

Transmission routes of chronic hepatitis C and their relation to HCV genotypes

Kronik hepatit C virus enfeksiyonlu hastalarda enfeksiyon kaynakları ile hepatit C virus genotipleri arasındaki ilişki

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Background/aims: Hepatitis C virus transmission routes in chronic hepatitis C patients, the relationship between the viral genotype and the transmission routes were studied. **Material and Methods:** Genotyping was performed by using a commercial reverse hybridization method, Line Probe Assay. **Results:** Genotyping of 108 HCV RNA positive patients revealed four different types (1,2,3, and 4) and some mixed types. Subtype 1b was the most common (n=82). Subtype 1a and 3a were detected in six patients, 2a/2c was detected in seven patients, and 4c/4d was detected in one patient respectively. Six subjects revealed mixed infections. Three of them were 1a+1b, two of them were 1b+4a, and one of them was 1b+2a/2c. Genotype 1b was most common in all groups. In 38,8% of the 108 patients with a history of blood or blood product transfusions, 16,6% of patients with a history of surgery, 15,7% of patients had an anamnesis of dental treatment and, 12,9% of patients receiving dialysis. **Conclusion:** Before the routine screening of blood donor practices became mandatory, the most common route of HCV infection was blood transfusions. The other risk factors of transmission such as tattoos, piercings, iatrogenic infections and intravenous drug usage have not been recorded for any of these patients in our study. The patient with a history of surgery had the genotype 1b as the most common genotype. The genotype 1b was determined in 75,9% of the whole patient population of the study.

Key words: Chronic hepatitis C, transmission routes, hepatitis C virus, genotype

Amaç: Kronik hepatit C'li hastalarda hepatit C virusunun bulaş yolları araştırılmış ve virusun genotipleri ile bulaş yolları arasındaki ilişki incelenmiştir. **Yöntem ve Gereç:** Genotipleme, revers hibridizasyon, Line Probe assay, yöntemi ile gerçekleştirilmiştir. **Bulgular:** 108 HCV RNA pozitif hastada, dört farklı genotip ve bazı mix genotipler saptanmıştır. 1b genotipi en yaygın genotip olarak bulunmuştur (n=82). Hastaların altısının 1a, altısının 3a, dördünün 2a/2c, üçünün 2a, birinin 4c/4d genotipinde olduğu belirlenmiştir. Altı hastada, üçü genotip 1a+1b, ikisi genotip 1b+4a, biri ise genotip 1b+2a şeklinde, mikst genotip saptanmıştır. Altı hastada mikst genotip belirlenmiştir. Bunların üçü 1a+1b, ikisi 1b+4a, biri de 1b+2a/2c genotipinde saptanmıştır. Bütün hasta gruplarında en yaygın genotip 1b idi. 108 hastanın %38,8'inde kan ve kan ürünleri transfüzyonu, %16,6'sında cerrahi girişim, %15,7'sinde diş tedavisi ve %12,9'unda diyaliz öyküsü mevcuttu. **Sonuç:** Kan donörlerinde rutin kan testleri yaptırılmaları zorunlu olmadan önce, HCV enfeksiyonunun en yaygın bulaşma yolu kan transfüzyonu ile olmaktaydı. Çalışmamızda, dövme, piercing, iatrojenik enfeksiyonlar ve damar içi ilaç kullanımı gibi, bulaş açısından risk faktörlerinin hiçbiri bu hastalarda kaydedilmemiştir. Cerrahi işlem öyküsü bulunan hastalarda en yaygın genotip 1b saptanmıştır. Tüm hasta popülasyonunda genotip 1b %75,9 oranında saptanmıştır.

Anahtar kelimeler: Kronik hepatiti C, bulaş yolu, hepatit C virusu, genotip

INTRODUCTION

Hepatitis C virus (HCV) is the prominent etiological agent of non-A non-B hepatitis occurring sporadically in the population (1). There are nearly 170 million people infected with HCV worldwide, and the infection is an important risk factor for chronic hepatitis, cirrhosis and hepatocellular carcinoma (HCC) (2, 3).

HCV is a single-stranded RNA virus that displays a high rate of genetic heterogeneity (4). At least six major HCV genotypes with many subtypes have been determined throughout the world. Regional differences exist regarding the frequencies of HCV genotypes. Although HCV genotypes 1, 2 and 3 have been identified throughout the world, their

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prevalences differ from one region to another (5-7). Determined in more than half of the patients, the parenteral route is the major transmission route of HCV infection. However, in 30-40% of the infected patients, other transmission routes, such as sexual intercourse, interfamilial contact or maternal-infant transmission, have also been reported (8).

The aim of this study was to investigate the transmission routes of HCV in a Turkish population and their possible relationship with genotypes for a better understanding of the epidemiology of the HCV infections in Turkey.

MATERIALS AND METHODS

A total of 108 (54 male, 54 female; age range: 9-74) patients, identified with chronic HCV infection at the Department of Gastro-entero-hepatology, Istanbul Faculty of Medicine, Istanbul University, were studied. During the patient interviews, the putative transmission routes, such as blood transfusion, surgical treatment, intravenous drug usage (IVDU), occupational risk, acupuncture, tattoo, dental treatment, high-risk sexual behavior, and family history of chronic hepatitis, were queried in order to determine the risk factors implicated in HCV transmission. The presence of anti-HCV antibodies in serum was detected by enzyme linked immunosorbent assay (ELISA: Innogenetics, Ghent, Belgium). RNA was extracted using the method of Boom et al. (9). HCV genotypes were detected by Inno-Lipa (Line Probe Assay) HCV kit (Innogenetics, Ghent, Belgium) after obtaining cDNA using reverse transcriptase enzyme. 5'-Non-coding region of HCV was amplified. Obtained amplicons were hybridized with different type- and subtype-specific nucleotide probes on nitrocellulose bands. Results were visualized with a colorimetric enzymatic assay.

Statistical Analysis

Data was analyzed with SPSS 10.0 version software (SPSS Inc., Chicago, IL, USA). Chi-square test was used for the analysis of the categorical variables. The statistical level of significance was set as $p < 0.05$ and $p < 0.01$.

RESULTS

Patient Grouping According to Transmission Routes

All patients were evaluated according to transmission routes. Forty-two (38.8%) of the 108 patients had a history of blood and blood product transfusi-

ons, 18 (16.6%) had a history of surgery that was thought to be their transmission route, 17 (15.7%) recalled previous dental treatment, 14 (12.9%) had received dialysis, 5 (4.6%) had a HCV-infected partner, and 3 (2.7%) each reported IVDU or receiving frequent injections. Intrafamilial transmission and social circumstances were cited as the transmission route in 2 patients (1.8%) each. The distribution of the patients for whom the transmission route was determined is shown in Table 1.

The Genotype Distribution of HCV-Positive Patients

Genotyping of the 108 HCV RNA-positive patients revealed four different types (1, 2, 3, and 4) and some mixed types. Genotype 1b was the most frequent genotype among the patients that had blood transfusion before 1996, when the routine blood screening for anti-HCV was introduced in blood banks in Turkey. Genotype 1b was determined in 75.9% of the whole patient population of the study. Within the same group, genotypes 1a, 3a, 2a/2c, 1a+1b and 1b+2a/2c (mixed) were also determined. In patients with a history of surgery, the most common genotype was 1b. Genotype 1b was also the most common genotype in the group of patients with prior dental treatment; genotype 4c/4d was also detected in one of the patients in the dental treatment group. Among the 14 patients receiving dialysis, 9 genotype 1b, 4 genotype 1a and 1 genotype 3a were determined. All of the patients who were frequent injection users or who had a HCV-infected partner were genotype 1b. In the IVDU group, genotypes 1a, 3a and 1a+1b (mixed) were determined in 1 patient each. The remainder of the patients studied had only genotype 1b.

DISCUSSION

Even though HCV is not as common as HBV, clinically, most of the cases tend to become chronic.

Table 1. Patients with determined transmission routes

	n	%
Blood and blood product exposure	42	38.8
History of surgery	18	16.6
Anamnesis of dental treatment	17	15.7
Dialysis	14	12.9
HCV-infected partner	5	4.6
Intravenous drug usage (IVDU)	3	2.7
Frequent injection (e.g. diabetes, other than IVDU)	3	2.7
Intrafamilial transmission	2	1.8
Social circumstances	2	1.8
Other	2	1.8
Total	108	~100

Table 2. Genotype distribution among HCV-positive patients according to transmission routes

Transmission routes/Genotype distribution	1a	1b	2a/2c	3a	1b+2a/2c	1a+1b	1b+4a	4c/4d	Total
Blood and blood product transfusion	1	30	5	2	1	1	2		42
History of surgery		17		1					18
Prior dental treatment		12	2	1		1		1	17
Dialysis	4	9		1					14
HCV-infected partner		5							5
Intravenous drug usage (IVDU)	1			1		1			3
Frequent injection (e.g. diabetes, except IVDU)		3							3
Intrafamilial transmission		2							2
Social circumstances		2							2
Other		2							2
Total	6	82	7	6	1	3	2	1	108

Since the retrieval type is important, there are a large number of studies aimed at detecting the route of contamination. Recently, studies on the relationship of genotypes with the paths of contamination have gained in importance.

In our study, when the HCV genotypes of patients who received blood or blood products were investigated, genotype 1b was found to be the most common (38.8%), but different genotypes were also detected (n:5, 2a/2c; n:2, 3a; n:2, 1b+4a; n:1, 1a; n:1, 1b+2a/2c; n:1, 1a+1b). In a study carried out in volunteer blood donor patients, genotype 2 (49%) was the dominant genotype. Beside genotype 2, 1b (35%), 3a (7%), 1a (6%), and 4 (2%) genotypes were also detected (10). In a study in which patients with a blood transfusion history were evaluated, genotype 1b (52%) was detected to be the dominant genotype (11).

All types of surgery performed under inadequate sterilization conditions present an important risk factor for HCV infections. Contamination may be from patient to patient (12), from surgeon to patient intraoperatively or vice versa (13,14). In our study, 17 of the 18 patients were established to be infected with genotype 1b (94%), whereas the remaining 1 patient was infected with the genotype 3a.

The RNA of HCV has been detected in many body fluids such as sweat, urine, tears, and saliva (15). The concentration of HCV is much lower in the saliva than in the blood. In our study, there were 17 patients with a history of dental treatment and no other known contamination history. Among these patients, 12 were infected with 1b, 2 with 2a/2c, 1 with 3a and 1 with 4c/4d genotypes. One of the patients was found to be infected with a mixed type genotype, 1a+1b. The patient who was found to be infected with 4c/4d stated that he was living in Syria and utilized a dentist in that country. Genotype 4 is common especially in the Middle East,

Egypt and some African countries (16); therefore, this patient was probably infected during his dental treatment in Syria.

HCV infection is a serious problem in dialysis units (17). From 14 HCV-positive dialysis patients, 9 were infected with genotype 1b, 4 with genotype 1a, and 1 with genotype 3a. In a study carried out by Schneeberger *et al.* (18), genotypes 1a and 1b were found to be the dominant genotypes among hemodialysis patients. In the same group of patients, Benani *et al.* (19) similarly found genotype 1b to be the dominant genotype. Our study is consistent with the results of these two studies.

Even though HCV infection is known to be sexually transmitted, this phenomenon is not clarified yet. In sexual transmission, the number of partners, sexual intercourse frequency, lack of condom usage, and presence of syphilis infection or human immunodeficiency virus (HIV) are the main risk factors (20,21). In our patient group, no syphilis infection or HIV positivity was detected. In our study, all 5 patients of this group were found to be infected with genotype 1b. When the genotypes of these patients' partners were investigated, except for one, all were found to be infected with genotype 1b as well. One of our patient's partner's anti-HCV was found to be positive, but the HCV RNA test was found to be negative; thus, genotyping could not be done. Medeiros-Filho *et al.* (22) performed a study with the same purpose, and they detected that the couples usually had the same genotypes; only one of the couples had a different genotype (n:4, 1a; n:4, 1b; n:3, 3a; n:1, 2b).

HCV is seen frequently in intravenous drug users. The most important cause of this, along with sharing of the syringe needles, is the different social lives of these patients, leading them to face a high incidence of risk factors. In our study, 3 of the HCV-positive patients with IVDU had 1a, 3a and 1a+1b (mixed) genotypes. Pawlotsky *et al.* (11) de-

tected 3a and Ross et al. (23) detected 1a as the dominant genotype among the intravenous drug users. Three of our patients who were not intravenous drug users but probably had shared a glass syringe were found to be infected with genotype 1b. One of these patients revealed that the syringe was not changed during the typhoid fever vaccination during his military duty, and the other two cited a history of injections with old-fashioned glass syringes in a pharmacy.

Like in HBV, intra-familial transmission is also possible in HCV, and this is especially more important in the middle level endemic areas. Besides the presence of epidemiological findings showing the intra-familial transmission, there is a lack of knowledge on how this occurs. Close contact, body secretions and the usage of shared tools (razor blades, toothbrushes, etc.) may play an important role in transmission (24). In our study, findings of an intra-familial transmission were detected in two patients; they were infected with genotype 1b. Both of these patients were females, and they were responsible for the care of the infected family member, but were unaware of the affected individual's genotype.

The gathering of individuals in places such as prisons, schools and military buildings increases the risk for transmission of HCV infection. In our study, both of the patients in this group (social circumstances) were found to be infected with genotype 1b. One of the patients revealed that he had commonly used the razor blades of his friends

during his military duty, while the other stated that several of his friends at his place of employment were infected with HCV.

Lack of sterilization, especially in places such as barber shops or hairdressers, is an important risk factor for HCV infection. Tumminelli et al. (25) found the transmission rate to be 38% in their study involving Sicilian barbers. In our study, two patients had a history of utilizing barbers who did not change the razor blades between shavings; both of these patients were infected with genotype 1b.

When the general spectrum of genotypes of our study population was examined, it could be seen that 75% of the patients were infected with genotype 1b. The studies carried out in Turkey reveal that 1b is the dominant genotype throughout the country (16, 26).

In conclusion, when transmission routes of HCV were evaluated for Turkey, the most important route was found to be via blood transfusions in the period before donor bloods were screened for the presence of HCV. Along with this finding, genotype 1b was determined to be the most dominant genotype in all of the patient groups of our study, which also parallels the widespread genotype distribution. This phenomenon being statistically significant in patients with a history of surgery is especially notable. In Turkey, there is a need for a greater number of studies on this topic, which will enlighten our understanding of the transmission routes of HCV and also help explain the relationship between genotypes and transmission routes.

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