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Human T-Lymphotropic Virus Type-I Infection, Antibody Titers and Cause-specific Mortality among Atomic-bomb Survivors

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There have been few longitudinal studies on the long-term health effects of human T-lymphotropic virus type-I (HTLV-I) infection. The authors performed a cohort study of HTLV-I infection and cause-specific mortality in 3,090 atomic-bomb survivors in Nagasaki, Japan, who were followed from 1985–1987 to 1995. The prevalence of HTLV-I seropositivity in men and women was 99/1,196 (8.3%) and 171/1,894 (9.0%), respectively. During a median follow-up of 8.9 years, 448 deaths occurred. There was one nonfatal case of adult T-cell leukemia/lymphoma (incidence rate=0.46 cases/1,000 person-years; 95% confidence interval [CI] 0.01–2.6). After adjustment for sex, age and other potential confounders, significantly increased risk among HTLV-I carriers was observed for deaths from all causes (rate ratio [RR]=1.41), all cancers (RR=1.64), liver cancer (RR=3.04), and heart diseases (RR=2.22). The association of anti-HTLV-I seropositivity with mortality from all non-neoplastic diseases (RR=1.40) and chronic liver diseases (RR=5.03) was of borderline significance. Possible confounding by blood transfusions and hepatitis C/B (HCV/HBV) viral infections could not be precluded in this study. However, even after liver cancer and chronic liver diseases were excluded, mortality rate was still increased among HTLV-I carriers (RR=1.32, 95% CI 0.99–1.78), especially among those with high antibody titers (RR=1.56, 95% CI 0.99–2.46, *P* for trend=0.04). These findings may support the idea that HTLV-I infection exerts adverse effects on mortality from causes other than adult T-cell leukemia/lymphoma. Further studies on confounding by HCV/HBV infections and the interaction between HCV/HBV and HTLV-I may be required to analyze the increased mortality from liver cancer and chronic liver diseases.

Key words: Cohort study — HTLV-I antibody — Liver neoplasms — Lymphoma — Mortality

Human T-lymphotropic virus type-I (HTLV-I)[1)] is a retrovirus that causes adult T-cell leukemia/lymphoma (ATL)[2)] and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP).[3, 4)] This virus is also an etiologic agent in a variety of chronic inflammatory diseases, such as rheumatoid arthritis, bronchitis, uveitis, polymyositis and Sjögren's syndrome.[5–8)] Production of cytokines by HTLV-I-infected cells is considered to be a biological mechanism involved in the development of these HTLV-I-associated syndromes.[9)] Other diseases or conditions in whose pathogenesis the virus has been suggested or hypothesized to play a role include chronic renal failure, mild suppression of cellular immunity, opportunistic infections, and cancers other than ATL.[8, 10, 11)]

However, most earlier observations on the association between HTLV-I infection and disease were based on case reports and cross-sectional or case-control studies. Few longitudinal studies have investigated the long-term health effects of HTLV-I infection. In our previous cohort study performed at A-Island, an HTLV-I endemic area of Nagasaki, Japan, HTLV-I seropositivity was associated with an approximately 1.8–1.9-fold increased risk of mortality, even after the effect of ATL was excluded.[12)] However, the small number of deaths precluded detailed analysis of cause-specific mortality, and the generalizability of the results remained unclear.

It has been reported that the serum titers of HTLV-I antibodies are closely correlated with the HTLV-I proviral DNA levels in peripheral blood mononuclear cells from asymptomatic HTLV-I carriers.[13, 14)] This suggests that HTLV-I antibody titer can be used as an alternative biomarker in cohort studies of HTLV-I proviral load and the subsequent occurrence of diseases among symptomless HTLV-I-infected individuals.

Matsuo *et al.*[15)] measured the HTLV-I antibody in the Adult Health Study (AHS) sample of atomic-bomb (A-bomb) survivors in Nagasaki between 1985 and 1987 and reported that the virus infection was endemic, with about 9% of the population testing seropositive. The AHS cohort provided an opportunity to investigate the long-

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term health effects of HTLV-I infection because the health status of this population has been examined biennially and vital status has been followed by the Radiation Effects Research Foundation (RERF, formerly the Atomic Bomb Casualty Commission [ABCC]). The purpose of the present study was to investigate longitudinally the association between HTLV-I seropositivity, HTLV-I antibody titer and the risk of cause-specific deaths in the AHS cohort.

MATERIALS AND METHODS

Study population A total of 7,564 subjects (3,374 men and 4,190 women) have received biennial examinations in Nagasaki since 1958 as a part of the ABCC-RERF follow-up program. A detailed description of this program has been published elsewhere.[16] The population for the present study comprised 1,196 men and 1,894 women aged 39–92 years at baseline, who had received AHS examinations and HTLV-I antibody assay during an approximately 2-year period beginning January 1985.

Assay of serum antibody to HTLV-I The indirect immunofluorescence (IF) method was used to detect anti-HTLV-I antibody, using the ATL cell line (MT-1 and MT-2) as antigen cells.[15] Cell smears were treated with diluted human serum at 37°C for 30 min and subsequently with fluorescein isothiocyanate-labeled goat anti-human IgG at 37°C for 30 min. The cell smears were then examined by fluorescence microscopy. The antibody titer was defined as the maximum dilution at which fluorescence-positive cells were identified. The antibody titer could not be measured in one woman.

The current standard for detection of HTLV-I antibodies usually uses at least two assay systems.[17] The IF method is known to have high specificity, but some researchers reported that it may provide a small number of false-negative reactions.[18] On the other hand, an early study using the IF method successfully prevented transfusion-mediated HTLV-I infection in all of the 252 recipients of blood containing cell components from seronegative donors.[19] A validation study also showed that the presence of HTLV-I proviral DNA in peripheral blood mononuclear cells, as detected by the polymerase chain reaction method, was closely correlated with serum HTLV-I antibodies tested by the IF method.[20] Thus, we concluded that any misclassification of HTLV-I carrier status would have been very small and did not pose a problem in this study.

Information on other variables Blood pressure, body mass index, and serum total cholesterol levels were measured at the time of AHS health examinations. Trained nurses asked study participants about smoking habits and alcohol intake. Unfortunately, data on these health habits were not available for 219 subjects who were not interviewed. Therefore, data from the initial survey of the ABCC-RERF follow-up program were used for 181 of these 219 subjects. We could not obtain any information on tobacco and alcohol use for the remaining 38 individuals.

Matsuo *et al.*[15] showed that there was no relationship between the anti-HTLV-I seropositivity and the A-bomb radiation dose (the Dosimetry System 1986) in the AHS cohort. This indicates that radiation dose is not a confounder in the association between HTLV-I infection and mortality, even though it is an independent risk factor of deaths.[21] Thus, the effect of radiation exposure was not controlled in the present study. Additional adjustment for radiation dose produced similar results for all calculations.

Confirmation of end points RERF has followed the vital status of all AHS members using the family registration system of Japan. Follow-up began on the date of the AHS health examination (1985–1987) and ended on the date of death or January 1, 1995, whichever came first. No individuals were lost during the follow-up. Death certificates were collected for all AHS members who had died, and the primary cause of death was classified according to the coding rules of the 9th revision of the International Classification of Diseases (ICD-9). Coders were unaware of serum HTLV-I antibody status. One of the authors (K. A.) reviewed all death certificates for those who had died.

Cases of ATL were identified by periodic health examinations and by checking the data of the Nagasaki Cancer Registry. Subjects were considered as having ATL when they satisfied the following four conditions: clinical diagnosis of ATL or malignant lymphoma with T-cell surface marker; seropositivity to anti-HTLV-I antibody; presence of atypical lymphoid cells in peripheral blood or lymphoid tissues; and exclusion of pre- or smoldering ATL.

Statistical analysis The statistical significance of the difference in the baseline characteristics between HTLV-I carriers and noncarriers was assessed by Wilcoxon's rank sum test, Student's t test, or the χ^2 test. The exact confidence interval for ATL incidence was calculated using a Poisson distribution. Time-to-event models were used to estimate the relative hazards and their 95% confidence intervals (CI) for cause-specific deaths associated with HTLV-I infection.[22] Proportionality was checked by assessing the significance of the interaction between HTLV-I seropositivity and log (time). Deaths from other causes were treated as censored cases. The potential confounding effects of sex, age (continuous), blood pressure (normal, borderline hypertension, established hypertension), serum cholesterol levels (<150, 150–219, ≥220 mg/dl), and smoking and drinking habits (nonsmoker and nondrinker, current or exsmoker and nondrinker, nonsmoker and current or exdrinker, current or exsmoker and current or exdrinker, missing) were adjusted by including

indicator variables as covariates, when considered necessary. Subjects with missing data on smoking and drinking habits were not excluded to avoid the loss of generalizability. The statistical significance of the sex difference in hazard ratios (HR) associated with HTLV-I seropositivity was assessed by incorporating a product term in the model. All statistical analyses were performed using SAS software (version 6.12),[23] and all *P*-values reported are two-tailed.

RESULTS

Table I shows the baseline study population characteristics according to sex and serum HTLV-I antibody status. The prevalence of HTLV-I seropositivity among men and women was 99/1,196 (8.3%) and 171/1,894 (9.0%), respectively. The age at baseline was somewhat higher among HTLV-I carriers than noncarriers, with a statistically significant difference in men. In men, HTLV-I carriers had significantly higher systolic blood pressure and significantly lower serum total cholesterol levels than did noncarriers. However, when the effect of age was adjusted, the significant difference in systolic blood pressure disappeared. In women, seropositive individuals had significantly lower serum cholesterol levels than seronegative persons. In both sexes, the proportion of current or exsmokers was higher among carriers than among noncarriers. In men, the proportion of current or exdrinkers was lower among seropositives. The association between HTLV-I seropositivity and lower serum total cholesterol

Table I. Baseline Characteristics of Study Population according to Serum HTLV-I Antibody Status

	Noncarriers		Carriers		*P*-value	
	Men	Women	Men	Women	Men	Women
Total No.	1,097	1,723	99	171		
Age at baseline (years)						
Median (range)	57 (39–92)	58 (39–90)	60 (46–87)	59 (40–92)	0.01[a]	0.21[a]
30–39 (%)	2 (0.2)	2 (0.1)	0 (0.0)	0 (0.0)		
40–49	189 (17.2)	207 (12.0)	5 (5.1)	21 (12.3)		
50–59	457 (41.7)	766 (44.5)	43 (43.4)	67 (39.2)		
60–69	198 (18.0)	471 (27.3)	25 (25.3)	45 (26.3)		
70–79	196 (17.9)	195 (11.3)	15 (15.2)	25 (14.6)		
80–89	50 (4.6)	79 (4.6)	11 (11.1)	11 (6.4)		
90–99	5 (0.5)	3 (0.2)	0 (0.0)	2 (1.2)		
Systolic blood pressure (mmHg)						
Mean (SD)	139 (23)	138 (24)	143 (23)	140 (25)	0.05[b]	0.48[b]
Unknown (%)	0 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)		
Diastolic blood pressure (mmHg)						
Mean (SD)	85 (12)	84 (12)	86 (13)	83 (12)	0.62[b]	0.54[b]
Unknown (%)	2 (0.2)	2 (0.1)	0 (0.0)	0 (0.0)		
Body mass index (kg/m^2)						
Mean (SD)	22.0 (2.9)	22.9 (3.3)	22.1 (2.2)	22.8 (3.7)	0.57[b]	0.80[b]
Unknown (%)	13 (1.2)	26 (1.5)	4 (4.0)	4 (2.3)		
Serum total cholesterol (mg/dl)						
Mean (SD)	183 (32)	204 (35)	174 (32)	197 (33)	<0.01[b]	0.02[b]
Unknown (%)	1 (0.1)	1 (0.1)	0 (0.0)	0 (0.0)		
Smoking status (%)						
Nonsmoker	274 (25.0)	1,524 (88.5)	16 (16.2)	137 (80.1)	0.08[c]	<0.01[c]
Current or Exsmoker	813 (74.1)	175 (10.2)	83 (83.8)	30 (17.5)		
Unknown	10 (0.9)	24 (1.4)	0 (0.0)	4 (2.3)		
Drinking status (%)						
Nondrinker	298 (27.2)	1,428 (82.9)	37 (37.4)	135 (78.9)	0.07[c]	0.35[c]
Current or Exdrinker	789 (71.9)	271 (15.7)	62 (62.6)	32 (18.7)		
Unknown	10 (0.9)	24 (1.4)	0 (0.0)	4 (2.3)		

a) By Wilcoxon's rank sum test.
b) By Student's *t* test.
c) By χ^2 test.

levels remained statistically significant even after adjustment for age and smoking and drinking habits in both men and women.

During a median follow-up of 8.9 years (25,913 person-years), 232 men (19.4%) and 216 women (11.4%) died (Table II). Of these 448 deaths, 162 were attributed to neoplasms, 274 to non-neoplastic diseases, and 11 to injuries or poisonings. The three most frequent causes of cancer deaths were gastric cancer (*N*=34), lung cancer (*N*=27), and liver cancer (*N*=24). There were seven deaths

Table II. Causes of Death according to HTLV-I Antibody Status

Causes of deaths (ICD-9)	Men		Women	
	Noncarriers	Carriers	Noncarriers	Carriers
All deaths	201	31	188	28
All neoplasms (140–239)	74	11	66	11
Gastric cancer (151)	20	3	11	0
Liver cancer (155)	14	3	5	2
Lung cancer (162)	14	0	12	1
Breast cancer (174–175)	0	0	6	1
Malignant lymphoma excluding ATL (200–202)	1	0	0	0
Other neoplasms	25	5	32	7
All non-neoplastic diseases	120	20	117	17
Infectious diseases (001–139)	5	0	5	0
Heart diseases (390–429)	30	7	38	10
Cerebrovascular diseases (430–438)	31	3	30	3
Respiratory diseases (460–519)	35	6	18	0
Chronic liver diseases (571)	3	2	2	0
Renal diseases (580–589)	3	1	5	0
Other non-neoplastic diseases	13	1	19	4
Injury or poisonings (800–999)	7	0	4	0
Unknown	0	0	1	0

Table III. Age-adjusted Relative Hazards of Deaths Associated with HTLV-I Infection, according to Sex and Antibody Titer

	Person-years (No. of events)	HR	95% CI	*P*-value	
Men					
Noncarriers	8,965 (201)	1.0			
Carriers (total)	747 (31)	1.53	1.05–2.23	0.03	
0<titer<320	473 (20)	1.32	0.83–2.10	0.23	
titer≥320	274 (11)	2.11	1.15–3.89	0.02	*P* for trend=0.03
Women					
Noncarriers	14,765 (188)	1.0			
Carriers (total)	1,436 (28)[a)]	1.41	0.95–2.10	0.09	
0<titer<320	869 (15)	1.23	0.72–2.08	0.45	
titer≥320	556 (13)	1.71	0.97–2.99	0.06	*P* for trend=0.04
Total					
Noncarriers	23,730 (389)	1.0			
Carriers (total)	2,183 (59)[a)]	1.47[b)]	1.12–1.93	0.006	
0<titer<320	1,342 (35)	1.26[b)]	0.89–1.79	0.19	
titer≥320	831 (24)	1.92[b)]	1.27–2.90	0.002	*P* for trend=0.003

a) Antibody titer was missing in one subject.
b) Adjusted for sex and age at baseline.

from breast cancer among women. Of 274 deaths due to non-neoplastic diseases, 85 were from heart diseases, 67 from cerebrovascular diseases, and 59 from respiratory diseases. Ten subjects died from infectious diseases, seven from chronic liver diseases and nine from renal diseases. The cause of death was not known for one woman.

Among HTLV-I carriers, there was one nonfatal case of ATL (male, 56 years old at baseline, antibody titer=160). By using the number of cases and the person-years of follow-up, the crude incidence rate of ATL was estimated at 1/2,183=0.46 cases/1,000 person-years (95% CI 0.01–2.6), which was in agreement with those reported previously (0.58–1.50/1,000 a year).[17] There were no deaths attributed to HAM/TSP.

After adjustment for age at baseline, the relative hazard of all-cause mortality associated with HTLV-I infection was 1.53 (95% CI 1.05–2.23) for men and 1.41 (95% CI 0.95–2.10) for women, with no significant sex difference in the age-adjusted hazard ratios (Table III). When both sexes were combined, HTLV-I seropositivity was associated with a 1.47-fold increased risk of total mortality (95% CI 1.12–1.93). The linear trend between the logarithm of the antibody titer and the mortality rate was statistically significant (P=0.003), suggesting that the relative

Table IV. Relative Hazards of Cause-specific Deaths Associated with HTLV-I Seropositivity

	No. of events	HR[a)]	95% CI	*P*-value	No. of events	HR	95% CI	*P*-value
All deaths	448	1.47	1.12–1.93	0.006	445	1.41[b)]	1.07–1.86	0.01
All neoplasms	162	1.62	1.03–2.53	0.04	162	1.64[c)]	1.04–2.57	0.03
Gastric cancer	34	1.01	0.31–3.32	0.98	34	0.96[c)]	0.29–3.16	0.95
Liver cancer	24	3.01	1.12–8.08	0.03	24	3.04[c)]	1.12–8.24	0.03
Lung cancer	27	0.39	0.05–2.85	0.35	27	0.38[c)]	0.05–2.84	0.35
Breast cancer (women)	7	1.77	0.21–14.7	0.60	7	1.73[d)]	0.21–14.5	0.61
All non-neoplastic diseases	274	1.44	1.02–2.03	0.04	273	1.40[b)]	0.98–1.99	0.06
Heart diseases	85	2.27	1.33–3.87	0.003	85	2.22[b)]	1.29–3.82	0.004
Cerebrovascular diseases	67	0.93	0.40–2.16	0.87	67	0.83[b)]	0.35–1.93	0.66
Respiratory diseases	59	1.00	0.43–2.34	1.00	59	1.08[c)]	0.46–2.53	0.86
Chronic liver diseases	7	4.49	0.87–23.2	0.07	7	5.03[c)]	0.95–26.6	0.06
Injuries or poisonings	11	0.00			11	0.00		

a) Adjusted for sex and age at baseline.
b) Adjusted for sex, age at baseline, blood pressure, serum cholesterol levels, and smoking and drinking habits. Six subjects with missing data on blood pressure or serum cholesterol levels were excluded.
c) Adjusted for sex, age at baseline, and smoking and drinking habits.
d) Adjusted for age at baseline, and smoking and drinking habits.

Table V. Relative Hazards of Cause-specific Deaths according to HTLV-I Antibody Titer[a)]

	No. of events	Titer<320		Titer≥320		
		HR	95% CI	HR	95% CI	*P* for trend
All deaths	445	1.25	0.88–1.78	1.74	1.14–2.64	0.009
All deaths[b)]	414	1.21	0.84–1.74	1.56	0.99–2.46	0.04
All neoplasms	162	1.60	0.92–2.77	1.72	0.84–3.51	0.04
All neoplasms[c)]	138	1.43	0.77–2.65	1.49	0.65–3.39	0.16
All non-neoplastic diseases	273	1.15	0.73–1.81	1.95	1.16–3.26	0.03
All non-neoplastic diseases[d)]	266	1.17	0.75–1.84	1.74	1.01–3.02	0.06
Heart diseases	85	2.14	1.12–4.10	2.38	1.02–5.57	0.003

a) Reference category was seronegative subjects. Numbers of subjects and covariates included in the model were the same as those in the right half of Table IV, except that one woman with missing information on antibody titer was excluded.
b) Deaths from liver cancer and chronic liver diseases were excluded.
c) Deaths from liver cancer were excluded.
d) Deaths from chronic liver diseases were excluded.

risk of death increased as the HTLV-I antibody titer increased.

Table IV shows the relative hazards of cause-specific deaths associated with HTLV-I seropositivity. Since the hazard ratios were not heterogeneous between men and women, both sexes were combined in all subsequent analyses. After adjustment for sex and age, a significant or marginally significant increase in risk was found for deaths from all causes, all cancers, liver cancer, all non-neoplastic diseases, heart diseases, and chronic liver diseases.

To exclude potential confounding by other variables, we controlled for blood pressure, serum levels of total cholesterol, and smoking and drinking habits when considered necessary. Significantly increased risks were observed for deaths from all causes (HR 1.41; 95% CI 1.07–1.86), all cancers (HR 1.64; 95% CI 1.04–2.57), liver cancer (HR 3.04; 95% CI 1.12–8.24), and heart diseases (HR 2.22; 95% CI 1.29–3.82). This high mortality from heart diseases resulted mainly from diseases other than heart failure. The association of anti-HTLV-I seropositivity with mortality from all non-neoplastic diseases (HR 1.40; 95% CI 0.98–1.99) and chronic liver diseases (HR 5.03; 95% CI 0.95–26.6) was of borderline significance.

Table V presents the relative hazards of cause-specific deaths according to the HTLV-I antibody titer. There was a significant dose-response relationship between the logarithm of the antibody titer and mortality from all causes (P=0.009), all cancers (P=0.04), all non-neoplastic diseases (P=0.03) and heart diseases (P=0.003). After excluding deaths from liver cancer and chronic liver diseases, mortality rate was still increased among HTLV-I carriers (HR=1.32, 95% CI 0.99–1.78), in particular among those with high antibody titers (HR=1.56, 95% CI 0.99–2.46, P for trend=0.04). However, the association between the serum titer of HTLV-I antibody and deaths from neoplasms was no longer significant (P=0.16).

DISCUSSION

The prevalence of HTLV-I seropositivity in this study population was 8–9%, a percentage similar to that of blood donors in Nagasaki City reported during the early 1980's (40–64 years, 9%).[24] There was no significant sex difference in HTLV-I seropositivity. This result was somewhat different from that reported for other HTLV-I endemic areas with higher seroprevalences.[25] In general, the HTLV-I seropositivity is higher in women than in men over 50 years of age, and this is explained by the more frequent occurrence of sexual transmission from male to female than *vice versa*.[25] In both sexes, seropositive persons had significantly lower mean serum total cholesterol levels than seronegative persons. One explanation for this may be transactivation of the interleukin-2 (IL-2) gene by the Tax protein encoded by HTLV-I, resulting in increased circulating IL-2 levels among HTLV-I carriers.[26] IL-2 is known to decrease serum levels of HDL- and LDL-cholesterol.[27] In other HTLV-I infected populations, a higher prevalence of mild anemia[28] and lower body mass index[29] have been reported. However, there was no significant difference in body mass index according to HTLV-I seropositivity in the present study population.

Mortality rates from liver cancer and chronic liver diseases were increased among HTLV-I carriers. One explanation for this result is the confounding by hepatitis C virus (HCV) and, to a lesser extent, hepatitis B virus (HBV) infections. Both of these hepatotropic viruses are strong risk factors for chronic hepatitis, liver cirrhosis, and liver cancer, and share similar routes of infection with HTLV-I, such as blood transfusion and mother-to-child transmission.[30] Kamihira *et al*.[31] reported that anti-HCV seropositivity in HTLV-I carriers was 1.7 times higher than that in noncarriers (1.9% vs. 1.1%) among blood donors in Nagasaki City. Another possible explanation is the interaction between HCV/HBV and HTLV-I. Okayama *et al*.[32] noted that HTLV-I seropositivity of male patients with HCV-associated hepatocellular carcinoma in Miyazaki Prefecture was significantly higher than that of patients with HCV-chronic hepatitis (odds ratio=12.8), suggesting that HTLV-I played a role in the evolution from chronic hepatitis to hepatocellular carcinoma. An experimental study also showed that an HTLV-I infected T-cell line (MT-2) was fairly susceptible to HCV infection.[33] However, because anti-HCV antibodies and HBV surface antigens were not determined for every subject, we could not directly examine the confounding by HCV/HBV or the interaction between HCV/HBV and HTLV-I in the present study.

There was a significantly increased risk of death from heart diseases among HTLV-I carriers. It is intriguing that higher proportions of those who had histories of cardiac diseases and abnormal findings of electrocardiograms have been reported among HTLV-I carriers in the Miyazaki Cohort Study.[34] We also investigated the history and the prevalence of heart diseases using data of the 1985–1987 AHS examinations. The prevalence of arrhythmia was somewhat higher (1.1% vs. 0.4%) among HTLV-I carriers (unpublished data). However, we could not find any plausible biological explanation for the strong association between HTLV-I seropositivity and increased mortality from heart diseases. We believe that our results should not be overinterpreted and require further investigations.

Several limitations of this study should be addressed. First, we used old information on smoking and drinking habits collected at the initial survey of the ABCC-RERF follow-up program for approximately 6% of the

study population. The ϕ correlation coefficients between repeated assessments, made twice at the initial survey of the ABCC-RERF program and the recent AHS health examinations, were 0.67 (N=398) for smoking and 0.55 (N=390) for alcohol consumption. Therefore, there may have been some random misclassification of these health habits. In addition, information on smoking and drinking habits used in this study was rather crude. Since nondifferential misclassification of a confounding variable leads to inadequate control of confounding,[35] it is possible that the influence of smoking and drinking habits could not be completely removed even after statistical adjustment. However, the residual confounding may not be strong, because the relations of HTLV-I seropositivity to smoking and drinking habits were rather weak in this study population. Second, disease diagnosis was based on death certificates. A validation study using autopsy cases of A-bomb survivors showed that the sensitivity and the positive predictive value are generally high for neoplasms, but low for non-neoplastic diseases.[36] However, misclassification of causes of death may not be serious when broad categories such as all neoplasms, all non-neoplastic diseases, and external causes are used. It should also be noted that the misclassification of causes of death is independent of the HTLV-I seropositivity. Therefore, the direction of the effect would be to bias the relative risks of cause-specific deaths toward that of all cause mortality. Furthermore, it is unlikely that deaths from ATL were misclassified as liver or cardiac diseases, since incident cases of ATL were identified by checking the data of the Nagasaki Cancer Registry. Third, we could not eliminate the possible confounding by blood transfusions. HTLV-I is transmitted by transfusions of contaminated cellular components[19] and many other pathogens can also be transmitted through transfusions. In this study, mortality rates among seropositive subjects were increased even after excluding deaths from liver cancer and chronic liver diseases, which are closely linked to HCV and HBV infections. However, the possibility still remains that some portion of excess risk of deaths observed among HTLV-I carriers resulted from infection with blood-borne pathogens other than HTLV-I. Moreover, the general health status of subjects who have received blood transfusions may be poorer than that of those who have not. This possible confounding by transfusions could have been controlled through restriction of study subjects or statistical modelling. Unfortunately, however, the history of transfusions at baseline was not available for every subject. Fourth, it is possible that the ecological and/or genetic backgrounds of HTLV-I seropositive subjects differ from those of seronegative persons. Generally, HTLV-I endemic foci are well preserved in very isolated villages and islands even within the Kyushu district,[25] and some fraction of HTLV-I carriers in Nagasaki City may have originated from such isolated areas. If such ecological/genetic differences were closely associated with occurrence of diseases, they might become a source of bias.

Despite the above limitations, the present results were in agreement with those of our previous study in A-Island in Nagasaki Prefecture, where blood transfusion was not a major route of HTLV-I infection and liver cancer accounted for only 1.7% of all deaths.[12] In addition, it is noteworthy that there was a significant dose-response relationship between the antibody titer, a correlate of HTLV-I proviral load,[13, 14] and mortality rate. The findings from the present study may support the idea that HTLV-I infection exerts an adverse effect on mortality which is greater than that caused only by the development of ATL. Further studies on confounding by HCV/HBV infections and the interaction between HCV/HBV and HTLV-I may be required to analyze the increased mortality from liver cancer and chronic liver diseases.

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