



Original Research Article

A Study of Prevalence and Associated Risk Factors of SEN Virus in Haemodialysis Patients

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ABSTRACT

Keywords

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Haemodialysis (HD) patients are at increased risk of parenterally transmitted viral infections such as Hepatitis B, Hepatitis C and HIV. SEN virus (SENV) is a recently discovered single-stranded DNA virus of Anelloviridae family and is believed to be transmitted parenterally. We conducted this study to identify the prevalence of SEN virus transmitted to patients of chronic renal disease (CKD) on renal replacement therapy. Clinical significance of SENV was assessed. Hundred CKD patients and 30 healthy controls were included in the study. Serum was separated and stored at -20°C till further use. Hepatitis B Virus, Hepatitis C Virus and HIV were detected by enzyme immunoassay. Nested PCR was performed for detection of SEN V. Four cases (4%) were positive for SEN virus. Two (2%) were HCV, 1(1%) was HBV and 1(1%) was HIV positive. Risk factors associated with SENV were repeated hospitalization, multiple episodes of blood transfusion and haemodialysis. No co-infection was found between SENV, HBV, HCV and HIV. Almost 4% prevalence of SENV in chronic renal disease patients was observed. We conclude that SENV appears to be not only hepatotropic but also one of the most commonly found viral infection in haemodialysis patients.

Introduction

Viral hepatitis and human immunodeficiency virus (HIV) infections are important causes of morbidity and mortality in haemodialysis patients. The use of Haemodialysis (HD) for chronic kidney disease (CKD) has expanded tremendously in the past few decades. Although the treatment has led to increased longevity of patients it also predisposes to some infections especially blood borne viruses.

Patients with chronic kidney disease have impaired host defenses against both viral and bacterial infections (Wong *et al.*, 2005). The haemodialysis procedure per se as well as disturbances in both innate (Lewis *et al.*, 1988) and adaptive immunity (Eleftheriadis *et al.*, 2004) render HD patients even more susceptible to infections.

HD patients are at high risk of acquiring parenterally transmitted viral diseases such as

Hepatitis B, Hepatitis C and HIV. Patients on HD represent a high risk group for infection by blood borne viruses because the HD materials used in different centers are not completely disposable and also the fact that the therapeutic procedures are frequently associated with bleeding and blood transfusion.

HD patients may also show hepatic dysfunctions consistent with viral hepatitis, even in the absence of documented hepatitis B, hepatitis C, hepatitis D infections and indeed potentially hepatotropic viral agents such as hepatitis G virus and TTV have been isolated in these patients (Wreghitt *et al.*, 1999).

In 1999, a DNA virus was detected in the blood of a HIV infected intravenous drug abuser and named SEN Virus (SEN V). SEN V are genetically heterogenous and likely are the members of Circoviridae (Tanaka *et al.*, 2001; Umemura *et al.*, 2001). The virus is subgrouped into eight genotypes, SEN-A to H. The ninth genotype has been also identified (Fiordalisi *et al.*, 2000).

SEN V is a single stranded, non-enveloped circular DNA virus with a size of 26nm. The mean genome consists of 3900 nucleotides and at least three open reading frames (ORF) have been identified (Yoshida *et al.*, 2001). The proportion of SENV-H positive haemodialysis patients in Germany was 12.8% (Schröter *et al.*, 2003) and 38% in Japan (Kobayashi *et al.*, 2006). The study was designed to assess the prevalence and risk factors of SEN V infections in patients on haemodialysis.

Materials and Methods

Study group

A prospective study was carried out on patients of chronic kidney disease (CKD)

requiring renal replacement therapy (RRT) admitted in the Nephrology Unit, Department of Medicine, Jawaharlal Nehru Medical College and Hospital, AMU, Aligarh. The present study was done on Consecutive CKD patients on maintenance HD or on patients with CKD proceeding for maintenance HD for the first time. The study was conducted after obtaining permission from Institutional Ethics Committee of Jawaharlal Nehru Medical College and the procedure followed in the study was in accordance with institutional guidelines and predesigned proforma.

The patients in the present study were enrolled after taking informed consent from them. One hundred cases and thirty healthy age and sex matched blood donors with no clinical evidence of present or past involvement of liver disease and kidney disease, having normal liver function test and screened negative for viral markers (HIV, HBV and HCV) were taken as controls for the study.

Patients who were already reactive for Hepatitis A, Hepatitis B, Hepatitis C or HIV and patients who failed to give valid consent were excluded from our study.

Detailed clinical history was elicited from patients. These patients were evaluated on the basis of various investigations such as serum creatinine, blood urea and liver function tests (alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Ultrasound was performed when required.

Sample collection

Around 7–10 ml of blood was collected, serum separated, aliquoted and stored at -20°C till further use.

Serology

All patients were screened for HBV, HCV, and HIV at the time of presentation. HbsAg for HBV was detected by ELISA kits from SD Biostandard diagnostic, India.

Anti HCV antibodies for HCV were detected by using ELISA kits from SD Bio standard diagnostic, India and J. Mitra & Co. Pvt. Ltd., India. Rapid HIV kit by SD Bioline and HIV ½ Trispot Test by AidsCan were utilized for detection of HIV.

After initial HBsAg screening, all the HBsAg nonreactive subjects were advised to receive immunization against Hepatitis B if not already vaccinated.

In cases that required repeated use of items (eg., dialyzers and tubings), the items were decontaminated and reserved for the individual patients for subsequent use. When required, the patients received blood transfusions involving blood units that were stringently screened for HBsAg, HIV and HCV.

Detection of SENV DNA

To identify the putative role of SEN V as a possible nosocomial pathogen in haemodialysis patients, SEN V viremia was detected by amplification in these patients. The presence of SEN V viremia was assessed in relation to their clinical findings.

DNA extraction was performed by using Phenol Chloroform Isoamylalcohol method (Saiki *et al.*, 1988).

Nested PCR was employed for detection of SEN Virus (349 bp) (Tang *et al.*, 2008). Primers were ordered from Fermentas, Life Sciences, USA. The sequences of SEN primers are as follows (W = A or T, Y = C or T, M = A or C).

For SENV common primers: P1, 5'-TWCYCMAACGACCAGCTAGACCT-3'; P2, 5'-GTTTG TGGTGAGCAGAACGGA-3'.

After extraction amplification was done by using Nested Polymerase Chain Reaction. The Master Mix used were bought commercially from FERMENTAS, Life sciences, USA Standardization of reaction mixture both for SEN Virus was done by using different amount of master mix, primers and template several times. Cycling conditions were also standardized by applying varying gradients of annealing temperature and different numbers of cycles in the amplification SEN Virus, to get the best results out of it. The final reaction mixture and cycling conditions are mentioned here. The thermal cycler used was Gradient thermo cycler named Le cycler of LABNICS, USA.

PCR reaction was carried out with a 25 µl reaction mixture containing 0.75µl Primer (Forward) SP1, 0.75µl Primer (Reverse) SP2, 6µl of DNA Template, 5µl of nuclease free water, 12.5 µl of Master Mix. The first round PCR was carried out for 40 cycles, each cycles consisting of denaturation at 94°C for 15 seconds, primer annealing at 55°C for 50 seconds and extension at 72°C for 50 seconds, followed by an additional extension at 72°C for 7 minutes.

After amplification, end product was run on agarose gel by electrophoresis. Horizontal electrophoretic apparatus from Genei TM, Bangalore, India was used. The PCR products (5µl), positive (5µl) and negative control (5µl) were mixed with the 6µl of 1X loading dye, and 11 µl was loaded in each well according to prepared master chart. 100 base pair and 50 base pair ladder (Fermentas, USA) were used.

Statistical analysis was performed with the IBM SPSS Statistics 19. Data expressed as mean values \pm SD or as percentage.

Results and Discussion

The study comprised of 100 Chronic Kidney Disease patients who were on haemodialysis and 30 age matched healthy controls. Of 100 patients in the study group, 55% were males and 45% were females. The mean age distribution was 36.71 ± 14.23 years in cases and 40.20 ± 6.42 years in the controls.

Most common symptom in CKD patients undergoing haemodialysis was decreased urinary output (81%) followed by swelling (62%), fever (52%), abdominal discomfort (27%) and haematuria (15%). All the CKD patients in the study group required repeated episodes of haemodialysis. 11% had 5 or more episodes of haemodialysis. 45% of the patients had 3–4 episodes of haemodialysis and 44% had 1–2 episodes of haemodialysis monthly

In this study, 41% of the patients of CKD required blood transfusion, out of them 33% required 1–2 transfusions and 8% required multiple transfusions. In the study, 67% of the patients on haemodialysis had low haemoglobin levels which ranged from 5–7.9 g/dl while 9% of the CKD patients were severely anaemic (<5). All the patients had deranged serum creatinine and blood urea levels.

4 cases (4%) were positive for SEN virus DNA. 2 (2%) were HCV, 1 (1%) was HBV and 1 (1%) was HIV positive.

Most of the patients on haemodialysis (75%) who were SEN Virus positive belonged to 21–30 age groups. There was no difference in the sex distribution among SEN Virus positive patients on haemodialysis. Most of the SEN Virus positive patients on

haemodialysis had chronic glomerulonephritis (50%).

Most of the SEN Virus positive patients who were on haemodialysis presented with fever 3 (75%) and swelling 3 (75%). Seventy-five percent of the SEN Virus positive patients had severely deranged AST levels (>60 IU/L), 25% had moderately deranged AST levels (40–60 IU/L). Fifty percent of SEN Virus positive patients have moderately deranged ALT levels (30–45 IU/L). Twenty-five of SEN Virus positive patients have severely deranged ALT levels (>45).

Fifty percent of the SEN Virus positive patients had history of episodes of prior hospitalization

Number of haemodialysis episodes per month ranged from 3 to 4 in all the SEN Virus positive CKD patients in the entire period of follow up of the cases in the study group.

Number of blood transfusions among haemodialysis patients ranges from 1–2 in 66.67% of all the SEN Virus positive CKD patients, multiple in 33.34% of SEN Virus positive CKD patients.

No co-infection was found among SEN positive CKD patients on haemodialysis.

SENV has been recently identified as a candidate agent of non A-E hepatitis virus (Umemura *et al.*, 2001). In a prior study we had analysed the prevalence of SENV in patients with acute and chronic liver disease. (Rizvi *et al.*, 2013). In this study we evaluated the association of SENV with haemodialysis in order to assess the risk of transmission of the virus by this route. Patients on haemodialysis represent a high risk group for infection by blood borne

viruses such as HBV, HCV and HIV because the hemodialysis materials used in different centres are not completely disposable and also due to the fact that the therapeutic procedures are frequently associated with bleeding and blood transfusion. (Schröter *et al.*, 1999). Pirovano *et al.* (2002) found that the patients who undergo hemodialysis can be at high risk of SEN-V transmission.

In our study, 4 % patients were SEN V positive while all the healthy controls were negative for this virus. This is the first study of its kind from India. A survey conducted in Japan tested 189 patients on maintenance hemodialysis for SEN-V (Kobayashi *et al.*, 2006). Of the 189, 153 were followed up for 2 years. Although SEN-V infection is almost frequent among Japanese general population, the prevalence in patients on maintenance hemodialysis (38%) was significantly higher than that of control group (22 %). A study conducted by Ismail *et al.* (2011) in Egypt concluded that SEN-V is commonly present in blood transfused and hemodialysis patients attending Assuit University Hospital as well as in blood donors at comparable rates. SEN V infection has been found in only 20% of blood donors but in 46.7 % of these patients ($p < 0.02$). In consonance with the general trend in the study group, most of the SEN Virus positive patients (75%) were in the 21–30 age group and were quite young. A study done by Shibata *et al.* (2001) reported mean age between 40.3 ± 17.3 to 65.5 ± 9.9 which is not in accordance with our study. In our study 50% were male and 50% were female. Yoshida *et al.* (2001) also have reported that there were no significant difference in age and sex between SEN V negative and SEN-V positive chronic liver disease and hepatocellular carcinoma patients.

Prevalence of SEN V was 25.18% in liver disease patients in a previous study conducted from here (Rizvi *et al.*, 2013). The low prevalence in haemodialysis patients suggests that transmission through this route is low.

While the prevalence of SENV isolated from serum samples of otherwise healthy persons has been reported to be 1.8% in the United States (Umemura *et al.*, 2001), 10–22% in Japan (Shibata *et al.*, 2001; Kobayashi *et al.*, 2006), 15–51% in Taiwan (Kao *et al.*, 2002), 5% in Thailand (Tangkjanich *et al.*, 2003), 8–17% in Germany (Umemura *et al.*, 2003, Schröter *et al.*, 2002), 24% in Greece (Umemura *et al.*, 2003) and at least 13% in Italy (Pirovano *et al.*, 2002). The above data suggest that SEN virus has a global distribution with marked geographic differences in its prevalence. The explanations for these differences are unknown, but they may result from interactions among behavioral, social and biological factors (Pfeiffer *et al.*, 2003).

Among the SENV positive individuals, 2 each were males and females. Hence there was no difference in the prevalence of SEN Virus infection among males (50%) and females (50%). This was in accordance with studies of Yoshida *et al.* (2002) who mentioned no significant differences in age and gender between SEN V positive and SEN V negative patients with non B and non C chronic liver disease.

In contrast, Kobayashi *et al.* (2006), Chiou *et al.* (2006) and Schröter *et al.* (2006) found notable difference in SEN V prevalence according to gender with higher prevalence of males among SEN V positive patient.

Table.1 Distribution of patients on haemodialysis according to study group

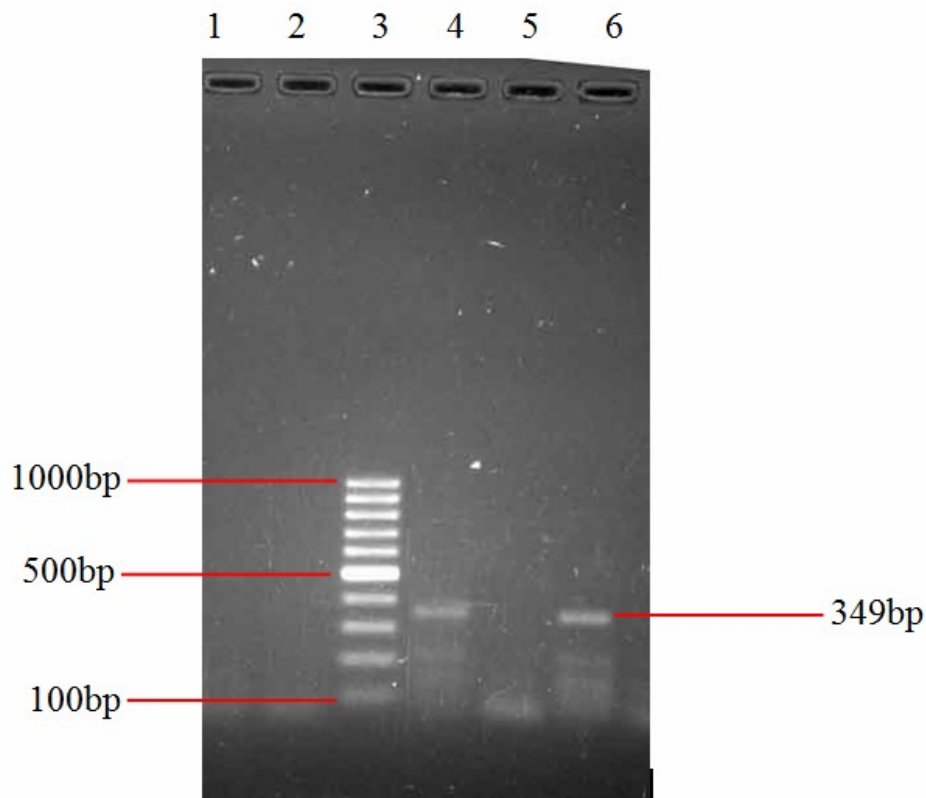
Study groups	Cases n(%)
Diabetic Nephropathy (DN)	28 (28)
Hypertensive Nephropathy (HN)	26 (26)
Chronic Glomerulonephritis (CGN)	22 (22)
Others*	24 (24)
Total	100

*Others – Obstructive Uropathies, Reflux Nephropathy, Adult Onset Polycystic Kidney Disease, Tubulointerstitial Diseases, Vascular diseases.

Table.2 Demographic, clinical and laboratory data in 4 haemodialysis patients who were SENV DNA positive

CHARACTERISTICS	SEN V(cases)
Number of patients	4/100
Male/Female ratio	1:1
Age(years)	
21–30	3
10–20	1
ALT(>45IU/L)	1
AST(>60IU/L)	3
Blood Transfusions	
1-2	2
multiple	1
No. of Episodes of Haemodialysis/month (3-4)	4
Renal Disorders	
Diabetic Nephropathy	0
Chronic Glomerulonephritis	2
Hypertensive Nephropathy	1
Adult Polycystic Kidney Disease	1

Figure.1 PCR amplification for SENV; Lane 3 showing 100 bp DNA Ladder, Lane 5 showing negative control for SENV, Lane 4 and 6 showing amplified SEN V PCR product of 349bp



Among the risk factors, all the patients positive for SENV had history of blood transfusion. 25% of them were associated with history of multiple episodes of blood transfusion. This is consistent with the findings of Schréter *et al.* (2006) who reported transmission of SENV by blood transfusion. In a study done by Zheng-Hao Tang *et al.* (2008) SENV positive non A-E hepatitis patients had no blood transfusion history, indicating that blood transfusion transmission is not the only way for people to get infected with SENV.

Also, since patients who have been on haemodialysis received more blood transfusions, one could expect that the presence of SENV DNA correlates with the Haemodialysis treatment.

In our study, 50% of the SEN Virus positive patients had a history of episodes of prior hospitalization. Patients during their course of admission go for multiple exposure to intravenous drugs and blood transfusions. Thus the possible route of SEN V infection might be mostly parenteral e.g. transmission by blood transfusion, intravenous drug use or hemodialysis (Umemura *et al.*, 2001)

SENV was unexpectedly the most prevalent blood borne virus in the study followed by HCV, HBV and HIV. However the prevalence of all four viruses was considerably lower compared with other reports pointing to effective infection control measures. More studies are required to confirm disease association.

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