Hepatitis C virus infection in urban and rural Natal/KwaZulu

S. S. ABDOOL KARIM, D. R. TAIT

Abstract This study was undertaken to estimate the prevalence of hepatitis C virus (HCV) infection in urban and rural blacks in Natal/KwaZulu. Sera from representative community-based samples comprising 176 urban and 441 rural black adults were tested for the presence of anti-HCV. The prevalence of HCV infection was 1.7% (95% confidence interval 0 - 3.6%) among urban and 0.9% (95% confidence interval 0.1 - 1.7%) among rural blacks. Four (0.9%) of the 466 subjects with evidence of hepatitis B virus (HBV) infection and 3 (2%) of the 151 with no evidence of HBV infection were anti-HCV-positive. The prevalence of HCV infection was low in contrast to the high prevalence of HBV infection among urban and rural blacks in Natal/KwaZulu. This suggests that HCV does not have the same main routes of transmission as HBV in this region. Larger scale studies are needed to explore this hypothesis.


Hepatitis C virus (HCV) is considered an important cause of non-A, non-B hepatitis.1 After extensive research4 which demonstrated that HCV is transmitted via blood and blood products, routine blood donor screening has been instituted in several countries. However, common risk factors for HCV and hepatitis B virus (HBV) infection extend beyond blood transfusions to include haemodialysis,5 intravenous drug use,6 sexual contact and health care employment.7 Although there are no reliable data on non-A, non-B hepatitis, the high prevalence of HBV infection in Natal/KwaZulu8 (4.4% among females and 7.1% among males) suggests that HCV infection is also likely to be common in this region. An indication of the importance of blood transfusions as a mechanism for the spread of HCV is the 3% prevalence of HCV infection among black blood donors in Natal/KwaZulu. It was undertaken to assess the feasibility of a larger project to study transmission mechanisms responsible for the spread of HCV in the community.

Materials and methods

In 1985, community-based studies to estimate the prevalence of HBV infection among black adults were undertaken in Mseleni, a rural area,9 and in Umlazi, an urban township.

Urban sample

Umlazi, a large black township just south of metropolitan Durban, comprises 26 sections each denoted by a letter of the alphabet. In 3 randomly selected sections, sequential sampling was used to identify a total of 200 houses to be visited. Houses were visited during working hours and over weekends. If no one was present at a selected house after two visits, then the next nearest house was visited. One randomly selected person over the age of 12 years from each house was invited to participate in this study. After consent was obtained, demographic information was recorded and 5 ml blood were collected by means of antecubital venepuncture. The blood specimens were centrifuged and tested for hepatitis B surface antigen (HBsAg) and antibodies to hepatitis B core antigen (anti-HBc) within 72 hours of being collected (AUSRIA and CORAB; Abbott Laboratories, Chicago, USA).

Rural sample

Mseleni, a rural area in northern Natal/KwaZulu, had approximately 1 000 households in 1985.10 About 60% of these were accessible by means of a 4-wheel drive vehicle. At these households one randomly selected person above the age of 12 years was invited to participate in the study. A total of 441 subjects agreed to participate. After consent was obtained, they were interviewed and a 5 ml tube of blood was drawn. Blood specimens were centrifuged within 24 hours and the sera stored at -20°C until transported to Durban on dry ice for HBV serological tests.

Testing for anti-HCV

After tests for HBV serological markers were performed, the sera were stored at -20°C until they were tested for anti-HCV in June 1991. Two commercially available enzyme immunoassay (EIA) kits were used and the manufacturer's instructions were followed (Abbott HCV EIA — 2nd generation and HCV Neutralisation EIA; Abbott Laboratories, Chicago, USA). All specimens found to be reactive by HCV EIA initially were retested. Repeatedly reactive specimens were then subjected to the HCV Neutralisation EIA. The manufacturer's criteria were used to interpret the results of HCV Neutralisation EIA. Specimens which produced a positive result on the HCV Neutralisation EIA were regarded as anti-HCV-positive; the remainder were regarded as false-positives.

Statistical tests

EPI-INFO Version 5 was used to analyse the data. χ²-tests and 95% confidence intervals (CI) were calculated by means of standard methods.
Results

The prevalence of HCV infection was 1.7% (CI 0 - 3.6%) among urban and 0.9% (CI 0.1 - 1.7%) among rural blacks. This finding was not statistically significant.

Twenty-four of the 200 subjects from Umlazi declined to participate, giving a response rate of 88% for the urban sample. Thirty-five (19.9%) of the 176 specimens from rural subjects were repeatedly reactive on HCV EIA. Of these, only 4 produced positive HCV Neutralisation EIA results, in 3 women aged 50, 52 and 65 years.

The response rate for the rural sample could not be calculated accurately but was estimated to be between 60% and 70%. Thirty-eight (8.6%) of the 441 specimens from rural subjects were repeatedly reactive on HCV EIA. Of these, only 4 produced positive HCV Neutralisation EIA results, in 3 women aged 50, 52 and 62 years and a 75-year-old man.

Women comprised 59.7% and 77.6% of the urban and rural subjects respectively. The mean age of the urban subjects was 34.2 years (14.4, range 13-69) and that of the rural subjects 43.9 years (16.9, range 13-78).

Five (2.8%) of the 176 urban subjects were HBSAg-positive while another 100 subjects (56.8%) had evidence of past HBV infection. Evidence of past HBV infection was present in 81.9% of rural subjects.

When data from urban and rural subjects were viewed jointly, 4 (0.9%) of the 466 subjects with evidence of HBV infection (current and past) and 3 (2%) of the 151 with no evidence of HBV infection were anti-HCV-positive, a finding not of statistical significance.

Discussion

Compared with the high prevalence of HBV infection, HCV infection is relatively uncommon in both urban and rural areas of Natal/KwaZulu. The prevalence of HCV infection in urban (1.7%) and rural (0.9%) areas of Natal/KwaZulu is similar to the 0.7% and 0.9% reported in studies involving 25,137 French11 and 11,117 Iranian12 blood donors, respectively. Further, the prevalence of HCV infection in Natal/KwaZulu is similar to the 0.6% reported from Ga-Rankuwa, Bophuthatswana.13

While the small number of anti-HCV-positive subjects prevented more detailed analysis of the association between HCV and HBV infections in this survey, anti-HCV was not more common among patients with HBV infection. Our failure to demonstrate an association between HBV and HCV infections in this survey implies that HCV may not share the as yet unknown predominant risk factors responsible for the rampant spread of HBV infection in these communities.

Because this was a serological survey, risk factors for HCV were not specifically investigated. Other studies have identified several risk factors for HCV infection and high prevalences of HCV infection have been documented among haemophiliacs13 and patients on haemodialysis14 in South Africa. Other risk groups for HCV infection have been identified on the basis of parenteral or sexual transmission, and include homosexuals, prostitutes, prisoners, institutionalised mentally retarded subjects and attenders at sexually transmitted disease clinics.14

Infected blood and blood products are a major transmission mechanism for HCV.14 In a follow-up study of transfusion recipients, Alter et al.1 demonstrated that HCV could be transmitted via infected blood and that anti-HCV remained detectable in blood for a long period after HCV infection. However, a survey in the Orange Free State did not detect anti-HCV in any one of 35 multitransfused patients,16 a finding which may be a result of the small number of subjects studied.

Heterosexual contact is considered a risk factor for HCV since heterosexual partners of intravenous drug users have been shown to be at risk.17 The prevalence of HCV infection among antenatal clinic attenders in Spain18 was 1.2% and in Australia19 0.4%. Two small non-random samples of 100 and 25 antenatal clinic attenders from Natal20 and the western Cape21 respectively, did not find any anti-HCV-positive subjects.

A survey22 comparing 216 hospitalised rural black men and 298 urban black donors found a higher, though not statistically significant, prevalence of HCV in the hospitalised patients (3.8% v. 1.2%). The trend in our study, which was also not statistically significant, was in the opposite direction (0.9% v. 1.7%). In the absence of statistical significance, the possibility that there is no difference between urban and rural blacks cannot be excluded: this makes the results of both studies difficult to interpret.

The false-positive rate on the HCV EIA was 91.4% (32/35) in the urban sample and 89.5% (34/38) in the rural sample. Heat inactivated and repeatedly frozen and thawed specimens have been found to produce false positives.9 Although the sera we tested had been thawed once only in the case of the urban sample and twice in the case of the rural sample, the number of false positive results was high and possibly associated with the prolonged storage of the sera.9 Confirmatory testing was therefore considered9-20,21 in the determining of anti-HCV status.

In conclusion, representative community-based samples from urban and rural blacks in Natal/KwaZulu indicate that the prevalence of HCV infection in this region is low. The contrast between this and the high prevalence of HBV infection in this region suggests that HCV does not have the same main routes of transmission as HBV. Larger scale studies, in which sufficient numbers of anti-HCV-positive subjects can be recruited, are needed to explore this hypothesis.

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REFERENCES

The persistence of hepatitis B antigen in the bloodmeal of the potential medicinal leech, *Asiaticobdella buntonensis*

G. B. WILKEN, C. C. APPLETON

**Abstract**

The persistence of the hepatitis B virus surface antigen (HBsAg) was used as an index of the survival time of this virus within the gastro-intestinal tract of the potential southern African medicinal leech, *Asiaticobdella buntonensis*. HBsAg was tested for in blood/faecal material at five intervals over 15 weeks. Samples from both the midgut and the rectum remained positive for the entire test period, although with decreasing strength. The results are compared with reports on other arthropods which indicate increasing antigen persistence with increasing body size. The findings implicate medicinal leeches as mechanical vectors of HBV and possibly of other medically important viruses, and argue against using leeches of suspect or unknown origin in the alleviation of venous congestion in failing microsurgical procedures.


Since the medicinal leech came back into use to alleviate venous congestion in failing microsurgical procedures, there have been several reports of leech-related nosocomial infections, with *Aeromonas hydrophila* the causative agent. Bacteriological investigation of the gut flora of leeches has been performed on *Hirudo medicinalis* and other bacteria, notably waterborne varieties, have been isolated, but not as yet implicated in leech-related infections. However, the presence of diverse gut flora in medicinal leeches and their occasional role in leech-associated infection reveal little about the risk of cross-infection between patients with infective agents associated with the previous bloodmeal. For this reason, and because of limited data on the fate of viruses in the bloodmeal of leeches, the persistence of hepatitis B surface antigen (HBsAg) in the bloodmeal of the leech, *Asiaticobdella buntonensis*, was investigated.

Hepatitis B virus (HBV) was chosen because its hardness makes it a likely candidate for transmission by leeches, and one against which the persistence of less robust viruses can be inferred. In addition, HBV's persistence and potential for transmission by various arthropods have been studied by several authors, making for interesting comparisons with regard to the persistence of this virus in sanguivorous leeches.

**Material and methods**

Leeches that weighed between 0.50 g and 1.0 g, housed under laboratory conditions (temperature 26 + 1°C, 12:12 hours light/dark) without a bloodmeal for more than 4 months were fed HBV-infected blood through a membrane feeder until satiated. Blood was obtained from the Natal Blood Transfusion Services and its HBsAg-positivity confirmed by radio-immunooassay (RIA). Positivity was expressed using the positive ratio (specimen count/cut-off value). The leeches were divided into batches of five, and housed separately in plastic ice-cream containers covered with fine gauze netting.

A pilot study revealed that HBsAg remained strongly positive for more than 28 days. In the present study, batches were killed by freezing at intervals of 3, 6, 9, 12 and 15 weeks (21, 42, 63, 84 and 105 days) respectively so as to document the duration of the viral marker more completely. The gut was exposed by a mid-dorsal incision and swabs of blood/faecal material were taken from the midgut and the rectum. The swabs were eluted in 1% bovine serum albumin (BSA) (pH 7.0) and tested for HBsAg.

**Results and discussion**

The survival time of the HBsAg in the bloodmeal of *A. buntonensis* is shown in Table I. Because the gut contents of most of the leeches (48/50) remained positive for HBsAg as determined by RIA for the duration of the trial, the results were broken down according to the categories used by the Department of Virology at King Edward VIII Hospital, Durban. These are negative (-), weak positive (+), strong positive (+++) and very strong positive (++++).