

Viral hepatitis infections in Basrah haemodialysis unit: Serological diagnosis and viral loading

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ABSTRACT

The liver disease caused by hepatitis B (HBV) and C viruses (HCV) has become an important cause of morbidity and mortality in patients with chronic renal insufficiency. Our study aimed to evaluate the seroprevalence and viral loading of HBV and HCV infections among patients at Basrah dialysis unit. A147 individuals, comprising 122 patients on maintenance hemodialysis attending to Basrah haemodialysis unit and 25 individual's staff members. Serological testing for HBsAg, IgM anti-HBc, Total anti-HBc and anti-HCV antibody were performed using ELISA kits, and then patients with positive samples were extracted of nucleic acid and viral loaded by using Real-Time PCR. Of 122 patients, HBV infections seroprevalence were 61(50%) positive, whereas HCV seroprevalence were 70(57.4%) anti-HCV negative and 52(42.6%) anti-HCV positive. HBV-DNA was detected in 38/61(62.29%) patients, all examined 61 positive patients were divided in four groups included, acute infections (34.4%), chronic infections (26.3%) past infections (37.7%) and early infection (1.6 %). Seroprevalence of HCV RNA was detected in 32/52 (61.53%) patients, all examined 52 anti-HCV positive patients were divided into three groups, 26(50%) patients > 2 ×10⁶ copies/ml, 6(11.5%) patients < 2 ×10⁶ copies/ml and 20(38.5%) patient's undetectable HCV RNA. we concluded that HD program patients were in high percentage infected with HBV and HCV.

Keywords: Viral Hepatitis, HBV, HCV, haemodialysis

INTRODUCTION

End-stage renal disease (ESRD) is a significant problem in almost all countries and the prevalence has increased considerably in developing countries [1]. The liver disease caused by hepatitis B (HBV) and C viruses (HCV) has become an important cause of morbidity and mortality in patients with chronic renal insufficiency [2]. Risk factors for spread include a history of transfusion, number of blood products transfused, and number of years on hemodialysis (HD) therapy [3].

HBV infection is a major clinical problem as it can lead to many serious consequences, including acute and chronic hepatitis, cirrhosis, hepatocellular carcinoma and hepatic failure [4]. Universal precaution measures should be strictly observed and the segregation of HBsAg positive patients on HD should be practiced. Early vaccination against HBV before the start of ESRD remains the best way to secure immunological protection against HBV infection in dialysis patients [5]. Hepatitis C virus infection is especially problematic in patients with ESRD who are undergoing HD [6]. The high prevalence of HCV infection in dialysis patients is of great concern because these patients have a higher mortality than HCV negative patients [7]. The prevalence of HCV infection is higher among HD patients than in the general population, and several routes of transmission are

thought to originate from HD units [8]. Although HCV transmission through blood products transfusion previously was a significant source of infection, current cases are more likely related to nosocomial exposure [9], previous blood transfusion [10], mode of dialysis therapy [11], and duration of hemodialysis [12]. The National Institutes of Health (NIH) workshop on management of Hepatitis B recommended that anti-viral treatment be considered in patients with HBeAg positive or HBeAg negative chronic hepatitis and HBV DNA 10^5 copies/ml [13]. Several studies were the basis for the establishment of a clinically relevant HCV quantitative threshold of 2,000,000 copies/ml for use in the determination of the length of treatment with alpha IFN–Ribavirin combination therapy [14]. The aims of the administered therapy for hepatitis B viral infection (interferon, lamivudin adefovir) as well as for hepatitis C viral infection pegylated interferon) are stable eradication of virus, regression of chronic hepatitis, prevention of liver cirrhosis and hepatocellular carcinoma [15]. In this study we aimed to evaluate the seroprevalence and viral loading of HBV and HCV infections among patients at Basrah dialysis unit, southern of Iraq.

MATERIALS AND METHODS

Across sectional study during the period between 1/10/2012 into 1/2/2013 was conducted on 147 individuals, comprising 122 patients on maintenance hemodialysis, 70 of them were males and 52 females with age range 12-74 years, attending to haemodialysis unit of a general Basrah hospital, Basrah - Iraq, furthermore, a 25 individual's staff members, 18 of them were males and 7 females with age range 21-48 years.

A sample of 5 ml blood from each patient and staff member was collected by vein puncture in sterile plain tube. The blood sample was left to clot at room temperature, and then centrifuged at 3000 rpm for 5 min. The serum of each sample was divided into several 0.25 μ L aliquots and immediately stored at -20° C until used.

Serological testing for HBsAg, IgM anti-HBc, Total anti-HBc and anti-HCV antibody were performed on the recruited HD patients using third generation enzyme linked immunosorbent assay (ELISA) kits (Biokit, Spain, Biokit, Spain, Biokit, Spain, Foresight kit, USA), respectively, all ELISAs were performed according to the manufacturers' instructions. Consecutively, patients with positive samples were extracted of nucleic acid by using automated Maxwell[®] 16 (Promega, USA), then tested by using Real-Time PCR (Applied Biosystems, USA) according to a sensitive commercially available Real-Time PCR kits (Real Time Kit for the Quantitative detection of HBV and HCV (Sacace Biotechnologies, USA) for detecting HBV-DNA and HCV RNA. Information on the age, gender and duration of haemodialysis was obtained from patient records and interviews.

SPSS was used to analyze data. Data of this study was analyzed by the Chi square test. Quantitative variables were expressed as min.-max. (Mean \pm SD). A p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Of 122 patients studied, the present results showed that 70 (57.4%) were males with significantly elevated ($P < 0.01$) than 52 (42.6%) females. The mean age of patients was 37 years. Based on the duration of HD, patients were divided into four groups of < 3, 3–6, 6–9, and > 9 months. In hepatitis B virus, of 38 patients (Figure, 1) the first groups were 18 (47.36 %) patients that showed significantly elevated ($P < 0.001$) than others studied groups, the second groups were 7 (18.43 %) patients, the third groups were 2 (5.27%) patients and the fourth groups were 11 (28.94%) patients. In hepatitis C virus, of 52 patients (Figure, 2) the first groups were 23 (44.23 %) patients that showed significantly elevated ($P < 0.001$) than others studied groups, the second groups were 13 (25%) patients, the third groups were 5 (9.61%) patients and the fourth groups were 11 (21.16%) patients. In a study by Assarehzadegan, et al, the duration of treatment with HD was significantly associated with HBV- and HCV-positivity ($p < 0.001$) [16]. Sekkat, et al, reported a seroprevalence rate of HCV of 68.3%. HCV seropositivity was associated with longer duration of dialysis ($p < 0.001$) [17].

The results from this study showed that HBV seroprevalence for 122 patients were 61 (50%) positive (Table, 1.), included 38/61 (62.29%) HBsAg positive, 21/61 (34.42%) IgM anti-HBc positive and 60/61 (98.36%) Total anti-HBc positive that showed significantly elevated ($P < 0.001$) than others markers (Figure, 3). In all, seroprevalence of HBsAg was 38/122 (31.14%). Whereas the results of HCV seroprevalence for 122 patients were 70 (57.4%) anti-HCV negative that showed significantly elevated ($P < 0.001$) than 52 (42.6%) anti-HCV positive (Figure, 4). So that in total, from 113 viral infected patients, infections were 54% HBV and 46% HCV, included 30 (26.5%) patients were coinfecting with HBV and HCV. Regarding the results, HBV-DNA was detected in 38/61 (62.29%) patients (with significant differences, $P < 0.05$), in hepatitis B virus, all examined 61 positive patients were divided by screening tests (ELISA test) in four groups (Figure, 5) on the basis of the presence of HBsAg and anti-HBc antibodies. The first groups (acute infections) were 21 (34.4%) patients, included HBsAg, IgM anti-HBc and Total anti-HBc was

positive, with viral load, 350-100000000 copies/ml ($48626211.54 \pm 50231976.47$). The second groups (chronic infections) were 16(26.3%) patients, included HBsAg and Total anti-HBc was positive, with viral load, 1210000-100000000 copies/ ml (87651250 ± 34927539). The third groups (past infections) were 23(37.7%) patients and only Total anti-HBc was positive. The fourth group (early infection) was 1(1.6 %) patient and only HBsAg was positive, with viral load 149×10^{11} copies/ml that showed significantly elevated ($P < 0.001$) than others viral load studied.

Analysed patients showed very heterogenic group. Most of them had 113 monoviral infections included 61(54%) HBV and 52(46%) HCV, but 30 had the so-called dual infections. In hepatitis B virus, the patients made heterologous group, Most of the patients (37.7%) belonged to past infections group, the smallest patients (1.6 %) belonged to early infection group. The high prevalence of HBV patients in our dialysis unit was associated with dialysis duration and the sharing of dialysis machines. Even though multiple measures are often implemented to reduce new infection such as HBV vaccination, execution of infectious disease control measures ,isolation of HBV-positive patients, and the use of dedicated dialysis machines[18]. However, the relatively low response rates to HBV vaccination in this group of patients might contribute, under some specific circumstances, to the ongoing HBV transmission in this setting [19].In our study, seroprevalence of HBsAg was 31.14% in HD patients which was higher than that reported in other studies such as 1.4% in north of Iran [20], 2.4% in Tehran, Iran [21], 5.1% in Khuzestan, southwest Iran [16], 12% in Kosova [22].In a study conducted by Boulaajaj, et al, on 186 chronic HD patients, the prevalence of HBV infection was 2 % [23]. The prevalence of anti-HBc (37.7%) was relatively high in our study. Anti-HBc antibodies are markers of acute, chronic, or resolved HBV infection and remain detectable for life. These can be present in the absence of both HBsAg and anti-HBs antibodies, during the convalescent period following acute hepatitis B before the appearance of anti-HBs antibodies, or in patients who have resolved infections but lost detectable anti-HBs antibodies. Anti-HBc antibodies are, therefore, detected in anyone who has been infected with HBV [24].As anti-HBc positivity indicates previous exposure to HBV infection, this finding in our HD patients in association with the 31.14% prevalence of HBsAg might suggest contact with HBV during Hemodialysis program.

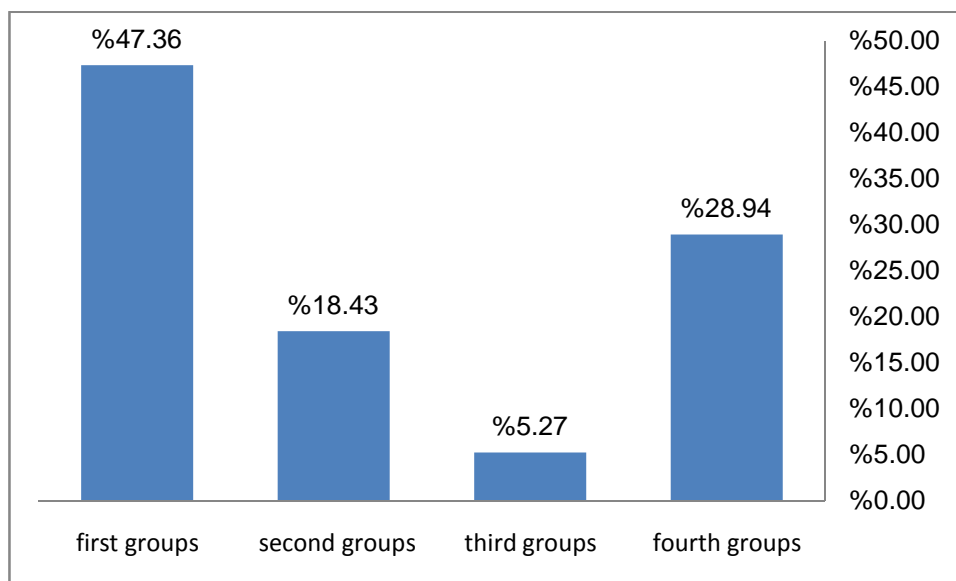


Figure (1) Duration of HD of HBV patients

HCV infection remains highly prevalent both in developed and less-developed countries [25]. In hepatitis C virus, the patients made heterologous group, Most of the patients (50%) belonged to high viral load group. The seroprevalence of HCV infection found in our HD patients (42.6%) was agreement with 43% in Kosova[22] , 41.9% in Saudi Arabia [26], 45% in Tunisia [27] and 45% in Syria [28], and was lower than that seen in other studies such as 10.5% in Salvador [29] and 34.6% in Jordan [30]. In a descriptive study conducted by Alavian, et al, the prevalence of positive HCV antibodies decreased from 14.4% in 1999 to 4.5% in 2006 [31]. Joukar, et al , found 11.9% patients positive for anti-HCV in north of Iran[20] .A retrospective study performed by Boulaajaj,et al, on 186 chronic HD patients, reported a high prevalence of HCV infection (76%) [23]. The sharing of dialysis machines for anti-HCV positive and negative patients has been clearly associated with transmission of hepatitis C. Use of dedicated machines has been linked to a significantly lower incidence of HCV infection. The possible routes of HCV infection within the HD unit include dialyzer reuse, sharing of HD machine and instruments, and environmental contamination (6).The transmission of HCV has been reported to occur between patients receiving hemodialysis in the same unit[32] .Risk factors for HCV infection in dialysis patients include number of

blood transfusions, duration of HD, mode of dialysis, prevalence of HCV infection in the dialysis unit, previous organ transplantation, intravenous drug use, male gender, older age, previous HBV infection and nosocomial transmission of HCV in HD units [33].

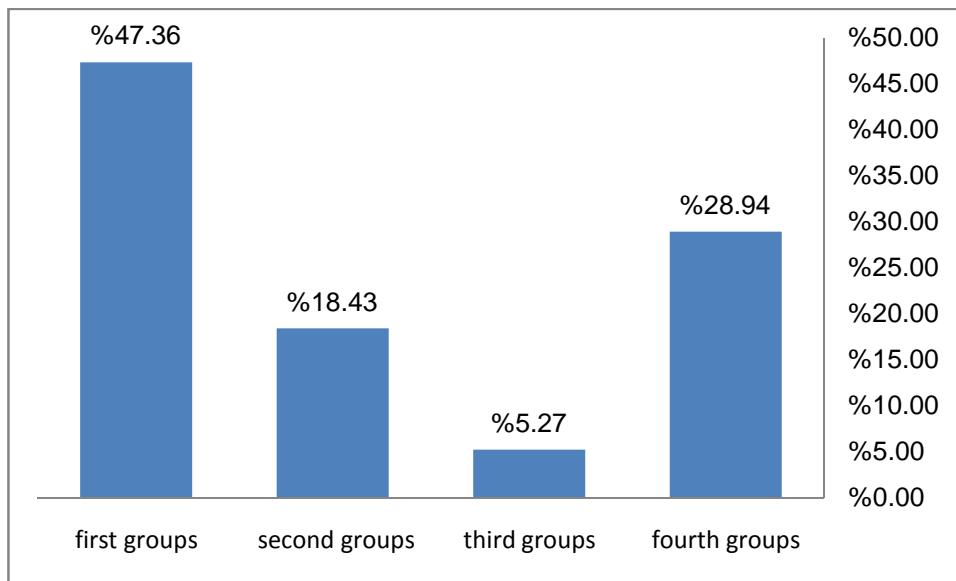


Figure (2) Duration of HD of HCV patients

It is crucial to find the best measures for early diagnosis, by which we can track the potential transmission and prevent further spread of the virus [32]. Therefore, the quantitative detection of HBV-DNA has been shown to be the most efficient method to evaluate viral replication in HD patients infected with HBV [34]. The present study demonstrated that 38/61(62.29%) patients had detectable HBV-DNA by real-time PCR. In a study performed by Mina, et al, HBV-DNA was detected in 4.1% patients [19].

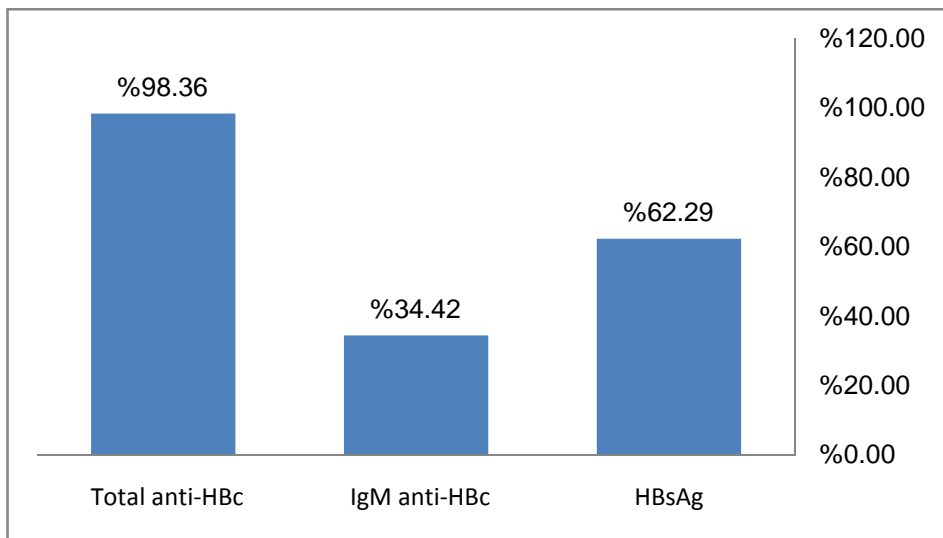


Figure (3) Percentage of HBV biomarkers of patients studied

Table (1) Distribution and percentage of biomarkers of studied patients

Negative	HBV						HCV				
	Positive (61, 50%)			Viral load			Anti-HCV Negative	Anti-HCV Positive	Viral load		
	HBsAg	Anti-HBc IgM	Total anti-HBc	>10 ⁵ copies/ml	<10 ⁵ copies/ml	Undetectable			> 2 ×10 ⁶ copies/ml	< 2 ×10 ⁶ copies/ml	Undetectable
61 (50%)	38 (62.29%)	21 (34.42%)	60 (98.36%)	26 (42.6%*)	21 (34.5%)	14 (22.9%)	70 (57.4%*)	52 (42.6%)	26 (50%*)	6 (11.5%)	20 (38.5%)

*P value < 0.05

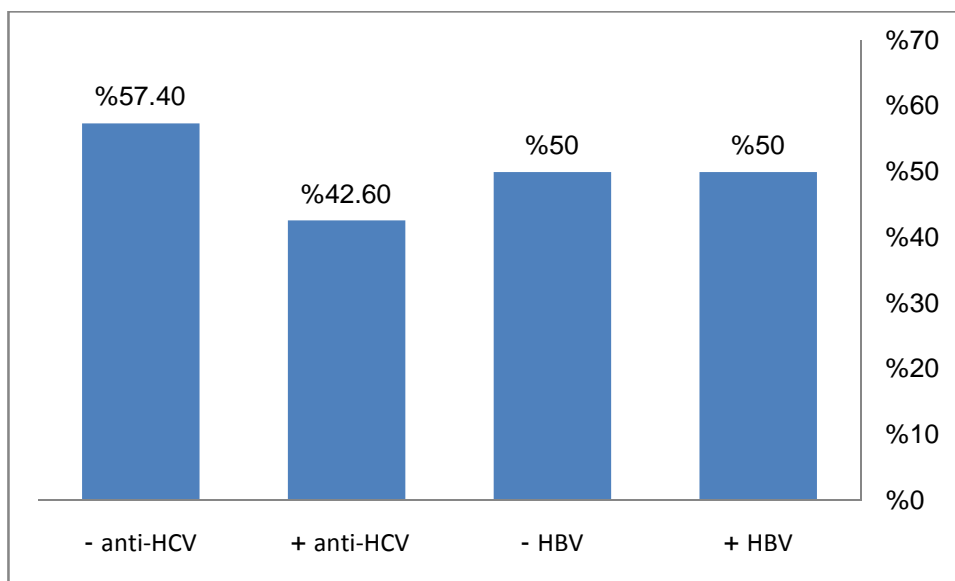


Figure (4) Percentage of HBV and HCV of patients studied

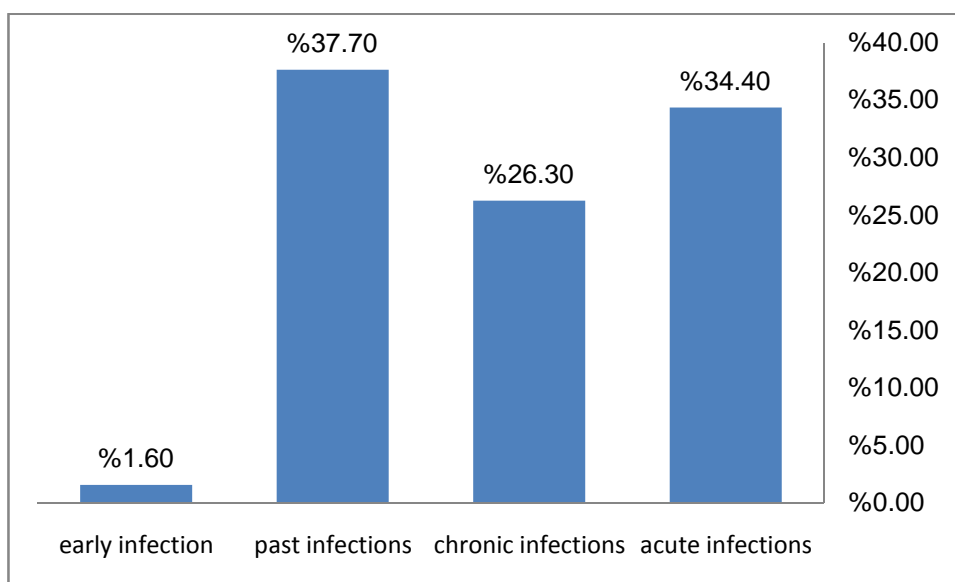


Figure (5) Percentage of HBV groups of patients studied

In our study, prevalence of HCV RNA was detected in 32/52 (61.53%) patients (with significant differences, $P < 0.05$), all examined 52 anti-HCV positive patients were divided according to viral load into three groups. The first groups were 26(50%) patients $> 2 \times 10^6$ copies/ml, with viral load 2470000- 435 $\times 10^6$ copies/ ml (373423070 \pm 201923000) that showed significantly elevated ($P < 0.001$) than others viral load studied. The second groups were 6(11.5%) patients $< 2 \times 10^6$ copies/ml, with viral load 6060-1860000 copies/MI (856676.7 \pm 630836.5). The third groups were 20(38.5%) patient's undetectable HCV RNA. Furthermore group 30/113 patients were coinfecting with HBV and HCV with viral load 350 -100000000 copies/ml (66673545.83 \pm 49226435.79) and 742000- 435 $\times 10^6$ copies/ml (102603650 \pm 234119616) respectively.

In fact, screening for antibodies to HCV in combination with PCR appears to be the safest way to identify all HCV-infected individuals. Several studies were performed round the globe which focused on HCV -RNA in HD patients and its relationship with risk factors. These studies had variable results. In a study performed by Silva, et al, HCV-RNA was detected in 92 (73.6%) of 125 antiHCV-positive patients [29].Dattolo, et al, showed that HCVRNA was positive in 18 (75%) of 24 anti-HCV-positive subjects [35]. Mansour-Ghanaei, et al, showed that 10.42% of 163 HD patients were positive for HCV RNA by PCR[36]. Ocak, et al reported a HCV RNA prevalence of 10.1%[37]. Patients who were HCV RNA positive or those who are positive for anti-HCV antibody were at increased risk for death compared with patients who were negative[38].

The present study showed that 30/113 patients were coinfecting with HBV and HCV. Moreover, viral interactions can maintain HBV infection in a latent state, as in ESRF patients with chronic HCV infection and occult HBV viremia[39]. In these cases, a negative interference has been considered between the two viruses, leading to low HBV-DNA levels[40]. Furthermore, our study showed that seroprevalence of HBsAg for 25 individual's staff members was 1(4%) patient's undetectable HBV DNA and negative for others HBV markers and anti-HCV. Basrah haemodialysis unit use hypex and citric acid to disinfect the haemodialysis machines at the end of each session. Filters and tubes were discarded after each use. Nurses do not regularly wear gloves when dealing with patients. To limit the spread of viral infections in haemodialysis unit, precautionary aseptic measures should be improved. Furthermore, education programmes for staff on the risk of transmission of blood-borne viruses should be considered. Administration of recombinant alfa-2a interferon or pegylated interferon is indicated in the treatment of patients in terminal stage of renal insufficiency [41]. Patients with transplanted kidneys as well as with HBV infections should be treated by lamivudine [13]. New preliminary results have shown that interferon application is safe in patients coinfecting with HBV and HCV infections until Ribavirin administration is contraindicated [41].

CONCLUSION

In our study, we concluded that Hemodialysis program patients were in high percentage infected with hepatitis B virus, hepatitis C virus, or both, suggesting possible nosocomial transmission between patients. In the present study, the route by which the haemodialysis patients acquired viral infections was not determined. There was low prevalence of HCV and HBV infection in HD population of different regions and it can be decreased by HBV vaccination of ESRD patients before setting chronic HD, antiviral treatment and isolation of infected individuals. A reliable diagnostic tool was necessary to make accurate diagnosis and isolate the infected patients to be effective in preventing the spread of the infection.

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