The Influence of Age on the Prevalence of Hepatitis C Virus Subtypes 1a and 1b

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The distribution of hepatitis C virus (HCV) genotypes was determined in isolates of 447 chronically HCV-infected German patients by nucleotide sequencing. Of these, 206 (46.1%) were infected with the subtype 1a, 215 (48.1%) with subtype 1b, 2 (0.4%) with subtype 1c, 9 (2.0%) with subtype 3a, and 15 (3.4%) with subtype 4a. Subtype 1a was predominant in those <40 years old (62.6%) and was associated with the risk factor of intravenous drug addiction and with shorter duration of disease. Conversely, subtype 1b was more frequent in patients >50 years old (84.7%; P < .001) and was associated with the risk factor of blood transfusions and with longer duration of disease. These data suggest that a shift from subtype 1b to subtype 1a occurred in the population studied. An increase in HCV infection with subtype 1a and a diminution of subtype 1b in the future can be expected.

Hepatitis C virus (HCV) has been identified as the major causative agent of posttransfusion and sporadic community-acquired non-A, non-B hepatitis [1]. It is a single-stranded RNA virus, closely related to the genera of flavi- and pestiviruses [2]. Until now, HCV infection can be diagnosed by the presence of specific antibodies in second-generation EIA, confirmed by strip immunoblot assay (UKE [Universitätskrankenhaus Eppendorf]-SIA) [3]. The most sensitive method for detection of HCV viremia is the reverse transcriptase-polymerase chain reaction (RT-PCR). Apparently, different HCV isolates show variability in nucleotide sequences of their genomes [4, 5]. These variations fall into a series of specific patterns, which enables classification of HCV isolates into different genotypes and subtypes [6, 7]. These distinct genotypes may differ in their clinical outcomes concerning disease severity and response to interferon (IFN) therapy [8, 9]. In the United States and western Europe, the HCV genotype 1, with its subtypes 1a and 1b, is the most frequent cause of HCV infection, as shown by recently published studies [10–12]. Little is known about the influence of age, risk factors, and duration of infection on the distribution of HCV subtypes. Therefore, we examined 447 HCV-infected patients taking particular account of this issue.

Patients and Methods

Patients. Between 1994 and 1995, sera from 447 clinic in- and outpatients with chronic HCV infection were included in our study. Of these, 430 were from northeastern Germany, around the city of Hamburg. Seventeen patients from northeastern Africa (Egypt and Sudan) had been living in Germany for only a few years and were chronically HCV-infected before they immigrated. The patients were between 7 and 84 years old, with a mean age of 39 years; 258 (57.8%) were male and 189 (42.2%) were female. All patients were positive for anti-HCV antibodies when tested by a second-generation EIA (Abbott, Abbott Park, IL) confirmed by
Table 1. Distribution of HCV genotypes in relation to age of 447 patients.

<table>
<thead>
<tr>
<th>HCV subtype (no. infected with that subtype)</th>
<th>Age group (years)</th>
<th>&lt;20</th>
<th>20–29</th>
<th>30–39</th>
<th>40–49</th>
<th>50–59</th>
<th>&gt;60</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a (206)</td>
<td></td>
<td>9</td>
<td>45</td>
<td>83</td>
<td>53</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>1b (215)</td>
<td></td>
<td>2</td>
<td>20</td>
<td>40</td>
<td>42</td>
<td>49</td>
<td>62</td>
</tr>
<tr>
<td>1c (2)</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3a (9)</td>
<td></td>
<td></td>
<td>4</td>
<td>4</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4a (15)</td>
<td></td>
<td></td>
<td></td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

NOTE. In group studied, subtypes 1a and 1b were most causative agent (94.2%) of chronic HCV infection. In patients aged <50 years, HCV subtype 1a (190 patients) was more frequent than 1b (104). Conversely, patients >50 years were infected more frequently with HCV subtype 1b (111 patients) than with 1a (16).

Results

HCV RNA was amplified by RT-PCR from 447 chronically infected persons. The genotypes and subtypes of these isolates were determined by sequencing the viral RNA polymerase coding region (NS5) according to a recently published classification scheme [6].

Of these 447 patients, 206 (46.1%) were infected with subtype 1a and 215 (48.1%) were infected with subtype 1b. An infection with subtype 1c could be detected in 2 (0.4%) subjects and with subtype 4a in 15 (3.4%). These 17 subjects infected with subtype 1c or 4a had acquired HCV infection in Egypt or Sudan. Nine (2.0%) of the 447 patients carried subtype 3a. Subtype 3a was found exclusively in persons with a history of IVDA.

Coinfection with a second genotype or subtype could not be detected in any of these patients.

A comparison between the age of the 447 patients and the HCV subtype they were infected with is shown in table 1. In patients who were <39 years old, the rate of infection with subtype 1a (n = 137) was at least two times more frequent than with subtype 1b (n = 62). In the group aged 40–49 years, the number infected with subtypes 1a (n = 53) and 1b (n = 42) was nearly identical. In patients >50 years, the number infected with subtype 1b (n = 111) was seven times higher than with subtype 1a (n = 16) (P < .001).

As shown in figure 1, HCV subtype 1a was present in 81.8% of patients <20 years of age, 63.4% of patients 20–29 years, 60.6% of patients 30–39 years, 54.6% of patients 40–49 years, 17.7% of patients 50–59 years, and 7.2% of patients >60 years of age. In contrast, HCV subtype 1b was present in 18.2% of patients <20 years of age, 28.8% of patients 20–29 years, 29.2% of patients 30–39 years, 43.3% of patients 40–49 years, 79.0% of patients 50–59 years, and 89.9% of patients >60 years of age.

Subtype 1a was more prevalent in persons with a history of IVDA (71/116; 61.2%) than subtype 1b (36/116; 31.0%). In contrast, subtype 1b was more frequent in patients with a his-

UKE-SIA [3] and positive for plasma HCV-RNA when tested by PCR targeting the 5' noncoding region [12, 13].

Of these 447 patients, 144 (32.2%) had a history of transfusion with blood products (mean age: 53 years), 116 (26.0%) had a history of intravenous drug addiction (IVDA) (mean age: 26 years), and 48 (10.7%) had undergone hemodialysis (mean age: 40 years) over a period of several years. The remaining 139 subjects (31.1%) had no known risk factors (mean age: 35 years), and it was assumed that the HCV infection was community-acquired.

For the 144 patients with a history of transfusion with blood products, the time of HCV infection was considered to be the date of blood transfusion; for the 116 patients with a history of IVDA, it was considered to be the date when intravenous drug use began.

Nucleotide sequencing. Part of the HCV RNA polymerase coding region (NS5) in isolates of 447 patients was analyzed by nucleotide sequencing. HCV RNA extraction was performed by the guanidinium thiocyanate–phenol–chloroform method as described [13]. For cDNA synthesis, primer 51 (5'-AGTCATAGCTTCTCGTGAA; nt 8290–8273 as described [2]) was used. After reverse transcription, HCV cDNA was amplified by nested PCR. For the first round of PCR, primers 50 (5'-ATGGGCAAAAGGACGTCCG; nt 7567–7585) and 51 were used. In a second PCR, 3 μL of the solution from the first round was amplified using the inner primer pair 52 (5'-ACTGAAATTCTCGTATGATACCCGC; nt 7911–7925) and 53 (5'-GTAAGCTTCACAGATAACG; nt 8233–8222). Amplified HCV cDNA fragments were purified by agarose gel electrophoresis. DNA fragments were eluted from the agarose gels using the Geneclean Kit II (Bio 101, Dianova, Germany). The isolated DNA was cloned into Bluescript SK+ vector (Stratagene, La Jolla, CA) after pretreatment with restriction enzymes EcoRI and HindIII (Boehringer Mannheim, Mannheim, Germany). After transformation into competent Escherichia coli DH5α (Life Technologies Gibco BRL, Gaithersburg, MD), the nucleotide sequences of at least three clones were determined by the dideoxy chain-termination method using the modified T7 DNA polymerase (Sequenase version 2.0 kit; United States Biochemicals, Cleveland).

Data were analyzed with the χ² test.

The nucleotide sequences obtained in this study were submitted to the International Sequence Databases (EMBL, accession numbers Z35502–Z35594, X88564–X88770, and Z70362–Z70515).
Figure 1. Distribution of HCV subtypes 1a and 1b by age of 447 patients.

tory of blood transfusion (93/144; 64.6%) than subtype 1a (51/144; 35.4%). The mean value for duration of HCV infection was 7.3 years in patients with subtype 1a and history of IVDA compared with 19.2 years in patients with subtype 1b and history of blood transfusion.

Discussion

We found that the subtypes 1a and 1b are the most frequent causative agents (94.2%) of HCV infection in patients from northeastern Germany. These results agree with other studies that examined the distribution of HCV genotypes in patients from other countries in western Europe and the United States [9-12]. In 447 patients, we found no significant difference between the rate of infections with subtype 1a (46.1%) and subtype 1b (48.1%). However, related to the age of infected patients, there was a surprisingly high prevalence of HCV subtype 1a in younger patients (<40 years; 62.6%) and of subtype 1b in older patients (>50 years; 84.7%) (figure 1). Not only did the age of the patients correlate closely with the prevalence of HCV subtypes, but also risk factors for HCV infection (IVDA, blood products) and duration of infection differed in both groups of patients. Therefore, we suggest that 1b was the predominant subtype for HCV infection 15–25 years ago.

Regarding the mean value of duration of HCV infection in persons infected with subtype 1a, it is apparent that there has been an alteration in prevalence that has favored subtype 1a in the last 10 years. The increase in infections with HCV subtype 1a may also be caused by new risk factors such as IVDA in younger patients. In contrast to a previously published study [14], we found that HCV subtype 1a is the predominant subtype in our patients with a history of IVDA. We found subtype 3a exclusively in this group of persons, but subtype 3a was found in only 7.8% (9/116) of our patients with history of IVDA.

We assume that younger persons will contribute to the spreading of HCV infection to a higher degree than the older patients. Therefore, we expect that the prevalence of those with HCV subtype 1a will rise and there will be a diminution of the subtype 1b prevalence.

Even after determining the nucleotide sequence of at least three clones from each patient, we did not observe any case of coinfection with a second genotype or subtype of HCV. However, coinfection with different HCV types has been found in 1%–6% of HCV-infected subjects, especially when genotyping was performed by restriction fragment length polymorphism (RFLP) analysis and by hybridization methods [10, 14]. This could be due to mutation of the initial strains infecting the patients or incorrect incorporation of nucleotides during PCR or reverse transcription. For these reasons it seems that use of RFLP analysis and hybridization might lead to an incorrectly high figure for prevalence of coinfection with different HCV types. In contrast, point mutations do not influence the results of genotyping by nucleotide sequencing. Furthermore, if superinfection with different subtypes occurs in 1 patient, it seems that after 3–6 months, only one of the two subtypes will be predominant [15]. Therefore, it is not surprising that we did not find any coinfection by nucleotide sequencing in our collective.

Regarding IFN-α treatment, we detected only a small difference in sustained complete response between subtypes 1a and 1b, as described previously [12]. There is disagreement in other studies whether subtype 1a or 1b is linked to a poorer response to treatment and a higher severity of liver disease [8, 9]. In the majority of these studies, subtype 1b has a poorer prognosis in treatment and course of disease than subtype 1a. This may...
be caused by longer duration of HCV subtype 1b infections
and by higher ages of the patients compared with persons
infected with subtype 1a. In further studies it should be investi­
gated whether response to treatment depends more on the dura­
tion of HCV infection than on the genotype responsible for
HCV infection.

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