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Research Article

Distribution of hepatitis C virus genotypes in Ordu province

Ordu ilinde hepatit C virüsü genotiplerinin dağılımı

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Abstract

Aim: Hepatitis C virus (HCV) infection is a major health problem worldwide and leads to chronic liver disease, cirrhosis, and hepatocellular carcinoma. Genotype 1, the most common genotype worldwide, usually requires longer and more complex treatment regimens compared to other genotypes. HCV infection is closely associated with changes in liver enzyme levels, especially alanine aminotransferase (ALT). This study aims to determine the distribution of HCV genotypes in chronic HCV patients and to investigate the relationship between HCV genotype and serum ALT levels.

Material and Methods: In this retrospective study, the outcomes of patients diagnosed with chronic liver disease due to HCV at Ordu University Medical Faculty, Education and Research Hospital between May 2021 and October 2023 were analyzed. HCV genotyping was performed using the Bosphore HCV Genotyping Kit v5 (Anatolia Geneworks, Türkiye) according to the manufacturer's instructions. Serum ALT levels were measured using the electrochemiluminescence immunoassay method (Cobas e 601, Roche, Germany).

Results: A total of 219 HCV-RNA positive patients were included in the study. Of all patients, 125 (57.1%) were female and 94 (42.9%) were male. When genotype distribution was examined, it was seen that 200 patients (91.3%) had genotype 1b, 9 patients (4.1%) had genotype 1a, 7 patients (3.2%) had genotype 3, 2 patients (0.9%) had genotype 1, and 1 patient (0.5%) had genotype 4. A total of 9 patients had serum ALT levels above 40 IU/L.

Conclusion: Genotype 1b remains the most frequently detected genotype among our patients, while the prevalence of Genotype 3 has changed over the years. No significant differences were found in serum ALT levels, mean age, and gender distribution between patients infected with Genotype 1 and other genotypes.

Keywords: Hepatitis C Virus, HCV genotype, HCV-RNA, ALT

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Öz

Amaç: Hepatit C virüsü (HCV) enfeksiyonu dünya çapında büyük bir sağlık sorunudur ve kronik karaciğer hastalığı, siroz ve hepatoselüler karsinomaya yol açar. Dünya çapında en yaygın genotip olan genotip 1, genellikle diğer genotiplere kıyasla daha uzun ve daha karmaşık tedavi rejimleri gerektirir. HCV enfeksiyonu, özellikle alanin aminotransferaz (ALT) olmak üzere karaciğer enzim seviyelerindeki değişikliklerle yakından ilişkilidir. Bu çalışma, kronik HCV hastalarında HCV genotiplerinin dağılımını belirlemeyi ve HCV genotipi ile serum ALT seviyeleri arasındaki ilişkiyi araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Bu retrospektif çalışmada, Ordu Üniversitesi Tıp Fakültesi Eğitim ve Araştırma Hastanesinde Mayıs 2021 ile Ekim 2023 tarihleri arasında HCV'ye bağlı kronik karaciğer hastalığı tanısı konulan hastaların sonuçları analiz edildi. HCV genotipleme, üreticinin talimatları doğrultusunda Bosphore HCV Genotyping Kit v5 (Anatolia Geneworks, Türkiye) kullanılarak gerçekleştirildi. Serum ALT düzeyleri elektrokemilüminesans immünoassay yöntemi (Cobas e 601, Roche, Almanya) kullanılarak ölçüldü.

Bulgular: Çalışmaya toplam 219 HCV-RNA pozitif hasta dahil edildi. Tüm hastaların 125'i (%57,1) kadın, 94'ü (%42,9) erkekti. Genotip dağılımına bakıldığında 200 hastanın (%91,3) genotip 1b, 9 hastanın (%4,1) genotip 1a, 7 hastanın (%3,2) genotip 3, 2 hastanın (%0,9) genotip 1 ve 1 hastanın (%0,5) genotip 4 olduğu görüldü. Toplam 9 hastanın serum ALT seviyesi 40 IU/L'nin üzerindeydi.

Sonuç: Genotip 1b hastalarımız arasında en sık tespit edilen genotip olmaya devam etmekte olup, Genotip 3'ün yaygınlığı yıllar içinde değişmiştir. Genotip 1 ile enfekte olan hastalar ile diğer genotipler arasında serum ALT düzeyleri, ortalama yaş ve cinsiyet dağılımı açısından anlamlı bir fark bulunmamıştır.

Anahtar Kelimeler: Hepatit C Virüsü, HCV genotip, HCV-RNA, ALT

Introduction

Hepatitis C virus (HCV) infection is a major health concern worlwide, affecting an estimated 58 million people worldwide and leading to chronic liver disease, cirrhosis, and hepatocellular carcinoma [1]. First identified in 1989, HCV is an enveloped, single-stranded RNA virus belonging to the Flaviviridae family. The virus primarily targets hepatocytes, leading to inflammation, fibrosis, and, in severe cases, cirrhosis and hepatocellular carcinoma (HCC). Globally, it is estimated that approximately 60 million people live with chronic HCV infection, with around 1.5 million new infections occurring annually. A defining feature of HCV is its genetic diversity. Understanding the genetic diversity of HCV is crucial for effective diagnosis, treatment, and epidemiological surveillance [2]. The virus is classified into seven major genotypes (1-7), with multiple subtypes within each genotype [3]. This genetic variation has important implications for the clinical management of HCV, as different genotypes exhibit varying responses to antiviral therapies. Genotype 1, the most prevalent genotype globally, often requires longer and more complex treatment regimens compared to other genotypes. The identification of HCV genotype in infected individuals is therefore critical for guiding appropriate treatment strategies [4]. HCV is a complex and evolving global health issue. Understanding its virology, epidemiology, and clinical management is essential for developing effective strategies to reduce the burden of disease and achieve the goal of HCV elimination.

HCV infection is closely associated with alterations in liver enzyme levels, particularly alanine aminotransferase (ALT) [5]. ALT is an enzyme found predominantly in the liver, and its levels in the bloodstream are commonly used as a biomarker for liver health [6]. When liver cells are damaged or inflamed, as occurs during HCV infection, ALT is released into the bloodstream, leading to elevated serum ALT levels [7]. Elevated serum ALT levels are frequently observed in HCV-infected individuals, indicating hepatocellular damage. The relationship between HCV genotype and ALT levels can provide insights into the pathogenicity and clinical progression of the infection [8]. Certain genotypes may be associated with more severe liver damage and higher ALT levels, reflecting a more aggressive disease course.

This study aims to determine the distribution of HCV genotypes in a specific population and to investigate the relationship between HCV genotype and serum ALT levels. By elucidating these associations, we can enhance our understanding of HCV pathogenesis and improve the management strategies for patients with chronic HCV infection.

Material and Methods

In this retrospective study, the results of blood samples sent for HCV genotyping from HCV-RNA positive patients diagnosed with chronic liver disease due to HCV, between May 2021 and October 2023, at the Molecular Microbiology Laboratory of Ordu University Faculty of Medicine Education and Research Hospital were analyzed. Demographic data of the patients, such as age and

gender, along with alanine transaminase (ALT) levels, and HCV genotypes, were recorded from the hospital information system.

HCV-RNA levels were measured using real-time PCR (Cobas TaqMan HCV, Roche Diagnostics, Germany) for viral load determination. The dynamic range of the test was 15 IU/ml, with a linear range of 15-1x10^8 IU/ml. HCV genotyping was performed using the Bosphore HCV Genotyping Kit v5 (Anatolia Geneworks, Türkiye) following the manufacturer's instructions. This kit is capable of detecting genotypes 1, 1a, 1b, 2, 3, 4, 5, and 6 individually and targets the NS5B region of the HCV genome with specific primers. Serum ALT and Anti-HCV levels were measured using the electrochemiluminescence immunoassay (ECLIA) method (Cobas e 601, Roche Diagnostics, Mannheim, Germany). Samples with Anti-HCV levels ≥ 1 (S/CO) were considered reactive. The normal reference range for ALT was defined as 10-40 IU/L.

Statistical Analysis

Statistical analyses were performed using the MedCalc (version 20.009; Ostend, Belgium) statistical package program. In the statistical description of the data, numerical data were expressed as number (n), percentage, arithmetic mean (mean) and standard deviation (SD).

Results

A total of 219 HCV-RNA positive patients were included in the present study. ALT values were available for 84 of these patients. Among all patients, 125 (57.1%) were female and 94 (42.9%) were male. The mean age of the patients was 64 years (age range 20-90), and 193 patients (88.1%) were over the age of 50. Genotype distribution revealed that 200 patients (91.3%) had genotype 1b, 9 patients (4.1%) had genotype 1a, 7 patients (3.2%) had genotype 3, 2 patients (0.9%) had genotype 1c-k, and 1 patient (0.5%) had genotype 4. Both of the two foreign patients were found to have genotype 1b. Among genotype 1b patients, 59.5% were female; in genotype 1a, 66.7% were male; in genotype 3, 71.4% were male; in genotype 1c-k, 50% were male; and in genotype 4, all patients were male (Table 1).

The mean ALT level was 26.5 IU/L in female patients and 29.6 IU/L in male patients. A total of 9 patients had serum ALT levels above 40 IU/L. The mean age of patients with genotype 1b was 65.6 ± 11.4 years, genotype 1a patients 48.2 ± 19.2 years, genotype 3 patients 46.3 ± 13.3 years, genotype 1c-k patients 56.5 ± 31.8 years, and the genotype 4 patient 54 years.

Table 1. Age and gender distribution according to genotypes.

Genotype	n	%	Age		Gender			
			Mean	SD	Male		Female	
						%	n	%
HCV Genotype 1a	9	4.1%	48.2	19.2	6	66.7%	3	33.3%
HCV Genotype 1b	200	91.3%	65.6	11.4	81	40.5%	119	59.5%
HCV Genotype 1c-k	2	0.9%	56.5	31.8	1	50.0%	1	50.0%
HCV Genotype 3	7	3.2%	46.3	13.3	5	71.4%	2	28.6%
HCV Genotype 4	1	0.5%	54,.0		1	100.0%	0	0.0%

The average age of patients with genotype 1 was higher compared to other genotypes. The mean ALT level was 27.8 IU/L in genotype 1b patients, 31 IU/L in genotype 1a patients, and 32.2 IU/L in genotype 3 patients (Table 2).

Table 2. Evaluation of ALT levels according to genotypes.

Genotype	n	%	ALT	
			Mean	SD
HCV Genotype 1a	4	4.8%	31.0	21.0
HCV Genotype 1b	73	86.9%	27.8	27.6
HCV Genotype 1c-k	1	1.2%	11.0	
HCV Genotype 3	5	6.0%	32.2	27.0
HCV Genotype 4	1	1.2%	34.0	

Discussion

Hepatitis C virus (HCV) remains a global public health issue due to its high chronicity rate, potential to lead to cirrhosis and hepatocellular carcinoma, and the absence of an effective vaccine. HCV genotypes are distinguished by variations in nucleotide and amino acid sequences in different regions of the virus genome resulting from mutations [9]. The distribution of HCV genotypes and subtypes varies by geographic region [10]. Determining the HCV genotype is essential for guiding the selection of treatment, determining the duration of therapy, and monitoring treatment response in patients with chronic hepatitis C. Cases infected with HCV genotypes 1 and 4 respond less favorably to interferon therapy and require longer treatment durations compared to those infected with genotypes 2 and 3. Additionally, the risk of developing hepatocellular carcinoma is reported to be higher in genotype 1b cases. Therefore, identifying HCV genotypes not only informs treatment but also provides prognostic insights [11]. Given that epidemiological data are influenced by human activities such as war, migration, and tourism, regularly updating regional data is crucial. This study aims to determine the distribution of HCV genotypes, which are pivotal in shaping treatment and prognosis, and to

evaluate changes in genotype distribution over the past three years, contributing to the epidemiological data of our region.

As seen globally and in our country, the most common genotype in our hospital in Ordu province is type 1b (91.3%). Genotype 1a ranks second (4.1%), followed by genotype 3 (3.2%). Genotype 4 was detected in only one patient. One of the main findings of our study is the lack of significant correlation between genotype variation and serum ALT levels in chronic HCV patients. However, larger-scale studies are required for more conclusive results. Similar to global genotype distribution, genotype 1 is also the most prevalent in our country [12]. Genotypes 2, 3, and 4 are reported less frequently in Türkiye, although their frequency may vary by region. For instance, in a study conducted in Nevşehir, the most common genotype was genotype 1 (45.1%), followed by genotype 2 (14.5%) [13]. In studies by Bulut and Sayar, genotype 1b was also found to be the most common type, consistent with the findings of our study [14,15]. In a study involving 119 patients in Adana, 71.4% were infected with genotype 1 (12.6% genotype 1a, 58.8% genotype 1b), 16.8% with genotype 3, 7.6% with genotype 2, and 3.4% with genotype 4 [16]. Similarly, a study from Gaziantep involving 160 patients reported that 98% of patients had genotype 1b, with only one patient having genotype 2 and two having genotype 3 [17]. In a study from Konya involving 480 patients, 396 (82.6%) had genotype 1b, 17 (3.5%) had genotype 1a, and 15 (3.1%) had genotype 3a [18]. In Giresun, the most frequently detected genotypes were 1b, followed by 1a, 3, and 2, with no detections of genotypes 4, 5, or 6 [19]. Consistent with these findings, genotype 1b was the most frequently identified genotype in 91.3% of our study group.

The frequency of genotype 3 in Türkiye has been reported to range between 6.7% and 14%, often ranking as the second most common genotype [20–24]. Due to its prevalence in neighboring countries (especially Syria), its resistance, and its longer treatment duration, monitoring genotype 4 is important [14]. Recent studies in Türkiye indicate that genotype 4 is more commonly observed in regions with high populations of Middle Eastern immigrants, particularly due to migration [25–27]. In our study, the frequencies of genotypes 3 and 4 were 3.2% and 0.5%, respectively. In a study from our region conducted in 2019, the frequencies of genotype 3 and genotype 4 were 2.5% and 0.5%, respectively [28].

Several studies have also shown that HCV genotype ratios differ by gender. In one study, genotypes 1a and 1b were found to be more common in female patients, while another study reported that genotype 1b was more prevalent in females, with genotypes 1a and 3 more common in males [29–31]. However, Arici et al. found no significant difference in genotype distribution between genders [21]. Bulut and Sayar also observed a similar distribution of genotypes between men and women [15]. In our study, genotypes 1a, 3, and 4 were more common in male patients, while genotype 1b was more frequently observed in female patients. In a study involving 160 patients, Harman and colleagues reported a mean age of 56 years, with 25% of patients in the 18-50 age group and 75% over 50 years of age [17]. In Izmir, the mean age of patients infected with genotype 1b was higher than those infected with genotypes 1a, 3, and 4 [32]. Similarly, in Giresun, the mean age of genotype 1b patients was significantly higher than those with genotypes 1a and 3 [19]. In our study, the mean age was 64, and 85.8% of patients were over the age of 50. Consistent with the literature, the mean age of patients infected with genotype 1b was higher than that of patients with genotypes 1a and 3. Borcak et al. found ALT levels to be between 13-352 UI/ml in patients with Genotype 1 and 4, and 18-218 IU/ml in patients with Genotype 2 and 3 [13]. Çizmeci also found serum ALT levels to be 68.02±43.6 IU/L in genotype 1b patients and 49.5±39.51 IU/L in genotype 1a patients [29]. This study found no significant association between different genotypes and ALT levels, which is consistent with previous findings [13,21,29]. However, Kirişçi et al. found that ALT values ranged between 10-215 UI/ml in Genotype 1 patients and 11-1085 IU/ml in genotype 3 patients. Kirişçi and colleagues reported a statistically significant relationship between genotypes and serum ALT levels in their study [33]. In their study, Tezcan et al. determined the median value of ALT levels in patients identified as Genotype 1b as 46.14 IU/L in women and 63.9 IU/L in men, and found the difference to be statistically significant [31]. Studies suggest that serum ALT levels are a significant indicator of liver fibrosis and can guide treatment decisions in patients with chronic hepatitis C [34].

In conclusion, based on initial data from our hospital, genotype 1b remains the most frequently detected genotype among our patients, maintaining its leading position. According to the study conducted by Çalgın and Çetinkol in Ordu province, the prevalence of Genotype 3 has changed over the years [28]. No significant difference was found between patients infected with genotype 1 and other genotypes regarding serum ALT levels, average age, and gender distribution.

Ethical Approval

This study is approved by Ordu University Institution's Clinical Research Ethics Committee (270/10.11.2023).

Conflict of Interest

The authors have no conflict of interest to declare.

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