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Seroprevalence of hepatitis B virus, hepatitis C virus and GB virus-C infections in Siberia

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SUMMARY

We studied the seroprevalence of hepatitis B virus (HBV), hepatitis C virus (HCV) and GB virus-C (GBV-C) infections in 348 Siberian natives who lived in the Kamchatka Peninsula of Russia. Of 348 samples studied, the seroprevalence of HBsAg and anti-HBs were 11·8% (41 of 348 samples) and 35·9% (125 of 348 samples), respectively. The prevalence of HCV infection was 1·4% (5 of 348 samples), and that of GBV-C RNA, using RT–PCR methods, was 7·5% (26 of 348 samples). In Siberia, the prevalences of HBV and GBV-C infections were about tenfold higher than those in Japan. The prevalence of HBsAg in subjects under 50 years of age was significantly higher than that in those over 50 years old ($P < 0·05$). Because HBV infection is highly endemic in Siberia, we propose that the community-based mass immunization must be conducted as soon as possible in this area.

INTRODUCTION

Hepatitis B virus (HBV) infection is a great public health concern in many countries. In some parts of Asia, Africa and the South Pacific, the prevalence of hepatitis B virus surface antigen (HBsAg) exceeds 10%. Barret and colleagues reported that Alaskan natives had a high prevalence of HBV infection, and the seroprevalences of HBsAg and antibody to HBsAg (anti-HBs) were 13·9 and 40·9%, respectively [1, 2]. The population of the Peninsula Kamchatka in Russia is made up of Siberian natives (Evens, Koryaks, Chukchi, Itel'mens and others) thought to be of the same ethnic origin as Alaskan natives as well as Russians, but, to date, there is no report of the seroprevalence of hepatitis virus infections in Siberia. Here we report the prevalence of HBV and hepatitis C virus (HCV) infections in Siberian natives of the Kamchatka Peninsula, Russia, We also report the prevalence of the tentatively called GB virus-C/hepatits G virus (GBV-C/HGV) infection in Siberia [3, 4].

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MATERIALS AND METHODS

We held many meetings with local leaders in each area to explain the purpose of the survey and to encourage maximum voluntary participation. After informed consent of all participants in this study, we collected serum samples at random from 348 Siberian natives, who were symptomless volunteers and who lived in

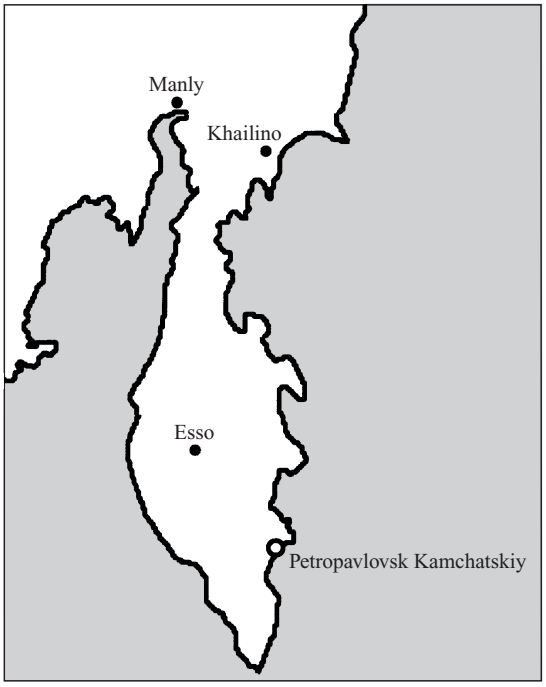

Fig. 1. Maps of Kamchatka Peninsula, Russia, and of the three villages (Esso, Khailino and Manyly villages) where the samples were collected for this study.

Esso, Khailino and Manyly villages in the Kamchatka Peninsula of Russia (Fig. 1), in 1994 and 1995. These comprised 85 samples from men and 263 samples from women; the mean age of the natives was $39{\cdot}3 \pm 13{\cdot}8$ (range 16–82) years old.

Serum alanine aminotransferase (ALT) levels in all samples was measured.

Serum samples were analysed for HBsAg by particle agglutination (PA) (Serodia HBs PA, Fujirebio, Tokyo) and for anti-HBs by passive hemagglutination (Serodia anti-HBs PA kit, Fujirebio, Tokyo). The HBsAg subtypes of the HBsAg positive samples were also determined by enzyme immunoassay (HBsAg Subtype EIA, Institute of Immunology Co. Ltd., Tokyo). Samples were tested for anti-HCV by PA (HCV PA test II, Fujirebio, Tokyo), and the anti-HCV-positive sera were tested for HCV RNA by the reverse transcription–polymerase chain reaction (PT–RCR) using primers derived from the highly conserved 5′-untranslated region (5′-UTR), as previously reported [5]. The HCV genotypes were determined by RT–PCR using type-specific primers for the core region of the viral genome [6].

It has been reported independently by two groups, that GBV-C/HGV is associated with acute and chronic persistent hepatitis, and a transfusion-transmissible agent [3, 4]. The pathogenicity and clinical significance of GBV-C/HGV remains unknown and most reports lack evidence of a causal link between GBV-C/HGV infection and chronic liver diseases [7–12]. For this reason, we refer to this virus as GBV-C in this report. The GBV-C RNA was detected by RT–PCR and the GBV-C genotypes were determined using restriction fragment length polymorphism (RFLP) analysis on GBV-C RNA-positive samples, as described previously [13]. RNA was extracted from 100 μl of serum using a SepaGene-RVR (Sanko, Tokyo). Then cDNA was synthesized from the extracted RNA at 37 °C for 1 h using Moloney murine leukaemia virus reverse transcriptase (MMLV–RT, Gibco BRL, Gaithersburg, MD) with random primers. GBV-C RNA was detected using nested PCR with specific primer sets derived from the 5′UTR of the GBV-C genome. The second PCR product was analysed by electrophoresis on 3% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. The GBV-C genotyping was performed by RFLP analysis. Restriction digestions were carried out on the second PCR product for 3 h after adjustment with either *ScrFI* (New England BioLabs, MA) or *BsmFI* (New England Biolabs). The digested PCR products were electrophoresed and the RFLP pattern was then evaluated under ultraviolet light. With the use of this method, GBV-C was classified into three genotypes, GB type (African type), HG type (Europe-US type), and Asian type [13, 14].

For statistical analysis, χ^2 and Fisher's exact probability test were used.

RESULTS

Of the 348 samples studied, HBsAg and anti-HBs were detected in 41 (11·8%) and 125 samples (35·9%), respectively (Table 1). The total seroprevalence of HBsAg and anti-HBs was 47·7%, relatively consistent in all age groups (Fig. 2). The seroprevalence of HBsAg in those under 50 years of age was significantly higher than in older age groups ($P < 0{\cdot}05$). Of 85 men and 263 women tested, 19 (22·4%) and 22 (9·1%), respectively, were HBsAg positive. The seroprevalence of HBsAg was significantly higher in males than in females ($P < 0{\cdot}05$). The HBsAg subtype was determined in 40 samples of the 41 HBsAg positive sera. The 40 HBsAg subtypes comprized 25 ayw, 9 adr, 5 ay and 1 adyr.

Ten (2·9%) of the 348 samples by PA were anti-HCV positive. HCV RNA was detected in 5 (1·4%) of

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Table 1. *The prevalence of HBV, HCV, and GBV-C infections in Siberian Natives of the Peninsula Kamchatka, Russia*

Village	No.	Mean age ± S.D. [M:F]	HBsAg	Anti-HBs	Anti-HCV	HCV RNA	GBV-C/ HGV	BTF*	ALT* [> 25 IU/L]
Esso	179	39·2 ± 15·4 [47:132]	25	56	0	0	15	27	7
Khailino	83	37·4 ± 12·3 [20:63]	10	36	3	2	9	15	17
Manyly	86	39·8 ± 13·8 [18:68]	6	33	7	3	2	16	8
Total	348	39·3 ± 11·6 [85:263]	41(11·8)	125(35·9)	10(2·9)	5(1·4)	26(7·5)	58 (16·6 %)	32(9·26)

* BTF, blood transfusion; ALT, alanine aminotransferase.

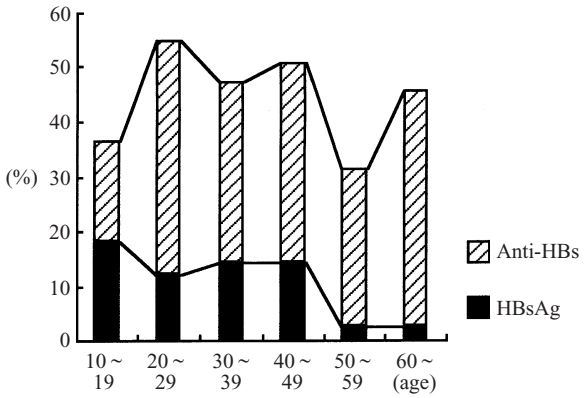

Fig. 2. Age-distribution of the seroprevalence of HBsAg and anti-HBs.

the 10 anti-HCV-positive sera, by RT–PCR. When anti-HCV was detected by PA in sera diluted at 2^{-12}, HCV RNA was positive by RT–PCR. If the subjects who tested positive both anti-HCV positive and HCV RNA are assumed to be infected with HCV, the prevalence of HCV infection was 1·4 %. Of the 5 HCV RNA-positive sera, 3 revealed genotype 1b and 2 revealed genotype 2a.

Using RT–PCR, GBV-C RNA was detected in 26 (7·5 %) of the 348 samples. Of the 26 GBV-C RNA-positive sera, 3, 13 and 2 samples were also HBsAg-positive, anti-HBs-positive, and HCV RNA-positive, respectively (one sample was both HBsAg and anti-HBs-positive). In 2 of 9 subjects with only GBV-C infection, the serum ALT levels were elevated over 25 IU/L (ALT = 33, 59); remaining 7 subjects had normal ALT levels.

Of the 348 subjects studied, 58 (l6·6 %) had a history of blood transfusion and of these 22 tested positive for anti-HBs, 7 for HBsAg, 3 for anti-HCV, 2 for HCV RNA and 2 for GBV-C RNA. Only 2 of the 26 GBV-C RNA-positive subjects had a history of blood transfusion. The GBV-C genotypes of the 26 GBV-C RNA-positive samples were all HG type (Europe-US type). Eight of 32 subjects with serum ALT levels elevated over 25 IU/L did not test positive for any virus markers.

DISCUSSION

It is well known that in the Alaskan natives Eskimos, the prevalence of HBV infection is high. Since the early 1970s, numerous epidemiological surveys of the prevalence, incidence, transmission and sequelae of HBV infection among Alaskan natives have established that the average seroprevalences of HBsAg and anti-HBs were 13·9 and 40·9 % [1], respectively, that the transmission of HBV occurred primarily from HBV carrier mothers to infants and from carrier individuals to susceptible children, and that HBV infection seemed to spread among household contacts [16].

We studied the prevalence of HBV, HCV and GBV-C infections in Siberia. In Siberian natives of Kamchatka Peninsula, the prevalence of HBV infection was very high, especially in younger persons, under 50 years old. The seroprevalence of HBsAg was 11·8 %, which is tenfold higher than that in the Japanese population, and the seroprevalence of anti-HBs was 35·9 %. Children have a significantly higher risk of becoming chronic carriers of HBsAg when infected than adults. In adults, HBV infection is probably acquired both from children with HBeAg and through heterosexual contact [16]. Although the total seroprevalence of HBsAg and anti-HBs was relatively consistent in all age groups, the prevalence of HBsAg was significantly higher in individuals under 50 years old than in those over 50 years old

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($P < 0{\cdot}05$). In the early 1970s, Alaskan natives Eskimos had a high prevalence of HBV infection. In 1983, as a part of the comprehensive Alaska-wide HBV control program, community-based mass immunization was conducted to immunize the high-risk population and all newborn Alaskan natives. Subsequent to this successful vaccination program, the incidence of acute symptomatic HBV infection in Alaskan natives dramatically decreased [2]. A similar programme is urgently needed in Siberia.

HBsAg subtyping has been used for tracing the route of HBV transmission and the geographical migration of HBV carriers [17, 18]. It has been reported that subtype ayw of HBsAg was mainly distributed in East Europe, the Mediterranean, Northern Africa, the Near East and Central Asia. And subtype adr of HBsAg was mainly distributed in Northern China, Korea and Japan [17]. The most prevalent HBsAg subtype in Siberia was ayw. Among Alaskan natives, it has been reported that all chronic HBV carriers had adw subtype of HBsAg. Although Siberian natives and Alaskan natives Eskimos, are thought to have the same ethnic origin, the prevalent subtypes of HBsAg were different between the two populations. The high seroprevalence of HBV infection markers may indicate that an epidemic of HBV infection probably occurred in this community 40–50 years ago or relatively more recently, rather than that individuals over age 50 years old seroconverted from HBsAg to anti-HBs, because the clearance ratio of HBsAg during the course of chronic HBV infection appeared to be very low [19].

Although HBV, HCV and GBV-C are all transfusion-transmissible agents, the prevalence of HCV infection (1·4%) was nearly the same as that in Japan (1·3%), but the prevalence of HBV and GBV-C infections was about tenfold higher than that in Japan [6, 20]. Moreover, the genotype in all the 26 GBV-C RNA-positive subjects was determined to be HG type (Europe-US type) which was not a prevalent type in Asia [13 14]. The routes of infection could not be determined in most of the GBV-C RNA-positive individuals. Among the 5 samples which were positive for HCV RNA, 3 were genotype 1b and 2 were genotype 2a, although there were few HCV RNA-positive samples. To clarify the distribution of HCV genotypes in Siberia, more extensive epidemiological surveys will be required. On the basis of the pattern of prevalence of HBsAg subtype and GBV-C genotype, we supposed that HBV and GBV-C infections were probably introduced relatively recently or that they replaced the European subtype or genotype of HBV and GBV-C, rather than that both infections were hyper-endemic from the ancient times in this area.

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