Prevalence of hepatitis C virus RNA in patients with chronic renal diseases

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انتشار الحمض النووي الريبي لفيروس التهاب الكبد ج في المصابين بأمراض كلوية مزمنة

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خلاصة: تم تحديد معدل انتشار الحمض النووي الريبي لفيروس التهاب الكبد ج (HCV) في ثمانين من المصابين بأمراض كلوية مزمنة. فاستعملت لهذا الغرض مجموعتان للبرمجة الأولية من المنطقة غير الشفرية بالفيروس HCV. وبعد تضخيم النواتج (188 bp) بواسطة تفاعل سلسلة البوليمراز، تم إظهارها بالرحلان الهلامي للأغاروز 2% الملون ببروميد الإثيديوم. وتم تصنيف المرضى في أربع مجموعات. شملت المجموعة الأولى أربعين مريضاً بالغاً يعانون المرحلة الأخيرة من الفشل الكلوي، وكان 31 مريضاً منهم (77.5%)، إيجابيين للحمض النووي الريبي للفيروس HCV. وشملت المجموعة الثانية 22 طفلاً يعانون اعتلالات في كبيبات الكلى، وكان 15 طفلاً منهم (68.2%) إيجابيين للحمض النووي. أما المجموعة الثالثة فقد شملت تسعة أطفال يشكون من فشل كلوي مزمن، وكان ستة منهم (66.6%) إيجابيين للحمض النووي. وشملت المجموعة الرابعة تسعة أطفال يعانون فشلاً كلوياً مزمناً ناجماً عن اعتلال بولي انسدادي، وكان ثلاثة منهم (33.3%) إيجابيين للحمض النووي. ونستنتج من ذلك أن الفيروس HCV يمكن أن يصيب نسبة مئوية كبيرة من مرضى الفشل الكلوي المزمن أو المصابين بأمراض في لباب الكلى.

ABSTRACT The prevalence of hepatitis C virus (HCV) RNA in 80 patients with chronic renal diseases was determined. Two sets of primers from the non-coding region of the hepatitis C virus were used. The products (188 bp) amplified by polymerase chain reaction were visualized by 2% agarose gel electrophoresis stained with ethidium bromide. The patients were classified into four groups. Group I comprised 40 adult patients with end-stage renal disease, 31 of whom were positive for HCV-RNA (77.5%); group II, 22 children with glomerulopathies, 15 of whom were positive (68.2%); group III, 9 children with chronic renal failure of unverified etiology, 6 of whom were positive (66.6%); group IV, 9 children with chronic renal failure due to obstructive uropathy of whom 3 (33.3%) were positive. We conclude that HCV may infect a high percentage of patients with chronic renal failure or renal parenchymatous disease.

Prévalence de l'ARN du virus de l'hépatite C chez des patients souffrant de maladies rénales chroniques

RÉSUMÉ La prévalence de l'ARN du virus de l'hépatite C (VHC) chez 80 patients souffrant de maladies rénales chroniques a été déterminée. Deux séries d'amorces de la région non codante du virus de l'hépatite C ont été utilisées. Les produits (188 bp) amplifiés par PCR (réaction de polymérisation en chaîne) ont été visualisés par électrophorèse sur gel d'agarose à 2% coloré avec du bromure d'éthidium. Les patients ont été classés en quatre groupes. Le groupe I consistait de 40 adultes au stade d'insuffisance rénale terminale dont 31 se sont révélés positifs pour l'ARN du VHC (77,5%). Le groupe II comprenait 22 enfants atteints de glomérulopathies dont 15 étaient positifs (68,2%). Le groupe III comportait 9 enfants atteints d'insuffisance rénale chronique d'étiologie non vérifiée dont 6 étaient positifs (66,6%). Le groupe IV consistait de 9 enfants atteints d'insuffisance rénale chronique due à une uropathie obstructive dont 3 (33,3%) étaient positifs. Nous en concluons que le VHC peut infecter un fort pourcentage de patients souffrant d'insuffisance rénale chronique ou de néphropathie parenchymateuse.

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Introduction

Hepatitis C virus (HCV) is a weak virus that infects immunocompromised hosts suffering another coexistent infection, such as acquired human immunodeficiency syndrome, or with chronic debilitating diseases, such as chronic renal failure and chronic active or persistent hepatitis B virus infection. Patients given blood products are at a high risk of acquiring this virus, such as haemophiliacs, haemodialysis patients and organ transplantation patients [*1*].

The incidence of hepatitis C virus infection in Egypt is being studied on a community scale by several investigators as well as by the hepatology committee of the Ministry of Health. In the general population, anti-HCV has been reported in 12% of children in a rural primary school, in 18% of the general population in a rural area (increasing with age) and in 16% of children with endemic hepatomegaly. In multitransfused children and patients on maintenance haemodialysis, the frequency has been found to be 44.5% and 46.0% respectively [*2*].

HCV is the most frequent cause of liver disease in dialysis and renal transplant recipients [*3*]. Approximately 20%–30% of dialysis patients are infected with HCV [*2*]. HCV is also recognized as a cause of membranoproliferative and membranous glomerulonephritis [*3*]. A possible relationship between HCV infection and transplant glomerulopathy has been described; therefore in renal transplant patients with proteinurea, serology for HCV infection, HCV-RNA and immunological tests should be performed as part of the differential diagnosis [*4*].

Many authors have described the progression of renal disease [*5,6*]. Hammoud et al. reported that terminal renal failure is the outcome of type I membranoglomerulonephritis (MPGN) in renal allograft [*5*]. Fornaisieri et al. reported that in one-third of their patients, remission of renal symptoms occurred and 20% of patients experienced nephritic or nephrotic flare-up during the course of the disease [*6*]. Uraemia was observed in only 10% of the patients 10 years after renal disease onset; however, 50% of patients died from cardiovascular diseases, infectious liver failure or neoplasia during those 10 years.

The aims of the study were to determine the prevalence of HCV-RNA in patients with chronic renal disease using polymerase chain reaction (PCR), the most sensitive technique for detection of HCV-RNA in serum.

Patients and methods

In all, 80 patients were included in the study. They were classified into four groups. Group I: 40 adult patients with end-stage renal disease; group II: 22 children with glomerulopathies, mostly nephrotic syndrome; group III: 9 children with chronic renal failure of unverified etiology; and lastly group IV: 9 children with chronic renal failure due to obstructive uropathy. The clinical and biochemical data of the four groups are shown in Table 1.

RNA was extracted from 100 µl serum by RNA zol solution (Cinna/Biotex Bulletin Incorporated, USA). It was reverse transcribed using 1 µl Moloney murine leukaemia virus reverse transcriptase (MMLV RT) enzyme (Bohringer Manheim), 1 µl ribonuclease blocker, 2 µl deoxynucleotide triphosphates (dNTPs) and 5 µl primer 1CH. The incubation time was 1 hour at 37 °C followed by 5 minutes at 90 °C. The first PCR mixture was formed of 10 µl (10 × PCR buffer), 5 µl of each primer (1CH and 2CH), 0.8 µl dNTPs, 2–5 units

Table 1 **Clinical and biochemical data of the four groups**

Group	No.	Male: female	Age range (years)	Clinical diagnosis	No.	Positive HCV	Creatinine level (mg/dl)
I	40	1:1	22–84	*ESRD*			6.9–14.0
				Unverified etiology	22	16	
				Diabetes mellitus	11	9	
				Obstructive uropathy	6	6	
				Amyloidosis	1	–	
II	22	1:1	3–18	*Nephrotic syndrome (NS)*			0.3–0.8
				Nephrotic nephritis	4	3	
				NS late onset	4	1	
				NS early onset	3	2	
				NS frequent relapses	3	3	
				NS steroid-dependent	6	5	
				NS steroid-resistant	2	1	
III	9	4:5	3–14	*Renal failure due to unverified etiology*			1.6–12.5
				Chronic renal failure	4	2	
				ESRD	5	4	
IV	9	7:2	5–12	*Renal failure due to obstructive uropathy*			1.3–12.0
				Chronic renal failure	6	2	
				ESRD	3	1	

HCV = hepatitis C virus *ESRD = end-stage renal disease*

Thermus aquaticus (Taq) polymerase and 5 µl of previously formed cDNA. The PCR cycling condition was one cycle at 94 °C for 4 minutes, 50 °C for 1 minute and 72 °C for 2 minutes followed by 34 cycles of 94 °C for 1 minute, 50 °C for 1 minute and 72 °C for 2 minutes. The nested PCR was formed in the same way as the first PCR, but we used two nested primers (4CH and 1TS) (Table 2). Only 5 µl of the first amplification was added to the PCR mixture and the cycling condition was the same but with 24 cycles only. The amplified product was visualized using 2% agarose gel electrophoresis stained with ethidium bromide. The positive samples showed bands at 188 bp (Figure 1). Student's *t*-test was used for statistical comparisons between proportions.

Table 2 Sequence of oligonucleotide primers

Primer 1CH	GGT GCA CGG TCT ACG AGA CCTC
Primer 2CH	AAC TAC TGT CTT CAC GCA GAA
Primer 4CH	ATG GCG TTA GTA TGA GTG
Primer 1TS	GCG ACC CAA CAC TAC TCG GCT

Results

Table 3 shows the incidence of HCV-RNA in the different groups. In group I, 31 of the

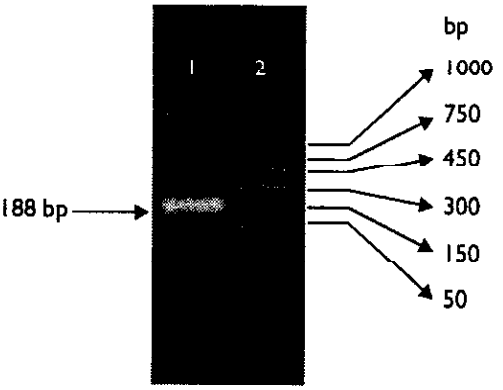

Figure 1 **A garose gel electrophoresis stained with ethidium bromide. Lane I shows a positive case of HCV and lane 2 shows PCR marker.**

Table 3 **Incidence of HCV-RNA in the different groups**

Group	No. positive	%
I	31/40	77.5
II	15/22	68.2
III	6/9	66.6
IV	3/9	33.3

40 adult patients were positive for HCV. In group II, 15/22 were positive. Patients in group II were classified according to their pathological findings into focal segmental glomerulosclerosis (FSGS) (nine cases), mesangioproliferative glomerulonephritis (GN) (five cases), no change under light microscopy (five cases) and one case each of membranoproliferative, membranoglomerulonephritis and focal proliferative GN. Of these, five with FSGS, five with mesangioproliferative GN, two with no change in their pathology, the three patients with membranoproliferative, membranoglomerulonephritis and focal proliferative GN were all positive for HCV-RNA. In group III, six patients were positive, five of whom had a history of blood transfusion as a risk factor. In group IV, only three patients were positive for HCV-RNA, two of whom had a history of blood transfusion.

Discussion

HCV infection has been described in association with various types of glomerular disease, usually type I membranoproliferative glomerulonephritis and rarely membranous glomerulonephritis [*4*].

In our study, group I gave positive results in 31 patients out of 40 (77.5%). All patients were diagnosed as having end-stage renal disease. Risk factors for developing HCV infection include a history of blood transfusion, a history of dialysis and a history of endoscopy and immunosuppressive drugs. Most of the 31 patients had one or more of these risk factors. Machine dialysis rather than blood transfusion was largely responsible for this high prevalence of hepatitis C in this group. All patients in this group were candidates for renal transplantation. Genesca et al. concluded that HCV infection was extremely prevalent in renal transplant recipients in Spain and was the main cause of chronic liver disease in such patients.

In the second group, 15 patients were positive. The positive patients were diagnosed pathologically as five with FSGS, five with mesangioproliferative GN, two with no change under light microscopy and one case of membranoproliferative, one of membranoglomerulonephritis and one of focal proliferative GN. Our results concur with those of Gallay et al. who reported that chronic infection with HCV had been iden-

tified as a cause of type I membrano-proliferative glomerulonephritis [*8*]. They also concluded that HCV infection was common in patients with end-stage renal disease and may persist in renal allograft recipients. Garcia-Valdecasas et al. found that the prevalence of HCV antibodies was higher (16.6%) in patients with glomerulonephritis compared with that of patients diagnosed as interstitial nephritis [*9*].

In group II patients with nephrotic syndrome, we found there was no statistically significant difference between those with idiopathic nephrotic syndrome (no change under microscopy), mesangioproliferative glomerulonephritis or follicular sclerosing glomerulonephritis and pathological forms of nephrotic syndrome ($P = 0.295$). Although all cases of frequently relapsing nephrotic syndrome (3/3) and most cases of steroid-dependent nephrotic syndrome (5/6) were HCV positive, no statistical conclusions could be drawn because of the relatively small numbers of patients in each subgroup. Similar to our findings, Sansonno et al. demonstrated the presence of HCV protein in the mesangium and suggested that mesangial cells were susceptible to HCV infection [*10*]. It has been reported that HCV replicates in both peripheral and bone marrow lymphomonocytes [*11*].

In our study, HCV-RNA was detected in six patients out of nine (66.6%) in group III with chronic renal failure secondary to an idiopathic chronic renal failure; five of these patients had a history of blood transfusion, two had chronic renal failure and four had end-stage renal disease. In group IV with chronic renal failure secondary to obstructive uropathies, only three patients were positive, two patients also had chronic renal failure and one suffered from end-stage renal disease.

There was no significant difference in the prevalence of HCV among patients with identified etiology (obstructive or diabetic-induced glomerulosclerosis) and other groups with unidentified etiology (atrophic kidneys or idiopathic glomerulonephritis) ($P = 0.939$).

Our data show that predialysis patients with chronic renal failure should be considered a specific risk group for HCV infections. Blood transfusion history and certain types of chronic renal failure are risk factors for acquiring HCV infection which may play a role in the development of liver disease in this clinical setting.

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The causative agent of hepatitis C was only identified in 1989. Its identification and characterization led to the understanding of its primary role in post-transfusion hepatitis and its tendency to induce persistent infection: percutaneous exposure to blood is the predominant mode of transmission of hepatitis C. In developed countries it is estimated that 90% of cases of hepatitis C are in current and former intravenous drug users and those with a history of transfusion of unscreened blood or blood products, such as haemophiliacs. In developing countries, it is believed that unsterile injections and unscreened blood are the predominant modes of transmission.

Source: World Health Organization Press Office. Press release WHO/36, 1 May 1998.