

Clinicopathological features and genotype distribution in patients with hepatitis C virus chronic liver disease

Rachel Abraham · Banumathi Ramakrishna · Avinash Balekuduru · Hubert Darius J. Daniel · Priya Abraham · C. Eapen Eapen · George Kurian

Abstract

Background and Objective Hepatitis C virus (HCV) genotype influences the severity of disease and response to therapy. This retrospective study examined the clinical and histological features and the genotype distribution in biopsied patients with HCV related chronic liver disease.

Methods Of 105 biopsies from patients with HCV infection, 96 from patients with chronic liver disease were reviewed. The Ishak scoring system was used for histological analysis.

Results Genotype 3 was most common accounting for 77.1%, and genotype 1 for 9.4% of cases. There was no significant association of transaminase levels, viral load or necro-inflammatory activity score with genotype. A severe degree of fibrosis was seen in 77.8% cases of genotype 1 and in 63.5% of genotype 3 ($p=0.76$). Variable degrees of steatosis were noted in 68.8% of cases. However, severe steatosis was noted only in genotype 3 (7 cases). Serum transaminase levels did not correlate with either histological activity ($p=0.43$) or degree of fibrosis ($p=0.72$). Severe fibrosis / cirrhosis was seen in 74.24% of patients above 40 years of age as compared to 33.3% of patients below 40 years ($p=0.001$). The frequency of Mallory hyaline was significantly different between genotypes 1 and 3 infection ($P<0.001$).

Conclusions This study confirms the preponderance of genotype 3 in Indian patients with HCV related chronic liver disease. Severe steatosis was seen only in genotype 3 and Mallory hyaline was very common in genotype 1. The small numbers

of patients in non genotype 3 could be a reason for the apparent lack of histological differences between different HCV genotypes. Severe fibrosis seen in older age groups confirms that HCV infection is progressive and major acceleration of the disease process occurs after 40 years of age.

Keywords Fibrosis Genotype Hepatitis C Histological activity Steatosis

Introduction

Hepatitis C virus (HCV) infection accounts for approximately 25% of all chronic liver disease in India.^{1,2} At least 6 genotypes and several subtypes of HCV have been identified based on the variation in nucleotide sequence. There is a marked geographic variation in the distribution of the HCV genotypes.³ In studies carried out in different regions of India, genotype 3 has been the most commonly identified genotype with 3a/b being the most common subtype.^{4,5,6} This study was undertaken to determine the genotype distribution, and to assess the clinico-pathological features, in biopsied patients with HCV-related chronic liver disease, in a tertiary care hospital in South India.

Methods

Liver biopsies from 162 patients with hepatitis C virus infection were received at the Department of Pathology, of our Institute, during the period January 2003 to December 2006. Of the 162 cases, liver biopsies from 105 patients in whom HCV genotyping was done constituted the material for this study. All biopsy specimens were fixed in 10% formalin, paraffin embedded, and 3 micron thick sections were cut. Hematoxylin–eosin, periodic acid Schiff with and without diastase, van Gieson, reticulin, Perl's iron and orcein stains were done. The type of liver biopsies were percutaneous (77), transjugular (20) and ultrasound-guided (2). One patient had both percutaneous and wedge biopsy. In 3 patients who underwent liver biopsies elsewhere, slides alone in 2 cases and both slides and paraffin block in 1 case, were available. Hepatectomy specimens were received from 2 patients who underwent liver transplantation (OLT).

R. Abraham¹ · B. Ramakrishna¹ · A. Balekuduru² · H. D. J. Daniel³ · P. Abraham³ · C. E. Eapen² · G. Kurian²

¹Departments of Pathology, ²Gastrointestinal Sciences, and ³Clinical Virology, Christian Medical College, Vellore, India

B. Ramakrishna (✉)
e-mail: banu@cmcvellore.ac.in

Received: 17 November 2008 / Revised: 27 February 2009 / Accepted: 28 February 2009

© Indian Society of Gastroenterology 2009

The clinical details regarding possible routes of transmission and liver function tests were obtained from hospital records. Clinical features were analyzed with respect to age, sex, duration of disease in months, and duration from the date of exposure to the development of the disease in years. The genotype was determined by performing polymerase chain reaction (PCR) using primers specific for the core region of the HCV genome.⁴ If there was any ambiguity, sequencing of the core region was done. The Ishak scoring system was used to assess the grade / histological activity index (HAI) and stage / degree of fibrosis.⁷ Since the necroinflammatory activity in HCV is usually mild, the final HAI score was given a verbal assessment as minimal (0–3), mild (4–6), moderate (7–9) and severe (>9), for statistical analysis. The degree of fibrosis was verbally graded as absent (0), mild (1–2), moderate (3), severe (4–5) or cirrhosis (6, probable / definite). The frequency of the following histological features i.e., presence of lymphoid aggregates or follicles, bile duct damage, bile ductular proliferation, sinusoidal lymphocytosis, acidophil bodies, Mallory hyaline, epithelioid cell granulomas and type and degree of steatosis were noted. Stainable iron present in parenchymal and/or mesenchymal location was noted and graded according to the method of Sciot *et al.*⁸ Steatosis was semiquantitatively graded as mild, moderate or severe degree according to the percentage of hepatocytes involved.⁹ The assessment of histological features was carried out without knowledge of the genotype of the patients.

Therapy and follow up

Information regarding the response to therapy was collected from the clinical records. A negative PCR for HCV RNA at the end of 6 months of therapy for non-1 genotype and at the end of 1 year therapy for genotype 1 was considered as end of treatment viral response (ETVR). The minimum follow up period available for assessment of sustained viral response (SVR) was 9 months after the RNA became negative and the maximum was 24 months.

Statistical analysis

Data were analyzed using SPSS software. Statistical significance was tested using the Chi-square or Fisher's exact test. The analysis for the association of age with the degree of fibrosis and that for HCV RNA titer with histological activity was done using the Spearman rank correlation test. A two tailed 'P' value of less than 0.05 was considered significant.

Results

Of the 105 liver biopsies, 4 were from patients who were on haemodialysis for end stage renal disease, 3 from patients with beta-thalassaemia and 2 from patients who had co-infection with hepatitis B virus. The remaining 96 (including 2 native hepatectomies) were from patients with chronic liver disease and were taken for this study.

HCV genotypes and clinical correlations

Genotype 3 was most common accounting for 74 cases, with 3a being the most common. The age at presentation was not significantly different between the genotypes (Table 1).

Table 2 shows the identifiable risk factors for HCV infection. No significant differences were noted between different genotypes. The alanine aminotransferase levels (ALT) ranged from normal in 15 cases to five-fold or greater levels in 22 cases. HCV RNA titer was available in 51 cases. The lowest viral titer was 100 IU/ml and the highest was 2×10^7 IU/mL with a median value of 65000 IU/mL. There was no significant association between viral load and HCV genotype ($p = 0.23$).

Biopsy sample characteristics

The biopsies were fragmented in 26 cases. In the remaining 68 needle biopsies, the average number of cores was 1.66. The core length ranged from 1–1.5 cms. Of the 2 native hepatectomy specimens from patients who underwent OLT, in one, the external and cut surfaces were diffusely

Table 1 Demographic features of patients with chronic hepatitis C

	Genotypes					Total
	1	2	3	4	6	
Number	9	1	74	7	5	96
Subtype	a-3, b-6	a-1	a-32, b-18, a/b-12, b/ g-8, i-1, g-1, f-2	d-1,6*	5*	
Age (years)	49.2 (12.3)	59.0	45.2 (12.1)	45.6 (2.5)	46.2 (12.6)	45.8 (12.0)
Sex M: F	3.5 : 1	1 M	1.74 : 1	6 : 1	4 : 1	2.09 : 1
Duration of disease (years)**	11.7 (7.6)	30	10.9 (6.3)	13.5 (2.1)	3	11.4 (6.8)

Data are as mean (SD)

*This genotyping method identifies genotype 4 and not any of its subtypes. Samples identified as genotype 6 were also sequenced – closest homology with GenBank strains of genotype 6 (subtype not specified in database)

**Information available for 42 patients

Table 2 Risk factors in chronic hepatitis C patients in relation to genotype

Mode of transmission	Genotypes					Total (n [%])
	1	2	3	4	6	
Number	9	1	74	7	5	96
Surgery	2	1	19	2	1	25 (26)
Transfusion**	-	-	19	-	-	19 (19.8)
I/V drug abuse	-	-	7	2	-	9 (9.4)
Needle stick injury	2	-	2	-	-	4 (4.16)
Unprotected exposures	-	-	-	-	1	1 (1.04)
Unidentifiable	5	-	27	3	3	38 (39.6)

**Includes 8 patients who had transfusion associated with a surgical procedure

Table 3 Histological features in relation to hepatitis C virus genotype

		Genotype					Total (n [%])
		1	2	3	4	6	
Number		9	1	74	7	5	96
Grade of inflammation							
	Median (Range)	6 (4–8)	-	5 (1–9)	5 (4–6)	4 (4–6)	
	Minimal (0–3)	-	-	8	-	0	8 (8.3)
	Mild (4–6)	6	-	44	7	5	62 (64.6)
	Moderate (7–9)	3	1	22	-	-	26 (27.1)
Degree of fibrosis	Median (range)	6 (2–6)	-	6 (0–6)	3 (0–6)	1 (1–6)	
	Mild (1–2)	1	1	11	2	3	18 (18.7)
	Moderate (3)	1	-	9	1	0	11 (11.4)
	Severe (4–5)	1	-	6	0	0	7 (7.3)
	Cirrhosis (6)	6	-	41	3	2	52 (54.2)
Steatosis	Macrovesicular	3	-	29	5	3	40 (41.7)
	Microvesicular	-	-	5	-	-	5 (5.2)
	Mixed	3	-	18	-	-	21 (21.9)
Degree	Mild	1	-	21	5	1	28 (29.2)
	Moderate	5	-	24	-	2	31 (32.3)
	Severe	-	-	7	-	-	7 (7.3)
Lymphoid aggregates		7*	1	33*	4	2	47 (48.9)
Bile duct damage		6*	1	26*	-	3	36 (37.5)
Bile ductular proliferation		6*	1	45*	4	1	57 (59.3)
Acidophil bodies		3*	1	18*	-	1	23 (23.9)
Sinusoidal lymphocytosis		4*	1	30*	5	1	41 (42.7)
Mallory hyaline		7**	-	10**	1	-	18 (18.7)

Fisher's exact test * p= NS, **p= 0.0001

nodular with nodules ranging from 0.1–0.4 cm and also had 2 circumscribed nodules measuring 2 and 1.6 cm in maximum diameter. The other showed multiple nodules of varying size measuring 0.1–0.7 cm in diameter. Of 42 cases in which portal tracts were counted, the average number of portal tracts present was 11.

Histological features

Of 96 cases, the severity of chronic hepatitis was classified as minimal in 8, mild in 62 and moderate in 26 cases. None of the cases showed severe necroinflammation. There was no correlation between the degree of histological activity and genotypes (Table 3).

Interface hepatitis was present in 93 cases (96.9%). Bridging necrosis was not seen in any of the cases. The distribution of portal and lobular inflammation was similar in all genotypes.

Cirrhosis was micronodular in 34 (65.4%), mixed type in 5 (9.6%) and untypable in 13 cases (25%) due to the fragmented nature of the biopsy samples. There was no correlation between the degree of fibrosis and genotype ($p=0.76$). One hepatectomy specimen of a patient with genotype 3 infection showed cirrhosis with well-differentiated hepatocellular carcinoma.

Varying degree of steatosis was present. The most severe grade of steatosis was seen only in genotype 3, in 7 cases. There was no difference in the frequency of various histological features between genotypes 1 and 3, except for Mallory hyaline ($p=0.0001$). Epithelioid cell granulomas were noted in 3 cases belonging to genotype 3. One had a history of treatment for pulmonary tuberculosis, another presented with abdominal tuberculosis later and the 3rd patient had systemic lupus erythematosus with vasculitis and was on treatment with steroids. Steatohepatitis was noted in 3 cases and one patient was a diabetic.

Hepatic iron deposition was seen in a total of 14 biopsies (5 grade I, 8 grade II and 1 grade III), 10 of which belonged to genotype 3.

ALT levels did not correlate with histological activity or severity of fibrosis (Table 4). HCV RNA levels (in the 51

patients where available) did not correlate with histological activity ($p=0.07$). A significant association was noted between the age of the patient and the severity of fibrosis ($p=0.001$) (Table 5).

Treatment response

Information regarding response to therapy was available in 50 patients; 22 patients were tested for ETVR alone, 17 for SVR alone and 11 for both ETVR and SVR. Twenty five of 33 patients achieved ETVR, and 19/28 patients achieved SVR. There was no difference in treatment response between the genotypes.

Discussion

This study reconfirmed that genotype 3, particularly subtype 3a, was the most common HCV genotype in Indian patients with chronic liver disease due to hepatitis C virus infection. Genotype 1 was noted in a small number of patients. Genotype 6, found in 5 cases, has earlier been described only in isolated case reports from India.¹⁰ Mixed genotype or genotype 5 was not seen in this study, the latter being restricted to Hong Kong and Vietnam.^{3,4}

Genotype did not correlate with age in this study, in contrast to other studies suggesting that genotype 3a and 1a occur in younger individuals and 1b in an older age group.^{11,12} The correlation of increasing age with severe fibrosis, as observed in previous studies confirms that HCV is a pro-

Table 4 Comparison of grade and stage of liver disease with serum alanine aminotransferase levels

ALT	Grade				Fibrosis score					
	Minimal (0-3)	Mild (4-6)	Moderate (7-9)	Total (n [%])	0	Mild (1-2)	Moderate (3)	Severe (4-5)	Cirrhosis (6)	Total (n [%])
Normal	2	10	3	15 (15.6)	1	2	2	2	8	15 (15.6)
2 X ULN	3	21	5	29 (30.2)	3	8	2	1	15	29 (30.2)
3 X ULN	2	8	5	15 (15.6)	2	3	3	2	5	15 (15.6)
4 X ULN	1	7	7	15 (15.6)	1	-	2	1	11	15 (15.6)
5 X ULN or more	-	15	7	22 (22.9)	1	5	1	2	13	22 (22.9)

ALT – alanine aminotransferase. Normal ALT – 8-40 IU/mL. ULN – upper limit of normal. Grade vs ALT: chi-square test, $p = 0.43$. Stage vs ALT: chi-square test, $p=0.72$

Table 5 Correlation of patients' age with degree of fibrosis

Age (years)	Score				
	0	1-2	3	4-5	6
18-29	3	3	2	1	-
30-39	3	5	4	2	7
40-49	2	1	2	1	15
50-59	1	7	2	2	25
>60	-	1	1	1	5
	9 (9.4%)	17 (17.7%)	11 (11.5%)	7 (7.3%)	52 (54.2%)

Spearman's correlation test, $p=0.001$

gressive disease with major acceleration in the rate of fibrosis after 50 years.^{13,14} A definite male predominance was noted among all genotypes, with no difference in the male to female ratio across genotypes 1 and 3, as has been documented in other studies.^{4,12} A history of previous surgery was the most common risk factor identified in this study followed by blood transfusion, in concordance with a previous study.¹⁶ The route of transmission was unknown in nearly 40% of cases, as reported by other studies.¹⁷ These cases are hence referred to as 'sporadic' or 'community acquired'.

Past studies of HCV infection have variably documented an association of histological severity with higher ALT levels^{12,18} or no association between these two parameters.^{19,20} In the present study, serum ALT levels correlated poorly with necroinflammatory activity and fibrosis, and did not correlate with genotype.

HCV RNA levels were not different in patients infected with different HCV genotypes, in keeping with some studies.^{12,18} An Italian study in a non-cirrhotic population with chronic hepatitis C had observed a significantly higher viral load in genotype 3.²¹ HCV RNA levels did not correlate with HAI scores in the present study. It has been suggested that since the serum HCV load may fluctuate, it does not reliably reflect the degree of liver injury.^{18,22}

Higher necroinflammatory activity and fibrosis on initial biopsy have been associated with more rapid progression to fibrosis in HCV infection.²³ Since there are differences between HCV genotypes in their response to interferon and ribavirin therapy, several studies have tried to assess the relationship between HCV genotype and the histopathological manifestations. Some of these studies observed a higher HAI score and higher portal, lobular or periportal inflammation in infection with 2a/c, 3a or mixed genotypes as compared to genotype 1^{11,12,21} while others showed a higher percentage of moderate to severe activity and a significantly higher grading score in genotype 1.^{15,16} As reported in previous studies,^{23,24} the majority of biopsies in the present study (64.6%) demonstrated only mild activity. There was no difference in the histological severity of liver disease, or in individual components of the necro-inflammatory score, in patients infected with different genotypes in this study. These findings are in keeping with other studies.^{5,25,26} In chronic hepatitis C, interface hepatitis is usually a lesion of mild severity while fibrosis and cirrhosis are common. Hence lobular necrosis/ inflammation is suspected to be of major importance in the prediction of liver fibrosis.²⁷ There was no difference in the frequency of other histological features between genotypes, except for an increased presence of Mallory hyaline in genotype 1. Granulomas were seen only in cases of genotype 3.

Steatosis, mostly macrovesicular, was a common finding in this study (68.7%). Several studies have shown a definite association of genotype 3 with both the prevalence and the severity of steatosis independent of sex, age, BMI and alcohol consumption.^{9,28,29} In the present study, although

the highest grade of steatosis was seen only in genotype 3, the distribution of moderate/ severe steatosis in genotype 1 (55.6%) and 3 (41.9%) was not different. Other studies from India also have not found any difference in steatosis between different genotypes.^{5,30}

Hepatic iron deposition was noted in 14 cases, of which 10 belonged to genotype 3. This is in accordance with other studies which have shown that hepatic iron deposition was either absent⁶ or usually mild in patients with chronic hepatitis C infection^{31,32} and was found to be more frequent in HCV genotype 3.³² The significance of iron deposition on the natural history of chronic HCV infection is still controversial, but it has been proposed as one of the factors that might enhance histological damage and reduce the efficacy of antiviral therapy.^{31,32}

Pegylated interferon has been shown to be more effective than plain interferon in treating patients with HCV infection for both genotype 1 and non 1.³³ But in this study, since treatment response was available only in a small number of patients, no difference was noted.

In conclusion, HCV genotype 3 predominated in biopsied patients with HCV related chronic liver disease. Serum transaminase levels correlate poorly with grade of activity or degree of fibrosis; steatosis occur in approximately two-thirds of patients and severe grades of steatosis are seen only in genotype 3 infection. HCV genotype does not correlate with serum ALT levels, viral load, histological activity, grade or degree of fibrosis. However, the predominance of genotype 3, along with the limited number of other genotypes, could have contributed to a failure to find clinical and histological differences between different HCV genotypes.

References

1. Issar SK, Ramakrishna BS, Ramakrishna B, *et al.* Prevalence and presentation of hepatitis C related chronic liver disease in southern India. *J Trop Med Hyg* 1995;98:161–5.
2. Chakravarti A, Verma V. Prevalence of hepatitis C and B viral markers in patients with chronic liver disease: A study from northern India. *Indian J Med Microbiol* 2005;23:273–4.
3. Guillemette HLG, Vallet S, Graffin CG, *et al.* Genetic diversity of the hepatitis C virus: impact and issues in the antiviral therapy. *World J Gastroenterol* 2007;13:2416–26.
4. Raghuraman S, Shaji RV, Sridharan G, *et al.* Distribution of different genotypes of HCV among patients attending a tertiary care hospital in south India. *J Clin Virol* 2003;26:61–9.
5. Hissar SS, Goyal A, Kumar M, *et al.* Hepatitis C virus genotype 3 predominates in north and central India and is associated with significant histopathologic liver disease. *J Med Virol* 2006;78:452–8.
6. Verma V, Chakravarti, Kar P. Genotypic characterization of hepatitis C virus and its significance in patients with chronic liver disease from Northern India. *Diagn Microbiol Infect Dis* 2008;61:408–14.
7. Ishak K, Baptista A, Bianchi L, *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696–9.

8. Burt AD, Macsween RNM. Fat, alcohol and iron. In: Symmers W, Wight DGD, eds. Liver, biliary tract and exocrine pancreas. Systemic Pathology 3rd edn. London, UK: Churchill Livingstone; 1994:237–85.
9. Brandt LR, Leandro G, Spahr L, et al. Liver steatosis in chronic hepatitis C: a morphological sign suggesting infection with HCV genotype 3. *Histopathology* 2001;39:119–24.
10. Raghuraman S, Abraham P, Sridharan G, Ramakrishna BS. Hepatitis C virus genotype 6 infection in India. *Indian J Gastroenterol* 2005;24:72–3.
11. Mihm S, Fayyazi A, Hartmann H, Ramadori G. Analysis of histopathological manifestations of chronic hepatitis C virus infection with respect to virus genotype. *Hepatology* 1997; 25:735–9.
12. Adinolfi LE, Utili R, Andreana A, et al. Relationship between genotypes of hepatitis C virus and histopathological manifestations in chronic hepatitis C patients. *Eur J Gastroenterol Hepatol* 2000;12:299–304.
13. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. *J Hepatol* 2001;34:730–9.
14. Ryder SD. Progression of hepatic fibrosis in hepatitis C: a prospective repeat liver biopsy study. *Gut* 2004;53:451–5.
15. Nousbaum JB, Pol S, Nalpas B, et al. Hepatitis C virus type 1b(II) infection in France and Italy. *Ann Intern Med* 1995;122:161–8.
16. Roffi L, Redaelli A, Colloredo G, et al. Outcome of liver disease in a large cohort of histologically proven chronic hepatitis C: influence of HCV genotype. *Eur J Gastroenterol Hepatol* 2001;13:501–6.
17. Karmochkine M, Carrat F, Santos OD, Cacoub P, Raguin G. A case control study of risk factors for hepatitis C infection in patients with unexplained routes of infection. *J Viral Hepat* 2006;13:775–82.
18. Lee YS, Yoon SK, Chung ES, et al. The relationship of histological activity to serum ALT, HCV genotype and HCV RNA titres in chronic hepatitis C. *J Korean Med Sci* 2001;16:585–91.
19. Persico M, Persico E, Suozzo R, et al. Natural history of hepatitis C virus carriers with persistently normal aminotransferase levels. *Gastroenterology* 2000;118:760–4.
20. Gholson CF, Morgan K, Catinis G, et al. Chronic hepatitis C with normal aminotransferase levels: a clinical histologic study. *Am J Gastroenterol* 1997;92:1788–92.
21. Saracco G, Sostegni R, Ghisetti V, et al. Hepatitis C virus genotypes in a non – cirrhotic Italian population with chronic hepatitis C: correlation with clinical, virological and histological parameters. Results of a prospective multicentre study. *J Viral Hepat* 2000;7:124–9.
22. McCormick SE, Goodman ZD, Maydonovitch CL, Sjogren MH. Evaluation of liver histology, ALT elevation and HCV RNA titer in patients with chronic hepatitis C. *Am J Gastroenterol* 1996;91:1516–22.
23. Kleiner DE. The liver biopsy in chronic hepatitis C: a view from the other side of the microscope. *Semin Liver Dis* 2005;25:52–64.
24. Rozario R, Ramakrishna B. Histopathological study of chronic hepatitis B and C: a comparison of two scoring systems. *J Hepatol* 2003;38:223–9.
25. Silva GF, Nishimura NF, Coelho KIR, Soares EC. Grading and staging chronic hepatitis C and its relation to genotypes and epidemiological factors in Brazilian blood donors. *Braz J Infect Dis* 2005;9:142–9.
26. Ramalho F, Costa A, Pires A, et al. Correlation of genotypes and route of transmission with histological activity and disease stage in chronic hepatitis C. *Dig Dis Sci* 2000;45:182–7.
27. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* 1996;24:289–93.
28. Negro F. Mechanisms and significance of liver steatosis in chronic hepatitis C infection. *World J Gastroenterol* 2006; 12:6756–65.
29. Westin J, Norlinder H, Lagging M, Norkrans G, Wejstal R. Steatosis accelerates fibrosis development over time in hepatitis C virus genotype 3 infected patients. *J Hepatol* 2002; 37:837–42.
30. Amarapurkar DN, Patel ND, Rane P, Kamani P. Do different hepatitis C virus genotypes behave differently? *Trop Gastroenterol* 2007;28:99–104.
31. Metwally MA, Zein CO, Zein NN. Clinical significance of hepatic iron deposition and serum iron values in patients with chronic hepatitis C infection. *Am J Gastroenterol* 2004;286–91.
32. Sebastiani G, Vario A, Ferrari A, Pistis R, Noventa F, Alberti A. Hepatic iron, liver steatosis and viral genotypes in patients with chronic hepatitis C. *J Viral Hepat* 2006;13:199–205.
33. Zhao S, Liu E, Yu H, et al. Comparison of peginterferon and interferon in treating Chinese patients with chronic hepatitis C. *Hepatogastroenterology* 2008;55:1047–54.