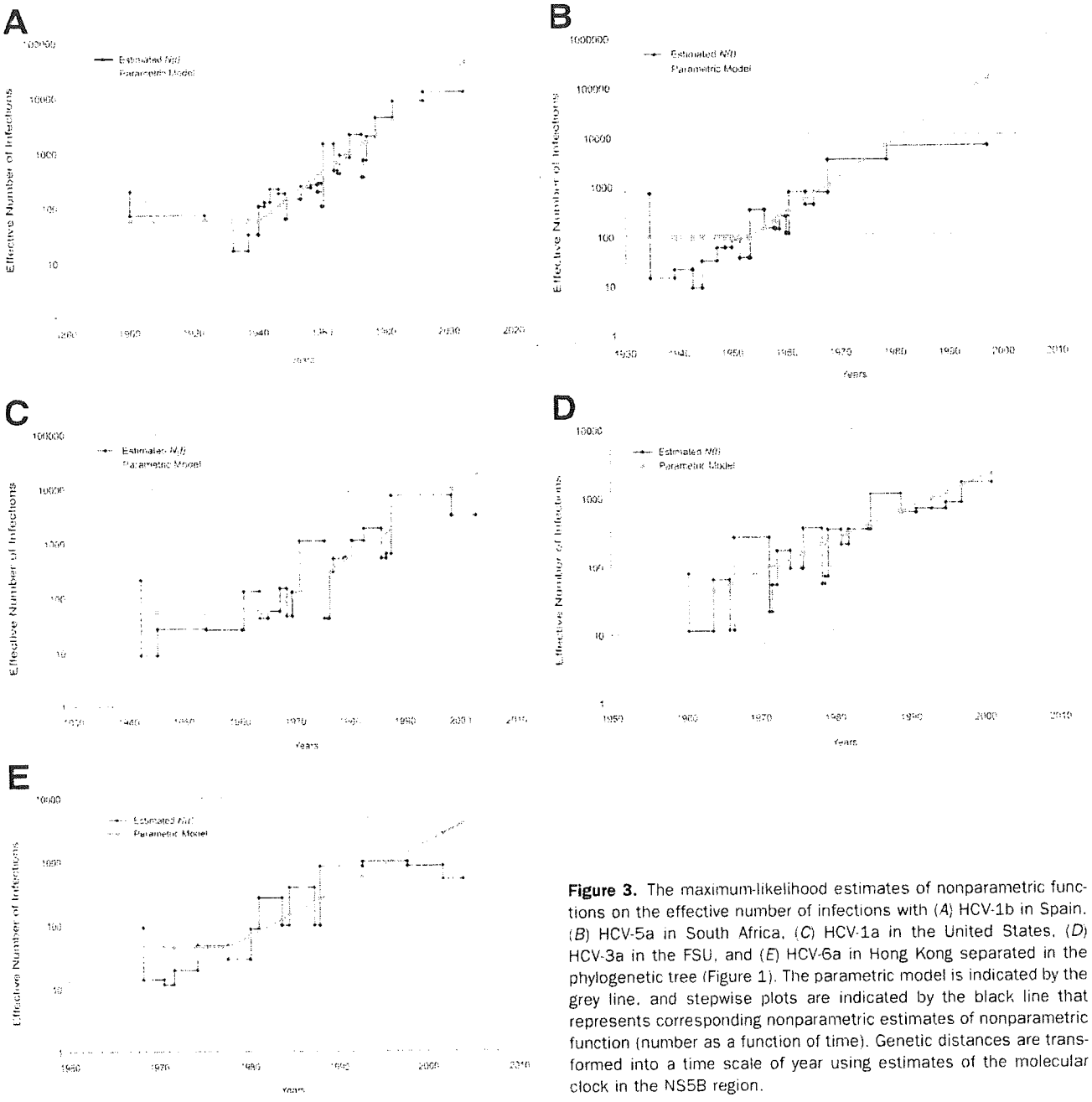

Figure 3. The maximum-likelihood estimates of nonparametric functions on the effective number of infections with (*A*) HCV-1b in Spain, (*B*) HCV-5a in South Africa, (*C*) HCV-1a in the United States, (*D*) HCV-3a in the FSU, and (*E*) HCV-6a in Hong Kong separated in the phylogenetic tree (Figure 1). The parametric model is indicated by the grey line, and stepwise plots are indicated by the black line that represents corresponding nonparametric estimates of nonparametric function (number as a function of time). Genetic distances are transformed into a time scale of year using estimates of the molecular clock in the NS5B region.

genetic model called *the coalescent theory*.[21] The coalescent framework requires a demographic model, denoted $N(t)$, that describes the effective population size through time. A demographic model based on neutral theory, which infers that a constant-size population in the past changes to grow exponentially starting at a specific point in time,[13,24,25] was applied to investigate the HCV population history worldwide. Various HCV genotypes and subtypes circulating in the geographic regions were studied. However, in each country we selected a single subtype representative of the subpopulation conforming to the largest indigenous phylogenetic cluster; this cluster would play an important role in the particular epidemic network (see later), and at the same time fulfill the requirements for calculations by the methods used.[13,14]

In Japan, HCC incidence is exceedingly high and comparatively well studied. Chronic HCV infection is responsible for the majority of HCC cases in Japan even though the overall HCV seroprevalence is relatively low at 1.4%. Notably, however, the highest incidence of HCC occurs in persons older than age 70 (>150 per 100,000 men), in whom HCV prevalence is correspond-

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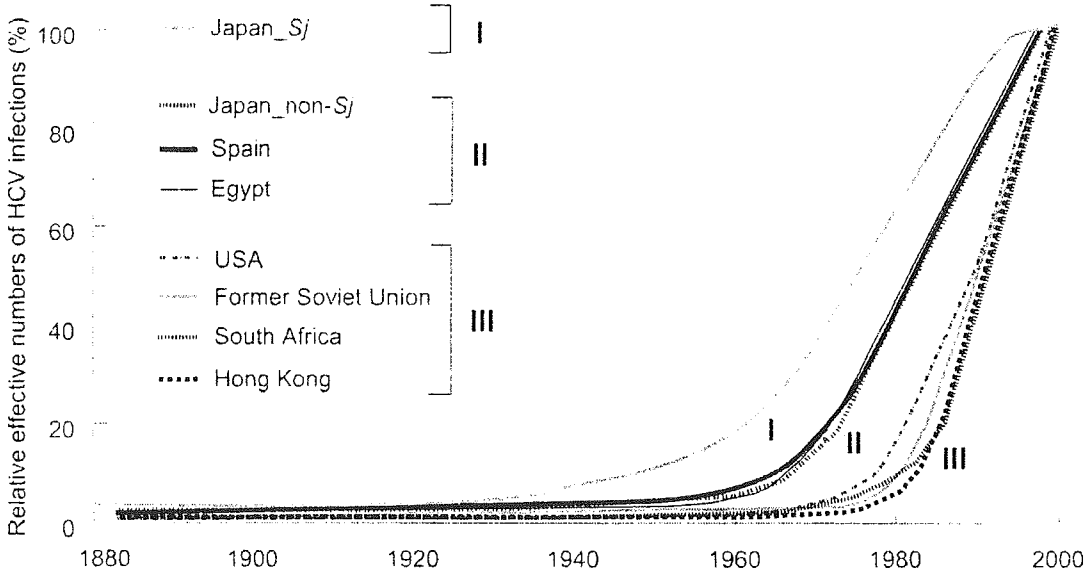

Figure 4. Relative effective numbers of HCV infections in each country. Three different growth patterns were defined as I, II, and III.

ingly high at approximately 7%.[32] HCV-1b is the predominant genotype in Japan and 2 relatively distinctive waves of its spread were described recently in detail.[15] Briefly, the first wave was associated with treatments for *Sj* beginning in 1921,[33] and the second wave coincided with World War II (1940s) when war-associated injection drug use (IDU), blood transfusions, and medical procedures intensified and contributed to HCV transmission.[15,32,34]

In Europe, HCC mortality rates are highly variable in different countries,[35] and a positive correlation with HCV seroprevalence was documented recently (summarized in Figure 5).[29] Detailed data were obtained in France using a model based on epidemiologic analyses of HCV-infected patients and mortality data from national statistics surveys that allowed tracing of the HCV epidemic back to the 1940s.[27] These data were similar to our results estimated for the epidemic in Spain, where exponential growth of the virus population began after 1940. These data are consistent with the likelihood of HCV transmission during and after the Spanish Civil War in the late 1930s, and with the widespread use of shared needles for penicillin treatments in the early 1940s. In Italy, as in Japan, the prevalence of anti-HCV was the highest among elderly people (age, 75–79 y) suggesting a cohort effect dating to exposure during World War II.[28]

Indeed, in the United States, prevalent subtypes in the general population are HCV-1a (57%) and 1b (17%)[36]; an association of the HCV-1a epidemic with IDU has been reported.[37] Our estimates of the epidemic history of HCV-1a in the United States are consistent with the onset of injection opiate use between 1950 and 1960 and more widespread use in the late 1960s and 1970s.[38] The relative importance of IDU and blood transfusion associated with HCV transmission in the United States has changed over time,[39] and IDU has been the predominant mode since the 1970s.[40]

HCV-1a and 3a are the most prevalent among patients with a history of IDU and appear to be increasing in prevalence worldwide.[10,16,41] In the FSU, HCV-1b was predominant in all population groups until the late 1970s, and was associated primarily with medical procedures and unscreened blood products during and after World War II. After blood screening started in the 1990s, an HCV genotypic shift occurred first in the drug-addicted population and, more recently, has been observed in the general population (Musabaev EI, personal communication). HCV-3a represents a comparatively recent and growing epidemic associated with IDU in the FSU.[16,41] Our estimates traced that HCV-3a spread from the 1960s, coinciding with the start of mass vaccination campaigns against measles when there were no disposable syringes in the FSU. However, a rapid increase of the estimated viral population size started in the late 1970s, probably associated with the expanded IDU network that was stimulated dramatically by the Afghan War (Ruzibakiev R, personal communication, web information-analytical recourses: http://druglibrary.org/ and http://www.irinnews.org/).

Similar data were obtained from Hong Kong where 2 relatively distinct HCV epidemics were observed: HCV-1b was predominant among older patients with chronic hepatitis whereas HCV-6 was detected predominantly in

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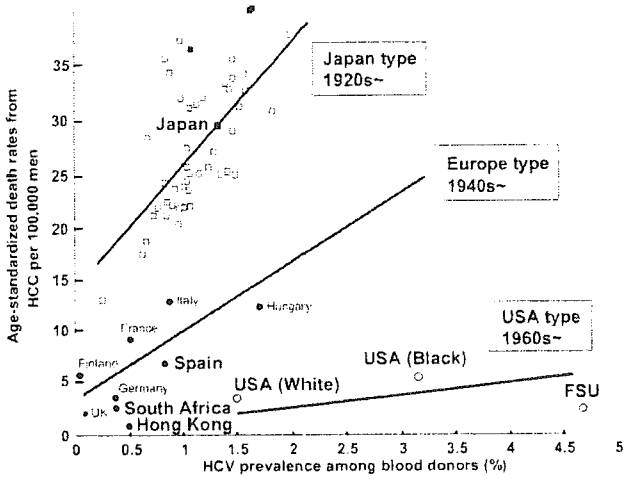

Figure 5. Linear relationship between HCV seroprevalence and HCC annual mortality rates constructed using available data from different countries. Three different patterns were observed: Japan type, European type, and the USA type. Data from European countries were obtained from a previous report.[29] Japan: HCV seroprevalence was approximately 1.4% in the general population from 1988 to 1992. The age-adjusted mortality rate of HCC among Japanese men was approximately 30 per 100,000 during 1990 (■),[32] which is close to the mean age-adjusted death rates of HCC among the 48 prefectures (27.3 per 100,000 persons in 2001). □ and ■, (S)-endemic areas) show the relationship between the age-adjusted annual mortality rates of primary liver cancer and anti-HCV among the general population older than 40 years of age in 2002 and a positive significant correlation was found.[50] Spain: data were obtained mainly from a previous European study,[29] which was consistent with another recent study performed in Valencia (Spain) (2,172,796 inhabitants in 1998: 1,060,156 males and 1,112,640 females) in 2000[51]; the estimated incidence of HCC was 8.2 cases per 100,000 inhabitants. United States: HCV seroprevalence was 3.2% among non-Hispanic blacks and 1.5% among non-Hispanic whites from 1988 to 1994.[36] The age-adjusted mortality rate of HCC among black men was 6.0 per 100,000 in 1991–1995, and 3.4 per 100,000 among white men.[52] FSU: HCV seroprevalence was approximately 5% in blood donors, and the HCV-related HCC mortality rate was estimated at approximately 2.5 per 100,000 men in 1990 (Ruzibakiev R and Musabaev M, personal communication). South Africa: The prevalence of HCV infection among blood donors was .41% during 1992 and 1994.[53] The HCV prevalence was .75% among blacks and .16% among non-Hispanic whites.[54] The age-adjusted incidence of HCC was approximately 30 per 100,000,[47] HCV-related HCC was approximately 10%, indicating 3 per 100,000 from HCV-related HCC. Hong Kong: the prevalence of HCV was .5% in the general population, and the estimated HCV-related HCC mortality was much rarer than HBV-related HCC mortality.[42,47]

younger patients with a history of IDU and in young patients with thalassemia major.[12] Age-related prevalence and results of our estimation suggested that HCV-6 infection represents a recent growing epidemic that will have an increasing influence on HCC incidence in the future.

In South Africa (sub-Saharan Africa) and Hong Kong, hepatitis B virus (HBV) infection is much more prevalent than HCV infection and the epidemiology of HCV infection is less well characterized. Our results indicate that both genetic diversity and the growth rate of the HCV-infected population in South Africa are higher than would be expected. This may be explained by the fact that most studied samples were obtained from elder patients in this region, including 8 of 24 patients with HCC (all male blacks; mean age, 65.3 ± 3.3 y). The major risk factors for HCV transmission in South Africa include contaminated blood transfusions and medical procedures performed with inadequately sterilized shared instruments.

In analyzing data on the association between HCC mortality and HCV seroprevalence, 3 general patterns were observed (Figure 5). The first pattern was observed in Japan (Japan type), where the data presented from all geographic parts of the country indicated that HCC mortality was the highest in the world, whereas HCV seroprevalence was comparatively low. The second pattern was observed in European countries (Europe type), where the HCC incidence had a more direct association with HCV prevalence as previously reported,[29] although recent reports in some European countries such as Spain indicated that HCC mortality in men older than 60 years overlapped with the high mortality rate observed in Japan.[45,14] The third pattern was observed in the United States (≈5 million HCV-infected) and the FSU (USA type) with HCV seroprevalence comparable with Japan, but low HCC mortality rates. The greatest progression to HCC in Japan, indicated by the steep ascent in Figure 5, is consistent with the previously reported high annual incidence of HCC (7.9%) among patients with stage F4 fibrosis.[15] Because the HCV epidemic in Japan began early, resulting in a large cohort with a very long duration of infection, more patients in Japan have reached the stage of advanced fibrosis that increases their likelihood of developing HCC. Thus, the slope of the curve is influenced strongly by the age-specific prevalence and the duration of HCV infection. This would predict that as age and the duration of infection increases in other populations, including the United States, the slope of the HCC mortality curve will increase steeply. The patterns observed in South Africa and Hong Kong, characterized by comparatively low HCV prevalence and low HCV-related HCC mortality, were intermediate between that in Europe and the United States. However, precise data regarding the association of HCV with HCC incidence are not available in these regions, and most cases of HCC in Hong Kong and South Africa probably were associated with HBV infection, which is highly prevalent in these countries.[16,17] A total of 80.3% and 62.7%–70.5% were positive for hepatitis B surface antigen in Hong Kong and South Africa, respectively, whereas a relatively low prevalence of HBV-related HCC (≈20%) was reported in the United States, Europe, and Japan.[18] The relative

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estimated viral population growth dynamics obtained in different countries are shown in Figure 4. All estimated data were separated into 3 groups according to the time virus exponential spread began: the first rapid spread was associated with schistosomiasis treatments in Japan (*Sj* group) in the 1920s; the second wave occurred in Japan (non-*Sj* group), Spain, and Egypt in the 1940s; and the third wave occurred in the 1960s involving the United States, the FSU, South Africa, and Hong Kong. When we combine these data (Figures 4 and 5), a putative picture of the global HCV epidemic emerges that potentially would allow predictions of HCC dynamics in any region of the world. However, no data on HCC mortality were available from Egypt, where the high general HCV prevalence and the estimated spread time suggest that Egypt might have a very high HCC mortality rate, comparable with that in Japan. A recent report from Egypt showing a high HCC incidence among chronic liver disease patients (4.7%)[19] is consistent with our hypothesis.

It could be argued that our results may not represent the community distribution of HCV strains because of the vast predominance of tertiary institution referral. However, we found no significant difference in the sequences of HCV isolated from blood donors and patients with chronic liver diseases, indicating that little bias would be expected to occur in our molecular evolutionary analyses. Hence, an advantage of the coalescent approach to molecular epidemiology used here is that the entire history of a transmission cluster can be investigated using a relatively small sample of gene sequences. In addition, this approach allows a more complete analysis of global HCC dynamics and a prediction of HCC occurrence rates over time. The implications are that Japan has set the model for HCV-related HCC and that the high HCC incidence in Japan may be replicated by the rest of the world as their HCV-infected population ages and the duration of HCV infection approaches that currently observed in Japan. Clearly, there is a need not only to prevent new HCV infections, but also to eradicate chronic infections with appropriate treatment strategies. Unfortunately, the current costs of antiviral therapy are prohibitive for many of the regions where HCC is likely to escalate in the future. High priority must be given to global prevention of HCV-related cirrhosis, the almost universal predecessor to HCV-induced HCC.

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Exacerbation of oral erosive lichen planus by combination of interferon and ribavirin therapy for chronic hepatitis C

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Abstract. Hepatitis C virus (HCV) induces extrahepatic manifestations such as oral lichen planus (OLP) as well as chronic liver diseases. The treatment of HCV-related chronic liver disease has evolved from the use of a single agent, mainly interferon (IFN), to the combination of IFN and ribavirin. We present a case of erosive OLP, cutaneous lichen planus (CLP), and leukoplakia of the vocal cord in a man with chronic hepatitis C infection treated with IFN and ribavirin. A 65-year-old man suffered from OLP before undergoing combination of IFN and ribavirin therapy for chronic hepatitis C. He was initially treated with IFNß (6 million units (MU) /day for 2 weeks), then a combination of IFNα-2b (6 MU/day for 2 weeks and 3 times a week for 14 weeks) and ribavirin (400-600 mg/day). The OLP lesion was not aggravated by application of steroids during the 7 weeks after the treatment, but after 18 weeks, the combination of IFN and ribavirin was stopped because of aggravation of the OLP. Elevated aminotransferase levels returned to normal during the therapy. But 7 weeks after discontinuation, aminotransferase levels rose to 10 times the normal range. Five months after discontinuation, the papules of CLP appeared. Eight months after discontinuation, the OLP erosion had gradually reduced, but some erosion remained. Aminotransferase levels were decreased, but serum HCV RNA had not disappeared. Caution should be exercised when IFN or ribavirin therapy is given to chronic hepatitis C patients with prior erosive OLP.

Introduction

It is thought that about 170 million people are infected with hepatitis C virus (HCV) worldwide, and 2 million people in Japan (1,2). HCV is recognized as a main major threat to global public health. HCV is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC) (3,4). Moreover, HCV induces extrahepatic manifestations such as oral lichen planus (OLP) as well as chronic liver diseases (5,6). In some patients, treatment with a single agent, mainly interferon (IFN), or with a combination of IFN and ribavirin, leads to sustained eradication of the HCV (7). As regards the effects of IFN therapy on lichen planus (LP) lesions, there are several reports (8-22). We report the course of a patient with chronic hepatitis C who experienced the exacerbation of a severe OLP by combination therapy of IFN and ribavirin.

Materials and methods

On February 18, 2003, a 65-year-old Japanese man consulted the Digestive Disease Center of Kurume University for examination of chronic liver disease. He had chronic hepatitis at age 49, and had periodical blood tests and abdominal ultrasound exams by a family doctor, but did not receive treatment. Concerning his past history, the patient underwent an appendectomy at age 23. At ages 37 and 47, the patient underwent hemorrhoidectomy. Hypertension was noted at age 50, and antihypertensive treatment was started. There was no habitual alcohol drinking or smoking. The patient had discontinued smoking 13 years prior to presentation. There was no history of blood transfusion or tattoo, and his family history was not contributory. In 2002 (at age 64), the patient noted contact pain at in the left buccal mucosa, consulted a local otolaryngologic clinic and was treated under a diagnosis of ulcer, but there was repeated aggravation and resolution of the oral lesion without the patient ever recovering completely.

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Abbreviations: HCV, hepatitis C virus; LP, lichen planus; OLP, oral lichen planus; CLP, cutaneous lichen planus; IFN, interferon; anti-HCV, antibodies to HCV; HBsAg, hepatitis B virus surface antigen; HCC, hepatocellular carcinoma; MU, million units; RBC, red blood red cell; Hb, hemoglobin; WBC, white blood white cell; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gammaglutamyl transpeptidase; LDH, lactate dehydrogenase; ZTT, zinc sulfate turbidity test

Key words: hepatitis C virus, oral lichen planus, cutaneous lichen planus, interferon, ribavirin

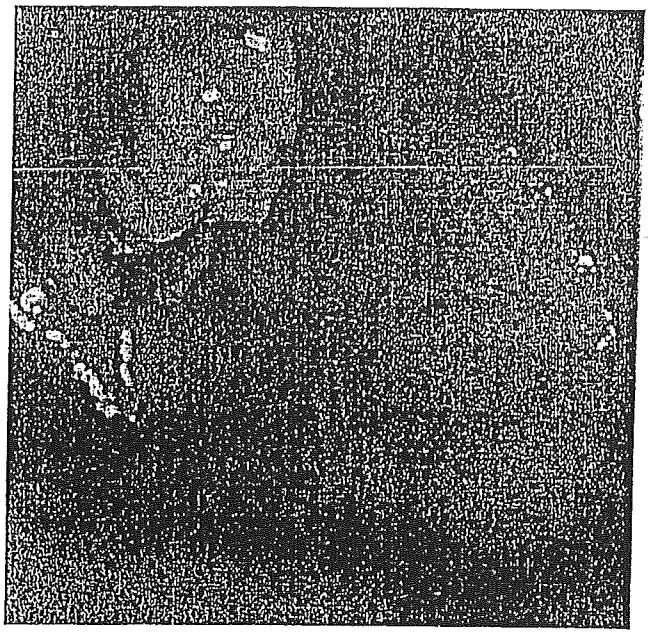

Figure 1. Lichen planus of the left buccal mucosa before administration of interferon and ribavirin as shown in Fig. 5A.

Among his physical findings, characteristic observations were an erosive type with white papules on his bilateral buccal mucosae, lower lip, and gingivae of the upper and lower molars (Fig. 1). Contact with most of the lesions caused mild contact pain. A biopsy specimen of his left buccal mucosa showed LP with parakeratosis and band-like lymphocytic infiltration. A physical examination by a dermatologist indicated that he did not have cutaneous LP (CLP) and genital LP.

Other findings were as follows: blood pressure, 150/100 mm Hg; pulse, 60/min; no anemia in the palpable conjunctiva; no jaundice in the bulbus conjunctiva; no findings such as palmar erythema, vascular spider and pitting edema of either leg or foot; no abnormalities in the heart and breath sounds; and no distention or fluctuation of the abdomen. The liver was palpable by one finger's breadth below the right costal margin, the liver edge was blunt and smooth, and its consistency was soft. The spleen was not palpable and the area of splenic dullness was widened.

Clinical examinations were as follows: the results of peripheral blood examination included red blood cells (RBC), $448 \times 10^4/mm^3$; hemoglobin (Hb), 15.9 g/dl; hematocrit, 44.3%; white blood cells (WBC), $6000/mm^3$ (Seg, 59.7%); and platelets, $14.4 \times 10^4/mm^3$. Blood chemistry tests on serum showed aspartate aminotransferase (AST), 57 IU/l; alanine aminotransferase (ALT), 96 IU/l; γ-glutamyl transpeptidase (γ-GTP), 44 IU/l; lactate dehydrogenase (LDH), 149 IU/l; zinc sulfate turbidity test (ZTT), 21.8; total bilirubin, 1.13 mg/dl; total protein, 7.68 g/dl; and albumin, 4.47 g/dl. Antibody to HCV (anti-HCV) was positive and serum HCV RNA was detected. The serum HCV RNA level quantified by Roche Amplicor Monitor assay and HCV genotype was 660 kIU/ml, and 1b, respectively. The serum was negative for hepatitis B surface antigen (HBsAg) and anti-nuclear antibody. Ultrasonographic examination, computerized tomography, and MRI scans of the abdomen performed in the outpatient clinic revealed a hepatic shape similar to that in chronic hepatitis, while multiple hemangiomas in segments eight (S8), five (S5), and six (S6), and liver cysts in S5 and S6 were observed as intrahepatic space-occupying lesions, but HCC was not observed. The spleen was enlarged. Regarding this patient who underwent liver biopsy, grading and staging of liver tissues were diagnosed as F2A1 according to the new Inuyama classification (23).

The patient was admitted to the Second Department of Medicine in our university hospital for treatment of chronic hepatitis C on April 9, 2003. The schedule of his treatment for chronic liver disease was as follows: initial administration of IFNß [Feron®, at a dose of 6 million units (MU)/day] for 2 week, then administration of IFNα-2b (Intron A®, at a dose of 6 MU/day for 2 weeks and thereafter 3 times a week for 22 weeks) and ribavirin (600 mg/day for 24 weeks). On April 18, 2003, the patient started to receive injections of IFN, and started to take ribavirin at a dose of 600 mg/day from May 2. The dose of ribavirin was reduced to 400 mg/day from May 15 because of vomiting and anorexia, and ribavirin was discontinued for 3 weeks, from May 27 to June 17, because of anemia. At the beginning of May, hoarseness developed and it was diagnosed histopathologically as leukoplakia of the right vocal cord. He underwent inhalation therapy by nebulizer. The erosive OLP lesion was not widely aggravated by application of steroids agent during the 7 weeks after IFN

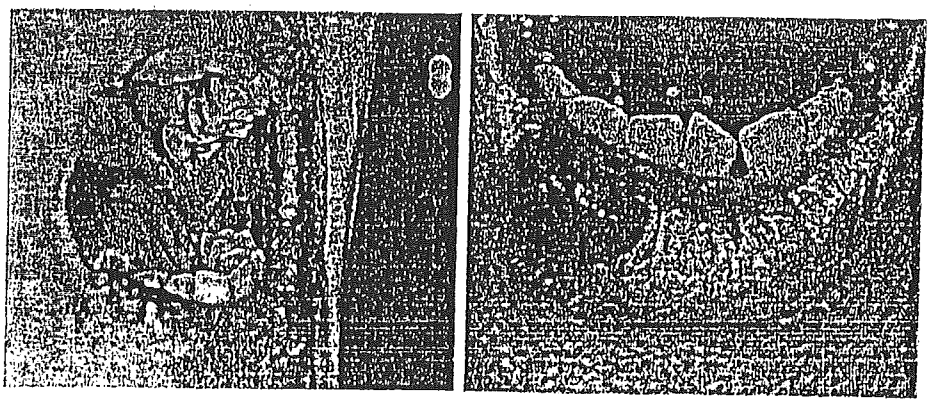

Figure 2. Exacerbation of lichen planus of the buccal mucosa and lip during administration of interferon and ribavirin as shown in Fig. 5B. The patient's oral pain and hemorrhagic crusts on the lower lip became severe and impaired his intake of food.

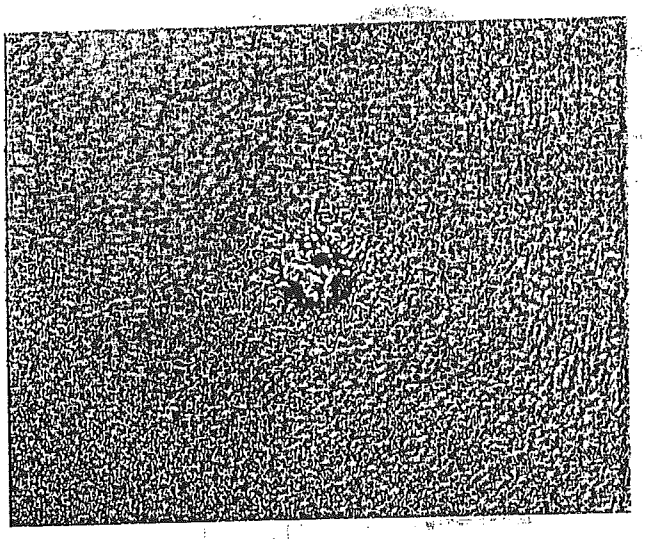

Figure 3. Cutaneous lichen planus of the legs and arms 6 months after stopping administration of interferon and ribavirin as shown in Fig. 5D.

treatment, but aggravation began at the beginning of the 8th week of administration. Hence, after 18 weeks and 4 days (on August 25), IFN and ribavirin were stopped completely because of aggravation of the OLP (Fig. 2). His oral pain and hemorrhagic crusts on the lower lip became severe and impaired his intake of food. A steroid for external use (Salcoat®) and gargles with an enzyme drug (Elase®) were used to treat the site showing severe inflammation. We also instructed him in tooth brushing. Elevated aminotransferase levels returned to normal during IFN and ribavirin therapy, then and serum HCV RNA disappeared. But during the 7th week after the discontinuation, aminotransferase levels increased to 10 times the normal range and the level of serum HCV RNA rose to 354 kIU/ml (on January 13, 2004). The patient then received the medication for his liver disease other than IFN treatment, such as glycyrrhizin (Stronger Neo-Minophagen C®) and ursodeoxycholic acid (Urso®) as conservative (anti-inflammatory) therapy, and his elevated aminotransferase levels decreased, but serum HCV RNA became positive. Papules of CLP with pruritic violaceous papules developed on the skin of his arms, legs, and trunk at the beginning of February 2004, and treatment of CLP was started (Fig. 3). At this writing, eight months have passed since IFN and ribavirin therapy were discontinued, and the erosion of OLP has reduced gradually. The pain has relieved, but the erosion of the lower lip remains (Fig. 4). The leukoplakia of the vocal cord was resected under general anesthesia on May 6, 2004. Fig. 5 illustrates the results of liver function tests and the clinical course of the patient.

Discussion

Many studies have shown that IFN results in biochemical improvement, viral suppression, histologic improvement, regression of fibrosis and reduced incidence of HCC (24,25). Moreover, therapy with IFN and ribavirin for patients with chronic hepatitis C is more effective than IFN alone in inducing virologic and histologic improvement (26).

On the other hand, it is well known that HCV induces not only chronic liver diseases but also extrahepatic manifestations (5,6). Subsequently, it has been reported that therapeutic effects of IFN alone or IFN plus ribavirin have also been confirmed in the treatment of extrahepatic lesions such as membranoproliferative glomerulonephritis (27), cryoglobulinemia (28), and porphyria cutanea tarda (29). With regard to the effects of IFN therapy on the LP lesion, one of the extrahepatic manifestations, there are reports of improvement in LP lesions (8,9), reports of LP manifestations triggered by IFN (10-18), and reports of aggravation of LP (19,20). Recently, Harden *et al* reported 5 cases (4 with CLP, 1 with CLP and OLP) that were treated with IFNα and ribavirin for chronic hepatitis C (22). The authors reported that 3 patients who became HCV negative as a result of therapy of with IFNα and ribavirin after 4 weeks showed improvement in their LP. In the remaining two patients the eruption worsened initially, but improved later, near the end of therapy, and one of these patients was a non-responder. However, the clinical course of CLP or OLP and the details about the inflammation of chronic hepatitis are unknown because long follow-up on the 5 patients was not carried out. We observed long-term histologic changes in Japanese patients with OLP and chronic hepatitis C (21). Over 3 years or longer, some OLP lesions (all reticular types) were improved, not only macroscopically, but also on histo-

Figure 4. Lichen planus of the buccal mucosa and lip at the 7 months after stopping the administration of interferon and ribavirin as shown in Fig. 5C. Exacerbation of the oral erosion was reduced gradually.

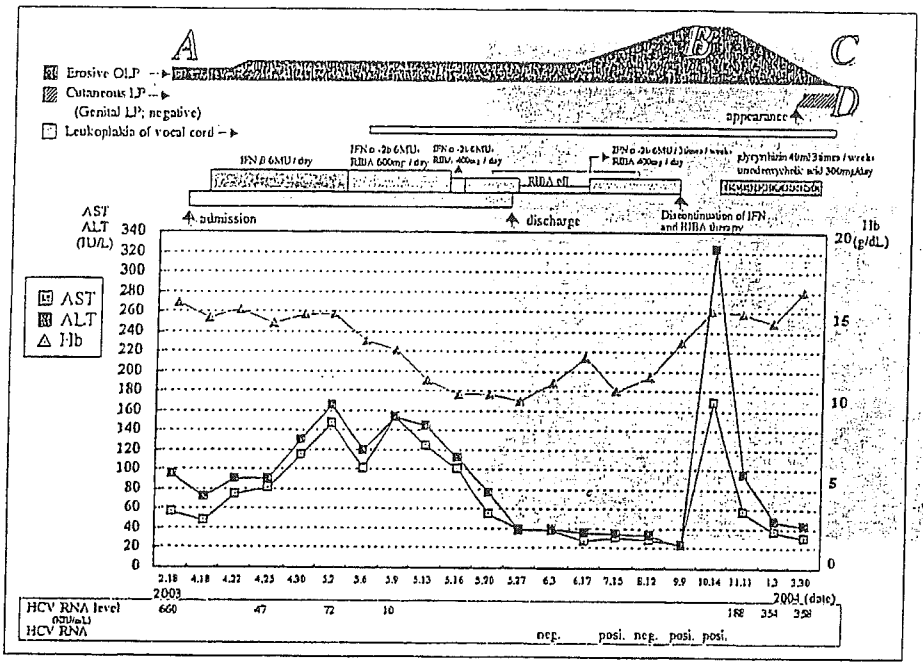

Figure 5. Clinical course of the patient. The photograph at point A shows oral lichen planus (OLP) before administration of interferon and ribavirin therapy (Fig. 1). The photograph at point B shows exacerbation of OLP during the therapy (Fig. 2). The photograph at point C shows reduced OLP after discontinuation of the therapy (Fig. 4). The photograph at point D shows the appearance of cutaneous lichen planus after discontinuation of therapy (Fig. 3).

pathologic examination. This finding resembles a histologic cure that has been confirmed in cases of chronic hepatitis C for which IFN treatment is markedly effective. The ability of IFN to eradicate HCV and improve liver damage may contribute to the improvement of OLP because histologic improvement of OLP was observed in our cases of OLP with chronic hepatitis C (21).

Dalekos *et al* prospectively evaluated dermatological side-effects during IFN therapy for chronic hepatitis B or C (15). Their study, which was done in northwestern Greece, demonstrated that IFNα only rarely (3.3%) induces immune-mediated dermatological disorders, especially LP in patients with chronic viral hepatitis. The authors reported that the development of these disorders may reflect a subclinical or covert autoimmune background of the patients. In our observations of oral lesions made before, during and after IFN treatment, OLP occurred in 16.7% (4/24 patients) of Japanese subjects (13). Some OLP lesions that appeared during IFN treatment and were aggravated temporarily were improved by symptomatic therapy, so that IFN treatment was continued. Two had OLP before treatment, 1 during treatment and 1 after treatment. Oral leukoplakia was seen in 4 patients before treatment and oral cancer in one patient 6 months after completing treatment (30).

In general, it is has been reported that caution should be exercised when IFN therapy is applied to chronic hepatitis C patients with prior OLP manifestations (5). It is difficult to predict which HCV cases will show IFN/ribavirin-induced OLP or cutaneous LP. However, caution is required in administering IFN or ribavirin to the HCV carriers who already suffer from LP, especially severe OLP of the erosive type. A close inspection of LP is essential before administering antiviral drugs to HCV carrier patients. When oral or cutaneous symptoms are exacerbated during IFN or ribavirin therapy, even if an oral surgeon or a dermatologist treats OLP or CLP, IFN or ribavirin should be reduced or discontinued immediately.

IFN may induce the expression of previously hidden surface antigens on keratinocytes, similar to the probable mechanism for achieving virus elimination from hepatocytes (19). Prolonged courses of IFN were reported to induce autoantibodies as well as autoimmune disorders (31). Garcia-Buey *et al* reported that 7 female patients developed features of autoimmunity during IFN therapy for chronic hepatitis C, suggesting a triggering by immune-stimulating effects of IFN (31). IFN contributes to the exacerbation of autoimmune phenomenon. HCV infection can be a trigger to an underlying immunologic abnormality that can worsen with IFN immunomodulation.

In conclusion, we report a case of chronic hepatitis C patient with exacerbation of prior erosive OLP, appearance of CLP and leukoplakia by treatment with IFN and ribavirin. The aggressive OLP has gradually diminished after discontinuation of the therapy, aminotransferase levels decreased, but serum HCV RNA levels remained elevated. Aggressive treatment with IFN or with a combination of IFN plus ribavirin for eradication of HCC may increase the chance of complications with various extrahepatic manifestations such as lichen planus. It is therefore important to examine oral membranes and skin before administering IFN or IFN plus ribavirin for patients with HCV infections. Accumulation of a larger study cohort and long-term follow-up is now needed to elucidate the therapeutic effects of IFN therapy on extrahepatic lesions.

Acknowledgements

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Causal relationship between hepatitis C virus core and the development of type 2 diabetes mellitus in a hepatitis C virus hyperendemic area: A pilot study

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Abstract. Hepatitis C virus (HCV) infection may contribute to the development of type 2 diabetes mellitus. However, this association at the population level remains unclear. The aim of this pilot study is to examine the relationship between HCV infection and the development of type 2 diabetes mellitus in an HCV hyperendemic area where we conducted health screenings in 1995. After 7 years of follow-up, we evaluated the relative risk of the development of type 2 diabetes mellitus in anti-HCV-inhabitants. Among 71 subjects free of diabetes mellitus in 1995, 7 developed type 2 diabetes mellitus during the 7-year follow-up evaluation. Overall, anti-HCV-positive subjects were nearly 3-fold as likely as anti-HCV-negative subjects to develop diabetes mellitus, but this difference was not statistically significant (p=0.08). After stratification of the anti-HCV-positive group according to serum HCV core titer, a significant increase in the incidence of diabetes was seen in subjects with high titer of HCV core compared to anti-HCV-negative subjects (p=0.02; relative hazard, 5.60; 95% confidence interval, 1.41 to 37.42). In conclusion, HCV infection potentially has a significant role in the development of type 2 diabetes mellitus at the population level. Further large-scale studies are needed to confirm these preliminary findings.

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Abbreviations: anti-HCV, antibody to hepatitis C virus; BMI, body mass index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HOMA-IR, insulin resistance determined by homeostasis model assessment; IRS, insulin receptor substrate; Tg, transgenic; SOCS, suppressor of cytokine signaling

Key words: hepatitis C virus carrier, manifestation, insulin resistance, homeostasis model assessment for insulin resistance, epidemiology

Introduction

Nearly 170 million people worldwide are infected with the hepatitis C virus (HCV) (1), which can lead to liver cirrhosis and hepatocellular carcinoma (HCC), as well as to other various manifestations (2-4). Since 1990, we have conducted health screenings of the residents of 'H town' (adult population, 7,389), Fukuoka prefecture in Northern Kyushu, Japan, which is known for its high prevalence of liver disease. The prevalence of antibody to HCV (anti-HCV) among the local residents of 'H town' in 1990 was 23.6% (120/509) (5). In terms of the area, we previously reported that the town had a high prevalence of HCV carriers (5), HCV infection was the principal cause of liver disorders (5-11), and most HCV carriers died of HCC or liver cirrhosis (9,10). We also reported that early detection and treatment of HCC should be carried out as the age of HCV carriers (10,11), and the prevalence of various extrahepatic manifestations such as oral lichen planus, rheumatoid arthritis, abnormal thyroid function, hypertension, and heart disease was higher in HCV carriers than those without HCV infection (12,13). In the same area, we recently demonstrated an increased prevalence of diabetes mellitus among subjects with HCV infection (13).

Insulin resistance precedes the onset of diabetes mellitus by 10 years and is a consistent finding in patients with type 2 diabetes mellitus (14,15). Thus, insulin resistance plays a crucial role in the development of type 2 diabetes mellitus. Several studies and our previous reports proposed that HCV infection antedates insulin resistance; however, these studies were based in referral centers, and the association may be restricted to patients with more severe forms of the disease (16-19). Insulin resistance in the general population with HCV infection remains unclear.

An increased prevalence of insulin resistance among subjects with HCV infection in the general population, including asymptomatic healthy HCV carriers, may suggest a unique mechanism other than the severity of hepatitis. HCV proteins, especially HCV core, affect many signalling pathways (20,21). We recently showed molecular mechanisms for HCV core-induced insulin resistance (19). HCV core up-regulates the suppressor of cytokine signaling (SOCS) 3 and

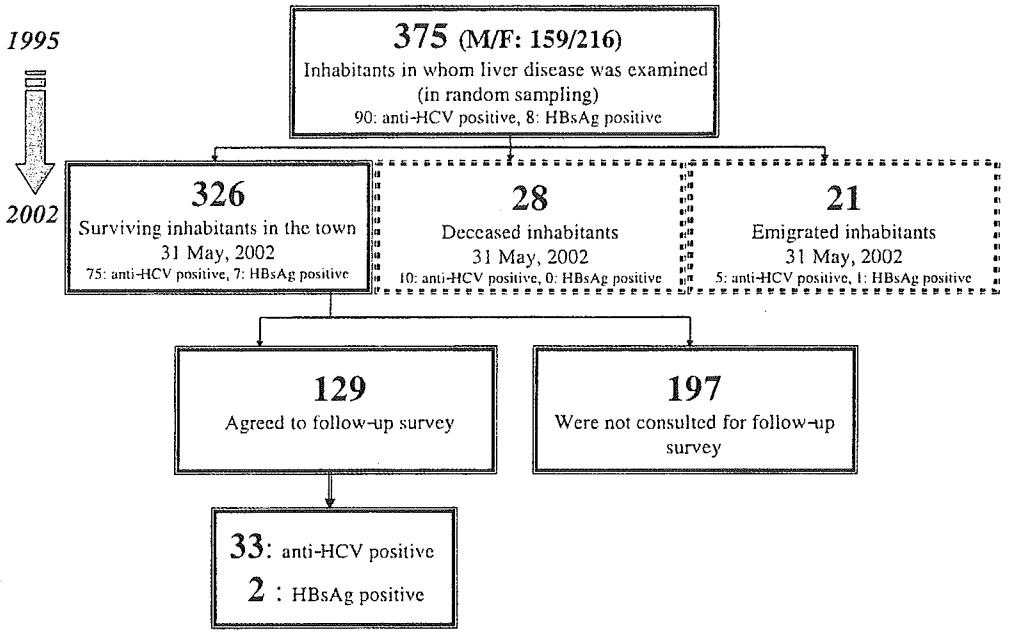

Figure 1. Diagram for pursuing the prognostic investigation of 375 inhabitants.

Table I. Characteristics of the subjects.

	Anti-HCV		
	Negative	Positive	p-value
Number	44	31	
Age (years)	59.6±9.8	59.7±10.6	NS
Sex ratio (male/female)	14/30	8/23	NS
Body mass index (kg/m^2)	22.7±2.7	22.1±1.9	NS
Lifetime use of alcohol (kg/life)	111.3±254.7	77.3±228.4	NS
Aspartate aminotransferase (U/l)	20.1±5.3	32.0±21.2	<0.01
Alanine aminotransaminase (U/l)	11.4±6.5	24.9±20.7	<0.01
γ-glutamyltranspeptidase (U/l)	10.9±6.4	13.8±9.3	NS

Data are expressed as mean ± SD or number of subjects. A p-value <0.05 was considered significant. Anti-HCV, anti-HCV antibody; NS, not significant.

inhibits insulin signaling by down-regulation of insulin receptor substrate (IRS) 1 and IRS2 in hepatocytes (19).

In this study, we evaluated insulin resistance among inhabitants with HCV infection in an HCV hyperendemic area of Japan. An association between HCV core and insulin resistance was also examined. Since association does not prove causality, we performed a case-cohort analysis to examine if inhabitants with HCV infection were at increased risk for type 2 diabetes mellitus after 7 years of follow-up.

Materials and methods

Study population. Since 1990, we have conducted health screenings of the residents of an HCV hyperendemic area (5). In 1995, 375 adult residents (159 men and 216 women) participated in this study. In the present study, we evaluated the insulin resistance of residents by using plasma and serum samples taken in 1995 and performed a case-cohort analysis to examine the relationship between HCV infection and the development of type 2 diabetes mellitus after 7 years of follow-up. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in prior approval by the Institutional Review Board at Kurume University School of Medicine. All participants provided written informed consent.

By 2002, 28 of 375 residents had died and 21 people had moved to other regions (Fig. 1). Thus, 326 residents of the original 375 residents remained in the area, and 129 residents agreed to participate in the medical follow-up survey, while the remaining 197 inhabitants did not declare their intention

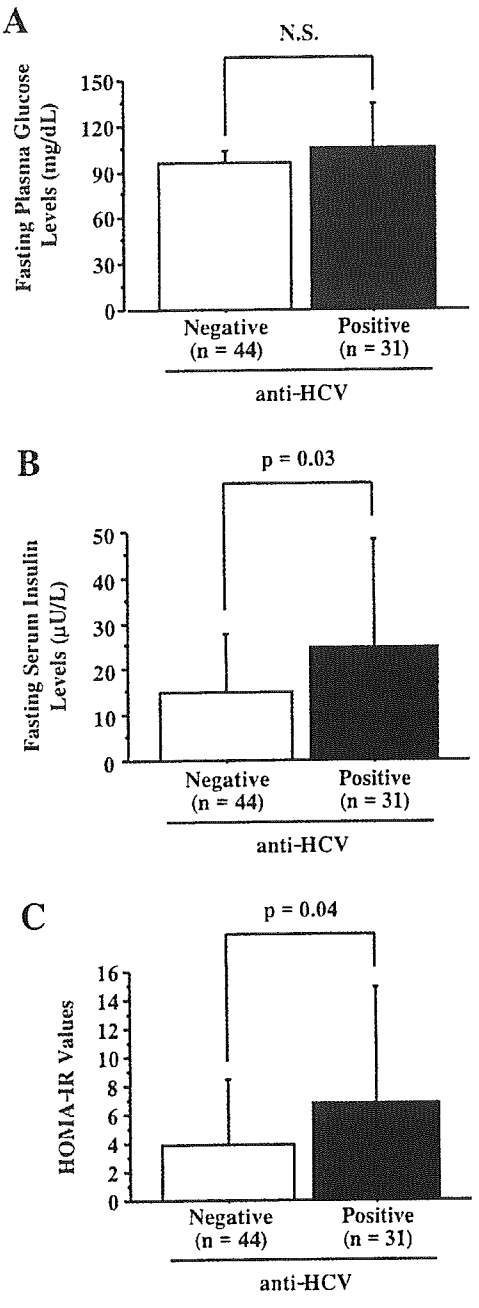

Figure 2. Glucose homeostasis in subjects with anti-HCV negative (n=44) and positive (n=31). (A) Fasting plasma glucose levels, (B) fasting serum insulin levels, and (C) insulin resistance determined by the homeostasis model assessment (HOMA-IR) values. Values are expressed as mean ± SD. NS, not significant.

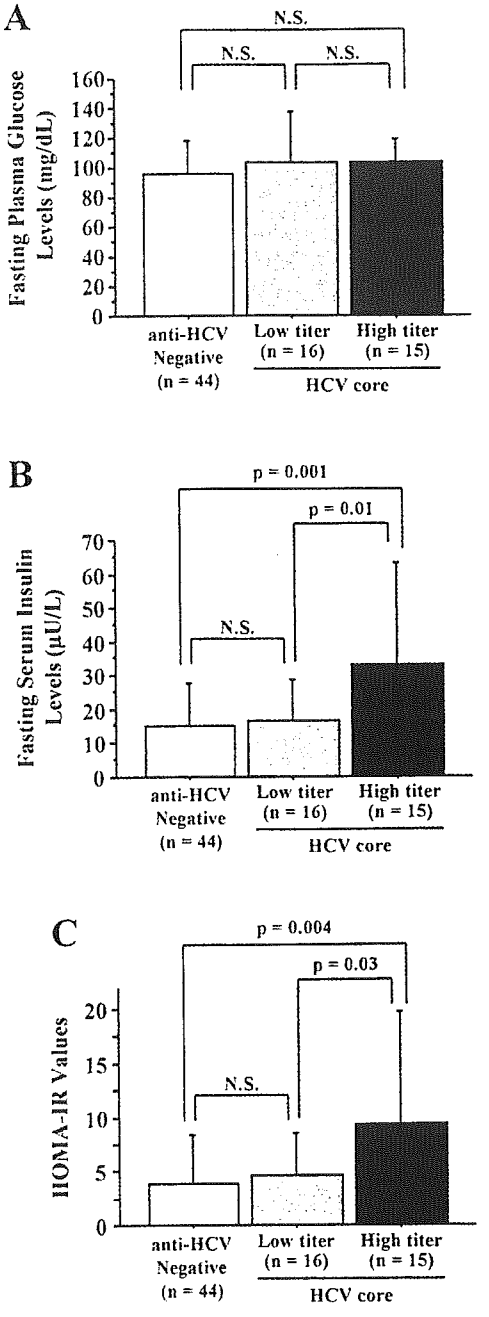

Figure 3. Glucose homeostasis in subjects with anti-HCV negative (n=44), low titer (<300 fmol/l; n=16), and high titer (>300 fmol/l; n=15) HCV core. (A) Fasting plasma glucose levels, (B) fasting serum insulin levels, and (C) insulin resistance determined by the homeostasis model assessment (HOMA-IR) values. Values are expressed as mean ± SD. NS, not significant.

either way in 2002. Among the surviving 129 residents who agreed to participate, 33 were anti-HCV positive. Of these 33, 2 residents were excluded from analysis because of indeterminate fasting glucose or insulin levels. The remaining 31 anti-HCV-positive residents and 44 randomly selected anti-HCV negative residents were enrolled in this study. All were hepatitis B virus surface antigen negative.

Clinical and serological assessment. Data including age, sex, and alcohol use were collected. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). Venous blood samples were taken in the morning after a 12-h overnight fast. Sera were examined for the presence of anti-HCV by using a second-generation enzyme immunoassay test (Lumipulse II HCV, Fujirebio Inc., Tokyo, Japan). Sera were also assayed for HCV core by using a newly developed HCV Core Antigen ELISA Test system (Ortho-Clinical Diagnostics K.K., Tokyo, Japan), which has high stability and reproducibility under all conditions, and a detection limit of 44 fmol/l (22). The concentrations of serum HCV core antigen and serum HCV RNA correlated significantly. Based on the concentration of serum HCV RNA, <300 fmol/l of serum HCV core is classified as the low titer group and >300 fmol/l of

Table II. Relative risk of type 2 diabetes mellitus.

Anti-HCV	HCV core titer	Total number	No. of people with diabetes	p-value	Relative risk	95% Confidence interval
Negative		42	2		1	
Positive		29	5	0.08	3.62	0.83 to 20.89
	Low	14	1	0.73	1.50	7.66 to 18.11
	High	15	4	0.02	5.60	1.41 to 37.42

Subjects with anti-HCV positive were categorized according to HCV core titer into 2 groups: low titer, <300 fmol/l; and high titer, >300 fmol/l.

serum HCV core is classified as the high titer group. Plasma glucose levels were measured by a glucose oxidase method. Serum insulin levels were measured by using a sandwich enzyme immunoassay kit (Eiken Chemical, Tokyo, Japan). Insulin resistance was calculated on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA-IR) method (23). The formula for the HOMA-IR is: HOMA-IR = fasting glucose (mg/dl) x fasting insulin (μU/ml)/405.

Diagnosis of type 2 diabetes mellitus. Type 2 diabetes mellitus was classified according to the 1997 American Diabetes Association criteria (24). Subjects who had a fasting plasma glucose level of ≥126 mg/dl were considered to have type 2 diabetes mellitus. Cases of type 2 diabetes mellitus were also defined by self-reported use of hypoglycemic medications. Subjects in whom diabetes was diagnosed at <30 years of age and who reported insulin use at the time of survey were categorized as having type 1 diabetes mellitus and excluded from this study.

Statistical analysis. All data are expressed as mean ± SD. Differences between 2 groups were analyzed using a Student's *t*-test. Statistical comparisons among multiple groups were performed by ANOVA followed by Scheffe's post-hoc test. Where indicated, simple correlation and linear regression analyses were done. A case-cohort analysis of the relationship of HCV infection and the development of type 2 diabetes mellitus was performed. The relative hazard for the development of type 2 diabetes mellitus, 95% confidence limit, and significance of the associations were evaluated based on 2 tests using Stat View® for Macintosh, version 5.0 (SAS Institute, Cary, NC). p-values <0.05 were considered significant.

Results

Characteristics of all subjects in an HCV hyperendemic area. In an HCV hyperendemic area, we enrolled 31 inhabitants who were anti-HCV-positive and 44 subjects who were anti-HCV-negative. Characteristics and laboratory data of these subjects are summarized in Table I. All subjects were Japanese. There was no significant difference in age, sex, BMI, lifetime alcohol use, and γ-glutamyltranspeptidase between the 2 groups. Although serum aspartate aminotransferase and alanine aminotransferase levels showed significant differences between the groups, both mean values were within normal range.

Insulin resistance in subjects with HCV infection. There was no significant difference in fasting glucose levels between anti-HCV-positive and anti-HCV-negative subjects (Fig. 2A). Anti-HCV-positive subjects showed increased fasting insulin levels compared to those subjects who were anti-HCV-negative (p=0.03; Fig. 2B). Values of HOMA-IR, an indicator of insulin resistance, were also increased in anti-HCV-positive subjects compared to those who were anti-HCV negative (p=0.04; Fig. 2C).

Association between HCV core and insulin resistance. Among 31 anti-HCV-positive subjects, 16 subjects showed low titer (<300 fmol/l) of serum HCV core and 15 subjects showed high titer (>300 fmol/l) of serum HCV core. Stratification of the anti-HCV-positive group according to serum HCV core titer revealed significant differences in fasting serum insulin levels, but not in fasting glucose levels between subjects with high titer of HCV core and subjects with anti-HCV negative (p=0.001; Fig. 3A and B). Similarly, HOMA-IR values were increased in subjects with high titer of HCV core compared to those in subjects with anti-HCV negative (p=0.004; Fig. 3C). A significant increase in fasting serum insulin levels and HOMA-IR values were also seen in subjects with a high titer of HCV core compared to those in subjects with a low titer of HCV core (Fig. 3B and C).

Correlations between fasting insulin levels of AST and ALT levels. Among anti-HCV-positive subjects, correlations between fasting insulin levels of AST and ALT levels were not statistically significant (r=0.33 and r=0.43, respectively). Similarly, correlations between HOMA-IR values of AST and ALT levels were not statistically significant (r=0.32 and r=0.45, respectively).

HCV infection and type 2 diabetes mellitus. To ascertain if HCV infection causes type 2 diabetes mellitus, we performed a case-cohort analysis in an HCV hyperendemic area. Of 75 subjects, 4 residents (2 anti-HCV-positive subjects and 2 anti-HCV-negative subjects) were excluded from the case-cohort analysis because the development of type 2 diabetes mellitus was at the baseline. Among 71 subjects free of diabetes mellitus at the baseline, 7 subjects developed type 2 diabetes mellitus during the 7-year follow-up evaluation.

Overall, anti-HCV-positive subjects were nearly 3-fold as likely as anti-HCV-negative subjects to develop diabetes mellitus, but this difference was not statistically significant (p=0.08; relative hazard, 3.62; 95% confidence interval, 0.83 to 20.89) (Table II). After stratification of the anti-HCV-positive group according to serum HCV core titer, a significant increase in the incidence of diabetes was seen in subjects with high titer of HCV core compared to anti-HCV-negative subjects (p=0.02; relative hazard, 5.60; 95% confidence interval, 1.41 to 37.42) (Table II).

Discussion

In this study, we described an association of HCV infection and the development of type 2 diabetes mellitus in an HCV hyperendemic area. Subjects with high titers of HCV core showed more severe insulin resistance than subjects with low titers of HCV core and anti-HCV negative subjects. Our pilot study showed a significant increase in the incidence of type 2 diabetes mellitus in subjects with high titers of HCV core compared to anti-HCV-negative subjects.

Although there was no significant difference in fasting glucose levels between subjects who were anti-HCV-positive and -negative, fasting insulin levels and HOMA-IR values (an indicator of insulin resistance) were significantly increased in anti-HCV-positive subjects compared to anti-HCV-negative subjects (Fig. 2A-C). These findings indicate that HCV infection-induced insulin resistance and fasting glucose levels were compensated by hyperinsulinemia.

Several studies and our previous reports proposed that HCV infection antedates insulin resistance (16-19,24). However, these studies were based in referral centers, and the association may therefore be restricted to subjects with more severe forms of the disease. In this study, we enrolled all anti-HCV-positive inhibitants, including asymptomatic HCV carriers, and showed increased serum fasting insulin levels and HOMA-IR values in subjects who were anti-HCV-positive compared to subjects who were anti-HCV-negative. The development of severe insulin resistance in the general population among subjects with HCV infection and less severe hepatitis may suggest an alternative mechanism to hepatitis-related insulin resistance. Our hypothesis is also supported by the fact that fasting insulin levels were not significantly correlated with AST and ALT levels.

HCV consists of envelope and core proteins. Although HCV envelope protein transgenic (Tg) mice develop no pathological changes in the liver (25), Shintani *et al* and our previous study showed that HCV core Tg mice developed hepatic insulin resistance (18,19). Thus, since HCV core is involved in the development of insulin resistance in Tg mice, we examined an association between HCV core and insulin resistance in an HCV hyperendemic area. By stratification of the anti-HCV-positive group according to serum HCV core titer, differences in fasting serum insulin levels and HOMA-IR values between subjects with high titer of HCV core (>300 fmol/l) and anti-HCV-negative subjects were made clear (Fig. 3B and C). Our previous study demonstrated one of the molecular mechanisms for HCV-induced insulin resistance; HCV core up-regulates SOCS3 and inhibits insulin signaling by the down-regulation of IRS1 and IRS2 in hepatocytes (19), and this molecular mechanism may account for an association of HCV core with insulin resistance. Thus, we provided additional evidence for an association between HCV core and insulin resistance.

Since it is also possible that subjects with type 2 diabetes mellitus are at increased risk for acquiring HCV infection, the above data were insufficient to conclude that HCV infection causes type 2 diabetes mellitus. We assessed whether subjects with HCV infection were at increased risk for type 2 diabetes mellitus. Although the difference in the development of type 2 diabetes mellitus between anti-HCV-positive and negative subjects was not statistically significant (p=0.08; Table II), stratification by serum HCV core titer revealed that the pre-existing high titer of HCV core increased the risk for type 2 diabetes mellitus (p=0.02; Table II). Mehta *et al* reported a preliminary causal relationship between HCV infection and type 2 diabetes mellitus (26); their data, however, were derived from a community with a low prevalence of HCV infection (0.8% residents were anti-HCV-positive). On the other hand, we performed this study in a community with a high prevalence of HCV infection (23.6% residents were anti-HCV-positive). In this study, we confirmed the preliminary causal relationship between HCV infection and type 2 diabetes mellitus and identified a new factor, 'HCV core,' responsible for the development of type 2 diabetes mellitus.

A limitation in this study was the absence of multivariate analysis. Since 197 subjects did not agree to the follow-up study, the small sample size limited our ability to perform multivariate analysis. However, this study had sufficient statistical power to detect an association between HCV core and type 2 diabetes mellitus because of high prevalence of anti-HCV and similar environmental factors, such as the eating habits and lifestyle of H-town residents.

In conclusion, HCV infection may cause the development of type 2 diabetes mellitus in an HCV hyperendemic area. The interaction between HCV infection and development of type 2 diabetes warrants further study.

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Extrahepatic manifestations and insulin resistance in an HCV hyperendemic area

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Abstract. Hepatitis C virus (HCV) causes extrahepatic manifestations as well as liver diseases, and contributes to insulin resistance and type 2 diabetes mellitus. The purpose of the present study was to evaluate the relationship of extrahepatic manifestations and insulin resistance in an HCV hyperendemic area. We investigated the incidence of extrahepatic manifestations among 139 inhabitants living in an HCV hyperendemic area in 2002 and compared it to 1999 data for the same inhabitants. Insulin resistance was tested for some non-HCV or HCV-infected inhabitants we had identified during mass screenings in 1999 and 2002. For some of the inhabitants in 2002, we examined records on the prevalence of insulin resistance seven years earlier. The prevalence of extrahepatic manifestations among individuals with positivity for anti-HCV antibodies was higher than among those without HCV in both 1999 and 2002. The prevalence of each extrahepatic manifestation which we identified in 2002 was higher than in 1999. Moreover, in some non-HCV or HCV-infected inhabitants, insulin resistance in 2002 was significantly higher than in 1999. Among inhabitants who had HCV infection with extrahepatic manifestations, fasting insulin levels or HOMA-IR findings seven years prior was significantly higher than for inhabitants who had neither HCV infection nor extrahepatic manifestations (p=0.03, p=0.01, respectively). Insulin resistance induces HCV infection, which causes an increase in the incidence of extrahepatic manifestations in HCV-infected individuals.

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Abbreviations: HCV, hepatitis C virus; HBV, hepatitis B virus; anti-HCV, antibodies to HCV; HBsAg, hepatitis B virus surface antigen; HCC, hepatocellular carcinoma; OLP, oral lichen planus; DM, diabetes mellitus

Key words: hepatitis C virus, extrahepatic manifestations, insulin resistance, lichen planus, diabetes mellitus

Introduction

It is common knowledge that hepatitis C virus (HCV) causes chronic hepatitis, cirrhosis, and even hepatocellular carcinoma (HCC). Of the HCC cases in Japan, approximately 16% are caused by hepatitis B virus (HBV) infection and approximately 80% by HCV infection. Therefore, the following are now implemented as emergency measures for hepatitis C in the Ministry of Health, Labour and Welfare in Japan. In our country, as part of a 5-year program from 2002 to 2007, candidates who reach certain turning points during every 5-year period from age 40-70, or those in whom abnormalities in liver function were pointed out during the basic health checkup according to the Health and Medical Service Law for the Aged will undergo examination for HCV and HBV infection.

On the other hand, HCV infection is known to be associated with extrahepatic manifestations including membranoproliferative glomerulonephritis, cryoglobulinemia, and oral lichen planus (OLP) (1,2). Many recent reports have described the relationship of LP to HCV, such as the incidence of HCV infection in LP patients (3-6) or the incidence of LP in subjects with HCV-related liver diseases (7-10). Diabetes mellitus (DM) is another extrahepatic manifestation attributed to HCV infection. Epidemiologic studies of liver disease patients (11-14) and of type 2 DM patients (13-15) have reported that HCV-related liver disease increases the risk of type 2 DM. One to two decades before type 2 DM is diagnosed, reduced glucose clearance is already present (16). Therefore, insulin resistance plays an important role in the development of type 2 DM. We recently showed molecular mechanisms for HCV core-induced insulin resistance (17). HCV core up-regulates the suppressor of cytokine signaling (SOCS) 3 and inhibits insulin signaling by down-regulation of insulin receptor substrate (IRS) 1 and IRS2 in hepatocytes.

We screened for HCC among inhabitants of 'H town' (adult population: 7,389), Fukuoka prefecture in northern Kyushu, Japan, which has been known for its high prevalence of liver diseases since 1990 (18-21). Anti-HCV positivity among H town residents in 1990 was 23.6% (18). We also screened for OLP in the same town in 1994 (7) and investigated the incidence of extrahepatic manifestations in 1999 (8) and 2002. Recently, we reported community-based evidence for an association between HCV core, insulin resistance, and the development of type 2 DM (22).

Table I. Characteristics of extrahepatic manifestation in 2002.

	Total	OLP[a]	Abnormal thyroid function	Rheumatoid arthritis	DM	Hypertension
Subjects (%)	139 (100)	15 (10.9)	3 (2.2)	3 (2.2)	13 (9.4)	33 (23.7)
Age (years) (mean ± SD)	60.6±13.1	62.7±12.5	51.3±6.6	66.3±11.8	66.5±8.8	66.9±10.7
Sex (M/F)	51/88	8/7	0/3	0/3	5/8	18/15
Anti-HCV (+) (%)	35 (25.2)	6/35 (17.1)	2/35 (5.7)	2/35 (5.7)	5/35 (14.3)	12/35 (34.3)
HCV RNA (+) (%)	21 (15.1)	5/21 (23.8)[b]	2/21 (9.5)[c]	1/21 (4.8)	4/21 (19.0)	7/21 (33.3)
Anti-HCV (-) and HCV RNA(-) (%)	104 (74.8)	9/104 (8.7)[b]	1/104 (1)[c]	1/104 (1)	8/104 (7.7)	21/104 (20.2)

[a]Examination of oral mucosa was performed for 138 subjects. [b]$p<0.05$, [c]$p=0.01$.

In the current study, we investigated the incidence of extrahepatic manifestations in 2002 in an HCV hyperendemic area of Japan and compared it with results for 1999. We also investigated insulin resistance and extrahepatic manifestations in some non-HCV and some HCV-infected inhabitants whom we had identified in both 1999 and 2002.

Finally, we examined insulin resistance for the prior seven years in some of the inhabitants in 2002.

Patients and methods

The 139 adult inhabitants we studied included 51 men and 88 women with a mean age of 60.6±13.1 years (mean ± SD). These subjects were inhabitants of H town, an area that was hyperendemic for HCV infection. These subjects participated in a liver disease examination in 2002, and 138 of 139 people (50 men and 88 women; mean age ± SD, 60.5±13.2) participated in an oral mucosa examination as well. These 139 subjects were interviewed in person by two trained interviewers. We inquired about the following information: present health condition, regular hospital visits, medical treatment received in the hospital, name of the family doctor, the kind of medicine taken, and the presence of extrahepatic manifestations of HCV infection such as thyroid dysfunction, articular rheumatism, DM, and hypertension. Informed consent was obtained from all subjects after the purpose and methods of the study were explained.

Examination for oral mucosa. Almost all (138 of 139) received oral mucosa medical examination by an oral surgeon. The diagnosis of OLP was made on the basis of clinical features. We used the headband fiber (50-100-10, Daiichi Medical Co., Ltd.) that had a brightness of 15,000 luxes for stomatic examination. When a lesion was found, we created a photograph as an intraoral record. A topographic map of the oral mucosa was precisely classified into 56 areas according to classification of Roed-Petersen *et al* (23) and examination of the oral mucosa was based on the 'Guide to epidemiology and diagnosis of oral mucosal diseases and conditions' published by the World Health Organization (WHO) (24). The OLP diagnosis was made on the basis of clinical features and WHO criteria.

Diagnosis of extrahepatic manifestations except for OLP. We asked the family doctors of the subjects about the presence of extrahepatic manifestations including thyroid dysfunction, articular rheumatism, type 2 DM, and hypertension. Moreover, for the subjects who did not have a family doctor, diagnosis of DM was based on the American Diabetic Association (ADA) criteria of 1997 (25). Persons in whom diabetes was diagnosed before 30 years of age and used insulin were categorized as type 1 DM and were excluded from this study.

Serological assay. Body mass index (BMI) was calculated as body weight in kilograms divided by the square of height in meters (kg/m^2). Sera from the 139 residents were evaluated for the following liver function tests: serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gammaglutamyl transpeptidase (γ-GTP), lactate dehydrogenase (LDH), total bilirubin (T.Bil), total protein (TP), albumin (Alb) and gamma-globulin (γ-glob.). Sera were also examined for the presence or absence of HCV or HBV infection. Anti-HCV was measured by a chemiluminescent enzyme immunoassay (CLEIA) kit (Lumipulse II HCV, Fujirebio Inc., Tokyo, Japan). HCV RNA in the sera was detected using the Amplicore HCV test (Nippon Roche, Tokyo, Japan). Hepatitis B virus surface antigen (HBsAg) was assayed by a CLEIA kit (Architect™, HBsAg QT, Dainabot Co. Ltd., Tokyo, Japan). Ultrasonographic examination of subjects with abnormalities in the liver function tests and positive for anti-HCV or HBsAg was performed in order to investigate the shape of the liver and lesions occupying the hepatic space.

Plasma glucose levels were measured by a glucose oxidase method for all subjects. In 80 subjects (anti-HCV positive: 35 cases, anti-HCV negative: 45 cases), serum insulin levels were measured using a sandwich enzyme immuno assay kit (Eiken Chemical, Tokyo, Japan). Insulin resistance (IR) was calculated

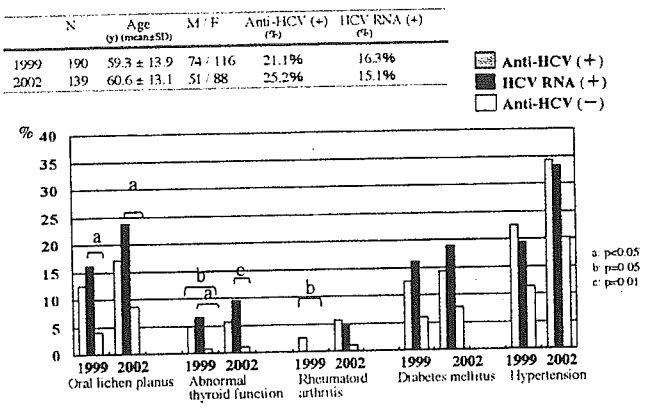

Figure 1. Prevalence of extrahepatic manifestations in HCV infection and in non-infected inhabitants. Note that the incidence of each extrahepatic manifestation was higher in those with HCV infection than in those without HCV. Note also that the incidence of each extrahepatic manifestation was higher in 2002 than in 1999. Y axix label should read: '% of total cases'.

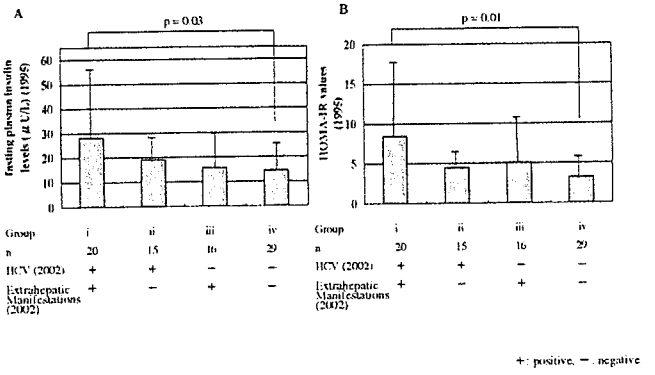

Figure 2. Association of insulin resistance in 1995 with the presence of HCV infection and extrahepatic manifestations in 2002. (A) Fasting serum insulin levels and (B) HOMA-IR values.

on the basis of fasting levels of plasma glucose and insulin, according to the homeostasis model assessment (HOMA-IR) method (26). The formula for the HOMA-IR is: HOMA-IR = fasting glucose (mg/dl) x fasting insulin (μU/ml)/405.

Comparison of prevalence of extrahepatic manifestation in 1999 and 2002. We reported the incidence of extrahepatic manifestations of HCV infection, including LP, for inhabitants of the same town in 1999 (8). We compared incidence of extrahepatic manifestations in 2002 with those in 1999.

Insulin resistance seven years prior in some inhabitants. The 80 of 139 people whom we followed-up in 2002, were divided into four groups: (i) extrahepatic manifestations with HCV infection, (ii) no extrahepatic manifestations with HCV infection, (iii) extrahepatic manifestations without HCV infection, (iv) no extrahepatic manifestations without HCV infection. We examined insulin resistance seven years prior in these four groups because we had surveyed HCC in the same town in 1995.

Statistical analysis. The chi-square test, the unpaired Student's t-test and Welch's t-test were used for statistical analyses. Differences were judged significant when $p<0.05$ (two-tailed). This study was approved by the Institutional Review Board/ Ethics Committee of our Institution.

Results

Extrahepatic manifestation in 2002. Anti-HCV antibodies and HCV RNA were detected in the sera from 35 (25.2%) and 21 (15.1%) of 139 inhabitants, respectively. HBsAg were detected in the sera from 4 subjects (4/139, 2.9%). The prevalence of OLP among all subjects was 10.9% (15/138), among HCV RNA positive subjects it was 23.8% (5/21), as shown in Table I. The incidence of OLP in those subjects who were serum HCV RNA-positive (23.8%, $p<0.05$ vs the OLP-HCV RNA negative group) was significantly higher than those without HCV RNA (Table I). The clinical appearances of 15 OLP subjects were: 5 with reticular, 5 with erosive, 4 with plaque-type, and 1 with atrophic OLP. Most (13/15 cases) of OLP had no pain. The most frequent site of OLP was the buccal mucosa. The diagnosis of all OLP subjects was made on the basis of clinical features. However, one OLP subject was biopsied and subsequently diagnosed as OLP histopathologically. There was no oral cancer. Besides OLP, the prevalences of extrahepatic manifestations among individuals with positivity for anti-HCV antibodies were higher than among those without HCV. The prevalence of thyroid dysfunction, articular rheumatism, DM, or hypertension in anti-HCV antibody positive subjects was 5.7, 5.7, 14.3, and 34.3%, respectively. Thyroid dysfunction, articular rheumatism, DM, or hypertension in HCV RNA positive subjects were 9.5, 4.8, 19.0, and 33.3, respectively (Table I). The prevalence of abnormal thyroid function among subjects with HCV infection was significantly higher than those without HCV infection.

Comparison with extrahepatic manifestations in 1999 and 2002. We had investigated the prevalence of extrahepatic manifestations for 190 people in the same town in 1999. Of the 190, 139 in 2002 participated in a second interview regarding extrahepatic manifestations. The prevalence of each extrahepatic manifestation in 2002 was higher than in 1999 (Fig. 1).

Characteristics of 35 subjects who were anti-HCV positive and 45 who were anti-HCV negative. We compared characteristics of 35 subjects who were anti-HCV positive (group 1) and 45 subjects who were anti-HCV negative (age and gender-matched controls, group 2). Table II indicates a comparison of characteristics of the 1999 and 2002 groups. For group 1 subjects, there were no significant differences between the two groups in age, sex, BMI, AST, ALT, gammaglutamyl transpeptidase or fasting plasma glucose between 1999 and 2002. Fasting serum insulin and HOMA-IR in 2002 levels were significantly increased over values in 1999. For group 2 subjects, fasting plasma glucose, fasting serum insulin, and HOMA-IR levels in 2002 were significantly increased over values in 1999, although there were no significant differences in age, sex, BMI, AST, ALT, and gammaglutamyl transpeptidase between 1999 and 2002.

Table II. Comparison of 35 HCV infected and 45 non-infected inhabitants in 1999 and 2002.

	Anti-HCV positive (35 cases)		
	1999	2002	p-value
Age (years) (mean ± SD)	62.4±10.0	67.1±10.6	ND
Sex (M/F)	9/26	9/26	ND
BMI	22.9±2.7	22.6±3.0	ND
AST	38.0±24.1	35.3±20.9	ND
ALT	37.9±27.7	31.7±26.9	ND
γGTP	36.8±25.9	34.3±25.4	ND
Fasting plasma glucose (mg/dl)	87.0±13.1	115.3±63.8	ND
Fasting serum insulin (μU/l)	12.6±7.3	29.3±28.1	0.04
HOMA-IR	2.8±1.9	8.8±9.0	0.02
	Anti-HCV negative (45 cases)		
	1999	2002	p-value
Age (years) (mean ± SD)	63.8±9.8	67.0±10.1	ND
Sex (M/F)	14/31	14/31	ND
BMI	22.9±3.5	22.9±3.5	ND
AST	22.4±5.6	21.6±4.9	ND
ALT	18.8±8.5	17.3±6.0	ND
γGTP	26.7±18.7	24.0±18.2	ND
Fasting plasma glucose (mg/dl)	92.6±26.6	109.6±46.8	0.04
Fasting serum insulin (μU/l)	14.0±12.3	22.3±17.1	0.01
HOMA-IR	3.5±4.5	6.4±5.7	0.01

ND, not statistically different.

Insulin resistance seven years prior for some of the inhabitants. Of the 139 people we examined on follow-up in 2002, 80 (35 anti-HCV positive and 45 anti-HCV negative; groups 1 and 2, respectively) were further studied, 20 had extrahepatic manifestations and HCV infection (group i), 15 had no extrahepatic manifestations and HCV infection (group ii), 16 showed extrahepatic manifestations without HCV infection (group iii), and 29 had no extrahepatic manifestations and no HCV infection (group iv). Fasting insulin levels seven years prior in these four groups were: 28.3±30.1, 19,2±9.6, 15.8±15.1, or 14.5±11.9, in groups (i), (ii), (iii), and (iv), respectively. Fasting insulin levels of group (i) was significantly higher than that of group (iv) (p=0.03, Fig. 2). HOMA-IR values seven years prior in groups (i), (ii), (iii), and (iv) were, respectively, 8.5±10.1, 4.5±2.3, 5.1±6.7, and 3.2±2.9. HOMA-IR values for group (i) was significantly higher than for group (iv) (p=0.01, Fig. 2).

Discussion

HCV is the cause of about 80% of HCC in Japan and contributes to various extrahepatic manifestations as well as chronic liver diseases. However, the mechanism through which various HCV-related extrahepatic manifestations develop has barely been elucidated. Detection of the HCV RNA negative strand has been reported not only in hepatocytes but also in many other cells, suggesting the extrahepatic replication of HCV (27). The fact that HCV replicates in tissue from OLP or oral cancer patients, both extrahepatic manifestations, has been demonstrated (28-31).

Diabetes mellitus has also been linked to HCV. Multiple studies have confirmed the suspicion that patients with HCV infection have a significantly higher prevalence of DM compared with patients with other liver diseases (11-14). An association of HCV and DM has also observed in cohorts of patients with type 2 DM (13,15), and in a large cross-sectional national survey (32). We recently showed molecular mechanisms for HCV core-induced insulin resistance. HCV core up-regulates suppressor of cytokine signaling (SOCS) 3 and inhibits insulin signaling by down-regulation of insulin receptor substrate (IRS) 1 and IRS2 in hepatocytes (17).

In the current study, we conducted a follow-up survey (in 2002) for the inhabitants who had been examined in 1999 for liver disease in an HCV hyperendemic area (18-20). We previously reported that medical treatment was considered to be a causative route of HCV transmission (18), that most HCV carriers died from HCC or liver cirrhosis (19), that the prevalence of OLP in HCV carriers was higher than in those without HCV (7,8), and that it is necessary to continuously provide medical treatment to recognized cases of HCV carriers (21). In the current study, we found: 1) a higher prevalence of extrahepatic manifestations in HCV positive subjects than in