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# **Nosocomial hepatitis C virus infection in a renal transplantation center**

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## **Abstract**

Nosocomial hepatitis C virus (HCV) infections were recorded in the renal transplantation unit of the university hospital. There were cases of acute HCV infection with aggressive clinical courses diagnosed from a positive HCV RNA test in the early post-transplantation period and which remained anti-HCV negative. Their anti-HCV seronegativity was attributed to them having acquired HCV under intense immunosuppressive therapy and suggested that the aggressive clinical course could be due to the deficient immune response resulting in an inability to limit viral replication. There were also donors diagnosed as having acute HCV infection in the early post-operative period. Genotyping and sequence analysis for HCV were performed on the isolates of eight of these patients who were consecutively transplanted and of three donors whose recipients were infected with HCV prior to transplantation, and who acquired acute HCV infection after transplantation. Of the eight recipients in the first group three were genotype 1a, three were genotype 1b, one was genotype 3a, and the last one was genotype 4 according to Simmond's classification. Of the three donor–recipient couples both the HCV isolates from one couple were genotyped as 1b and the phylogenetic analysis indicated that the patients were infected with a common variant of HCV, but the genotypes of HCV isolates from the other couples were different. Recipients were genotype 1b and the donors were genotype 1a in these couples. Genotype results of the first group and donor–recipient couples, and sequence analysis of genotype 1b and 1a isolates, showed that the source of infection was not a unique strain and there were multiple breaks in universal precautions while managing these patients.

Hemodialysis patients and renal transplantation recipients are at high risk for hepatitis C virus (HCV) infection. Known sources for this infection in these groups of patients are blood transfusions, hemodialysis and receiving kidneys from infected donors, but other nosocomial routes of transmission have also been reported [1–4].

Three hundred and fifty-two renal transplantations were performed in the Ege University Organ Transplantation Centre between 1988 and 1998. All donors and recipients are screened for HCV antibody before transplantation, however, HCV-positive recipients are accepted for transplantation in this center. It was noticed that there were cases with acute C hepatitis in the early post-transplantation period in a group of these patients. They were diagnosed as acute C hepatitis by HCV RNA test (Amplicor, Roche Diagnostics, Branchburg, NJ, USA), since their antibodies for HCV (UBI-HCV, United Biomedical Inc., Hauppauge, NY, USA) were negative. These were evaluated as nosocomial infections and it was planned to determine the nature of this infection.

The recognition of nosocomial HCV infection in this unit was of acute hepatitis C cases in the early post-operative period who underwent an aggressive clinical course and progressed rapidly to cirrhosis as previously reported by Ok *et al.* [5]. These patients were seronegative for anti-HCV, but positive for HCV RNA in serum. The lack of an antibody response in these recipients may have resulted from them acquiring HCV under intense immunosuppression, and the aggressive clinical course could be due to deficient immune response. The finding of anti-HCV negativity in 34% of the HCV RNA-positive recipients supports the first hypothesis [6].

HCV infections in the early post-transplant period were evaluated as pre- or post-transplantation acquired infections. It had been questioned if those patients had been infected during hemodialysis and were seronegative because of immunosuppression due to renal deficiency. The preoperative anti-HCV status of each recipient was known, but HCV RNA status was not known. Anti-HCV positivity in the hemodialysis unit was 45.7% and seronegative HCV RNA positivity was not observed unless there was a recent infection as previously reported [7]. HCV RNA screening of pre-transplant recipients was also started after the recognition of these cases. There were three donors who became HCV infected. These observations led to a conclusion that those nosocomial HCV infections could have been acquired during or after transplantation.

Two interesting groups of patients with nosocomial HCV infection were recognized during follow-up. The first group consisted of renal transplant recipients who were transplanted consecutively every other week after a pre-operatively HCV-infected patient, and had acute HCV hepatitis at the early post-transplant period. In the first group there was a total of eight recipients; four of them were transplanted between 15 November and 6 December 1993 and four others were transplanted between 3 March and 4 April 1994. The second group consisted of three donor–recipient couples whose recipients were HCV-infected pre-transplant and the donors became HCV positive. These two representative groups were chosen for investigation by genotyping and phylogenetic analysis.

The study was designed to investigate the phylogenetic relationship of HCV sequences responsible for nosocomial infection in two groups of patients. HCV isolates were genotyped using restriction fragment length polymorphism (RFLP) analysis of nested 5' non-coding region (NCR) reverse transcription–polymerase chain reaction products as previously described [8]. Amplification of NS5B from HCV sequences utilized outer primers 1203 and 1204, and biotinylated inner primers 123 and 122 [9]. Biotinylated products were immobilized on streptavidin-coated magnetic beads (Dynabeads M280, Dynal A/S, Oslo, Norway) and single strands were sequenced directly using T7 DNA polymerase (SEQUENASE version 2.0, USB, Cleveland, OH, USA) according to the manufacturer's instructions. Sequencing was performed in the University of Edinburgh. Phylogenetic analysis of sequences was carried out on a 248-base pair fragment in the NS5B region corresponding to nucleotide positions 7964–8211 in the HCV type 1a clone, HCV-PT [10]. The nucleotide sequences of group I were aligned with other sequences from GenBank by CLUSTAL W version 1.7. Phylogenetic analysis of sequences from the NS5 region of type 1a and 1b sequences using neighbor-joining on nucleotide distances was calculated using Jukes–Cantor correction for multiple substitution. The phylogenetic tree constructed by TREECON for Windows version 1.3b.

Of the eight recipients who were consecutively transplanted in group I, three genotype 1a, three genotype 1b, one genotype 3a, and one genotype 4 HCV sequences were identified (Table 1).

Of the three donor–recipient couples, one couple was infected with genotype 1b, whereas the remaining two recipients were infected with type 1b while the donors were infected with type 1a (Table 2). Predominant genotypes were 1b and 1a in our study. Genotype distribution in Turkish renal transplant recipients was previously reported as being 73.4% genotype 1b,

24.4% genotype 1a and 2.2% genotype 4 [11]. The phylogenetic tree analysis showed two type 1b sequences from patients 4 and 7 and type 1a sequences from patients 2, 3 and 5 in group I were clustered closely with high bootstrap values (Figure 1). Type 1b sequences isolated from patients 1 and 4 seem not to be related, while type 1b sequences from patients 4 and 7 showed some evidence of linkage. However, patient 7 was transplanted 4 months after patient 4. Type 1a sequences isolated from patients 2 and 3 grouped together with sequence 5, but there were 4 months between the operation dates of patient 3 and 5. It is not possible to define a traceable source of infection among those sequentially transplanted recipients; there were different genotypes and unrelated HCV sequences in phylogenetic analysis.

Type 1b sequences in group II: 9, 10 and 13 clustered together despite the inclusion of a large dataset of epidemiologically unrelated type 1b sequences indicating that the patients were infected with a common variant of HCV. Patients 10 and 13 were a donor–recipient couple as shown in Table 2.

It had been questioned if there was any carry-over between the donor and recipient such as surgeons going between the recipient and donor with the same gloves. Genotypes and nucleic acid sequences of HCV isolates from the donor and the recipient were compared to answer this question. However, recipients and donors in two couples were found to be infected with different HCV genotypes (1b and 1a, respectively). It is likely that only one couple was infected with a common variant of HCV (patients 10 and 13). One recipient (patient 9) who underwent transplantation a week before the couple of 10 and 13 was also infected with this variant. However, it is not possible to tell the origin of this virus; both of the recipients had been infected before the transplantations. It is apparent that the other donors (patients 12 and 14) were infected with other HCV variants.

In conclusion, concerning these strains of nosocomial HCV infection in this centre there was no traceable source of infection among sequentially transplanted recipients. There were different genotypes and unrelated HCV isolates in phylogenetic analysis. There was one couple with a related sequence, but this sequence was also found in another unrelated couple's recipient. These data suggest that multiple breaches in universal infection control precautions could be responsible for these HCV infections. None of the staff was infected with HCV. Infection control procedures were evaluated by personal interviews with the medical staff; some poor practices were found concerning the sterilization, disinfection and

universal infection control procedures used in managing these patients, including not changing gloves between patients and insufficient sterilization of some re-used equipment.

The Organ Transplantation Centre responded by redoubling educational efforts among the staff, improving the practice of universal infection control measures and strictly preventing re-use of disposable material. This lowered the HCV infection rate. In 1999, the Central Sterilization Unit started to function in the hospital under the control of the Infection Control Committee and no new cases have been detected since then.

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Tables and figures

Tab.1

Genotyping results of group I

Patient no	Date of transplantation	Genotype
1	15-11-1993	1b
2	22-11-1993	1a
3	29-11-1993	1a
4	06-12-1993	1b
5	21-03-1994	1a
6	23-03-1994	3a
7	28-03-1994	1b
8	04-04-1994	4

Tab.2

Genotyping results of group II

Couple no	Recipient		Donor	
	Patient no	Genotype	Patient no	Genotype
1	9	1b	12	1a
2	10	1b	13	1b
3	11	1b	14	1a

Fig.1

Phylogenetic tree of 1b and 1a HCV NS5B region sequences of the isolates from six recipients in group I. The GenBank accession numbers of the HCV 1a and 1b sequences used in phylogenetic analysis are: genotype 1a sequences, AF071979, AF071966, AF071964, AF071975, AF071982, AF071986, AF071963; genotype 1b sequences, AF071976, AF071957, AF071962, AF071978, AF071973, AF071963.



