RESEARCH COMMUNICATION

An Epidemiological Study of HBV, HCV and HTLV-I in Sherpas of Nepal

Hitoshi Chiba[1], Toshiro Takezaki[2], Dhanapati Neupani[3], Jinbo Kim[4], Shigeru Yoshida[1], Emi Mizoguchi[1], Jun Takeuchi[5], Junko Suzuki[6], Yuuki Tanaka[1], Keiko Ito[1], Tadashiro Kitamura[1], Kiyonori Kuriki[7], Kenji Wakai[7], Kunihiko Samejima[4], Shunro Sonoda[2], Kazuo Tajima[7]

Abstract

An epidemiological study of hepatitis viruses type B (HBV) and type C (HCV) and human T-cell leukemia virus type I (HTLV-I) was carried out among 103 residents (male:female=61:42) regarded as Sherpas, at Lukla (Solukhumbu district), Nepal in 2004. Blood was drawn from apparently healthy volunteers at ages of 28.8±12.3 (range 15-66) years. HBsAg, HBsAb, HBcAb, and HCV Ab were measured by microparticle enzyme-immunoassay, and HTLV-I Ab was measured by particle agglutination. Prevalence of HBsAg(+), HBsAb(+), HBcAb(+), and HBsAb(+) or HBcAb(+) were 1.9% 22.3%, 24.3%, and 28.2%, respectively. For HCV Ab, only a borderline reaction was observed in one sample, and for HTLV-I Ab all samples were negative. Nucleotide sequencing of the PreS1, PreS2, and S genes revealed that HBV among Sherpas to be of the A' (or Aa) genotype, which is prevalent among Nepalese but rare in native Tibetans, suggesting transmission within Nepal rather than association with ancestors' migration from Tibet as the origin. This is the first report of Himalayan Sherpas' state of infection with HBV, HCV, and HTLV-I.

Key Words: HBV - HCV - HTLV-I - prevalence - Sherpas - Nepal - Himalayas

Asian Pacific J Cancer Prev, **5**, 370-373

Introduction

Epidemiological information on hepatitis viruses is essential for strategic prevention of chronic hepatitis, liver cirrhosis, and liver cancer. So far, no information on the prevalence of hepatitis B and C viruses (HBV, HCV) or the HBV genotype has been reported in the accessible literature for the Sherpas. According to a certain estimate (Fisher, 1990), about 35,000 Sherpas presently live in Nepal, and also in India, Bhutan, and Tibet. Since the majority still inhabit isolated villages at high altitudes, only few medical surveys for them have been reported.

The Sherpas are a people indigenous to the Himalayan region, with a close affinity to Tibetan religion and language. Their ancestors are deemed to have migrated from eastern Tibet about 400-500 years ago and they are settled mainly in the eastern Himalaya region of what is now Nepal (Fischer, 1990). In 2000, we conducted an epidemiological study of HBV at four isolated villages in eastern and western remotes of Tibet (China), and reported that HBV was highly prevalent among native Tibetans in these regions; 19.1% were positive for HBsAg and 29.0% for HBsAb or HBcAb (Zhao, et al., 2001). In particular, one of the villages in eastern Tibet exhibited an extremely high prevalence (37.5% for HBsAg and 45.0% for HBsAb or HBcAb).

The strains of HBV have been classified into seven major genotypes (type A to G) based on the nucleotide sequence, with distinct geographical distributions (Bowyer et al., 1997; Bowyer and Sim, 2000; Stuyver et al., 2000; Nakano et al., 2001). We earlier reported that genotype C is predominant

[1]Department of Laboratory Medicine, Hokkaido University Hospital, Sapporo, Japan; [2]Department of International Island and Community Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan; [3]Tribhuvan University, Dharan, Nepal; [4]Department of Food Science, Rakuno Gakuen University, Ebetsu, Japan; [5]The Second Department of Internal Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan; [6]Department of Nutrition, Tenshi University, Sapporo, Japan; [7]Division of Epidemiology and Prevention, Aichi Cancer Center Research Institute, Nagoya, Japan
Address correspondence to: Hitoshi Chiba, Department of Laboratory Medicine, Hokkaido University Hospital, North-14, West-5, Sapporo 060-8648, Japan. Fax: (81)-11-706-5703. E-mail: chibahit@med.hokudai.ac.jp

among native Tibetans (Zhao et al., 2001), as well as in Central and East Asia, including Korea, China and Japan. Of interest, in Nepal, the genotypes A and D are dominant, whereas the genotype C is rare. Genotype A is also prevalent in South and South East Asia including India and the Philippines, as well as in North-West Europe, North America, and Central Africa. Genotype D is most common in the Middle East through to India and the Mediterranean. Thus, the Himalayas appear to have been preventing diffusion and mixing of HBV genotypes through prevention of human migration.

Here we report the prevalence of HBV and HCV among Sherpas living in Himalaya, with information on the HBV genotype. We also describe the prevalence of human T-cell leukemia virus (HTLV-I) among Sherpas. Regarding this latter retrovirus, foci are known to be present in Japanese southwestern and northern regions and in the Andes of South America (Tajima and Takezaki, 2003). Of interest, a 1500-year-old Andean mummy and contemporary Japanese and Chileans were found to share similar HTLV-I provirus sequences, indicating that the Andean HTLV-I was carried from Asia by populations with genetic link to the contemporary Japanese with HTLV-I (Li et al., 1999). Thus, investigating the prevalence of HTLV-I among Sherpas might possibly add our knowledge concerning ancient migration of Mongoloids.

To the best of our knowledge, this is the first report as to Himalayan Sherpas' state of infection with HBV, HCV, and HTLV-I.

Materials and Methods

Subjects

EDTA blood and information were collected from apparently healthy volunteers (n=103; male:female=61:42), living in Lukla or nearby in the district of Solukhumbu. This region is thought to be the earliest settlement place for Sherpas' ancestors, and is inhabited by their descendants now. We confirmed that every volunteer had a typical Sherpa surname. The volunteers' ages were distributed as: n=27 at <20 yr, n=55 at 20-39 yr, n=21 at $\pm$40 yr, and overall, 28.8$\pm$12.3 yr (mean$\pm$SD, range 15-66). Plasma was separated at Kathmandu and was kept frozen until use. All measurements were done at Hokkaido University Hospital using automated analyzers.

Ethics

To perform this study, we obtained permission from the Nepal Health Research Council, and also from the ethical committee of Hokkaido University Graduate School of Medicine. Informed consent was obtained from each volunteer after providing information concerning this research in their native language.

Methods

HBsAg, HBsAb, HBcAb, and HCV Ab were measured by micropaticle enzyme-immunoassays (Abbott). HTLV-I infection was serologically screened using a commercial test kit (SERODIA HTLV-I, FUJIREBIO Inc., Tokyo) based on passive particle-agglutination (Fujiyama et al., 1995).

For HBV genotyping, nucleic acids were extracted from plasma using a QIAamp MinElute Virus Spin kit (Qiagen, Germany). The polymerase chain reaction (PCR) was done under standard conditions: pre-cycle 94˚C/2min, denaturing 94˚C/30 sec, annealing 55˚C/30 sec, extension 72˚C/2min, 35 cycles, final extension 72˚C/7 min. The primers used in this study are summarized in Table 1. First, a segment of HBV DNA (nt 1824-1803) spanning the PreS1, PreS2, and S regions was amplified. The obtained fragment (1.5 kb) was amplified into four overlapping fragments using the four sets of nested primers. The obtained fragments were directly sequenced with the same primer sets, using BigDye Terminator v3.1 Ready Reaction Cycle Sequencing kit (ABI) in an ABI Prism 3100 genetic analyzer. The nucleotide sequences were analyzed using the HBV genotyping software provided by NCBI.

Results

Prevalence

Prevalences of HBV, HCV, and HTLV-I among Sherpas are summarized in Table 2, in comparison with data for native Tibetans from our previous report (Zhao et al., 2001). Despite the similar rates of positive HBV antibodies between the

Table 1. Primer Sets Used

	nucleotide numbers		nucleotide sequence
1st PCR	1824 - 1803	sense	5'-TTCACCTCTGCCTAATCATC-3'
		antisense	5'-AACAGACCAATTTATGCCTA-3'
Nested PCR	2637 - 3156	sense	5'-ATGCCTGCTAGGTTCTATCCT-3'
		antisense	5'-TCTTCCTGACTGCCGATTGGT-3'
	3002 - 376	sense	5'-GGCCAGAGGCAAATCAGGTAG-3'
		antisense	5'-CCAGCGATAGCCAGGACAAAT-3'
	156 - 618	sense	5'-TGGAGAGCACAACATCAGGA-3'
		antisense	5'-GATGATGGGATGGGAATACA-3'
	496 - 1032	sense	5'-AACTACCAGCACGGGACCAT-3'
		antisense	5'-TGTAAAAGGGGCAGCAAAGC-3'

two groups, the rate of positive HBsAg among Sherpas was 10-fold less than that for Tibetans. Definite HCV or HTLV-I infection was not detected, with only a borderline HCV Ab reaction observed in one subject. The liver function was normal in the two subjects positive for HBsAg.

HBV Gene Analysis

The nucleotide sequences in the PreS1, PreS2 and S genes from the two plasma samples with positive HBsAg, nominated as Sherpa 1 and Sherpa 2, were classified as genotype A, according to the NCBI genotyping software. The deduced amino acid sequences in the PreS1 and PreS2 regions are shown in Fig. 1, these being known to contain the most useful information for genotyping. The wild-type amino acid sequence obtained from NCBI (Index No. D50517) is shown for reference. Of 18 amino acid changes observed, sixteen were shared by Sherpa 1 and Sherpa 2, indicating their proximate origins. Among the 16 amino acid changes, five (54Q, 74V, 86A, 91V in the PreS1 region and 32L in the PreS2 region) have been reported to be shared by genotype A isolates from both Asia and Africa (subtype A' or Aa), but undetectable in genotype A isolates from Europe (or subtype Ae) (Sugauchi et al., 2004).

Discussion

Previous reports as to the prevalence of HBV, HCV or HTLV-I among Nepalese are based on the data from regions where Sherpas are only a small minority. In one report (total n=458), positive HBsAg, HBcAb, and HCV Ab were, 1.1%, 7.2%, and 1.7%, respectively, in Bhadraka and Kotyang in 1996 (Sawayama et al., 1999). In our present report, the positive rate of HBsAg among Sherpas was 1.9%, which is similar to that for non-Sherpa Nepalese described above. On the other hand, for Tibetans, we have reported a strikingly high HBsAg positive rate (19.1%) (Zhao et al., 2001), indicating a high risk of HBV infection in perinatal period or early childhood in Tibet.

Table 2. Prevalence of HBV, HCV, and HTLV-I Infection Among Sherpas: Comparison with Native Tibetans

	Sherpas (total n=103) n (%)	Tibetan[a] (total n=262) n (%)
HBsAg (+)	2 (1.9%)	50 (19.1%)
HBsAb (+)	23 (22.3%)	-
HBcAb (+)	25 (24.3%)	-
HBs Ab (+) or HBc Ab(+)	29 (28.2%)	76 (29.0%)
HBV genotype	A (2/2 cases)	C (20/20 cases)
HCV Ab (+)	1 (1.0%)[b]	-
HTLV-I Ab (+)	0 (0%)	-

[a]Zhao, et al (2001). [b]borderline reaction.

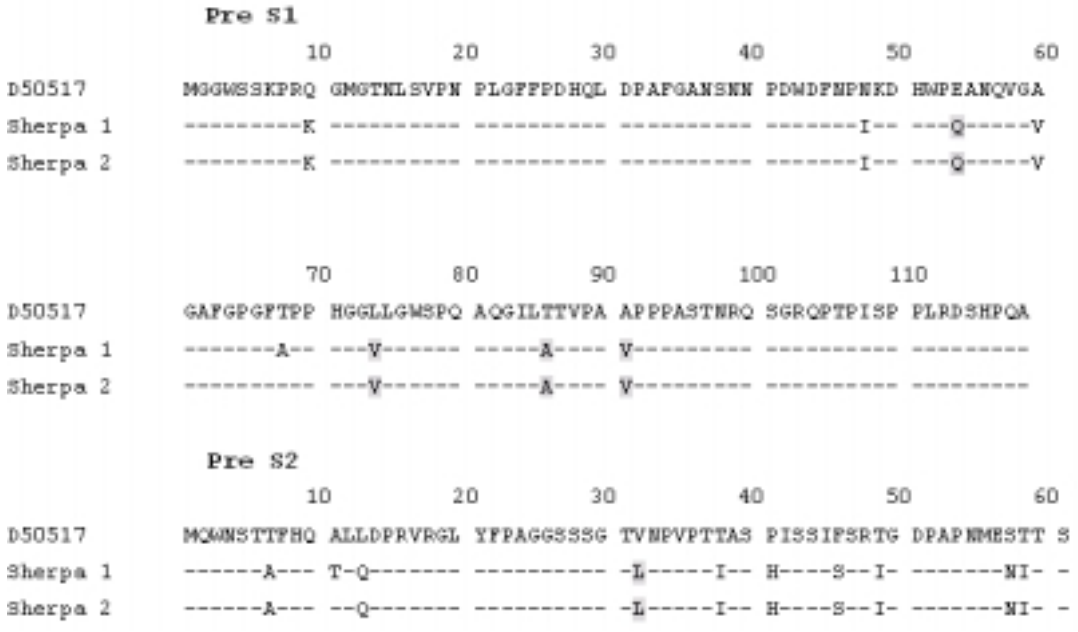

Figure 1. PreS1 and PreS2 Amino Acid Sequences Obtained from Himalayan Sherpas. The wild type sequence (D50517) was obtained from the NCBI data base. The hatched amino acids are reported to be shared by the genotype A from both Asia and Africa (subtype A' or Aa), but undetected in that from Europe (or subtype Ae) (Sugauchi et al., 2004).

The divergent HBsAg positive rates between Tibetans and Sherpas might partly be explained by the difference in HBV genotype. Although no information on clinical and virological comparisons between genotypes C (Tibetans) and genotype A (Sherpas) is available, the former is known to be associated with higher HBeAg positive rates as compared with genotype B (Orito et al., 2001), and might consequently be associated with higher perinatal infection rates.

The A genotype of HBV has been divided into two subtypes, on the basis of nucleotide sequence; one is for Asia/Africa (Aa or originally A') and the other is for Europe (Ae or originally A) (Kramvis et al., 2002; Sugauchi et al., 2004). The subtype Aa has been reported in three HBV isolates from Nepal (Sugauchi et al., 2004). This indicates a shared origin of HBV of Nepalese and Sherpas. The subtype Aa is reported to be associated with reduced serum HBV DNA levels and a low rate of HBeAg positivity (Kramvis et al., 1997, 1998), which might also explain the low rate of HBsAg positivity among Sherpas in comparison with Tibetans.

In a previous survey in Chitwan, Dhapakhel, and Kathmandu, HTLV-I was documented to be negative in all of 413 residents tested (Ishida et al., 1992). The results, however, are representative of non-Sherpa Nepalese, since large Sherpa populations do not live in these regions. Our present study, for the first time, revealed that Himalayan Sherpas lack HTLV-I. This might suggest that the Sherpa is genetically remote from Upper Paleolithic Mongoloids to whom contemporary Japanese and Chilean populations carrying HTLV-I are closely related.

We conclude that HCV and HTLV-I are negligible among Sherpas, and that HBV with the Aa genotype probably from the Nepal side is present among Sherpas, but is not prevalent.

Acknowledgment

This work was supported by Grants-in-Aid from the International Scientific research Program of the Japan Society for Promotion of Science (JSPS) in 2003-4.

References

Bowyer SM, van Staden L, Kew MC, Sim JGM (1997). A unique segment of the hepatitis B group A genotype identified in isolates from South Africa. *J Gen Virol*, **78**, 1719-29.

Bowyer SM, Sim JG (2000). Relationships within and between genotypes of hepatitis B virus at points across the genome: footprints of recombination in certain isolates. *J Gen Virol*, **81**, 379-92.

Fischer J (1990). Sherpas: Reflections on Change in Himalayan Nepal, University of California Press, Los Angeles.

Fujiyama C, Fujiyoshi T, Matsumoto D, Tamashiro H, Sonoda S (1995). Evaluation of commercial HTLV-I test kits by a standard HTLV-I serum panel. *Bull World Health Organ*, **73**, 515-21.

Ishida T, Takayanagi K, Shotake T, Hirai K, Yuasa I (1992). A seroepidemiological study of HTLV-I infection in Nepal. *Scan J Infect Dis*, **24**, 399-400.

Kramvis A, Bukofzer S, Kew MC, Song E (1997). Nucleic acid sequence analysis of the precore region of hepatitis B virus from sera of southern African black adult carriers of the virus. *Hepatology*, **25**, 235-40.

Kramvis A, Kew M, Bukofzer S (1998). Hepatitis B virus precore mutants in serum and liver of Southern African Blacks with hepatocellular carcinoma. *J Hepatol*, **28**, 132-41.

Kramvis A, Weitzmann L, Owired WKBA, Kew MC (2002). Analysis of the complete genome of subgroup A' hepatitis B virus isolates from South Africa. *J Gen Virol*, **83**, 835-9.

Li HC, Fujiyoshi T, Lou H, et al (1999). The presence of ancient human T-cell lymphotropic virus type I provirus DNA in an Andean mummy. *Nat Med*, **5**, 1428-32.

Nakano T, Lu L, Hu X, et al (2001). Characterization of hepatitis B virus genotypes among Yucpa Indians in Venezuela. *J Gen Virol*, **82**, 359-65.

Orito E, Mizokami M, Sakugawa H, et al (2001). A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotype B and C. Japan HBV Genotype Research Group. *Hepatology*, **33**, 218-23.

Sawayama Y, Hayashi J, Ariyama I, et al (1999). A ten year serological survey of hepatitis A, B and C viruses infections in Nepal. *J Epidemiol*, **9**, 350-4.

Stuyver L, Gendt SD, van Geyt C, et al (2000). A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. *J Gen Virol*, **81**, 67-74.

Sugauchi F, Kumada H, Surbat A, et al (2004). Epidemiological and sequence differences between two subtypes (Ae and Aa)of hepatitis B virus genotype A. *J Gen Virol*, **85**, 811-20.

Tajima K, Takezaki S (2003). Ethnoepidemiology of ATL/HTLV in Mongoloids with special reference to Mongoloid dispersal. *Gann Mongor Cancer Res*, **50**, 277-88.

Zhao SM, Li HC, Lou H, et al (2001). High Prevalence of HBV in Tibet, China. *Asian Pacific J Cancer Prev*, **2**, 299-304.