Use of Polymerase Chain Reaction for Early Detection and Management of Hepatitis C Virus Infection after Needlestick Injury

Teh Y. Wang, Hsing T. Kuo, Lien C. Chen, Yu T. Chen, Chun N. Lin, and Matthew M. Lee
1 Clinical Laboratories and 2 Gastroenterology Department, Sinlau Christian Hospital, Tainan, Taiwan; 3 Center For Drug Evaluation, Taipei, Taiwan, Republic of China

Abstract. Infection with hepatitis C virus (HCV) is a matter of great concern because of its potentially grave consequences. Instead of relying on the conventional anti-HCV antibody test to detect HCV infection after needlestick incidents, we used the polymerase chain reaction (PCR) to achieve earlier detection, to manage a patient more effectively, and to exclude possible infection more quickly. Fourteen incidents were studied in which the source patients were positive for both the anti-HCV antibody and HCV RNA, and the exposed subjects were negative for anti-HCV antibody at the time of the incidents. In one of the exposed subjects, a nurse, the result of the PCR test for HCV RNA was positive at 2 wk after the needlestick incident; the nurse’s viral load was very low (800 copies/ml) and she responded well to immediate medical treatment. She never developed acute hepatitis C; her serum anti-HCV antibody level and alanine aminotransferase (ALT) activity did not become elevated, and results of her PCR test for HCV RNA were negative following treatment. In the other 13 needlestick incidents, the results of PCR tests of the exposed subjects were negative for HCV RNA throughout the study and possible infections were quickly ruled out.

Introduction

Hepatitis C virus (HCV) is the major cause of non-A and non-B hepatitis associated with blood transfusion. It is also a major agent of sporadic hepatitis in certain parts of the world [1]. HCV infection induced by needlestick incidents that involve health care workers, or by other parenteral exposures that involve intravenous drug abuse, is a matter of great concern because of the potentially grave consequences of the infection (ie, high rates of progression to chronic hepatitis and cirrhosis) [2-6]. Furthermore, there is no vaccine against HCV [3,7]. It is therefore critical to develop an effective strategy to monitor and manage needlestick incidents in health care workers.

Although the transmission of HCV infection by needlestick injury may be infrequent, a prevalence of positive anti-HCV antibody (HCV Ab) levels as high as 8.8% has been reported in persons with multiple-needlestick injuries [8]. Needlestick injuries are associated with increased risk of acquiring hepatitis C virus infection, since a significant number of patients in hospitals are positive for anti-HCV Ab [6,9].

Conventionally, after a needlestick incident, if the source patient has tested positive for anti-HCV Ab, the exposed person is tested for anti-HCV Ab and elevated serum alanine aminotransferase (ALT) activity at monthly intervals up to 1 year, in order to detect or rule out infection [10]. Although the anti-HCV Ab may appear <6 weeks after the onset of symptoms in acute cases, it may not appear for as long as 1 year thereafter. Some patients never develop the anti-HCV Ab. By the time an infection is detected by this approach or after acute hepatitis C has developed, the viral load of the exposed person
may already be high and intractable for treatment. Early detection of HCV infection is especially important since a low viral load is associated with a more favorable response to therapy [11].

Measurement of HCV RNA level has been reported to be an advantageous method for early detection of HCV infection [12]. In the present study, the polymerase chain reaction (PCR) was used for qualitative and quantitative assays of HCV RNA to achieve early detection and effective management of needlestick exposures to HCV infection and to rule out such infections early on.

Methods

HCV PCR, anti-HCV Ab, and ALT assays were performed on serum specimens from subjects with a needlestick injury immediately, at 2 weeks, and monthly for 12 months post-incident, if the source patient was anti-HCV Ab-positive and the exposed subject was anti-HCV Ab-negative. If the source patient was positive for HCV RNA, he or she was further tested for viral load. Genotyping of HCV was also performed for comparison purposes.

HCV RNA was measured qualitatively according to the manufacturer’s recommendations (Amplicor HCV Test, version 2, Roche Diagnostics, Indianapolis, IN), and quantitatively (Amplicor HCV Monitor Test and Quanti-Path Kit, Roche Diagnostics). In our experience, the Amplicor HCV Test reliably detects HCV qualitatively as low as 100 copies/ml and the Amplicor HCV Monitor Test quantifies HCV RNA as low as 100 copies/ml and linearly from 500 to 500,000 copies/ml. Linearity was tested by serial dilutions on HCV RNA-positive specimens with >500,000 copies/ml.

HCV genotyping was carried out with sense and antisense primers deduced from the core gene as described by Okamoto et al [13]. Assays of serum anti-HCV Ab were performed using the Axsym HCV kit 3.0 version) with the Axsym automated analyzer (Abbott Laboratories, Abbott Park, IL) following the manufacturer’s recommended procedure. Serum ALT assays were performed using a Vitros 750 automated analyzer (Ortho Diagnostics, Paritan, NJ) following the manufacturer’s recommended procedure.

Results

During a period of 2 years, 35 needlestick incidents were documented at our hospital. Among them, 14 incidents were available for study in which the source patient was positive for both the anti-HCV Ab and HCV RNA, and the exposed subject was negative for anti-HCV Ab at the time of the incident.

One exposed subject, a nurse, initially tested negative for HCV RNA. However, at 2 weeks after the incident, she tested positively. The positive PCR assay was repeated and the viral load was determined to be 800 copies/ml. The viral load of the source patient was >500,000 copies/ml at the time of incident. Genotype testing showed that the HCV of the source patient and exposed subject were both type 1b (Okamoto’s type II) [14].

Following rapid and intense treatment with interferon alpha-2a (Roferon-A 5 MIU, twice/wk for 6 months) [11,15,16], the exposed subject became HCV RNA-negative within 1 month and remained negative for 12 months. She never developed acute hepatitis C, she never had a positive test for anti-HCV Ab, and her serum ALT activity remained normal throughout the 12 months.

In the 13 other exposure incidents with source patients who tested positive for both anti-HCV Ab and HCV RNA, the exposed subjects never became positive for HCV RNA throughout the study. Eleven of the source patients had low viral loads (<500,000 copies/ml). The other 2 source patients were positive for anti-HCV Ab but were negative for HCV RNA for 3 consecutive months. These 2 source patients were deemed negative for HCV infection and the corresponding exposed subjects (who had tested negative all 3 times) were withdrawn after 3 months from monitoring for potential HCV infection. We consider the positive anti-HCV Ab tests in the 2 source patients either to be false positive results or to reflect resolved HCV infections.

As noted above, the HCV genotype was type 1b in the exposed subject who became infected and in the corresponding source patient. In the 13 other exposure incidents (discussed in the previous paragraph), HCV genotypes were investigated in 8 source patients. The HCV genotype was type 1b in 7 of these patients and type 2a in 1 patient.
Discussion

In previously reported cases who developed HCV infection after needlestick injury, serum HCV RNA became detectable as early as 3 days and usually within 1 to 2 weeks post-inoculation, although the time to detection could be as long as several weeks. Serum anti-HCV Ab, however, was not detected until much later [12,17]. The anti-HCV Ab test alone, without PCR measurement of HCV RNA, did not provide adequate information, since the viral load and the correlation of viral subtypes could not be measured with the Ab test. No correlation was evident between the first appearance of HCV viremia and the time of Ab seroconversion [18,19]. Thus, testing for anti-HCV Ab is neither rapid nor effective as a tool for managing needlestick incidents. Detection of HCV RNA in serum by PCR is the only available means to establish an early diagnosis of primary HCV infection. It is also an effective procedure for monitoring the efficacy of antiviral therapy [20].

In the present study, among 14 needlestick incidents that involved HCV-positive patient sources, only 1 of the exposed subjects became infected (7.1%). This low rate of transmission is comparable with other reports of 3.3, 5.4, and 6.0% [21-23]. The infected nurse was found to be positive using the PCR technique as early as 2 weeks after the incident. She was evaluated continuously for 12 months in order to rule out possible intermittent viremia [24]. Serum ALT activity was also assayed to assist in excluding a possible intermittent viremia [25,26]. In this subject, recurrence of viremia and rise of ALT activity were not observed after the initiation of therapy.

In this study, we show that the PCR assay for HCV RNA can be used to monitor and manage needlestick cases rapidly and effectively. Medical intervention was introduced immediately after a positive HCV RNA result was obtained. In theory, early detection enables interferon therapy to be initiated early, while the viral load is still very low, thus making the therapy more effective. Patients with low pre-treatment HCV levels should respond better to treatment [11,18,25]. Once pretreatment HCV levels go up, treatment becomes more intractable [27]. We do not intend here to discuss the efficacy of interferon treatment, except to comment that early detection allows for the early introduction of medical intervention and may promote a more favorable therapeutic response.

Although it is possible that spontaneous clearance of HCV virus can occur without treatment, early detection of the virus and its subsequent follow-up with a quantitative HCV RNA assay can add more insight into the infection and facilitate the decision about treatment. The positive RNA result was confirmed by a quantitative RNA test and by genotyping. Even more convincing evidence might have been obtained if the nucleic acid sequence-based amplification (NASBA) test or branched-chain DNA test had been available to confirm the titer.

Fortunately, in 13 of the 14 needlestick incidents whose source patients were positive for both anti-HCV Ab and HCV RNA, the exposed subjects did not become infected. We consider that negative PCR tests during 3 consecutive months are sufficient to rule out a potential infection. This is better than the conventional 12 months of monitoring using anti-HCV Ab. Interestingly, 11 of the 13 source patients that did not cause infections had viral loads of less than 500,000 copies/ml, while the one that caused an infection had >500,000 copies/ml. Obviously, the number of cases is inadequate to show a relationship, but it may be worthwhile to explore this issue further.

It has been suggested that the low rate of transmission of HCV in needlestick incidents may reflect the low quantity of viral material in blood and secretions [28]. An advantage of testing for HCV RNA is that when the source patient and exposed subject are both anti-HCV Ab positive, but the exposed subject is HCV RNA negative, the exposed subject can be monitored for potential infection using HCV RNA. If the results of the PCR test for HCV RNA are negative for 3 consecutive months, an infection can be ruled out. If the exposed subject is initially positive for HCV RNA but has an HCV genotype that differs from that of the source patient, genotyping can be used to rule out a superimposed infection.
In the present study, the HCV genotype of 8 source patients was type 1b and only 1 was type 2a. This finding is consistent with the report that type 1b is predominant in patients with hepatic cirrhosis or hepatocellular carcinoma in Taiwan [29]. Although some reports indicate that patients with HCV genotypes 1a and 1b respond poorly to treatment, compared to those with genotypes 2 and 3, others have found no correlation [25,30,31]. We do not suggest that genotyping be necessarily included in a needlestick protocol; it was performed here primarily for investigative purposes.

In summary, we have shown that PCR methods for HCV RNA can be used for early detection and effective management of HCV infection in needlestick cases in hospitals. The PCR technique can also be used to rule out an infection quickly. Measurements of HCV RNA by PCR are becoming common in clinical laboratories, with the availability of commercial PCR test kits and automation. We suggest that the conventional approach using anti-HCV Ab testing to manage needlestick incidents be modified to incorporate HCV RNA testing. Our testing protocol, which uses both qualitative and quantitative HCV RNA measurements, can serve as an example of such a modification. More cases will need to be studied to define the relationship, if any, between viral dose and infectivity.

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References


