HIGH PREVALENCE OF HEPATITIS C VIRUS INFECTION IN ABORIGINES IN TAIWAN

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SUMMARY: In Taiwan, the epidemiological status of HCV infection is similar to those observed in other areas of the world, with 1.0% prevalence among adult volunteer blood donors and high prevalences among the high risk groups, by the detection of anti-HCV with synthetic peptide antigens. However, unusually high prevalences, 35.1%, 15.8% and 14.2%, were observed among adult populations in three of the five aboriginal communities. No difference in sex specificity was noted. In 37 (75.0%) of the 48 anti-HCV-positive cases, HCV-RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) assay. None of such particular risk factors as tattooing, sexual promiscuity, operation, blood transfusion, nor intravenous drug abuse could be accounted for this high prevalence of HCV infection. No helpful supporting evidence for ethnic specificity was noted, either. Although a possible sexual transmission between spouses was observed, it is unlikely to be the main cause of the high prevalence in these aboriginal communities. We conclude that the unusually high prevalence of HCV infection observed in some aboriginal communities in Taiwan could be ascribed to poor anti-septic medical practice derived from insufficiency of medical personnel and facilities in these communities as compared with the other regions in Taiwan at the time before twenty years ago.

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INTRODUCTION

In Taiwan, an area with high endemicity of hepatitis B virus infection, chronic liver diseases (chronic hepatitis, cirrhosis and hepatocellular carcinoma) with more than 80% of patients associated with HBV infection are common and counted among the 10 leading causes of death (1-3). In the meantime, the epidemiological status of hepatitis C virus (HCV) infection is similar to other areas of the world, with 0.8% prevalence among adult volunteer donors and high prevalences among intravenous drug users, hemodialysis patients, cases positive for antibodies to human immunodeficiency virus, patients with chronic liver disease, and female prostitutes, determined with anti-C100 (4-15). However, all these data were obtained with the descendents of those having immigrated from the mainland China and been living in the developed plain region of western Taiwan since several hundred years ago, and no data concerning the aboriginal ethnic tribes residing in the underdeveloped mountain regions of this island have been obtained. In this paper, we tried to study the prevalence of HCV infection among aboriginal tribes in Taiwan and clarified the difference from our previous report, of which the study subjects were confined to the population living in the developed plain region of western Taiwan (13).

MATERIALS AND METHODS

Subjects: A total of 3,709 residents of five aboriginal communities were studied, including 1,523 adults and 2,186 subjects under the age of 19. They were 765, 479, 1,172, 426 and 867 from Tau-yuan, Juo-Hsi, Hsiu-Lin, Tah-Tuong and Nan-Au communities, respectively. The residents of the first two communities belong to Bunon tribe and those of the remaining three belong to Atayal tribe.

For a reference and comparison, subjects living in the developed plain region of western Taiwan, the descendents of those from mainland China, including normal population and high risk groups were studied. They consisted of 1,134 adult volunteer blood donors, 64 blood donors with abnormal alanine aminotransferase (ALT), 63 hemodialysis patients, 71 chronic hepatitis cases (58 HBsAg-negative and 13 HBsAg-positive), and 703 intravenous drug abusers.

More detail information including physical examination, blood chemistry (ALT, AST and GGTP) assay, and personal history were obtained from 82 subjects (60 adults and 22 children) of the residents in the Tau-Yuan community, the most isolated mountain village among the five communities. History of jaundice, alcohol consumption, history of illness, and other complaints were
obtained on face to face questioning with the subjects by a physician of our team. Physical characteristics of chronic liver diseases such as palmer erythema, spider angiomata, superficial venous engorgement, ascites, hepatomegaly, and lower leg pitting edema were examined.

Methods: The serum samples were separated immediately after drawing, and stored at 0 °C for 1-3 days, then stored at −80 °C until test.

Anti-HCV was tested with EIA-III (United Biomedical, Inc., Lake Success, NY) derived from immunodominant regions of both structural and nonstructural HCV proteins and anti-C100 (Ortho Diagnostics, Raritan, NJ) recombinant antigen (7,16). For markers for HBV infection, commercial kits from Abbott Laboratories (Chicago, IL) were used. All the tests were performed according to the manufacturer's instructions.

HCV-RNA was extracted from 200 μl of serum by the acid-guanidinium-phenol-chloroform (AGPC) method (17). During the extraction of RNA, glycogen was added as a carrier. Five microliters of the RNA solution was mixed with 1 μl of random primers (0.5 mg/ml) and it was heat-treated for 5 min at 70 °C. The cDNA was synthesized in a 20-μl solution for 90 min at 37 °C, after adding 40 units of RNAse inhibitor, 5X reaction buffer and 200 units of Mooney murine leukemia virus reverse transcriptase. An aliquot of cDNA reaction solution (5 μl) was directly amplified by polymerase chain reaction (PCR) with a pair of primers 464 S (5′-GGCTATACCGGACTTCGA-3’) and 526 A (5′-GACATGCATGTGATGATGTA-3’), encompassing the 623 base pairs (bp) (18). Amplification was done in a 100-μl reaction volume and on a DNA Thermal Cycler (Perkin Elmer Cetus, Norwalk, CN) for 5 min at 94 °C, 1 min at 50 °C and 1 min at 72 °C for 10 min. The products of the reaction were electrophoresed through a 2% agarose gel in tris-acetate-EDTA buffer and visualized by ethidium bromide staining. The amplified DNA fragment was then hybridized with the digoxigenin-labeled probe by the southern hybridization and the immunodetection was followed the procedure recommended by the manufacturer's instruction (Boehringer Mannheim, Biochemicals, Indianapolis, IN). The specific DNA probe for NS3 region was cut and eluted from the pUI 4f652 which was provided by T. Miyamura (National Institute of Health, Tokyo).

RESULTS

Unusually high prevalence of HCV infection among adult populations were obtained in three of the five aboriginal communities (Table I, Fig. 1). They were 35.1% (67/193), 15.2% (40/253) and 14.2% (71/501) for Tau-Yuan, Juo-Hsi and Hsiu-Lin communities, respectively. They were significantly higher than that
(1.1%) of the volunteer blood donors. Sex-specific prevalence of HCV infection studied in Tau-Yuan community showed no significant difference (M:F 31.8%: 34.9%). The prevalences in comparable adult populations from the other two communities and in the subjects under the age of 19 from all five communities were similar to that of the general population. None of the 82 residents (60 adults, 22 children) in the Tau-Yuan community had a history of definite icterus, though one case of chronic liver disease with icterus was found on physical examination. Most adults (90%) had history of heavy alcohol consumption (more than 80 g of alcohol per day for more than 10 years). Seven had hepatomegaly, while five showed higher elevation of GGTP than ALT, consistent with alcohol-induced liver damage. Abnormal serum ALT, AST or GGTP levels were noted in 16 of the 60 adult subjects, 10 and six cases each for sero-positive and negative anti-HCV subjects. No apparent difference was observed in the level of ALT or AST between sero-positive and sero-negative subjects for anti-HCV, nor was any correlation noted between HBV and HCV infections.
Table I. Prevalence of anti-HCV antibodies in aborigines in Taiwan*

<table>
<thead>
<tr>
<th>Community</th>
<th>(1)</th>
<th>(2)</th>
<th>(3)</th>
<th>(4)</th>
<th>(5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tau-Yuan</td>
<td>Juo-Hsi</td>
<td>Hsiu-Lin</td>
<td>Tah-Tuong</td>
<td>Nan-Au</td>
</tr>
<tr>
<td>Age (Yrs)</td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
<td>Number</td>
</tr>
<tr>
<td>20-39</td>
<td>16/ 85(18.8)</td>
<td>6/113(5.3)</td>
<td>10/205(4.9)</td>
<td>2/101(2.0)</td>
<td>5/181(2.8)</td>
</tr>
<tr>
<td>40-59</td>
<td>33/ 72(45.8)</td>
<td>19/ 85(22.4)</td>
<td>37/164(22.6)</td>
<td>4/ 77(5.2)</td>
<td>1/106(0.9)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>18/ 34(52.9)</td>
<td>15/ 55(27.3)</td>
<td>24/132(18.2)</td>
<td>2/ 35(5.7)</td>
<td>0/ 78(0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>67/191(35.1)*</td>
<td>40/253(15.8)*</td>
<td>71/501(14.2)*</td>
<td>8/213(3.8)</td>
<td>6/365(1.6)</td>
</tr>
</tbody>
</table>

0-19        | 8/574(1.4) | 7/226(3.1) | 9/671(1.3) | 4/213(1.9) | 6/502(1.2) |

*P<0.001 compared with general populations. #Determined with UBI-III diagnostic kit.

Table II. Positive rate of serum HCV-RNA detected by RT-PCR in an aboriginal community in Taiwan

<table>
<thead>
<tr>
<th>HCV-RNA +ve (%)</th>
<th>HCV-RNA -ve (%)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HCV +ve Cl00-3</td>
<td>34 (75.6)</td>
<td>11 (24.4)</td>
</tr>
<tr>
<td>UBI-III</td>
<td>37 (77.1)</td>
<td>11 (22.9)</td>
</tr>
<tr>
<td>Anti-HCV -ve Cl00-3</td>
<td>3 (10.3)</td>
<td>26 (89.7)</td>
</tr>
<tr>
<td>UBI-III</td>
<td>0 (0.0)</td>
<td>26 (100.0)</td>
</tr>
<tr>
<td>Total</td>
<td>37 (50.0)</td>
<td>37 (50.0)</td>
</tr>
</tbody>
</table>
Table III. Comparison of prevalence of HCV infection tested by anti-C100 (Ortho) and synthetic peptide antigens (UBI-III)

<table>
<thead>
<tr>
<th>Tests</th>
<th>Number Tested</th>
<th>Ortho +ve Number (%)</th>
<th>LBI +ve Number (%)</th>
<th>Either +ve Number (%)</th>
<th>LBI -ve Number (%)</th>
<th>Ortho -ve Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volunteer blood donors</td>
<td>1,134</td>
<td>8 (0.7)</td>
<td>12 (1.1)</td>
<td>19 (1.7)</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Hemodialysis patients</td>
<td>65</td>
<td>23 (35.4)</td>
<td>37 (56.9)</td>
<td>39 (60.0)</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>Chronic hepatitis HBsAg -ve</td>
<td>58</td>
<td>17 (29.3)</td>
<td>20 (34.5)</td>
<td>22 (37.9)</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>HBsAg +ve</td>
<td>13</td>
<td>1 (7.7)</td>
<td>2 (15.4)</td>
<td>2 (15.4)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>IVDUs</td>
<td>703</td>
<td>578 (82.2)</td>
<td>666 (94.7)</td>
<td>670 (95.3)</td>
<td>4</td>
<td>92</td>
</tr>
<tr>
<td>Aboriginal adults</td>
<td>220</td>
<td>53 (24.1)</td>
<td>74 (33.6)</td>
<td>79 (36.0)</td>
<td>5</td>
<td>26</td>
</tr>
</tbody>
</table>

IVDUs: Intravenous drug users.

In 37 (77.1%) of the 48 anti-HCV positive cases but none of the anti-HCV-negative cases, HCV-RNA was detected by RT-PCR (Table II).

The comparison of prevalence of anti-HCV positivity detected by both assay methods among volunteer donors and other high risk groups was shown for reference in Table III, which disclosed a higher sensitivity of synthetic peptide antigen assay than by anti-C100 assay.

**DISCUSSION**

An unusually high prevalences (35.1%, 15.5% and 14.2%) of HCV infection, which was confirmed by the detection of HCV-RNA in 37 (77.1%) of the 48 anti-HCV-positive sera and sequencing the nucleotide peptides of C/E and NS3 regions, were observed among adult populations in three of the five aboriginal
communities inhabited by Bunon and Atayal tribes, two of the nine aboriginal tribes in Taiwan.

On physical examination and history reviewing, we failed to obtain any finding or information on tattooing, sexual promiscuity, operation, or blood transfusion which could elucidate the high prevalence of HCV infection in these aboriginal communities. Most of the cases with hepatomegaly and impairment of the liver function could be ascribed to the habit of heavy alcohol consumption among the aborigines. Possible infection caused from a contaminated needle prick has also been taken into consideration, however, the same condition had been very common everywhere in Taiwan before 1980 when the use of disposable syringes began available in medical practice, and we have not observed such an unusually high prevalence elsewhere up to now. Therefore, this aspect of concerns seems to be negligible.

In the previous report, we disclosed that no serum cross-reactivity was present between HCV and flavivirus infections, dengue fever and Japanese encephalitis, so that the possibility of false positivity due to flavivirus infection could be ruled out (13). Wong (19) reported an apparently high prevalence (76.5%) of anti-HCV among the general populations in the Solomon Islands, and mentioned a long-term preservation of serum samples for the possible influencing factors for high anti-HCV positivity. Aceti (20) also reported a high rate of false-positive results (63%) in malaria patients attributing to non-specific reaction to serum anti-extractable nuclear antigen autoantibodies. In this study, the blood samples were kept at 4°C for the first 2 days after drawing, followed by storage at -80°C until use within four months; besides, none of the subjects was suffering from malaria; therefore, there would not be of any possible influence from long-storage of serum, nor non-specific reaction observed in malaria infection. In our laboratory, we have also observed of false positivities attributed to long storage of serum, especially, in those sera from cirrhosis and hepatocellular carcinoma cases when tested with the first generation kit of anti-C100, but not with synthetic peptide antigens (unpublished data).

Although Taiwan is a hyperendemic area of HBV infection and the HCV has the same blood-borne route of transmission as HBV, the epidemiology of these two hepatitis virus infections showed no correlation, because the chronic HBV infection in Taiwan has mostly come from vertical transmission, but vertical transmission in HCV infection has not been verified.

Cloning and sequencing 583 nucleotides of NS3 region from the isolate of one of the HCV-RNA-positive cases verified 93% and 97% homology for
nucleotides and amino acids, respectively, with that of Japanese type HCV (21). For clarifying the possibility of household transmission, serum anti-HCV and HCV-RNA detection were performed among 59 subjects, seven pairs of spouses and 45 members of eight families. The results showed that 27% (16/59) were positive for anti-HCV with detectable HCV-RNA in 10/16 and both of spouses were positive for anti-HCV in five of seven pairs of spouses. Comparison of nucleotide sequences (C/E and NS3, 1,184 nt) among three anti-HCV positive member of a seven-member family showed 2.6-3.5% and 1.5-2.5% heterogeneity among them, which is greater than that compared with another isolate from outside of this family (to be published). These results seem to suggest the likelihood of sexual transmission between spouses as reported by Tajima (22) but less possibility of other routes of horizontal intrafamilial transmission.

A high rate of detectable HCV-RNA among anti-HCV-positive cases and sequencing nucleotide peptides of C/E and NS3 regions confirm the fact of high prevalence of HCV infection among the aboriginal communities in Taiwan. The verification of high homology with a Japanese-type HCV would also suggest that the HCV infection in this aboriginal tribe is not a matter of an isolated unique origin. The results of intrafamilial study only suggest the possible transmission between spouses and no other ethnic specificity could be ascribed to.

The higher sensitivity for anti-HCV by synthetic peptide antigens assay than by anti-C100 assay is comprehensible because the former contains more conservative antigen from the C region in addition to antigen from the non-structure region.

It is concluded that because of lack of any particularly traceable risk factors, and the results of a greater anti-HCV-positive rate in the villages of less improved sanitation of underdeveloped region, we would rather ascribe the high prevalence of HCV infection among the aboriginal communities in Taiwan to poor antiseptic medical practice derived from insufficiency of medical personnel and facilities in these aboriginal communities as compared with the other regions in Taiwan before twenty years ago. Although a possible sexual transmission between spouses was observed, it is unlikely to be the main cause of high prevalence in these aboriginal communities.
REFERENCES


