を Fig. 2 に示す. 母親および弟も HBV キャリアである. 16 歳時にはじめて HBV キャリアと指摘