

# GENOTYPING OF HEPATITIS C VIRUS ISOLATES FROM SAUDI PATIENTS BY ANALYSIS OF SEQUENCES FROM PCR-AMPLIFIED CORE REGION OF THE VIRUS GENOME

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We investigated the genotype distribution of hepatitis C virus (HCV) among Saudi patients with chronic hepatitis C. Serum specimens from 119 native Saudi Arabian patients with chronic hepatitis C, as documented by serology and polymerase chain reaction (PCR) for HCV RNA, were used. Genotyping was performed by reverse transcription-PCR, using specific primers at the core region of HCV genome, and DNA sequencing of the resultant amplicons. It was found that the majority of samples (47.9%) belong to genotype 4, followed by subtype 1b (16.8%), and subtype 1a (10.1%). Twenty samples (16.8%) were not able to be typed by our method. We confirmed the results by cloning at least one PCR amplicon from each genotype, and determining the nucleotide sequence of the clones. Our findings suggest that genotype 4 is the most common among native Saudi Arabian patients with chronic hepatitis C infection. Genotypes 1b and 1a were also prevalent. *Ann Saudi Med* 1997;17(6):601-604.

Hepatitis C virus (HCV) is globally recognized as a major causative agent of posttransfusion and sporadic non-A, non-B hepatitis.<sup>1</sup> Infection with HCV could lead to chronicity, with possible progression to hepatocellular carcinoma (HCC), or complications requiring liver transplantation, such as liver cirrhosis.<sup>2</sup> HCV genome is a single-stranded RNA with positive polarity. It is approximately 9400 bases, with a long open reading frame encoding four structural proteins and at least six nonstructural proteins. The genome is flanked by untranslated, conserved regions at both the 5' and 3' ends.<sup>3</sup> HCV genome exhibits a high degree of sequence variability from one isolate to another.<sup>4</sup> As such, HCV has been classified into a number of genotypes and subtypes.<sup>5-7</sup> Genotyping of HCV is of clinical relevance to follow-up interferon therapy,<sup>8-11</sup> and of epidemiological importance in order to investigate transmission routes and geographical clustering.<sup>12,13</sup>

In Saudi Arabia, HCV infection is considered endemic, reaching a serological prevalence of up to 5% in individuals over 50 years of age.<sup>14</sup> So far, only three studies have described the distribution of HCV genotypes among Saudi blood donors and patients with liver disease or on hemodialysis.<sup>15-17</sup> However, most of these studies

have used a limited number of specimens, or those from patients with various conditions and blood donors. In this study, we report genotype distribution among 119 serum from Saudi patients with chronic HCV infection, by identifying genotype-specific sequences in the core region of HCV RNA.

## Materials and Methods

Serum samples were sent to the Virology, Immunology and Infectious Diseases Research Laboratories at the Research Centre of King Faisal Specialist Hospital and Research Centre, for the detection of HCV RNA by the polymerase chain reaction (PCR). All sera showed elevated liver enzymes, tested positive for anti-HCV antibodies by second-generation ELISA, and were confirmed reactive by RIBA-2. The serum samples were from patients diagnosed to have chronic hepatitis C infection. By using our in-

TABLE 1. *Oligonucleotide primers used for amplification of HCV cDNA regions.*

| Genomic region      | Primer sequence (5'-3') | Genomic location  |
|---------------------|-------------------------|-------------------|
| 5' noncoding region |                         |                   |
| Outer primers       | CGTTAGTATGAGTGTCTCGTGC  | 90-109 (S)        |
|                     | CGGTGTACTIONCACCGGTTCC  | 171-153 (AS)      |
| Inner primers       | AGTGTCTGTGCAGCCTCCAGG   | 100-119 (S)       |
|                     | CGGTCCCGCAGACCACTATG    | 159-140 (AS)      |
| Core region         |                         |                   |
| Outer primers       | AGTGTGGGTTCGCGAAAGGC    | 248-267 (HC1, S)  |
|                     | CCCCATGAGGTCGGCGAAGC    | 734-715 (HC2, AS) |
| Inner primers       | ACTGCCTGATAGGGTGCTTG    | 276-295 (HC3, S)  |
|                     | AAGGGTATCGATGACCTTAC    | 707-688 (HC4, AS) |

S=sense; AS=antisense.

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house, nested PCR method with primers at the 5' noncoding region (Table 1), as described by Al-Ahdal and Kessie,<sup>18</sup> PCR-positive serum samples from various individuals were used for this genotyping study.

Reverse transcription-PCR was performed on sera from all HCV RNA-positive samples, as previously described by Hotta et al.<sup>19</sup> Briefly, HCV RNA core region sequences were amplified, using primers HC1 and HC2 in the first round, and HC3 and HC4 in the second round (Table 1). Each round was for 35 cycles, consisting of 1 minute at 94°C, 1 minute at 40°C, and 2 minutes at 72°C. The PCR products were electrophoresed in a 2% agarose gel containing ethidium bromide, and visualized by UV illumination.

Amplified cDNA fragments from the core region were sequenced by a direct sequencing method with a *Taq* Dye Deoxy Terminator Cycle Sequencing Kit (Applied Biosystems GmbH, Weiterstadt, Germany), and an ABI 373A Autosequencer (Applied Biosystems GmbH). Each sequence obtained was compared with those of reported types and subtypes, by using the Lasergene Navigator computer program (DNA Star Sequence Analysis Software Package for Apple MacIntosh, Madison, WI). On the basis of percent homologies, each isolate was assigned a type or a subtype. In order to validate the sequencing results, representative amplicon from each genotype or subtype were subcloned into TA vector (Invitrogene, San Diego, CA) and sequenced as above.

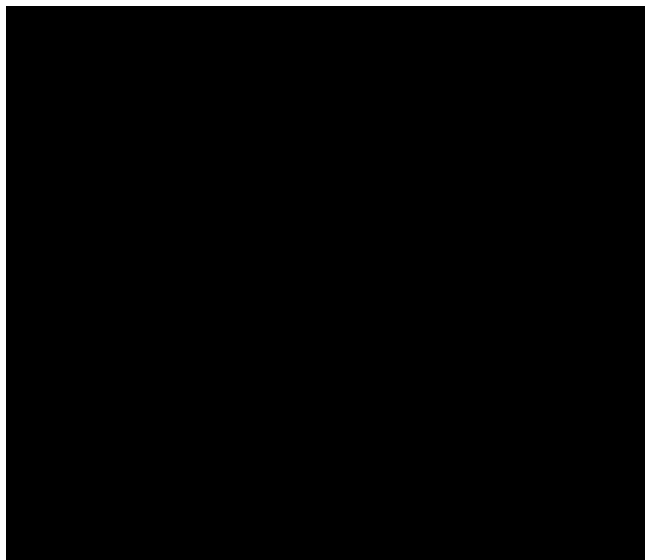


FIGURE 1. Agarose gel electrophoresis of PCR-amplified product (60 bp) of the 5' noncoding region of HCV RNA after serum extraction and reverse transcription. M is  $\phi$ 174/*Hae*III molecular weight markers. P1 and P2 are positive controls of plasmid-cloned HCV cDNA sequences and a documented case of HCV, respectively. N1 and N2 are negative controls of serum from a normal blood donor and reagent control, respectively. Representative serum samples are in Lanes 1-8. Samples 1, 4, 7, and 8 were negative by PCR. Samples 2, 3, 5 and 6 were positive.

## Results

Our research laboratories have been performing HCV RNA detection in patients' sera by the PCR technology, using both the in-house method<sup>18</sup> and recently, the Roche Amplicor<sup>TM</sup> PCR assay. Samples that were HCV RNA-positive by PCR amplification of the 5' noncoding region were processed for amplification of core region sequences. One hundred and nineteen specimens exhibited positive amplification of the core region. Figures 1 and 2 show representative positive and negative samples of amplification of the 5' noncoding region and core region, respectively. Table 2 shows that genotype 4 was the most common (47.9%) among Saudis, followed by genotype 1b (16.8%) and 1a (10.1%). Genotypes 2a (0.84%), and 2b and 3a (1.68% each), were the least common. Each of five samples (4.2%) contained two genotypes (1a+1b, 1a+3b, 1b+3a, 2b+3a, and 3b+4). Twenty samples (16.8%) did not show enough homology to enable assigning a genotype to any of them. These samples may require genotyping by using other genomic regions.

## Discussion

As HCV infection is common in Saudi Arabia,<sup>20</sup> we investigated the distribution of HCV genotypes in sera from Saudi patients with chronic hepatitis C. Several methods are used to type HCV isolates,<sup>21</sup> including

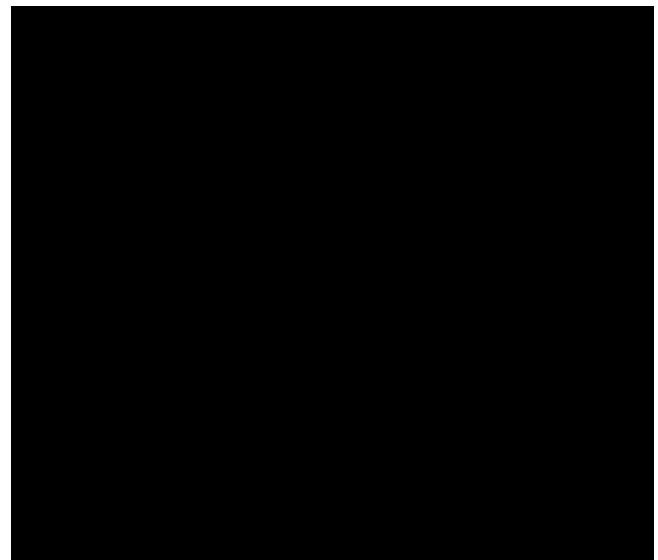


FIGURE 2. Agarose gel electrophoresis of PCR-amplified product (432 bp) of the core region of HCV RNA after serum extraction and reverse transcription. M is  $\phi$ 174/*Hae*III molecular weight markers. P is a positive serum control. N1 and N2 are negative controls of serum from a normal blood donor and reagent control, respectively. Representative, core-positive PCR results on serum samples are in Lanes 1-6.

TABLE 2. Genotype distribution of HCV among 119 Saudi patients with chronic hepatitis C.

| Genotype       | Number of samples (%) |
|----------------|-----------------------|
| 1a             | 12 (10.1)             |
| 1b             | 20 (16.8)             |
| 2a             | 1 (0.84)              |
| 2b             | 2 (1.68)              |
| 3a             | 2 (1.68)              |
| 4              | 57 (47.9)             |
| Mixed*         | 5 (4.2)               |
| Not typable ** | 20 (16.8)             |

\*Mixed infection is the presence of two or more subtypes in the same sample; \*\*sequences do not show homology with any published genotype sequence available to us.

restriction fragment length polymorphism, type-specific oligonucleotide primers and probes, direct sequencing and analysis of consensus sequences in the C, E1 and NS5 regions of the virus genome, and a recently commercialized line probe assay. Of these, the direct sequencing is the most precise method. Aside from being cumbersome, it may not be readily available to many laboratories. We used direct sequencing of PCR-amplified product in the core region of HCV genome, since it has previously been successfully used.<sup>19,22,23</sup> The first study on HCV genotypes of isolates from Saudis was that of Saeed et al.<sup>15</sup> They found that among four Saudi blood donors who were positive for anti-HCV antibodies by RIBA-2, three donors had HCV genotype 1, and one donor had HCV genotype 4. In other studies,<sup>24,25</sup> samples from Saudi Arabia were included among samples from the Middle East. Although the sample number was not known, the results of these studies collectively indicated that genotype 4 is the most prevalent. HCV genotype distribution in 28 and 32 Saudi patients with chronic liver disease (CLD) and chronic renal failure (CRF), respectively, was investigated by Al-Faleh et al. using the line probe assay method.<sup>16</sup> They reported that genotype 4 was the most prevalent in both conditions, followed by type 1b in CLD patients, and type 1a in CRF patients. A recent study which included serum samples from 32 Saudi hepatitis patients<sup>17</sup> reported that 10/32 samples were type 1b, 2/32 were 1a, and 20/32 were unclassified. The results of this and previous reports demonstrate the existence of the major HCV genotypes in the HCV-infected Saudi population, with genotype 4 being the most common, and 1a and 1b being prevalent. Other genotypes could have been introduced to the community through the importation of a foreign strain, as has been documented in South Africa.<sup>26</sup> In other parts of the world, the most prevalent genotypes are 1, 2 and 3 in European and North American

countries, and 1, 2, 3, and 6 in countries of the Far East.<sup>27</sup> In the Middle East, Africa and South Africa, genotypes 4 and 5 are the most common.<sup>28</sup> Three additional genotypes (7, 8, and 9) were recently reported from Southeast Asia.<sup>6</sup> Further molecular studies with large sample numbers are necessary to determine the major genotypes that cause HCV infection in various clinical conditions. Such studies will produce useful information on potential association with disease severity, and will suggest the future course of management and treatment of HCV disease in Saudi Arabia.

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