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Mutation at 243-G of 5′ untranslated region of HCV and its correlation with viral load in HCV infected individuals

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Abstract
The 5′untranslated region (UTR) of hepatitis C virus genome is highly conserved. Two distinct RNA elements, a longer internal ribosomal entry site and short 5′ proximal stem loop RNA are present within it. The 243-G nucleotide of HCV 5′ UTR present in the longer element is important for viral replication and translation. The aim of this study was to determine the mutation present at 243-G nucleotide of HCV genome among different population groups and its correlation with patient's viral load, an indication of in-vivo HCV replication. The variations associated with this mutation among host gender, viral genotype and different populations were also studied. 110 HCV sero-reactive blood samples were subjected to RNA detection and amplification of complete 5′ UTR followed by sequencing. The sequences were used for mutation, genotyping and phylogenetic analysis. It was found that HCV genotype 3a (56%) was the predominant type followed by 3b (20%), 1a (17.33%) and 1b (6.67%). Viral load was maximum (4.64 ±10.15) X 10^5 IU/ml in case of genotype 1b infected patients. In presence of G-243-A mutation, viral load decreased to 6.55 and 2.54-fold for genotype 1 and 3 respectively. Viral 243-G mutation within male individuals showed the highest decrease (2.51-fold) in viral load than females (1.7-fold). The overall decrease in viral load with G-243-A mutation was observed maximum (2.46-fold) within the thalassemia population. Thus, from our observations, viral load is host and viral genotype dependent and G-243-A mutation reduces the viral replication in-vivo.

Importance
The importance of our study is provided below:
- This is the first in-vivo report stating that G-243-A mutation of 5´ UTR has a direct effect on viral load among the infected individuals.
- Individuals irrespective of the population group with G-243-A mutation had a decreased viral load compared with the wild type.
- Association between the G-243-A and viral load was dependent upon the individuals genotype and host gender.
- Mutation rate was maximum among HCV genotype 1 (72.22%) than in genotype 3 (36.84%).
- In presence of G-243-A mutation, viral load decreased to 6.55 and 2.54-fold for genotype 1 and 3 respectively.
- Male individuals with G-243-A mutated virus showed the highest decrease (2.51-fold) in viral load compare to females (1.7-fold).

Viral load was higher among HCV genotype 1 especially in genotype 1b infected individuals compared to genotype 3.

Keywords: individuals, HCV, importance, NS5A-NS5B

Introduction
Hepatitis C virus (HCV) infects around 185 million individuals throughout the world [1]. Among them 70-80% develops chronic hepatitis of which 20-30% shows severe liver complications like cirrhosis and hepatocellular carcinoma (HCC) [2]. HCV is mainly transmitted percutaneously through the exposure of infected blood and blood products [3]. Combination therapy with pegylated-interferon (Peg-IFN) and ribavirin is used for better management of this disease as no vaccine is available for HCV.

HCV is an enveloped, single stranded positive sense RNA virus of the genus *Hepacivirus* and a member of *Flaviviridae* family with genome size ~9.6 kb [4]. HCV genome is divided into 2 parts, structural and non-structural region. Structural region is divided into 3 parts and non-structural region into 7 parts [5]. It has only one open reading frame (ORF) i.e Core-E1-E2-P7-NS2-NS3-NS4A-NS4B-NS5A-NS5B which is flanked by 5′ and 3′ untranslated region (UTR) [6]. A single polyprotein of ~ 3010 amino acids is synthesized during translation and is processed into 10 individual proteins with distinct function [6]. Proteolytic effects of NS2 and NS3 in combination with host protease help in viral protein processing. Both of these proteases are directly involved in HCV genome replication during the viral life cycle that leads to virus multiplication within the host [7].

HCV is divided into seven major genotypes [1, 7] and 67 subtypes with a specific pattern of geographical distribution [8]. Genotype 1 and genotype 3 are common throughout the world including India, while others are limited to specific regions of the world [9, 10]. In India, genotype 3 is the most common with subtype 3a and 3b being predominant followed by genotype 1 [11]. The distribution of HCV genotypes has a north-south divide with genotype 1 being the predominant form in the south whereas genotype 3 is

common in the north, east and west of India infecting around 1% of total population [11].

Phylogenetic, computational, biochemical and enzymatic study have identified the structural elements in the 5′ and 3′ UTR of HCV genome [12]. Internal ribosome entry site (IRES) present in the 5′ UTR is critically involved in the cap independent translation of HCV RNA [13]. Functional and structural studies of the HCV IRES have been carried out in different laboratory and most of these studies have drawn the model of secondary structure of the 5' UTR of HCV [12, 14, 15]. This model was modified by Wang *et al* in 1995 following the demonstration of a pseudoknot present in the 5′ UTR essential for translation [14]. HCV 5' UTR is also play an important role in HCV RNA replication [16]. The tertiary structure of 5′ UTR of HCV RNA shows several domains (I, II, III and IV) by cryoelectron microscopy [17]. These domains are further divided into different sub domains. Domain I of HCV IRES is mainly responsible for HCV RNA replication. Domain II and III are the major domains of HCV IRES, which play crucial role for translation initiation of HCV [18]. Domain II consists of two stem loop structures II-a and II-b. Domain III is the largest domain consisting of branching hairpin stem-loops (III a, b, c, d, e, f) [18]. The basal part of domain III contains a small stem loop III-e and a predicted pseudoknot III-f [18]. Domain IV is present in downstream of domain III and surrounds the AUG start codon with its small stem-loop structure [18]. It has been shown that the first 157 nucleotides of 5′ UTR is the minimal sequence required for HCV RNA replication [16].

Previous studies showed that the mutation present in 243th nucleotide in 5′ UTR has an effect on HCV replication [7]. The G-243-A mutation of 5′ UTR reduces replication and modulates translation efficiency with the help of different viral and host proteins and the alteration of the structure. Previous study reported that nucleotide substitutions in the HCV IRES affects lymphoid replication [19]. Long-term cultures of HCV in lymphoblastoid lines showed nucleotide substitution G-243-A is responsible for increase in translational activity in lymphoid cell lines [20]. The role of 243-G is to maintain the IRES structure in HCV genotype 1b and the viral load alteration was found when this particular nucleotide position encountered changes [21]. The viral load among different genotypes and host population with respect to G-243-A mutation has not been studied. Thus, the aim of this study was to evaluate the sequence variation of the 5′ UTR 243 G among different genotypes and its correlation with HCV viral load in different HCV infected population groups.

Materials and methods

Study population

110 HCV seroreactive samples were collected from different Liver Clinics in Kolkata, India from April 2011 to March 2013. The study is in accordance with the Helsinki declaration and International Conference on Harmonization Good Clinical Practices guidelines (1961). All inclusions were approved by the Institutional Ethical Committee and written consent was obtained from each individual who participated in the study.

RNA extraction

RNA was extracted from 140µl of serum collected from HCV seroreactive individuals using QIAamp viral RNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The viral RNA was eluted in 50µl elution volume and the RNA aliquot was stored in -80ºC for further use.

Detection of viral RNA by nested RT-PCR of 5′ UTR of HCV genome

Detection of HCV RNA was done by nested RT-PCR based on 5′ UTR of the HCV genome. The primers used in this study were designed in house (Table 1) using primer 3 software (http://gmdd.shgmo.org/primer3/?seqid=47). First round RT-PCR was performed in a 20µl reaction volume containing 2µl of Taq 10X buffer II (ABI, USA), 0.8mM of dNTPs (ABI, USA), 1.5mM of magnesium chloride, 5mM dithiotheritol (DTT) (Sigma-Aldrich, USA), 0.25µM of forward and reverse primers (Table 1), 0.4U of AMV reverse transcriptase (Promega, USA), 0.5U Taq DNA polymerase (ABI, USA) and 2µl of extracted RNA. The RT-PCR conditions were 42°C for 60 minutes followed by 94°C for 5 minutes, 94°C for 1 minutes, 55°C for 1 minutes and 72°C for 1 minutes for 35 cycles, the final extension was at 72°C for 5 minutes in an ABI 9700 thermal cycler (ABI, USA). Second round nested PCR was performed in a total volume of 25µl containing 2.5µl Taq 10X buffer II, 0.8mM of dNTPs, 2.5mM of magnesium chloride, 0.25µM of forward and reverse primers (Table 1), 0.5U of Taq DNA polymerase and 2µl of the first round RT-PCR product. PCR conditions were same as first round except the reverse transcription step. The amplified product was electrophoresed in 1.5 % agarose gel stained with ethidium bromide and observed under a gel documentation system (Bio-rad, USA). The size of the PCR amplicon was 395bp.

DNA sequencing and mutational analysis

Nested PCR amplified product of 5′UTR of HCV genome were gel purified using QIAquick PCR purification kit (Qiagen, Germany) according to the manufacturer's protocol and directly used for sequencing in an automated DNA sequencer (3130XL, ABI, USA) using Big-Dye terminator 3.1 kit (ABI, USA). The sequenced region was analyzed for mutations bioinformatically using alignment tool Clustal omega. All the edited and raw sequences were managed using the freely available Bio-Edit sequence editor [http://www.mbio.ncsu.edu/bioedit/bioedit.html].

Genotyping and phylogenetic analysis

The sequences obtained were edited using BioEdit software and aligned together with other HCV reference sequences using MEGA5.0 software. The reference sequences were downloaded from the Los Alamos HCV database (http://hcv.lanl.gov/content/index). The phylogenetic tree of the 5' UTR regions was constructed using neighbour-joining method of the MEGA5.0 software and the feasibility of the tree was calculated using 1000 bootstrap replications. Divergence less than 2% was regarded as one coming from potential common source [22]. Monophyletic clusters and phylogenetic trees were derived from those sequences using neighbour-joining method with p distance algorithm of MEGA 5.0 software.

Quantitative HCV RNA estimation

Quantitative HCV RNA was determined in-house using ABI real time RT-PCR Kit (AgPath-ID^{TM} One Step RT-PCR Kit). The HCV primers and probe sequences were directed

against the 5′ UTR of the HCV genome and were designed in house (Table 1). Amplification was performed in a total volume of 25µl containing 4µl extracted RNA, 12.5µl of 2X PCR buffer, 1.5µl of detection enhancer, 0.5µl of 10µM forward and reverse primers, 0.5µl of 3µM probe and 1µl of 25X enzyme mix. The PCR conditions were 45°C for 15 minutes, followed by 95°C for 10 minutes, 95°C for 20 seconds and 62°C for 1 minute for 45 cycles in a 7500 real time PCR system (ABI, USA). The 4th WHO International Standard for HCV, NIBSC code 06/102, was used as standard. The viral loads in serum were expressed as international units per millilitre (IU/ml**)**.

Statistical analysis

The statistical tests were performed using Chi-square test. The corresponding mean values were calculated and the observed standard deviations were also computed based on the data present within the studied population. Categorical data and p values ≤0.05 were considered as statistically significant.

Result

HCV RNA detection, viral load and genotype distribution

Out of 110 seroreactive HCV samples, 75 were RNA positive (Table 2, 3). The pattern of genotype distribution of 75 RNA positive samples were 3a (56%), 3b (20%) as the predominant subtypes, followed by 1a (17.33%) and 1b (6.67%) (Table 2, Fig 1). Individuals infected with genotype 1b showed the highest mean viral load (4.64 ± 10.15) X 10^5 IU/ml followed by 3a (1.11 ± 2.55) X 10^5 IU/ml, 1a (0.71 ± 1.17) X 10^5 IU/ml and 3b (0.35 ± 0.6) X 10^5 IU/ml respectively (Table 2). The accession numbers of the 75 sequences, which were submitted to Gene-bank, are KF279419-KF279493 respectively.

Demographic characteristics of the studied population

HCV genotyping was done with HCV RNA positive samples and divided into two groups genotype 1 and 3. In addition, study population was divided according to their gender and mode of HCV transmission (Table 3). The study population had an age range of 5–72 yrs, with the mean age of the population being 30.72 yrs. Among the genotypes (1 and 3) the mean age of the individuals were 27.61 and 31.7 yrs respectively. Male individuals accounted for a higher percentage among the genotype groups with 18.67% and 62.67%, while female individuals were 5.33% and 13.33% for genotype 1 and 3 respectively (p<0.0001) (Table 3).

Of the 75 HCV positive individuals, 48% (n=36) belonged to IVDUs (Gen-1= 8, Gen-3 = 28) followed by thalassemic individuals 26.67% (n=20) (Gen-1 = 6, Gen-3 = 14). Chronic liver disease infected patients were 21.33% (n=16) (Gen-1 = 3, Gen-3 = 13) and dialysis patients were 4% (n=3) (Gen-1 = 1, Gen-3 = 2) respectively (Table 3).

G-243-A mutation

The percentage of G-243-A mutation was 46.67% (35 out of 75) within our study population. This G-243-A mutation in genotype 1 was 72.22% (n=13), which was almost double with respect to genotype 3 (36.84%) (Table 4). HCV mutation within male individuals showed higher G-243-A mutation rate (54.1%) compared to female individuals (14.29%). Male individuals infected with genotype 1 showed the highest mutation rate (85.71%) compared to genotype 3 (44.68%), whereas female individuals infected with genotype 1 showed 25% mutation rate in contrast to genotype 3 at 10% (Table 4). IVDUs infected with genotype 1 showed the highest mutation rate (87.5%) followed by chronic liver disease HCV infected patients (66.67%), thalassemic patients (50%) and the least cases of mutation within the dialysis patients (33.33%) (Table 5). Genotype 3 infected chronic patients (68.54%) showed the maximum mutation rate compared to others (Table 6).

Correlation between viral load, HCV genotype, population gender and G-243-A mutation

Correlation between HCV viral loads and genotypes were analyzed with or without G-243-A mutation by univariate analysis. The mean viral loads for the population with G-243-A mutation were (0.54 ± 0.89) X 10^5 IU/ml compared to the wild type (1.63 ± 4.31) X 10^5 IU/ml as shown in Table 4 (p<0.0001). The mean viral load were significantly low for both genotypes (genotype 1 and 3) in G-243-A mutant versus wild type with p<0.0001 (Table 4). The mean viral load among the mutant strain in male host individuals were (0.49 ± 0.81) X 10^5 IU/ml compared to wild type (1.23 ± 3.06) X 10^5 IU/ml. Whereas in female patients, the viral load of the mutant and wild types strain were (1.46 ± 2.03) X 10^5 IU/ml and (2.56 ± 6.45) X 10^5 IU/ml respectively. This study showed a statistically significant level of correlation between the G-243-A mutant and wild type strain with host gender and mean viral load (p<0.0001). Between the two genotypes (genotype 1 and 3), a significant difference in the mean viral load for the mutant and the wild type were also observed (p<0.0001).

Among the different HCV infected study populations, like IVDUs, patients with chronic liver disease, thalassemic individuals and dialysis patients, the mean viral load were low in mutant type than the wild type (Table 5). A significant difference of viral load was observed between the mutants and wild types (p<0.0001) (Table 5). The study population was further divided into two genotypes (genotype 1 and 3) and their respective mutation was also analyzed along with statistical correlations (Table 6).

Table 1: Primers for detection of HCV genome

Primer[@]	Start	Stop	Sequence (5′-3′)	Product size (bp)[$]
U10 OF	10	29	TTACGAGGCGACACTCCGCC	434
U443 OR	443	421	GGTTATACTCCGCCAACGATCTG	434
18 IF	18	41	GGCGACACTCCACCATAGATCACT	395
U412 IR	412	393	GGAACTTAACGTCCTGTGGG	395
130S	130	146	CGGGAGAGCCATAGTGG	161
290R	290	272	AGTACCACAAGGCCTTTCG	161
PROBE	148	168	CTGCGGAACCGGTGAGTACAC	21

[@] U10 OF, outer forward primer; U443 OR, outer reverse primer; 18 IF, inner forward primer; U412 IR, inner reverse primer; 130S, forward primer for quantitative PCR; 290R, reverse primer for quantitative PCR [$] bp, base pair

Table 2: HCV genotypes and viral load distribution in this study[!]

	GENOTYPE					
	1a	**1b**	**3a**	**3b**	**Total**	**p-value[*]**
Samples	13 (17.33)	5 (6.67)	42 (56)	15 (20)	75 (100)	
Viral load Mean±SD[#] IU/ml (10^5)	0.71±1.17	4.64±10.15	1.11±2.55	0.35±0.6	1.12±3.23	<0.0001

! Data are No. (%) of subjects unless otherwise stated
SD, standard deviation
*p value, probability value

Table 3: Demographic and HCV characteristic of the study participants among different genotype groups[€]

	Genotype			
Category	**1**	**3**	**Total**	**p-value[b]**
No. of Individuals	18(24)	57(76)	75(100)	
AGE (Mean±SD) [a]	27.61±12.32	31.7±14.25	30.72±13.84	0.844
GENDER	1	3		
Male	14(18.67)	47(62.67)	61(81.34)	<0.0001
Female	4(5.33)	10(13.33)	14(18.66)	
POPULATION	1	3		
IVDUS[c]	8(10.67)	28(37.33)	36(48)	<0.0001
THALASSEMIA	6(8)	14(18.67)	20(26.67)	
CLD[d]	3(4)	13(17.33)	16(21.33)	
DIALISYS	1(1.33)	2(2.67)	3(4)	

[€] Data are No. (%) of subjects unless otherwise stated
[a] SD, standard deviation
[b] p value, Probability value
[c] IVDUs, Intravenous Drug Users
[d] CLD, Chronic liver disease

Table 4: Univariate gender based viral load analysis within genotypes with G-243-A mutation[@]

Factor	**No. of Individuals**	**Viral Load (IU/ml) Mean±SD[!] (10^5)**		**p-value[$]**
		Wild type (%)	**Mutation (G-243-A) (%)**	
Total	(n=75)	1.63±4.31 (53.33)	0.54±0.89 (46.67)	<0.0001
Genotype	1(18)	4.65±10.15 (27.77)	0.71±1.17 (72.23)	<0.0001
	3(57)	1.17±2.74 (63.16)	0.46±0.69 (36.84)	<0.0001
Gender	Male(n=61)	1.23±3.06 (45.9)	0.49±0.81 (54.1)	<0.0001
	Female(n=14)	2.56±6.45 (85.71)	1.46±2.03 (14.29)	<0.0001
Male				
Genotypes	1(n=14)	0.11±0.1 (14.29)	0.77±1.21 (85.71)	<0.0001
	3(n=47)	1.32±3.16 (55.32)	0.33±0.42 (44.68)	<0.0001
Female				
Genotypes	1(n=4)	7.68±13.09 (75)	0.02 (25)	<0.0001
	3(n=10)	0.85±1.12 (90)	2.89 (10)	<0.0001

[@] Data are No. (%) of subjects unless otherwise stated
[!] SD, standard deviation
[$] p value, Probability value.

Table 5: Variation of HCV viral load in presence of G-243-A mutation in different population groups[$]

Population category	**No. of Individuals**	**Viral Load (IU/ml) Mean±SD (10^5)**		**p-value**
		Wild type	Mutation (G-243-A)	
IVDUs [@]	36	1.91±0.98 (47.22)	1.52±1.01 (52.78)	<0.0001
THALASSEMIA	20	2.53±59.31 (80)	1.03±11.87 (20)	<0.0001
CLD[!]	16	1.36±13.98 (37.5)	1.17±12.64 (62.5)	<0.0001
DIALISYS	3	134.01 (33.33)	1.82±0.21 (66.67)	<0.0001

[$] Data are No. (%) of subjects unless otherwise stated
[@] IVDUs, Intravenous Drug Users
[!] CLD, Chronic liver disease

Table 6: Mutation analysis of G-243-A among different HCV genotypes in different population groups[@]

Population	**Genotype**	**Viral Load (IU/ml) Mean±SD[!] (10^5)**		**p-value[$]**
		Wild type	**Mutation(G-243-A)**	
IVDUs[a]	1(22.22)	0.18 (12.5)	0.11±0.08 (87.5)	<0.0001
	3(77.78)	0.19±0.1 (57.14)	0.17±0.1 (42.86)	<0.0001
THALASSEMIA	1(30)	7.62±13.15 (50)	0.76±1.29 (50)	<0.0001
	3(70)	1.35±2.68 (92.86)	1.85 (7.14)	<0.0001
CLD[b]	1(18.75)	0.22 (33.33)	2.99±0.0004 (66.67)	<0.0001

	3(81.25)	1.59±1.43 (38.46)	0.71±0.93 (68.54)	<0.0001
DIALISYS	1(33.33)	-	0.17 (100)	<0.0001
	3(66.67)	13.4 (50)	0.19 (50)	<0.0001

[@] Data are No. (%) of subjects unless otherwise stated
[!] SD, standard deviation
[$] p value, Probability value
[a] IVDUs, Intravenous Drug Users
[b] CLD, Chronic liver disease.

Discussion

Molecular mechanisms that control the HCV IRES functions are not fully understood. The IRES functions primarily as structural elements, which directs the proper folding of the HCV RNA molecule and helps in its replication and translation [16, 23]. IRES also direct the unpaired nucleotide of loops and bulges at the correct three-dimensional position which helps ribosomal subunit to recognize and bind to it. According to this model, the structure of the loops contains the critical nucleotide sequences for protein as well as RNA interactions [23]. This hypothesis, is further supported by the conservation of the RNA primary structure of most of the loops and bulges. The experimental evidences are limited to support this hypothesis whether all conserved loops and bulges in the HCV IRES element are essential for the IRES function or not [24]. Studies have also shown that co-evolutionary sites of the HCV NS2 and NS3 proteins in humans has a close association with 5′ UTR 243-G, these covariance during HCV genome evolution is presumed to be beneficial for HCV replication [25].

HCV 5′ UTR 243-G is located between the domain IIIc and IIId, which is at a non-Watson-Crick base pair position and at the domain IIIe of the replicative strand [18]. Both the positive and negative stranded domains of 5′ UTR regulate translation and replication by its host protein binding sites [16]. Previous studies has shown that the 5′ UTR 243-A and 5′ UTR 243-G had the same IRES translation activities for both rabbit reticulocyte and hepatic lysates in-vitro [7]. However, G-243-A mutation of 5′ UTR showed preferential translation functions in lymphoblastoid cell lines and primary dendritic cells [7], which suggests that the 5′ UTR 243-G might be a viral RNA translation determinant in a cell specific manner. In our study, viral loads were analyzed with respect to the mutation found within the different host population groups. Based on our observation, G-243-A mutation is frequently found in host and showed the highest rate of mutation for genotype 1 compared to genotype 3, which was almost double (Table 4). Similar results were observed with respect to host gender but when correlated with both the parameters of gender and genotype, viral load were higher within genotype 1 for males and genotype 3 for females. Thus, from our study, we can conclude that mutation of G-243-A affects HCV viral load or HCV replication in-vivo and is dependent specifically on HCV genotype and gender.

HCV genotypes are helpful in deciding the duration of IFN therapy [26]. The therapy with IFN-alpha alone or combination of IFN-alpha and ribavirin are more effective on HCV genotype 3 infected individuals than genotype 1 [27]. One possible reason for treatment failures in case of genotype 1 could be its efficient replication ability that leads to high viral load among HCV infected patients [28]. In our study, HCV genotype 1b showed the highest viral load than other genotypes such as genotype 1a, 3a and 3b. Thus, our observation corroborates with previous observation that genotype 1 infected patient had higher viral load than those individuals infected with genotype 2 and 3 [29].

In this study, we have tried to find a correlation between the viral load i.e. HCV replication in vivo and G-243-A mutation among different genotype in different populations such as IVDUs, chronic liver disease, thalassemia and dialysis patients. Host HCV viral load was low in case of G-243-A mutation when compared with the wild type virus within the different HCV genotypes.

In summary, this is the first in-vivo report stating that G-243-A mutation of 5′ UTR has a direct effect on viral load among the infected individuals. It was observed that, individuals irrespective of the population group with HCV 5′ UTR G-243-A mutation had a decreased viral load compared with individuals with wild type virus. On the contrary, association between the G-243-A mutation and viral load was dependent upon the HCV genotype and host gender. Mutation rate was higher among HCV genotype 1 (72.22%) than in genotype 3 (36.84%) infected populations. HCV mutation within host male individuals showed higher rate (54.1%) than in females (14.29%). Among the different population groups, patients with chronic liver disease infected with HCV showed the highest mutation rate (62.5%) than others (Table 5). It was observed that the viral load was high among HCV genotype 1 especially in genotype 1b infected individuals. Although the sample size, of HCV genotype-1, is small in this study but provides a real time correlation of HCV viral load and G-243-A mutation in vivo.

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References

1. Negro F. HCV causes systemic disorders that can be cured. Nat. Rev. Gastroenterol. Hepatol. 2013; 11:77-78.
2. Alter MJ, Margolis HS, Krawczynski K, Judson FN, Mares A. The natural history of community-acquired hepatitis C in the United States, The Sentinel Counties Chronic non-A, non-B Hepatitis Study Team. N. Engl. J Med. 1992; 327:1899-1905.
3. Beltrami EM, Williams IT, Shapiro CN, Chamberland ME. Risk and Management of Blood-Borne Infections in Health Care Workers. Clin. Microbiol. Rev. 2000; 3:385-407.
4. Honda M, Beard MR, Ping LH, Lemon SM. A phylogenetically conserved stem-loop structure at the 5′ border of the internal ribosome entry site of hepatitis C virus is required for cap-independent viral translation. J Virol. 1999; 73:1165-1174.
5. Prabhu R, Garry RF, Dash S. Small interfering RNA targeted to stem-loop II of the 5' untranslated region

effectively inhibits expression of six HCV genotypes. Virol. J. 2006; 3:100-110.
6. Guangxiang L, Shaojie X, Zhaohui C. Role of the 5′ - Proximal Stem-Loop Structure of the 5′ Untranslated Region in Replication and Translation of Hepatitis C Virus RNA. J Virol. 2003; 77:3312-3318.
7. Sun HY, Ou NY, Wang SW, Liu WC, Cheng TF, Shr SJ, *et al*. Novel nucleotide and amino acid covariation between the 5'UTR and the NS2/NS3 proteins of hepatitis C virus: bioinformatic and functional analyses. PLoS One, 2011; 6:e25530.
8. Smith DB, Bukh J, Kuiken C, Muerhoff AS, Rice CM, Stapleton JT, *et al*. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment Web resource. Hepatology. 2014; 59:318-27.
9. Pybus OG, Markov PV, Wu A, Tatem AJ. Investigating the endemic transmission of the hepatitis C virus. Int. J Parasitol. 2007; 37:839-849.
10. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G, *et al*. Steatosis accelerates the progression of liver damage of chronic hepatitis C patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001; 33:1358-64.
11. Mukhopadhya A. Hepatitis C in India. J Biosci. 2008; 33:465-473.
12. Tsukiyama KK, Iizuka N, Kohara M, Nomoto A. Internal ribosome entry site within hepatitis C virus RNA. J Virol. 1992; 66:1476-1483.
13. Brown EA, Zhang H, Ping LH, Lemon SM. Secondary structure of the 5′ non-translated regions of hepatitis C virus and pestivirus genomic RNAs. Nucleic Acids Res. 1992; 20:5041-5045.
14. Wang C, Le SY, Ali N, Siddiqui A. An RNA pseudoknot is an essential structural element of the internal ribosome entry site located within the hepatitis C virus 5′ noncoding region. RNA. 1995; 1:526-537.
15. Kieft JS, Zhou K, Jubin R, Murray MG, Lau JY, Doudna JA, *et al*. The hepatitis C virus internal ribosome entry site adopts an ion-dependent tertiary fold. J Mol. Biol. 1999; 292:513-529.
16. Wang L, Jeng KS, Lai MMC. Poly(C)-Binding Protein 2 Interacts with Sequences Required for Viral Replication in the Hepatitis C Virus (HCV) 5′ Untranslated Region and Directs HCV RNA Replication through Circularizing the Viral Genome. J Virol. 2011; 85:7954-7964.
17. Piñeiro D, Martinez-Salas E. RNA Structural Elements of Hepatitis C Virus Controlling Viral RNA Translation and the Implications for Viral Pathogenesis. Viruses. 2012; 4:2233-2250.
18. Lukavsky PJ. Structure and function of HCV IRES domains. Virus Res. 2009; 139:166-171.
19. Forton DM, Karayiannis P, Mahmud N, Taylor-Robinson SD, Thomas HC. Identification of Unique Hepatitis C Virus Quasispecies in the Central Nervous System and Comparative Analysis of Internal Translational Efficiency of Brain, Liver, and Serum Variants. J Virol. 2004; 78:5170-5183.
20. Lerat H, Shimizu YK, Lemon SM. Cell Type-Specific Enhancement of Hepatitis C Virus Internal Ribosome Entry Site-Directed Translation due to 5' Nontranslated Region Substitutions Selected during Passage of Virus in Lymphoblastoid Cells. J Virol. 2000; 74:7024-7031.
21. Mostafa KEA, Hassan MA, Ahmed MF, Sherif MS, Noha GB, Samar SY, *et al*. Positional effect of mutations in 5'UTR of hepatitis C virus 4a on patients' response to therapy. World J Gastroenterol. 2009; 15:1480-1486.
22. Pham ST, Bull RA, Bennett JM, Rawlinson WD, Dore GJ, Lloyd AR, *et al*. Frequent Multiple Hepatitis C Virus Infections Among Injection Drug Users in a Prison Setting. Hepatology. 2010; 52:1564-1572.
23. Lemon S, Honda M. Internal ribosome entry sites within the RNA genomes of hepatitis C virus and other Flaviviruses. Semin. Virol. 1997; 8:274-288.
24. Psaridi L, Georgopoulou U, Varaklioti A, Mavromara P. Mutational analysis of a conserved tetra loop in the 5′ untranslated region of hepatitis C virus identifies a novel RNA element essential for the internal ribosome entry site function. FEBS Letters. 1999; 453:49-53.
25. Donlin MJ, Cannon NA, Aurora R, Li J, Wahed AS, Di Bisceglie AM, *et al*. Contribution of Genome-Wide HCV Genetic Differences to Outcome of Interferon-Based Therapy in Caucasian American and African American Patients. PLoS One, 2010; 5:e9032.
26. Slavenburg S, Weggelaar I, van Oijen MG, Drenth JP. Optimal length of antiviral therapy in patients with hepatitis C virus genotypes 2 and 3: a meta-analysis. Antivir. Ther. 2009; 14:1139-1148.
27. Pujol FH, Loureiro CL. Replacement of hepatitis C virus genotype 1b by genotype 2 over a 10-year period in Venezuela. J Clin. Gastroenterol. 2007; 41:518-520.
28. Pang PS, Planet PJ, Glenn JS. The evolution of the major hepatitis C genotypes and correlates with clinical response to interferon therapy. PLoS One, 2009; 4:e6579.
29. Soriano V, Mocroft A, Rockstroh J, Ledergerber B, Knysz B, Chaplinskas S, *et al*. EuroSIDA Study Group. Spontaneous Viral Clearance, Viral Load, and Genotype Distribution of Hepatitis C Virus (HCV) in HIV-Infected Patients with Anti-HCV Antibodies in Europe. J Infect. Dis. 2008; 198:1337-1344.