

Hepatitis C virus among hemodialysis patients in Northern part of Palestine

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ABSTRACT: Hepatitis C virus (HCV) has been considered a significant problem for hemodialysis patients (HD). Most studies show a significant correlation between anti-HCV seropositivity and the number of blood transfusions and duration of dialysis. However infection with HCV has declined in regions where the screening for anti-HCV in blood banks and hemodialysis-specific infection control measures were adopted. The aim of the study was to evaluate the prevalence and the incidence of seroconversion for HCV in our hemodialysis unit during the period from June 1999 to Nov. 2000. Ninety five HD patients (64 M and 31 F) mean age 44.6 yrs (range, 14-70) with mean duration of dialysis 43 months (range, 13-124 months) were studied. All patients were interviewed and serum samples tested for HCV antibodies and ALT. Anti-HCV prevalence increased from 52.11% to 65.15% in the 1st period (6 months), however a decrease in positivity was observed by the end of the follow up study (46.32% final vs. 52.11% initial) when infection control measures were implemented. ALT test was used as an indicator for the episode of hepatitis; it seems to be a valuable indicator for those in the acute phase of infection since out of 10 cases with elevated ALT levels 7 were seroconverted to anti-HCV. The present data confirmed previous studies that duration of dialysis and blood products are risk factors for HCV infection. High prevalence rate among our HD patients suggested nosocomial transmission of HCV, and requires implementation of universal precautions in HD units.

Key Words: Palestine, HCV, Hemodialysis, ALT, Risk factors and HCV incidence.

INTRODUCTION

Hepatitis C virus (HCV) was first identified in 1989¹. This virus is of clinical importance because it is commonly associated with chronic liver disease and cirrhosis² and is possibly associated with hepatocellular carcinoma³.

Haemodialysis patients (HD) have a high risk of acquiring hepatitis C virus (HCV) infection. Transfusion of unscreened blood⁴, duration of dialysis⁵ and nosocomial transmission within haemodialysis units^{6,7} are implicated as the main transmission routes of HCV in HD patients. In these patients, HCV infection is usually asymptomatic⁸ and can be diagnosed by serological methods and by amplification of HCV RNA by reverse transcriptase-PCR (RT-PCR)⁹, which distinguishes between viraemic and non-viraemic HCV patients and also is used for HCV genotyping¹⁰.

The prevalence of HCV infection in HD patients is usually greater than that found in general population¹¹. The prevalence was found to be high and varies between countries (3.4- 70%) and between dialysis units within a single country^{12,13}.

The aims of the study were: 1) to evaluate the prevalence of anti- HCV among HD patients, 2) to study the risk of infection in relation to the duration of dialysis, 3) to assess the influence of virological and host factors on serum ALT levels, and 4) to study the acute phase and the incidence of seroconversion. Such a study would help to develop practical measures to minimize the spread of HCV infection among those patients and improve the management of HD operation.

MATERIALS AND METHODS

Patients'

The study was conducted during the period of June

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1999 to November 2000. A total of 95 HD patients (64 M, 31 F; mean age 44.6 years [range, 14-70 years]) enrolled at AL-Watani hospital dialysis unit in the city of Nablus, West Bank, Palestine. The mean duration of dialysis was 43 months (range 13-124 months). The patients were distributed on three daily shifts. The causes of their renal failure were chronic glomerulonephritis (n = 41), diabetic nephropathy (n = 28), polycystic kidney disease (n = 2) and obstructive nephropathy (n = 24). Sera samples were tested for anti-HCV at enrollment. On follow up, patient's sera were tested on weekly basis for alanine aminotransferase (ALT), and aliquots of the sera samples were stored at -80°C for further analysis. ALT test was used as an indicator for the episode of hepatitis. The episode was defined as elevation of serum ALT (normal <32 IU/L) in more than two sequential weekly tests, with a peak value of greater than 64 IU/L. Patients with previous history of elevated ALT levels due to other hepatic diseases were excluded. Hepatitis events were calculated to clarify its relationship with seroconversion to anti-HCV. By the end of 18 months all stored sera samples from all patients were tested for HCV. Information regarding sex, age, and duration of dialysis for all subjects involved was collected from patient's charts.

Dialysis Procedure

Dialysis machines (Fresenius AG, Germany) are located in a single room. No dedicated areas or machines were used for HCV infected subjects. Anti-HCV positive patients were not isolated, as they constitute a high proportion of the patients and because of undesirable psychological effects. It is reasonable to assume that the same staff treated all involved patients.

Hemodialysis has been performed with bicarbonate and Cuprophan or polysulfone dialyzers. No dialyzers were reused. Heparin was given as a bolus dose followed by an individually determined continuous infusion. All dialysis machines were disinfected by citrosteril and heat between treatments.

Serology

Anti-HCV for all sera samples was determined using Abbot IMx HCV Version 3.0 (Abbot Diagnostic Division, USA). All positive results were confirmed

using Immunocomb II (organics, Israel) according to manufacturer's instructions.

Biochemical Assays

ALT values were determined by a kinetic method using Cobas Mira (Roche, Germany) analyzers.

RESULTS

Patients were studied in three consecutive periods; each period lasted for 6 months. This strategy was adopted due to the complications generated by the high mortality rate and the introduction of new cases to the dialysis unit at various stages of the study. It is important to note that due to the limited number of cases we were obliged to include newly admitted cases.

Data presented in table I shows that 6 new cases were infected with HCV within the first period of the follow up. Out of the 71 studied patients, 5 HCV negative and 1 HCV positive cases died during this period. In period II none of the 41 HCV negative (18 newly admitted) cases were seroconverted to anti-HCV. By the end of this period 7 HCV negative and 2 HCV positive cases died. In the last period that included 40 HCV positive and 40 HCV negative (6 newly admitted cases), seroconversion to HCV positive was observed in 1 case only. Out of the 40 HCV negative cases 5 died. On the other hand 5 of the HCV positive cases died.

HD patients with acute hepatitis and peak ALT level are shown in table II. Out of 10 patients with clear elevated ALT levels (ranged 213-1200 IU/ml), 7 were seroconverted into anti-HCV positive. The conversion required time in days ranged about 21-109. Based on Immunocomb II assay, 4 of the seroconverted subjects expressed anti-core in an earlier date ranging between about 21-30 days, while the rest 3 expressed anti-NS on a later date ranging between about 56-109 days. It is worth noting that of the 7 seroconverted subjects, 4 showed hepatitis episodes within the same month (Aug. 1999), 2 were seroconverted in Nov. 1999 and 1 case in August 2000. The data also shows that most (6 out of 7) of the seroconversion was observed within the first 6 months of the study. Seroconversion into positive HBsAg was observed in one case.

ALT levels for positive/negative HD patients in

	Period I (June 99-Nov. 1999)			Period II (Dec. 99-May 2000)			Period III (June 2000-Nov. 2000)		
HCV - VE	Initial reading 34 (47.89%)	Final reading 23 (34.85%)	Deaths 5 (83.33%)	Initial reading 41 (49.4%)	Final reading 34 (45.95%)	Deaths 7 (77.78%)	Initial reading 40 (50%)	Final reading 34 (48.57%)	Deaths 5 (50%)
HCV + VE	37 (52.11%)	43 (65.15%)	1 (16.67%)	42 (50.6%)	40 (54.05%)	2 (22.22%)	40 (50%)	36 (51.43%)	5 (50%)
Total	71 (100%)	66 (100%)	6 (100%)	83 (100%)	74 (100%)	9 (100%)	80 (100%)	70 (100%)	10 (100%)

Table 1. HCV prevalence among HD patients during the three studied period.

correlation with anti HCV patterns are shown in table III. ALT elevation seems to be associated with both markers as they appear in the acute phase of the infection. It is worth noting that subjects expressing anti-NS at early stages of infection (before the appearance of anti-core) seems to show a much higher elevated ALT levels and such levels persist for a much longer time than that observed for anti-core (data not shown).

Data presented in table IV shows the association between dialysis duration and HCV infection and its markers. Infection rates of 14.29%, 71.61%, 83.33% and 100% for those on dialysis for 0-20, 21-40, 41-60 and above 60 months, respectively. This table also shows that with increased duration of dialysis, a shift from anti-core or anti-NS to both markers and with a clear longer persistence for anti-NS marker compared to anti-core.

The general characteristic and clinical picture of all cases (95) by the end of the follow up study of HD patients are presented in table V. Overall, 46.32% and 53.68% were observed for HCV positive and HCV negative cases, respectively. Compared to the initial findings a clear decrease in HCV seropositivity was observed (52.11%) as shown in table I, period I. With respect to gender, males showed a much higher percentage of HCV seropositivity compared to females (63.64% and 36.36% for males and female, respectively). Association between HCV infection and ALT levels showed 5-fold increase in HCV positive cases compared to HCV negative subjects (38.64% to 7.84%).

DISCUSSION

HD patients are at high risk for hepatitis viral infections due to the high number of blood transfusions, prolonged vascular access and the potential for exposure to infected patients and contaminated equipment¹⁴.

In the northern Palestine, Al-Kurdi 1998 reported the prevalence of 0.24%, 34.4%, 10.8%, and 39.6% for blood donors, kidney transplant, transfusion dependent and hemodialysis patients, respectively. The findings of Alkurdi, 1998 encouraged us to evaluate HCV status among HD patients¹⁵.

During period I of the study a clear increase (13%) in HCV rate was observed. This sudden increase took place within three months as 4 subjects were seroconverted during August 1999 and the other 2 in November of the same year. Such a finding clearly indicates a nosocomial infection within the unit. To confirm this proposed hypothesis it was essential to carry out genotyping analysis for the involved subjects. Unfortunately, such techniques are not available in the area.

During period II of the study none of the 41 HCV negative cases were seroconverted to Anti-HCV, providing evidence in support of the nosocomial mode of transmission within the unit during the first period of the study. The observed reduction in HCV prevalence rate compared to that observed during the first phase is most likely due to the strict recommendations of this study based on the initial findings in period one. It is worth mentioning that the recommendations included the use of separate heparin vials, sharing of equipments and the frequent change of gloves¹⁶.

In period III, the data clearly indicates drop in the

No.	Sex	Age	Onset of hepatitis	Peak ALT IU/L	Seroconversion/days	Antibody Type
1.	F	53	Aug. 1999	580	56	NS*
2.	M	53	Aug. 1999	488	21	Core
3.	F	39	Aug. 1999	213	70	NS
4.	M	70	Aug. 1999	332	28	Core
5.	M	35	Nov. 1999	313	109	NS
6.	M	14	Nov. 1999	599	30	Core
7.	M	50	Feb. 2000	437	No serocon.	----
8.	M	14	Feb. 2000	246	No serocon./D*	----
9.	M	30	Aug. 2000	600	23	Core
10.	F	65	Feb. 2000	1200		

HBV infection (one case)

*NS: Non structural

*D: Dead

Table 2. Patients with acute hepatitis and peak ALT levels.

Anti-HCV patterns					
ALT level	Core-ve/NS-ve	Core+ve/NS-ve	Core-ve/NS+ve	Core+ve/NS+ve	Total
Normal	47 (92.16%)	0 (0%)	0 (0%)	27 (84.38%)	74 (77.89)
Elevated	4 (7.84%)	4 (100%)	8 (100%)	5 (15.6%)	21 (22,11%)
Total	51 (100%)	4 (100%)	8 (100%)	32 (100%)	95 (100%)

Table 3. Correlation between anti-HCV pattern and ALT levels among HD patients.

prevalence of HCV infections, although the majority of the study cases were on maintained hemodialysis for longer than 12 months within the same unit. Thus, confirming that the adopted hygiene policy for the control of HCV is effective.

ALT status could be of a limited diagnostic value in chronic patients; however it seems to be a valuable indicator for those in the acute phase of infection. Monitoring of ALT levels, among follow up patients, seems to provide more support to the significant association between elevated ALT levels and HCV seropositivity¹⁷. Thus, it seems reasonable and essential to take in account that one ALT measuring is not a good enough indicators for the association between HCV infection and ALT.

In this study, monitoring of ALT levels during the first phase (3 times a week) showed a significant increase in ALT levels among HCV negative cases (10 out of 34). Out of those subjects, 7 entered the acute phase of HCV, 1 case seroconverted into HBsAg positive (data not shown) and 2 showed elevated levels without any signs of seroconversion to either HCV or HBV. Such data is in agreement with previous reports on the association between HCV and ALT elevation¹⁸. It also confirms that ALT monitoring is a valuable tool in HCV diagnosis. Reason(s) for the observed fluctuation and abnormality in ALT levels in the other 2 cases is not known, however, other viral infections and or drug interactions may account for such elevation¹⁹.

Anti-HCV markers	Duration of dialysis/months				Total
	0-20	21-40	41-60	>60	
Core-ve/NS-ve	42 (85.71%)	8 (27.58%)	1 (16.67%)	0 (0%)	51 (53.68%)
Core+ve/NS-ve	3 (6.12%)	1 (3.45%)	0 (0%)	0 (0%)	4 (4.21%)
Core-ve/NS+ve	3 (6.12%)	2 (6.9%)	1 (16.67%)	2 (18.18%)	8 (8.42%)
Core+ve/NS+ve	1 (2.05%)	18 (62.07%)	4 (66.66%)	9 (81.82%)	32 (33.69%)
Total	49 (100%)	29 (100%)	6 (100%)	11 (100%)	95 (100%)

Table 4. Association between HCV markers, infection and duration of dialysis.

Patient characteristic	Patients	
	Anti-HCV + ve	Anti-HCV - ve
No. of patients	44 (46.32%)	51 (53.68%)
Male	28 (63.34%)	36 (70.59%)
Female	16 (36.36%)	15 (29.41%)
Median age (yr)	43.07	45.92
Age range (yr)	14-70	14-70
Median duration of dialysis (mo)	43	13.92
Range of duration of dialysis (mo)	13-124	4-57
No. HBsAg positive	6 (13.63%)	2 (3.92%)
ALT patterns		
Normal	27 (61.36%)	47 (92.16%)
Elevated	17 (38.64%)	4 (7.84%)
Causes of renal failure		
- Diabetic nephropathy	8	20
- Obstructive nephro.	10	14
- Glomerulonephritis	25	16
- Polycystic kidney disease	1	1

Table 5. General characteristic and the clinical data for HCV positive/negative HD patients by the end of the follow up period.

With respect to HCV related antigens (core and NS antigens), our findings are in agreement with previous observations²⁰ in which antibodies to the core appeared earlier (range 21-30 days) than NS antigens (range 56-109 days). Anti-core antibody seems to be closely associated with HCV RNA and considered as

a reliable marker for viral replication²¹. On the other hand, anti-NS antibodies may appear first²², as observed among 3 out of 7 HCV seroconverted subjects in our study. Subjects expressing anti-NS during the acute phase and before the onset of anti-core seems to express a much higher ALT levels. Such levels

persist for a much longer time compared to that observed when anti-core expressed first.

A shift from anti-core or anti-NS to both markers was observed in association with increased dialysis duration. These findings suggest that immune response changes are not influenced by dialysis duration and could be a consequence of HCV infection duration. Such finding is in agreement with previous study²³. The findings of 14.29%, 71.61%, 83.33% and 100% HCV seropositivity rates among those with dialysis duration of 0-20, 21-40, 41-60 and above 60 months, respectively, strongly indicate that dialysis is a major risk factor for HCV infection.

By the end of the study period, a decrease in HCV infection rate was observed (46.32% final/ 52.11% initial). This could be attributed to: first, the observed nosocomial infection at the first phase of the study and second, to the fact that 18 and 6 newly admitted cases were enrolled during the second and third phases of the study. The adoption of new hygiene strategies based on our recommendations by the end of phase 1 might be another factor that account for such decrease in HCV infection rate. The finding of 38.64% and 7.84% with elevated ALT levels among HCV positive and HCV negative cases, strongly link ALT elevation with HCV seropositivity. This finding is in agreement with previous report on the behavior of ALT marker²⁴.

With respect to gender, males showed a much higher rate (63.64%) of HCV infection compared to females (36.36%) among dialysis patients. Reasons behind such variations are not clear, however, findings of much higher rate of diabetic males compared to females, which seems to lead to further complications including renal failure may account for this. On the other hand, renal obstruction due to prostate cancer, congenital reflux and gouty may contribute to such variations. One should point out in this respect that such complications are usually the result of lack of good follow up regimes.

In conclusion, the prevalence of HCV infection in Palestinian HD patients is high (52.11%). This indicates a nosocomial infection in the dialysis unit, however, mode of transmission is not clear and further investigation at the molecular level, seems to be essential at this stage. In addition, this study showed a significant decline of HCV infection among HD

patients, concentrating on the importance of health control measurements such as screening for anti-HCV in blood bank and infection control measures for control and prevention of HCV in HD environment.

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Ο ιός της ηπατίτιδας C μεταξύ ασθενών σε αιμοδιάλυση στο βόρειο τμήμα της Παλαιστίνης

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ΠΕΡΙΛΗΨΗ: Η λοίμωξη από ιό της Ηπατίτιδας C (HCV) θεωρείται ένα σημαντικό πρόβλημα για τους ασθενείς που υποβάλλονται σε αιμοδιάλυση (HD). Οι περισσότερες μελέτες δείχνουν σημαντική συσχέτιση μεταξύ οροθετικότητας για anti-HCV αντισώματα και αριθμού μεταγγίσεων αίματος και διατήρησης της διάλυσης. Εν τούτοις, η λοίμωξη από τον ιό HCV έχει υποχωρήσει σε περιοχές όπου έχει εφαρμοστεί εξέταση για anti-HCV αντισώματα στις Τράπεζες Αίματος και όπου έχουν υιοθετηθεί ειδικά μέτρα για τον έλεγχο των λοιμώξεων σε μονάδες Αιμοδιάλυσης. Σκοπός της παρούσης μελέτης ήταν η εκτίμηση του επιπολασμού και της επίπτωσης (συχνότητας) της ορομετατροπής για τον ιό HCV στη δική μας μονάδα αιμοδιάλυσης κατά τη διάρκεια της περιόδου από τον Ιούνιο του 1999 μέχρι το Νοέμβριο του 2000. Μελετήθηκαν 95 ασθενείς σε αιμοδιάλυση (64 Α και 31 Θ) με μέση ηλικία 44.6 έτη (εύρος 14-70 έτη) και μέση διάρκεια αιμοδιάλυσης 43 μήνες (εύρος 13-124 μήνες). Από όλους τους ασθενείς ελήφθησαν ιστορικό και δείγματα αίματος για την ανίχνευση αντισωμάτων και ALT. Ο επιπολασμός των anti-HCV αυξήθηκε από 52.11% σε 65.15% κατά την πρώτη περίοδο (6 μήνες), αλλά το ποσοστό της θετικότητας ελαττώθηκε κατά το τέλος της μελέτης (τελική τιμή 46.32% προς 52.11% αρχικά) όταν εφαρμόστηκαν μέτρα ελέγχου των λοιμώξεων. Η παράμετρος ALT χρησιμοποιήθηκε ως δείκτης επεισοδίου ηπατίτιδας. Φαίνεται πως αποτελεί έναν αξιόλογο δείκτη για όσους βρίσκονται στην οξεία φάση της λοίμωξης καθότι από τις 10 περιπτώσεις με αυξημένη ALT, 7 παρουσίασαν ορομετατροπή σε θετικότητα για anti-HCV αντισώματα.

Τα παρόντα δεδομένα επιβεβαιώνουν προηγούμενες μελέτες που δείχνουν ότι η διάρκεια της αιμοδιάλυσης και τα προϊόντα του αίματος αποτελούν παράγοντα κινδύνου για HCV λοίμωξη. Ο υψηλός επιπολασμός μεταξύ των ασθενών μας που βρίσκονται σε αιμοδιάλυση συνηγεί υπέρ νοσοκομειακής μετάδοσης του HCV και απαιτεί εφαρμογή ειδικών μέτρων πρόληψης στις δικές μας μονάδες Αιμοδιάλυσης.

Λέξεις Κλειδιά: Παλαιστίνη, HCV, Αιμοδιάλυση, ALT, Παράγοντες κινδύνου και HCV.

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