A Ten Year Serological Survey of Hepatitis A, B and C Viruses Infections in Nepal

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Background: In 1987, we reported that the prevalence of hepatitis B virus (HBV) and hepatitis C virus (HCV) infection in Nepal was low, as compared to hepatitis A virus (HAV) infection, and that no human T-lymphotropic type-1 (HTLV-1) infection was found in Nepal.

Objectives: To determine changes in the prevalence of HAV, HBV, and HCV infections between 1987 and 1996 in inhabitants of Bhadrakali (suburban) and Kotyang (rural) villages in Nepal.

Study design: We did a cross-sectional survey of 458 inhabitants of two Nepalese villages, to assess the prevalence of antibody to HAV (anti-HAV), antibody to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg), antibody to HCV (anti-HCV), and antibody to HTLV-I (anti-HTLV-I).

Results: Anti-HAV was detected in 454 (99.1%), HBsAg in 5 (1.1%), anti-HBc in 33 (7.2%) and anti-HCV in 8 (1.7%) of serum samples tested in 1996. Statistically significant differences by gender or age group were nil. The prevalence of HCV infection was significantly higher in 1996 than in 1987 after adjusting for age of subjects living in the two villages (p<0.01). The prevalence of HBsAg was significantly higher in 1996 than 1987 in Bhadrakali after adjusting for the factor of age (p<0.05). Between 1987 and 1996, evidence for HTLV-1 positive residents was nil.

Conclusion: These results suggest that HAV has been endemic in Nepal for long time while not of HBV, and that HCV infection tends to be increased recently.

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Hepatitis A virus (HAV) infection is transmitted primarily by the fecal oral route 2. The prevalence of HAV infection is influenced mainly by general hygiene, especially in relation to toilet facilities, water supplies, and food preparation, factors reflecting living standards and socioeconomic status 2,3.

Hepatitis B virus (HBV) 4-5, hepatitis C virus (HCV) 6-8, and human T lymphotropic virus type 1 (HTLV-1) 9,10 are spread by infected blood, sexual intercourse, and transmission from mother to child 10,11. These viruses can either be latent or persistent, and are the most significant viruses causing malignant diseases, including hepatocellular carcinoma caused by HBV and HCV 12 and adult T-cell leukemia/lymphoma associated with HTLV-1 13 and which is characterized by striking geographic clustering in limited areas. In 1987, we reported that the prevalences of HBV and HCV infections in Nepal was low in comparison with HAV infection, and that no HTLV-1 infection was never detected in this population 14.

The present study concerned an assessment of changes in the prevalence of HAV, HBV, and HCV infections since 1987. A cross sectional seroepidemiologic study was done among the residents of these same two villages in Nepal.

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MATERIALS AND METHODS

Study Population
The present study was a cross-sectional survey of inhabitants of two villages in Nepal. Between August and September 1996, 458 inhabitants (Mean age 43.1 years; range 15-90 years), including 222 from Bhadrakali (112 males, 110 females; mean age 46.3 years) and 236 from Kotyang (117 males, 119 females; mean age 39.8 years) were available for study. We examined HAV, HBV, HCV and HTLV-1 markers in these areas between 1987 and 1996. The results of surveys carried out on the two villages of Nepal in 1987 were documented\(^{10}\). Blood samples were screened for HAV, HBV, HCV and HTLV-1 infections. The two villages are about 1,200 km above sea level and 75 km apart. Kotyang, located on the eastern side of a mountain separating Kathmandu and Sun Chose Valleys, is an isolated rural village about 30km east of Kathmandu, and the inhabitants have to walk many hours to reach the nearest roads. Most residents are engaged in agriculture and the population is approximately 1500. In this village, no dwelling has treated municipal water; all the villagers drink spring water and there is no sewage system. Bhadrakali is located on terraces that extend downward from Burhanikanth to Kathmandu and is easily accessible by public transportation. The main occupation is agriculture, but some villagers are office workers. The population is approximately 3900. This village has both piped and tanked water and there exists a simple sewage system. The boiling of drinking water is not customary in either village\(^{15}\). The study protocol, including blood sampling, was explained by Nepalese doctors to all subjects, the purpose was explained to leaders of the villages and their consent was obtained prior to start of the survey. A study had been done to assess health status of the residents and to determine the prevalence of hypertension and the possible factors influencing the pathogenesis of hypertension\(^{16,17}\).

Approximately 10ml of blood was withdrawn from subjects in a supine position following an overnight fast. After centrifugation, the sera were frozen within a few hours, stored at -20°C and kept frozen until just before testing. The frozen serum kept in freezer was transported to Japan in dry ice.

Laboratory Methods
Anti-HAV was tested using radioimmunoassay methods (HAVAB, Abbott Laboratories, North Chicago, IL). Serum samples were tested for HBsAg by passive hemagglutination (MyCell, Institute of Immunology Co., Tokyo, Japan) and anti-HBc was tested using commercially available RIA (CORAB, Abbott Laboratories, North Chicago, IL). Anti-HCV was assayed using a second-generation passive hemagglutination assay (HCV PHA; Abbott, North Chicago, IL). Anti-HTLV-1 was examined using a gelatin particle agglutination test kit (SERODIA ATLA, Fujirebio, Tokyo), and specimens reactive by the particle agglutination test were confirmed by indirect immunofluorescence assay using MT-1 cells\(^{18}\).

Statistical Analysis
Differences in frequencies were compared using chi-square test as appropriate, and the Cochran-Armitage’s test was used to determine the relationship between increase or decrease in the prevalence of disease with advancing age. The Mantel-Haenszel test was used to compare the overall prevalence in the two groups in which the distribution differed. For all tests, a p value of less than 0.05 was considered to have statistical significance.

RESULTS
The age and sex-specific prevalences of anti-HAV, HBsAg, anti-HBc and anti-HCV in 1996 are shown in TABLE 1. Anti-HAV was detected in 454 (99.1%), HBsAg in 5 (1.1%), anti-HBc in 33 (7.2%) and anti-HCV in 8 (1.7%). Statistically significant differences by gender or age group were nil. No one in either village was positive for anti-HTLV-1.

<table>
<thead>
<tr>
<th>Age group</th>
<th>No. tested</th>
<th>Male Anti-HAV (%)</th>
<th>Male HBsAg (%)</th>
<th>Male Anti-HBc (%)</th>
<th>Male Anti-HCV (%)</th>
<th>Female Anti-HAV (%)</th>
<th>Female HBsAg (%)</th>
<th>Female Anti-HBc (%)</th>
<th>Female Anti-HCV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤24</td>
<td>44</td>
<td>43 (97.7)</td>
<td>1 (2.3)</td>
<td>1 (2.3)</td>
<td>43 (100.0)</td>
<td>1 (2.3)</td>
<td>1 (2.3)</td>
<td>0</td>
<td>87 (86.9)</td>
</tr>
<tr>
<td>25-34</td>
<td>40</td>
<td>37 (92.5)</td>
<td>2 (5.0)</td>
<td>1 (2.5)</td>
<td>37 (92.5)</td>
<td>1 (2.5)</td>
<td>2 (5.0)</td>
<td>0</td>
<td>77 (77.0)</td>
</tr>
<tr>
<td>35-44</td>
<td>49</td>
<td>41 (83.7)</td>
<td>1 (2.0)</td>
<td>1 (2.0)</td>
<td>41 (83.7)</td>
<td>1 (2.0)</td>
<td>1 (2.0)</td>
<td>0</td>
<td>90 (90.0)</td>
</tr>
<tr>
<td>45-54</td>
<td>29</td>
<td>24 (82.8)</td>
<td>2 (6.9)</td>
<td>0</td>
<td>24 (82.8)</td>
<td>2 (6.9)</td>
<td>0</td>
<td>73 (72.3)</td>
<td></td>
</tr>
<tr>
<td>55-64</td>
<td>29</td>
<td>28 (96.6)</td>
<td>5 (17.2)</td>
<td>0</td>
<td>28 (96.6)</td>
<td>5 (17.2)</td>
<td>0</td>
<td>68 (68.9)</td>
<td></td>
</tr>
<tr>
<td>≥65</td>
<td>38</td>
<td>25 (65.8)</td>
<td>7 (18.4)</td>
<td>0</td>
<td>25 (65.8)</td>
<td>7 (18.4)</td>
<td>0</td>
<td>63 (63.2)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>228</td>
<td>226 (99.6)</td>
<td>17 (7.4)</td>
<td>6 (2.6)</td>
<td>226 (99.6)</td>
<td>17 (7.4)</td>
<td>6 (2.6)</td>
<td>226 (99.6)</td>
<td>17 (7.4)</td>
</tr>
</tbody>
</table>

The overall prevalence of HAV in 1996 (99.1%) was nearly as high as in Table 1.
as in 1987 (99.3%). There were no significant differences in the overall anti-HBc and HBsAg prevalences between 1996 (1.1%, 7.2%) and 1987 (0.3%, 7.5%).

The overall anti-HCV prevalence was significantly higher in 1996 (1.7%) than in 1987 (0.1%)(p<0.01; Mantel-Haenszel test). Although there was no evidence of anti-HCV in the subjects under 55-64 years in 1987, there were a few cases of anti-HCV all age groups, except for the ≥65 age group in 1996. Statistically significant differences for these infections by age group were nil.

TABLE 3 shows a comparison of the prevalence of the tested viral markers between in 1987 and 1996. There were no significant differences in the prevalence of HAV, HBV and HCV infections between the two village in 1987 or in 1996. Although anti-HCV did increase in both villages, there were no significant differences between age groups.

In Bhadrakali, the prevalence of HBsAg was significantly higher in 1996 (2.3%) than in 1987 (0.3%)(p<0.05; Mantel-Haenszel test). Moreover, the prevalence of HBsAg in 1987 and 1996 was 0.5% (1/188) and 0% in residents under 34 years age and 3.0% (2/66) and 3.9% (3/77) in those over 55 years age, respectively, but the difference was not statistically significant. Thus, the prevalence of HBsAg tended to increase in the younger age groups (under 34 years age) and in the older age groups (over 55 years age) in 1996, as compared to findings in 1987. Moreover, there was no significant difference in the prevalence of anti-HBc between 1987 and 1996. In Kotyang, the prevalence of HBsAg and anti-HBc decreased from 0.4% and 7.8% in 1987 to 0% and 4.2% in 1996, but with no statistical significance.

### Table 2. Age and sex-specific prevalence of anti-HAV, HBsAg, anti-HBc and anti-HCV between 1987 and 1996 in Nepal.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>1987</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>Anti-HAV %</td>
</tr>
<tr>
<td>≤ 24</td>
<td>145</td>
<td>100.0</td>
</tr>
<tr>
<td>25 - 34</td>
<td>179</td>
<td>99.4</td>
</tr>
<tr>
<td>35 - 44</td>
<td>125</td>
<td>99.2</td>
</tr>
<tr>
<td>45 - 54</td>
<td>118</td>
<td>97.5</td>
</tr>
<tr>
<td>55 - 64</td>
<td>72</td>
<td>100.0</td>
</tr>
<tr>
<td>≥ 65</td>
<td>37</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>676</td>
<td>99.3</td>
</tr>
</tbody>
</table>

* p<0.01(Mantel-Haenszel test; 1987 vs 1996)

### Table 3. Age and sex-specific prevalence of anti-HBc and anti-HCV between Bhadrakali and Kotyang between 1987 and 1996, in Nepal.

<table>
<thead>
<tr>
<th>Age group</th>
<th>1987</th>
<th>1996</th>
<th>1987</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested</td>
<td>HBsAg %</td>
<td>Anti-HBc %</td>
<td>Anti-HCV %</td>
</tr>
<tr>
<td>≤ 24</td>
<td>84</td>
<td>0.0</td>
<td>5.8</td>
<td>0.0</td>
</tr>
<tr>
<td>25 - 34</td>
<td>104</td>
<td>1.0</td>
<td>4.7</td>
<td>0.0</td>
</tr>
<tr>
<td>35 - 44</td>
<td>73</td>
<td>0.0</td>
<td>5.4</td>
<td>0.0</td>
</tr>
<tr>
<td>45 - 54</td>
<td>68</td>
<td>0.0</td>
<td>11.7</td>
<td>0.0</td>
</tr>
<tr>
<td>55 - 64</td>
<td>43</td>
<td>0.0</td>
<td>13.9</td>
<td>0.0</td>
</tr>
<tr>
<td>≥ 65</td>
<td>22</td>
<td>0.0</td>
<td>4.4</td>
<td>4.5</td>
</tr>
<tr>
<td>Total</td>
<td>394</td>
<td>0.3*</td>
<td>7.6</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* p<0.05(Mantel-Haenszel test; 1987 vs 1996)
DISCUSSION

This study revealed serological evidence of a high prevalence of HAV infection in contrast to a low prevalence of HBV infection between 1987 and 1996. Differences in anti-HAV prevalence among countries is probably related to differences in environmental and socioeconomic conditions. We reported that the prevalence of anti-HAV in Okinawa 30 years ago was significantly higher than in Kyushu, Japan. We also reported that fewer subjects had HAV infection as sanitary conditions had improved, a decision based on analysis using a catalytic model. Therefore, our results suggest that hygienic and sanitary conditions may have facilitated the fecal-oral spread of HAV infection not seem to have been much improved in Nepal since 1987.

In developed nations, HBV infection is usually acquired through parenteral, sexual, or perinatal exposure. However, horizontal transmission is predominant in the less developed nations, though changes in customs, lifestyle, and socioeconomic conditions may influence the relative contributions of the different modes of HBV transmission. Although overall HBV infection did not change as did HAV infection from 1987 to 1996, the prevalence of HBV infection in Bhadrakali significantly increased compared to that in Kotyang, which is a more isolated area. Bhadrakali is located in a suburban area near Kathmandu and is readily accessible by public transport. Thus, many people earn their living in Kathmandu. There are more opportunities for transmission by the percutaneous route as a result of medicine interventions, drug use or tattooing, and sexual intercourse than in Kotyang, which is a much more isolated area. We reported that HBV infection among nursery school children was markedly reduced in Okinawa, Japan. This is attributed to a combined administration of hepatitis B immunoglobulin and hepatitis B vaccine which protects against maternal transmission. This approach to immunization may be effectively in these villages in Nepal. Because our study was a cross-sectional survey and may have included bias, a cohort study will be necessary for evaluation of the prevalence of HBV infection.

Major routes of HCV transmission are percutaneous exposure to blood through blood transfusion or contaminated needles. We found the prevalence of HCV infection to be significantly increased from 1987 to 1996. Although the anti-HCV prevalences in each villages tended to increase in different age groups, this trend was not statistically significant, possibly because numbers were too few. We reported that medical interventions probably played a more important role in the spread of the HCV infection in a village in Japan than did familial transmission. Our results suggest that percutaneous medical treatment has increased in these villages in Nepal. However, the question of whether or not HCV is actually transmitted by the percutaneous route as a result of medical treatment or drug use or by tattooing in these villages is difficult to answer. Further study with a longer observation period is required to show the precise route of HCV infection.

The HTLV-1 virus has two major routes of transmission, percutaneous exposure via blood transfusion and familial transmission (mother-to-child transmission via breast feeding and male-to-female transmission through sexual contact). We found no anti-HTLV-1 positive inhabitants and HTLV-1 has not been spread to the surveyed area in Nepal.

These villages in Nepal had a continuously high prevalence of HAV infection, in contrast to HBV infection, as seen in 1987 and 1996. Moreover, the results of this study showed that HCV infection significantly increased, indicating that there is a high risk of HCV infection.

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REFERENCES


