Research Article

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Hepatitis C Virus Genotype Distribution in Forensic Cases

Adli Olgularda Hepatit C Virüs Genotip Dağılımı

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ABSTRACT

Objectives: In this study, we aimed to determine the hepatitis C virus (HCV) genotype and subtypes in blood samples that were determined by polymerase chain reaction (PCR) in autopsy cases.
Materials and Methods: The blood samples of autopsy cases that was sent to serological screening to post-mortem microbiology laboratory between years 2014-2018 were recruited. Forty blood HCV-PCR positive autopsy cases were further evaluated including demographic, clinic, laboratory and autopsy features.
Results: Thirty-five 35 (87.5%) of the patients were male and 5 (12.5%) were female. The mean age of the patients was 43.1±11.8 years. Of the 40 cases, 18 (45%) were Turkish citizens and 16 (40%) were other nationals. The identity information of 6 cases (15%) could not be determined. Among 40 HCV-positive cases by PCR, the genotype 3 was determined in 11 (27.5%) of the cases, genotype1a in 9 (22.5%) cases, genotype-1b in 7 (17.5%) cases, genotype-2 in 2 (5%) cases and genotype-4 in 2 (5%) cases. In 9 (22.5%) cases, the genotype could not be determined.
Conclusion: The most common HCV genotype in our study population was determined to be genotype-3 and the most common genotype in Turkish origin cases was found to be genotype-1a. Post-mortem PCR analysis for HCV infection is feasible and relevant for demonstrating the ongoing infections at death. Monitoring the change in HCV genotype distribution is critical for the development of effective strategies for HCV elimination.
Keywords: Hepatitis C Virus, genotype, molecular epidemiology, post-mortem microbiology

ÖZ

Amaç: Bu çalışmada, otopsi olgularından polimeraz zincir reaksiyonu (PCR) ile hepatit C virüs (HCV) pozitif saptanan kan örneklerinde HCV genotip ve alttiplerinin belirlenmesi amaçlanmıştır.
Gereç ve Yöntemler: 2014-2018 yılları arasında post-mortem mikrobiyoloji laboratuvarına serolojik tarama için gönderilen otopsi olgularının kan örnekleri alındı. Demografik, klinik, laboratuvar ve otopsi özellikleri de dahil olmak üzere 40 HCV-PCR pozitif otopsi olgusunun kan örnekleri değerlendirildi.
Bulgular: Çalışmaya alınan olguların 35'i (%87,5) erkek, 5'i (%12.5) kadın olup, olguların yaş ortalaması 43,1±11,8 yıl olarak belirlenmiştir. 40 olgunun 18'i (%45) Türk vatandaşı olup, 16'sı (%40) yabancı uyrukludur. Altı (%15) olgunun da kimlik bilgilerine ulaşılamamıştır. Real time PCR analiz sonuçlarına göre örneklerin 11'inde (%27,5) genotip 3, 9'unda (%22,5) genotip 1a, 7'sinde (%17,5) genotip 1b, 2'sinde (%5) genotip 2 ve 2'sinde (%5) genotip 4 tespit edilmiştir. Örneklerin 9'unda (%22,5) ise genotip tayini yapılamamıştır.
Sonuç: Çalışmamızda, genel popülasyonda en sık HCV genotip 3 saptanırken, Türk vatandaşlarında ise en sık saptanan genotip 1a olmuştur. HCV enfeksiyonu için post-mortem PCR analizi uygulanabilir ve ölümde devam eden enfeksiyonları göstermek için önemlidir. HCV genotip dağılımındaki değişikliğin izlenmesi, HCV eliminasyonu için etkili stratejilerin geliştirilmesi için kritik öneme sahiptir.
Anahtar Kelimeler: Hepatit C Virüs, genotip, moleküler epidemiyoloji, post-mortem mikrobiyoloji

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Introduction

Hepatitis C has been a global health problem with a well-known importance since the identification of the Hepatitis C virus (HCV) in 1989. During the last 15 years seroprevalance of HCV has been increasing and more than 185 million people are tought to be infected with this virus all around the world (1,2,3). Initially, HCV was thought to be the most common cause of post-transfusion

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related hepatitis and almost not changing the life expectancy in infected individuals but later on studies shown that 85% of the HCV cases become chronic and HCV is one of the most common cause of deaths due to liver cirrhosis and the reason for liver transplantation. In our country, HCV is the second most common cause of chronic viral hepatitis after Hepatitis B virus (4).

After the full-length genome sequences of HCV strains up to date 7 genotype and more than 100 subtypes have been defined based on on phylogenetic and sequence analyses (5,6). HCV genotypes are expressed in numbers from 1 to 7 and subtypes are expressed in lowercase letters such as a, b, c, etc. Each HCV genotype differs from each other by at least 20% at the nucleotide level and by more than 15% at the amino acid level (6). Moreover, base sequence changes within the same genotype can be observed at a rate of 5-8% in the nucleotide sequence and at a rate of 4-5% in the amino acid bases. The global geographical distribution of HCV genotypes is various. Some genotypes appear to be seen in a particular region of the world. In North America, genotype 1a predominates, whereas genotype 1b, which is more commonly associated with aggressive liver disease, is more common in Western Europe and Japan. Genotype 2 is less common in Europe than Asian countries such as China, Japan and Taiwan. Genotype 3 is usually found in the UK and Thailand, while genotype 4 is seen in Middle East region and Central Africa. Genotype 5 is observed in South Africa and genotype 6 in Hong Kong (7). Geneotype 7 has been reported to from Democratic Republic of Congo in Central Africa (6,8). The most common observed genotype in Turkey is the genotype 1b, followed by genotype 1a (9,10). Besides 7 genotypes, there are 67 confirmed and 20 possible subtypes of HCV identified with at least 15% genomic variation; genotypes 5 and 7 have a single subtype, while at least seven subtypes are observed in other genotypes (11).

The most important problem of post-mortem serological analysis is that its validity. Higher positive results was observed in some studies more than expected (12,13,14). For this reason, it is recommended to use sensitive kits for post-mortem serological examinations (15). Unfortunately, most of the kits used in routine microbiology laboratory for post-mortem serological examinations were not validated for post-mortem samples. Due to loss of specific reactions, the post-mortem samples show decreased sensitivity and increased false-negative results (15). For these reasons, anti-HCV antibody tests was not used in the blood samples of autopsy cases. The success of post-mortem microbiological investigations depends on the adequacy of the post-mortem sampling protocol and strategy (16). Firstly, it is recommended to perform the autopsy preferably within the first 24 hours after death. Microbiological samples should be obtained as soon as possible. In particular, it is emphasized that blood samples should be drawn at the beginning of autopsy (17). As a general principle, the samples should be obtained at room temperature as soon as within 2 hours, stored in suitable transport and storage environments, and if samples was needed to transferred to the study laboratory should be done within 48 hours at 2 to 8 °C (18). The most common drawbacks of post-mortem serological and molecular examinations occurs because of insufficient quantity or bad quality of blood samples, or samples obtained at inappropriate time intervals (19,20).

The aim of this study was to determine the distribution of HCV genotypes and subtypes in blood samples that were determined by polymerase chain reaction (PCR) in autopsy cases in Turkey.

Materials and Methods

This study was done in Turkish Ministry of Justice Council of Forensic Medicine Post-mortem Microbiology Laboratory, in İstanbul. Forty HCV positive autopsy cases were included in this study between years 2014 to 2018. This study was approved by the Ethics Commitee and Research and Scientific Research Commission of the Ministry of Justice Council of Forensic Medicine (approval number: 21589509/2019/125).

Blood samples were drawn from large vessels (femoral artery, femoral vein, jugular vein) for serological and PCR evaluations of autopsy cases. The blood samples were transferred to EDTA tubes and sent to the laboratory as fast as possible. After centrifuging the post-mortem blood samples at 10.000 rpm for 10 minimum, the resulting plasma samples were aliquoted and stored at -80 °C until the assay. 400 µl of the plasma samples were removed and RNA isolation was performed on the QIA symphony device with the QIA symphony DSP Virus/Pathogen Midi kit, and the amplification of the RNA was performed on the Rotor-Gene® Q device (Qiagen, Germany) by the RT PCR method using the Artus® HCV-PCR kit. For determination of the HCV genotypes (RTA) HCV Genotyping qPCR Kit [targeting NS5b and NS3 of viral 5'-UTR region obtained by real time (RT)-PCR] (RTA, Kocaeli, Turkey) was used. The Kit identifies the six major and most common HCV genotypes (1, 1a, 1b, 2, 3, 4, 5, 6). Analysis was carried out on the CFX C1000 Touch instrument (Bio-Rad, Hercules, USA) and genotypes were determined.

Statistical Analysis

The statistical software program of SPSS (Version 16) was used in the data analysis of the study. The descriptive statistics were expressed as numbers and percentages.

Results

Of the 40 cases included in the study, 35 (87.5%) were male and 5 (12.5%) were female, and the mean age was 43.1±11.8 years (range: 23-69 years). Among the 40 cases, 18 (45%) were Turkish citizens and 16 (40%) were foreign nationals. We could not reach nationality of 6 (15%) cases (Table 1). Demographic features, HCV genotype distribution, clinical history, laboratory results and the autopsy results of the study population were shown in Table 2.

Table 1. The distribution of the autopsy cases according to nationality

	Number of cases	%
Turkish	18	45
Turkmenistan	4	10
Georgia	3	7.5
Pakistan	3	7.5
Syria	2	5
Uzbekistan	2	5
South Africa	1	2.5
Tanzania	1	25
Unknown	6	45
Total	40	100

Table 2. Demographic, clinical, laboratory and autopsy results of the study cases

	Gender	Age	HCV genotype	Clinical history	Co-morbidity status	Autopsy results
1	Male	35	1a	HCV positive, cocaine intoxication, intensive care unit admission, intubation due to low glascow coma score and respiratory distress,	-	Death due to drug intoxication.
2	Male	38	2/1a	Hospitalized with unconsciousness, death after hospitalization.	Pulmonary tuberculosis	Death due to multiple drug intoxication and complications.
3	Female	47	1b	Hospitalized with the complaint of abdominal pain a day before, sent after the intervention and died in next day.	Cirrhotic liver, hepatic encephalopathy	Death due to peritonitis and complications.
4	Male	43	3	Death at home	-	Death due to drug intoxication.
5	Male	38	3	History of drug intake, taken to emergency room by the foreigners' office, death after hospitalization	-	Sent to 1th forensic expertise board of the council of forensic medicine
6	Male	29	1a	Death after hospitalization	-	Death due to drug intoxication.
7	Male	38	1a	Found dead on the street	-	Death due to drug intoxication.
8	Male	25	1b/3	Admission to the emergency room after fainting on the street, respiratory distress, intensive care unit admission	-	Death due to Lung Infection and related complications and due to drug intoxication.
9	Male	64	1a	Chronic schizophrenia, 34-40% body burn due to fire in hospital ward	-	Death due to body burn and related complications.
10	Male	36	1b	Hospitalized on the deterioration of the general condition, death after hospitalization.	-	Death due to drug intoxication.
11	Male	56	3	Operated for cerebral hemorrhage due to hypertension, 15 days intensive care unit stay	-	Death due to Non-traumatic cerebral hemorrhage and developing complications
12	Male	52	1a	Tuberculosis, 2 months anti-TBC treatment, complained of chills, chest pain, hospitalization due to deterioration of the general condition	Tuberculosis	Lung infection and died due to drug intoxication
13	Male	37	2	Drug intake? Hospitalization due to unconsciousness, exitus	-	Death due to drug intoxication and complications
14	Male	25	N/A	Hospitalized on the deterioration of the general situation at home, drug intake?	-	Death due to drug intoxication and complications
15	Male	37	3	Treatment for substance abuse, hospitalization due to deterioration of the general condition at home	-	Death due to drug intoxication.
16	Male	28	2/1b	Found dead on the street	Growth of *Streptococcus pyogenes* in blood, lung, spleen, and pleural fluid	Died due to systemic infections and drug intoxications
17	Female	58	3	Death at the care center	DM, cirrhosis, COPD	Death due to Self-existing disease (DM, cirrhosis, COPD) and complications
18	Female	31	1b	One month ago intensive care unit admission due to traffic accident, ARDS, exitus	-	Sent to 1st forensic expertise board of the council of forensic medicine
19	Male	45	3	No history of disease before, admitted to the emergency department after the use of bonzai, unconscious, intubated, 1 week ICU stay.	-	Death due to drug intoxication.
20	Male	35	4	Found dead on the street	HIV positivity	Death due to drug intoxication and complications

Table 2. contiuned

21	Male	63	1a	Hospitalization due to non-vehicle traffic accident, operated due to hypertension, ICU admission	-	Died due to trauma to the general body, brain hemorrhage and complications.
22	Female	65	N/A	Body burn due to fire at home	-	death due to body burn and related complications (lung infection, sepsis).
23	Male	55	N/A	Death at home	-	Death due to blunt head trauma and brain hemorrhage
24	Male	33	N/A	Three weeks hospitalization after gunshot injury	-	Death due to Spinal cord injury, gunshot injury and spinal cord injury related complication
25	Female	44	1b	Hospitalized on the deterioration of the general condition, death after 3 day hospitalization.	-	Methyl alcohol intoxication and related complications
26	Male	45	3	Found dead on the street	-	Death due to drugs and inhalant intoxication.
27	Male	52	3	Hospitalized on the deterioration of the general condition, death after hospitalization, drug intake?	-	Death due to gastrointestinal bleeding and gastric ulcer complications and drug intoxication,
28	Male	52	3	Death at home, liver cirrhosis and cancer.	Cancer, liver cirrhosis	Death due to cirrhosis and related complications.
29	Male	69	1a	A history of subarachnoid hemorrhage after a fall from stairs, post op exitus.	-	Sent to 1th forensic expertise board of the council of forensic medicine
30	Male	42	N/A	A history of AIDS and drug abuse, death in police custody.	HIV positivity	Death due to AIDS disease and related complications.
31	Male	50	2/1b	Death at home.	-	Sent to 1st forensic expertise board of the council of forensic medicine.
32	Male	45	1b	Drug intake. Found wounded roadside with unconscious, death after hospitalization,	Cardiovascular disease	Death due to methyl alcohol intoxication.
33	Male	34	3	Hospitalized after falling, 1 week ICU stay.	-	Died of skull damage, facial bone fractures, brain hemorrhage, and brain tissue damage due to traumatic body trauma.
34	Male	23	4	Bronchitis history, hospitalized on the deterioration of the general condition at home	Bronchitis	Sent to 8th forensic expertise board of the council of forensic medicine
35	Male	28	1a	Prisoner, death outside the prison when he was on leave.	-	Death due to drug intoxication.
36	Male	58	1b	Heart attack at home, death after hospitalization	-	Death due to cardiovascular disease.
37	Male	44	3	Found dead on the street, had drugs in pocket	-	Death due to lung infection and cardiovascular disease.
38	Male	37	1a	Found dead on the street, history of COPD, alcohol and drug usage,	HBV positivity	Death due to pulmonary tuberculosis and its complications
39	Male	41	1b	Death after 15 day hospitalization.	Tuberculosis culture and Tbc-PCR positivity in lung, spleen, and HBV-HIV positivity	Death due to systemic tuberculosis and related complications
40	Male	50	2	Found dead on the street	-	Death due to drug intoxication.

*N/A could not be genotyped.
HCV: Hepatitis C virus, DM: Diabetes mellitus, COPD: Chronic obstructive pulmonary disease, ICU: Intensive care unit, ARDS: Acute respiratory distress syndrome, PCR: Polymerase chain reaction, HIV: Human immunodeficiency virus

By using the RT-PCR, HCV genotype 3 was determined in 11 (27.5%) of the samples, HCV genotype 1a in 9 (22.5%) samples, HCV genotype 1b in 7 (17.5%) samples, HCV genotype 2 in 2 (5%) samples, and HCV genotype 4 in 2 (5%) samples. HCV genotype could not be determined in 9 (22.5%) samples. The most common HCV genotype in the study cases was genotype 3, while the most common HCV genotype in Turkish citizens was genotype 1a. The genotype distribution of Turkish citizens and foreign nationals was shown in Table 3.

We found that 22 (55%) autopsy cases had a history of intravenous drug usage. All of the 22 cases were male, 10 of them were Turkish citizens. The HCV genotype distribution of 22 samples was as follows: genotype 3 in 7 (31.8%), genotype 1a in 6 (27.3%), genotype 2 in 2 (9.1%), genotype 1b in 1 (4.5%) and genotype 4 in 1 (4.5%) sample. The genotype could not be determined in 5 samples (22.7%). Among the 10 Turkish citizens with history of intravenous drug usage; genotype 1a was found in 5 (50%) of them, genotype 3 in 2 (20%) of them, and genotype 1b in 1 (10%) of them, and genotype could not be detected in 2 (20%) of them.

Study Limitations

There are also several limitations to our study, the rate of genotype 3 was found to be which is higher than the rates reported previously from our country. So, it is not possible to reach a definitive conclusion due to the low number of cases in our study. The second limitation, in our study, we used an automated PCR based method. Although the RT-PCR method has advantages such as being user independent, standard, automatic and yielding fast results, but in our study, the genotype of HCV subtypes could not be determined in 22.5% of cases.

Conclusion

The most common detected HCV genotype has been reported to be genotype 1, at a rate of 46% all around the world (21). In our country, while the most common HCV genotype in hepatitis C patients between years 1995 and 2014 was found to be genotype 1b (22,23), but most recently, genotype 3 was found to be the most common genotype in intravenous substance addicts, and in prisoners in different two studies (24,25). Although genotype 3 has been reported at low rates (0-4.5%) in studies conducted in different centers in Turkey, after year 2010, genotype 3 detection rates has been increasing significantly (26). In our study, the most common genotype detected in study samples was genotype 3 (27.5%), and the genotype 1a (44%) took the first place in samples from Turkish citizens. Our findings show that the presence of foreign nationals in the HCV genotype distribution conflicts with our country data. Events that cause social changes such as war, migration and tourism affect the epidemiology of infections (23). In our study, the heterogeneity among genotypes may be related to demographic changes in our country due to its geographical location. We thought that the presence of high number of foreign nationality in our cases may be responsible for excessive detection of genotype 3 (27.5%). On the other hand, the rate of genotype 3 was found to be 22.2% in Turkish nationality cases which is higher than the rates reported previously from our country (27,28). Although it is not possible to reach a definitive conclusion due to the low number of cases in our study, it may be concluded that genotype 3 is becoming more common in our country. The main route of transmission of HCV is parenteral. After initiation of screening programs in blood and blood products, intravenous drug usage has become the main parenteral route of transmission. In European countries such as France, Germany, Italy and Sweden 30-59% of all HCV infections are associated with intravenous drug usage. In America, this rate reaches 68% (29). The anti-HCV antibody positivity prevalence among intravenous drug users in Turkey has been reported to be 28.9% (30). In another study, HCV infection was determined in 47% of intravenous drug users that were mostly adolescents and young adults (31). In addition, the lack of attention to sterilization and disinfection during the medical procedures is also one of the most common causes of HCV transmission in our country (32). In a study conducted in our country in young people with intravenous drug addiction, the most common genotype was determined to be genotype 1a (26). In our study, 22 (55%) patients had a history of intravenous drug use and all were male. Ten (45%) of these drug users were Turkish citizens. In our cases among the IV drug users genotype 3 (7/22; 31.8%) was found to be the most common genotype. On the other hand, among the Turkish IV drug users

Table 3. Hepatitis C virus genotypes distrubution of autopsy cases with respect to nationality

		HCV Genotype								
Nationality	**Number (n)**	**1A (n,%)**	**1B (n,%)**	**1B/3 (n,%)**	**2 (n,%)**	**2/1A (n,%)**	**2/1B (n,%)**	**3 (n,%)**	**4 (n,%)**	**N/A (n,%)**
Turkey	18	8 (44.4)	1 (5.6)	1 (5.6)	-	-	-	4 (22.2)	-	4 (22.2)
Turkmenistan	4	-	2 (50)	-	-	-	-	2 (50)	-	-
Georgia	3	-	1 (33.3)	-	1 (33.3)	-	-	1 (33.3)	-	-
Pakistan	3	-	1 (33.3)	-	-	-	-	1 (33.3)	-	1 (33.3)
Syria	2	-	-	-	-	-	-	1 (50)	1 (50)	-
Uzbekistan	2	-	2 (100)	-	-	-	-	-	-	-
South Africa	1	-	-	-	-	-	-	1 (100)	-	-
Tanzania	1	1 (100)	-	-	-	-	-	-	-	-
Unknown	6	-	-	-	1 (16.7)	1 (16.7)	2 (33.3)	1 (16.7)	1 (16.7)	-
Total	40 (100)	9 (22.5)	7 (17.5)	1 (0.5)	2 (5)	1 (2.5)	2 (5)	11 (27.5)	2 (5)	5 (12.5)

HCV: Hepatitis C virus

genotype 1a (5/10; 50%) was found to be the most common genotype in our study. Of the all our autopsy cases cause of death was found to be related drug intoxication in 20 (50%) cases. For those reasons, much more prospective studies should be focused on people with HCV infection who are intravenous drug addicts.

There are various methods available for HCV genotyping. Techniques such as restriction fragment length polymorphism, allele-specific PCR and line probe assay are widely used to identify the HCV major and subtypes that are very common in North America, Europe and Japan. However, it has been stated that the standard reference and gold standard method comprise sequence analysis of HCV NS5, core, E1 and 5'-UTR regions and subsequent phylogenetic analysis (12). In our study, we used an automated PCR based method. Although the RT-PCR method has advantages such as being user independent, standard, automatic and yielding fast results, but in our study, the genotype of HCV subtypes could not be determined in 22.5% of cases. Like our study result, the genotype could not be determined in 9%, 25% and 27.3% of study population done in our country (13,14,33).

To our knowledge, this is the first HCV study with post-mortem cases in our country. More comprehensive molecular epidemiological studies are needed to understand the ways in which HCV enters our country and how it spreads. We think that import cases may affect the HCV biodiversity, and the detection of genotype 3 frequency in our country. Therefore, an effective HCV surveillance system should be established.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Commitee and Training and Scientific Research Commission of the Ministry of Justice Council of Forensic Medicine (approval number: 21589509/2019/125).

Informed Consent: It wasn't obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: N.Z., N.E., Design: N.Z., Ö.Y., M.N.A., Data Collection or Processing: N.Z., Ö.Y., Analysis or Interpretation: N.Z., N.E., Ö.Y., Literature Search: N.Z., N.E., Writing: N.Z., Ö.Y., N.E., M.N.A.

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References

1. Mohd Hanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57:1333-1342.
2. https://www.who.int/campaigns/world-hepatitis-day/2019 Access: 11.09.2019
3. 3.http://www.worldhepatitisday.org/wp-content/uploads/2019/02/WHD-report-2018.pdf.Access:11.09.2019.
4. Mıstık R, Balık İ. Türkiyede viral hepatitlerin epidemiyolojisi. Bir metaanaliz. Kılıçturgay K, editör. Viral Hepatit 98. 1. Baskı. Ankara: Viral Hepatitle Savasım Derneği Yayını; 1998.p.1-40.
5. Türkoğlu S. Hepatit C virüsü: Viroloji ve seroloji. Viral Hepatit 2003. Viral Hepatitle Savasım Derneği. 2003; 186-198.
6. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, Simmonds P. Expanded classifiatin of hepatitis C virus into 7 genotypes and 67 subtypes: Updated criteria and genotype assignment web resource, Hepatology. 2014;59:318-327.
7. Webster G, Barnes E, Brown D, Dusheiko G. HCV genotypes-role in pathogenesis of disease and response to therapy. Baillieres Best Pract Res Clin Gastroenterol. 2000;14:229-240.
8. Murphy DG, Sablon E, Chamberland J, Fournier E, Dandavino R, Tremblay CL. Hepatitis C virus genotype 7, a new genotype originating from Central Africa. J Clin Microbiol. 2015;53:967-972.
9. Erensoy S, Göksel S, Akarca US, Özkahya M, Canatan D. Hepatit C cirüsünün polimeraz zincir reaksiyonu ürünlerinin doğrudan dizi analizi ile genotiplemesi. Flora 2002;7:104-111.
10. Akpınar H, Abacıoğlu YH, Tankurt E, Şimşek İ, Yuluğ N, Ersöz G, Batur Y, Gönen Ö. Prevalence and genotyping of hepatitis C virüs RNA in Turkish patients with chronis non-A, non-B liver disease. Turk J Gastroenterol. 1998;9:208-212.
11. Galli A, Bukh J. Comparative analysis of the molecular mechanisms of recombination in hepatitis C virus. Trends Microbiol. 2014;22:354-364.
12. Elahi E, Pourmand N, Chaung R, Rofoogaran A, Boisver J, Samimi-Rad K, Davis RW, Ronaghi M. Determination of hepatitis C virus genotype by Pyrosequencing. J Virol Methods. 2003;109:171-176.
13. Türkoğlu S, Bozacı M, Çakaloğlu Y. İkinci kuşak core genotiplemesi ile hepatit C virus genotiplerinin araştırılması. 3. Ulusal Hepatoloji Kongresi Kongre Kitabı. İstanbul. 1999: 41.
14. Sönmez E, Taşyaran MA, Kızılkaya N, Korkut H, Tombul Z, Akçam ZC, Yılmaz Ş, Köksal F, Leblebicioğlu H, Ekici H. Hepatit C virus ile infekte 59 hastada HCV genotiplerinin dağılımı: Çok merkezli bir çalışma. Flora 1996;2:92-95.
15. Kitchen AD, Gillan HL. The serological screening of deceased tissue donors within the English Blood Service for infectious agents-a review of current outcomes and a more effective strategy for the future. Vox Sang 2010;98:193-200.
16. Caplan MJ, Koontz FT (2001) Post-mortem microbiology. In: McCurdy BW (ed) Cumitech 35. ASM Press, Washington DC.
17. Fernández-Rodríguez A, Cohen MC, Lucena J, Van de Voorde W, Angelini A, Ziyade N, Saegeman V. How to optimise the yield of forensic and clinical post-mortem microbiology with an adequate sampling: A proposal for standardisation. Eur J Clin Microbiol Infect Dis. 2015;34:1045-1057.
18. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gillian PH, Thomson Jr RB, Bourbeau P, Carroll KC, Kehl SC, Michael Dunne W, Robinson-Dunn B, Schwartzman JD, Chapin KC, Snyder JW, Forbes BA, Patel R, Rosenblatt JE, Pritt BS. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Disease Society of American (IDSA) and the American Society for Microbiology (ASM). Clin Infect Dis. 2013;57:22-121.
19. Kitchen AD, Newham JA. Qualification of serological infectious disease assays for the screening of samples from deceased tissue donors. Cell Tissue Bank. 2011;12:117-124.
20. Kitchen AD, Gillan HL. The serological screening of deceased tissue donors within the English Blood Service for infectious agents- a review of current outcomes and a more effective strategy for the future. Vox Sang 2010;98:193-200.
21. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E. Global distribution and prevalence of hepatitis C virus genotypes. Hepatology. 2015;61:77-87.
22. Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modeling study. Lancet Gastroenterol Hepatol. 2017;2:161-176.
23. Sağlik İ, Mutlu D, Öngut G, İnan D, Öğünç D, Can Sarinoğlu R, Özhak Baysan B, Gültekin M, Çolak D. Distribution of hepatitis C virus genotypes among patients with chronic hepatitis C infection in Akdeniz University Hospital, Antalya, Turkey: A five-year evaluation. Mikrobiyol Bul. 2014;48:429-437.

24. Keten D, Emin Ova M, Sirri Keten H, Keten A, Gulderen E, Tumer S, Caliskan A, Kulotu S. The prevalence of hepatitis B and C among prisoners in Kahramanmaras, Turkey. Jundishapur J Microbiol. 2016;9:e31598.
25. Akyar E, Seneca KH, Akyar S, et al. Linkage to care for suburban heroin users with hepatitis C virus infection, New Jersey, USA. Emerg Infect Dis. 2016;22:907-909.
26. Çizmeci Z. Kronik hepatit C enfeksiyonlu hastalarda hepatit c virüs genotiplerinin dağılımı. Türk Mikrobiyol Cem Derg. 2016;46:27-32.
27. Küçköztaş MF, Ozgünes N, Yazici S. Investigation of the relationship between hepatitis c virus (HCV) genotypes with HCV-RNA and alanine aminotransferase levels in chronic hepatitis c patients. Mikrobiyol Bul. 2010;44:111-115.
28. Çekın Y, Gür N, Çekın AH, Altuğlu İ, Yazan Sertöz R. Investigation of hepatitis C virus genotype distribution in patients with chronic hepatitis C infections in Antalya Training and Research Hospital, Turkey. Mikrobiyol Bul. 2014;48:484-490.
29. Alter MJ. Prevention of spread of hepatitis C. Hepatology 2002;36(5 Suppl 1):93-98.
30. Abacıoğlu YH, Öktem MA. Hepatit C Virusu. Us AD, Ergünay K, editor. Moleküler, Klinik ve Tanısal Viroloji Ankara: Bilimsel Tıp Yayınevi 2012;335-371.
31. Alaei A, Alaei K, Waye K, Tracy M, Nalbandyan M, Mutlu E, Cetin MK. Hepatitis C infection and other drug-related harms among inpatients who injected drugs in Turkey. J Viral Hepat. 2016;24:496-505.
32. Barut HŞ, Günal Ö. Dünyada ve Ülkemizde Hepatit C Epidemiyolojisi. Klimik Dergisi 2009;22:38 43.
33. Gökahmetoğlu S, Atalay MA, Kılınç A. Hepatit C virüs genotiplerinin pirosekanslama yöntemi ile belirlenmesi. Erciyes Tıp Dergisi (Erciyes Medical Journal) 2011;33:99-102.