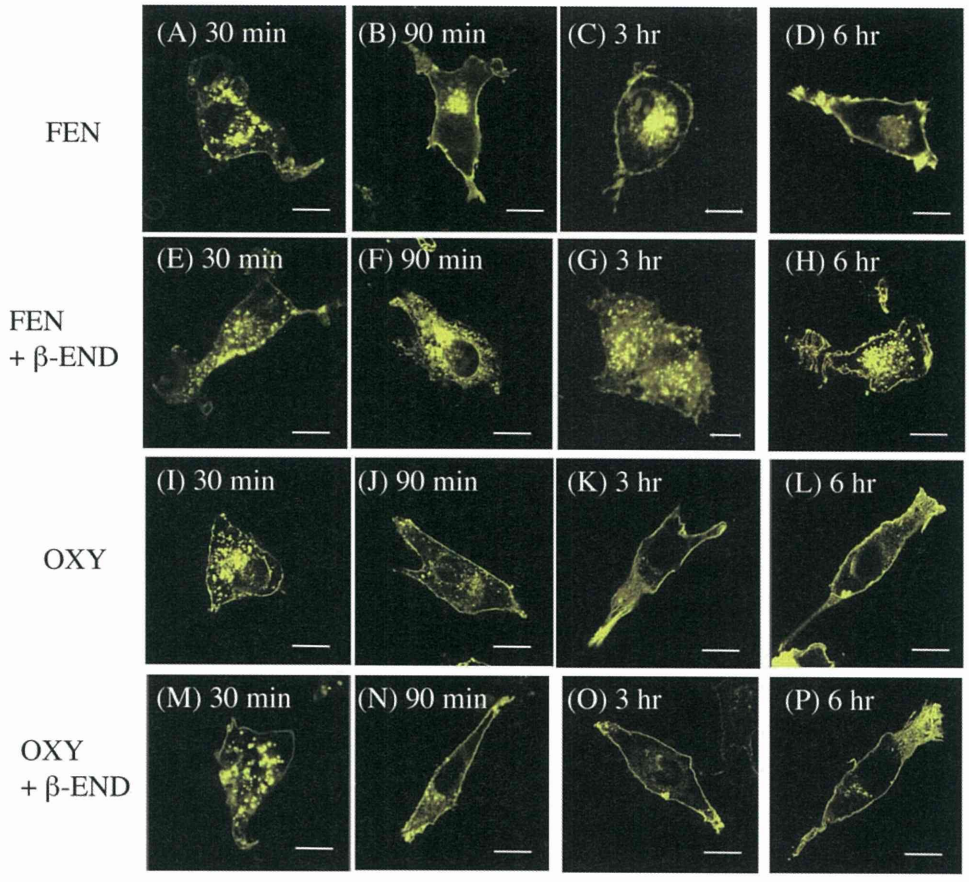

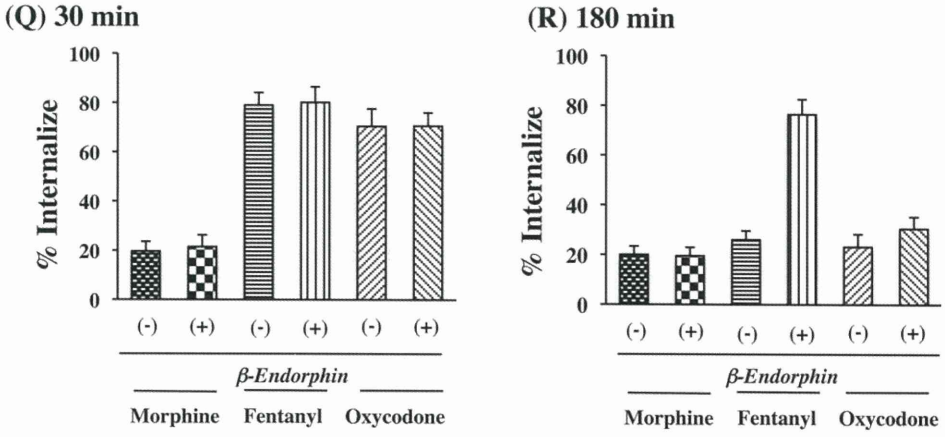

Fig. 3. Confocal imaging of resensitization of MORs in BHK cells expressing Venus-fused MORs. Cells were incubated with 100 nM fentanyl (**A–H**) or 10 μM oxycodone (**I–P**) in the absence (A-D and I-L) or presence (E-H and M-P) of β-endorphin, and then apposed for 30 min, 90 min, 3 h, or 6 h at 37°C. The cells were then fixed and counted by confocal microscopy. Yellow fluorescence from Venus indicates the cellular localization of MOR in BHK cells. Scale bars, 10 μm. Quantitative analysis of the % of the internalized cells expressing Venus-fused MORs treated with the drugs for 30 min (**Q**) or 180 min (**R**), respectively. The agonist concentrations represent the dose required to induce the maximal effect on receptor endocytosis for each drug. Each value represents the mean ± SEM of six separate experiments.

remained in the cytosolic fraction at 3–6 h after the washing-out of β-endorphin and fentanyl (Figs. 3F–3H). However, in both the presence and absence of β-endorphin, the internalized MOR induced by oxycodone returned to the plasma membrane after the washing-out of agonist in a time-dependent manner (Figs. 3I–3P). We performed quantitative analysis of the agonist-induced internalization of MORs after the washing-out of each agonist shown in Materials and Methods. At 30 min after the washing-out of agonists,

cells treated with fentanyl or oxycodone showed robust internalization of MORs (fentanyl: 79.0 ± 5.14%, β-endorphin fentanyl: 80.2 ± 3.7%, oxycodone: 70.5 ± 7.09%, β-endorphin oxycodone: 70.7 ± 5.35%), which was not seen in morphine-treated cells (morphine: 19.67 ± 3.93%, β-endorphin morphine: 21.5 ± 4.76%; Fig. 3Q). However, while there was no difference in the degree of oxycodone-induced MOR internalization between the presence and absence of β-endorphin 3 h after washing-out (oxycodone: 23.17 ± 5.12%, β-endorphin oxycodone: 30.5 ± 4.72%), in fentanyl-treated cells, β-endorphin caused the prolonged internalization of MORs and fluorescence was stayed in the cytosolic fraction (fentanyl: 27.67 ± 5.47%, β-endorphin fentanyl: 76.5 ± 6.02%; Fig. 3R).

It has been widely accepted that receptor desensitization, internalization and trafficking appear to play a key role in the development of opioid tolerance (Claing et al., 2002; Gainetdinov et al., 2004). The initial process in these events is the phosphorylation of intracellular domains of MOR. Phosphorylated MORs are mostly internalized via clathrin-coated pits into early endosomes and subsequently dephosphorylated by intracellular protein phosphatases. The dephosphorylated MORs might either be recycled to the plasma membrane or transported to lysosomes for degradation. A growing body (Smalheiser and Lugli) of evidence suggests that among diverse serine/threonine (Thr) residues of the intracellular domain of MOR, the phosphorylation of Ser 375 in the mouse MOR is essential for the internalization of MORs (Schulz et al., 2004). In a previous study, we found that repeated treatment with fentanyl, but not morphine, resulted in an increase in the levels of phosphorylated-MOR (Ser 375) associated with the enhanced inactivation of protein phosphatase 2A and a reduction in Rab4-dependent MOR resensitization in the spinal cord of mice that showed inflammatory pain (Imai et al., 2006). However, several lines of evidence indicate that, in response to pain stimulus, endogenous β-endorphin is released within some brain regions (Zubieta et al., 2001). We previously reported that β-endorphin released in the ventral tegmental area is a key factor in regulating the dysfunction of MOR to negatively modulate opioid reward under a neuropathic pain-like state (Niikura et al., 2008, 2010). Taken together, although further studies are still needed, these findings support the idea that inhibition of the resensitization system of MOR following chronic treatment with fentanyl in the presence of β-endorphin may be associated with antihyperalgesic tolerance to fentanyl under a chronic pain-like state.

In conclusion, we demonstrated here that unlikely morphine, either fentanyl or oxycodone induced a robust MOR internalization and, in turn, its resensitization. In the presence of β-endorphin, the internalized MOR induced by fentanyl, but not oxycodone, remained within the cytosolic fraction even after washing out. These findings strongly support that idea that fentanyl has different pharmacological profile form that of morphine or oxycodone.

REFERENCES

Claing A, Laporte SA, Caron MG, Lefkowitz RJ. 2002. Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. Prog Neurobiol 66:61–79.

Corbani M, Gonindard C, Meunier JC. 2004. Ligand-regulated internalization of the opioid receptor-like 1: A confocal study. Endocrinology 145:2876–2885.

Gainetdinov RR, Premont RT, Bohn LM, Lefkowitz RJ, Caron MG. 2004. Desensitization of G protein-coupled receptors and neuronal functions. Annu Rev Neurosci 27:107–144.

Haberstock-Debic H, Kim KA, Yu YJ, von Zastrow M. 2005. Morphine promotes rapid, arrestin-dependent endocytosis of μ-opioid receptors in striatal neurons. J Neurosci 25:7847–7857.

Imai S, Narita M, Hashimoto S, Nakamura A, Miyoshi K, Nozaki H, Hareyama N, Takagi T, Suzuki M, Suzuki T. 2006. Differences in tolerance to anti-hyperalgesic effects between chronic treatment with morphine and fentanyl under a state of pain. Nihon Shinkei Seishin Yakurigaku Zasshi 26:183–192.

Koch T, Widera A, Bartzsch K, Schulz S, Brandenburg LO, Wundrack N, Beyer A, Grecksch G, Hollt V. 2005. Receptor endocytosis counteracts the development of opioid tolerance. Mol Pharmacol 67:280–287.

Minnis JG, Patierno S, Kohlmeier SE, Brecha NC, Tonini M, Sternini C. 2003. Ligand-induced mu opioid receptor endocytosis and recycling in enteric neurons. Neuroscience 119:33–42.

Nagai T, Ibata K, Park ES, Kubota M, Mikoshiba K, Miyawaki A. 2002. A variant of yellow fluorescent protein with fast and efficient maturation for cell-biological applications. Nat Biotechnol 20:87–90.

Niikura K, Narita M, Nakamura A, Okutsu D, Ozeki A, Kurahashi K, Kobayashi Y, Suzuki M, Suzuki T. 2008. Direct evidence for the involvement of endogenous β-endorphin in the suppression of the morphine-induced rewarding effect under a neuropathic pain-like state. Neurosci Lett 435:257–262.

Niikura K, Narita M, Butelman ER, Kreek MJ, Suzuki T. 2010. Neuropathic and chronic pain stimuli downregulate central μ-opioid and dopaminergic transmission. Trends Pharmacol Sci 31:299–305.

Schulz S, Mayer D, Pfeiffer M, Stumm R, Koch T, Hollt V. 2004. Morphine induces terminal micro-opioid receptor desensitization by sustained phosphorylation of serine-375. EMBO J 23:3282–3289.

Smalheiser NR, Lugli G. 2009. microRNA regulation of synaptic plasticity. Neuromolecular Med 11:133–140.

Zubieta JK, Smith YR, Bueller JA, Xu Y, Kilbourn MR, Jewett DM, Meyer CR, Koeppe RA, Stohler CS. 2001. Regional μ opioid receptor regulation of sensory and affective dimensions of pain. Science 293:311–315.

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Mental health conditions in Korean atomic bomb survivors: a survey in Seoul

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More than 60 years have elapsed since the atomic bombings to Hiroshima and Nagasaki, and since all of the atomic bomb survivors have become old, the importance of caring their mental health has become increasing in Japan. Although approximately 70% of overseas atomic bomb are living in Korea, there have been quite few studies on their mental health. The objectives of the present study were to elucidate whether the mental health conditions of atomic bomb survivor in Korea are similar to those in Japan. The subjects were 181 Korean atomic bomb survivors living in Korea (cases) and 209 outpatients of a hospital in Seoul who were not exposed to atomic bombs (controls). Interviewers administered them at the hospital a questionnaire with Impact of Event Scale-Revised, General Health Questionnaire 12 (GHQ-12), Korean version of short form Geriatric Depression Scale and the K scale of the Minnesota Multiphasic Personality Inventory. Excluding subjects with incomplete responses we analyzed 162 cases and 189 controls. The proportion of subjects with high score of GHQ-12 (≥ 4) was significantly higher in cases (78/162 or 48.1%) than in controls (42/189 or 22.2%) ($p < 0.0001$, Fisher's exact test). The present results, though preliminary, indicate that atomic bomb survivors in Korea have also mental health problems similar to those observed in Japanese atomic bomb survivors, indicating the necessity of a larger study.

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Keywords: Korean A-bomb survivors, mental health, traumatic stress

Introduction

Two atomic bombs (A-bombs) were dropped on Hiroshima on 6 and on Nagasaki on 9 August 1945, respectively. The bombs instantaneously destroyed almost all areas of the respective cities, resulting in a total of 194,000 deaths by the end of 1945 and about 158,000 injured people. As of the end of March 2007, there were approximately 240,000 atomic bomb survivors in Japan. Hereafter, the atomic bomb survivor (A-bomb survivor) designates an individual who has officially been issued a so called A-bomb survivor's handbook. The A-bomb survivors are classified into 4 groups: Category 1-individuals exposed to the A-bombs in designated areas in Hiroshima or Nagasaki, who are called directly exposed; Category 2-individuals who had not been exposed to A-bombs but entered into the designated areas in either city

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within 2 weeks after the bombing; Category 3-individuals who were engaged in rescue and burial; and Category 4-individuals who were in uteri of those in Categories 1-3.

In addition to these survivors, there are many so-called 'overseas atomic bomb survivors' who had been exposed to the A-bombs and immigrated to foreign countries or returned to their home country. As of the end of March 2007, there were approximately 4,300 overseas atomic bomb survivors. Of those survivors, about 2,930 were in the Republic of Korea (called hereafter Korean A-bomb survivors), 970 were in the United States, and 160 were in Brazil, so that 90% of overseas survivors were in these 3 countries.

As Japan is the unique country that has experienced atomic bombing, health effects of exposure to the A-bomb have been investigated extensively. The results of these studies have demonstrated that many survivors are still experiencing physical problems due to the effects of A-bomb radiation, 65 years after exposure. Known physical effects include an increased risk of leukemia and various other types of cancer, and many researches have been carried out to elucidate the role of radiation in developing these diseases.[1-3]

Studies on overseas A-bomb survivors include a mail survey of the health status of 1,256 Korean A-bomb survivors,[4] an investigation of the health status of 4,079 children of 1,115 Korean A-bomb survivors,[5] and studies comparing Korean A-bomb survivors and controls with respect to the physical conditions using blood tests and other data.[6-7] The study of Korean survivors' children[5] demonstrated a relatively high frequency of mental retardation (0.18%) and congenital bone disorders (0.18%) among them. Jhun et al[7] reported that blood pressure, white blood cell count, serum total cholesterol, and aspartate aminotransferase level were higher in Korean A-bomb survivors than in controls, while hemoglobin concentration, hematocrit, and red blood cell count were lower in the former than in the latter. Although the reports on physical illness in Korean A-bomb survivors are increasing, their number is still small compared to those on Japanese A-bomb survivors.

Though less compared to the study on physical conditions in Japanese A-bomb survivors, several researches have been carried out about their mental health conditions. The study by Okumura et al[8] is the first one regarding the psychological impact on A-bomb survivors: they randomly selected 3 months after the Nagasaki atomic bombing 50 patients among 192 A-bomb survivors having been hospitalized at Omura Naval Hospital, examined them, reviewed their medical history, and diagnosed 3 patients psychogentic disorder. Nishikawa et al[9] reported that they observed 533 neurosis cases among 7297 A-bomb survivors in Nagasaki who underwent an extensive health examination and that the proportion of neurosis cases was higher by twofold or more in those with acute symptoms due to radiation exposure (9.7%) than in those without such symptoms (3.9%). Ohta et al[10] reported that psychological distress measured half a century after exposure to the A-bomb on the basis of the GHQ-30, 30-item version of the original General Health Questionnaire (GHQ) consisting of 60 items,[11] was greater in the A-bomb survivors than in the controls. A study by Honda et al[12] on the mental health of Nagasaki A-bomb survivors revealed that 8.4% of them scored 4 points or higher in GHQ-12 (12-item version of the original GHQ),[11] suggesting that A-bomb survivors had mental health problems. The analysis[13] of 35,035 responses to the mail survey which Nagasaki city administered to 49,867 A-bomb survivors in 2003 showed that 4,503 (28.2%) scored 25 points or higher on the Impact of Event Scale-Revised (IES-R).[14] Furthermore, Yamada et al[15] demonstrated that the proportion of A-bomb survivors showing anxiety disorder or somatoform disorder was significantly higher in those who had acute symptoms than in those who did not.

Since little is known about the mental health of overseas atomic bomb survivors, and since most of them living in areas other than Korean Peninsula are Japanese, we carried out the present study to elucidate the mental health conditions in Korean A-bomb survivors. The study was approved by the Nagasaki University School of Medicine Ethics Committee (08061978-2).

Subjects and Methods

Study subjects were Korean A-bomb survivors living in Seoul and outpatients of a hospital in Seoul not exposed to the A-bomb, who were included as controls.

Information on subjects was collected using anonymous self-administered questionnaire written in Korean with the help, if necessary, by Korean interviewers who were not healthcare professionals; however, they received, prior to commencement of the survey, one-day training enough to understand the content of the questionnaire. The questionnaire administered to controls included basic demographic items, e.g. gender and age, the GHQ-12,[14] the Korean version of short-form Geriatric Depression Scale (K-SGDS),[16] and the Minnesota Multiphasic Personality Inventory (MMPI),[17,18] while the questionnaire administered to Korean A-bomb survivors included furthermore the I-ESR,[18] the questions about circumstances due to the atomic bombing, e.g. injuries and deaths of family members, questions about the

experience of blast, heat and light from the bomb, and questions about official category as an A-bomb survivor.

The IES-R was used to assess the presence of posttraumatic stress disorder (PTSD) in A-bomb survivors. All items in the IES-R are scored on a 5-point scale (0, 1, 2, 3, 4) and the degree of PTSD is measured as the sum of all scores. The IES-R was assessed on the basis of the score distribution.

The GHQ-12 was used to assess the current mental health status. There are two methods for calculating the GHQ scores: the one method called the Likert scale method scores respective responses to each item by 4-point scale of (0, 1, 2, 3) and sums up all the scores to make up the total score, while the other method scores respective responses to each item by 4-point scale (0, 0, 1, 1) and sums up all the scores to make up the total score. The latter method was used in this study to calculate the GHQ score; the GHQ-12 score of 4 or higher was designated as a high GHQ-12 score, and those with a high GHQ-12 score are said to be more likely to have a non-psychotic mental illness.[11]

The K-SGDS, the respective items being scored on a 2-point scale, was used to measure depression status. The total of the scores of all items was used for assessment, and subjects with total score of 8 or more were classified into a group of high scorers showing a tendency towards depression.

The MMPI K-scale (MMPIK), the respective items being scored on a 2-point scale, was used to assess respondent's demeanor during interviews related to attitudes towards personal problems. The total of the scores of all items was used for assessment, and subjects with total score of 20 or more were classified into a group of high scorers.

The frequency of subjects with high score was compared between A-bomb survivors and controls for GHQ-12, K-SGDS and MMPIK on the basis of Fisher's exact test. Similar comparison was also made in A-bomb survivors between those exposed within 2.5 km from the hypocenter and those exposed at further place. The effects of radiation exposure, gender, age and MMPIK score on the frequency of high GHQ-12 score was assessed by logistic regression analysis. The necessary calculations were performed using PROC FREQ and PROC LOGISTIC of the SAS system® version 9.1. The results were called (statistically) significant if the p-value was less than 0.05.

Results

The questionnaire was administered to 181 individuals (66%) among 274 people who received health consultations for Korean A-bomb survivors in Seoul between June 23 and July 5, 2008; they provided written consent to participate in the present study. Of 181 participants, 19 people were excluded because they cancelled participation in the course of interview or because their responses were incomplete. A total of 162 A-bomb survivors (86 men and 76 women) remained for the analysis. A slightly different questionnaire was administered to 225 controls who provided written consent to participate in the present study. After excluding 36 participants who cancelled participation in the course of interview, whose responses were incomplete or who required hospitalization in the course of interview189 controls (89 men and 100 women) remained. Thus a total of 351 people (175 men and 176 women) remained for the analysis.

The male-female ratio was 1.13 in A-bomb survivors and 0.89 in controls, and no statistically significant difference was observed in the male-female ratio between A-bomb survivors and controls ($p = 0.29$, Fisher's exact test). The ages of A-bomb survivors ranged from 62 to 88 years with quartiles of 65, 68, and 73 years, while those of controls ranged from 59 to 89 years with quartiles of 66, 71, and 76 years. Though a statistically significant difference was observed in the median of age distribution between the two groups ($p = 0.0039$, Wilcoxon rank-sum test), the difference was rather small.

Among 162 A-bomb survivors, 148 (91.4%) were of Category 1, 3 (1.8%) were of Category 2, 7 (4.3%) were of Category 4, and categories in 4 (2.5%) were unknown. Regarding the A-bomb survivors' health conditions before the bombing, 118 (72.8%) responded good, 17 (10.5%) responded fair, 2 (1.2%) responded not good, and 25 (15.4%) responded nothing. Twenty-five A-bomb survivors (15.4%) had been treated after the bombing, while 129 (79.6%) did not, and 8 (4.9%) responded nothing. Approximately half of the A-bomb survivors (80 or 49.4%) lost their family members or relatives. Among 160 A-bomb survivors who responded to the questions about flash, blast and heat, 8 (5.0%) responded that they had not feel any of them, 42 (26.3%) responded that they had felt all, 63 (39.4%) responded that they remember any of them, 21 (13.1%) and 24 (15.0%) responded that they felt one and two, respectively, 2 (1.2%) responded that although they felt flash, they didn't remember other two.

The mean (standard deviation, SD) of IES-R score in A-bomb survivor was 19.9 (14.3), and the proportion of those with score of 25 or higher was 30.25%.

The proportion of subjects with high GHQ-12 score was

larger in A-bomb survivors (48.1% or 78/162) than in controls (22.2% or 42/189); the difference was statistically significant ($p < 0.0001$, Fisher's exact test) (Table 1). The mean (SD) of GHQ-12 score calculated by Likert scale method was 14.4 (6.4) in A-bomb survivors and 10.7 (4.3) in controls, respectively.

Table 1. Comparison of the A-bomb survivors and controls regarding the frequency of subjects with high scorers in the GHQ-12, K-SGDS, and MMPIK scales

Scale	Score	Group		*P*-value
		A-bomb survivors (n = 162)	Controls (n = 189)	
GHQ-12	≥4	78 (48.1%)	42 (22.2%)	< 0.0001
K-SGDS	≥8	56 (34.6%)	56 (29.6%)	0.3586
MMPIK	≥20	39 (24.1%)	24 (12.7%)	0.0077

GHQ-12: General Health Questionnaire (GHQ) is a self-administered screening instrument developed by Goldberg[11] to detect psychiatric disorders in community settings and non-psychiatric clinical settings such as primary care or general practice. The full version consists of 60 items. In the present study, we used a quick, reliable and short form consisting of 12 items named GHQ-12.
K-SGDS: The Korean version of SGDS, which is a short form of self-evaluating scale GDS (Geriatric Depression Scale) designed specifically to identify depression in the elderly. Although the full version of GDS consists of 30 items, the number of items in SGDS is decreased to 15 for not fatiguing elderly testee.
MMPIK: Abbreviation of The Minnesota Multiphasic Personality Inventory (MMPI) K scale. MMPI was developed in the late 1930's by psychologist S.R. Hathaway and psychiatrist J.C. McKinley at the University of Minnesota. The K scale of the MMPI was an attempt to assess more subtle distortion of response, particularly clinically defensive response.

The proportion of subjects with high K-SGDS score was larger in A-bomb survivors (34.6% or 56/162) than in controls (29.6% or 56/189); the difference, however, was not statistically significant ($p = 0.3586$, Fisher's exact test) (Table 1). The mean (SD) of K-SGDS score was 5.8 (4.5) in A-bomb survivors and 5.0 (4.2) in controls, respectively.

The proportion of subjects with high MMPIK score was larger in A-bomb survivors (24.1% or 39/162) than in controls (12.7% or 24/189); the difference was statistically significant ($p < 0.0077$, Fisher's exact test) (Table 1).

Table 2 presents the results of the logistic regression analysis regarding the frequency of subjects with high GHQ-12 score; we see from this Table, for example, that the odds of the frequency of subjects with high GHQ-12 score in A-bomb survivors will be about 3.3 times higher than those in controls after adjustment for gender, age and MMPI K scale score.

Table 3 summarizes the comparison of 69 A-bomb survivors directly exposed within 2.5 km from the hypocenter and 58 those directly exposed at 2.5 km or further from the hypocenter with respect to the frequency of subjects with high IES-R, GHQ-12, K-SGDS, and MMPIK scorers. No statistically significant difference was indicated between the two groups of A-bomb survivors regarding these three scales. No adjustment for circumstances in A-bomb survivors was made because they correlate with the distance of the exposed place from the hypocenter.

Table 2. The results of the logistic regression analysis regarding the frequency of subjects with high GHQ-12 score

Factor	Comparison	Estimated odds ratio (95% CI)
Exposure to A-bombing	Yes vs No	3.3 (2.09-5.50)
Gender	Female vs Male	1.8 (1.14-2.97)
Age	≥ 70 years vs < 70 years	1.2 (0.78-2.03)
MMPI K scale score	≥ 20 vs < 20	1.8 (1.04-3.35)

Table 3. Comparison of the A-bomb survivors exposed within 2.5 km from the hypocenter and those exposed at 2.5 km or further from the hypocenter regarding the frequency of subjects with high scorers in the IES-R, GHQ-12, K-SGDS, and MMPIK scales

Scale	Score	Distance of the exposed place from the hypocenter		*P*-value
		< 2.5 km (n = 69)	≥ 2.5 km (n = 58)	
IES-R	≥ 24	24 (34.8%)	25 (43.1%)	0.8523
GHQ-12	≥ 4	34 (49.3%)	25 (43.1%)	0.5924
K-SGDS	≥ 8	21 (30.4%)	21 (36.2%)	0.5711
MMPIK	≥ 20	14 (20.3%)	16 (27.6%)	0.4032

IES-R: Abbreviation of The Impact of Event Scale-Revised developed by Weiss and his colleagues, which is a self-administered 22-item questionnaire based on three clusters of symptoms identified in the Diagnostic and Statistical Mannual of Mental Disorders, third edition (DSM-III), as indicators of posttraumatic stress disorder (PTSD).
See the footnote of Table 1 for other scales.

Discussion

In the present study carried out in 2008, over 60 years after the atomic bombings, 30.25% of Korean A-bomb survivors scored 25 or more in IES-R with the mean (SD) of 19.9 (14.3).

The following results have been reported for studies related to PTSD in Koreans using the IES-R. In a survey on Koreans conducted by Yoon et al[19], the mean (SD) of IES-R score among 65 individuals diagnosed with PTSD was 40.6 (16.9).

Studies by Bahk et al[20] and Kim et al[21] reported the mean (SD) of IES-R score as 53.1 (13.0), and 49.8 (11.9), respectively, for individuals diagnosed with PTSD. These studies are subject to people got the PTSD was evaluated immediately after exposure to stress reactions (1 month). The IES-R levels of the precedent studies were higher than that of our study. We guess the reason as follows: the subjects of survey being patients given a diagnosis of PTSD, and the evaluation time was one month later exposed to severe stress in the precedent studies.

IES-R questionnaires given to survivors of the Nagasaki atomic bombing[12] showed that 28.2% were high scorers (25 points or higher). This study results, though preliminary, indicate that A-bomb survivors in Korea have also mental health problems similar to those observed in Japanese A-bomb survivors,

The study by Honda et al[12] reported that 8.4% of Nagasaki survivors were in the GHQ high scoring group (4 points or higher). In the present study, 48.1% of Korean survivors were in the GHQ-12 high scoring group (4 points or higher). These percentages are higher than those of Japanese survivors, suggesting that Korean survivors have some mental health problems.

Surveys conducted in Korea related to the GHQ-12 scale revealed the mean (SD) of GHQ-12 score calculated by Likert scale method as 17.7 (5.5) in subjects who showed a tendency towards depression.[22] A Korean study conducted by Han et al[23] on patients with depression or anxiety disorder who were receiving primary care reported mean GHQ-12 scores calculated by Likert scale method of 13.8 for those with depression and 15.3 for those with panic disorder. In the present study, the mean (SD) of GHQ-12 score calculated by Likert scale method in Korean survivors was 14.1 (6.0), which is roughly equal to the score in the above study. This study results were similar to the group that any psychiatric problems.

When we calculated odds ratios to test the influence of various factors on GHQ-12 score, we found that gender and age had little effect on the rise of GHQ-12 scores, but the effect from presence/absence of A-bomb exposure was large. As a result, it was suggested that a being bombed experience adversely affected the mental health of the A-bomb survivors.

Research conducted by Bae et al[24] on K-SGDS of older Korean psychiatric outpatients revealed the mean (SD) of the score as 10.82 (3.00) in those with major depression and 5.71 for those without depression. The mean (SD) of K-SGDS score found in this study was 5.8 (4.5). However, in other study,[25] the mean (SD) of K-SGDS score in patients with early dementia was 5.5 (4.0). Therefore, it cannot be said that a mean of K-SGDS in results of this study is low unconditionally. The percentage of high scorers in the K-SGDS questionnaire with scores of 8 points or higher was 34.6% (56 people) for survivors. From the above, the percentage of depression among Korean A-bomb survivors was high.

In the MMPI K scale as well, the percentage of high scorers in the MMPI K questionnaire with scores of 20 points or higher was 34.1% (39 people) for survivors of Korean survivors were high scorers. High MMPI K scorers are said to be defensive towards the investigation, while low scorers are said to be frank and self-critical.[16,17] The fact that scores for this scale were high in Korean survivors suggests that they may be leading their lives without being aware of their own psychological confusion.

When we tested the difference between those within or outside of a 2.5 km perimeter from the hypocenter during exposure, we did not find any significant differences in any items of the GHQ-12, K-SGDS, or MMPI scales, suggesting that distance from the hypocenter did not affect psychiatric health. Other studies related to the mental health of survivors[10,13,15] have reveal the influence of distance from the hypocenter on psychiatric health of survivors. In those studies, survivors who were within 2.0 km of the hypocenter when exposed to the A-bomb presented with acute symptoms due to radiation from exposure. Following exposure, they felt anxiety regarding their own health, as well as anxiety from such experiences as deaths in their family and destruction of their home. Such anxiety is considered to be a factor that leads to poorer mental health. 51.2% of subjects of this study were 5 years old or younger at the time of A-bomb exposure. Therefore, memory at being bombed is vague, and the possibility that affected the anxiety about the being bombed is inferred. We think that more detailed examination will be necessary about the association between being bombed distance and mental health.

The present study has several limitations, including the probable biases stemming from that the Korean A-bomb survivors participated in the study were only those who participated in consultation program, and the smallness in the sample size. Addressing these issues in further research is important for increasing our understanding of psychiatric health problems in Korean survivors.

The above-mentioned results, in the present that passed from atom bomb being bombed more than 60 years, it was found that the A-bomb survivors residing in Korea had a problem in mental health associated with a being bombed experience.

Conclusion

This study is the first to focus on psychiatric health problems in Korean A-bomb survivors. The results demonstrated the poor psychiatric health of Korean survivors, and suggested that atomic bomb exposure may be a major causal factor. Further studies are needed to provide detailed examinations of mental health problems among Korean survivors.

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References

1. Iwanaga M, Tagawa M, Tsukasaki K et al. Relationship between monoclonal gammopathy of undetermined significance and radiation exposure in Nagasaki atomic bomb survivors. *Blood* 113: 1639-1650, 2009
2. Furukawa K, Preston DJ, Lonn S et al. Radiation and smoking effects on lung cancer incidence among atomic bomb survivors. *Radiat Res* 174: 72-82, 2010
3. Iwanaga M, Hsu WL Hsu, Soda M et al. Risk of myelodysplastic syndromes in people exposed to ionizing radiation: a retrospective cohort study of Nagasaki atomic bomb survivors. *J Clin Oncol* 29: 428-434, 2011
4. Jhun HJ, Ju YS, Kim JB, Kim JK. Present Status and self-reported diseases of Korean atomic bomb survivors: a mail questionnaire survey. *Med Confl Surviv* 21: 230-236, 2005
5. Jhun HJ, Ju YS, Kim JB, Kim JK. Korean atomic bomb survivors' report on the present status and health of their children: a mail questionnaire survey. *Med Confl Surviv* 22: 275-282, 2006
6. Ju YS, Jhun HJ, Kim JB, Kim JK. Non-Cancer Diseases of Korean Atomic Bomb Survivors in Residence at Hapcheon, Republic of Korea. *J Korean Med Sci* 21: 385-390, 2006
7. Jhun HJ, Kim BG, Park JT, Kim SY, Koo BM, Kim JK. Biological Profiles of Korean Atomic Bomb Survivors in Residence at Daegu and Kyungbuk, Republic of Korea. *J Korean Med Sci* 23: 1090-1093, 2008
8. Okumuara N, Hikita H. Results of Psycho-neurological study on atomic bomb survivors. *Kyusyu Shinkei Seishin Igaku* 1: 50-52, 1949 (in Japanese)
9. Nishikawa T, Tuiki S. Psychiatric investigation of atomic bomb survivors. *Nagasaki Igakkai Zasshi* 36: 717-722, 1961 (in Japanese)
10. Ohta Y, Mine M, Wakasugi M et al. Psychological effect of the Nagasaki atomic bombing on survivors after half a century. *Psychiatry Clin Neurosci* 54: 97-103, 2000
11. Goldberg DP. *The Detection of Psychiatric Illness by Questionnaire.* Oxford University Press, London, 1972
12. Honda S, Shibata Y, Mine M el al. M. Mental health conditions among atomic bomb survivors in Nagasaki. *Psychiatry Clin Neurosci* 56: 575-583, 2002
13. Yamakawa D, Shibata Y. Post-traumatic stress disorders and influencing factors among Nagasaki atomic bomb survivors. *Nagasaki Igakkai Zasshi* 81: 210-212, 2006 (in Japanese)
14. Weiss DS. *The Impact of Event Scale-Revised.* The Guilford Press, New York, 2004
15. Yamada M, Izumi S. Psychiatric sequelae in atomic bomb survivors in Hiroshima and Nagasaki two decades after the explosions. *Soc Psychiatry Psycguatr Epidemiol* 37: 409-415, 2002
16. Briley M. *Diagnosis and Treeatment of Depression in Late Life.* Informa Healthcare Press, London, 1999
17. Graham JR. *MMPI-2 Assessing Personality and Psychopathology, 4th ed.* Oxford University Press, New York, 2006
18. Graham JR. *The MMPI: A Practical Guide.* Oxford University Press, New York, 1978
19. Yoon SJ, Jun CS, An HY, Kang HR, Jun TY. Patterns of temperament and character in patients with posttraumatic stress disorder and their association with symptom severity. *Compr Psychiatry* 50: 226-231, 2009
20. Bahk WM, Pae CU, Tsoh J et al. Effects of mietazapine in patients with post-toraumatic stress disorder in Korea: a pilot study. *Human Psychopharmacology* 17: 341-344, 2002
21. Kim W, Pae C, Chae JH, Jun TY, Bahk WM. The effectiveness of mirtazapine in the treatment of post-toraumatic stress disorder: A 24-week continuation therapy. *Psychiatry Clin Neurosci* 59: 743-747, 2005
22. Lim HE, Lee MS, Ko YH el al. Assessment of the Type D Personality Construct in the Korean Population:A Validation Study of the Korean DS14. *J Korean Med Sci* 26: 116-123, 2011
23. Han C, Pae CU, Patkar A, Masand P, Kim KW, Joe SH. Psychometric Properties of the Paient Health Questionnaire-15 (PHQ-15) for Measuring the Somatic Symptoms of Psychiatric Outpatients. *Psychosomatics* 50: 580-585, 2009
24. Bae JN, Cho MJ. Development of the Korean version of the Geriatric Depression Scale and its short form among elderly psychiatric patients. *J Psychosom Res* 57: 297-305, 2004
25. Lee KS, Hong CH, Cheong HK, Oh BH. Difference in nutritional risk between mild cognitive impairment group and normal cognitive function elderly group. *Arch Gerontol Geriatr* 49: 49-53, 2009

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Total Numbers of Undiagnosed Carriers of Hepatitis C and B Viruses in Japan Estimated by Age- and Area-Specific Prevalence on the National Scale

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Key Words
Hepatitis C virus · Hepatitis B virus · Blood donors · Liver cirrhosis · Hepatocellular carcinoma · Healthcare · Japan

Abstract
Objective: To estimate total numbers of undiagnosed carriers of hepatitis C virus (HCV) and hepatitis B virus (HBV) in Japan. ***Methods:*** Area- and age-specific prevalence of HCV as well as HBV was determined in the first-time blood donors [20–39 years (n = 2,429,364)] and examinees of periodical health check-ups [40–74 years (6,204,968 for HCV and 6,228,967 for HBV)] in Japan. Prevalence in adolescents [5–19 years (79,256 for HCV and 68,792 for HBV)] was determined in a single prefecture, and that of HCV in the elderly (≥75 years) was estimated by the exponential model. HBV infection was determined by the detection of hepatitis B surface antigen, and HCV infection by either the algorithm or assuming persistent infection in 70% of the individuals with antibody to HCV. ***Results:*** Of the total population of 127,285,653 in 2005, 807,903 (95% CI 679,886–974,292) were estimated to be infected with HCV at a carrier rate of 0.63%, and 903,145 (837,189–969,572) with HBV at that of 0.71%. ***Conclusion:*** Accurate estimation of undiagnosed HCV and HBV carriers in the general population would help to predict the future burden of liver disease, and take appropriate measures for improving healthcare.

Introduction

Hepatitis C virus (HCV) and hepatitis B virus (HBV) are estimated to infect 170 and 350 million people over the world, respectively [1, 2]. Most infections with HCV or HBV do not induce clinical liver disease, while ~30% of them develop severe liver disease such as cirrhosis and hepatocellular carcinoma [3, 4]. Hence, there is a pressing need to identify the individuals who have undiagnosed HCV or HBV infection, and take effective measures for terminating viral infections and preventing the progression of liver disease.

For management of persistent HCV and HBV infections in a given country, it is necessary to know their exact numbers for assessing medical and financial needs in the foreseeable future. Prevalence of undiagnosed HCV or HBV

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infection has been estimated by survey of blood donors in Japan [5] and a representative population in the USA [6].

In the present study, area- and age-specific prevalence of HCV or HBV infection was determined in 8 jurisdiction areas of the Japanese Red Cross Blood Center. Then, the total numbers of undiagnosed HBV and HCV infections were estimated by compiling the results in the first-time blood donors and examinees of the periodical health check-up program. Of the 127,285,653 Japanese registered in 2005, 807,903 (0.63%) were estimated to be infected with HCV and 903,145 (0.71%) with HBV.

Materials and Methods

Japanese Population

Japan is divided into 8 areas, along its north-to-south axis, according to jurisdiction of the Japanese Red Cross Blood Center, into Hokkaido, Tohoku, Kanto, Hokuriku/Tokai, Kinki, Chugoku, Shikoku and Kyushu. Populations in 5-year age groups in each jurisdiction area were obtained from the registry at the National Census 2005.

First-Time Blood Donors

During 6 years from January 2001 to December 2006, 3,748,422 individuals (aged 16–64 years) donated whole blood or apheresis products for the first time, and their sera were tested for markers of HCV and HBV infections. Ongoing HCV infection was estimated by assuming the detection of HCV RNA in 70% of individuals with the antibody to HCV (anti-HCV), in accordance with a previous report [5].

Examinees of Hepatitis Virus Infections

Since the fiscal year 2002 in Japan, individuals who turned 40, 45, 50, 55, 60, 65 and 70 years were offered to take tests for hepatitis viruses at periodical health check-ups by a 5-year national project. During 5 years through 2006, 6,204,968 individuals received tests for HCV and 6,228,967 for HBV, corresponding to ~30% of the eligible Japanese, and their area- and age-specific prevalence of HCV or HBV infection was determined.

School Children and Adolescents

In the Iwate prefecture located in the north of Japan, biochemical markers of diseases dependent on the lifestyle were examined in children and adolescents at the entrance to schools. Their serum samples had been stored frozen, and were tested for markers of hepatitis virus infections. Carrier rates of HCV and HBV among them were calculated, with their ages adjusted to those in 2005; infants aged <5 were represented by the children aged from 5 to 9 years. Designs and procedures of this investigation were approved by the Ethics Committee of Hiroshima University.

Simulation of HCV and HBV Infections in the Elderly

By its age-specific profile, the prevalence of HCV was deduced to be an exponential function of the age. Accordingly, age-specific prevalence of HCV in the individuals aged ≥75 years was simulated by an exponential function model; it was constructed on the prevalence of HCV in each age group ≥50 years.

The formula was constructed as:

$$\log y(x) = a + bx$$

where x is the 5-year age code, $y(x)$ is an estimator of HCV prevalence in x, and a and b are coefficients.

The equation is transformed into:

$$y(x) = e^a e^{bx}$$

in which e^a represents the HCV prevalence when $x = 0$ (in the group aged 0–4 years), since $y(0)$ is equal to e^a. By replacing x for $x + 1$ in the above equation, it is converted to $y(x + 1) = e^a e^{b(x+1)}$.

Then, the following equation can be constructed:

$$y(x + 1) = e^b y(x)$$

where e^b is the slope of HCV prevalence increasing with age. Thus, the HCV prevalence is multiplied by a factor e^b for an increment of the age code by 1.

The simulation model was applied to estimate age-specific prevalence of HCV in each of 8 areas in the individuals ≥75 years.

Prevalence of HBV in the individuals ≥75 years was represented by that in those aged 70–74 years, since it stayed constant from 65 through 75 years.

Markers of Hepatitis Virus Infections

In blood donors, anti-HCV was determined by passive hemagglutination of the second generation with commercial assay kits (HCV PHA; Abbott Laboratories, North Chicago, Ill., USA) with a cutoff limit set at 2^5, as well as by particle agglutination with commercial assay kits (HCV PA Test-II; Fujirebio, Inc., Tokyo, Japan). HBsAg was determined by reversed passive hemagglutination with reagents prepared by the Japanese Red Cross.

In examinees of periodical health check-ups, ongoing HCV infection was determined by the algorithm with anti-HCV and HCV RNA [7]. Anti-HCV was determined by passive hemagglutination of the second generation with commercial assay kits (HCV PHA; Abbott Laboratories), and since 2002, it was determined by enzyme immunoassay with commercial assay kits (AxSYM HCV Dinapack-III; Abbott Laboratories). Samples with high anti-HCV titers contain HCV RNA, and therefore, only those with low and middle titers were examined for HCV RNA. HBsAg was determined by reversed-passive hemagglutination with commercial assay kits (Institute of Immunology Co., Ltd, Tokyo, Japan).

Statistical Analyses

Statistical analyses for the evaluation of R^2 values were performed with JMP 8.0 (SAS Institute, Inc., Cary, N.C., USA) and DeltaGraph 5.5 (RedRock Software, Inc., Salt Lake City, Utah, USA). A p value > 0.05 was considered significant.

Results

Age-Specific Prevalence of HCV in the First-Time Blood Donors and Examinees of Periodical Health Check-Ups

Figure 1 illustrates age-specific prevalence of HCV in the first-time blood donors (aged 15–69 years in 2005) and examinees of periodical health check-ups (39–73 years in 2005); 70% of individuals with anti-HCV were considered

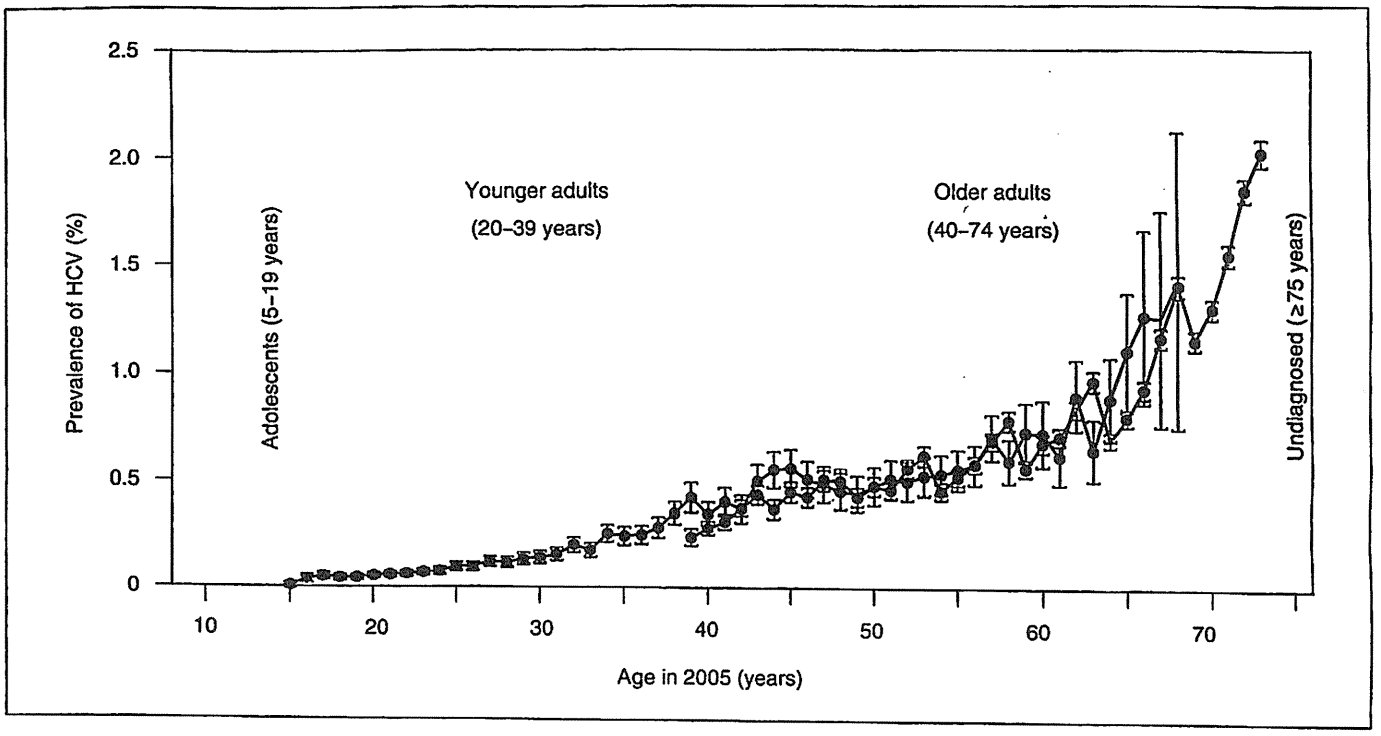

Fig. 1. Age-specific prevalence of HCV in Japan. The prevalence of HCV was determined in the first-time blood donors aged from 15 to 68 years (blue dots) and examinees of periodical health check-ups aged from 39 to 73 years (red dots). Their ages were adjusted to those in the year 2005. Bars indicate ranges of 95% CI.

to possess HCV RNA in serum [5]. Results of two distinct populations were well in accord. For the first-time blood donors, however, the variation (95% CI) widened increasingly with age. It would have reflected decreases in the first-time blood donors with age, since the majority of these (83.5%) were aged ≤39 years. As the prevalence of HCV in blood donors ≥40 years was unreliable in them, that in examinees of periodical check-ups was adopted for estimating the national prevalence of HCV.

Area-Specific Prevalence of HCV in Eight Jurisdiction Areas

In view of distinct geographic distribution of HCV, the prevalence of HCV in the general population would not be applicable to every area in Japan. Figure 2 compares results in the first-time blood donors and recipients of health check-ups among 8 jurisdiction areas spanning from north (Hokkaido) to south (Kyushu). They unfolded a wide variety in the age-specific prevalence of HCV. Although the prevalence of HCV increased with age in all areas, the slope of increase differed widely among them. Hence, it was necessary to employ a distinct age-specific prevalence in each of the 8 areas for estimating HCV carriers precisely.

Table 1. Age-specific prevalence of HCV in three different populations

Age in 2005	n	HCV-positive, n	Prevalence, % (95% CI)
School children			
5–9	17,390	2	0.012 (0.000–0.027)
10–14	29,817	3	0.010 (0.000–0.021)
15–19	32,049	7	0.022 (0.006–0.038)
Blood donors			
20–24	1,205,966	1,122	0.065 (0.061–0.070)[a]
25–29	536,560	874	0.114 (0.105–0.123)[a]
30–34	408,814	1,089	0.186 (0.173–0.200)[a]
35–39	278,024	1,190	0.300 (0.279–0.320)[a]
HCV screening			
40–44	611,146	2,127	0.348 (0.333–0.363)
45–49	495,032	2,292	0.463 (0.444–0.482)
50–54	675,350	3,485	0.516 (0.499–0.533)
55–59	947,438	5,974	0.631 (0.615–0.646)
60–64	1,081,854	8,423	0.779 (0.762–0.795)
65–69	1,264,496	13,722	1.085 (1.067–1.103)
70–74	1,054,472	17,649	1.674 (1.649–1.698)

[a] The prevalence in blood donors was based on an assumption of HCV infection persisting in 70% of those with anti-HCV [5].

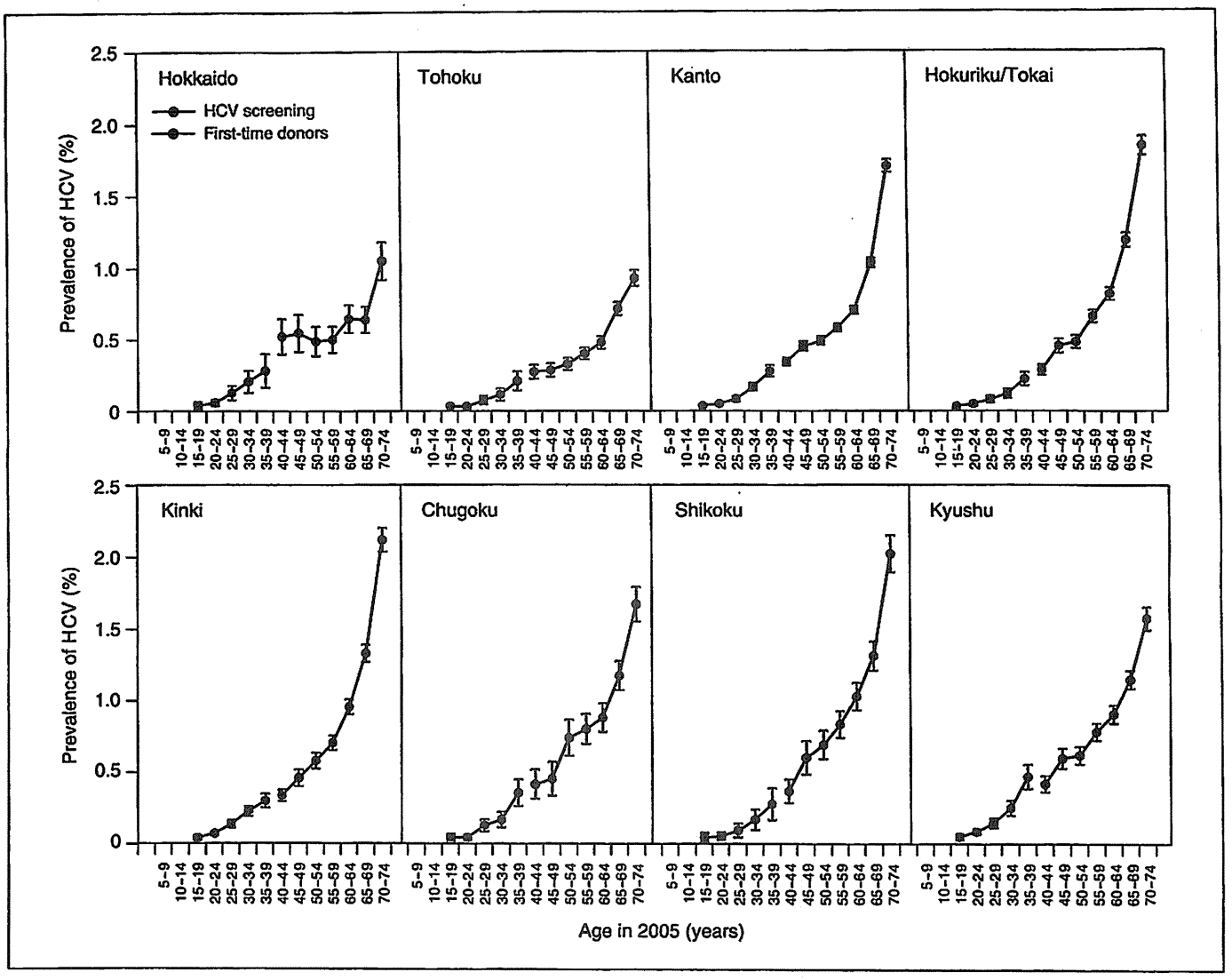

Fig. 2. Age-specific prevalence of HCV in 8 jurisdiction areas in Japan. The prevalence of HCV is calculated in each of twelve age groups notched by 5 years. The prevalence in five groups ≤39 years was represented by the first-time blood donors, and that in seven groups ≥40 years by recipients of HCV screening. Bars indicate ranges of 95% CI.

Prevalence of HCV in Adolescents

Since blood donors were restricted to 16–64 years of age, and health examinees were targeted on 40–70 years, they did not cover individuals aged ≤15 or ≥75 years in the year 2005. To fill in an opening on the younger side, the age-specific prevalence of HCV was determined in school children and adolescents in the Iwate prefecture (table 1). The prevalence in infants aged 0–4 years was assumed similar to that in the children aged 5–9 years; an extremely low prevalence of HCV (0.012%) would support such an assumption.

Simulating Prevalence of HCV in the Elderly

The prevalence of HCV appeared to be an exponential function of the age, according to its profiles in the first-time blood donors and examinees of health check-ups (fig. 1). Based on this assumption, a formula was constructed to simulate the prevalence of HCV in age groups ≥75 years for each of the 8 jurisdiction areas in Japan (see Materials and Methods).

Figure 3 compares actual (dots) and simulated data (red line) of five age groups from 50 to 74 years (corresponding to age codes 10–14) among the 8 areas. There was a high coefficient of determination between them,

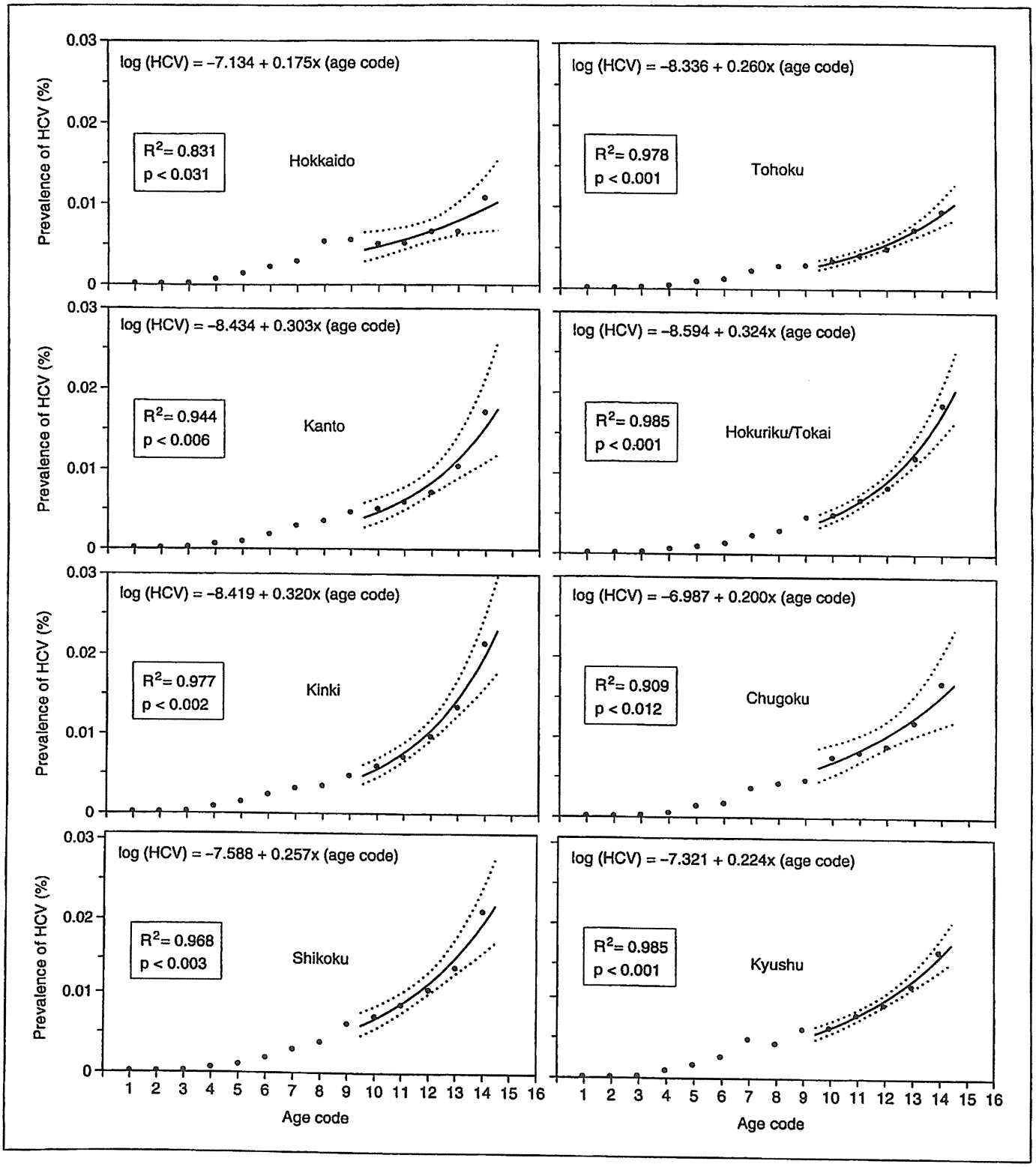

Fig. 3. Simulation of age-specific prevalence of HCV in the elderly. Prevalence of HCV in the first-time blood donors as well as examinees of periodical health check-ups (dots) and that simulated by formulation (red line with ranges of 95% CI in dotted line) are shown for 8 jurisdiction areas in Japan. Formula is shown at the top of each area. Age codes are: 1, 5–9 years; 2, 10–14 years; 3, 15–19 years; 4, 20–24 years; 5, 25–29 years; 6, 30–34 years; 7, 35–39 years; 8, 40–44 years; 9, 45–49 years; 10, 50–54 years; 11, 55–59 years; 12, 60–64 years; 13, 65–69 years; 14, 70–74 years, and 15, 75–79 years.

Table 2. Regional and total HCV carriers in Japan

Areas	Population	HCV carriers (95% CI)	Carrier rate
Hokkaido	5,620,813	26,097 (19,356–34,413)	0.46%
Tohoku	12,047,975	50,688 (42,754–59,953)	0.40%
Kanto	41,247,892	235,328 (195,408–293,611)	0.57%
Hokuriku/Tokai	19,294,443	132,434 (114,216–154,446)	0.69%
Kinki	22,657,542	173,808 (147,548–207,173)	0.52%
Chugoku	7,650,977	53,296 (42,299–67,698)	0.70%
Shikoku	4,083,698	35,159 (28,746–43,004)	0.86%
Kyushu	14,682,313	101,092 (89,379–113,993)	0.80%
Total	127,285,653	807,903 (679,886–974,292)	0.63%

Table 3. Age-specific prevalence of HBV in three different populations

Age in 2005	n	HBV-positive, n	Prevalence, % (95% CI)
School children			
5–9	17,363	3	0.017 (0.000–0.037)
10–14	29,817	14	0.047 (0.022–0.072)
15–19	32,049	12	0.037 (0.016–0.059)
Blood donors			
20–24	1,205,966	1,826	0.151 (0.144–0.158)
25–29	536,560	1,650	0.308 (0.293–0.322)
30–34	408,814	1,759	0.430 (0.410–0.450)
35–39	278,024	1,327	0.477 (0.452–0.503)
HBV screening			
40–44	613,960	5,491	0.894 (0.871–0.918)
45–49	497,589	5,373	1.080 (1.051–1.109)
50–54	679,893	8,700	1.280 (1.253–1.306)
55–59	950,508	12,891	1.356 (1.333–1.379)
60–64	1,085,119	13,282	1.224 (1.203–1.245)
65–69	1,268,304	12,406	0.978 (0.961–0.995)
70–74	1,057,469	9,545	0.903 (0.885–0.921)

with R^2 values ranging from 0.831 to 0.985 ($p < 0.031$ and $p < 0.001$, respectively), attesting to the validity of this simulation. Of note, the factor *b* in formula (by which age codes were multiplied) varied broadly among the 8 areas. Thus, it was the highest in Hokuriku/Tokai at 0.324 and lowest in Hokkaido at 0.175, with close to twofold differences between them.

Estimation of Undiagnosed HCV Carriers in Eight Areas and the Entire Nation

Based on age- and area-specific prevalence of HCV, numbers of undiagnosed HCV carriers were calculated for 8 jurisdiction areas, and they were compiled in the entire nation (table 2). The prevalence of HCV in each of three age groups (75–79, 80–84 and ≥85 years) was simulated by the formula, while that of HBV was represented by the prevalence in the group of 70–74 years. As of the year 2005, 127,285,653 were registered in the national census of Japan, and 807,903 of these are estimated to have undiagnosed HCV infection at an overall carrier rate of 0.63%. There was an increasing gradient in the prevalence of HCV along the north-to-south axis of Japan.

Age-Specific Prevalence of HBV

Figure 4 depicts age-specific prevalence of HBV in 2005. It was deduced from HBsAg in the first-time blood donors (15–69 years) and examinees of periodical health check-ups (39–73 years). Since the prevalence of HBV in the elderly did not increase with age so sharply as that of HCV (fig. 1), it was presumed not to increase further and stay around 1% in the individuals ≥75 years. The age-specific prevalence of HBV tabulated in three different

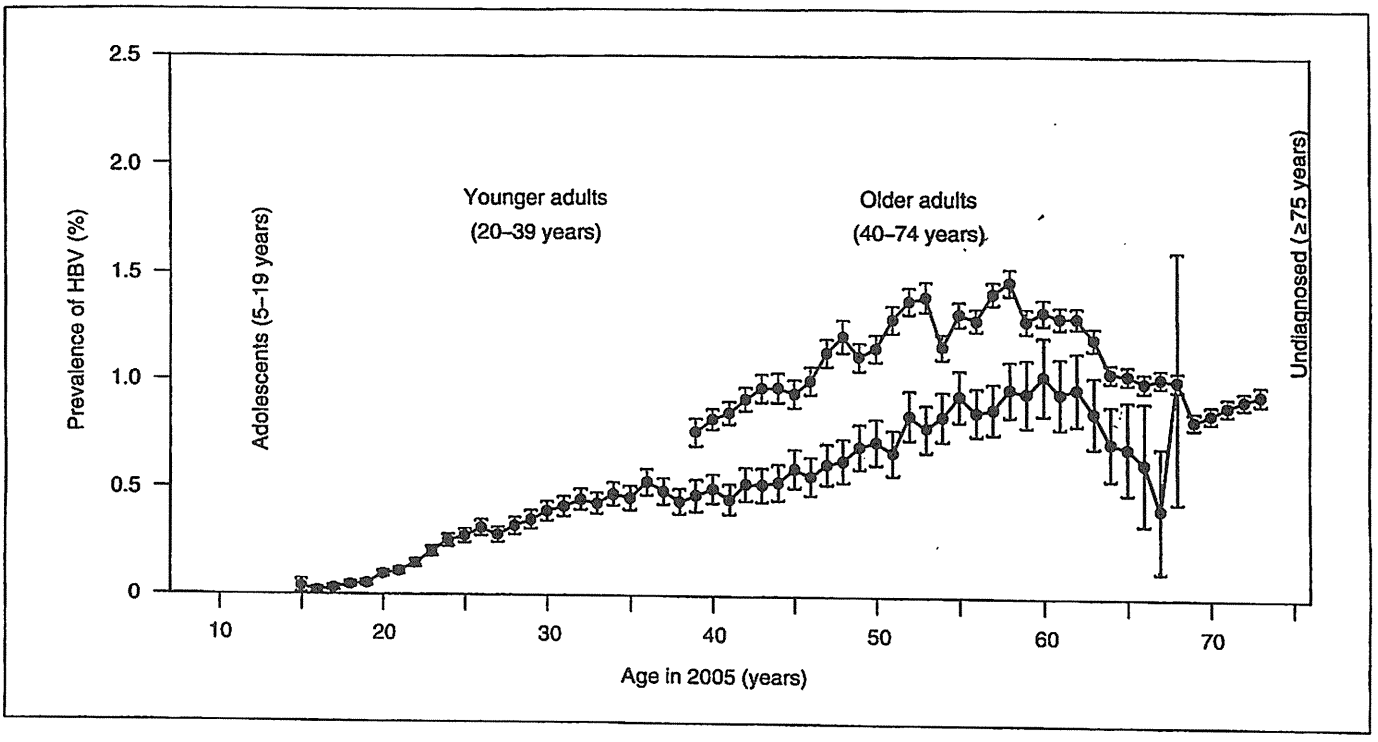

Fig. 4. Age-specific prevalence of HBV in Japan during 2002–2006. The prevalence of HBV was determined in the first-time blood donors aged from 15 to 68 years (blue dots) in the year 2005 and examinees of periodical health check-ups aged from 39 to 73 years (red dots) in the year 2005. Bars indicate ranges of 95% CI.

populations is listed in table 3. There was a constant decline with decreasing age in the frequency of HBV in individuals ≤39 years, and it was particularly low in children ≤9 years (0.017%).

In examinees of periodical health check-ups, the age-specific prevalence of HBV did not diverge and stayed within a narrow 95% CI (fig. 4). By contrast, that in the first-time blood donors dispersed widely. Such a variation in the age-specific prevalence of HBV would have been ascribed to the first-time blood donors who clustered in age groups ≤40 years.

Area-Specific Prevalence of HBV in Eight Jurisdiction Areas

The age-specific prevalence of HBsAg varied widely among 8 jurisdiction areas (fig. 5). HBsAg was most frequent in the age group of 55–59 years in every area, and reached 3.1% in the northern-most Hokkaido. The peak frequency decreased in central Japan (1.1% in Kanto and Hokuriku/Tokai), and increased towards the southern end (1.9% in Kyushu). Thus, the prevalence of HBsAg was determined individually along the axis of Japan in estimating the total number of HBV carriers in Japan.

Estimation of Undiagnosed HBV Carriers in Eight Areas and the Entire Nation

Numbers of undiagnosed HBV carriers were complied by multiplying age-specific prevalence of HBsAg by corresponding subpopulations in 8 jurisdiction areas (table 4). In total, 903,145 of the 127,285,653 (0.71%) individuals are estimated to have undiagnosed HBV infection in Japan in 2005.

Shift of Undiagnosed HCV and HBV Carriers during 5 Years (2000–2005) in Japan

Table 5 compares numbers of HCV and HBV carriers aged 15–69 years between 2000 and 2005 for 8 jurisdiction areas in Japan. Data for the year 2000 were extracted from a previous survey [5]. Data for the year 2005 were obtained in the first-time blood donors during 2001–2006 in this study by the same method as in the previous survey [5]. Undiagnosed HCV and HBV carriers decreased during 5 years by 55 and 47.5%, respectively. The overall carrier rate of HCV declined sharply from 0.95 to 0.44%, and that of HBV from 1.04 to 0.55% in Japan.

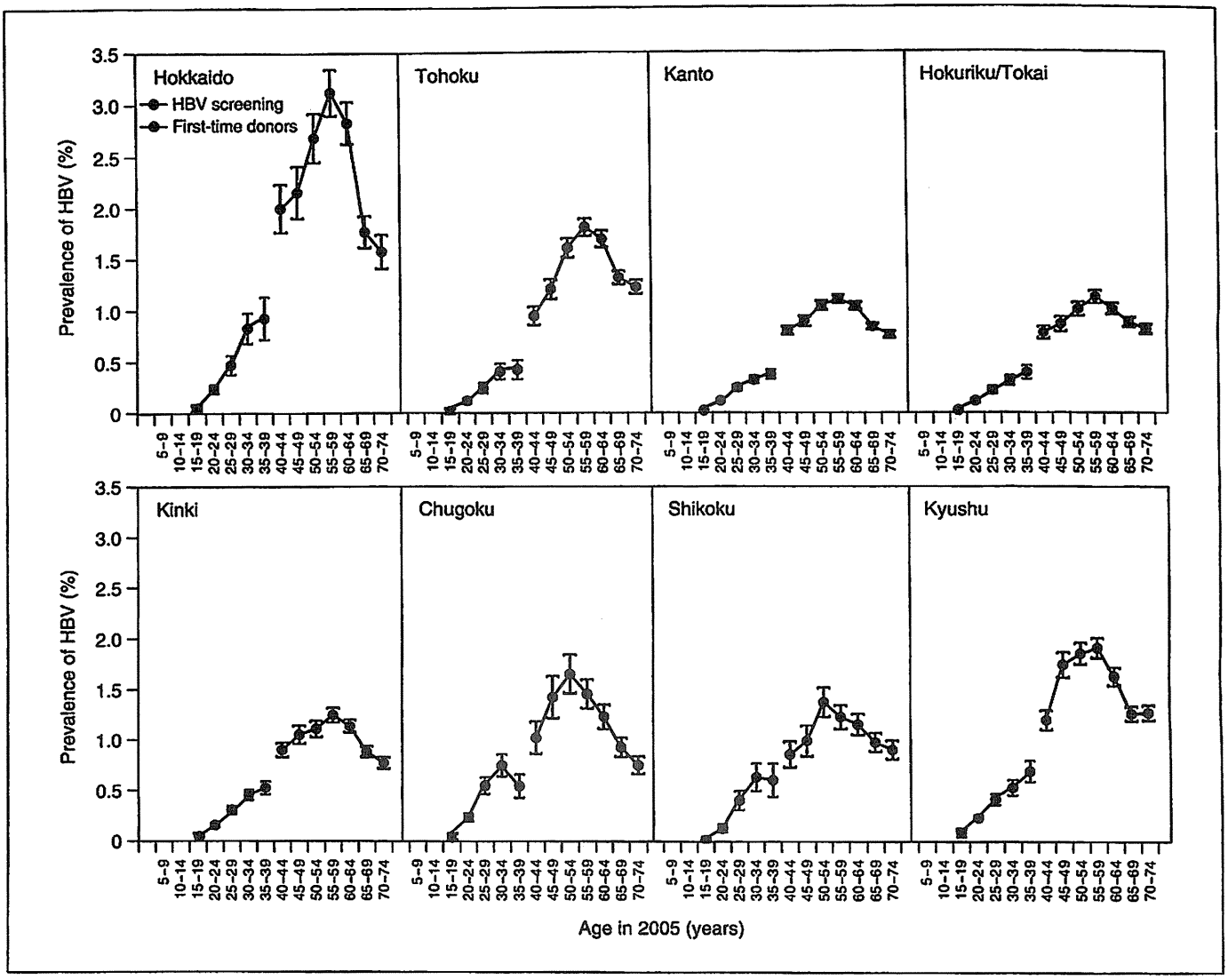

Fig. 5. Age-specific prevalence of HBV in 8 jurisdiction areas in Japan. The prevalence of HBV is calculated in each of twelve age groups notched by 5 years. The prevalence in five groups ≤39 years was represented by the first-time blood donors and that in seven groups ≥40 years by recipients of HCV screening. Bars indicate ranges of 95% CI.

Table 4. Regional and total HBV carriers in Japan

Areas	Population	HBV carriers (95% CI)	Carrier rate
Hokkaido	5,620,813	80,573 (72,314–88,765)	1.43%
Tohoku	12,047,975	104,736 (97,742–111,816)	0.87%
Kanto	41,247,892	231,799 (220,129–244,105)	0.56%
Hokuriku/Tokai	19,294,443	109,709 (101,722–117,581)	0.56%
Kinki	22,657,542	144,965 (134,387–155,464)	0.64%
Chugoku	7,650,977	59,948 (52,705–67,121)	0.78%
Shikoku	4,083,698	29,776 (26,080–33,437)	0.73%
Kyushu	14,682,313	141,639 (132,111–151,282)	0.96%
Total	127,285,653	903,145 (837,189–969,572)	0.71%

Table 5. Decrease of undiagnosed HCV and HBV carriers in the 15- to 69-year-old population in Japan

	Survey in 2000[a]		Survey in 2005		Difference	
	number estimated	carrier rate in area[b]	number estimated	carrier rate in area[b]	number estimated	balance
Shift of HCV carriers during 5 years from 2000 to 2005						
Hokkaido	41,139	0.99%	17,658	0.44%	−23,481	−57.1%
Tohoku	61,658	0.71%	30,525	0.37%	−31,133	−50.5%
Kanto	277,644	0.90%	126,283	0.41%	−151,361	−54.5%
Hokuriku/Tokai	88,724	0.64%	48,360	0.35%	−40,364	−45.5%
Kinki	178,871	1.06%	70,526	0.43%	−108,345	−60.6%
Chugoku	72,431	1.32%	24,595	0.47%	−47,836	−66.0%
Shikoku	43,497	1.49%	16,504	0.59%	−26,993	−62.1%
Kyushu	120,989	1.16%	64,115	0.63%	−56,874	−47.0%
Total	884,954	0.95%	398,567	0.44%	−486,387	−55.0%
Shift of HBV carriers during 5 years from 2000 to 2005						
Hokkaido	106,896	2.56%	54,557	1.35%	−52,339	−49.0%
Tohoku	104,923	1.21%	48,490	0.58%	−56,433	−53.8%
Kanto	255,207	0.83%	132,414	0.43%	−122,793	−48.1%
Hokuriku/Tokai	78,481	0.56%	51,477	0.37%	−27,004	−34.4%
Kinki	165,915	0.98%	85,083	0.52%	−80,832	−48.7%
Chugoku	90,041	1.64%	37,706	0.71%	−52,335	−58.1%
Shikoku	38,411	1.32%	19,162	0.69%	−19,249	−50.1%
Kyushu	127,879	1.23%	77,941	0.77%	−49,938	−39.1%
Total	967,753	1.04%	506,830	0.55%	−460,923	−47.6%

[a] Data for the year 2000 were extracted from a previous survey of hepatitis virus infections in Japan [5].
[b] The carrier rate specific for respective jurisdiction area was applied.

Discussion

There are many constraints in estimating total HCV and HBV infections in a given nation. Since it is not feasible to test every member for serological markers of hepatitis virus infection, populations representative of the entire nation have served for the estimation. Volunteer blood donors are recruited, but they have a restricted age range (16–64 years in Japan). Students attending schools and universities can close the opening in younger generations, but infants younger than the school age are not enrolled. Moreover, there are no means of estimating carrier rates of hepatitis virus infections in the individuals aged beyond the eligibility of blood donation. In addition, blood donors are selected individuals who are leading healthy lives above the average. In the survey of inhabitants in sentinel counties of the USA [6], who represent the average Americans, patients with liver disease and persons with restricted activities, such as those incarcerated or institutionalized, are not included.

Patients with clinical liver disease, as well as individuals found with HCV or HBV infection by health check-ups, can receive the medical care. However, many blood donors found with viral infections have developed severe liver disease already, and therefore, cannot receive efficient medical interventions [7, 8]. Hence, it is necessary to detect undiagnosed HCV and HBV infections hidden in the society. For this purpose, periodical health check-ups for screening hepatitis virus markers were started in April 2002 on the individuals, who turned 40, 45, 50, 55, 60 and 70 years, by a 5-year national project in Japan. The target age range (40–70 years) was selected due to a high incidence of hepatocellular carcinoma [9]. Since by far the majority of the first-time blood donors were younger than 40 years, the prevalence of HCV or HBV beyond that age dispersed widely (fig. 1, 4). In this study, therefore, the coverage by the first-time blood donors was confined to 20–39 years of age, and it was taken place by examinees of health check-ups aged 40–74 years; they left age groups $\leq$15 and $\geq$75 years uncovered, however.

The national prevalence of hepatitis virus infections in individuals ≤19 years was presumed to be similar to that in the Iwate prefecture situated in northern Japan. Since the prevalence of HCV or HBV infection in them was extremely low and stayed between 0.01 and 0.02%, such an assumption would not have affected the overall results to any significant extent. The prevalence of HCV in age groups ≥75 was simulated by a premise that it would be an exponential function of the age. Consequently, the formula based on profiles in five age groups from 50 to 74 years (at a 5-year notch) was extrapolated to three age groups ≥75 years. The simulation matched closely with the prevalence determined in corresponding age groups, with R^2 values ranging from 0.83 to 0.99 ($p < 0.05$ and $p < 0.01$, respectively) throughout 8 jurisdiction areas in Japan (fig. 3).

Japan has an axis spanning 2,000 kilometers from the north-east towards the south-west over the four major islands (Hokkaido, Honshu, Shikoku and Kyushu). Within a rather small land, the prevalence of HCV or HBV is not uniform all over Japan. The prevalence of HCV had an increasing gradient from north to south, and was the highest in Kyushu (table 2), while that of HBV was the highest in Hokkaido, decreased in between and then increased towards Kyushu (table 4). Reflecting such local differences, age-specific prevalence of HCV or HBV differed widely among 8 jurisdiction areas (fig. 2, 5).

Based on the results obtained on the area- and age-specific prevalence of HCV or HBV, carriers of these hepatitis viruses in 8 jurisdiction areas were tabulated separately over age groups from 20 to 74 years. Those in age groups ≤19 years were represented by the Iwate prefecture. The prevalence of HCV in age groups ≥75 years was simulated by the formula, and that of HBV was represented by individuals aged 70–74 years. Japan was populated by 127,767,994 people in 2005. Of these, 807,903 (95% CI 679,886–974,292) were estimated to have undiagnosed HCV infection at an overall prevalence of 0.63%, and 903,145 (837,189–969,572) to possess undiagnosed HBV infection at that of 0.71%. These estimates are much less than publically inferred numbers of HCV and HBV carriers in Japan at 1.5–2.0 million each. Leaving aside HCV and HBV carriers who have developed liver disease and stayed outside the scope of the present study, our estimates based on reasonable scientific grounds are much smaller; they add up barely half of generally referred figures around 1.5–2.0 million in Japan.

Based on the sex- and age-specific prevalence of hepatitis virus markers in the 3,478,422 first-time blood donors during 2001–2006, with the same criteria used in the previous study [5], we have estimated the number of undiagnosed HCV carriers aged 15–69 years in the year 2005 to be 398,567 (95% CI 295,410–501,453) and that of undiagnosed HBV carriers to be 506,830 (95% CI 398,115–616,113). In the previous study [5], undiagnosed HCV and HBV carriers aged 15–69 years in the year 2000 were assessed to be 884,954 (95% CI 725,082–1,044,826) and those with HBV to be 967,753 (95% CI 806,760–1,128,745). They decreased by 55.0 and 47.6%, respectively, during 5 years (table 5). In support of this view, the incidence of HCV or HBV infection during 10 years (1994–2000) in Japan is very low and estimated at 1.86 (95% CI 1.06–3.01) or 2.78 (1.87–4.145) per 100,000 person-years [10]. Decreases in undiagnosed HCV and HBV carriers in Japan would have been attributed to increased chances of receiving tests for hepatitis virus infections at health checkups and medical institutions, as well as increased awareness due to educational programs or other healthcare campaigns or screening programs in high-risk individuals. Additionally, there would have been a cohort effect in individuals aged 15–69 years who have shifted by 5 years during the observation period.

The results of the Third National Health and Nutrition Survey (HANES III, 1988–1994) [11] and those of more recent HANES (2001–2002) [6] in the USA are essentially similar with respect to age-specific profiles of HCV infection, and shifted by 10 years. The incidence of de novo HCV and HBV infections may have decreased substantially both in the USA and Japan, driven partly by the introduction of the nucleic acid amplification test and a more stringent questionnaire on donors to exclude blood donations in the window period of infection [12–17]. The national burden of HCV infection has been reported in Great Britain [18], where the prevalence of anti-HCV in hospitalized patients was 3.4% and that in the first-time blood donors was 0.03% in the year 2008.

In spite of many improvements in the control of hepatitis virus infections, there are many HCV and HBV carriers buried in the society who need immediate identification for receiving timely and efficient medical interventions. Treatment of viral hepatitis keeps improving, especially for liver disease induced by HCV. The sustained virological response in the patients infected with HCV of genotype 1, who have received triple therapy with pegylated interferon, ribavirin and protease inhibitors, has increased to 70% or higher, from 50% with the state-of-care therapy with pegylated interferon and ribavirin [19, 20]. With the advent of new antiviral drugs that will enter the scene in the foreseeable future, the virological response is expected to increase further. There would be

nothing like early detection of HCV and HBV infections for appropriate and timely medical care to prevent the progression of liver disease. Such a rational strategy will benefit not only patients themselves, but also merit the society and government, which are going to be burdened with ever-increasing morbidity and mortality along with skyrocketing costs.

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References

1 World Health Organization: Hepatitis C (Global Alert and Response, 2002). Geneva, WHO, 2002. Updated February 2010 (http://www.who.int/csr/disease/hepatitis/whocdscsrlyo2003/en/index.html).

2 World Health Organization: Hepatitis B (Factsheet No. 204). Geneva, WHO, Revised August 2008 (http://www.who.int/ mediacentre/factsheets/fs204/en/index.html).

3 Lok AS: Chronic hepatitis B. N Engl J Med 2002;346:1682–1683.

4 Seeff LB: Natural history of chronic hepatitis C. Hepatology 2002;36:S35–46.

5 Tanaka J, Kumagai J, Katayama K, Komiya Y, Mizui M, Yamanaka R, Suzuki K, Miyakawa Y, Yoshizawa H: Sex- and age-specific carriers of hepatitis B and C viruses in Japan estimated by the prevalence in the 3,485,648 first-time blood donors during 1995–2000. Intervirology 2004;47:32–40.

6 Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ: The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. Ann Intern Med 2006;144:705–714.

7 Yoshizawa H, Tanaka J: A national project for the management of viral hepatitis toward prevention of hepatocellular carcinoma in Japan; in Morrisey RF (ed): International Kilmer Conference Proceedings. Laval, Polyscience Publications, 2004, vol 8, pp 247–264.

8 Mizui M, Tanaka J, Katayama K, Nakanishi T, Obayashi M, Aimitsu S, Yoshida T, Inoue J, Yokoyama T, Tsuji K, Arataki K, Yamaguchi S, Miura T, Kitamoto M, Takezaki E, Orimen S, Sakata T, Kamada K, Maruhashi A, Tamura T, Nakamura T, Ishida K, Teramen K, Miyakawa Y, Yoshizawa H: Liver disease in hepatitis C virus carriers identified at blood donation and their outcomes with or without interferon treatment: study on 1,019 carriers followed for 5–10 years. Hepatol Res 2007;37:994–1001.

9 Yoshizawa H: Hepatocellular carcinoma associated with hepatitis C virus infection in Japan: projection to other countries in the foreseeable future. Oncology 2002;62(suppl 1):8–17.

10 Tanaka J, Mizui M, Nagakami H, Katayama K, Tabuchi A, Komiya Y, Miyakawa Y, Yoshizawa H: Incidence rates of hepatitis B and C virus infections among blood donors in Hiroshima, Japan, during 10 years from 1994 to 2004. Intervirology 2008;51:33–41.

11 Wong JB, McQuillan GM, McHutchison JG, Poynard T: Estimating future hepatitis C morbidity, mortality, and costs in the United States. Am J Public Health 2000;90:1562–1569.

12 Busch MP, Glynn SA, Stramer SL, Strong DM, Caglioti S, Wright DJ, Pappalardo B, Kleinman SH: A new strategy for estimating risks of transfusion-transmitted viral infections based on rates of detection of recently infected donors. Transfusion 2005;45:254–264.

13 Busch MP, Glynn SA, Wright DJ, Hirschkorn D, Laycock ME, McAuley J, Tu Y, Giachetti C, Gallarda J, Heitman J, Kleinman SH: Relative sensitivities of licensed nucleic acid amplification tests for detection of viremia in early human immunodeficiency virus and hepatitis C virus infection. Transfusion 2005;45:1853–1863.

14 Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K: HBV NAT positive [corrected] blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77–86.

15 Biswas R, Tabor E, Hsia CC, Wright DJ, Laycock ME, Fiebig EW, Peddada L, Smith R, Schreiber GB, Epstein JS, Nemo GJ, Busch MP: Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788–798.

16 Kleinman SH, Busch MP: Assessing the impact of HBV NAT on window period reduction and residual risk. J Clin Virol 2006; 36(suppl 1):S23–S29.

17 Yugi H, Mizui M, Tanaka J, Yoshizawa H: Hepatitis B virus screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing – Japanese experience. J Clin Virol 2006; 36(suppl 1):S56–64.

18 http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/125915222116.

19 Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, Bronowicki JP, Bourliere M, Gharakhanian S, Bengtsson L, McNair L, George S, Kieffer T, Kwong A, Kauffman RS, Alam J, Pawlotsky JM, Zeuzem S: Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839–1850.

20 McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, McNair L, Alam J, Muir AJ: Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360: 1827–1838.

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Interferon Alone or Combined with Ribavirin for Acute Prolonged Infection with Hepatitis C Virus in Chimpanzees

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Abstract
Infection with hepatitis C virus (HCV) persisted for longer than 29 weeks in 2 chimpanzees after they had been inoculated with it experimentally. One of them (C-210) received short-term subcutaneous interferon-α (IFN-α) 6 million units (MU) daily for 7 days at week 29. He cleared HCV RNA from the serum and remained negative for it during 25 weeks after the withdrawal of IFN. The other (C-224) did not respond to 2 courses of a short-term IFN monotherapy at weeks 20 and 23. Twelve weeks thereafter, he received IFN-α 3 MU daily for 2 weeks and then 3 times a week for 14 weeks combined with oral ribavirin 600 mg daily during 16 weeks. HCV RNA disappeared from the serum and stayed negative until the last follow-up 24 weeks after the completion of combination therapy.

Due to a very narrow species-specificity of hepatitis C virus (HCV), chimpanzees remain the only animal that can be infected with it. Once they served as the sole means of identifying the infection with HCV that had been referred to as non-A, non-B hepatitis virus until its discovery in 1989 [1]. HCV infection can persist in chimps at rates ranging from 30 to 60%, depending on the age and gender as well as viral strains in inocula they have received [2, 3]; the persistence rate is comparable to that of 55–85% in humans [4, 5]. The long-term outcome of chimpanzees infected with HCV is not known, nor have there been any attempts to treat them with either interferon (IFN) alone or IFN in combination with ribavirin.

Two chimps with acute prolonged HCV infection received antiviral treatment. They were chimps No. 210 (male, 14 years old and weighing 62.8 kg) and No. 224 (male, 14 years old and weighing 59.1 kg). Both of them were kept in individual cages and received humane care, in accordance with all relevant requirements for the use of primates in an approved facility. Chimp No. 210 participated in the experimental transmission study for determining the minimum infectious dose of HCV [6]. He received 1 ml of fresh-frozen plasma from a donor in the window period of HCV infection with mixed genotypes (1b plus 2a) containing 7.0×10^6 copies/ml of HCV RNA. Chimp No. 224 was inoculated with 1 ml of fresh-frozen plasma from another donor in the window period of HCV infection with genotype 1b containing 8.4×10^6

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