EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 10
392-398
Year: 2018

DISTRIBUTION, PREVALENCE AND PHYLOGENETIC SIGNIFICANCE OF HCV GENOTYPES IN INDIAN CASES: A RECENT CRITICAL ANALYSIS

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Article Received on 20/08/2018 **Article Revised on 10/09/2018** **Article Accepted on 30/09/2018**

ABSTRACT
Analysis of 1099 referral cases to Hepatitis C Virus (HCV) during 2014-2017 revealed 817 valid genotypes/subtypes with a detection rate of 74.3%. HCV genotypes 1,3 including subtypes 1a,3a subtypes were maximum (31.1%) in 2016 followed by 25% in 2017,23.4% in 2015 and 20.6% in 2014. These infected cases were correlated epidemiologically with prevalence, geographic distribution and other factors like age, gender and viral load in Indian subcontinent. The genotypes of HCV detected were same (1 and 3) and were dominant in old age groups (41-50, 51-60 years) and 3a and 1a subtypes also showed similar pattern as compared to other HCV types. Further, males were significantly (P<0.001) affected by these (1, 3, and 3a, 1a) HCV genotypes. Moreover their prevalence was dominant in Delhi and Gujarat. These genotypes correlated with higher viral load volumes i.e. higher (>500000 IU/ml) and intermediate (100000-500000 IU/ml) levels.Thus, our epidemiological data concluded that India is affected currently by HCV genotypes of 1, 3 and their 3a, 1a subtypes and are well interactive with age, gender and high viral load countsindicating phylogenetic significance. These variations of HCV from genotype to subtypes are well explained by their genetic heterogeneity and other environmental factors. Accordingly typeof hepatic disease and its severity in population differ. The drugs or vaccines related to these changing genotypes and subtypes levels now are refined and suggested to control liver diseases.

KEYWORDS: HCV genotypes/subtypes; Epidemiology; Viral load; Sequencing; Phylogeny; Indian cases.

INTRODUCTION
An established 170-200 X 10^6 individuals are infected globally with Hepatitis C virus (HCV). In India its burden of carrier is about 12-13 X 10^6.[25,26,6] Hepatitis C virus is a major cause of chronic liver disease, frequently progresses to cirrhosis with increased risk of hepatocellular carcinoma. HCV enveloped by positive stranded RNA virus belongs to genus *Hepacivirus* in the family *Flaviviridae*. The RNA genome consists of approximately 9500 ribonucleotides with a single open reading frame (ORF) coding a polypeptide precursor of 3000 amino acids and flanked by the non-coding regions (NCR) at both the 5' and 3' terminal. The above polypeptide precursor is fragmented by host signal peptidases to yield other core, envelope and non-structural proteins. The genotype based classification of HCV was proposed by Simmonds etal.[21] due to the genetic heterogeneity observed. Currently, 6 major HCV genotypes are identified but many are predicted[23] with almost 80 closely related subtypes. This genetic heterogeneity of HCV has been known to affect treatment outcomes. However differential outcomes based on genotypes have been established only in certain HCV genotypes.[4] About 350,000 people die annually then number.[17] The disease caused by the HCV depends on type, subtype of the virus, geographic dissimilarities, drugs, age, gender, environmental and other risk factors.[2,20,19,24] No much data reports are available about viral loads and HCV genotypes.[18,9] All these factors are necessary to cause the severity of the disease and its management in Indian population, though few genotypes are worldwide epidemic.[18] Hence, this systematic study was undertaken with respect to age, sex, viral load, duration, genotype, subtypes and geography dissimilarities in Indian subcontinent in relation to this disease and HCV changing pattern. This study, thus may be important for proper management, anti-retroviral therapy at subtype level and control of disease.[4]

MATERIALS AND METHODS
Patients
This prospective study included 1099 total patients during the year 2014-2017 at our Supratech Micropath Laboratory and Research Institute, Ahmedabad (India).

Out of which, there were 817 positive cases and 282 were unidentified cases due to no viral copies or the copies were lower than the detection limit of the assay. The ethical committee of Gujarat University (HEC001GU), Ahmedabad and Internal Review Board of this Institution approved the protocol. Informed consent was obtained from individual patients.

RNA extraction

Five ml blood was collected from each patient. The HCV RNA was extracted from patient's plasma or serum using Chemagic Prepito based automated as per manufacturer's instructions (PerkinElmer, United States). The eluted RNA was stored at -80°C until use.

Quantitative detection of HCV RNA

The extracted RNA was initially used for viral load quantification. Quantification of HCV viral load was carried out in the ABI 7500 Fast Real Time PCR utilizing a Taqman probe based chemistry. The HCV probes obtained for this assay were procured from ThermoFisher Scientific, United States (P171208-D05 H10). The HCV Taqman probe is a short linear oligonucleotide which is labeled with a FAM fluorophore at the 5′ end and a MGB quencher at the 3′ end. The primer probe targets the conserved regions of the 5′ UTR of the HCV genome. In absence of HCV target, the fluorescence is quenched and in presence of the HCV target sequence, the HCV Probe hybridizes target sequence, allowing fluorescence detection.

HCV Genotyping

Identification of HCV genotype was carried out by Sanger Sequencing. One step reverse transcriptase PCR was performed with the extracted RNA in a reaction mixture containing Express Universal Master Mix, Express One Step Superscript for conversion of RNA to c-DNA and 20 pmol custom designed primers (Eurofins, Bangalore), in a total reaction volume of 20 µl. The target region for this reaction was the 5' untranslated region (5'UTR) with a thermal cycling protocol of 50°C for 45 min (Reverse transcription), 95°C for 2 min (Initial denaturation), followed by a further 45 cycles at 95°C for 15 sec(denaturation) and 60°C for 60 sec (annealing with extension) followed by a 4 degree Celsius hold. The amplified PCR product was electrophoresed in ethidium bromide stained 2.5 % agarose gel (Alliance, India) and visualized in a Gel-Doc System (Syngene, India) for identifying the desired 350 bp fragment with a 100bp DNA ladder. Internal positive and negative controls were also included.

Sanger Sequencing

The PCR products purified with exosap-IT are used for sequencing with the dideoxynucleotide chain termination method with the ABI BigDye® Terminator v3.1 Sequencing Reaction Kit (Applied Biosystems, Foster City, CA, USA). Sequence analysis is performed with the ABI 3500 Genetic Analyzer.

HCV genotype analysis

HCV type/subtype analysis was performed after Sanger Sequencing with the primary analysis being performed with Codon Code Aligner (CodonCode Corporation, Massachusetts.USA). HCV genotype classification was performed utilizing the HCV Sequence Database at http://www.webcitation.org/getfile?fileid=ad4613c457300aae8ac3cebb8d3bb95631173c46

Statistical analysis

MedCalc is the flagship product of MedCalc Software, a developer of medical and statistical software. It was used for statistical analysis. A value of $p<0.05$ was considered significant.

RESULTS

A total number of 1099 referral cases were analyzed for HCV infection, where 817 were detected for 1, 2, 3, 4, 5, 6 genotypic and their subtype variants of virus (817/1099;74.3%). Significantly higher frequency of genotype 1 was identified (9.8%) followed by HCV-3 (7.8%). Genotype 5 was zero. But viral subtype 3a was maximum (52.5%) followed HCV-1a (15.3%); 1b (3.7%) and 3b (3.2%) and other types respectively and unidentified cases were 282 (282/1099; 25.7%) (Fig.1).

Genotype Vs Age

We analyzed viral genotypes and subtypes in six age groups. Genotypes 1, 3 of HCV were higher (9.81%, 7.8%) followed by maximum 3a, 1a subtypes amongst all age groups (52.5%, 15.3%) respectively. Higher age groups i.e. 41-50, 51-60 and >60 years had higher frequency of genotypes and subtypes (1, 3 and 3a, 1a) comparatively (Table.1).

Genotype/Subtype Vs Year-wise distribution

We analyzed 1099 samples since four consecutive years i.e. 2014-2017, where viral genotypes were higher in 2016 (31%) followed by 2017 (25%), 2015 (23.4%) and 2014 (20.6%). In all these years variants 1 and 3 types were maximum (9.8%; 7.8%) followed by others. Zero variant was genotype 5 (0%). Similarly, HCV subtypes were 3a (52.5%) and 1a (15.3%) followed by HCV-1b and 3b (3.7% and 3.1%) and other types respectively (Table. 2).

Genotype Vs Gender

Males were more infected ($p<0.001$) significantly than the opposite sex (57%, 43%). Further, the viral genotypes those had were 1 and 3 types (60%; 62.5%) and the subtypes 3a and 1a (51.3%, 71.2%) respectively in males comparatively (Table.3).

Genotype vs Geographic region

Geographically, Delhi is significantly ($p<0.001$) affected with high percent of HCV genotype (71.5%) followed by Gujarat (23.6%) in our study. High genotype was HCV - 1 (9.8%) followed by HCV-3 (7.7%) comparatively (Table.4).

Genotype Vs Viral load

Viral load detected cases were 430 (52.6%). High viral load (>500000 IU/ml) had maximum percent of genotypes (59%), followed by intermediate level (100000-500000 IU/ml) with 22.5%. Less percent of genotypes was at low viral load range (10000-100000 IU/ml) with only 18.4%. The maximum viral types were 1 and 3 (10%, 7.4%) and subtypes HCV- 3a and 1a (54.1% and 14.6%) followed by other variants respectively in our cohort. (Table.5).

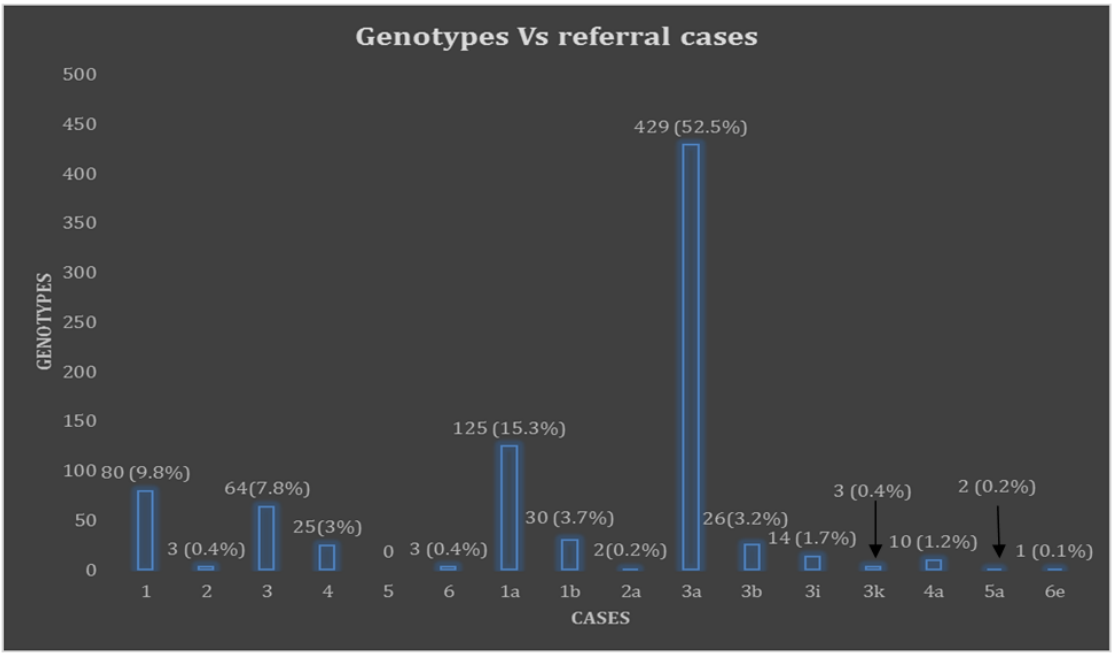

Fig.1: Genotype and referral cases in our study.

1, 3 genotypes and 3a, 1a subtypes were maximum.

Total cases-1099; Infected/ Identified=74.3% (817/1099)

Unidentified=25.7% (282/1099)

Table: 1 Genotypes and age groups (years) in our study cohort.

Genotypes	1-20	21-30	31-40	41-50	51-60	>60	Specific type (%)
1	2(2.5%)	12(15%)	13(16.2%)	25(31.2%)	19(23.7%)	9(11.2%)	80(9.8%)
2	0	2(66.7%)	1(33.3%)	0	0	0	3(0.4%)
3	4(6.2%)	5(7.8%)	10(15.6%)	12(18.7%)	21(32.8%)	12(18.7%)	64(7.8%)
4	0	4(16%)	3(15%)	8(32%)	4(16%)	6(24%)	25(3.0%)
5	0	0	0	0	0	0	0
6	0	0	1(33.3%)	0	2(66.7%)	0	3(0.4%)
1a	4(3.2%)	25(20%)	19(15.2%)	29(23.2%)	29(23.2%)	19(15.2%)	125(15.3%)
1b	0	5(16.7)	5(16.7%)	6(20%)	6(20%)	8(26.7%)	30(3.7%)
2a	0	0	2(100%)	0	0	0	2(0.2%)
3a	5(1.2%)	72(16.8%)	54(12.6%)	114(26.6%)	117(27.3%)	67(15.6%)	429(52.5%)
3b	0	4(15.4%)	3(11.5%)	8(30.8%)	4(15.4%)	7(27.0%)	26(3.2%)
3I	1(7.1%)	3(21.4%)	1(7.1%)	1(7.1%)	4(28.6%)	4(28.6%)	14(1.7%)
3K	0	1(33.3%)	1(33.3%)	0	0	1(33.3%)	3(0.4%)
4a	0	1(10%)	2(20%)	3(30%)	2(20%)	2(20%)	10(1.2%)
5a	0	0	0	1(50%)	0	1(50%)	2(0.2%)
6e	0	0	0	0	1(100%)	0	1(0.1%)
Total	28(2.6%)	191(17.4%)	159(14.5%)	273(24.9%)	272(24.8%)	175(16.0%)	817

Total cases: 1099; Identified=817/1099=74.3%

Unidentified: 282/1099= 26.7%

Genotype Pattern: 1>3>4>2&6>5

Subtypes: 3a>1a>1b>3b>3i>4a>3k>2a&5a>6e

Table 2: Genotypes during four consecutive years in our cohort.

Genotypes/Subtypes	2014	2015	2016	2017	TOTAL
1	23 (28.7)	18 (22.5%)	34 (42.5%)	5 (6.2%)	80 (9.8%)
2	3 (100%)	0	0	0	3 (0.4%)
3	18 (28.1%)	15 (23.4%)	15 (23.4%)	16 (25%)	64 (7.8%)
4	9 (36%)	2 (8%)	11 (44%)	3 (12%)	25 (3.0%)
5	0	0	0	0	0
6	0	0	3 (100%)	0	3 (0.4%)
1a	23 (18.4%)	20 (16%)	43 (34.4%)	39 (31.2%)	125 (15.3%)
1b	6 (20%)	8 (26.7%)	4 (13.3%)	12 (40%)	30 (3.7%)
2a	2 (100%)	0	0	0	2 (0.2%)
3a	63 (14.7%)	124 (28.9%)	129 (30%)	113 (26.3%)	429 (52.5%)
3b	7 (26.9%)	2 (7.7%)	6 (23%)	11 (42.3%)	26 (3.1%)
3i	5 (35.7%)	0	7 (50%)	2 (14.3%)	14 (1.7%)
3k	2 (66.7%)	0	0	1 (33.3%)	3 (0.4%)
4a	6 (60%)	2 (20%)	0	2 (20%)	10 (1.2%)
5a	0	0	2 (100%)	0	2 (0.2%)
6e	1 (100%)	0	0	0	1 (0.1%)
Total	168 (20.6%)	191 (23.4%)	254 (31.1%)	204 (25%)	817

HCV types and subtypes were same as in Table 1

Table 3: Genotypes and Gender.

Genotypes	Female	Male
1	32 (40%)	48 (60%)
2	3 (100%)	0
3	24 (37.5%)	40 (62.5%)
4	5 (20%)	20 (80%)
5	0	0
6	3 (100%)	0
1a	36 (28.8%)	89 (71.2%)
1b	18 (60%)	12 (40%)
2a	0	2 (100%)
3a	209 (48.7%)	220 (51.3%)
3b	10 (38.5%)	16 (61.5%)
3i	6 (42.8%)	8 (57.2%)
3k	1 (33.3%)	2 (66.7%)
4a	4 (40%)	6 (60%)
5a	0	2 (100%)
6e	0	1 (100%
TOTAL	351 (43%)	466 (57%)*

*P<0.001

Table 4: State wise distribution of HCV genotypes.

Genotyping	Gujarat	West Bengal	Karnataka	Tamilnadu	Rajasthan	Maharashtra	Delhi	Haryana	Madhya pradesh	Total
1	24	0	0	0	2	0	55	0	0	81 (9.9%)
2	0	0	0	0	0	0	3	0	0	3 (0.4%)
3	18	2	1	1	1	0	42	0	0	65 (7.9%)
4	4	0	0	0	0	0	20	0	0	24(3.0%)
5	0	0	0	0	0	0	0	0	0	0 (0.0%)
6	1	1	0	0	0	0	1	0	0	3 (0.4%)
1a	35	0	3	1	1	0	84	0	1	125 (15.3%)
1b	7	1	0	4	0	0	18	0	0	30 (3.7%)
2a	0	0	0	0	0	0	2	0	0	2 (0.24%)
3a	77	3	4	2	5	0	337	1	0	429 (52.5%)
3b	10	0	0	1	1	0	14	0	0	26 (3.2%)

3I	8	0	0	0	0	0	6	0	0	14 (1.7%)
3K	0	0	0	0	0	0	3	0	0	3 (0.4%)
4a	1	0	0	0	0	0	9	0	0	10 (1.2%)
5a	1	0	0	0	0	0	1	0	0	2 (0.2%)
TOTAL	185 (22.6%)	7 (0.9%)	8 (1.0%)	9 (1.1%)	9 (1.1%)	0 (0.0%)	587 (71.8%)	1 (0.1%)	1 (0.1%)	817

Delhi > Gujarat >Tamilnadu& Rajasthan > Karnataka > West Bengal > Haryana & Madhya Pradesh > Maharashtra
HCV types: same as in Table.1
Subtypes: 3a>1a>1b>3b>3i>4a>3k>2a & 5a

Table 5: Distribution of HCV genotypes depending on viral load.

Genotype	10000-100000*	100000-500000*	>500000*	TOTAL
1	8	10	26	44 (10.2%)
2	0	0	0	0 (0%)
3	7	10	15	32(7.4%)
4	2	1	10	13(3.0%)
5	0	0	0	0(0%)
6	1	0	1	2 (0.5%)
1a	13	16	34	63 (14.6%)
1b	4	4	9	17 (4.0%)
2a	0	0	1	1 (0.2%)
3a	40	48	145	233 (54.1%)
3b	2	6	7	15 (3.5%)
3i	2	1	3	6 (1.4%)
3k	0	0	1	1 (0.2%)
4a	0	1	0	1 (0.2%)
5a	0	0	2	2 (0.5%)
TOTAL	79 (18.4%)	97(22.5%)	254 (59.0%)	430 (100%)

Genotype Pattern: 1>3>4>6>2 & 5
Subtypes Pattern: 3a> 1a> 1b> 3b> 3i> 5a>3k, 4a & 2a
*Viral copies (IU/ml)

DISCUSSION

Hepatitis C Virus (HCV) causes chronic hepatitis in most of the cases (80%) leading cirrhosis or hepatocellular carcinoma. It also induces liver fibrosis.[1,22] This HCV has predominantly 1-6 genotypes, though several variants are identified. Each group contains several subtypes like a, b, c etc. These genotypes and subtypes vary depending upon the clinical manifestations, severity of disease and susceptibility to drug therapy.[8] Further, its types of distribution vary depending on region, gender, age, viral load and other factors.[1,14]

In our study cohort, we analyzed the referral cases of 1099 during four years i.e. 2014-2017. In these four years the infected cases were 74.3% consisting higher HCV- 1 followed by type HCV- 3. But 3,1 genotypes were reported by Christdas et al.[6] and Narahari et al.[14] in Eastern India, North- Eastern regions and South India of this subcontinent respectively. Our analysis (Fig.1) indicated that viral genotypes were 1 and 3 and subtypes detected were HCV-3a and 1a in India corroborating with global prevalence and distribution of HCV genotypes recorded by Blach et al.[5] These collaborators globally preferred modelling study on HCV infection in 2015. Further, it is known that Indian subcontinent including Pakistan, the variants of HCV types are well demonstrated.[1,17,18,26,4] But our report demonstrated distribution, prevalence and evolution pattern of genotypes and subtypes and their interaction with various factors in India during last four consecutive years i.e. 2014-2017.

The percentage of viral genotypes 1 and 3 (7.3%; 5.8%) are more followed by other types, whereas HCV subtypes 3a (39%) and 1a (11%) are maximum showing their predominance respectively with age. The infected age groups were 41-50, 51-60 and >60 years respectively. Therefore, older age groups are more affected. Most of the earlier workers have also reported the higher age groups were targets of HCV infection. Mohammed et al.[12] in Pakistan contrarily reported higher rates of infection in young age groups suggesting early appearance of disease. Riaz et al.[17] and Aziz et al.[3],further found 30-40 years old infected were more males, in contrast with the finding by Nafees et al.[13]who found more prevalence of disease in 45-50 years old cases in support of our observation.In our report, males were more affected by this viral infection where HCV-1,3 and their subtypes (3a,1a) were maximum. This could be due to active mobility of the males exposing to environment. In support of our data, Liu et al.[10]reported high risk of males in contrast to females who belong to higher age groups.

Currently, number of studies have been reported to detect the frequency of genotypes in different geographical regions of Pakistan and HCV-3a was found prevalent in Hazara region[1]. In our study cohort, Delhi was highly infected with genotypes 1 and 3 followed by Gujarat and other states of India. In Pakistan, South India, North and Southern India HCV-1 and 3 and their subtypes are well reported to support our data. This regional difference with respect to these genotypes was thus prevalent in Indian subcontinent.[14,1,22,6] The year-wise distribution of viral genotypes 1 and 3 and their subtype (3a,1a) observed were more during 2016 followed by other three years (Table:2). Similar trend was observed with respect to genotype infection as noted in our total cases analyzed. This could be related to genetic diversity and environmental factors affecting viral genome in the patients. This refined study also revealed that the infection was correlated with viral loads. Hence, we found high viral load (>500000 IU/ml) was related to maximum number of cases (59%). Further, HCV-1,3 genotypes and subtypes (3a,1a) were also correlated with high viral loads. Rong et al.[18] also reported higher viral load which is associated with viral genotype and subtypes. Higher RNA levels were too related with viral genotypes. [4] Multicentric study further may be in help to ascertain the correlation with load and genotypes. A certain basal level of RNA is important for viral therapies for a population genotypes of virus causing security of liver disease.

In conclusion, viraemia caused by HCV has multiple types and subtypes inducing multiple liver diseases. In our study cohort genotypes 1 and 3 and subtypes are 3a and 1a are able to cause infection in our population. These viral types are variable with age, gender, geographical regions and viral load. Males are significantly infected by HCV-1 and 3 and their subtypes (3a and 1a). This study also confirmed that Indian subcontinent is infected mostly by HCV-1 and 3 genotypes, followed by subtypes of virus (3a,1a) and are associated with these factors causing multiple liver diseases. These hepatic infections are well variable depending upon type of variant generated by evolutionary factors like mutation and other environmental factors observed in this study. Thus, the genotype/subtype differs in causation of severity, degree of intensity of infection and other clinical malformations. The severity of disease, its progression and response to therapy for HCV infection might vary according to genotype/ subtype in light of earlier observation.[7,11,15,16] Accordingly, drugs and vaccines are to be developed in future for treating such hepatic infection of HCV. Hence, our study documented that year-wise distribution, age, geographical region, sex and viral load including genetic variability are responsible for evolving new variants of HCV to cause varieties of liver infections. These variation in viral types in our refined report indicate changing trend of viral genotypes to subtypes due to multiple factors to support the global genotype records. In light of the developing and changing national hepatitis elimination strategies to expand prevention, screening and treatment, a more rapid decline in total new viraemic conditions is forecasted around the globe including India.

ACKNOWLEDGEMENT

We thank all Staff of Supratech Genopath Laboratories for their timely assistance in this work.

CONFLICT OF INTEREST

No conflict of interest is expressed by the authors.

REFERENCES

1. Ali A, Nisar M, Ahmad H, Saif N, et al.. Determination of HCV genotypes and viral loads in chronic HCV infected patients of Hazara Pakistan. Virol J., 2011; 8: 466-1.
2. Altuglu I, Soyler I, Ozacar T and Erensoy S. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in Western Turkey. Int J Infect Dis., 2008; 12(3): 239-4.
3. Aziz H, Raza A, Murtaza S, Waheed Y et al. Molecular epidemiology of hepatitis C virus genotypes in different geographical regions of Punjab Province in Pakistan and a phylogenetic analysis. Int J Infect Dis., 2013; 17(4): e247-3.
4. Bhattacharjee D, Mukherjee K, Chakroborti G, Ghosh R et al. Correlation Study Between HCV Genotypes Distribution Pattern and Viral Load in a Tertiary Care Hospital in Kolkata, India. J ClinDiagn Res., 2015; 9(5): DC15-7.
5. Blachs, Zeuzems, manns M, Ibrahim, et al. The Polaris Observatory HCV Collaborators. Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol, 2017; 2(3): 161-76.
6. Christdas J, Sivakumar J, David J, Daniel HD et al. Genotypes of hepatitis C virus in the Indian sub-continent: a decade-long experience from a tertiary care hospital in South India. Indian J Med Microbiol, 2013; 31(4): 349-353.
7. Dusheiko G, Schmilovitz-weiss H, Brown D, McOmish F, et al. Hepatitis C virus genotypes: an investigation of type-specific differences in geographic origin and disease. Hepatology, 1994; 19(1): 13-8.
8. Ikeda K, Saitoh S, Koida I, Arase Y, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology, 1993; 18(1): 47-3.
9. Larney S, Grebely J, Hickman M, De angelis D et al. Defining populations and injecting parameters among people who inject drugs: Implications for the assessment of hepatitis C treatment programs. Int J Drug Policy, 2015; 26(10): 950-7.
10. Liu J, Valencia-sanchez MA, Hannon GJ and Parker R. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat Cell Biol., 2005; 7(7): 719-3.

11. Mahaney K, Tedeschi V, Maertens G,Adrian M. Di Bisceglie, et al. Genotypic analysis of hepatitis C virus in American patients. Hepatology, 1994; 20(6): 1405-1.
12. Muhammad N and Jan MA. Frequency of hepatitis "C". J Coll Physicians Surg Pak, 2005; 15(1): 11-4.
13. Nafees M, Bhatti M and Haq I. Sero-Prevalence of HCV Antibodies in Population Attending Madina Teaching Hospital, Faisalabad. Ann King Edward Med Univ, 2007; 13(4): 260-3.
14. Narahari S, Juwle A, Basak S and Saranath D. Prevalence and geographic distribution of Hepatitis C Virus genotypes in Indian patient cohort. Infect Genet Evol, 2009; 9(4): 643-5.
15. Nousbaum JB, Pol S, Nalpas B, Landais P et al. Hepatitis C virus type 1b (II) infection in France and Italy. Collaborative Study Group. Ann Intern Med., 1995; 122(3): 161-8.
16. Pozzato G, Kaneko S, Moretti M, Croce L, et al. Different genotypes of hepatitis C virus are associated with different severity of chronic liver disease. J Med Virol, 1994; 43(3): 291-6.
17. Riaz S, Bashir MF, Haider S and Rahid N. Association of genotypes with viral load and biochemical markers in HCV-infected Sindhi patients. Braz J Microbiol, 2016; 47(4): 980-6.
18. Rong X, Lu L, Wang J,Xiong H, et al. Correlation of viral loads with HCV genotypes: higher levels of virus were revealed among blood donors infected with 6a strains. PLoS ONE, 2012; 7(12): e52467-4.
19. Rose R, Lamers SL, Dollar JJ,Grabowski MK, et al. Identifying Transmission Clusters with Cluster Picker and HIV-TRACE. AIDS Res Hum Retroviruses, 2017; 33(3): 211-8.
20. Salehi moghadam F, Mohebbi SR, Hosseini SM, Romani S, et al. Phylogenetic analysis of hepatitis C virus strains and risk factors associated with infection and viral subtypes among Iranian patients. J Med Virol, 2014; 86(8): 1342-9.
21. Simmonds P, Holmes EC, Cha TA,Chan SW, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. J Gen Virol, 1993; 74(Pt 11): 2391-9.
22. Singh S, Malhotra V and Sarin SK. Distribution of hepatitis C virus genotypes in patients with chronic hepatitis C infection in India. Indian J Med Res, 2004; 119(4): 145-8.
23. Stuyver L, Wyseur A, Van arnhem W, Lunel F, et al. Hepatitis C virus genotyping by means of 5'-UR/core line probe assays and molecular analysis of untypeable samples. Virus Res., 1995; 38(2-3): 137-57.
24. Thrift AP, El-serag HB and Kanwal F. Global epidemiology and burden of HCV infection and HCV-related disease. Nat Rev Gastroenterol Hepatol, 2017; 14(2): 122-2.
25. WHO. Hepatitis C-global prevalence (update). WklyEpidemiol Rec., 1999; 74: 425-7.
26. WHO. Hepatitis C Fact Sheet. Geneva, Switzerland, 2011.