

Distribution of hepatitis C virus genotypes among intravenous drug users in the Çukurova region of Turkey

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Background/aim: The most common hepatitis C virus (HCV) genotype in Turkey is genotype 1. However, there has not been a study about the distribution of HCV genotypes among intravenous drug users (IVDUs) in the Çukurova region of Turkey. This study was planned to understand if there is a difference between IVDUs and the normal population.

Materials and methods: Between May 2010 and May 2014, anti-HCV positive IVDUs who applied to the 6 hospitals in the Çukurova region of Turkey were included in this study. Their HCV genotypes were studied.

Results: Ninety-seven anti-HCV positive IVDUs were screened in terms of HCV RNA and genotype. Ten were excluded from the study because their HCV RNA results were negative. Fifty-one of the 87 patients (58.6%) had genotype 3. Genotype 2 was detected in 26 (29.9%) and genotype 1 was detected in 10 (11.5%) patients.

Conclusion: HCV genotypes seem to be different between the normal population and IVDUs according to studies worldwide. Among IVDUs, we detected a dominance of genotype 3 and genotype 2, which is apparently different from the normal population. The reason for this difference can be simply explained by infection through shared needles. However, there may still be a different immunological response in IVDUs, the investigation of which may lead to further studies.

Key words: Hepatitis C, HCV genotypes, intravenous drug users

1. Introduction

The hepatitis C virus (HCV) is a blood-borne virus and an important cause of chronic liver disease, liver cirrhosis, and hepatocellular carcinoma. It is also one of the most prominent causes of liver transplantation, especially in Western countries (1,2). Its worldwide prevalence is about 2%, which means that almost 180,000,000 individuals are infected with HCV. However, among different countries the prevalence shows extensive variation; for instance, while its prevalence is 1.3%–1.6% in the United States, it increases up to 15% in Egypt (3,4). In Turkey, the subject of this study, the prevalence is around 0.4%–1% (5,6).

The highest risk groups for HCV infection consists of intravenous drug users (IVDUs), patients who have a history of blood transfusion, and hemodialysis patients, since the virus is blood-borne (7). Due to the fact that

with today's technology blood transfusion can be done with much safer methods, the percentage of transfusion-related infections has considerably decreased. However, unfortunately, intravenous drug usage is still one of the most important causes of viral transmission due to shared needles; this fact results in IVDUs being the highest risk group for HCV infection (7). HCV prevalence among IVDUs ranges from 30% to 90% worldwide (8–10).

The HCV genome includes 9400 nucleotides and reveals a high genetic heterogeneity, which results in the emergence of multiple genotypes (11). So far, 6 major genotypes and multiple subtypes have been identified, the distribution of which also varies in different geographical regions (12,13). Genotype 1 is the dominant genotype in North Europe, North America, South and East Europe, and Japan, whereas genotype 2 is detected less frequently

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in these regions. On the other hand, genotype 3 is most common in Southeast Asia, genotype 4 in Egypt and Middle Africa, and genotype 5 in South Africa (14–16).

In Turkey, studies show that genotype 1 (with subtype 1b) is the dominant type in the country, some studies even giving the resulting prevalence as up to 100%. However some recent studies indicate that, as of now, frequencies of other genotypes are increasing (17–23). It is well known that the HCV genotype directly affects the success of the treatment and the clearance rate; therefore, the diagnosis of the HCV genotype in the pretreatment phase is critical for medication.

During the treatment and tracking of IVDU patients in the Çukurova region, it was noticed that the frequency of genotype 3 had increased significantly. Since there have not been any studies about the distribution of HCV genotypes among IVUDUs in Turkey, this study was planned to investigate if there is a difference between IVDU and non-IVDU patients.

2. Materials and methods

The IVUDUs who applied to gastroenterology and infectious diseases clinics of the State Hospital of Adana Çukurova, the Dr. Ekrem Tok Psychiatric Hospital of Adana, the State Hospital of Adana, the State Hospital of Ceyhan, the State Hospital of Tarsus, the hospital of Mersin University Medical School, and the State Hospital of Kahramanmaraş between May 2010 and May 2014 with an HCV antibody positive diagnosis were examined and evaluated in terms of HCV RNA levels and genotypes.

Serological anti-HCV, HBsAg, and anti-HIV tests were done using the enzyme-linked immunosorbent assay (ELISA) method (Abbott Laboratories, USA). HCV RNA and hepatitis B virus (HBV) DNA levels were measured by the real-time polymerase chain reaction (RT-PCR) technique with the help of a Cobas TaqMan 48 kit (Roche Diagnostics, USA). Determination of the HCV genotypes was accomplished with another kit, an AMPLIQUALITY HCV-TS (AB Analitica, Italy), which is also referred to as

the “line probe assay” (LiPA). The LiPA quantifies the HCV genotypes by reverse hybridization of the amplification products that are already measured by Cobas TaqMan RT-PCR from the 5’UTR viral region. Special probes, made up of the unique oligonucleotides of HCV genotypes, are immobilized on nylon strips that contain these products.

The statistical analysis was executed by using the Mann–Whitney U test and differences were assumed to be significant at P < 0.05.

3. Results

Ninety-seven patients with positive HCV antibody diagnosis were involved in this study. After evaluating their HCV RNA levels and genotypes, 10 of them were excluded from the group of interest because their HCV RNA levels were negative. Of the remaining 87 patients, 2 of them were female (2%) and the other 85 patients were male (98%) with an average age of 27.4 ± 6.8 years. HBV–HCV coinfection was detected in only 1 patient and the HBV DNA level of this patient was found to be negative. None of the patients had HIV infection. While one of the patients had an acute HCV infection, the rest were chronic HCV patients.

After examining the genotype distribution, genotype 3 was identified in 51 (58.6%) of the 87 patients. Among these, genotype 3a was detected as a subtype in 2 patients while a subtype could not be assigned for the rest. Genotype 2 was detected in 26 (29.9%) patients, with 1 of them having genotype 2b, and genotype 1 was identified in 10 (11.5%) with 5 of them having genotype 1a.

HCV RNA levels of the patients ranged from 1.10 × 10² IU/mL to 3.3 × 10⁷ IU/mL, with an average HCV RNA level of 1.28 × 10⁶ ± 3.91 × 10⁶ IU/mL. Mean HCV RNA levels were 1.28 × 10⁶ ± 1.66 × 10⁶ IU/mL in genotype 1, 2.22 × 10⁶ ± 6.67 × 10⁶ IU/mL in genotype 2, 8.15 × 10⁵ ± 1.66 × 10⁶ IU/mL in genotype 3. Although HCV RNA levels were lower in genotype 3 patients, statistically there was not any significant difference between these groups in terms of this or binary HCV RNA comparisons (Table 1).

Table 1. Characteristics of the study population.

	Genotype 1	Genotype 2	Genotype 3	P-value
Frequency	11.5%	29.9%	58.6%	NA*
Sex (male/female)	10 / 0	26 / 0	49 / 2	NA
Age (years)	27.8	25.8	28.1	0.38
HCV RNA (IU/mL)	1.28 × 10 ⁶	2.22 × 10 ⁶	8.15 × 10 ⁵	0.36

*NA: Not available.

4. Discussion

Identification of the HCV genotype is an important parameter for the course and treatment of the disease. Studies so far have clarified that genotype 1 is the most frequent genotype in the Turkish population (17–23). Our previous research, focusing only on the genotype distribution in Mersin, is also consistent with this fact as genotype 2 and 3 were detected with rates of 2% and 4%, respectively, whereas genotype 1 and subtype 1b were diagnosed in 92% and 85% of the patients in the study group (17). However, when IVDUs come into question, the distribution changes completely, with genotype 3 (58.6%) in first place, followed by genotype 2 (29.9%), and genotype 1 only identified in a few patients (11.5%). Other genotypes were not detected in any of the patients.

Although the prevalence of genotype 1 is about 90% in Turkey, some recent studies have shown that the frequency of other genotypes is increasing in some regions of the country. Genotype 4 in Kayseri, genotype 2 in Gaziantep, and genotype 3 in İstanbul and Adana are being detected much more often compared to older studies (18,22,24,25). In a recent study from Adana and Antakya, genotype 1 turned out to be the most prevalent (59%), followed by genotype 2 (14%) and genotype 3 (26%) (18). In the latter study, it could not be clarified why these genotypes were seen at such high rates. However, our study may explain these unusual results since in the study of Öztürk et al. (18)

the route of transmission was not mentioned. Probably, IVDU were included in that study and they may have led to unexpectedly high rates.

Another study recently published reveals that there was no change in the distribution of genotypes in the past 10 years in Turkey, with genotype 1 being the most frequent genotype with 90% prevalence (26). Performed among 500 patients, the HCV genotypes and subtypes according to the NS5B, E1, and 5'UTR regions were diagnosed at rates of 92.5%, 93.5%, and 87.7% for genotype 1b and 6.7%, 5.6%, and 6.6% for genotype 1a, respectively, and also in all 3 analyses, genotypes 2 and 3 were detected at a rate of less than 1%. Eventually, in this study it was deduced that the phylogenetic analysis of NS5B and E1 regions indicated more accurate and consistent data compared to 5'UTR, because genotype 1 rates found with 5'UTR analysis are lower than the other two; however the deviation is still small. We used the 5'UTR viral region for determining the HCV genotypes in our study based on the fact that this method is being used in many regions of the world (3,24,27).

HCV genotypes in IVDU patients were found to be variable in different studies from various regions of the world (3,7,27–36) (Table 2). For instance, studies from China showed that the most prevalent genotype among all patients was genotype 1, followed by genotype 2. On the other hand, when focusing specifically on IVDUs,

Table 2. Genotype distribution among IVDU from various countries.

Study	HCV genotype (%)					
	1	2	3	4	5	6
China (24)	12	0	41	0	0	47
China (25)	11	0	35	0	0	46
China (26)	24	0	41	0	0	33
France (3)	52.9	2.3	36.8	7.5	0	0
Nigeria (29)	75	25	0	0	0	0
Greece (11)	22	2.4	65	10.4	0	0
Lebanon (33)	21.4	0	57.1	17.9	0	0
Belgium (27)	38	2	49	9	0	0
Iceland (28)	60	0	37.5	0	0	0
Cyprus (30)	43	0	57	0	0	0
Italy (31)	45.5	3	35	15	0	0
Slovakia (32)	41.3	0	57.7	0	0	0
This study	11.5	29.9	58.6	0	0	0

genotype 6 becomes dominant, followed by genotype 3 (28–30). In Europe, for all patients genotype 1 is the predominant genotype; however, an increase in genotype 3 frequency is noted among IVDUs according to some studies (3,7,27,31,33–35). For another example, in Lebanon, although the predominant genotype is genotype 4, genotype 3 was diagnosed at a high rate among IVDUs (36) (Table 2).

In this study, we determined that the most common genotypes among IVDUs are genotypes 3 and 2, whereas genotype 1 was identified with a very low rate. These findings are unexpected because even in geographically close regions to Turkey, like Cyprus or Greece, genotype 1 has not been identified with such a low rate (7,33). In addition, HIV is expected to be diagnosed at a high rate among IVDUs; however, in our study none of the patients had HIV infection (3,37). Lastly, HBV–HCV coinfection was determined only in 1 patient, whose HBV DNA was detected as negative. Another implication of the study is that IV drug usage among women is not common in Turkey compared to other countries, since only 2% of the patients were female in our study (38–42).

This research had some limitation due to the characteristics of the subject of interest. First, in IVDUs, adherence to HCV treatment is not at the desired level. A great number of patients cannot complete the therapy due to restarting IV drug usage or the side effects of the therapy (7). Besides, a lack of information about the history and duration of drug usage and also of the records of the progress in the treatment limits further investigation and therefore a variety of conclusions can be drawn. In addition, there is also a restricted amount of data about the effects of different HCV genotypes on transmission and replication

of the disease as the studies are focused on the effects of genotype on the treatment. For example, especially intermittent viremia can be observed by reinfection in IVDU, affecting further consecutive transmissions. Also, for some cases, the viruses that could initially escape from the immune response may develop rebound viremia. It is suspected that some human monoclonal antibodies may have a neutralizing effect; however, unfortunately, the correlation with the genotypes has not yet been clarified (43).

To sum up, it is not clear why the prevalence of HCV genotypes among IVDUs is different than in normal patients. This situation can be related to infection through sharing needles; however, that is far too simple an explanation for such a significant difference. Also, if that were the case, the IVDUs who were infected with genotype 1, the dominant genotype all over the country, would also spread the disease through needle sharing and cause an increase in genotype 1 percentage. Therefore, it seems likely that different immunologic reactions catalyze the fast spread or help the persistence of genotype 3 and 2 HCV RNA in IVDUs. This is not within the scope of this study but, nevertheless, it can be an inspiration for further research.

In conclusion, the prevalence of HCV genotypes among IVDUs is different from the normal population all over the world. It was found that rare genotypes, like genotype 3 and genotype 2, are seen commonly among IVDUs in the Çukurova region of Turkey. This study is important because this is the first of its kind in Turkey and it needs to be supported by new studies from other regions of the country.

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