Distribution of genotypes in 529 Iraqi patients with chronic hepatitis C virus infection

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ABSTRACT

INTRODUCTION: Hepatitis C virus (HCV) infection is a major cause of chronic liver disease and many dangerous complications. It affects about 3% of the world population. HCV is a positive-strand RNA virus and has been classified into six major genotypes with multiple subtypes. Genotyping is recognized as the primary tool for assessing the course of infection, determining type, and duration of treatment, and the response to treatment.

OBJECTIVE: This study was designed to identify the predominant HCV genotype in a sample of patients diagnosed with chronic hepatitis C infection in two biggest centre in Baghdad.

METHODS: This study is a descriptive cross-sectional study which was conducted at the outpatient clinics of Gastroenterology and Hepatology Teaching Hospital and Baghdad Teaching Hospital at Medical city in Baghdad from December 2014 to September 2016. We included 529 patients, 306 females and 223 male, diagnosed as chronic HCV infection. Anti HCV Ab, genotyping and viral load measurement had been done for all these patients.

RESULTS: In this study there is 254 (48%) of patients were infected with genotype 4; 127(24%) of patients infected with genotype 1a and 106 (20%) of patients infected with genotype 1b, so two major genotype distribution in this studied group genotype 1 and genotype 4.

CONCLUSION: Two predominant genotypes in Iraq genotype 4 followed by genotype 1a then genotype 1b. Genotype 1a more predominant in younger group than genotype 1b while genotype 4 equally distributed.

Key words: Hepatitis C Virus, Genotype, Iraq.

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major global health problem. Estimates indicate that three to four million individuals are newly infected each year, 185 million people are chronically infected and at risk of developing liver disease including cirrhosis and liver cancer, and 350,000 deaths occur each year due to all HCV-related causes.[1]

HCV is a small size (55-65 nm), enveloped virus, belong to a member of the family Flaviviridae. It is mainly transmitted by exposure to contaminated blood or blood products. HCV viral genome is positive sense RNA with approximately 9.4 kb in length. The sequence contained a 5' untranslated region (5' UTR) of 341 bases, a long open reading frame coding for a polyprotein of 3,011 amino acids, and a 3' untranslated region (3' UTR) of about 27 bases, this Consistent with the known functions of most flavivirus proteins, the three N-terminal HCV proteins are probably structural (C, E1, and E2/NS2) and the four C-terminal proteins (NS2, NS3, NS4, and NS5) are believed to function in viral replication.[2]

After the complete HCV genome was determined by Choo et al in 1991, several HCV isolates from different parts of the world were obtained and sequenced. Comparison of the published

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sequences of HCV has led to the identification of several distinct types that may differ from each other by as much as 33% over the whole viral genome. Sequence variability is distributed equally throughout the viral genome, apart from the highly conserved 5' UTR and core regions and the hyper variable envelope (E) region.[2]

The most widely accepted classification system of HCV genotyping is that of Simmonds et al in 1993. This system is based on the sequence variability of the 222 base pairs of the NS5B region and it divides HCV isolates into six phylogenetically distinct groups, and more than 80 subtypes. However, following the description of the new genotypes 7-11, Robertson et al in 1998, measured that it was more accurate for the genotypes 7, 8, 9 and 11 to be assigned as subtypes of genotype 6 and the genotype 10 to be a subtype of the genotype 3.[3]

Globally, genotype 1 is estimated to account for more HCV cases than any other genotype at 83.4 million (46.2%), with over one-third of genotype 1 cases located in East Asia. HCV genotype 3 is the next most common and is estimated to account for 54.3 million (30.1%) cases globally, approximately three-quarters of which occur in south Asia. Genotypes 2, 4, and 6 are responsible for the majority of the remaining cases of HCV worldwide, with an estimated 16.5 million (9.1%), 15.0 million (8.3%), and 9.8 million (5.4%) cases, respectively.

East Asia accounts for the greatest numbers of genotype 2 and genotype 6 HCV cases, while North Africa and the Middle East have the largest number of genotype 4 cases. Genotype 5 responsible for the fewest HCV cases globally (1.4 million, <1% of all HCV cases), the great majority of which occur in Southern and Eastern sub-Saharan Africa.[4]

Geographic distribution, disease outcome and response to antiviral therapy differ among the genotypes so the genotype determination is important for proper selection of antiviral therapy and for optimal patient management.[5]

The predominant genotype in Baghdad according to a study conducted on 50 patients in 2015 was genotype 4 (54%) followed by genotype 1 (46%),[6] another unpublished study conducted in 2012 has found that genotype 1 (48%) is slightly more common than genotype 4 (41.38%).[7] Update about the epidemiology of the HCV is always required taking in consideration that the sample size included in the above studies were relatively small, and the expected change in the epidemiology of the disease in Iraq due to increase intravenous drug use, immigration patterns, and the spread of unsafe sexual practices in sexually active age groups.[8] So this study was designed to identify the predominant HCV genotype in a sample of patients diagnosed with chronic hepatitis C infection in two biggest centres in Baghdad; Gastroenterology and Hepatology Teaching Hospital and Baghdad Teaching Hospital in Medial City Complex and to figure out the effect of gender, age and viral load on the distribution of genotypes.

METHODS

Setting and Study Design: This is a descriptive study in which 529 patients with chronic HCV infection were enrolled. The study was conducted at the Gastroenterology and Hepatology Teaching Hospital and the Centre of Gastroenterology and Hepatology at Baghdad Teaching Hospital/ Medial City Complex in Baghdad between December 2014 to September 2016.

Ethical Consideration: The study protocol was approved by the Research Committee of Medical City Directorate. All participants were asked to provide their written consent after being prepared to understand the aims of the study.

Definition of the cases, inclusion and exclusion criteria: Patients who were visiting the outpatient clinics of the above mentioned hospitals diagnosed as chronic HCV infection were enrolled in this study. The operational definition of chronic HCV infection which is used in this study was persistence of detection of hepatitis C RNA in the blood over 6 months. We included only naïve patients who were above age of 18 years. Patients who were co-infected with HBV, or using immune modulating drugs like systemic steroid, interferon, interleukins or cytokines were excluded from this study.

Sampling: Any patient who fulfil the enrolment criteria of this study and visiting the consultancy GIT outpatient clinic at the outpatient clinic at the Gastroenterology and Hepatology Teaching Hospital on Mondays, Wednesdays, and Thursdays or attending Baghdad Teaching Hospital on Sundays and Tuesdays during the studied period was enrolled in this study.

The procedure: Sample collection and processing: Blood sample from HCV patients were collected in sterile test tube. Five milliliters of blood samples, received in the serology section of GIT centres at medical city from patient, were analysed. The sera were separated and screened for HCV antibodies by using HCV ELISA test kit utilizes antigens from the core, NS3, NS4, and NS5 regions of the virus. Antigens have been carefully developed and selected to provide a sensitive and specific diagnostic test and positive serum was stored in frozen (-20°c) unit being tested for viral load. HCV viral load has been done in medical city and Dubai private laboratory in Baghdad while genotyping was done in Dubai private laboratories only.

HCV detection by polymerase chain reaction (PCR) is based on amplification of specific regions of the pathogen genome. In real-time PCR the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes that bind specifically to the amplified product. Monitoring of fluorescence intensities during the PCR run (i.e. in real time) allows the detection and quantification of accumulating product without having to re-open the reaction tubes after the PCR run. HCV RNA viral load was determined following the manufacturer's recommendations by a sensitive PCR based assay (COBAS amplicor; ROCHE diagnostic Kit: COBAS®AmpliPrep/ COBAS TaqMan® HCV Quantitative test, v2.0) manufactured by Roche /German.

HCV genotyping test done based on reverse-hybridization principle; biotinylated amplicons, generated by RT-PCR of the 5' UTR and Core regions of HCV RNA, are hybridized to specific probes that are bound to nitrocellulose strip by a poly-T tail; biotinylated hybrids are then detected using streptavidin bound to alkaline phosphatase; ampilicons that are not complemented are washed out. Then the substrate reacts with the streptavidin-alkaline phosphatase complex forming purple precipitate and coloring banding pattern on the strip (The instrument used was Rotor-Gen Q manufactured by Qiagen Hiden-Germany and the kit used (GEN-C 2.0 manufactured by nuclear laser medicine S.r.i/Italy).[9, 10]

The detection of genotyping variation of HCV was carried-out based on six deferent oligonucleotide probes corresponding to the six HCV genotypes as well as their different subtypes.

Table 1: Age and sex distribution of HCV infected patients

Characteristic		No.	%
Age in year	18-20	32	6.0
	20-39	237	44.8
	40-60	221	41.8
	>60	39	7.4
	Total	529	100.0
Gender	Male	223	42.2
	Female	306	57.8
	Total	529	100

Statistic: obtained data were analysed by using the SPSS software for window version 19. The comparison of data in respect of age groups and gender were performed by chi-square test. $P < 0.05$ was consider to be statistically significant.

RESULTS

Of the 529 patient enrolled in this study; 44.8% (237 patients) of them aged between 20-39 years; 41.8% (221 patients) of patients aged between 40-60 years; From those 529 patients,306 (57.8%) were females and 223(42.2%) were males, as shown in table 1.

Table 2: The genotype distribution among HCV infected patients

Genotype	No.	%
Total genotype 1 subtypes	263	49.7
1 (non-specified)	29	5.5
1a	127	24.0
1b	106	20.0
1a/b	1	0.2
2	2	0.4
3	7	1.3
Total genotype 4 subtypes	254	48.0
4 (non-specified)	134	25.3
4a	15	2.83
4b	13	2.45
4c	13	2.45
4c/d	1	0.18
4d	43	8.1
4e	34	6.4
4h	1	0.18
6	3	0.6
Total genotype number	529	100%

Table 3: Age range for each genotypes and subtypes in HCV infected patients

Genotype	Age in year No. (%)				Total No. (%)
	<20	20-39	40-59	>60	
1	1(3.4%)	21(72.4%)	5(17.2%)	2(6.9%)	29(100%)
2	0(0%)	1(50%)	1(50%)	0(0%)	2(100%)
3	0(0%)	3(42.9%)	3(42.9%)	1(14.3%)	7(100%)
4	11(4.3%)	108(42.5%)	110(43.3%)	25(9.8%)	254(100%)
6	0(0%)	2(66.7%)	1(33.3%)	0(0%)	3(100%)
1a	14(11%)	63(49.6%)	35(27.6%)	15(11.8%)	127(100%)
1b	5(4.7%)	39(36.8%)	50(47.2%)	12(11.3%)	106(100%)
1a/b	1(100%)	0(0%)	0(0%)	0(0%)	1(100%)
Total	32(6%)	237(44.8%)	205(38.8%)	55(10.4%)	529(100%)
Chi-square test	P-Value=0.002 (which is significant at alpha <0.05)				

Table 4: Binary logistic regression analysis of association of age with HCV genotype

Variable	p-value	Odd ratio	CI 95%	Note
G4 verus G1a	0.005	1.022	(1.007-1037)	Association with age > 40 years
G1b verus G1a	0.009	1.024	(1.006-1.043)	Association with age > 40 years
G1a verus G1b	0.006	2.020	(1.223-3.337)	Association with age < 40 years

In this study there is 254 (48%) of patients were infected with genotype 4 and 127(24%) of patients infected with genotype 1a and 106 (20%) of patients infected with genotype 1b; the total number of patient infected with all subtype of genotype 1 was 263(49.7%) as shown in table 2.

Mean age of patient in this study was 39.56 year ± 13.653 SD; there is 218(85%)of patients infected with genotype 4 affected age 20-59 years;63(49.6%) of patients infected with genotype 1a affected age 20-39 years;50(47.2%) of patients infected with genotype 1b affected age 40-59 years as and this difference was statically significant(p-value=0.002), table 3.

The study confirms that older age patients more than 40 years associated with one-fold increase in the incidence of infection with genotype 4 and genotype 1b, while the patients age younger than 40 years associated with two folds increase in the incidence of infection with genotype 1a compared to genotype 1b and these relations was statically significant, see table 4.

The major genotype in male patients was genotype 4 in 101(45.3%) of patients then genotype 1a in 58(26%) then genotype 1b 48(21.5%); the same sequence in female patients: Genotype 4 in 153(50%) then genotype 1a in 69(22.5%) patients, the genotype 1b in 58(19.0%) and this difference was statically non-significant (p-val-

Table 5: Distribution of the HCV genotype according to viral load

Genotype	Viral load<800000 iu/ml No (% within genotype)	Viral load≥800000 iu/ml No (% within genotype)	Total No (% within genotype)
1	20(69%)	9(31%)	29(100%)
1a	84(66.1%)	43(33.9%)	127(100%)
1b	73(68.9%)	33(31.1%)	106(100%)
1a/b	1(100%)	0(0)	1(100%)
Total genotype 1 subtypes	178(67.7%)	85(32.3%)	263(100%)
2	2(100%)	0(0%)	2(100%)
3	3(42.9%)	4(57.1%)	7(100%)
4	146(57.5%)	108(42.5%)	254(100%)
6	2(66.7%)	1(33.3%)	3(100%)
Total	331(62.6%)	198(37.4%)	529(100%)

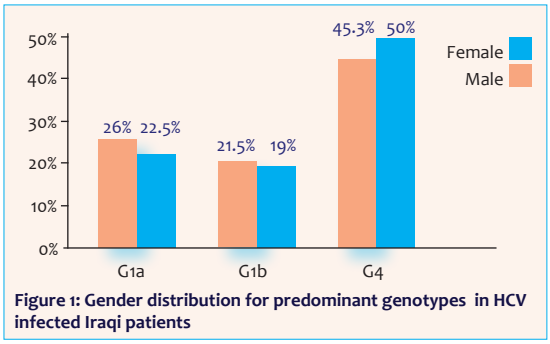

Figure 1: Gender distribution for predominant genotypes in HCV infected Iraqi patients

ue =0.373).

In this study there were 146 (57.5%) of patients infected with genotype 4 and 84 (66.1%) of patients infected with genotype 1a & 73 (68.9%) of patients infected with genotype 1b had low viral load (<800000 iu/ml) as shown in **table 5**.

DISCUSSION

Identification of HCV genotype is very important to determine the type, duration and response of the treatment. According to the obtained results from our study, genotype 4 is the predominant genotype found in 254 (48%) followed by genotype 1a in 127 (24%) then genotype 1b in 106 (20%). This result is supported by the results of many studies conducted in Iraq in the past few years. Al-Kubaisy et al[11] found that type 4 is the predominant genotype of HCV infecting children with thalassemia. In another study conducted on patients with haemodialysis in 2015, Al-Kubaisy et al[12] has shown that Genotype 4 was the most prevalent (33.3%), followed by genotype 1a (25.9%) and genotype 1b (22.2%). Similarly, Abdulhassan et al[6] in 2018 reported that genotype 4 is the predominant genotype (54%) followed by genotype 1(46%).

Other studies conducted in Iraqi governorates other than Baghdad have come to the similar conclusion; In Babylon middle part of Iraq, Tawfeeq et al[13] in 2014 found that 46.7% of chronic HCV is genotype 4, 22.2% had HCV genotype 1, and 6.6% had subtypes 1a, and 15.6% had subtype 1b. In Duhok north of Iraq, Othman et al[14] in 2014 found that the most frequent genotype detected was genotype 4 (52.9%) followed by 3a (17.1%), 1b (12.9%) and 1a (1.4%), while mixed genotypes (4 with either 3a or 1b) were detected in 7.1%. In Ninava north of Iraq, the predominant genotype was genotype 4.[15] In Basrah south of Iraq, Najim et al in 2018 showed that the most frequent genotype was 4 in 43 (56.5%), followed by 1 in 31 (40.7%) patients;1a: 21.1% and 1b: 19.6%.[16]

Similar study was performed in many adjacent countries and the accumulated data showed that there are two main patterns for the distribution of HCV genotypes in Middle east: one to the Arab countries were genotype 4 predominates, while the other pattern is characteristic of the non-Arab countries (Turkey and Iran) where genotype 1 predominates.[17]

Al-Sweedan et al in 2008 showed that the HCV genotype 4 is the commonest genotype in multi-transfused patients in Jordan.[18] However, another study from Jordan[19] has contradicted this rule and found that genotype 1a is the dominant subtype (40%) followed by 1b(33.3) and genotype 4(26.6%).

In asystematic review and meta-analysis Khodabandehloo et al found that genotype 1 is the most prevalent genotype in Iran with a rate of 54%, and followed by genotype 3 in 36%. Subtype 1a was the predominant subtype with a rate of 42%; followed by subtype 3a, 35%; and subtype 1b, 11%.[20]

Janahi et al in Bahrin found that 36.71 % of chronic HCV is genotype 1, and 15 % for each genotype 4 and 3.[21] In Saudi Arabia, Al-Zayed stated that genotype 4 is the commonest genotype found in 48.3 %, followed by genotype 1 in 34.1 %, mixed genotype in 10 %, genotype 3 in 5 %, and genotype 2 in 2.5 %.[22]

In a study conducted in Turkey in 2008, genotype 1b is the commonest found in 67.7 % of patient with HCV, followed by the non-specified genotype 1 in 7.7 %, genotype 4 in 7.3 %, and genotype 3 in 6.7 %.[23]

Abbas et al in a study conducted Lebanon published in 2006 found that Genotype 1 is the commonest found in 42.8%, Genotype 3 in 26.1%, Genotype 4 in 24.6%, Genotype 2 in 3.4%, and Genotype 5 in 2.96%. And these results represent a shift from previously documented genotype 4 to genotype 1.[24]

Antaki et al in Syria found that Genotype 4 was the most frequent genotype in 59%, followed by genotype 1 in 28·5%, and G5 in 10%. In is worth mentioning that The majority of G5 patients, 87%, were living in the north of Syria;

about a third from Azaz, a small city close to Turkey.[25]

Ryu et al from Egypt found that HCV genotype 4 was detected in 90.3%. The other 9.7% of the patient with HCV were having genotype 1g and 1a.[26] The widespread use of parenteral antihelminthics to combat schistosomiasis is believed to be predominately responsible for the scale of the epidemic.[27]

We found a significant relation between age and genotype and age group; 43% of patients infected with genotype 4 were at age group 40-59 years, 49.6% of patients infected with genotype 1a were at age group 20-39 years, and 47.2% of patients infected with genotype 1b were at age group 40-59 years with a p-value <0. 003.

In conclusion, it could be assumed that 1b is the original 'resident' genotype, spread in the 1970s, especially through iatrogenic transmission. The introduction of anti-HCV screening tests in the 1990s may have dramatically reduced this risk. In the meantime, the spread of intravenous drug use and active immigration could have led to a new wave of HCV infection among young people, with an increase of new 'emergent' genotypes (3a, 1a and 4).[8]

Altuglu et al (Turkey 2008) demonstrated that patient infected with genotype 1 were significantly older than patient infected with non 1 genotype with no significant difference in gender distribution.[28] The genotype 1b in this study was predominant and represented 87% of all genotype and this could explain its distribution in older patient. Similar to our results a study from Libya has shown that patient infected with genotype 1a are younger than genotype 1b and genotype 4.[29] Petruzziello et al (Italy 2012) showed a direct correlation between genotype and age, with a high frequency of genotypes 1b and 2a/2c in older patients and genotypes 1a and 3a in younger patients.[30]

In this study there is no significant difference in distribution of genotypes between male and female. Previous studies from different countries have analysed genotype distribution in relation to the gender. In Libya, The prevalence of HCV genotype 1 was found to be significantly associated with males, while genotype 4 has frequently been found in females.[29] In contrary Rouabhia et al in Algeria (2013) showed no effect of gender on type of HCV genotypes.[31]

In our study, the most predominant subtypes in genotype 4 was genotype 4d and 4e, while in Egypt, the predominant genotype 4 subtype was subtype 4a.[32] In Saudi Arabia the predominance subtypes were 4c/4d, followed by subtypes 4h, 4e, and 4a, suggesting that the origin and transmission of HCV-4 is different from that in Egypt while in western Europe, have a higher incidence of 4a and 4d.[32-34]

In this study, viral load does not correlate with HCV genotypes, a finding which is similar to that of Rouabhiain Algeria in 2013,[31] and Ucibilek et al in Turkey, [35] But contradicts that from China which found a significant correlation between genotype and viral load; HCV genotype 1 and 6 had significantly higher viral loads than genotype 2 and 3.[36]

CONCLUSIONS

The predominant genotype is genotype 4 followed by genotype 1a then genotype 1b, there is statistically significant difference in distribution of HCV genotypes according to age groups were genotype 1a more predominant in younger group than genotype 1b.

REFERENCES

1. Mohd Hanafiah K, Groeger J, Flaxman A, Wiersma S. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. ***Hepatology***. 2013;57(4):1333-134.
2. Zein NN. Clinical Significance of Hepatitis C Virus Genotypes. ***Clinical Microbiology Reviews***. 2000;13(2):223-235.
3. Hussain Z. Genomic heterogeneity of hepatitis viruses (AE): role in clinical implications and treatment. InPractical management of chronic viral hepatitis 2013 May 29. IntechOpen. DOI: 10.5772/55231.
4. Messina JP, Humphreys I, laxman A,Brown A,Cooke GS, Pybus OG , Barnes E, Global distribution and prevalence of hepatitis C virus genotypes. ***Hepatology*** 2015;61:77–87. doi:10.1002/hep.27259
5. Hnatyszyn HJ. Chronic hepatitis C and genotyping: the clinical significance of determining HCV genotypes. ***Antiviral therapy***. 2005;10(1):1-1.
6. Abdulhassan L, Abdulamir A, Al-Khalidi N, AlWaysi S. Frequency of Hepatitis C Virus Genotypes/ Subtypes Association with Response to Therapy in a Sample of HCV Infected Iraqi Patients. ***Iraqi JMS***. 2018;16(1):30-40
7. Abdullah AM. Hepatitis C virus prevalence Genotyping and some Cytokines profile in hemodialysis patients: a survey by polymerase chain Reaction and Serological Methods. PhD thesis. College of Medicine. Al-Nahrin University, 2012.

8. Petruzziello A, Coppola N, Diodato AM, Iervolino V, Azzaro R, Di Costanzo G, Di Macchia CA, Di Meo T, Loquercio G, Pasquale G, Cacciapuoti C. age and Gender Distribution of Hepatitis C Virus Genotypes in the Metropolitan Area of Naples. ***Intervirology***. 2013;56(3):206-12.
9. QIAamp® DNA isolation Mini kit 2016, Germany www.qiagen.com
10. Artus® HCV RG PCR Kit 2014, QIAGEN GmbH, QIAGEN Strasse 1, 40724 Hilden, GERMANY, www.qiagen.com.
11. Al-Kubaisy WA, Al-Naib KT, Habib MA. Seroprevalence of hepatitis C virus specific antibodies among Iraqi children with thalassaemia. ***East Mediterr Health J*** 2006;12(1-2):204-10.
12. Al-Kubaisy W. Prevalence and risk factors of hepatitis C virus infection in haemodialysis patients: testing, antibodies, RNA and genotypes. ***Journal Teknologi*** 2015;77(25) :115–119.
13. Al-Yousif AM, Al-Aouadi RF, Tawfeeq WF. Detection of Hepatitis C Virus Infection and Genotypes among Seropositive Blood Donors by Polymerase Chain Reaction in Babylon Governorate Iraq. ***Medical Journal of Babylon***. 2013;10(1):25-38. doi:1812-156X-10-1
14. Othman AA, Eissa AA, Markous RD, Ahmed BD, Al-Allawi NA. Hepatitis C virus genotypes among multiply transfused hemoglobinopathy patients from Northern Iraq. ***Asian J Transfus Sci***. 2014;8(1):32-4.
15. Khalid M D & Abdullah BA. Hepatitis C virus genotypes in Iraq. ***Iraqi J Biotech*** .2012;11 (2):475-480.
16. Najim OA, Hassan MK. Prevalence of hepatitis C virus seropositivity among multitransfused patients with hereditary anemias in Basra, Iraq. ***Iraqi Journal of Hematology***. 2018 Jan 1;7(1):39.
17. Sadeghi F, Salehi-Vaziri M, Almasi-Hashiani A, Gholami-Fesharaki M, Pakzad R, Alavian SM. Prevalence of Hepatitis C Virus Genotypes Among Patients in Countries of the Eastern Mediterranean Regional Office of WHO (EMRO): A Systematic Review and Meta-Analysis. ***Hepatitis Monthly***. 2016;16(4): e35558. doi:10.5812/hepatmon.35558.
18. Al-Sweedan S, Jaradat S, Amer K , Hayajneh W, Haddad H seroprevalence and genotyping of hepatitis C virus (HCV) in multiple transfused Jordanian patients with β-thalassemia major. ***Turk J Hematol*** 2011; 28: 47-51.
19. Bdour S. Hepatitis C virus infection in Jor¬danian haemodialysis units: Serological diagnosis and genotyping. ***J Med Microbiol*** 2002;51(8):700-4.
20. Khodabandehloo M, Roshani D. Prevalence of Hepatitis C Virus Genotypes in Iranian Patients: A Systematic Review and Meta-Analysis. ***Hepatitis Monthly*** 2014;14(12):e22915. doi:10.5812/hepatmon.22915.
21. Janahi EM, Al-Mannai M, Singh H, Jahromi MM. Distribution of Hepatitis C Virus Genotypes in Bahrain. ***Hepatitis Monthly*** 2015;15(12):30300.
22. Al Zayed RM, Hamdy NM, Al-Ajlan HH, Aref NM. Prevalence of HCV genotypes and viral load in Saudi Arabia. ***Int J Intern Med***. 2015;4(2):26-41. doi:10.5923/j.ijim.20150402.02
23. Altindis M, Dal T, Akyar I, Karatuna O, Gokahmetoglu S, Ulger ST, Kulah C, Uzun B, Şener AG, Ozdemir M, Aydogan S. Six-year distribution pattern of hepatitis C virus in Turkey: a multicentre study. ***Biotechnology & Biotechnological Equipment***. 2016 Mar 3;30(2):335-40.
24. Abbas F, Haddad J, Mahfouz R, Genotype distribution of hepatitis C virus among Lebanese patients: Results from a major tertiary care center. ***Gene Reports*** 2016; 4: 190–193.
25. Antaki N, Haddad M, Kebbewar K.The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province. ***Epidemiology and Infection*** 2008;137(1):79–84. doi: 10.1017/S095026880800054X.
26. Ryu SH, Fan X, Xu Y, Elbaz T, Zekri AR, Abdelaziz AO. Lack of association between genotypes and subtypes of HCV and occurrence of hepatocellular carcinoma in Egypt. ***J Med Virol***. 2009;81(5):844–7.
27. Hathorn E , Elsharkawy AM .Management of hepatitis C genotype 4 in the directly acting antivirals era. ***BMJ Open Gastroenterology*** 2016;3:e000112. doi: 10.1136/bmjgast-2016-000112.
28. Altuglu I, Soyler I, Ozacar T, Erensoy S. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in Western Turkey. ***International Journal of Infectious Diseases***. 2008 May 1;12(3):239-44.
29. Elasifer HA, Agnnyia YM, Al-Alagi BA, Daw MA. Epidemiological manifestations of hepatitis C virus genotypes and its association with potential risk factors among Libyan patients. ***Virol J***. 2010; 7:317.
30. Petruzziello A, Nicola Coppola N, Diodato AM, Iervolino V, Azzaro R, Costanzo GD, Macchia C, Meo T, Loquercio G, Pasquale G, Carmela Cacciapuoti C. Age and gender distribution of hepatitis C virus genotypes in the metropolitan area of Naples. ***Intervirology***.2013;56(3):206–212.
31. Rouabhia S, Sadelaoud M, Chaabna-Mokrane K, Toumi W, Abenavoli L. Hepatitis C virus genotypes in north eastern Algeria: a retrospective study. ***World J Hepatol***. 2013;5:393–397. doi: 10.4254/wjh. v5. i7.393
32. Kamal SM. Improving outcome in patients with hepatitis C virus genotype 4. ***Am J Gastroenterol*** .2007;102:2582–2588.
33. Kamal SM, Nasser IA. Hepatitis C genotype 4: what we know and what we don't yet know. ***Hepatology*** .2008;47:1371–1383. 10.1002/hep.22127.
34. Di Lello FA, Neukam K, Parra-Sanchez M, Hepatitis C virus genotype 4 in southern and central Spain does not originate from recent foreign migration waves. ***J Med Viro*** 2015;85(10):1734–1740. 10.1002/jmv.23657
35. Ucbilek E, Abayli B, Koyuncu MB, Midikli D, Gozukucuk S, Akdag A, et al. Distribution of hepatitis C virus genotypes among intravenous drug users in the Cukurova region of Turkey. ***Turk J Med Sci***. 2016;46(1):66-71.
36. Rong X, Lu L, Wang J, Xiong H, Huang J. Correlation of Viral Loads with HCV Genotypes: Higher Levels of Virus Were Revealed among Blood Donors Infected with 6a Strains. ***PLoS ON*** 2012; E 7(12): e52467. doi: 10.1371/journal.pone.0052467

Abbreviation list: Enzyme-linked immunosorbent assay (**ELISA**), Hepatitis B virus (**HBV**), Hepatitis c virus (**HCV**), International unit (**iu**), Milliliter (**ml**), Nanometer (**nm**), Polymerase chain reaction (**PCR**), Ribonucleic acid (**RNA**), Standard Deviation (**SD**), Statistical package for the social sciences (**SPSS**), Untranslated region (**UTR**).

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