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HCV Chronicity: Is True Cure Truly Rare? Long-Term Follow-Up in Two HCV Patients

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Abstract

Background: Serum anti-hepatitis C virus antibody (anti-HCV Ab) has multipronged bedside interpretation. Given the inefficient B-cell response and paucity of T-cell response in HCV infection, we hypothesize that anti-HCV Ab represents persistence of HCVRNA irrespective of HCV polymerase chain reaction (HCVPCR) status.

Case report: We present long-term follow-up of two patients, infected with HCV since nearly 1.5 decade. Case 1: A 35-year old female presented to us in July 2003 with dyspepsia. Anti-HCV Ab was positive. Qualitative PCR for HCV (HCV RT-PCR) was positive in May 2007. HCVPCR was negative after treatment with interferon (IFN) and ribavirin in 2008. HCV RT-PCR was continuously negative for 7 times after 2008. Serum anti-HCV Ab tested negative in 2014 but reappeared in 2017. Post-treatment ALT "flare" was observed twice. Case 2: A 24-year old married female reported to us in April 2004 with elevated ALT. Anti-HCV Ab was positive. HCV RT-PCR tested positive in December 2005. She was successfully treated with IFN and ribavirin in 2006. Re-treatment with sofosbuvir and ribavirin was required for HCV relapse in December 2016. After initial treatment in 2006, HCV RT-PCR has remained negative for 9 times, with normalization of ALT. Anti-HCV Ab is persistently positive. HCV RT-PCR is negative since re-treatment in 2017.

Conclusion: Since HCV re-activation may occur in seropositive persons, long-term follow up is recommended after treatment and on incidental detection of anti-HCV Ab. Persistence of anti-HCV Ab actually favours true seropositivity and non-detectable viremia.

Keywords: Anti-HCV antibody; Chronic hepatitis C; HCV; RT-PCR; Social impact

Abbreviations: Ag: Antigen; ALT: Alanine Aminotransferase; Anti-HCV Antibody: Anti-HCV Ab; AP: Alkaline Phosphatase; BM: Bone Marrow; EHM: Extra-Hepatic Manifestations; HBsAg: Hepatitis B Surface Antigen; Hb: Hemoglobin; HCC: "HCV Clearance" Card; HCV: Hepatitis-C; HD: Hemodialysis; HE: Hepatic Elastography; HEC: "HCV Exposed" Card; MCV: Mean Corpuscular Volume; PBMNs: Peripheral Blood Mononuclear Cells; RT-PCR: Qualitative Polymerase Chain Reaction; qPCR: Quantitative PCR; S:Co R: Signal to Cut-Off Ratio; TLC: Total Leukocyte Count; TSH: Thyroid Stimulating Hormone; SVR: Sustained viral response

Introduction

HCV was proposed in 1972 and cloned by Choo in 1989 [1]. Notwithstanding an "inefficient" blood borne transmission of HCV, the worldwide prevalence of chronic hepatitis-C is 200 million [2,3]. Yearly estimates of new infection and its associated death are 3-4 million and >350,000, respectively [4]. Approximately 7% of people in Pakistan are infected with HCV; 80-90% of the infected persons demonstrate HCV genotype-3. The mode of transmission remains undetermined in approximately 40% of infected persons [5]. Closely related but distinct virions, i.e., quasi species, are constantly generated in the hyper-variable region of viral glycoprotein E2 (E2HVR1). E2 blocks CD81 binding site on various neutralizing epitopes, especially the epitope at amino acid position 384-410 [6,7]. A defiantly high mutation rate of 10^3-10^4virions/replication cycle facilitates HCV to establish as "antibody escape mutants" [6,8]. HCV interaction with cellular Scavenger Receptor Class-B type (SCARB1) dissociates viral particles from their lipoprotein envelope i.e., the Lipo-viro-particles (LVPs) to expose CD81 binding epitopes on E2. CD81-HCV interaction and "lateral movement" of this complex are key steps to clathrin-mediated internalization of HCV. The virus has cytoplasmic life cycle and replicates in the acidic milieu of endocytic compartment producing 10^{12} virions/day [9-12]. Fast replication is determined by HCV binding to cytoplasmic mi-RNA in the hepatocytes [13]. The half-life of virions is 2-3 hours with an ability to infect 1-54% of hepatocytes [8,14]. HCV promotes growth and "overrides" cell death by modulating epithelial mesenchymal transition (EMT) related genes, tumor initiating stem-like cells (TISCs) and cytoplasmic miRNAs [9]. HCV specific immunity is incomplete due to inefficient antibody neutralization along with quantitatively insufficient cytotoxic and memory T-cell responses. Life-time infection thus results in more than 80% from persistence of HCV in the lymphocytes/macrophages with production of cytokines and cytokine receptor like proteins i.e., immune-modulation [13]. We attempt to elaborate several interesting features which include:

(a) 15 year follow-up.

(b) Atypical presentation.

(c) Delay in diagnosis.

(d) Unusual therapeutic response.

(e) Prolonged SVR.

However, the key observation that supports our hypothesis is the persistence of anti-HCV in both patients during the follow-up.

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Initial Laboratory Results	Current laboratory reports (24th-26th March 2018)
TLC 7000/mm^3	TLC 7300/mm^3
Hb 8.3 G/dL	Hb 2.1 G/dL
MCV 64.3 fl	MCV 83.4 fl
Platelet count 379000/mm^3	Platelet count 277000/mm^3
ALT 39 IU/mL	ALT 21 IU/mL
ALP 70 IU/mL	ALP 75 IU/mL
Aspartate aminotransferase 23 IU/mL	Anti-HCV positive
Gamma glutamyltransferase17 IU/mL	HBsAg negative
Prothrombin time 16:13 sec	serum albumin 4.1 g/dL
Anti-HCV positive	serum bilirubin 0.53 mg/dL
HBsAg negative	serum TSH1.75 micro IU/mL
serum bilirubin 0.4 mg/dL	serum cholesterol 190 mg/dL
serum TSH1.9 micro IU/L	serum HDL-cholesterol 49 mg/dL
serum FT4 7.8 pmol/L	serum LDL-cholesterol 101 mg/dL
serum T3 3.5 nmol/L	serum triglycerides 198 mg/dL
serum cholesterol 209 mg/dL	serum creatinine 0.69 mg/dL
serum HDL-cholesterol 58 mg/dL	serum Na+ 139 mEq/L
serum LDL-cholesterol 121 mg/dL	serum K+ 4 mEq/L
serum triglycerides 151 mg/dL	Blood urea 15 mg/dL
serum creatinine 0.76 mg/dL	Blood glucose (R) 106 mg/dL
Blood urea 9 mg/dL	Urinalysis: Unremarkable
Urinalysis: Unremarkable	Abdominal ultrasound: Diffuse hepatic steatosis
Anti-streptolysin O titer 200 IU/mL	HCV RNA was negative in PBMNs and BM aspirate
Rheumatoid arthritis factor negative	All her family members are sero-negative for anti-HCV
Anti-nuclear antibody negative	
Echocardiography: Normal study	
Abdominal ultrasound: Diffuse fatty infiltration of liver and gestational sac containing single fetus	

Table 1: Detailed initial and current laboratory results (Case 1).

Case Series

Case 1

A female of 35-years visited us on 14th July 2003 with dyspeptic symptoms and vomiting since 2-months. There was 12-week gestational amenorrhea and generalized muscle/joint pains. Between 1989-92, she had undergone 2 vaginal deliveries and 3 spontaneous abortions. Clinical findings included mild anemia and palpable, non-tender liver. Initial biochemistry and imaging have been detailed in Table 1. HCV RT-PCR was negative on 24th July 2003. She delivered a live baby girl on 2nd February 2004. Two months later, a local rheumatologist prescribed azathioprine and methotrexate for her musculo-skeletal complaints. Single elevation of ALT 183 IU/mL was observed on 14th May 2004. Another physician advised gluten-free diet and related work up on 7th June 2004. HCV RT-PCR was negative again on 15thJune, 2004. HCV RT-PCR was positive on 21st May 2007. After 10-month delay for financial reasons, we treated her with IFN (3 mU SQ 3/week) and ribavirin (per oral 400 mg bid) for 24-weeks. Viral counts dropped to 2088 IU/mL on 30th July 2008 with 12-week therapy. HCV RT-PCR and HCV qPCRs tested negative on 20th and 24th October 2008, respectively. She is constantly HCV PCR negative since more than 15-years although Anti-HCV has persisted until now barring one negative result on 28th January 2014. Our patient is randomly on silymarin and vitamin E. Apart from intermittent joint pain; she is largely asymptomatic and preserves good quality of life. Between 2008 and 2017, she had another 4 spontaneous miscarriages. Other salient observations during 15-year follow-up are given in Table 2. Current laboratory reports on 24th and 26th March, 2018 are shown in Table 1. All her family members are seronegative for anti-HCV.

Serial No	Date	Observations
1	14th July 2003	Dyspepsia; Pregnancy; ALT (24 IU/mL); anti-HCV positive; Fatty liver.
2	24th July 2003	HCV RT-PCR negative
3	2nd Feb 2004	Parturition
4	15th April 2004	Azathioprine and methotrexate
5	14th May 2004	ALT (183 IU/mL)
6	7th June 2004	Gluten free diet Trial
7	15th June 2004	HCV RT-PCR negative
8	6th Dec 2005	ALT (43 IU/mL)
9	17th Feb 2006	Analgesics for shoulder pain
10	20th Feb 2006	HCV RT-PCR negative
11	20th Dec 2006	Serum triglycerides (216 mg/dL)
12	21st May 2007	HCV RT-PCR positive; ALT (39 IU/mL)
13	11th June 2007	Interferon and ribavirin
14	20th July 2008	HCV qPCR (2088 IU/mL)
15	30th Oct 2008	HCV RT-PCR negative
16	24th Oct 2008	HCV qPCR negative
17	6th August 2009	ALT (48); serum triglycerides (287 mg/dL)
18	8th March 2010	Dental treatment
19	6th Aug 2010	Serum triglycerides (305 mg/dL)
20	12th Oct 2010	Hb (10.9 g/dL)
21	28th Jan 2014	Anti-HCV negative
22	8th June 2014	ALT (48 IU/mL)
23	1989-1992	3 spontaneous abortions
24	2008-2017	4 spontaneous abortions
25	October 2008-March 2018	HCV RT-PCR persistently negative
26	23rd May 2018	HCV RT-PCR negative in PBMNs and BM

ALT: Alanine Aminotransferase; Anti-HCV Antibody: Anti-HCV; AP: Alkaline Phosphatase; BM: Bone Marrow; Hb: Hemoglobin; HCV: Hepatitis-C; MCV: Mean Corpuscular Volume; PBMNs: Peripheral Blood Mononuclear Cells; RT-PCR: Qualitative Polymerase Chain Reaction; Qpcr: Quantitative PCR; TLC: Total Leukocyte Count

Table 2: Salient observations during 15-year follow-up (Case 1).

Case 2

A 24-year old mother of 4 reported to us on 8th April 2004 with 3-month history of maldigestion and joint pains affecting hands and knees. Symptoms responded well to proton pump inhibitors and analgesic support. Her physical examination was unremarkable. Initial biochemistry and imaging are given in Table 3. She was lost to follow-up before confirmatory HCV RT-PCR and re-visited us on 22nd December 2004. Further evaluation (27th December 2004 and 11th January 2005) is mentioned in Table 3. On 1st February 2006, she was advised IFN (3mU SQ 3/week) and ribavirin (per oral 400 mg bid) for 24-weeks. However, treatment was delayed for 2.5-months due to financial reasons. Pregnancy test became positive on 26th April 2006. Post-treatment HCV RT-PCR was negative on 16th September 2006. She delivered a live baby boy in January 2007. HCV RT-PCR remained negative for nearly ten years before detecting positive on 14th December 2016. HCV RT-PCR was negative on 22nd July 2017 after initiating 24-week therapy with

Initial laboratory results	Current laboratory reports (4th-14th April 2018)
TLC 8800/mm^3	TLC 6300/mm^3
Hb 12.5 G/dL	Hb 12.3G/dL
MCV 64.3 fl	MCV 76.4 fl
Platelet count 316000/mm^3	Platelet count 211000 mm^3
ALT 72 IU/mL	ALT 16 IU/mL
AP 224 IU/mL	AP 185 IU/mL
Anti-HCV positive	Anti-HCV positive
HBsAg negative	HBsAg negative
Aspartate aminotransferase 23 IU/mL	Protein C 157.7%
Prothrombin time 12.1:15.6 sec	Protein S 106.5%
serum bilirubin 0.2 mg/dL	Anti-thrombin III 128%
serum T3 1.52 nmol/L	Anti-cardiolipin IgG 2.3 GPL-U/mL
serum T4 106 nmol/L	Anti-cardiolipin IgM 4.7 MPL-U/mL
serum TSH 1.52 mIU/L	serum albumin 4.7 g/dL
serum cholesterol 231 mg/dL	serum bilirubin 0.7 mg/dL
serum triglycerides 143 mg/dL	serum T3 0.83 ng/mL
serum creatinine 0.6 mg/dL	serum T4 95.34 nmol/L
Blood urea 12 mg/dL	serum TSH1.75 mIU/mL
Urinalysis: unremarkable	serum prolactin 15.71 ng/mL
Anti-streptolysin O titer<200 IU/mL	serum cholesterol 230 mg/dL
Rheumatoid arthritis factor negative	serum triglycerides 205 mg/dL
Anti-nuclear antibody negative	serum creatinine 0.6 mg/dL
Abdominal ultrasound: fatty liver.	serum Na+ 143 mEq/L
	serum K+ 3.3 mEq/L
	Blood urea 12 mg/dL
	Blood glucose (R) 113 mg/dL
	Urinalysis: Unremarkable
	Abdominal ultrasound: diffuse hepatic steatosis, coarse liver; portal vein thrombosis with cavernous transformation.
Further evaluation 27th December 2004 to 11th January 2005	
ALT 41 and 78 IU/mL	
Anti-HCV Ab positive	
HCV qPCR <250 copies/mL	
HCV RT-PCR negative	
Abdominal ultrasound: hepatic steatosis	
Liver biopsy: Fragmented liver tissue without portal areas, swollen hepatocytes with granular cytoplasm, dilated sinusoids, no granuloma or malignancy	
Autoimmune work-up: Unremarkable	
Alanine aminotransferase 48	
Lost to follow up until 11th November 2005	
Alanine aminotransferase 78	
HCV RT-PCR negative	
Anti-HCV Ab positive	
HCV RT-PCR (genotype 3a) positive	
Liver biopsy: hepatic steatosis, piecemeal necrosis and portal triaditis	

ALT: Alanine Aminotransferase; Anti-HCV Antibody: Anti-HCV; AP: Alkaline Phosphatase; BM: Bone Marrow; HBsAg: Hepatitis B Surface Antigen; Hb: Hemoglobin; HCV: Hepatitis-C; MCV: Mean Corpuscular Volume; PBMNs: Peripheral Blood Mononuclear Cells; RT-PCR: Qualitative Polymerase Chain Reaction; qPCR: Quantitative PCR; TLC: Total Leukocyte Count; TSH: Thyroid Stimulating Hormone

Table 3: Detailed initial and current laboratory results (Case 2).

sofosbuvir (400 mg/day PO) and ribavirin (per oral 400 mg bid) on 21st January 2017. Significant findings between 2006-2017 are mentioned in Table 4: Miscarriage; full term pregnancy; hyperechoic liver; hepatic elastography score F1; portal vein thrombosis. Presently, our patient maintains a good functional status. All her immediate contacts are seronegative for anti-HCV.

Serial No	Date	Observations
1	8th April, 2004	Dyspepsia; arthralgia; ALT (72 IU/mL); anti-HCV positive; fatty liver.
2	Until 21st Nov. 2004	Lost to follow-up
3	22nd Dec 2004	ALT (41 IU/mL); anti-HCV positive
4	5th Jan 2005	HCV qPCR (<250 copies/mL)
5	11th Jan 2005	HCV RT-PCR negative
6	14th Jan 2005	Liver biopsy
7	26th April 2005	ALT (48 IU/mL)
8	Until 10th Nov 2005	Lost to follow-up
9	11th Nov 2005	HCV RT-PCR negative
10	28th Nov 2005	ALT (78 IU/mL); anti-HCV positive
11	12th Dec 2005	HCV RT-PCR positive
12	1st Feb 2006	Interferon and ribavirin
13	26th April 2006	Pregnancy test positive
14	16th Sep 2006	HCV RT-PCR negative
15	January 2007	Parturition
16	Sep 2006 to December 2016	HCV RT-PCR persistently negative
17	28th Dec 2007	Epigastric pain; barium study normal
18	2nd April 2008	Increased liver echogenecity
19	12th May 2010	Spontaneous Abortion (2-month Gestation)
20	31st July 2012	Vitamin-D deficiency; Articular degeneration (right knee and lumbar spine)
21	29th July 2013	ALT 24 IU/mL
22	26th Feb 2015	Pregnancy test positive; No varices on endoscopy
23	8th April 2015	8-week gestation; portal vein thrombosis
24	6th Dec 2016	ALT 14 IU/mL
25	14th Dec 2016	HCV relapse; Hepatic fibroscan score F1
26	22nd July 2017	HCV RT-PCR negative (re-treatment with sofosbuvir and ribavirin on 21st January 2017).
27	24th July 2017	Coarse liver

ALT: Alanine Aminotransferase; Anti-HCV Antibody: Anti-HCV; AP: Alkaline Phosphatas; HCV: Hepatitis-C; RT-PCR: Qualitative Polymerase Chain Reaction; qPCR: Quantitative PCR; TLC: Total Leukocyte Count; TSH: Thyroid Stimulating Hormone

Table 4: Salient observations during >14 years follow-up (Case 2).

Discussion

In both cases, anti-HCV test was performed on ARCHITECT ABOTT with state-of-the-art Chemiluminescent Micro particle Immune Assay (CMIA) technique as per manufacturer's standard protocol. Similarly, assay for genotyping was performed by automated real-time PCR system (CE, IVD and FDA approved). The assay can quantify 1a, 1b, 2, 3, 4, 5 and 6 genotypes by amplifying highly conserved 5'UTR and NSSb region/core region of the HCV genome. The lower detection limit of the assay was 15 IU/ml with 95.5% specificity (Tables 5 and 6).

This is the longest clinical follow-up of two female patients who seem to have acquired HCV during multiple obstetrical interventions. HCV infection implies the presence of anti-HCV or HCVRNA without prior treatment [15-20]. Confirmation of diagnosis was significantly delayed in both patients due to repeatedly negative HCVPCR (Case 1) and two conflicting results of HCVPCR in January 2005 (Case 2) [16,17]. With its "Lower Limit of Detection" (LLOD) at <15 IU/mL, qPCR has now replaced HCV RT-PCR (LLOD: <50 IU/mL) [12]. Viremia between 15-50 IU/mL is liable to be misdiagnosed as "aviremia" [18]. HCVRNA persistently <25 IU/mL ("low grade viremia") remains below

the LLOD of modern assays [19]. HCV infection may be transmitted from such cases and their treatment gets inadvertently delayed. HCV assays are usually unnecessary in advanced liver disease [21]. Diagnosis of HCV infection on history alone is often missed due to non-classical symptoms [20]. In Case 1, we attributed her dyspepsia to gestation and ignored 3 important clues: elevated ALT, hepatic steatosis and anti-HCV. ALT "flares" more often than "persistently elevated" ALT require aggressive testing for HCVRNA [19]. Interestingly Post-treatment ALT remained normal for nearly 10-years in Case 2 (Table 4). However, ALT "flared" twice after treatment in "apparently cured" Case 1 (Table 3), indicating erratic behavior of a reliable HCV marker. Hepatic steatosis results from cytopathic effect of $CD8^+$ T cells [22]. It resolves with HCV eradication [14]. Continued seropositivity and fatty acid, fatty change persists in both females despite extraordinary outcome of treatment, possibly reflecting $CD8^+$ response to "low grade viremia". SVR is the "absence of HCVRNA on at least one occasion after treatment" [23]. SVR is expected to translate into "sustained viral clearance" [19]. Continued seropositivity in both females and the emergence of serum HCVRNA after 10-years in Case 2, augment our hypothesis that HCV seropositivity represents persistence of HCV infection in the host. No "true viral clearance" exists as evident from "ongoing" liver damage in HCV treated subjects irrespective of ALT and HCVPCR status. Lee et al. maintain that non-detectable HCVRNA at the end of treatment does not represent "true viral clearance" [24]. HCVRNA appears in serum for up to 8 years after successful treatment [8]. Spontaneous clearance of HCVRNA occurs in merely 1.7% of infected persons [14]. The claims for complete resolution of acute HCV infection (20%) are not backed by long-term follow-up. However, we present the longest clinical follow up, which is concurrent with already published recommendations [25,26]. About 37-74 million people worldwide are anti-HCV positive and HCV PCR negative [15]. Anti-HCV cannot differentiate between acute and chronic HCV infection [25]. Between 74-86% of anti-HCV positive subjects are also HCVPCR positive [14]. Thus "true"

1	Patient Category	"Healthy Carrier"/Incidental detection (during evaluation for job/visa/travel/marriage!!)
		Enlisted for hemodialysis/operative intervention/blood donation
		Valid exposure and/or clinical evidence of liver disease
2	Purpose of Evaluation	Initial screening
		Confirmation
		Intention-to-treat
		Immediate follow-up
		Long-term follow-up
3	Essential Criteria	Anti-HCV
		Anti-HCV S: Co R
		HCV Core Ag
		HCVPCR (quantitative>qualitative)
		HCV genotype
		HCV detection in liver and extra-hepatic sites (PBMNs, lymphocytes, BM, etc.)
4	Supportive Criteria	Family history of HCV infection, type of exposure, injection drugs, alcohol intake, substance abuse, atypical symptoms, anti-viral treatment.
		Clinical findings (jaundice, ascites, visceromegaly and EHM)
		ALT elevation
		Hepatic steatosis
		HE scores>F1
		Liver biopsy (histopathology and PCR)
ALT: Alanine Aminotransferase; Anti-HCV Antibody: Anti-HCV Ab; EHM: Extra-Hepatic Manifestations; HE: Hepatic Elastography; HCV: Hepatitis-C; PBMNs: Peripheral Blood Mononuclear Cells; RT-PCR: Qualitative Polymerase Chain Reaction; qPCR: Quantitative PCR; S:Co R: Signal to Cut-Off Ratio; TLC: Total Leukocyte Count; TSH: Thyroid Stimulating Hormone		

Table 5: Mowahids' evaluation plan for HCV infection (algorithm).

A	"Healthy Carriers"/Job and visa Applicants	Anti-HCV positive (incidental detection); No Supportive Criterion: Confirm simultaneously with Anti-HCV S: Co R, HCV Core Ag and HCV qPCR with genotype; Baseline abdominal ultrasound (hepatic echogenicity) and HE score (liver stiffness); Consider as Inactive Carrier if evaluation non-revealing.
		"Versant HCVPCR" or 3 consecutive HCV qPCR at 6 month intervals for "low grade viremia".
		Issue "HCC" (15-year validity); and follow-up advice.
		Immediate follow-up (2 years): 6 monthly ALT, anti-HCV S: Co R and HCV Core Ag simultaneously; Yearly HCV qPCR, abdominal ultrasound (hepatic echogenicity) and HE score; Rising titers/scores important.
		Long-term follow-up (10 years): 2 yearly anti-HCV, anti-HCV S: Co R, HCV Core Ag and HCV qPCRs simultaneously; abdominal ultrasound and HE; Rising titers/scores important; 5 yearly HCVRNA detection in PBMNs and hepatocytes
B	Enlisted for HD/surgical intervention/blood donation	Anti-HCV positive (incidental detection) with absence of 3 or >3 supportive criteria: Confirm simultaneously with anti-HCV S: Co R, HCV Core Ag and HCV qPCR with genotype; Baseline abdominal ultrasound and HE score; Consider as Low grade viremia if evaluation non-revealing.
		Issue "HCC" (10-year validity); and follow-up advice.
		Initial Follow-up (3 years):6 monthly ALT, anti-HCV S: Co R, and HCV Core Ag simultaneously; Yearly HCV qPCR, abdominal ultrasound and HE score; Rising titers/scores important.
		Long term follow-up (10 years): 2 yearly OR on clinical suspicion: anti-HCV S: Co R, HCV Core Ag, HCV qPCR simultaneously, abdominal ultrasound and HE. Rising titers/scores important. 5 yearly OR on clinical suspicion HCVRNA detection in PBMNs and hepatocytes.
C	Valid Exposure with intention-to-treat	Anti-HCV positive on initial screening with presence of 3 or >3 supportive criteria: Confirm simultaneously with anti-HCV S: Co R, HCV Core Ag and HCV qPCR with genotype; Baseline abdominal ultrasound and HE score; Consider as true positive anti-HCV and treat.
		Issue "HEC" (Life time validity); and follow-up advice.
		Initial follow-up (5 years):6 monthly ALT, anti-HCV S: Co R and HCV Core Ag simultaneously; Yearly HCV qPCR, abdominal ultrasound and HE score; Rising titers/scores important. At least twice in 5 years OR on clinical suspicion HCV RNA detection in liver and extrahepatic sites.
		Long-term follow-up (15 years): 2 yearly OR on Clinical suspicion anti-HCV, anti-HCV S: Co R and HCV Core Ag simultaneously. 3 yearly OR on Clinical suspicion HCV qPCR, abdominal ultrasound and HE. Rising titers/scores important. 5 yearly OR on clinical suspicion HCVRNA detection in PBMNs and hepatocytes.
Anti-HCV Antibody: Anti-HCV Ab; AP: Alkaline Phosphatase; BM: Bone Marrow; EHM: Extra-Hepatic Manifestations; HE: Hepatic Elastography; HCV: Hepatitis-C; HCC: HCV Clearance Card; HD: Haemodialysis; HEC: HCV Exposure Card; PBMNs: Peripheral Blood Mononuclear Cells; RT-PCR: Qualitative Polymerase Chain Reaction; qPCR: Quantitative PCR; S:Co R: Signal to Cut-Off Ratio		

Table 6: Mowahid's evaluation plan for HCV infection (methodology).

seropositivity appears more common than "false" seropositivity [12], possibly because anti-HCV (unlike anti-HBs) is a weak and non-neutralizing antibody. Post-treatment seropositivity with negative HCVPCR is interpreted worldwide as "cure". Paradoxically though, incidental detection of anti-HCV in HCV carriers, blood donors and job/visa applicants are universally perceived as active HCV infection. This perception is possibly based on incessant B-cell response to "immunologically visible" HCVRNA. Stigmatization arising from an unclear relevance of anti-HCV has serious psychosocial impact. Our patients have encountered social segregation at home and in hospitals since >1.5-decade. Technical issues also emerge during enlistment of seropositive persons for hemodialysis and other indoor procedures with added risk of inadvertent HCV transmission [26,27].

Most patients do not clear HCV with IFN therapy [13]. An unusual treatment response to IFN in our patients is in sharp contrast to emerging resistance against Direct Acting Antivirals (DAAs) [28] with current focus on cellular mi-RNA-122 [9]. This observation portrays an unpredictable behavior of HCV. The credibility of "treatment-stopping rules [29,30] may well be questioned notwithstanding the influence of hepatic fibrosis [31] and "IFN-lambda protein 3" on "viral control" amongst females [23].

New diagnostic strategies for HCV infection include: simultaneous testing for anti-HCV and HCVRNA; detection of HCVRNA in PBMNs and hepatocytes [26]; 3 negative results of HCVqPCR [12,26], "Versant HCVRNA" [30], HCV Core Ag [32-35], anti-HCV S:Co R [12-41]. We have summed up these parameters to propose "Mowahids' Evaluation Plan" with emphasis on "simultaneous testing" and follow-up (Tables 5 and 6). Inspite of supportive data and 15 year follow-up, limitations in the present study are small number of patients, reliance on HCV RT-PCR, non-availability of initial results of HCV genotypes, and the need for liver biopsy.

Conclusion

There is ample evidence for persistence of anti-HCV and liver injury in subjects with normal ALT and aviremia. Anti-HCV, therefore, represents "true seropositivity" and merits uniform interpretation worldwide for a "true and ethical counseling" of the HCV infected persons. Seropositive individuals should be monitored for 5-years (HCV carriers) or 10-years (CHC patients).

Declaration

The authors of this study bear no conflict of interest

Authors contribution

QH: Designed the project, collected the data, analyzed and interpreted the data, wrote the first draft. KHH discussed and analyzed the data, interpreted the patient data, wrote the manuscript and submitted. All authors read and approved the final manuscript.

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