Original Article

Do different hepatitis C virus genotypes behave differently?

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ABSTRACT

Background: The natural history of hepatitis C genotype III infection, the predominant form in India, is not wholly understood. This study attempted to compare the natural history of diseases due to genotypes III and I.

Methods: This 10-year prospective follow-up study (mean follow-up period=3.6±1.4, range=1–10 years) included 108 patients of hepatitis C. Group1 comprised 65 patients with hepatitis C genotype III infection (mean age=46.1±11.3 years, male: female=1.8: 1) and group 2 comprised 43 patients with hepatitis C genotype I infection (mean age=44.2±8.2 years, male: female=2.1: 1). Demographic features, clinical presentation and course, response to treatment (either interferon-ribavirin or peginterferon-ribavirin combination) and complications were noted for all patients. Data were analysed using the chi-square test and Student's t-test.

Results: The number of steatosis cases was larger in group 1 (32.3%, 21/65 patients) than in group 2 (18.6%, 8/43 patients) although statistically not significant. There was no significant difference in the mode of infection, presence of diabetes, obesity or alcoholism, clinical presentation, extra-hepatic manifestations, stage of liver disease, complications like decompensation or hepatocellular carcinoma and mortality. Overall, the sustained treatment response was significantly greater in group 1 patients [(87.5%, 21/24 treated patients vs. 56.2%, 9/16 treated patients in group 2; p=0.0001)].

Conclusion: HCV with genotype III was associated with better treatment response. Although statistically not significant, more number of patients in genotype III had steatosis.

Key words: natural history, chronic hepatitis C, hepatitis C virus genotype III, hepatitis C virus genotype I

ABBREVIATIONS:

Hepatitis C virus (HCV), hepatocellular carcinoma (HCC), alanine aminotransferase (ALT)

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INTRODUCTION

Hepatitis C virus (HCV) infection is present worldwide, with a global prevalence of 3%; amounting to more than 170 million HCV-infected persons.[1] In India, the prevalence of HCV ranges from 0.3% to 4% in the general population in different parts of the country.[2] In industrialised countries, HCV accounts for 20% of acute hepatitis, 70% of chronic hepatitis, 40% of cirrhosis, 60% of hepatocellular carcinoma (HCC) and 30% of liver transplantation cases;[3] whereas in India, HCV infection is responsible for 0–12.8% acute hepatitis, 10–20% of chronic liver disease and 10–15% of HCC.[2,4]

HCV is a RNA virus and demonstrates genetic variability, which results in the emergence of various HCV genotypes.[5] There are at least six HCV genotypes and more than 50 subtypes.[6] HCV genotype distribution is different throughout the world, but genotypes I, II and III predominate in most areas.[5] Most studies in India have shown predominance of HCV genotype III with prevalence ranging from 12% to 64%.[7]

The HCV genotype is a strong and independent predictor of sustained response to therapy;[8–13] but its role in clinical presentation, severity and progression of HCV-related liver disease is still unclear.[5,14–16] There are a few reports which suggest that genotype I is associated with more severe liver disease with faster progression to HCV-related liver injury;[7,17–25] more frequent HCC,[26–28] and increased histological recurrence, and faster progression in the post-liver transplantation period;[29–31] whereas other reports have negated these observations.[32–42]

Most of the natural history studies have explained for HCV genotypes I vs. II,[22,24] or HCV genotypes Ia vs. Ib.[17] There is inadequate data available for genotype III. There are very few studies from India on the natural history of HCV and the impact of HCV genotypes on the course of infection.[4,7] This study aimed to compare the natural history of patients with genotypes III and I.

METHODS

Study population

This is a 10-year prospective follow-up study carried out at the Bombay Hospital & Medical Research Centre from January 1995 through December 2004. Indian patients with chronic HCV infection (i.e. presence of anti-HCV antibodies on third generation enzyme immunoassay and/or HCV RNA on polymerase chain reaction for more than 6 months) with genotypes III and I (genotypes determined by restriction fragment length polymorphism technique) were included in the study. Patients with HCV genotype III comprised group1 and genotype I comprised group 2.

Baseline evaluation

These patients underwent detailed evaluation including clinical history and examination with special emphasis on the mode of HCV detection (asymptomatic individuals detected on screening or during investigation for other diseases); the mode of acquiring HCV infection (blood transfusion, intravenous drug abuse, sexual contact, haemodialysis, surgery, dental treatment, tattooing/ body piercing, occupational exposure and

maternal HCV status); symptoms of chronic liver disease and decompensation; use of drugs (immunosuppressive therapy, diabetic medications and lipid-lowering drugs), tobacco and alcohol; history of diabetes, obesity, organ transplantation, chronic renal failure; past anti-HCV therapies; list of previous laboratory tests in chronological order; probable date of event leading to HCV infection (in case of multiple events, the date when the patient first tested positive for anti-HCV in cases where serial testing was available, or the date of first event was taken as the probable date); and stigmata of chronic liver disease. All patients underwent laboratory testing [including complete blood count, liver profile (comprising alanine aminotransferase [ALT], aspartate aminotransferase[AST], bilirubin, alkaline phosphatase, gamma glutamyltransferase, albumin, globulin and prothrombin time), lipid profile (including total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglyceride, apolipoprotein A1, apolipoprotein B and lipoprotein a), fasting and post-prandial plasma glucose levels, HBsAg, HIV, anti-HCV, HCV RNA quantitative and genotype estimation, serum á-fetoprotein], upper gastr-ointestinal endoscopy (to look for oesophageal varices), imaging (abdominal ultrasonography for evidence of fatty liver, shrunken and/or nodular liver, liver tumour and portal hypertension) and liver biopsy (to evaluate presence of cirrhosis and steatosis) whenever possible.

Follow-up schedule and evaluation

The same physician (Amarapurkar DN) conducted follow-up visits for all patients at six-month intervals and these visits included clinical evaluation, laboratory testing and imaging. Treatment was offered to eligible patients as combination therapy with either interferon and ribavirin (initial 5 years of the study period) or peginterferon and ribavirin (last 5 years of the study period) according to standard criteria. During follow-up, data comprising response to treatment, progression to cirrhosis, development of decompensation, development of HCC and cause of mortality, were collected.

Diagnostic criteria

1. Chronic hepatitis C was defined as the presence of anti-HCV and HCV RNA in the serum for a duration of more than 6 months.
2. Cirrhosis was diagnosed on the basis of presence of history of decompensation, stigmata of chronic liver disease, shrunken and nodular liver and/or portal hypertension on imaging, oesophageal varices on upper gastrointestinal endoscopy and/or presence of cirrhosis on histology (whenever possible).
3. Decompensation of liver disease was diagnosed on the basis of presence of at least one point of evidence for clinical decompensation (as ascites, variceal bleeding and/or encephalopathy).
4. Hepatic steatosis was defined in the non-alcoholic patient (alcohol consumption < 20 g/day) as the presence of fatty liver on imaging and/or the presence of macro-vesicular fatty change (>5%) with/without associated necro-inflammation, fibrosis and hepatocyte degeneration on histology (whenever possible).
5. Serum aminotransferase elevation was defined as an increase of more than or equal to twice the upper limit of normal on two separate occasions in a 6–month period.
6. Diabetes mellitus was diagnosed as per the American Diabetes Association criteria and included use of oral hypoglycaemic drugs; fasting plasma glucose level ≥126 mg/dl; 2–hour plasma glucose ≥200 mg/dl during an oral glucose tolerance test; and/or random or 2–hour post-prandial plasma glucose level ≥200 mg/dl.
7. Dyslipidaemia was diagnosed on the following criteria: fasting cholesterol level ≥200 mg/dl; fasting triglyceride level ≥150 mg/dl; fasting high-density lipoprotein cholesterol level < 40 mg/dl in men or < 50 mg/dl in women; and/or use lipid lowering agents.
8. Obesity was defined as past history or baseline data suggesting body mass index ≥25 kg/m^2; waist circumference > 90 cm in men or > 80 cm in women; and/or waist-to-hip ratio ≥0.9 in men or ≥0.85 in women.

STATISTICAL ANALYSIS

Statistical analysis was performed using the chi-square test and Student's t-test. P value less than 0.05 was considered statistically significant.

RESULTS

Study Population

A total of 108 patients were included in the study. Group1 comprised 65 patients (mean age=46.1± 11.3 years; range=11–69 years; sex ratio male: female=1.9:1) and group 2 comprised 43 patients (mean age=44.2 ± 8.2 years; range=10–68 years; sex ratio male: female=2.3:1). The demographic feaures in both the groups were comparable.

Baseline data in group 1 (65 patients) vs. group 2 (43 patients)

The mode of acquiring HCV infection was known in 61 (93.8%) vs. 40 (93%) cases : blood transfusion in 48 (78.6%) vs. 32 (80%); haemodialysis without blood transfusion in 8 (13.1%) vs. 5 (12.5%); surgery without blood transfusion in 5 (8.1%) vs. 3 (7.5%) in Group 1 and 2 respectively. Among the 80 patients who received blood transfusion, reasons for the transfusion were as follows: haemophilia 5 (10.4%) vs. 2 (6.2%), thalassaemia 3 (6.2%) vs. 2 (6.2%), surgery 25 (52%) vs. 16 (50%), haemodialysis for chronic renal failure 12 (25%) vs. 9 (28.1%), cause not known 3 (6.2%) vs. 3 (9.3%) in group 1 and 2 respectively. In none of the patients was HCV infection attributable to intravenous drug abuse, needle-stick injury, sexual transmission or intra-familial transmission. The duration of HCV infection before the presentation was as follows: 12.5±3.2 (7–24) years vs. 8.6±3.3 (4–22) years in group 1 and 2 respectively.

Modes of presentation were as follows: asymptomatic ALT elevation 18 (27.6%) vs. 11 (25.5%), asymptomatic anti-HCV positive individuals 3 (4.6%) vs. 2 (4.6%) , asymptomatic abnormal imaging 1 (1.5%) vs. 1 (2.3%) , symptomatic ALT

elevation 19 (29.2%) vs. 17 (39.5%) , decompensation 21 (32.3%) vs. 11 (25.5%) and HCC 3 (4.6%) vs. 1 (2.3%) in group 1 and 2 respectively.. Total patients with normal ALT at presentation were 26 (40%) vs. 12 (27.9%) in both the groups .

Stage of disease at baseline was as follows: chronic hepatitis 25 (38.4%) vs. 16 (37.2%) and cirrhosis 40 (61.5%) vs. 27 (62.7%) Among extra-hepatic manifestations of HCV, membranoproliferative glomerulonephritis was present in 1 (1.5%) vs. 2 (4.6%), essential mixed cryoglobulinaemia in 2 (3%) vs. 1 (2.3%), thyroid dysfunction in 1 (1.5%) vs. 1 (2.3%), lichen planus in 1 (1.5%) vs. 0 (0%) and type-2 diabetes mellitus in 14 (21.5%) vs. 8 (18.6%) in group 1 and 2 respectively. Co-factors at baseline were as follows: alcoholism in 8 (12.3%) vs. 5 (11.6%), smoking in 9 (13.8%) vs. 5 (11.6%), hepatitis B co-infection in 2 (3%) vs. 1 (2.3%), human immunodeficiency virus co-infection in 1 (1.5%) vs. 1 (2.3%), chronic renal failure in 20 (30.7%) vs. 14 (32.5%), obesity in 9 (13.8%) vs. 5 (11.6%) and hyperlipidaemia in 6 (9.2%) vs. 4 (9.3%) in group 1 and 2 respectively. None had undergone organ transplantation or treatment with immunosuppressive drugs. Baseline mean HCV viral loads were 4.6×10^6 copies/ml (range= 2.2×10^4–6.8×10^8 copies/ml) vs. 4.8×10^6 copies/ml (range= 3.1×10^4–7.4×10^8 copies/ml) in group 1 and 2 respectively.All these parameters were comparable in both the groups and there was no statistically significant difference.

Steatosis was present in higher number of patients, although not statistically significant, in Group 1 as compared to Group 2 [21 (32.3%) vs. 8 (18.6%) [p =0.11].

Follow-up data in group 1 (65 patients) vs. group 2 (43 patients)

Total follow-up was from 1 through 10 years with a mean follow-up period of 3.6 ±1.4 years. 25 (38.4%) patients in group 1 and 19 (44.1%) patients in group 2 were lost to follow-up.

Total patients with cirrhosis at baseline or at the end of follow up (where this information was available) were 41 (63%) vs. 29 (67.4%), time-to-cirrhosis was 13.3 ± 3.2 years (range=9–21 years) vs. 10.3 ± 2.4 years (range=5–18 years) , patients with decompensation at presentation or on follow-up (where this information was available) were 35 (53.8%) vs. 18 (41.8%),time-to-first-decompensation was 16.5 ± 3.3 years vs. 12.2 ± 2.8 years,on follow-up, 3 (4.6%) vs. 2 (4.6%) patients developed HCC and 11 (16.9%) vs. 8 (18.6%) patients had liver-related mortality in group 1 and group2 respectively. There was no significant difference in any of these parameters in both the groups.

Treatment data in group 1 (65 patients) vs. group 2 (43 patients)

Of the patients who received treatment (24 in group 1 and 16 patients in group 2), a combination of interferon and ribavirin was given to 10 patients in group 1 and 7 patients in group-2; the remaining patients received a combination of peginterferon and ribavirin. The end-of-treatment response rate was 22 (91.6%) vs. 10 (62.5%); whereas sustained treatment response was achieved in 21 (87.5%) vs. 9 (56.2%) (p=0.0001).

DISCUSSION

This is the first study from India exploring the natural history of HCV genotype III, the main genotype in most parts of India. In our study, HCV genotype III was associated with a better treatment response rate when compared with genotype I. Although there was shorter duration for development of cirrhosis and decompensation in HCV genotype I, difference was not statistically significant.

This prospective follow-up study was performed at a tertiary care centre on patients who had established liver disease due to HCV infection. In our study, at presentation, around 60% of patients were cirrhotic and more than 25% had had an episode of decompensation within 8–24 years of exposure to HCV infection. In previous series of patients presenting with chronic liver disease, cirrhosis was present in 17–55% cases, HCC in 1–23% and mortality in 4–15% within 10–30 years of exposure to HCV infection.[4, 43–50] In a recent analysis, risk of cirrhosis after 20 years was higher in patients with transfusion-related HCV infection and in patients evaluated at tertiary care centres as compared to patients in community-based cohort studies.[51] Prevalence of cirrhosis and decompensation, progression rate to cirrhosis and morbidity-mortality rates are likely overestimated in such studies.[4,50–52] Conclusions reached by the differing studies of natural history of disease necessarily depend on the time of initiation of study, i.e. at onset of infection, or when established chronic liver disease is already present.[4,50] In studies based on a 4–16 year follow-up of acute hepatitis C cases, cirrhosis was seen in 1–20%, HCC in 0–1.3% and liver-related mortality in 0–6%.[4, 49, 50, 52] In studies based on more than 20–year follow-ups, subsequent to established parenteral exposure, cirrhosis is seen in 0.3–15%, HCC in 0–1.9% and liver-related mortality in 0–2.8%.[4, 49, 50, 52]

Both groups in our study were comparable in terms of age, sex, race, and mode of acquiring HCV, HCV RNA viral load, alcohol, smoking, obesity, diabetes, HBV co-infection, HIV co-infection and immunocompromised states. Our study did not demonstrate any difference between HCV genotype III and I in terms of clinical presentation or clinical progression of disease. This is in accordance with previous series, which have also demonstrated that the natural history of HCV is independent of the genotype. [32–42]

Although time-to-cirrhosis or time-to-decompensation was lower with genotype I in our study, it was not statistically significant. Time-to-cirrhosis was 13.3 (range=9–21) years in HCV genotype III and 10.3 (range=5–18) years in genotype I. In the previous series, time-to-cirrhosis was measured as approximately 20 years and time-to-hepatocellular carcinoma as approximately 30 years.[4,43,44] In the literature, rapid progression in those who develop cirrhosis less than 20 years after HCV exposure is well-recognised.[41] In previous studies cirrhosis was reported in 15–20% of patients within less than 10 years of an acute hepatitis C episode.[53, 54]

There was no difference in the rate of various extrahepatic manifestations between the two genotypes in our study. It has been previously noted that a majority of the extra-hepatic manifestations are not genotype-specific.[55]

In our study, there was no significant difference in the ALT elevation between the two groups. This is in contrast to previous studies where it has been observed that HCV genotype III is associated with higher ALT elevation than genotype I.[42,56]

HCV genotype III in our study was associated with an increased rate of steatosis compared with HCV genotype although statistically not significant, despite comparable prevalence of alcohol intake and risk factors for nonalcoholic fatty liver/ insulin resistance i.e. diabetes mellitus, obesity (generalised or central) and dyslipidaemia in both the groups; thereby this might suggest an independent role for HCV genotype III in the onset of steatosis. Overall, the prevalence of steatosis varies from between 30% to 70% among HCV-infected patients in most series.[57] Previously it has been seen that hepatitis C genotype III is associated with significantly more steatosis and bile duct lesions than genotype I.[42] Steatosis is more common and severe in HCV genotype III,[57, 58] and the steatosis resolves once HCV is eradicated after successful anti-viral treatment.[59,60] Genotype III is associated with steatosis in non-obese individuals.[61] These evidences support direct involvement of HCV genotype III in the development of steatosis.[57] HCV infection induces steatosis consequent either to the direct cytopathic effect on the hepatocyte,[62,63] or to hypobetalipoproteinaemia induced by HCV,[64] or to the indirect effect mediated through insulin resistance.[63,65] HCV causes insulin resistance either by inducing tumour necrosis factor-á,[66–68] or by altering the insulin-signaling pathways.[69,70] Steatosis in turn increases insulin resistance,[71] and a vicious cycle sets in, which furthers the development of steatosis.

To conclude, in our study, treatment response to HCV genotype III was significantly better than that to HCV genotype I, even when baseline characteristics were comparable in both groups. Thus, our study reaffirms the hypothesis set forth by previous studies that HCV genotypes are indeed independent predictors for treatment response.[8–13]

REFERENCES

1. Yen T, Keeffe EB, Ahmed A. The epidemiology of hepatitis C virus infection. J Clin Gastroenterol. 2003;36:47–53.
2. Arankalle VA. Epidemiology of HCV infection in India: a comprehensive analysis. In: Sarin SK, Okuda K eds. Hepatitis B and C– carrier to cancer. Harcourt India Pvt. Ltd. India. 2002;201–18.
3. Alter MJ. World Epidemiology of Hepatitis C. Hepatitis Annual Update. 2004;77–92.
4. Amarapurkar D. Natural history of hepatitis C virus infection. J Gastroenterol Hepatol. 2000;15:105–110.
5. Pawlotsky JM. Hepatitis C virus genetic variability-pathogenetic and clinical implications. Clin Liver Dis. 2003;7:45–66.
6. Robertson B, Myers G, Howard C, et al. Classification, nomenclature and database development for hepatitis C virus (HCV) and related viruses-proposals for standardizations. Arch Virol. 1998;149:2493–503.
7. Amarapurkar D, Dhorda M, Kirpalani A, Amarapurkar A, Kankonkar S. Prevalence of hepatitis C genotypes in Indian Patients and their clinical significance. J Assoc Physicians India. 2001;49:983–5.
8. McHutchison JG, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, et al. Interferon alfa-2b alone or in combination with Ribavirin as initial treatment for chronic hepatitis C. N Engl J Med. 1998;339:1485–92.
9. Poynard T, Marcellin P, Lee SS, Niedarau C, Minuk GS, Ideo G, et al. Randomized trial of interferon alfa-2b plus Ribavirin for 48 weeks or for 24 weeks versus interferon alfa-2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. Lancet. 1998;352:1426–32.
10. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus Ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomized trial. Lancet. 2001;358:958–65.
11. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med. 2002;347:975–82.
12. Hadziyannis SJ, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, et al. Peginterferon alfa-2a (40 kilodaltons) and ribavirin combination therapy in chronic hepatitis C: randomized study of the effect of treatment duration and ribavirin dose. Ann Intern Med. 2004;140:346–55.
13. Davis GL, Estoban-Murn R, Rustgi V, Hoefs J, Gordon SC, Trepo C, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C– international hepatitis interventional therapy group. N Engl J Med. 1998;339:1493–9.
14. Feld JJ, Liang TJ. Hepatitis C identifying patients with progressive liver injury. Hepatology. 2006;43:194–206.
15. Webster G, Barnes E, Brown D, Dusheiko G. HCV genotypes role in pathogenesis of disease and response to therapy. Bailliere's Clin Gastroenterol. 2000;14:229–40.
16. Alberti A, Chemello L, Benvegnu L. Natural history of hepatitis C. J Hepatol. 1999;31:17–24.
17. Pozzato G, Moretti M, Franzin F, Croce LS, Tiribelli C, Masayu T, et al . Severity of liver disease with different hepatitis C virus clones. Lancet. 1991;338:509.
18. Pozzato G, Kaneko S, Moretti M. Different genotypes of hepatitis C virus are associated with different severity of chronic liver disease. J Med Virol. 1994;43:291–6.
19. Qu D, Li JS, Vitvivski L, Mechai S, Berby F, Tong SP, et al. Hepatitis C virus genotypes in France: comparison of clinical features of patients infected with genotype I and genotype II. J Hepatol. 1994;21:70–5.
20. Silini E, Bottelli R, Asti N, Bruno S, Candusso ME, Brambilla S, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis - a case controlled study. Gastroenterology. 1996;111:199–205.
21. Kobayashi M, Tanaka E, Sodeyama T, Urushira A, Matsumoto A, Kiyosawa K . The natural course of chronic hepatitis C – a comparison between patients with 1 and 2 hepatitis C virus. Hepatology. 1996;23:695–9.
22. Dusheiko G, Weiss HS, Brown D, McOmish F, Yap PL, Sherlock S et al. Hepatitis C virus genotypes - an investigation of type-specific differences in geographic origin and disease. Hepatology. 1994;19:13–18.
23. Yamada G, Tanaka E, Miura T, Kiyosawa K, Yano M, Matsushima T, et al. Epidemiology of genotypes of hepatitis C virus in Japanese patients with type C chronic liver diseases - a multi-institution analysis. J Gastroenterol Hepatol. 1995;10:538–45.
24. Ichimura H, Tamura I, Kurimura O, Koda T, Mizui M, Tsuchie H, et al. Hepatitis C virus genotypes, reactivity to recombinant immunoblot assay 2 antigens and liver disease. J Med Virol. 1994;43:212–5.
25. Zein NN, Rakela J, Krawitt EL, Reddy KR, Tominaga T, Persing DH. Hepatitis C virus genotypes in the United States - epidemiology, pathogenicity and response to interferon therapy.Collaborative Study Group.Ann Intern Med. 1996;125:634–9.
26. De Mitri MS, Poussin K, Baccarini P, Pontisso P, D'Errico A, Simon N, et al. HCV-associated liver cancer without cirrhosis. Lancet. 1995;345:413–5.

27. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis - a prospective study. Hepatology. 1997;25:754–8.
28. Hatzakis A, Katsoulidou A, Kaklamani E, Toulomi G, Koumantaki Y, Tassapoulos NC, et al. Hepatitis C virus 1b is the dominant genotype in HCV-related carcinogenesis - a case control study. Int J Cancer. 1996;68:51–3.
29. Gane EJ, Portman BC, Naumov N, Smith HM, Underhill JA, Donaldson PT, et al. Long term outcome of hepatitis C infection after liver transplantation. N Engl J Med. 1996;334:815–20.
30. Feray C, Caccamo L, Alexander GJ, Ducot B, Gugenheim J, Casanovas T, et al. European collaborative study on factors influencing outcome after liver transplantation for hepatitis C – European concerted action on viral hepatitis (EUROHEP) group. Gastroenterology. 1999;117:619–25.
31. Prieto M, Berenguer M, Rayon JM, Cordoba J, Arguello L, Carrasco D, et al. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation relationship with rejection episodes. Hepatology. 1999;29:250–6.
32. Roffi L, Ricci A, Ogliari C, Scalori A, Minola E, Coolaredo G, et al. HCV genotypes in northern Italy - a survey of 1368 histologically proven chronic hepatitis C patients. J Hepatol. 1998;29:701–6.
33. Mita E, Hayashi N, Kanazawa Y, Hagiwara H, Uedak K, Kasahara A, et al. Hepatitis C virus genotype and RNA titer in the progression of type C chronic liver disease. J Hepatol. 1994;21:468–73.
34. Lau JYN, Davis GL, Prescott LE, Maertens G, Lindsay KL, Qian K, et al. Distribution of hepatitis C in virus genotypes determined by line probe assay in patients with chronic hepatitis C seen at tertiary referral centres in the United States. Hepatitis Internentional Group Therapy. Ann Int Med. 1996;124:868–76.
35. Puoti C, Magrini A, Stati T, Rigato P, Montagnesse F, Rossi P, et al. Clinical, histological, and virological features of hepatitis C virus carriers with persistently normal or abnormal alanine transaminase levels. Hepatology. 1997;26:1393–8.
36. Vargus HE, Wang LF, Laskus T, Poutous A, Lee R, Demetris A, et al. Distribution of infecting hepatitis C virus genotypes in end-stage liver disease patients at a large American transplantation center. J Infectious Dis. 1997;175:448–50.
37. Reid AE, Koziel MJ, Aiza I, Jeffers L, Reddy R, Schiff E, et al. Hepatitis C virus genotypes and viremia and hepatocellular carcinoma in United States. Am J Gastroenterol. 1999;94:1619–626.
38. Zhou S, Terrault NA, Ferrell L, Hahn JA, Lau JY, Simmonds P, et al. Severity of liver disease in liver transplantation recipient with hepatitis C virus infection: relationship to genotype and level of viremia. Hepatology. 1996;24:1041–6.
39. Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchinson J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis C. J Hepatol. 2001;34:730–9.
40. Serfaty L, Chazouillires O, Poujol-Robert A, Morand-Joubert L, Dubois C, Chretian Y, et al. Risk factors for cirrhosis in patients with chronic hepatitis C virus infection - results of a case-control study. Hepatology. 1997;26:776–9.
41. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C – the OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups. Lancet. 1997;349:825–32.
42. 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.
43. Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, et al. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma - analysis by detection of antibody to hepatitis C virus. Hepatology. 1990;12:671–5.
44. Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med. 1995;332:1463–6.
45. Yano M, Kumada H, Kage M, Ikeda K, Shimamtsu K, Inone O, et al. The long-term pathological evolution of chronic hepatitis C. Hepatology. 1996;23:1334–40.
46. Gordon SC, Eloway RS, Long JC, Dmuchowski CF. The pathology of hepatitis C as a function of mode of transmission-blood transfusion versus intravenous drug use. Hepatology. 1993;18:1338–43.
47. Takayashi M, Yamada G, Miyamoto R, Doi T, Endo H, Tsuji T, et al. Natural history of chronic hepatitis C. Am J Gastroenterol. 1993;88:240–3.
48. Roberts JM, Searle JW, Cooksley WGE. Histological patterns of prolonged hepatitis C virus infection. Gastroenterol Jpn.1993;28:901–5.
49. Alberti A, Chemello L, Benvegnu L. Natural history of hepatitis C. J Hepatol. 1999;31:17–24.
50. Seeff LB. Natural history of hepatitis C. Hepatology Rev. 2005;2:88–96.
51. Dore GJ, Freeman AJ, Law MG, Kaldor JM. Is severe liver disease a common outcome for people with chronic hepatitis C. J Gastroenterol Hepatol. 2002;17:423–30.
52. Alter HJ, Seeff LB. Recovery, persistence and sequele in hepatitis C virus infection - a perspective on long-term outcome. Sem Liv Dis. 2000;20:17–35.
53. Di Bisceglie AM, Goodman ZD, Ishak KG, Hoofnagle JH, Melpolder JJ, Alter HJ. Long-term clinical and histopathological follow-up of chronic post-transfusion hepatitis. Hepatology. 1991;14:969–74.
54. Tremolada F, Casarin C, Albert A, Drago C, Tagger A, Ribero ML, et al. Long-term follow-up of non-A, non-B (type C) post-transfusion hepatitis. J Hepatol. 1992;16:273–81.
55. Hadziyannis SJ. The spectrum of extrahepatic manifestations in hepatitis C virus infection. J Viral Hepatitis. 1997;4:9–28.
56. McOmish F, Chan SW, Dow BC, Gillan J, Frame WD, Crawford RJ, et al. Detection of three types of hepatitis C virus in blood donors investigation of type-specific differences in serologic reactivity and rate of alanine aminotransferase abnormalities. Transfusion. 1993;33:7–13.
57. Patton HM, Patel K, McHutchinson JG. Hepatic steatosis in chronic hepatitis C virus infection-a review of mechanisms, influence on natural history, and treatment outcomes. Hepatology Rev. 2004;1:72–80.
58. Westin J, Nordlinder 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.
59. Kumar D, Farrell GC, Fung C, George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes- reversal of hepatic steatosis after sustained therapeutic response. Hepatology. 2002;36:1266–72.
60. Poynard T, Ratziu V, McHutchinson J, Manns M, Goodman Z, Zeuzem S, et al. Effect of treatment with peginterferon or interferon alpha-2b and ribavirin on steatosis in patients infected with hepatitis C. Hepatology 2003;38:75–85.
61. Adinolfi LE, Gambardella M, Andreana A, Tripodi MF, Utili R, Ruggiero G. Steatosis accelerates the progression of the liver damage of chronic hepatitis C Patients and correlates with specific HCV genotype and visceral obesity. Hepatology. 2001;33:1358–64.
62. Lerat H, Honda M, Beard MR, Loesch K, Sun J, Yang Y, et al. Steatosis and liver cancer in transgenic mice expressing the structural and nonstructural proteins of hepatitis C virus. Gastroenterology. 2002;122:352–65.
63. Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, Ishibashi K, Matsuura K, et al. Hepatitis C virus core protein induces hepatic steatosis in transgenic mice. J Gen Virol. 1997;78:1527–31.
64. Serfaty L, Andreani T, Giral P, Carbonell N, Chazouilleres O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia- a possible mechanism for steatosis in chronic hepatitis C. J Hepatol. 2001;34:428–34.
65. Hui JM, Sud A, Farrell GC, Bandara P, Byth K, Kench JG, et al. Insulin resistance is associated with chronic hepatitis C virus infection and fibrosis progression. Gastroenterology. 2003;125:1695–704.

66. Knobler H, Schattner A. TNF-alpha, chronic hepatitis C and diabetes- a novel triad. Q J Med. 2005;98:1–6.
67. Elsammak M, Refai W, Elsawaf A, Abdel-Fattah I, Abd Elatti E, Ghazal A. Elevated serum tumour necrosis factor alpha and ferritin may contribute to the insulin resistance found in HCV positive Egyptian patients. Current Med Research Opinion. 2005;21:527–34.
68. Shintani Y, Fujie H, Miyoshi H, Tsutsumi T, Tsukamoto K, Kimura S et al. Hepatitis C virus infection and diabetes- direct involvement of the virus in the development of insulin resistance. Gastroenterology. 2004;126:840–48.
69. Aytug S, Reich D, Sapiro LE, Bernstein D, Begum N. Impaired IRS-1/PI3-kinase signaling in patients with HCV- a mechanism for increased prevalence of type 2 diabetes. Hepatology. 2003;38:1384–92.
70. Kawaguchi T, Yoshida T, Harada M, Hisamoto T, Nagao Y, Ide T, et al. Hepatitis C virus down-regulates insulin receptor substrates 1 and 2 through up-regulation of suppressor of cytokine signaling 3. Am J Pathol. 2004;165:1499–508.
71. Chou CJ, Haluzik M, Gregory C, Dietz KR, Vinson C, Gavrilova O, et al. WY14,643, a peroxisome proliferatoractivated receptor alpha (PPARalpha) agonist, improves hepatic and muscle steatosis and reverses insulin resistance in lipoatrophic A-ZIP/F- 1 mice. J Biological Chem. 2002;277:24484–9.