Brain Microstructural Correlates of Cognitive Dysfunction in Clinically and Biochemically Normal Hepatitis C Virus Infection

Ajay Kumar^{*}, Amar Deep^{†,*}, Rakesh K. Gupta[‡], Virendra Atam[§], Samir Mohindra[¶]

^{*}Department of Medical Gastroenterology, King George's Medical University, Lucknow 226003, India, [†]Experimental and Public Health Lab, Department of Zoology, University of Lucknow, 226003, India, [‡]Department of Radiodiagnosis, SGPGIMS, Lucknow 226003, India, [§]Department of Medicine, King George's Medical University, India and [¶]Department of Gastroenterology, SGPGIMS, Lucknow, UP, India

Background/Aims: This study examined correlates of the brain's neurocognitive performance among clinically and biochemically normal adult patient with hepatitis C virus (HCV). We hypothesized that anti-HCV positive individuals would demonstrate structural brain abnormalities and neurocognitive dysfunction as well as the changes in cell component and extracellular space in the white matter regions of brain in asymptomatic HCV infection by using diffusion tensor tractography (DTT) metrics. **Methods:** Anti-HCV positive patient ($n = 40$), and healthy controls ($n = 31$), fulfilling inclusion criteria (incidentally detected anti-HCV positive) and able to provide informed consent were screened and recruited for the study. All these subjects and controls underwent subjective assessment of their quality of life related symptoms, neuropsychometric tests (NPT) and magnetic resonance imaging. **Results:** The patients were subjected to neuroimaging as well as psychological testing. There was no significant difference in basic laboratory parameters in these two groups. Independent *t*-test reveals significantly lower neuropsychological functioning as compared to healthy control. A significantly decreased FA values and myoinsitol were observed in HCV subjects on sensory, inferior longitudinal fasciculus, and STR fiber bundles as compared to healthy control. Bivariate correlation analysis reveals that neuropsychological scores are significantly positive. **Conclusion:** Our result show that HCV positive individuals would demonstrate structural brain abnormalities and neurocognitive dysfunction as well as the changes in cell component and extracellular space in the white matter regions of brain in asymptomatic HCV infection by using DTT metrics. (J CLIN EXP HEPATOL 2017;7:198–204)

The hepatitis C virus (HCV), the second most common blood-borne illness in the world, is a leading cause of cirrhosis and liver cancer. Studies have shown that it is a direct cytopathic virus.¹ Neuropsychiatric abnormalities and cerebral microstructural changes are well known to occur due to cirrhosis and are generally associated with malfunctioning of portosystemic shunt, high ammonia concentration and astrocyte swelling.^{2,3} Recent studies conclusively suggested impairment in attention,^{4–8} psychomotor speed,^{7,9,10} executive function,^{7,10–12} motor skill,^{7,13,14} visual construction ability,¹⁵

learning, and memory^{6,12} in cirrhotics. The majority of patients infected with hepatitis C virus suffer from cognitive impairment and mood disturbances.^{16,17} It is interesting to note that the basic cognitive functions are apparent prior to the development of cirrhosis and are unrelated to extent of liver dysfunction, viral load, or genotype.^{18,19} As we know that the cognitive impairment and quality of life (QOL) are interrelated, studies have shown a significant reduction in QOL in hepatitis C patients.^{20–23} The limitations of these studies are that most of them have not tried to combine functional as well as anatomical modalities to look for these changes in asymptomatic volunteers infected with HCV. Diffusion tensor imaging (DTI) is a novel MRI technique that measures different DTI metrics: mean diffusivity (MD) is an index of water movement across cell membranes and fractional anisotropy (FA) is an index of microstructural integrity of the brain white matter. It has been shown to be useful in assessment of microstructural integrity in a number of clinical situations.

To the best of our knowledge, there is no published study that has addressed the issue of cerebral metabolite changes as well as diffusion weighted image changes in clinically and biochemically asymptomatic HCV-infected volunteers. The present study aims to find out the microstructural correlates of the neuropsychological changes in

Keywords: hepatitis C virus, neuropsychometric tests, MRI, myoinsitol, STR fiber bundles

Received: 2.07.2016; Accepted: 14.03.2017; Available online: 4 April 2017

Address for correspondence: Ajay Kumar, Assistant Prof, Department of Medical Gastroenterology, K.G. Medical University, Lucknow, UP 226003, India. Tel.: +91 9455519306.

E-mail: drajaymd12345@gmail.com

Abbreviations: BD: block design; CC: central cortex; Cho: choline; DS: digit symbol; DTT: diffusion tensor tractography; FA: fractional anisotropy; FCA: figure connection test A; FCB: figure connection test B; Gln: glutamine; Glx: glutamate; ILF: inferior longitudinal fasciculus; Ins: inositol; MD: mean diffusivity; NAA: N-acetylaspartate; OA: object assembly; PA: picture arrangement; PC: picture completion; QOL: quality of life; SLF: superior longitudinal fasciculus; STR: superior thalamic radiations
<http://dx.doi.org/10.1016/j.jceh.2017.03.011>

HCV-infected asymptomatic subjects with the help of advanced magnetic resonance imaging (MRI) methods. Our hypothesis was that HCV affects brain much before the occurrence of liver dysfunction and related cerebral changes are related to direct effect of HCV. This study will enable us to understand and foresee the problems related to direct effect of HCV in humans that may be of value to improve their QOL and well-being.

METHODS

Incidentally detected anti-HCV-positive patients without evidence of cirrhosis ($n = 40$) and healthy controls ($n = 31$), fulfilling inclusion criteria and are able to provide informed consent were screened and recruited for the study. Exclusion criteria were alcohol misuse (>50 g/day for males and 20 g/day for females), consumption of psychotropic drugs, bad vision, interferon therapy, symptoms/signs of overt liver disease (hepatic/extrahepatic), head injury, and any abnormality in routine biochemical tests. Out of 40 HCV patients, 15 HCV patients were excluded from the study due to either their unwillingness to undergo MRI ($n = 2$), or to undergo neuropsychometric tests ($n = 4$), or lack of their complete laboratory data ($n = 9$). Later, 6 HCV patients were excluded due to elevated transaminases. Finally, in the present study, anti-HCV-positive, biochemically and clinically normal subjects ($n = 19$) and healthy controls ($n = 31$) were included in the present study for the purpose of data evaluation. All these subjects and controls underwent subjective assessment of their quality of life-related symptoms, neuropsychometric tests (NPT) and MRI. Institutional ethics committee approved the study. Complete hemogram, serum electrolytes, renal and liver function tests were done in both controls and HCV-positive patients, while HCV RNA (COBAS® TaqMan® HCV Test v2.0; Roche diagnostics, Basel, Switzerland), TSH (thyroid stimulating hormone), ANA (antinuclear antibody), upper gastrointestinal endoscopy, and abdominal ultrasound were done in only HCV-positive subjects. Cirrhosis was diagnosed when any of the findings was present: encephalopathy, varices, splenomegaly, ascites, dilated portal vein, portosystemic collaterals, liver showing irregular margins, nodular surface, shrunken small size.

Neuropsychological Test (NPT)

NPTs were performed on patients and controls. NP tests included trail making test, number connection tests (NCT A, B), and figure connection test (FCT A) as well as the performance subset of modified Wechsler Adult Intelligence Scale (WAIS-P, modified for Indian population), which included the picture completion (PC), digit symbol (DS), block design (BD), picture arrangement (PA), and object assembly (OA). PC assesses visual recognition and identification (long-term visual memory), DS measures

sustained attention, concentration, and psychomotor speed; BD assesses spatial visualization, nonverbal concept formation, and visual-motor coordination and perceptual organization; PA is a test of ability to plan, interpret, and anticipate social events within a cultural context; and OA measures visual-motor integration. In NCT and FCT A, lower scores represent better performance, whereas in WAIS-P, a higher score represents better performance. The trail-making test assesses visual motor coordination, concentration, mental speed, memory alteration, and attention.

World Health Organization Quality of Life, Brief Version (WHOQOL Bref)

To assess QOL WHOQOL-Bref in Hindi, developed by Saxena, Chandiramani and Bhargava (1998) was used. The scale had 26 items. These items were distributed under four domains. The four domains of QOL were: (a) physical health and level of independence (seven items assessed areas such as presence of pain and discomfort, dependence on substances or treatments, energy and fatigue, mobility, sleep and rest, activities of daily living, perceived working capacity); (b) psychological well-being (eight items assessed areas such as affect, both positive and negative self-concept, higher cognitive functions; body image and spirituality); (c) social relationships (three items assessed areas such as social contacts, family support and ability to look after family and sexual activity); and (d) environment (eight items assessed areas such as freedom, quality of home environment, physical safety, security, financial status, involvement in recreational activity, health and social care, and quality and accessibility). Two items were examined separately: one which asked about the individual's overall perception of QOL and the other which asked about the individual's overall perception of his or her health. Domain scores were scaled in a positive direction (higher scores denoted better QOL). In this scale, responses were to be given on a 5-point rating scale, where 1 indicated low, negative perception and 5 indicated high, positive perception.

MRI Protocol

MRI including conventional MRI and DTI data were acquired on a 3 T MR scanner (Signa HDxt, General electric, Milwaukee, USA), using 8 channel head coil. Conventional MRI included fast spin echo (FSE) T2-weighted with echo time (TE)/repetition time (TR)/number of averages (NEX) 95 ms/5660 ms/2, 3D Fast Spoiled Gradient Echo Brain Volume with following parameters: 1 mm thickness with TR = 7.8 ms and TE = 3.0 ms, inversion time 400 ms, number of averages 1, image matrix of 256×256 with flip angle 13 degree was performed.

The diffusion tensor encoding was used as a vendor supplied DTI scheme with 30 uniformly distributed

directions. The sequence was performed in axial plane. The diffusion weighting b-factor was set to 1000 s/mm² field of view (FOV) = 240 mm × 240 mm, slice thickness = 3 mm, interslice gap = 0, and number of slices = 46. Localized single voxel point resolved spectroscopy (PRESS) with TE/TR = 35 ms/3000 ms and number of averages = 64 were obtained from the right basal ganglion with and without water suppression. Voxel volume was 8 ml. After global shimming, voxel shimming was performed, and a full width at half maximum of 8–12 Hz was achieved in all cases.

¹H MR Spectroscopy Quantification

Quantification of cerebral metabolites ratios were done as described in detail earlier.^{25,26} The quantification of all individual ¹H-MR spectra was done with LC Model software package²⁷ (Version 6.0; Stephen Provencher, Oakville, ON, Canada).

Diffusion Tensor Tractography (DTT) and Data Quantitation

Reconstruction of fibers was done utilizing fiber assignment by continuous tracking algorithm.²⁸ For fiber tracking, FA threshold of 0.15 was used. DTI measures were calculated for the entire fiber. Different fiber bundles have characteristic signature segments on the SFM (segmented fiber mass) color maps, and these fiber bundles can be automatically and reproducibly reconstructed by providing these segments as regions of interest to the fiber assignment by a continuous-tracking algorithm.^{28,29} This reconstruction enables to investigate anatomy and identify the coordinates of specific white matter tracts. The central sulcus was identified and marked on sagittal surface image reconstructed by 3D surface rendering of the b0 image stack. The central sulcus was displayed on the axial images by using 3D cross connectivity. Freehand region-of-interests (ROIs) were drawn on axial T2-weighted images near the brain's vertex on the precentral and postcentral gyri,

and the fibers generated from those ROIs were defined as motor and sensory tracts, respectively.²⁹ White matter (WM) fiber tracts were generated by using in-house developed JAVA-based software.³⁰

Sample Size Estimation and Statistical Analysis

We assumed that mean value of FA and MD in HCV, infected group and healthy control were (0.3 ± 0.05 and 0.5 ± 0.05) and (0.7 ± 0.05 and 0.9 ± 0.05) respectively. Under above assumptions, the minimum numbers of cases required were 9 and 8. We used independent *t*-test to compare the difference at *P* = 0.05 and 80% power for a two-tailed hypothesis. Independent *t*-test was used to analyze the significant difference among two groups (healthy control and HCV-positive) on neuropsychological measures, QOL, DTT, and metabolites. Bivariate correlation was used to see possible correlation among these variables. A *P* value of less than 0.05 was considered to be statistically significant. All the statistical analyses were performed using the SPSS statistical package (V16; SPSS Inc, Chicago, USA).

RESULT

Subjects from two study groups, that is, (i) asymptomatic anti-HCV-positive, (ii) healthy control, having comparable demographic features (age, sex, education, etc.), were subjected to neuroimaging as well as psychological testing. There was no significant difference in basic laboratory parameters in these two groups (Table 1).

Independent *t*-test reveals that patients with HCV showed significantly lower neuropsychological functioning on PC (*P* < 0.001), DS (*P* < 0.001), BD (*P* < 0.001), PA (*P* < 0.001), OA (*P* < 0.04), FCA (*P* < 0.017), and FCB (*P* < 0.001) as compared to healthy control (Table 2).

A significantly decreased FA values were observed in HCV subjects on sensory (*P* < 0.005), ILF (*P* < 0.046), and STR (*P* < 0.001) fiber bundles as compared to healthy

Table 1 Basic Parameters of HCV-positive Patients and Healthy Controls.

Parameters	Healthy control (mean ± SD)	HCV-positive (mean ± SD)	<i>P</i> value
Hb (g/dl)	13.3 ± 1.3	13.1 ± 2.0	0.55
TLC (×1000 per cmm)	6.7 ± 1.5	5.9 ± 1.3	0.06
PLT (×1000 per cmm)	158.8 ± 56.6	132.5 ± 38.6	0.08
Creatinine (mg/dl)	0.7 ± 0.3	0.8 ± 0.2	0.50
SGOT (U/L)	50.6 ± 6.6	53.3 ± 7.0	0.17
SGPT (U/L)	50.6 ± 6.8	54.1 ± 10.0	0.14
ALP (U/L)	80.7 ± 37.2	126.6 ± 19.0	0.50
Alb (mg/dl)	4.1 ± 0.5	4.3 ± 0.3	0.08
HCV RNA (IU/ml)	–	5,511,387 ± 1.355	–

Table 2 Summary of Mean Neuropsychological Test Scores in HCV-positive Patients and Healthy Controls.

Group	PC	DS	BD	PA	OA	FCA	FCB
Healthy control	14.90 ± 1.39	11.13 ± 2.18	12.77 ± 1.31	13.26 ± 1.03	11.19 ± 1.13	60.68 ± 23.88	81.39 ± 26.01
HCV-positive	10.1 ± 2.3	8.1 ± 2.2	9.7 ± 2.5	10.4 ± 1.8	10.1 ± 2.0	78.5 ± 26.1	118.6 ± 37.7
P value	0.001	0.001	0.001	0.001	0.04	0.017	0.001

Table 3 Summary of Mean DTT Measures (FA) in HCV-positive Patients and Healthy Controls.

Group	FA			MR spectroscopy
	Sensory	ILF	STR	Ins
Healthy control	0.44 ± .01	0.43 ± .02	0.46 ± .02	0.49 ± 0.16
HCV-positive	0.42 ± .02	0.42 ± .02	0.43 ± .02	0.29 ± 0.13
P value	0.005	0.046	0.001	0.002

Table 4 Quality of Life in HCV-positive Patients and Healthy Controls.

Group	Physical	Psychological	Environmental	Social
Healthy control	14.87 ± 1.8	14.6 ± 1.87	13.6 ± 2.04	13.9 ± 2.2
HCV-positive	11.4 ± 1.5	11.1 ± 2.02	11.1 ± 1.9	11.6 ± 1.9
P value	<0.001	<0.001	<0.001	<0.001

control (Table 3 and Figure 1). A significantly decreased myoinositol value was observed in HCV-positive subjects as compared to healthy control (Table 3).

Significantly decreased QOL measures were observed in patients with HCV-infected subjects on physical ($P < 0.001$), psychological ($P < 0.001$), social ($P < 0.001$), and environmental ($P < 0.001$) assessment (Table 4).

Bivariate correlation analysis reveals that neuropsychological scores significantly positively correlated with FA value of sensory, motor, STR, SLF, and CC fiber bundles (Table 5). Glx significantly positively correlated with FA value of CC. Neuropsychological scores significantly positively correlated with all the domains of QOL (Table 5).

Figure 1 Summary of mean DTT measures (FA) in HCV positive patients and healthy controls.

DISCUSSION

To the best of our knowledge, this is the first quantitative DTT study showing major white matter tracts in clinically and biochemically normal subjects infected with HCV and their comparison with age- and sex-matched controls. Anti-HCV-positive individuals showed significantly lower neuropsychological functioning and QOL scores. A significantly decreased myoinositol and decreased FA values were found in certain fiber bundles.

While HCV is primarily a hepatotropic virus, the *flaviviridae* family includes a number of well-known neurotropic viruses (e.g., West Nile virus, yellow fever virus, dengue virus, and tick-borne encephalitis virus). HCV replicates through an RNA negative-strand intermediate, the presence of which is regarded as direct evidence of replication.³¹ Letendre et al.³² demonstrated the presence of HCV proteins in brain tissue by Western blotting and immunostaining. However, while there is convincing evidence of HCV neuro-invasion, it is not clear which cells in the brain harbor HCV. Wilkinson et al. showed that HCV infects brain microglia/macrophages and, to a lesser extent, astrocytes.³³ Seifert et al. described a case of tetraparesis with encephalopathy with detectable HCV-RNA in serum and brain tissues. MRI of the brain revealed leukoencephalopathy with signal alterations in bilateral white matter, splenium, pons, and medulla oblongata. Magnetic resonance spectroscopy revealed reduced N-acetylaspartate (NAA) and lactate.³⁴ Further evidence of HCV neuro-invasion is provided by recent laboratory investigations, where CSF is

Table 5 Pearson's Correlation of FA Values and Quality of Life with NPT and Metabolites in HCV-positive Patients and Healthy Controls.

	PC	DS	BD	PA	OA	FCA	FCB	NAA	Cho	Gln	Glx	Ins
(FA)												
Sensory	.38**	.50**		.34*	.30*	-.42**	-.52*					
Motor		.27*		.26*		-.31*	-.31*					
SLF	.38*	.42*		.34*		-.48**	-.49**	.30*				
STR	.34*	.29*		.28*			-.34**					
CC		.30*				-.45**	-.37**				.55**	
(QOL)												
Physical	.47**	.43**	.42**	.56**			-.39**					
Psychology	.50**	.33**	.43**	.53**			-.39**					
Social	.36**	.27*		.31*			-.28*					
Environmental	.32*	.31*	.31*	.42**				-.48**				

* and ** cited to denote level of significance of *r* values.

found to be HCV-positive in more than 50% of HCV-positive patients.^{28,35,36}

The exact biological mechanism by which HCV enters the central nervous system is unknown. Two major hypotheses were proposed to explain in the available literature which interpret neurocognitive impairment associated with HCV: first, HCV may cross the blood-brain barrier by infected monocytes using a Trojan horse mechanism and³⁷ second, a nonspecific reaction of brain cells to mediators of inflammation such as cytokines.¹⁸

DTT and MRS

In our study, significantly decreased FA values were observed in HCV on sensory, ILF, and STR fiber bundles as compared to healthy control. The most probable explanation for decreased FA values in above white matter tract could be due to the inflammatory changes induced by HCV. These types of changes in FA have been described in Alzheimers' disease,³⁸ subacute sclerosing panencephalitis,³⁹ and old age.⁴⁰ We observed a significantly decreased inositol values in anti-HCV-positive subjects as compared to healthy control which is in contradiction to the literature. Two separate studies have shown significant increase in myoinositol in advanced HCV disease⁴¹ as well as histologically proven mild chronic hepatitis C.⁴² Myoinositol is considered a glial marker; however, it has been shown to be present in neuronal cells. An increase in myoinositol on MR spectroscopy is considered to be due to neuroinflammation and gliosis while its reduction is noticed in hepatic encephalopathy as well in psychiatric patients on lithium therapy. However, it may be low in patients with bipolar disorders and may not show a decrease in patients who are on lithium therapy. HCV virus needs phosphoinositols for its replication inside the cells and use the host cell inositols for its replication. Studies have shown an increase in inositols in established

hepatitis C viral infection of the brain, and it is considered to be due to neuroinflammation. However, in the present study, we found a significant reduction in myoinositols in the brain. All our patients had asymptomatic anti-HCV-positive and did not have any biochemical and clinical abnormalities. It is likely that state of inflammation was not present.

Neuropsychological Test

Asymptomatic patients with HCV infection have shown lower NPT scores, which indicates poor visual recognition and identification (long-term visual memory), poor sustained attention, concentration and psychomotor speed, poor spatial visualization, nonverbal concept formation, visuoconstruction ability and perceptual organization, and inability to plan, interpret, and anticipate social events as compared to normal healthy control. Similar findings reported in other studies have shown cognitive impairment in patients infected with HCV.^{4-7,11,13,15}

In the present study, asymptomatic HCV-infected subjects reported deficits in attention. Similarly study conducted by Hilsabeck et al.⁶ in which they observed attention deficits in HCV infection with and without other medical comorbidities found that up to 82% of HCV-positive individuals with other medical co-morbidities had impaired performance on tests of sustained attention, and 59% showed impairment on tests of selective attention, whereas 49% of HCV-positive individuals without other medical co-morbidities had impairment on sustained attention and 25% had impairment on selective attention.⁶ Another study found poor selective attention in HCV-infected patients with cirrhosis,⁷ whereas other studies have demonstrated that HCV-infected patients at asymptomatic stage show deficits in attention.^{4,5,8}

In our study, we observed poor psychomotor speed among HCV-infected group as compared to healthy

control group. Previous studies reported impairment in psychomotor speed among HCV-positive individuals.^{4,7,9,11} Poor visuoconstruction abilities were observed in HCV-infected group as compared to healthy control group. However, Hilsabeck et al.¹⁵ found impaired vasoconstriction ability only in 9% of HCV-positive individuals. Most of the previous studies evaluating visuoconstruction skill in HCV have examined drawing, whereas in the present study, we measure this skill by using building and assembly tasks, which evaluated visuoconstruction as well as motor abilities. In the present study, individuals infected with HCV were found to have difficulty in planning, interpreting, and anticipating social events. Similarly, impaired executive functions were reported among individual infected with HCV.^{7,10–12}

Quality of Life (QOL)

In the present study, we observed that HCV-infected people reported poor QOL in all the domains, including physical, psychological, social, and environmental. Similarly, numbers of studies have reported that HCV has a negative impact on QOL even in the absence of symptoms.^{21,43–45} Reduction in QOL among asymptomatic HCV-infected people reflect general reduction in well-being associated with the knowledge of a serious illness.

Asymptomatic anti-HCV-positive patients include full gamut of patients including no fibrosis to advanced fibrosis and cirrhosis that may affect liver function and secondarily structural and functional brain changes. Since we have not included liver biopsy or Fibroscan to stage fibrosis, our data may not discriminate changes in different stages of fibrosis.

CONCLUSION

We conclude that it is possible to identify the changes in cell component and extracellular space in the white matter regions of brain in asymptomatic HCV infection by using DTT metrics. Significant correlation of FA values with NPT scores suggests that the changes in cell component and extracellular space in the white matter regions may be responsible for the impairment in cognitive functions. These DTT metrics may be used as an image biomarker of cognition in asymptomatic HCV infection. The important limitations of the study were that numbers of cases were small and we used normal transaminase values as surrogate marker of normal liver structure and function, while liver biopsy is the gold standard to rule out significant fibrosis or cirrhosis. In future studies, either liver biopsy or newer non-invasive tests such as transient elastography can be used to correlate MRI parameters and other variables used in our study.

CONFLICTS OF INTEREST

The authors have none to declare.

REFERENCES

1. Negro F. Hepatitis C virus-induced steatosis: an overview. *Dig Dis*. 2010;28:294–299.
2. Cudalbu C. In vivo studies of brain metabolism in animal models of Hepatic Encephalopathy using (1)H Magnetic Resonance Spectroscopy. *Metab Brain Dis*. 2012.
3. Lemberg A, Fernandez MA. Hepatic encephalopathy, ammonia, glutamate, glutamine and oxidative stress. *Ann Hepatol*. 2009;8:95–102.
4. Weissenborn K, Krause J, Bokemeyer M, et al. Hepatitis C virus infection affects the brain-evidence from psychometric studies and magnetic resonance spectroscopy. *J Hepatol*. 2004;41:845–851.
5. Forton DM, Hamilton G, Allsop JM, et al. Cerebral immune activation in chronic hepatitis C infection: a magnetic resonance spectroscopy study. *J Hepatol*. 2008;49:316–322.
6. Hilsabeck RC, Perry W, Hassanein TI. Neuropsychological impairment in patients with chronic hepatitis C. *Hepatology*. 2002;35:440–446.
7. Cordoba J, Flavia M, Jacas C, et al. Quality of life and cognitive function in hepatitis C at different stages of liver disease. *J Hepatol*. 2003;39:231–238.
8. Huckans M, Seelye A, Parcel T, et al. The cognitive effects of hepatitis C in the presence and absence of a history of substance use disorder. *J Int Neuropsychol Soc*. 2009;15:69–82.
9. McAndrews MP, Farcnik K, Carlen P, et al. Prevalence and significance of neurocognitive dysfunction in hepatitis C in the absence of correlated risk factors. *Hepatology*. 2005;41:801–808.
10. Fontana RJ, Bieliauskas LA, Lindsay KL, et al. Cognitive function does not worsen during pegylated interferon and ribavirin retreatment of chronic hepatitis C. *Hepatology*. 2007;45:1154–1163.
11. Fontana RJ, Bieliauskas LA, Back-Madruga C, et al. Cognitive function in hepatitis C patients with advanced fibrosis enrolled in the HALT-C trial. *J Hepatol*. 2005;43:614–622.
12. Karaivazoglou K, Assimakopoulos K, Thomopoulos K, et al. Neuropsychological function in Greek patients with chronic hepatitis C. *Liver Int*. 2007;27:798–805.
13. Bieliauskas LA, Back-Madruga C, Lindsay KL, et al. Clinical relevance of cognitive scores in hepatitis C patients with advanced fibrosis. *J Clin Exp Neuropsychol*. 2006;28:1346–1361.
14. Vigil O, Posada C, Woods SP, et al. Impairments in fine-motor coordination and speed of information processing predict declines in everyday functioning in hepatitis C infection. *J Clin Exp Neuropsychol*. 2008;30:805–815.
15. Hilsabeck RC, Hassanein TI, Carlson MD, Ziegler EA, Perry W. Cognitive functioning and psychiatric symptomatology in patients with chronic hepatitis C. *J Int Neuropsychol Soc*. 2003;9:847–854.
16. Kenny-Walsh E. Clinical outcomes after hepatitis C infection from contaminated anti-D immune globulin. Irish Hepatology Research Group. *N Engl J Med*. 1999;340:1228–1233.
17. Singh N, Gayowski T, Wagener MM, Marino IR. Quality of life, functional status, and depression in male liver transplant recipients with recurrent viral hepatitis C. *Transplantation*. 1999;67:69–72.
18. Forton DM, Allsop JM, Main J, Foster GR, Thomas HC, Taylor-Robinson SD. Evidence for a cerebral effect of the hepatitis C virus. *Lancet*. 2001;358:38–39.
19. Forton DM, Thomas HC, Murphy CA, et al. Hepatitis C and cognitive impairment in a cohort of patients with mild liver disease. *Hepatology*. 2002;35:433–439.

20. Carithers Jr RL, Sugano D, Bayliss M. Health assessment for chronic HCV infection: results of quality of life. *Dig Dis Sci*. 1996;41:75S–80S.
21. Foster GR, Goldin RD, Thomas HC. Chronic hepatitis C virus infection causes a significant reduction in quality of life in the absence of cirrhosis. *Hepatology*. 1998;27:209–212.
22. Rodger AJ, Jolley D, Thompson SC, Lanigan A, Crofts N. The impact of diagnosis of hepatitis C virus on quality of life. *Hepatology*. 1999;30:1299–1301.
23. Barrett S, Goh J, Coughlan B, et al. The natural course of hepatitis C virus infection after 22 years in a unique homogenous cohort: spontaneous viral clearance and chronic HCV infection. *Gut*. 2001;49:423–430.
25. Hasan KM, Gupta RK, Santos RM, Wolinsky JS, Narayana PA. Diffusion tensor fractional anisotropy of the normal-appearing seven segments of the corpus callosum in healthy adults and relapsing-remitting multiple sclerosis patients. *J Magn Reson Imaging*. 2005;21:735–744.
26. Srivastava A, Yadav SK, Lal R, et al. Effect of surgical portosystemic shunt on prevalence of minimal hepatic encephalopathy in children with extrahepatic portal venous obstruction: assessment by magnetic resonance imaging and psychometry. *J Pediatr Gastroenterol Nutr*. 2010;51:766–772.
27. Yadav SK, Saksena S, Srivastava A, et al. Brain MR imaging and 1H-MR spectroscopy changes in patients with extrahepatic portal vein obstruction from early childhood to adulthood. *AJNR Am J Neuroradiol*. 2010;31:1337–1342.
28. Mori S, Crain BJ, Chacko VP, van Zijl PC. Three-dimensional tracking of axonal projections in the brain by magnetic resonance imaging. *Ann Neurol*. 1999;45:265–269.
29. Trivedi R, Agarwal S, Shah V, et al. Correlation of quantitative sensorimotor tractography with clinical grade of cerebral palsy. *Neuroradiology*. 2010;52:759–765.
30. Trivedi R, Agarwal S, Rathore RK, et al. Understanding development and lateralization of major cerebral fiber bundles in pediatric population through quantitative diffusion tensor tractography. *Pediatr Res*. 2009;66:636–640.
31. Lanford RE, Sureau C, Jacob JR, White R, Fuerst TR. Demonstration of in vitro infection of chimpanzee hepatocytes with hepatitis C virus using strand-specific RT/PCR. *Virology*. 1994;202:606–614.
32. Letendre S, Paulino AD, Rockenstein E, et al. Pathogenesis of hepatitis C virus coinfection in the brains of patients infected with HIV. *J Infect Dis*. 2007;196:361–370.
33. Wilkinson J, Radkowski M, Laskus T. Hepatitis C virus neuroinvasion: identification of infected cells. *J Virol*. 2009;83:1312–1319.
34. Seifert F, Struffert T, Hildebrandt M, et al. In vivo detection of hepatitis C virus (HCV) RNA in the brain in a case of encephalitis: evidence for HCV neuroinvasion. *Eur J Neurol*. 2008;15:214–218.
35. Laskus T, Radkowski M, Bednarska A, et al. Detection and analysis of hepatitis C virus sequences in cerebrospinal fluid. *J Virol*. 2002;76:1006–1084.
36. Morsica G, Bernardi MT, Novati R, Uberti Foppa C, Castagna A, Lazzarin A. Detection of hepatitis C virus genomic sequences in the cerebrospinal fluid of HIV-infected patients. *J Med Virol*. 1997;53:252–254.
37. Forton DM, Taylor-Robinson SD, Thomas HC. Central nervous system changes in hepatitis C virus infection. *Eur J Gastroenterol Hepatol*. 2006;18:333–338.
38. Kiuchi K, Morikawa M, Taoka T, et al. White matter changes in dementia with Lewy bodies and Alzheimer's disease: a tractography-based study. *J Psychiatr Res*. 2011;45:1095–1100.
39. Trivedi R, Anuradha H, Agarwal A, et al. Correlation of quantitative diffusion tensor tractography with clinical grades of subacute sclerosing panencephalitis. *AJNR Am J Neuroradiol*. 2011;32:714–720.
40. Gao J, Cheung RT, Lee TM, et al. Possible retrogenesis observed with fiber tracking: an anteroposterior pattern of white matter disintegrity in normal aging and Alzheimer's disease. *J Alzheimers Dis*. 2011;26:47–58.
41. Nagarajan R, Sarma MK, Thames AD, Castellon SA, Hinkin CH, Thomas MA. 2D MR spectroscopy combined with prior-knowledge fitting is sensitive to HCV-associated cerebral metabolic abnormalities. *Int J Hepatol*. 2012;2012:179365.
42. Grover VP, Pavese N, Koh SB, et al. Cerebral microglial activation in patients with hepatitis C: in vivo evidence of neuroinflammation. *J Viral Hepat*. 2012;19:e89–e96.
43. Ware Jr JE, Bayliss MS, Mannocchia M, Davis GL. Health-related quality of life in chronic hepatitis C: impact of disease and treatment response. The Interventional Therapy Group. *Hepatology*. 1999;30:550–555.
44. Bonkovsky HL, Woolley JM. Reduction of health-related quality of life in chronic hepatitis C and improvement with interferon therapy. The Consensus Interferon Study Group. *Hepatology*. 1999;29:264–270.
45. Bernstein D, Kleinman L, Barker CM, Revicki DA, Green J. Relationship of health-related quality of life to treatment adherence and sustained response in chronic hepatitis C patients. *Hepatology*. 2002;35:704–708.