10.2478/abm-2010-0032

Asian Biomedicine Vol. 4 No. 2 April 2010; 253-262

Original article

Distribution of hepatitis C virus genotypes, hepatic steatosis and their correlation with clinical and virological factors in Pakistan

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Background: Due to the inherently unstable nature of HCV, various genotypes have been identified. Steatosis is a histological feature in the progression of HCV-associated liver disease and has been shown to alter the host lipid metabolism.

Objective: Assess the distribution of HCV genotypes in the two provinces of Pakistan, and determine the association of hepatic steatosis with altered clinical and virological factors in chronic HCV patients.

***Methods*:** One hundred twenty six chronic HCV patients (steatosis in 49 patients) were enrolled for qualitative analysis by PCR. Out of 126 ELISA and PCR positive samples, 119 (48 with hepatic steatosis) chronic HCV patients (mean age 42.0±13.3 years, mean body mass index (BMI) 24.2±4.1) were proved positive after PCR-based detection. Biochemical and virological factors such as HCV genotype, or glucose, in 119 CHC patients were determined and compared between patients with and without hepatic steatosis.

Results: Out of 126 samples, 119 were HCV positive, where 58 (48.7%) were genotype 3a, 24 (20.2%) were 3b, 12 (10.1%) were 1a, eight (6.7%) were 2a, six (5.0%) were 1b, and one (0.8%) was 4. Furthermore, seven (5.9%) had a co-infection and three (2.5%) were untypable. BMI (p=0.004), genotype 3a (p<0.001), and triglycerides (p=0.002) were significantly associated with steatosis. It is noteworthy that cholesterol (p=0.281), glucose (p=0.305), low-density lipoprotein (p=0.101), high-density lipoprotein (p=0.129), alanine amino transferase (p=0.099), aspartate transaminase (p=0.177), bilirubin (p= 0.882), and age (p=0.846) showed non-significant association.

Conclusion: Genotype 3a is the predominant genotype in Pakistan. Hepatic steatosis is quite frequent feature in HCV patients and strongly correlates with BMI, genotype 3a, and triglyceride contents in patients infected with HCV.

Keywords: Clinical parameters, genotypes; hepatitis C virus, steatosis, subtype 3a

Hepatitis C virus (HCV) infection presents severe health problem worldwide, particularly in the developing countries including Pakistan. HCV is positive stranded RNA virus and abstractedly related to the flaviviruses and pestiviruses. It has been classified into the genus hepacivirus of the virus family flaviviridae [1, 2]. The whole genome of HCV is 9.5Kb containing highly conserved untranslated regions (UTR) at both the 5' and 3' termini, which flank a large translational open reading frame encoding a polyprotein of 3,000 amino acids.

HCV has been categorized into different genotypes based on at least 67% similarity of nucleotide sequences [3]. Determination of HCV genotypes and subtypes is critical to a tailored clinical management and prognosis. Several studies have shown an association between HCV genotype and both the responsiveness to interferon treatment and the extent of clinical progression of chronic HCV infection [4-6]. At this time, six genotypes and above 100 subtypes of HCV have been identified [7]. Various

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studies have shown that there is a strong correlation of HCV genotypes with the grade and extent of steatosis, impaired glucose tolerance, body mass index (BMI), atherogenic lipid profile and insulin resistance [8, 9]. Therefore, genotype specific correlations are of high clinical relevance.

HCV is a major cause of chronic liver disease and various associated metabolic disorders, affecting approximately 170 million people worldwide [10]. Acute infection of HCV progresses to chronic hepatitis in up to 60 to 80% of patients and can progresses to steatosis [11, 12]. Hepatic steatosis is the excessive lipid accumulation within the hepatocyte cytoplasm and is recognized as having a strong association with the development of cirrhosis [13]. The prevalence of steatosis in chronic HCV-infected patients has been reported to be approximately 50% varying between 35 to 80% in chronic hepatitis C (CHC) patients [2, 8]. In another study, steatosis is found in 41 of 73 patients with CHC infection [14]. Infection with HCV has been associated with alterations in lipid metabolism. In some studies, lipid changes occur in HCV patients, and may be more common among patients infected with HCV genotype 3 who develop liver steatosis [9]. Studies suggest that the link between lipid changes may be more common in genotype 3 infected persons [9]. Severe and frequent steatosis has been seen in patients infected with genotype 3 as compared to those infected with other genotypes [2, 15].

Geographic distribution of HCV genotypes are well documented in different parts of the globe, but not so in Pakistan. Furthermore, steatosis is significantly associated with HCV genotype 3a, which has been reported in a study as most prevalent genotype in Pakistan, where no data exist about HCV-induced steatosis. The purpose of this study was to document the prevalence of HCV genotypes and their association with virological and biochemical markers. Such information is clearly relevant in addressing the management of HCV in this country.

Methods

Patients and samples

The present study was conducted at NUST Centre of Virology and Immunology (NCVI), National University of Science and Technology, in collaboration with Government Lady Reading Hospital (LRH) Peshawar, and Holy Family Hospital Rawalpindi Pakistan. The study was approved by the Ethics Committee of NUST Centre of Virology and Immunology.

This study included 126 consecutive patients with CHC infection who were referred to undergo liver studies (biopsy or abdominal ultrasound) between December 2008 and July 2009. During the course of the study, fasting venous blood samples were collected in the morning (74 from Government Lady Reading Hospital Peshawar, 29 from Government Holy Family Hospital Rawalpindi and 23 from NCVI diagnostics). After data analysis, 49 patients out of 126 patients who were referred to liver analysis were proved as steatotic and the remaining as non-steatitic.

Exclusion criteria

Patients who did not undergo liver studies (biopsy or abdominal ultrasound) and those carrying additional blood born viruses, such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis D virus (HDV) were excluded from the study. Data collected on these patients included presence or absence of hepatic steatosis, age, height, and weight.

Viral RNA extraction

Sera from 126 patients were subjected to viral RNA extraction from 140 mL serum samples using Qiagen (Germany) RNA isolation kit according to the manufacturer's protocols.

Qualitative analysis (PCR-based detection)

All these 126 ELISA and PCR positive samples with CHC infection were further tested for HCV presence or absence by PCR, as ELISA is not as reliable as PCR-based analysis. Viral RNA was taken to reverse transcribe the 5' NCR of HCV using Moloney murine leukemia virus reverse transcriptase (M-MLV RTase, Fermentas) in a total reaction volume of 20 μL. The reaction mixture contained 4 μL M-MLV (5X) buffer, 1 μL M-MLV reverse transciptase (RT) enzyme, 1 μL dNTPs (10mM), 0.5 μL Rnase inhibiter (Fermentas), 1.5 μL RNase free water, 1 μL specific antisence primer and 10 μL template (viral RNA). cDNA was synthesized using ABI Veriti 96 well thermocycler. Cycle conditions for cDNA were as follows: 42°C for 55 minutes followed by 70°C for 10 minutes.The cDNA produced was stored at 4°C for short-term storage or -20°C for prolonged storage.

cDNA product was used for qualitative analysis of HCV infection. The first round PCR was performed using sense and antisense primers followed by second round PCR (nested PCR), using the first

round product with inner sense and antisense primers. PCRs were carried out using Taq polymerase (Fermentas) for 35 cycles. Finally, 5 μL of the second round PCR product was mixed with 2ul of 6x loading dye and electrophoresed on 1.5% agarose gel with commercially available 100bp DNA marker (fermentas). Specific bands of HCV detections were visualized under UV light of Wealtec gel doc system (see **Fig. 1**). After analysis, out of 126 patients, 119 (with mean age 42.0±13.3 and mean BMI 24.2±4.1) proved positive. Seven samples were HCV negative (one of steatotic and six of non-steatotic patients).

HCV genotyping

One hundred nineteen PCR positive samples were then preceded for HCV genotyping by the method described by Ohno et al. [16] with slight amendment. In brief, 10 μL of viral RNA was used for the synthesis of cDNA through reverse transcription using 200 U of M-MLV RT at 42°C for 60 minutes. First round PCR was performed with 5 μL of the synthesized cDNA using 5'-GGGAGGTCTCGTAGACCGTGC ACCATG-3' primer as forward and 5'-GAGACGGG TATAGTACCCCATGAGAGTCGGC-3' as reverse primer. The first round product was amplified in the second round as Mix1 and Mix 2 using the primers presented as below [16]. Mix 1 contained primers for genotype 2a, 2b, 3b, 1b and genotype 1a, 3a, 4, 5a, and 6a in Mix 2. 5 μL of the second round PCR products were electrophoresed on 1.5% agarose gel with 100 bp DNA marker and analyzed under UV light.

Genotype confirmation was based on specific PCR bands (see **Fig. 2A** and **B**)

Biochemical, clinical and virological factors

After completion of the genotyping, many biochemical studies were undertaken. The fasting blood glucose, total cholesterol (TC), high-density lipoprotein (HDL), alanine amino transferase (ALT), aspartate transaminase (AST), bilirubin, and fasting triglyceride (TG) were measured by microlab 300 apparatus (Merck, Haarlem, Netherland).

Sera of all 119 chronic HCV patients were analyzed for these parameters. One-milliliter reagent containing 100 μL sample of each patient and 1mL reagent containing 100 μL standard were used to measure HDL. Blank contained only 1 mL reagent with no sample and standard. In the case of cholesterol, triglycerides, and other parameters, 10 μL sera of all 119 patients (in each case) were taken into 1 mL of the respective reagent with a blank having no sample and a standard having 10 μL standard in 1 mL reagent. All these mixtures were incubated at 37°C for 10 minutes and then readings were noted.

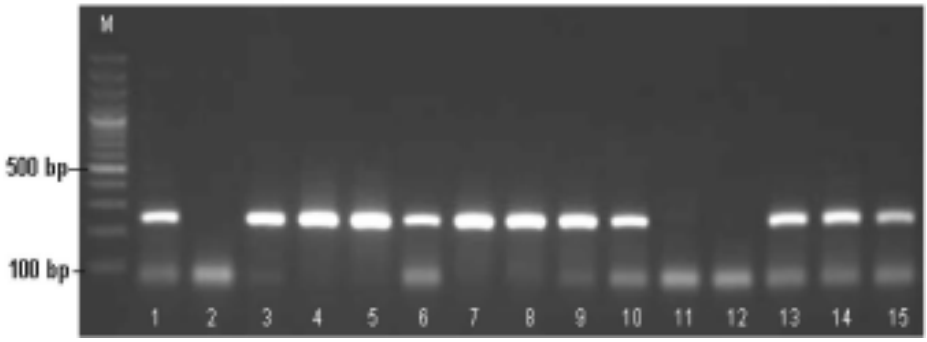

Fig. 1 Digital printout of gel electrophoresis of some samples, performed for qualitative analysis. Lane 1, 3-10, and 13-15 contain bands being HCV positive, lane 2 a negative control and lane 11 and 12 contain those samples which were HCV negative.

Name	Mix 1 primers	Name	Mix 2 primers
S7	AGACCGTGCACCATGAGCAC	S7	AGACCGTGCACCATGAGCAC
S2a	AACACTAACCGTCGCCCACAA	G1a	GGATAGGCTGACGTCTACCT
G1b	CCTGCCCTCGGGTTGGCTAAG	G3a	GCCCAGGACCGGCCTTCGCT
G2a	CACGTGGCTGGGATCGCTCC	G4	CCCGGGAACTTAACGTCCAT
G2b	GGCCCCAATTAGGACGAGAC	G5a	GAACCTCGGGGGGAGAGCAA
G3b	CGCTCGGAAGTCTTACGTAC	G6a	GGTCATTGGGGCCCCAATGT

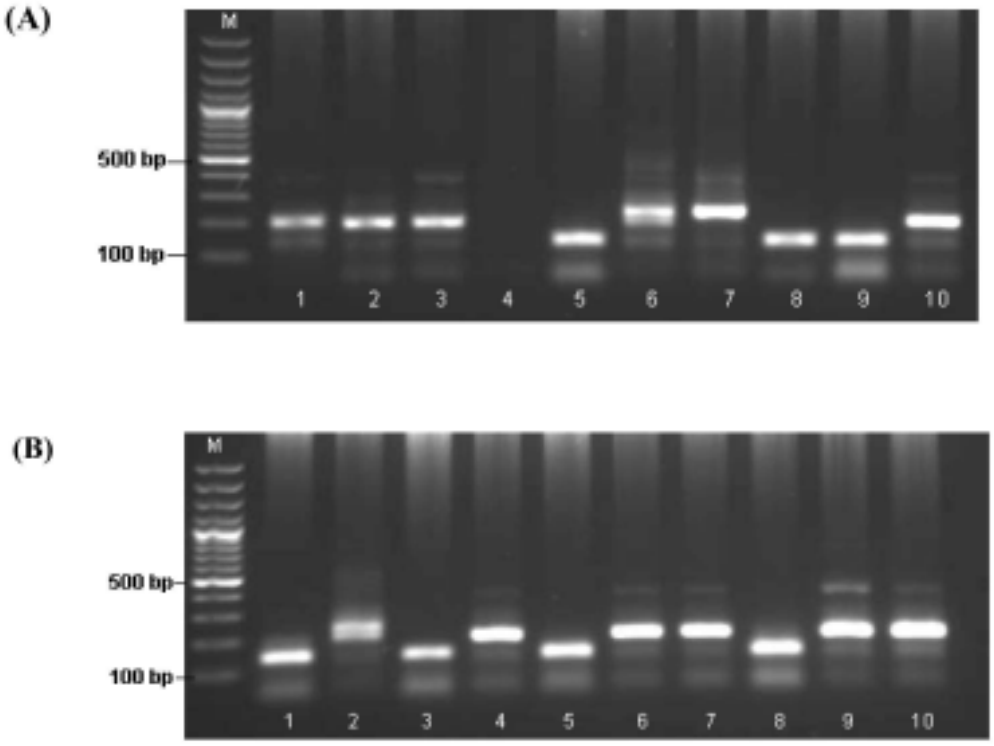

Fig. 2 Gels patterns of PCR products of different HCV genotypes using two sets of primers named Mix1 and Mix 2 [16]. Only one mix (either Mix1 or Mix2) of a specific sample which had genotype specific band is included. Mix 1 has primers that allow specific detection of PCR products for HCV genotypes 1b, 2a, 2b, and 3b, while Mix 2 primers reveal HCV genotypes 1a, 3a, 4, 5a, and 6a. (**A**) Lane 1, 2, 3 and 10 amplified bands represent genotype 1a (Fragment size 208), lane 4 untypable genotype, lane 5, 8, and 9 for genotype 3b (fragment size 176), and lane 6 and 7 indicate genotype 3a (fragment size 232). (**B**) Lane 1 represents genotype 3b (fragment size 176 bp), lane 2 genotype 1b (fragment size 234 bp), lane 3, 5 and 8 genotype 2a (fragment size 190 bp), while lane 4,6,7,9 and 10 show genotype 3a (fragment size 232 bp).

ALT, AST, and glucose levels were determined using the ALT and glucose kits by Merck (Darmstadt, Germany) while for TC, TG and HDL; Dialab GmbH kits (Austria) were used. Standards (Dialab GmbH) and commercially available control (PRO-LAB) were used for the precise calibration. For LDL estimation, we used Friedewald et al. formula [17] as follows:

$$LDL = TC - HDL - TG/5.0 \text{ (mg/dL)}$$

Statistical analysis

Clinical variables were presented as mean±SD (Standard Deviation). Mean, SD, p-value, confidence interval, (CI 95%) and standard error were calculated and compared by using independent t-test. In case of p-value, a significance of 5% was assumed. Body mass index (BMI) was calculated as kg/m^2. Data analysis was accomplished using the computer software, SPSS (version 12.0) for windows.

Results

Genotypes

Out of 126 HCV positive samples, 119 cases (64 males and 55 females) were proved as HCV positive after PCR based qualitative analysis. Seventy were from North West Frontier province (LRH Hospital) and the remaining 49 from Punjab province (Holy Family Hospital Rawalpindi and NCVI Diagnostics) (see Punjab and NWFP indicated by red dots in **Fig. 3**)

The distribution of HCV genotypes in 119 patients was determined (**Table.1**). Out of 119 PCR positive samples, 58 (48.7%) were genotype 3a, 24 (20.2%) were 3b, 12 (10.1%) were 1a, eight (6.7%) were 2a, six (5.0%) were 1b, and one (0.8%) was genotype 4. Additionally, seven (5.9%) had co-infection and three (2.5%) samples showed no result being untypable by the genotyping system used here.

Fig. 3 Map of Pakistan, in which red dots indicate areas for sample collection.

Distribution of HCV based on two provinces (Punjab and NWFP) is shown in **Table 1**. In total, 49 samples collected from Punjab regions, 27 samples (55.1%) were of genotype 3a, seven (14.3%) were 3b, six (12.2%) were 1a, two (4.1%) were 1b, three (6.1%) were 2a, three (6.1%) had a co-infection, and two (4.1%) were of unknown genotype. Among 70 patients from N.W.F.P, 31 (44.3%) were of genotype 3a, 17 (24.3%) were 3b, five (7.1%) were 2a, six (8.6%) were 1a, four (5.7%) were 1b, one (1.4%) was genotype 4, four (5.7%) had a co infection, and one (1.4%) was untypable. The distribution of genotype of the cohort with and without steatosis is presented in **Table 2** and **3**.

HCV genotypes, steatosis, and biochemical markers

In 119 PCR positive samples, 49% patients were genotype 3a, 20% were 3b, 10% were 1a, 5% were 1b, 7% were 2a, 0.8% were 4, 6% had a co-infection and 3% revealed no band (**Table 1**).

Biochemical parameters in patients with and without steatosis were shown in **Table 4**. We note that BMI, genotype 3a, and triglycerides were significantly associated with steatosis, while cholesterol, glucose, LDL, HDL, ALT, AST, bilirubin and age showed a non-significant association in HCV patients with and without steatosis.

Table 1. Distribution of HCV genotypes in two main provinces of Pakistan.

Genotypes	Total (119 samples)	NWFP province (70 samples)	Punjab province (49 samples)
Genotype 3a	58 (48.7%)	31 (44.3%)	27 (55.1%)
Genotype 3b	24 (20.2%)	17 (24.3%)	7 (14.3%)
Genotype 1a	12 (10.1%)	6 (8.6%)	6 (12.2%)
Genotype 2a	8 (6.7%)	5 (7.1%)	3 (6.1%)
Genotype 1b	6 (5.0%)	4 (5.7%)	2 (4.1%)
Genotype 4	1 (0.8%)	1 (1.4%)	-
Co infection	7 (5.9%)	4 (5.7%)	3 (6.1%)
Untypable	3 (2.5%)	1 (1.4%)	2 (4.1%)

Table 2. The genotype-wise presentation of various clinical parameters in patients without steatosis (N=71).

Parameters	HCV 3a (N=28)	HCV 3b (N=17)	HCV 1a (N=7)	HCV 1b (N=6)	HCV 2a (N=5)	HCV 4 (N=1)	HCV Co (N=4)	Untype (N=3)
Cholesterol (mg/dL)	168.8±18.3	155.4±30.3	145.2±14.4	158.6±17.6	145.4±56.7	59	153.5±47.4	126.6±52.3
Triglyceride (mg/dL)	142.7±23.6	130.9±33.6	117.2±56.4	119.0±22.5	128.2±9.2	138	129.5±24.7	113.3±17.0
LDL (mg/dL)	62.3±18.8	48.2±11.9	56.0±8.9	42.7±12.1	65.6±16.7	34	52.5±7.9	39.9±9.2
Glucose (mg/dL)	116.2±17.9	110.3±13.8	129.6±10.2	117.3±8.1	126.6±9.6	119	114.3±15.8	120.4±13.4
HDL (mg/dL)	82.6±11.9	67.1±16.3	87.2±17.6	61.9±8.4	50.7±14.1	78	74.5±15.1	65.8±13.6
Age(SD) (year)	42.1±14.2	40.5±22.7	40.8±10.8	37.0±7.3	43.6±13.1	40	38.7±9.1	41.9±12.3
BMI	23.2±2.9	22.7±3.5	24.5±2.8	22.6±2.5	21.6±1.7	24.8	23.6±2.4	24.5±1.4
Bilirubin (kg/m^2)	1.78 ± 0.81	1.97±0.76	1.91±0.33	1.84±0.09	1.87±0.44	1.80	1.79±0.56	1.81±0.68
ALT (mg/dL)	95.8±14.4	82.1±15.9	76.7±11.8	84.1±17.5	91.5±17.1	94	87.4±9.2	90.6±16.4
AST (mg/dL)	89.6±24.3	87.3±18.7	78.5±13.5	80.2±10.3	73.2±8.5	77	83.4±12.1	71.4±9.0

Data are expressed as mean±SD.

Table 3. The genotype-wise presentation of various clinical parameters in patients with steatosis (N=48).

Parameters	HCV 3a (N=30)	HCV 3b (N=7)	HCV 1a (N=5)	HCV 2a (N=3)	HCV Co (N=3)
Cholesterol (mg/dL)	166.2±21.9	175.8±15.0	140.3±14.0	147.5±55.9	146.5±42.7
Triglyceride (mg/dL)	148.7±28.3	159.2±31.3	143.3±28.0	150.5±26.2	145.7±31.5
LDL (mg/dL)	65.8±17.7	75.6±16.7	45.6±9.0	49.8±20.0	51.0±19.9
Glucose (mg/dL)	125.3±23.5	121.8±19.0	126.6±38.7	117.5±20.5	118.7±14.5
HDL (mg/dL)	71.3±18.0	65.3±9.4	78.3±14.2	56.0±14.1	61.5±16.6
Age (SD) (year)	40.9±15.5	40.2±8.7	37.6±17.7	41.5±7.8	41.7±18.4
BMI (kg/m^2)	25.8±4.5	24.9±3.4	25.7±2.3	22.9±2.5	25.9±5.2
Bilirubin (mg/dL)	1.88±0.80	1.78±0.32	1.96±0.56	1.90±0.42	2.02±0.31
ALT (mg/dL)	97.1±24.4	99.2±25.6	91.6±22.2	98.5±9.2	87.5±22.5
AST (mg/dL)	85.2±30.5.3	67.2±26.6	57.3±24.5	75.5±10.6	50.7±13.3

Data are expressed as mean±SD.

Table 4. The biochemical marker's assessment of HCV patients with and without steatosis.

	With steatosis (N=48) Mean ± SD	Without steatosis (N=71) Mean ± SD	P-value	95% CI
Cholesterol (mg/dL)	162.5±25.6	156.2±30.5	0.281	-5.2 to 17.7
Triglyceride (mg/dL)	149.6±27.8	132.3±22.2	0.002	6.7 to 27.1
LDL (mg/dL)	63.2±18.6	55.0±27.0	0.101	-1.6 to 17.7
Glucose (mg/dL)	124.0±22.7	117.0±16.2	0.305	-3.6 to 12.2
HDL (mg/dL)	69.6±16.7	74.1±17.5	0.129	-12.3 to 1.6
Age (SD)	40.5±14.3	41.0±14.2	0.846	- 6.4 to 5.3
BMI (Kg/m^2)	25.6±4.13	23.1±3.7	0.004	0.8 to 4.0
Bilirubin (mg/dL)	1.88±0.68	1.85±0.74	0.882	-0.3 to 0.3
ALT (mg/dL)	96.3±23.1	88.6±17.9	0.099	-1.4 to 15.8
AST (mg/dL)	76.9±29.6	84.7±33.3	0.177	-21.6 to 4.0

Data are expressed as mean±SD.

HCV genotypes and steatosis

As shown in **Table 3**, hepatic steatosis was present in 48 HCV patients, and was frequently detected in patients with genotype 3a. Among the genotypes other than 3a, seven were genotype 3b, five were genotype 1a, three were genotype 2a and 3 steatotic patients were infected with mix genotypes. Seventy-seven percent patients with steatosis were infected with genotype 3 and only 23% with other genotypes (genotype 1, 2, 4 and mix genotypes). This clearly indicates that there is a strong association between steatosis development and genotype 3 HCV. No tendency was found in the distribution of HCV genotypes among various age groups and gender. However, all the three patients having untypable genotypes were females.

Body mass index (BMI)

The BMI was calculated as weight (kilograms) divided by height squared (meters) in 119 HCV patients, and compared the values of patients having steatosis with those without steatosis (**Table 4**). We note that the BMI of HCV patients having steatosis was significantly higher than those without steatosis.

Discussion

Hepatitis C virus (HCV) infection is a major global health problem with increasing prevalence in developing countries, including Pakistan. An estimated 10 million persons in Pakistan are infected with HCV [18]. It is genetically heterogeneous virus with nucleotide substitution, which results its genome to undergo diversification and evaluation into different genotypes. In Pakistan, information about the genotypes and related risk factors in Hepatitis C virus infected patients is limited. Patients infected with different genotypes of HCV may have different clinical manifestations, severity of liver diseases, and response to interferon therapy [6, 19]. A multivariable logistic regression analysis in 755 chronic HCV patients was performed by Rubbia et al and reported that Steatosis affects chronic hepatitis C progression in a genotype specific way, most predominantly genotype 3a [20].

This study indicates that of the cohort studied from the two regions of Pakistan (The North West Frontier and Punjab Provinces) genotype 3 is the most prevalent form. A similar distribution has been demonstrated in other studies undertaken in the region (including Bangladesh, Northern and Southern India and Nepal). The prevalence of genotype 3 has been reported as greater than 45% by Idrees et al. [21], Afridi et al. [22], and 48.7% in our study. The apparent prevalence of 3a being most frequent subtype in Pakistani population, may render persons infected with steatosis at greater frequency than other parts of the globe where the predominant genotype is not 3a. Our study also confirms that in chronic HCV infection, risk factors including high TG contents, high glucose levels, and other biochemical parameter in steatotic patients are more frequent than non-steatotic patients, as shown in **Table 4**. High level of triglycerides and higher BMI are significantly linked to those patients who are at the same time steatotic. The distribution of gender mean age, LDL, HDL, AST, ALT and bilirubin are not significantly different in patients with and without steatosis. These findings are in accordance with previous work [23, 24]. However, in our 119 CHC patients, there were 21 patients with diabetes Type II (we did not make its correlation because of small number of patients). The high level of glucose in steatotic 3a patients might be due to the 16 out of 21 diabetic patients in this group. This also suggests that HCV 3a, diabetes and steatosis are closely linked. The remaining five diabetic patients were not of genotypes 3a.

Presence of untypable samples in our study indicates that 1) the genotyping methods of Ohno et al. [16] and Idrees cannot detect these genotypes, or 2) some novel genotypes are present in Pakistan. To solve this question, the cloning and sequencing of these samples may be very useful. It will also be helpful for health care workers to prescribe the therapy for patients with untypable genotypes. Our next goal is to find out the sequence of untypable HCV region to clarify the causes of untypable samples.

Our current understanding of the process leading to steatosis is limited, However, various studies have proposed some putative mechanisms where viral component leads to accumulation of lipids within hepatocytes [13]. Animal models [25] as well as *in vitro* investigations [26] propose that a virological effect induces steatosis, while clinical observational data suggest a direct virological effect with hepatitis C genotype 3 infection [27]. In cultured cells, as a minimum two HCV proteins (core and NS5A) are supposed to interact with the cell machinery. This is concerned with lipid metabolism because of the intracellular lipid buildup [26]. In fact, Sabile et al. [28] proposed that HCV core protein interacts with

apolipoprotein AII, contributing to the development of steatosis. Tsutsumi et al. [29] suggested an interaction between virus and retinoid X receptor α RxRα, which is a transcriptional regulator involved in the lipids metabolism. On the other hand, according to Okuda et al. theories [30], HCV protein induces an axidative stress in mitochondria, resulting in the lipid accumulation inside the liver [30]. In human, hepatic steatosis is also linked with a decrease in hepatic CYP3A activity through post-translational mechanism [31]. Thus, an impaired metabolism of lipids may lead to the pathogenesis of steatosis in chronic hepatitis C.

It is well known that there is an association between HCV 3a and steatosis and although the mechanism underlying this relationship currently is being unravelled, some research has been directed to explore why HCV 3a is predominantly involved in HCV induced steatosis. Hourioux et al. [32] reported that genotype 3-specific hepatitis c virus core protein residue phenylalanine 164 increases the chances of steatosis in an *in vitro* cellular model. According to Jackel-Cram et al. [33], the severity of steatosis in HCV-3a infections is due to the stronger effect of HCV-3a core protein on fatty acid synthase (FAS) promoter, leading to higher prevalence of steatosis. Up-regulation of FAS promoter probably leads to an increase in free fatty acids synthesis and consequently accumulation of lipids inside the hepatocytes. Romero-Gomez et al. [19] stated that hepatic steatosis is related to genotype, fibrosis degree, serum leptin levels and genotype 3 seems to have a viral specific steatogenic effect. Hence, we agree with the hypothesis of a direct pathogenic role of the HCV 3a in promoting liver steatosis, as describe by other researchers [34, 35].

Conclusion

HCV 3a was the leading genotype in Pakistan, posing more risk for the development of hepatic steatosis. Other major types are 1 and 2. Hepatic steatosis is frequently occurring feature with CHC in Pakistani population. Triglycerides, BMI, and genotype 3a are significantly associated with hepatic steatosis along with other altered biochemical markers in HCV-infected patient. Diagnosis, prognosis, interferon therapy, and clinical management of HCV are very poor due to the lack of knowledge about HCV genotypes. Our findings will help in anti viral treatment by knowing the genotypes of HCV and will enhance the understanding of hepatic steatosis in HCV patients that is largely genotype 3a specific.

Acknowledgements

The authors are thankful to Dr. Muhammad Tariq, Dr. Imran Bajwa (Holy Family Hospital Rwalpindi), and Muhammad Tahir for their cooperation in sampling.

The first author (SZS) is grateful of National University of Science and Technology (NUST) for financial support by S & T fund during this study. This work was supported by Higher Education Commission of Pakistan grant (No. 829) and US-Pakistan grant (Dr. Ishtiaq Qadri). The authors have no conflict of interest to declare.

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