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Transmission of hepatitis C virus to recipients of parenteral vitamin therapy in a primary care facility

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ABSTRACT

Background and objectives: The Australian prevalence of hepatitis C virus (HCV) is approximately 1%, with the majority of cases acquired through injecting drug use. However, occasionally HCV infection occurs in healthcare settings. Three new HCV infections were identified amongst patients attending a general practice in Sydney, Australia, specialising in parenteral vitamin therapy.
Study design: An investigation was conducted to identify the source of infection and mechanism of transmission. Molecular analysis was conducted by sequencing the HCV NS5A, Core and NS5B regions.
Results: Two sources were identified using molecular epidemiology – a genotype 3a case was the source for a case acquired in late 2004 and a genotype 1b case the source for one case acquired in late 2006 and another in early 2007. The common risk factor was parenteral vitamin C therapy.
Conclusions: Inadequate infection control was apparent and likely to have resulted in blood contamination of the healthcare workers, their equipment, the clinic environment and parenteral medications. Molecular and clinical epidemiology clearly identified parenteral transmission of HCV, highlighting the risks of blood contamination of parenteral equipment and use of multi-dose flasks on more than one patient.

1. Background

Hepatitis C virus (HCV) is a significant problem worldwide. In Australia the prevalence rate of antibodies to hepatitis C virus (anti-HCV) is approximately 1%, with a spontaneous clearance in only a quarter of new cases.[1] More than 80% of HCV transmission in Australia now occurs through sharing of injecting drug equipment.[2] Unfortunately, despite the existence of a nationally accepted minimum standard and a regulatory framework for infection control,[3,4] HCV infection still occurs in health care settings.

Most cases of newly acquired HCV infection are asymptomatic, and many go undetected. Healthcare associated transmission is well-recognised and reported, generally occurring in hospital facilities associated with a high frequency of parenteral procedures, such as renal dialysis units and oncology outpatient clinics.[5–7] Transmission in general practice settings has been reported less frequently.[8]

HCV is classified into six genotypes which differ in genomic sequence by more than 30%, with subtypes within each genotype differing by 20–25%.[9] Molecular analysis has emerged as an important tool in establishing transmission events, and has been used to provide evidence of transmission of infection in cases of clinically acquired HCV.[10–14]

2. Objectives

The setting reported here was a general practice in which around 80% of the clients attended for intravenous vitamin C (IVC) and other parenteral therapies. We describe the epidemiological and molecular epidemiological investigation which confirmed the existence of two HCV clusters.

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Table 1
Cases described in this study, date of first known positive HCV serology, HCV genotype identified in 2007 and type of parenteral vitamin treatment. 1a: genotype 1b cases; 1b: genotype 3a cases.

Case	1st positive HCV serology	Genotype	Parenteral vitamin therapy
S1	11 March 2003	1b	Commenced October 2006, regular and frequent
C3	4 January 2007	1b	Long-standing, and current at time of acquisition of HCV infection
C2	19 February 2007	1b	Long-standing, and current at time of acquisition of HCV infection
S2	Known pos November 2004	3a	Commenced November 2004, regular and frequent
C1	7 March 2005	3a	November 2004 (and only on two occasions, both dates on which S2 received vitamin treatment) after a break of 18 months

3. Study design

3.1. Recognition and notification of the suspected cluster

In January 2005, a patient, Case 1 (C1, Table 1) was referred to a Sydney, Australia, gastroenterologist for the management of acute HCV infection (genotype 3a). In February 2007, two other patients, Cases 2 and 3 (C2 and C3, Table 1), were referred to the same gastroenterologist, again with acute HCV infection (both genotype 1b). The gastroenterologist noted that all three index cases had been or were currently receiving IVC from the same general practitioner. The gastroenterologist advised the practitioner to suspend parenteral therapy and immediately inform the public health authority. The Public Health Unit began an investigation into the possible source(s) of infection and mechanism of transmission.

3.2. Infection control and epidemiological investigation

An on-site infection control audit was carried out by a public health infection control practitioner. Detailed interviews were held with the three index cases to establish risk factors, and with the general practitioner and nursing staff to gain an understanding of the practices performed at the surgery. A review of paper medical records focused on patients who received vitamin and mineral therapy at the practice between November 2004 and March 2007, the period from the earliest possible HCV exposure of C1 to the time when parenteral therapy was suspended. Patients who were known to the practice to be anti-HCV positive were identified from the medical records. The Notifiable Diseases Database, used in New South Wales, Australia for recording data on certain notifiable conditions, was used to identify other practice patients with HCV infection. Appointment records were reviewed to determine dates when the index cases had received parenteral therapy on the same day as a person known to be infected with HCV. Calculated exposure periods of the newly diagnosed cases, order of entry in the accounts book (a proxy for order of attendance – no appointments were made), and HCV genotype were taken into account.

In order to identify further possible cases of clinic-acquired HCV, a retrospective investigation was conducted for all patients who had received parenteral therapy on the relevant dates. Patients were contacted by telephone to offer testing for HCV, hepatitis B virus (HBV) and HIV.

3.3. Screening for HCV

Serum samples were taken from 162 patients between March and May of 2007 for serological testing. Screening for anti-HCV antibodies was performed using the ARCHITECT Anti-HCV kit (Abbott Diagnostics, ILL, USA,) and positive results confirmed using the Innotest HCV Ab IV kit (Innogenetics, Ghent, Belgium). Anti-HCV positive samples were tested for HCV RNA using transcription-mediated amplification (Versant HCV RNA Quantitative assay, Bayer, Pymble, Australia), or the COBAS Amplicor (Roche Diagnostics, Castle Hill, Australia). Viral load was quantified using a branch-chain-DNA (bDNA) signal-amplification assay (Versant HCV 3.0, Bayer) or the COBAS Taqman assay (Roche Diagnostics). HCV genotype was determined using a line probe assay (LiPA, Bayer) in association with an in-house RT-PCR targeting the 5′UTR of the HCV genome.

3.4. Sequencing

Sequencing and analysis was performed in parallel at two reference laboratories – one in Sydney, New South Wales and one in Melbourne, Victoria. Viral RNA was extracted from sera using a commercial RNA purification kit (QIAGEN, Doncaster, Australia) or total nucleic acid extracted using semi-automated extraction on robots (MagNA Pure, Roche Diagnostics, Germany). Amplification of HCV RNA was performed by nested PCR using primers targeting the Core, NS5A and NS5B protein genes.[15,16] A one-step RT-PCR reaction was performed using 3 U AMV reverse transcriptase (Promega, Alexandria, Australia), 1.5 U Taq DNA polymerase (Promega), 2 mM $MgCl_2$, 10 mM DTT, 4 μl PCR buffer (5×), 200 μM dNTP, 0.5 mM of each primer and 10 μl of RNA template. Amplification conditions for the first-round RT-PCR included reverse transcription at 42 °C for 40 min and denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, with a final extension step of 72 °C for 7 min. The second-round PCR consisted of 1.5 U Taq DNA polymerase, 2 mM $MgCl_2$, 8 μl PCR buffer (5×), 200 μM dNTP, 0.5 mM of each primer and 2 μl of first-round template with cycling conditions of 94 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s and a final single extension step of 72 °C for 7 min.

Second-round PCR products were purified by column chromatography (Millipore, North Ryde, Australia) or Polyethylene Glycol precipitation and sequenced using BigDye terminator mix (Applied Biosystems, Scoresby, Australia) at Monash University, Melbourne, and the Ramaciotti Centre for Gene Function Analysis at the University of New South Wales, Sydney.

3.5. Statistical analysis

Statistical and phylogenetic analyses were performed using the MEGA 4.0 DNA analysis package.[17] The ClustalW program[18] was used to determine nucleotide distances using a Kimura 2-parameter distance matrix and phylogenetic trees were constructed using the neighbour-joining method, including unrelated control sequences from the GenBank database made available through the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov).

4. Results

With the assistance of the general practitioner, a possible source case of genotype 1b (S1, Table 1) was identified. The medical record review identified a long-standing case of HCV genotype 3a infection as a possible source (S2, Table 1). The gastroenterologist who originally identified the cluster provided details of an additional case, diagnosed in early 2006 with genotype 1a (S3). Another case, brought to the attention of the investigators, had both 2a/2c and 1a HCV infections – the latter genotype diagnosed in early 2006, shortly after commencement of the case's vitamin therapy (S4). Another case (U3), tested by the physician during the investigation,