



# *Distribution of Hepatitis C Virus Genotypes in the Region of Istanbul Northern Anatolian Association of Public Hospitals*

Istanbul Anadolu Kuzey Kamu Hastaneler Birliđi Hizmet Bölgesinde Hepatit C Virüs Genotiplerinin Dağılımı

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## ABSTRACT

**Objective:** Hepatitis C virus (HCV) is responsible for 20% of acute hepatitis and 70% of chronic hepatitis. Determination of HCV genotype is important in the regulation of treatment and the follow-up of clinical course. In this study, we retrospectively evaluated the results of test performed in the Central Laboratory of Istanbul Northern Anatolian Association of Public Hospitals to determine the genotype distribution of hepatitis C patients in our region.

**Materials and Methods:** HCV genotypes were determined by real time polymerase chain reaction (Qiagen, Germany) and reverse hybridization line probe assay (Nim srl, Italy) methods.

**Results:** Among HCV RNA-positive 554 patients, 312 (56.5%) patients had genotype 1b, 127 (23.1%) - genotype 1a, and 94 (17.3%) patients had genotype 3a. A total of 10 samples were identified to be mixed genotype; 3 (0.5%) - genotype 4c/d, 3 (0.5%) - genotype 2a/c, 3 (0.5%) - genotype 1a/1b, and 1 (0.2%) - genotype 1b/4.

**Conclusion:** HCV genotype 1b is the most common genotype in our region similar to country-wide results. However, the rates are lower in our country than in the previous years. Probably the difference in this genotype distribution may depend on globalization, developments in technology, changes in human movements and social behaviors. Genotype determination is important for the regulation of treatment and prognosis of HCV infection.

**Keywords:** Hepatitis C virus genotypes, real time polymerase chain reaction, Istanbul

## ÖZ

**Amaç:** Hepatit C virüsü (HCV) akut hepatitlerin %20'si, kronik hepatitlerin %70'inden sorumludur. HCV'nin genotip tayini tedavide ve klinik sürecin takibinde önemlidir. Bu çalışmada bölgemizdeki HCV genotiplerinin dağılımını belirlemek amacıyla İstanbul Anadolu Kuzey Kamu Hastaneler Birliđi Merkezi Laboratuvarı'nda çalışılmış olan test sonuçları retrospektif olarak değerlendirilmiştir.

**Gereç ve Yöntemler:** HCV genotiplerinin tespiti için real time polimeraz zincir reaksiyonu (Qiagen, Almanya) ve reverse hibridizasyon line probe assay metodu kullanıldı (Nim srl, İtalya).

**Bulgular:** HCV RNA pozitif 554 hastada HCV genotiplerinin prevalansı genotip 1b: 312 (%56,5), genotip 1a: 127 (%23,1), genotip 3a: 94 (%17,3) ve genotip 4c/d: 3(%0,5), genotip 2a/c: 3 (%0,5), genotip 1a/1b: 3 (%0,5), genotip 1b/4: 1 (%0,2) olmak üzere 10 hastada (%1) mix tip olarak tespit edildi.

**Sonuç:** Bölgemizde ülkemizdeki oranlara benzer şekilde HCV genotip 1b en yaygın genotiptir. Ancak oranları ülkemizde önceki yıllarda yapılan çalışmalara göre daha düşüktür. Muhtemelen bu genotip dağılımındaki farklılık, günümüzdeki globalleşme, teknolojiye gelişim, insan hareketlerinin ve sosyal davranışların değişimine bağlı olabilir. Yeni tedavi protokollerinin uygulanabilmesi ve prognozun belirlenmesi açısından HCV hastalarında genotip tayini önemlidir.

**Anahtar Kelimeler:** Hepatit C virüs genotip, real time polimeraz zincir reaksiyonu, İstanbul

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## Introduction

Hepatitis C is a single-stranded positive RNA virus which is found in Flaviviridae family (1). Hepatitis C virus (HCV) is the most important cause of chronic liver disease, cirrhosis and hepatocellular carcinoma around the world (2). Studies using nucleic acid sequence analysis have identified 7 major genotypes and over 100 subtype of HCV (3). Several tests, such as polymerase chain reaction (PCR) amplification and sequence analysis, restriction fragment length polymorphism, reverse hybridization line probe assay (LIPA) and serological genotyping are used for the diagnosis of HCV genotypes (4).

There are differences in the distribution of genotypes of HCV by geographical region. In Europe, United States and Japan, genotype 1 and 2 are the most common genotypes (5). Genotype 3 is the most common genotype in Southeast Asia, genotype 4 in the Middle East, Egypt and Central Africa, genotype 5 in South Africa, and genotype 6 in Asia (6). Genotype 7 is the genotype found in Congo, Africa. It has been reported that the most prevalent genotype in the Mediterranean countries was 1b (7). The risk of developing cirrhosis and hepatocellular carcinoma in patients infected with HCV genotype 1b is higher and this genotype is more resistant to antiviral treatment compared to other genotypes. Genotyping of HCV, collection of epidemiological data, formation of antiviral therapy and the frequency of HCV genotypes in a population varies depending on the age of infection and the route of transmission (8). While preliminary results have shown that genotype 1b was strongly associated with previous blood products transfusion, the incidence of genotypes 1a and 3a due to intravenous drug use has increased (9). It has been found that mean intrahepatic HCV-RNA load was higher in patients infected with genotype 1b (10).

The Northern Anatolian Association of Public Hospitals consists of 11 hospitals, 6 of them are educational and research hospitals. The Central Laboratory services to 11 hospitals. This study was aimed to determine the distribution of HCV genotype in patients who were diagnosed with HCV infection in regions we serve.

## Materials and Methods

Patients with the diagnosis of or suspected HCV infection, whose blood samples were retrospectively evaluated for HCV genotypes from January 2016 to January 2017 from 11 hospitals at

the Central Laboratory of İstanbul Anatolian North Public Hospitals Association. Sample distribution was as follows, 77.5% chronic HCV infection, 10.5% liver function abnormalities, and 12% other clinic diagnoses.

HCV-RNA positivity was determined by real time PCR (artus HCV QS-RGQ Kit, Qiagen, Germany) method. Real time PCR and reverse hybridization line LIPA (NIm srl, Italy) methods for genotyping were applied and interpreted according to manufacturers' instructions. In LIPA method, cDNA synthesis was performed first. Hybridization of PCR products obtained from cDNA using biotin-labeled primers to membrane-bound genotype-specific HCV sequences was enzymatically demonstrated. The resulting bands were compared to the guidelines and were genotyped. This study was approved by Haydarpaşa Numune Training and Research Hospital Ethics Committee for Clinical Investigations [Approval number: 08.05.2017 (HNEAH-KAEK 2017/KK/70)].

## Results

HCV genotype was identified in a total of 554 HCV RNA-positive samples during a period of one year. Of the patients included in the study; 230 (41.3%) were male and 324 (58.6%) were female, with an average age of 57. HCV genotype 1b was identified as the most dominant genotype followed by genotype 1a and genotype 3a. Of the total of 554 cases, 312 (56.5%) were genotype 1b, 127 (22.9%) were genotype 1a, and 94 (17.3%) were genotype 3a. A total of 10 samples (1.8%) were identified as mixed type; genotype 4c/4d was found in 3 samples (0.5%), genotype 2a/2c in 3 (0.5%), genotype 1a/1b in 3 (0.5%), and genotype 1b/4 in 1 (0.2%).

HCV genotype 1b was found in 56.5% of samples (n=312), in the highest order, followed by genotype 1a with 23.1% (n=127) and genotype 3a with 17.3% (n=94), genotype 4c/4d with 0.5% (n=3) genotype 2a/2c with 0.5% (n=3), genotype 1a/1b with 0.5% (n=3) and genotype 1b/4 with 0.1% (n=1). Eleven (2%) samples were not genotyped (Table 1).

## Discussion

Knowing HCV genotypes, collection of epidemiological data, vaccine development studies, treatment design and prognosis are important (3). There are some difficulties in defining genotypes in all methods, including sequence analysis, which is used as the gold

Genotype	Genotype Distribution			Age		
	Number (%)	Female (%)	Male (%)	18-40	41-60	60+
1b	312 (56.3)	181 (32.6)	131 (23.6)	5 (0.9)	177 (31.9)	130 (23.4)
1a	127 (22.9)	72 (12.9)	55 (9.9)	7 (1.2)	71 (12.8)	49 (8.8)
3a	94 (16.9)	58 (10.4)	36 (6.4)	14 (2.5)	53 (9.5)	27 (4.8)
1a/1b	3 (0.5)	2 (0.3)	1 (0.1)	-	2 (0.3)	1 (0.1)
2a/2c	3 (0.5)	1 (0.1)	2 (0.3)	-	-	3 (0.5)
4c/4d	3 (0.5)	2 (0.3)	1 (0.1)	-	3 (0.5)	-
1b/4	1 (0.1)	1 (0.1)	-	-	1 (0.1)	-
Unknown	11 (1.9)	7 (1.2)	4 (0.7)	-	2 (0.3)	9 (1.6)
Total	554 (100)	324 (58.4)	230 (41.5)	26 (4.6)	309 (7)	219 (39.5)

standard method (11). The use of a second method in the laboratory increases sensitivity (12). Studies investigating the effectiveness of interferon and ribavirin combination therapy have shown that the cure rate with antiviral drugs is lower in patients infected with HCV genotype 1b than in those infected with other genotypes (13). In patients with genotype 1b infection, long-term, high-dose therapy results in a higher survival response (14). Furthermore, the rate of permanent response in combination therapy is higher than in interferon therapy alone (15). For this reason, determining HCV genotype may be useful in evaluating the response to treatment and in selecting the most effective treatment regimen (16).

There are geographical differences in the distribution of HCV genotypes. In studies conducted in Turkey, it was seen that genotype 1b was the first most frequently detected type in HCV genotypes with a rate of 66.7%. This is followed by genotype 1a at a rate of 5.8% (1). In our study, the dominant genotype sequence was found to be similar. It is observed that genotypes 2a, 3a, 4, 4c were reported less frequently (3). In their study investigating the distribution of HCV genotypes in 89 Turkish patients, Abacioglu et al. (15) reported that 75.3% of patients had genotype 1b, 19.1% had genotype 1a, 3.4% had genotype 2 and 2.2% had genotype 4. In a study including

Seventy-two patients with chronic HCV infection, conducted by Yarkin and Hafta (3) in 2000, using reverse transcriptase (RT) - PCR technique, it was found that 82.2% of patients had type 1b, 14.5% had type 1a, and 3.3% patients had type 2a. Erensoy et al. (17) in 2002, in their study of 45 genotypes in 2002 found genotype 1b in 66.7% of isolates and genotype 1a in 33.3%. In a study by Ural et al. (18) all the 80 HCV RNA-positive cases included in Konya region were found to be genotype 1b (100%). Sönmez et al. (19) analyzed 80 anti-HCV-positive samples using RT-PCR, and detected genotype 1b in 41 samples (69.5%) and mixed type (genotype 1a and 1b) in 3 samples (5.1%). Altuglu et al. (20) investigated serum samples collected from 345 patients with chronic HCV infection and reported that infection with subtype 1a and subtype 1b was observed in 9.9% and 87.2% of patients, respectively. Genotypes 2, 3, and 4 were determined in 0.9%, 1.4%, and 0.6% of the patients, respectively. Sağlık et al. (21) in their study including 422 HCV RNA-positive patients performed in 2014, it was determined that 63.3% of subjects (n=267) had genotype 1b, 14.7% (n=62) - genotype 1a, 11.1% (n=47) - genotype 3a, 0.9% (n=4) - genotype 2b, and 0.2% (n=1) of patients had genotype 4; genotype 1 and 4 were observed in 1 patient (0.2%). In genotype 1, 2 and 4 infected patients, subtyping could not be performed in 5.4% (n=23), 2.6% (n=11) and 1.4% (n=6), respectively. In a study performed in Manisa Region by Şanlıdağ et al. (22), a total of 100 HCV-RNA positive patients were included. Genotype 1 was found in 92% of patients (92%) and genotypes 2 and 4 were found in 7% of patients, while HCV genotype could not be identified in one patient (1%). When evaluating the subtypes, genotype 1b was determined in 90 patients (90%), genotype 4a in five patients (5%), genotype 1a in two patients (2%), and genotype 2a in two patients (2%). Kabakçı et al. (23) reported in their study including 500 HCV RNA-positive patients that the most frequent genotype was found to be 1b (93.5%) and the second most frequent genotype was 1a (6.7%). Öztürk et al. (24) found in their study performed in 2014 that the frequency of type 1a (0.31%), 1b (86.73%), 2 (9.26%), 3 (0.93%), and 4 (2.78%) in Antakya was compatible with the nationwide results in

Turkey. Altindis et al. (25) who investigated the distribution of HCV genotypes in 7 regions of Turkey reported in their study evaluating 7002 patients with chronic hepatitis C in a six-year period that genotype 1b was the most common genotype (67.7%) followed by untypeable genotype 1 (7.7%), genotype 4 (7.3%) and genotype 3 (6.7%). In 2014, genotype 3 was the second most common one (11.3%) and genotype 4 (9.8%) was the third most common one. A total of 96 (1.3%) patients were found to have mix genotypes. Genotypes 1a/1b were detected in 11 patients, genotypes 2a/2c in - 76 patients, and 9 other genotypes in 9 patients while 2 samples were not genotyped. Uzun et al. (26) reported that genotype 1 was observed in 271 of 308 patients (88%) with chronic HCV infection. Genotype 3 was determined in 15 patients (4.9% of all cases), mix genotype in 9 patients (2.9% of all cases); genotype 2 in 8 patients (2.6% of all cases), and genotype 4 in 5 patients (1.6% of all cases). Of those with mixed genotype, 8 patients had infection with genotype 1/4 and 1 patient with genotype 1/3.

In our study, genotype 1b was dominant genotype and genotype 1a was the second frequently observed genotype. Genotype 3a was the third genotype as reported in studies by Altuglu et al. (20) and Sağlık et al. (21). HCV genotype 1b is the most common genotype in our region similar to that in regions throughout the country. However, the rates are lower in our country than in previous years. Probably the difference in this genotype distribution may depend on globalization, developments in technology, the change of human movements and social behaviors. The incidence of mixed genotypes was 1.8 % and the rates were close to those of Altindis et al. (25) and Uzun et al. (26).

## Conclusion

As a result in this context, it is important to determine the molecular epidemiology of HCV infections in our region and to determine the treatment planning and prognosis of HCV infection in terms of follow-up of genotype profile with multi-center country-wide studies.

## Ethics

**Ethics Committee Approval:** This study was approved by Haydarpaşa Numune Training and Research Hospital Ethics Committee for Clinical Investigations [Approval number: 08.05.2017 (HNEAH-KAEK 2017/KK/70)].

**Peer-review:** Externally and Internally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: K.Ö., Concept: S.A., Ü.O.Z., Design: Ü.O.Z., Ş.D.D., Data Collection or Processing: Ü.O.Z., Ş.D.D., Analysis or Interpretation: Ü.O.Z., Literature Search: Ü.O.Z., F.M.Y, Ö.Y., Writing: Ü.O.Z.

**Conflict of Interest:** The authors declared no conflict of interest.

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