The prevalence of antibodies to hepatitis C virus at two haemodialysis units in South Africa

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The prevalence of antibodies against hepatitis C virus (HCV) was determined in 103 haemodialysis patients who attended two dialysis units in South Africa. With the use of a second-generation enzyme-linked immunosorbent assay (UBI HCV EIA, Organon Teknika, The Netherlands) and a 4-recombinant immunoblot assay (Chiron Corporation, USA), antibodies to HCV were found in 22 patients (21%). Statistically significant associations with anti-HCV carrier status were duration of dialysis (P = 0,0005) and number of blood transfusions received (P = 0,008). With stepwise logistic regression analysis it was not possible to separate the effects on HCV status associated with these two variables. A transient elevation in alanine aminotransferase (ALT) occurred in 8 of the 22 anti-HCV-positive patients, compared with 14 of the 81 anti-HCV-negative patients (P = 0,054). As yet, no patients have clinical evidence of ongoing liver disease or persistently elevated ALT levels. Of the 45 dialysis staff members tested, none was positive for anti-HCV.

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Regular screening of all dialysis patients for hepatitis B and isolation of positive patients remains a crucial factor in the prevention of spread of hepatitis B in haemodialysis (HD) units. Viral hepatitis, however, remains a health hazard in HD units and since the successful cloning of the HCV genome[1] and subsequent development of solid-phase assays for the detection of antibodies to HCV (anti-HCV), this virus has been identified as the main culprit.

With the use of the first-generation HCV C100-3 antibody test, reports of prevalence in unselected HD patients varied from 0% in a German unit[2] to 40,4% in Saudi Arabia,[3] with most units reporting between 20% and 30%. Concern has subsequently been voiced about both the sensitivity[4] and specificity[5] of the assay used initially which is based on a non-structural viral protein. Second-generation enzyme-linked immunosorbent assays (ELISAs) developed to identify additional antigens have proved more sensitive and specific[4,6] and a four-antigen recombinant immunoblot assay (RIBA) HCV test system, which detects antibodies to four separate hepatitis antigens, is gaining acceptance as a confirmatory test for HCV positivity.[7]

The aim of this study, which is the first published report from sub-Saharan Africa, was to determine the prevalence of HCV antibodies in two large HD units in the Western Cape by means of a second-generation ELISA and the RIBA assay. The association of anti-HCV with previous blood transfusions, duration of HD and liver dysfunction was also investigated.

Patients and methods

One hundred and three patients, who had been on HD for longer than 1 month, and 45 staff members were studied. After informed consent had been obtained, serum samples were taken and stored at –70°C or less until assayed for anti-HCV. At the time of the study routine HD at the two hospitals (Groote Schuur and Tygerberg) consisted of a 4-hour dialysis session 3 times a week that entailed the use of disposable cuprophane dialysers and acetate dialysate in the majority of patients. Fifty-five patients attended Groote Schuur Hospital and 48 attended Tygerberg Hospital. The mean age of the group was 42 years (range 30 - 79 years) and it consisted of 53 men and 50 women. Four patients were HbsAg-positive and 1 HIV-positive. The median duration of HD was 48 months (range 1 - 271). Ninety-four patients (91%) had received blood transfusions (median 11 units; range 1 - 115). Previous transplant history was also recorded. A total of 45 staff members who worked in the two dialysis units were also tested for anti-HCV.

Antibodies to HCV were detected with a commercial ELISA (UBI HCV EIA, Organon Teknika, The Netherlands). Positivity for HCV antibodies was defined as an absorbance value in the serum sample well reproducibly greater than the calculated cut-off value. The presence of antibodies was confirmed with a 4-RIBA assay (Chiron Corporation, USA).[7]

HBsAg was measured by radio-immunoassay (Ausria, Abbott Laboratories, North Chicago, USA). ALT levels were measured with standard auto analyser techniques and an ALT level more than twice the upper limit of normal for the two laboratories was regarded as abnormal.

Statistical analysis was performed by applying the Mann-Whitney test for differences in location of two distributions and a χ^2-test for 2 x 2 table of frequencies. Stepwise logistic regression analysis was performed to examine the effects of transfusion and of duration of dialysis between the anti-HCV-positive and negative groups. Programmes 3S, 4F and LR of the BMDP suite (Biomedical Data Processing Statistical Software, University of California, Berkeley, 1983) were used in this analysis.

Results

Twenty-four of the 103 patients screened tested positive for anti-HCV with ELISA, and 2 of these were negative on the confirmatory RIBA test. The prevalence of anti-HCV was

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therefore 21% and was similar in the two units (Groote Schuur Hospital 20% and Tygerberg Hospital 23%). The 4 patients (4%) who were positive for HBsAg and the HIV-positive patient were all negative for anti-HCV. The clinical characteristics of HD patients according to anti-HCV status is shown in Table I. There were no significant differences in age and sex between the two groups, although 9 of the 11 positive patients at Tygerberg Hospital were male ($P = 0,08$). No staff members were anti-HCV-positive.

Table I. Clinical findings in 103 haemodialysis patients

	ANTI-HCV+ ($N = 22$)	ANTI-HCV– ($N = 81$)	*P*-value
Male/female	13:9	40:41	NS
Age, years (mean and range)	44 (23 - 79)	42 (20 - 75)	NS
Months on dialysis (mean and range)	75 (3 - 196)	37 (1 - 271)	0,0005
No. of blood transfusions (mean and range)	18 (2 - 115)	9 (0 - 76)	0,008
Previous transplant (No. and %)	6 (27,3%)	33 (40,7%)	NS

The Mann-Whitney test for significant differences in median number of blood transfusions confirmed that the anti-HCV-positive group had generally had more transfusions than the anti-HCV-negative group ($P = 0,008$). In addition, the anti-HCV-positive group had been on dialysis for a higher median number of months ($P = 0,0005$). There was no statistical evidence that either group had had a greater number of renal allografts ($P = 0,3$). The prevalence of anti-HCV antibodies in relation to blood transfusions and duration of dialysis is shown in Table II.

Table II. Cumulative prevalence of anti-HCV antibodies (ELISA and RIBA) in relation to both number of blood transfusions and duration of haemodialysis

No. of blood transfusion	Duration of haemodialysis (months)			
	0 - 6	7 - 25	> 25	Total
0	0/4 (0)	0/2 (0)	0/3 (0)	0/9 (0)
1 - 10	1/6 (16,7)	1/13 (7,7)	6/20 (30)	8/39 (20,5)
11 - 20	0/1 (0)	0/4 (0)	4/22 (18,2)	4/27 (14,8)
>20	0/0 (0)	0/1 (0)	10/27 (37)	10/28 (35,7)
Total	1/11 (9,1)	1/20 (5)	20/72 (27,8)	22/103 (21,4)

No. anti-HCV-positive/total number in group (%).

The presence of anti-HCV antibodies was taken as the binary response variable for the stepwise logistic regression analysis. The explanatory variables available were the number of blood transfusions, months on dialysis and renal transplants received for each patient. In this data set the number of transfusions and the number of months on dialysis were strongly related, so that it was not possible to separate the effects on HCV status associated with these variables. HCV status was examined by means of the ELISA and the RIBA test results with and without the 4 patients who were HBsAg-positive and the 1 patient who was HIV-positive, as these 5 patients were dialysed in isolation. In all of these analyses it appeared to be necessary and sufficient to use either months on dialysis or number of transfusions as an explanatory variable for HCV status. No additional benefit from using both variables achieved statistical significance at the 5% level. Months, however, seemed a slightly better predictor than transfusions in all but 1 case.

The relationship of anti-HCV antibody positivity to ALT and HbsAg is shown in Table III. Eight (36%) of the 22 anti-HCV-positive patients had a single rise in ALT to over twice the upper limit of normal compared with 14 (17%) of the anti-HCV-negative patients ($\chi^2 = 3,75$; $P = 0,054$). In only 1 patient was an abnormal rise in ALT associated with a clinically significant episode of hepatitis. Multiple abnormal ALT elevations, characteristic of HCV infection, occurred in only 1 patient. In addition, no patient had persistently elevated ALT levels for longer than 6 months.

Table III. Relationship with alanine aminotransferase activity and HbsAg status

	ANTI-HCV+ ($N = 22$)	ANTI-HCV– ($N = 81$)
Abnormal ALT No. of patients		
Single peak	8 (36%)	14 (17%)
Multiple peaks	1	0
Persistently raised	0	0
HBsAg-positive	0	4

Discussion

The prevalence of anti-HCV-positive patients in our two HD units (21%) confirms that dialysis patients are at high risk of acquiring hepatitis C.

The fact that seroconverters were dialysed for a longer period suggests nosocomial transmission of HCV and is in keeping with other reports implicating duration of dialysis as a risk factor in the development of hepatitis C.[2,8-10] Interestingly, and also supportive of this, was the fact that all our HbsAg-positive patients, who are strictly isolated from the main units, were negative for anti-HCV. Isolation of anti-HCV-positive patients has also been effective in reducing spread,[11] and outbreaks of non-A, non-B hepatitis in HD units[12,13] underline the potential risk of nosocomial spread. Our staff, however, tested negative for anti-HCV, which would militate against nosocomial spread unless uraemia *per se* increased the risk of viral acquisition. This predisposition is probably the case as horizontal transmission to staff, household members and sexual partners of patients has previously been reported as uncommon.[12,14] To avoid spread within units it is clearly important to treat every HD patient as potentially infectious and to adhere strictly to the precautions recently published to prevent transmission of blood-borne pathogens.[15]

In this study there was a significant association between blood transfusion and anti-HCV antibodies. However, patients who had been dialysed for longer had received more blood transfusions and, using stepwise logistic regression analysis, we could not discern which of these variables had primary predictive power.

The current risk of transfusion-associated HCV hepatitis is not known. The incidence has decreased markedly in the USA since the implementation of donor screening for surrogate markers and antibodies to HCV and is estimated to be 3 per 100 000 units transfused.[16] At the time this study was performed, the Western Province Blood Transfusion

Service, from which blood was obtained, implemented strict exclusion criteria for donors with a past history of hepatitis in addition to excluding donors who had serological markers of hepatitis B, but did not screen for HCV antibodies. Published data document a prevalence in South Africa of anti-HCV antibodies in 1 498 urban blacks, Asians and whites as 1,2%, 0,8% and 0,6% respectively.[17] The cumulative number of units of blood given to all our patients was 1 760; the probability of all of our anti-HCV-positive patients acquiring HCV infection through transfusion is therefore miniscule, despite the fact that all of the anti-HCV-positive patients had received at least two transfusions.

The management of patients who are anti-HCV-positive but have no clinical or biochemical evidence of liver disease, as is the case in the majority of our patients, is far from clear. Hepatitis C is often asymptomatic and appears to be silent, yet 40 - 50% of patients will develop chronic hepatitis and 10 - 25% eventually progress to cirrhosis.[18,19] Although not yet approved as a standard treatment for hepatitis C, initial results with alpha interferon are encouraging.[20]

Testing positive for anti-HCV antibodies is not synonymous with persistent infection. In a study by Alberti and colleagues[21] of 23 non-uraemic, asymptomatic, anti-HCV-positive patients, 16 had histological evidence of chronic hepatitis. All of these patients had evidence of viraemia, as demonstrated by the finding of HCV-RNA in the blood with the polymerase chain reaction. However, 7 of the 23 patients (30%) were anti-HCV-positive without evidence of continuing viraemia and had presumably recovered from HCV infection. Antibodies may persist for years after resolution of active infection. In a study by Farci *et al.*,[22] which investigated the relationship between HCV replication and antibody levels, 1 of 5 patients cleared their viraemia 15 weeks after first detection but remained anti-HCV antibody-positive for 9 years.

Although we have not performed liver biopsies on any of our patients, none has persistently abnormal liver function tests, and in only 1 patient was a clinical episode of hepatitis documented. It is our clinical impression that chronic liver disease is not a common cause of morbidity or mortality among dialysis or renal transplant patients, although transient abnormalities in liver function tests have been noted incidentally in the latter group. This finding is in keeping with the low incidence of serious morbidity and mortality due to progressive liver disease in renal transplant patients recently reported from the UK.[23] In other countries this pattern may not be the case;[24,25] the reasons for this geographical difference remain to be elucidated but may in part be due to rigid screening for hepatitis B virus, and the varying prevalence and virulence of HCV in different countries. The indolent nature of HCV infection may be another reason why we are not recognising it as a major cause of morbidity and mortality in our patients; studies where follow-up has been of less than 10 years' duration have almost certainly underestimated the problem. Only 6 of our anti-HCV-positive patients have been on dialysis for longer than 10 years and the point at which they became HCV-positive is unknown.

Doubts have been cast upon the sensitivity[4] and specificity[5] of earlier tests for anti-HCV that employed a first-generation ELISA for HCV C100-3. In this study, we have used a second-generation ELISA and also the four-antigen RIBA system to confirm positive results. Of the 24 patients testing positive on ELISA, only 2 were negative on RIBA. There is still controversy about which test provides the most accurate results.

In conclusion, our study confirms the high prevalence of anti-HCV antibodies in haemodialysis patients. Both the number of blood transfusions and the duration of dialysis were significantly related to anti-HCV status. Although we were unable statistically to separate these two variables as predictors from the low incidence of anti-HCV antibodies in the general population, we infer that it is likely that nosocomial spread of HCV occurs in dialysis units. Further studies are required to determine HCV infectivity in these patients. Longer follow-up of anti-HCV-positive patients is also needed to determine the long-term effect of HCV infection.

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