Seroprevalence of hepatitis B and C in maintenance dialysis in a public hospital in a developing country

A E O Otedo, SO Mc'Ligeyo, F A Okoth, J K Kayima

Background. Patients with end-stage renal disease (ESRD) on maintenance dialysis are predisposed to hepatitis B virus (HBV) infection for a number of reasons. In a similar way, the prevalence of anti-hepatitis C virus (HCV) antibodies among patients on chronic haemodialysis and peritoneal dialysis is consistently higher than in healthy populations. There are few published data on these diseases in patients undergoing maintenance dialysis in sub-Saharan Africa.

Objective. To determine the seroprevalence of HBV and HCV in patients on maintenance dialysis.

Setting. Renal Unit, Kenyatta National Hospital, the largest public referral and teaching hospital in Kenya.

Design. Cross-sectional descriptive study.

Study population. All 100 patients on maintenance dialysis during the 9-month study period were evaluated.

Method. The following information was obtained from all the patients: socio-demographic data, date of diagnosis of ESRD and commencement of dialysis, and number of blood transfusions. Additionally, a history suggestive of hepatitis in spouses was looked for and physical examination for tattoos and other scars was carried out. Laboratory investigations included urea, electrolytes and serum creatinine, liver enzymes, hepatitis B surface antigen (HBsAg), immunoglobulin M anti-hepatitis B core antibody (IgM anti-HBc), hepatitis B e antigen (HBeAg) and anti-HCV antibodies. Student's *t*-test was used to assess the significance of the data collected.

Results. The results were expressed as mean (±SD). Fifty-seven males and 43 females were studied. Mean age was 44.3 ± 14.6 years. Ten patients (10%) had elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (> 40 U/l for both).

HBsAg was found in 8 patients (8%), IgM anti-HBc in 2%, and HBeAg in none. Anti-HCV antibody was found in 5%. Six of the HBsAg-positive patients were on haemodialysis, the other 2 on continuous ambulatory peritoneal dialysis (CAPD). There was no coexistence of HBV and HCV markers. Longer duration of dialysis and the number of blood transfusions were associated with an increased seroprevalence of HBV and HCV.

Conclusion. There is a low seroprevalence of HBV and HCV in our dialysis population. This should not lead to complaisance in screening for these potentially lethal complications.

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Patients with end-stage renal disease (ESRD) on maintenance dialysis are predisposed to hepatitis B virus (HBV) and hepatitis C virus (HCV) infection more than the general population because of haemodialysis, blood transfusions, exposure to invasive procedures, and nosocomial and iatrogenic transmissions.[1-3] Other risk factors may include duration of ESRD,[3,4] duration of dialysis[5] and mode of dialysis.[4]

Haemodialysis units have experienced outbreaks of HBV infection, with fatal cases among patients and staff. The HBV prevalence rate varies from one dialysis unit to another. In Europe the prevalence rate is reported to be 23 - 43%;[16] other reported rates are USA16 - 18%,[6] Tunisia 18%, [7] France 2.2%,[8] Russia 39 - 50%[9] and South Africa 17%.[6] Generally, more than half of the infected patients on dialysis become persistent, symptomless hepatitis B surface antigen (HBsAg) carriers without biochemical evidence of hepatitis.[6] In addition, the appearance of serological markers for HBV may be delayed by as long as 6 - 12 months. Because of the interpatient variation in response to HBV infection, liver involvement in patients on maintenance dialysis should be monitored using transaminase activity over the period of dialysis.[4-6] Some dialysis centres have reported outbreaks of fulminant HBV infection indicating that immunocompromised hosts, such as ESRD patients on dialysis, may develop severe disease from HBV infection.[10]

Studies carried out in various centres worldwide among dialysis patients have shown a prevalence of HCV of the order of 8 - 36% in North America,[6,11] 25 - 39% in South America,[3] 1 - 36% in Europe,[12] 17-51% in Asia,[11] 1.2 - 10% in New Zealand and Australia,[4-8] and 7 - 85% in South Africa.[13] False-negative tests for anti-HCV have been reported in up to 33% of ESRD patients on dialysis due to immunosuppression,[14] and per-

Department of Medicine, University of Nairobi, Kenya
A E O Otedo, MMed
S O Mc'Ligeyo, MMed
J K Kayima, MMed
Kenya Medical Research Institute, Ministry of Health, Nairobi, Kenya
F AOkoth, MMed

sistent viraemia after resolution of clinical hepatitis occurs in 80 - 90% of dialysis patients. The HCV-infected haemodialysis patients develop high alanine aminotransferase (ALT) levels in the months after exposure. This suggests progressive liver damage in this group of patients.[11] Therefore a high ALT level has been used as a surrogate marker of HCV infection in dialysis patients.[15] HCV infection is usually confirmed by demonstration of viral RNA. This is expensive, especially for centres such as Kenyatta National Hospital Dialysis Unit, which is not well established.

The seroprevalence of HBV in the general population in Kenya is reported to be 7 - 30% in studies using HBsAg as a marker, while the seroprevalence of HCV, using anti-HCV antibodies, is 0.2 - 0.9%.[16, 17] This makes the country highly endemic for HBV, with a probable low endemicity of HCV. Given that HBV and HCV seroprevalence is high in some well-developed dialysis centres, sometimes with grave consequences, and that there are scanty data on these issues in sub-Saharan Africa (Kenya included), we decided to carry out a study on the prevalence of some of the serological markers of the two viruses in our dialysis unit, which is one of the largest in the region.

Materials and methods

A total of 100 patients (57 males, 43 females) with ESRD on maintenance dialysis (haemodialysis and continuous ambulatory peritoneal dialysis (CAPD)) over a period of 9 months (March 1998 - November 1998) at the Kenyatta National Hospital's Renal Unit were recruited into the study. This comprised all patients on maintenance dialysis at that time. None of the patients had been vaccinated against HBV. Five patients were HBsAg-positive and were undergoing haemodialysis on an isolated machine and were excluded from the study.

The study was approved by both the Department of Internal Medicine, University of Nairobi, and the Kenyatta National Hospital Ethical and Research Committee. Signed consent was obtained from every patient.

The following information was obtained from patients and their follow-up records: socio-demographic data, date of diagnosis of ESRD, date of starting dialysis and mode of dialysis offered, duration of dialysis treatment and number of blood transfusions. Past surgical history was enquired about and patients were asked if their sexual partners had ever had evidence of presumed or confirmed hepatitis. The patients were also examined for tattoos and scarification.

Eight millilitres of blood were drawn from each patient and divided into two aliquots of 4 ml each. Serum was separated from one aliquot soon after collection and immediately stored at -15°C in a refrigerator for HBV and HCV serology. The other 4 ml of blood was used for analysis of creatinine, urea, electrolytes and liver enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)). These were done using the Technicon RA1000 machine (Technicon RAsystems No. SM-0034 D91 and No. SM 4-0137D91, 1996). HBsAg was analysed using reverse passive haemagglutination assay (RPHA). Immunoglobulin M (IgM) anti-HBc was assayed using the enzyme-linked immunosorbent assay (ELISA) method. HBeAg was analysed using the enzyme immunoassay (EIA). Anti-HCV was analysed using the AxSYM HCV version 3.0 microparticle enzyme immunoassay (MEIA) (Abbott Diagnostic Laboratories, Germany). The presence of liver dysfunction was defined as elevated AST or ALT.

The raw data were entered into a computer and analysed using the SPSS package. The prevalence of HBV markers and anti-HCV was obtained. The results were expressed as means (± standard deviation (SD)). Student's *t*-test was used to asses the significance of the raw data obtained, a *p*-value of less than 0.05 being taken as statistically significant. The relationship between the prevalence of the HBV markers, anti-HCV and risk factors for the transmission of HBV and HCV was determined.

Results

A total of 100 patients with ESRD on maintenance dialysis (82 haemodialysis, 18 CAPD) were recruited into the study. The results are expressed as mean (±SD). The mean age of patients was 44.0 ± 13.7 years for males and 42.74 ± 16.10 for females ($p > 0.05$)

There was no statistically significant difference between the mean age, duration of dialysis and number of blood transfusions for males and females ($p > 0.05$) (Table I). However, the patients on haemodialysis had been on dialysis for significantly longer periods and had been exposed to more blood transfusions than those on CAPD ($p = 0.02$, $p = 0.027$) respectively.

Three patients (2 males and 1 female) had received blood transfusions before developing chronic renal failure. No patient had tattoos or scarifications.

Table I. Demographic data, duration of dialysis and number of blood transfusions

Age (years ± SD)	42.35 ± 13.00	43.90 ± 16.75	0.0822
Male / female	43:37	12:6	0.0843
Duration of dialysis (yrs)	1.45 ± 1.41	1.40 ± 0.95	0.02
Blood transfusions during dialysis	5.48 ± 4.73	1.56 ± 0.87	0.027

Prevalence of HBV markers (HBsAg, IgM anti-HBc, HBeAg)

HBsAg was detected in 8 patients (8%) (4 males and 4 females). Two of these patients also tested positive for IgM anti-hepatitis B core antibody (anti-HBc), making HBsAg the only useful seroprevalence marker in this study because immunoglobulin G (IgG) anti-HBc could not be done. Six of the 8 patients were on haemodialysis and 2 on CAPD. The prevalence of the HBV markers did not vary with the mode of dialysis ($p = 0.44$). Only 2 patients on CAPD tested positive for HBsAg. This sample is, therefore, inadequate for any significant differences to be expected. The 8 patients with HBV markers did not have other pre-dialysis risk factors to exposure to HBV infection such as tattoos, history of surgery, the sexual partner having presumed or confirmed evidence of hepatitis or previous blood transfusions. All the patients studied tested negative for HBeAg. The mean age of patients on haemodialysis who tested positive for HBsAg was 42.90 ± 13.97 years. They had received 7.25 ± 6.10 units of blood compared with 2.31 ± 0.97 units received by the patients who tested negative for HBsAg ($p < 0.001$). They had also been on haemodialysis for 1.74 ± 0.9 years compared with 1.49 ± 1.10 years for patients who tested negative for HBsAg ($p = 0.015$).

Prevalence of anti-HCV

Anti-HCV was detected in 5 of the patients (5%) (4 males and 1 female, $p = 0.36$). Patients who were anti-HCV-positive had been on haemodialysis for 2.64 ± 1.20 years compared with 1.27 ± 0.93 years for patients who tested negative for anti-HCV ($p = 0.018$). They had received 2.60 ± 1.80 units of blood compared with 1.37 ± 0.93 units of blood for patients who tested negative for anti-HCV ($p = 0.0183$). No patient on CAPD tested positive for anti-HCV.

Relationship between number of blood transfusions and HBsAg and anti-HCV status

Table II shows the relationship between the number of units of blood transfused and the HBsAg and anti-HCV positivity.

The patients who tested positive for the viral markers had had more blood transfusions (5.44 ± 4.72 units) than patients who tested negative for the viral markers (2.10 ± 1.07 units) ($p < 0.001$). The majority of the patients who were sero-positive for HBV and HCV belonged to the group that had received 1 - 10 units of blood, but this was the largest group.

Table II. Number of blood transfusions and HBsAg and anti-HCV status (%)

No. of transfusions	No. of patients	HBsAg-positive	Anti-HCV-positive
0	2	0	0
1 - 10	75	7 (9.33)	4 (5.33)
11 - 20	23	1 (4.35)	1 (4.35)

Relationship between duration of dialysis and HBsAg, IgM antiHBc, HBeAg and anti-HCV

Table III shows the relationship between the duration of dialysis and the prevalence of HBV and HCV markers.

Table III. Duration of dialysis and seroprevalence of HBV and HCV (%)

Duration of dialysis (yrs)	No. of patients	HBsAg-positive	IgM anti-HBc-positive	HBeAg-positive	Anti-HCV
< 1	53	2 (3.8)	1 (1.9)	0	0
1 -5	44	6 (13.6)	1 (2.3)	0	4 (9.9)
5.1 - 10	3	0	0	0	1 (33.3)

The duration of dialysis for the patients who tested positive for the viral markers was longer than for those who tested negative for viral markers. Patients who tested positive for HBV markers (8 patients, 6 haemodialysis and 2 CAPD) had been on dialysis for 1.90 ± 0.97 years, while those who tested positive for anti-HCV (5 patients, all on haemodialysis) had been on dialysis for 2.64 ± 1.23 years. This duration was longer than that for patients who tested negative for viral markers, viz. 1.48 ± 1.32 years (87 patients, $p = 0.02$). This shows a significant statistical relationship between the duration of dialysis and the presence of viral markers.

Of the 8 patients on maintenance dialysis with evidence of exposure to HBV, none tested positive for anti-HCV, and the relationship between anti-HCV and HBV markers was not statistically significant ($p > 0.05$) (Table IV).

Table IV. Relationship of anti-HCV to HBV markers (HBsAg, IgM anti-HBc and HBeAg)

Markers	No. of patients	Anti-HCV-positive
HBsAg-positive	8	0
HBsAg-negative	92	5
IgM antiHBc-positive	2	0
IgM HBc-negative	98	5
HBeAg-positive	0	0

Prevalence of deranged liver enzymes in maintenance dialysis patients

Three of the 10 patients with deranged liver enzymes on maintenance dialysis were positive for viral markers (2 HBsAg and IgM anti-HBc and 1 anti-HCV-positive) and had elevated AST and ALT ($p = 0.04$) (Table V). Deranged liver enzymes

Table V. Correlation between elevated AST and ALT and the presence or absence of the viral markers

	Patients (*N*)	HBsAg / IgM anti-HBc-positive	Anti-HCV-positive	HBsAg / anti-HCV-negative	*p*-value
AST / ALT > 40 IU / l	10	2	1	7	0.04

was defined as elevated AST and ALT. Ten patients (10%) had evidence of elevated liver enzymes. Of these, 8 were on haemodialysis and 2 on CAPD. The 8 patients on haemodialysis had elevated AST and ALT of between 50 - 75 U / l. Two of the 8 patients tested positive for HBsAg and IgM anti-HBc. None tested positive for HBeAg. Five patients tested positive for anti-HCV and only 1 of them had elevated AST and ALT. The other 4 had normal liver enzymes.

Discussion

Despite the fact that maintenance dialysis has greatly improved the prognosis and quality of life of patients with ESRD, their impaired immune responses puts them at higher risk of infection, including HBV infection. Predisposition to the development of other liver diseases with consequent elevation of transaminases also occurs in these patients.[4,6,14]

In our study population, the prevalence of HBsAg was 8% (50% males). Our patients who were positive for HBsAg had tested negative pre-dialysis. The HBV infection correlated with the duration of dialysis and blood transfusions and may, therefore, have been acquired through haemodialysis or blood transfusion. Although blood for transfusion is screened for HBV, blood transfusion still remains a major risk factor for transmission of HBV infection. HBV DNAhas been detected in serum and peripheral blood mononuclear cells of HBsAg-negative haemodialysis patients and staff, and they are therefore potentially infectious to other patients and staff.[18] Absence of HBsAg does not therefore exclude the presence of HBV infection. Regular periodic screening for HBV markers in patients on dialysis is therefore important. We note in this study that subsequent screening revealed that previously viral hepatitis seronegative patients had now seroconverted. There could also be *de novo* HBV infection secondary to the immunodeficient state in these patients, as has been reported.[6] The prevalence of HBsAg in this patient population is much the same as that reported in the general Kenyan population, viz. 7 - 30%.[16] It should, however, be noted that the dialysis population is highly selected and usually screened pre-dialysis. The study population here was HBsAg-negative on entrance to the dialysis programme. Additionally, this prevalence is also lower than that reported in other parts of the world such as Europe, the USA, Russia, Tunisia and South Africa.[6,7,9,10] This may be related to the fact that patients in these better developed centres stay on dialysis for longer periods than in our centre.

The prevalence of anti-HCV was 5% (80% male). Males have been reported to have a higher prevalence of HCV infection than females[19] and DuBois and colleagues[20] observed that male haemodialysis patients infected with HCV had a significantly higher concentration of serum HCV RNAthan females. However, there are currently no other data available regarding gender-related differences in the natural history of HCV infection and our data are too small to allow for any reasonable conclusions. The prevalence of 5% is much higher than the 0.2 - 0.9% reported in our blood donors and is higher than the 0% reported in the dialysis population in 1995.[17] Despite the fact that the prevalence is much lower than that reported by other countries such as North America (8 - 36%),[4] South America (25 - 39%),[3] Europe (1 - 36%),[12] Asia (17 - 51%), [4] New Zealand and Australia (1.2 - 10%)[4] and South Africa (7 - 85%),[13] it shows a definite increase and should therefore not be treated lightly. Moreover, it is a well-recognised fact that the prevalence of HCV varies widely from country to country and from one dialysis centre to the next even within the same country.[4] All our 18 patients on CAPD tested negative for anti-HCV, the 5% positivity being restricted to the haemodialysis patients. Although our numbers are small, patients on CAPD have been reported to be at lower risk for HCV infection[4] and in contrast to haemodialysed patients, duration of dialysis does not appear to be a risk factor for acquiring HCV infection.[21]

It may be noted that blood donors were school and college students and army personnel; few individuals and relatives of patients voluntarily donated blood. The blood was screened using the ELISAmethod, with a sensitivity and specificity of 97% and 99% respectively. Viruses in the blood could have been in the window period or there could have been contamination from the other HBsAg-positive patients who were otherwise isolated within the dialysis unit but excluded from the study.

Our patients on haemodialysis for a short duration and those who had received few blood transfusions, tested negative for the viral markers. This suggests that the duration of haemodialysis and the number of blood transfusions are significant risk factors for HBV and HCV transmission, as been reported by others.[22] Thus, the risk of acquiring HCV infection on haemodialysis has been estimated at 10% per year. In our study, there was no relationship between HCV antibodies and HBV antigenaemia in patients undergoing maintenance haemodialysis as has recently been alluded to. The human immunodeficiency virus (HIV) causes significant impairment of the immune system and a higher rate of HBV replication.[23] Two of our patients on CAPD were HIV-infected and were positive for HBsAg. However, they had also received blood transfusions so we cannot be categorical about the role of their HIV status.

We found evidence of liver dysfunction manifested by elevated ALT and AST in 10% of our patients (8 on haemodialysis and 2 on CAPD). This compares with the figure of 10 - 44% reported by Pereira *et al*.[4] Two of the 8 patients on haemodialysis had elevated AST and ALT and also tested positive for HBsAg and IgM antiHBc, and 1 of the 8 patients tested positive for anti-HCV. This reached statistical significance, suggesting an association with elevated liver enzymes in the majority of viral marker-positive patients. The general prognosis of hepatitis in patients on maintenance dialysis appears to be benign. Most of these patients have no clinical symptoms but do have episodic mild increases in serum ALT and AST.[4,6,24] They therefore become carriers who are potentially infectious to other patients and staff. Their HBV and HCV infection is only discovered by routine serological or biochemical testing during blood donation or routine health screening.[24] They should therefore be monitored over the period of dialysis using liver transaminases as surrogate markers of HBV and HCV infection.[4,6]

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