Avicenna J Clin Microb Infec. 2014 May; 1(1): e19212. DOI: 10.17795/ajcmi-19212

Published online 2014 April 28. **Review Article**

New Insights Into the Relationships Among Alcohol Consumption, Hepatocellular Carcinoma and Hepatitis C Virus Infection

Masoud Sabouri Ghannad [1]; Avid Mohammadi [1]; Hamid Kazemian [1,*]

[1]Department of Microbiology, Research Center for Molecular Medicine, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran

*Corresponding author: Hamid Kazemian, Department of Microbiology, Research Center for Molecular Medicine, Faculty of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel: +98-8118380160, Fax: +98-8118380208, E-mail: hamid_8321@yahoo.com

Received: January 21, 2014; **Revised:** February 8, 2014; **Accepted:** March 13, 2014

Context: Viral hepatitis and the consumption of alcohol are recognized as important reasons for the development of liver disease throughout the world. It would also seem that chronic alcoholism causes more severe and rapid progression of liver disease in patients with chronic hepatitis C, leading to more frequent liver cirrhosis and hepatocellular carcinoma.

Evidence Acquisition: The data for this article were obtained through an initial Medline search and from the references of relevant articles, and used to provide updated information on the relationship between alcohol consumption and the hepatitis C virus.

Results: Excessive alcohol consumption among patients with chronic hepatitis C is likely to result in more severe hepatic injuries, promote pathologic progression to cirrhosis, and increase the risk of developing hepatocellular carcinoma. Although the exact mechanisms involved in the progression of chronic hepatitis C in alcoholic patients have not been definitely established, possible alcohol-induced enhancement of viral replication, iron overload, immunologic suppression, the role of NF-kappa B, and the signaling pathways involved in its activation, have been suggested. Significant correlations have been reported between hepatitis C virus RNA levels and the amount of alcohol consumed by an individual. Interferon therapy is less effective for alcohol patients, than non-alcoholic patients, even after a period of abstinence. The obtained data suggest that a hepatitis C virus infection is an important cofactor in the pathogenesis of liver disease among patients with an alcohol problem.

Conclusions: In light of a possible synergistic effect between alcohol and hepatitis C virus replication, total abstention ought to be recommended, and due to alcohol's inhibitory effect on interferon therapy, patients with alcohol problems should not be treated until they stop drinking.

*Keywords:*Hepatitis C; Carcinoma, Hepatocellular; Alcohols; Interferons

1. Context

Viral hepatitis is caused by a diverse number of viruses, including; hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), and hepatitis GB virus C (GBV-C), and these lead to varying degrees of chronicity and liver injury (1-3). The HCV remains a major health problem, and the prevalence of this virus varies from 1% to 10% (approximately 180 million people) worldwide (4). The HCV has a high propensity to cause lifelong, persistent infections that can progress to significant liver disease (3, 5). It is estimated that 2% of the world's population is infected with HCV, which can cause acute and chronic liver diseases, resulting in a large variety of symptoms, (6) and it is the cause of related morbidity and mortality (7). Mild and subclinical symptoms are usually seen in cases of acute HCV infection. However, in a large proportion of patients, currently estimated to be about 85%, the virus persists for more than six months (2), which is determined by persistent abnormal serum enzymes and/or the presence of viremia (8). Furthermore, these patients are at increased risk of developing chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC) (2). Every year 550000 cases of HCC are recognized and liver cancer is now known to be the fifth most common cancer in the world (9). Chronic hepatitis is mainly asymptomatic. In the first 20 years of infection, mortality and morbidity rates are modest, but these frequently increase in the third and fourth decades, post infection (8).

According to Wiley et al. 20% to 30% of all patients HCV infections, will gradually progress to cirrhosis after a period of 20 to 30 years. Viral hepatitis combined with the consumption of alcohol are recognized as significant reasons for the increase in liver disease throughout the world (10). Hepatitis C and alcohol consumption have a synergistic effect on the development of HCC. HCC is more prevalent in patients with HCV, who are also alcohol users, than either factor alone (9). Combined HCV

Implication for health policy/practice/research/medical education:
Chronic alcoholism in patients with chronic hepatitis C appears to cause more severe and rapidly progressive liver disease, leading more frequently to liver cirrhosis and HCC. In conclusion, patients with chronic hepatitis C should limit their alcohol intake and if cirrhosis is present or interferon therapy is planned, complete avoidance from alcohol should be encouraged.

infection and alcohol abuse, has been reported in 14% of patients with chronic liver diseases (11). In addition, the prevalence of HCV infection among patients with alcoholism is also high, and the related reported range is 4.6% to 55.5% (12). One reason for the high level of HCC in these patients is that alcohol metabolism increases HCV replication (13). The possible reason for high HCV prevalence in alcoholics could be that they are exposed to increased risk factors, as a result of polysubstance abuse. Moreover, patients with alcoholism are at greater risk of trauma, for example as a result of road accidents requiring blood transfusions, and they are also at greater risk of engaging in unsafe sexual behaviors (14, 15). Inhibition of cellular and humoral immunity by alcohol could be another reason for the high prevalence of HCV in patients with alcohol issues (13). Chronic alcoholism in patients with chronic hepatitis C appears to cause more severe and rapidly progressive liver disease, leading more frequently to cirrhosis of the liver and HCC. The aim of this survey was to assess the relationship between alcohol consumption and HCV related diseases, including HCC.

2. Evidence Acquisition

Data for this article were obtained by an initial Medline search and from the references of relevant articles. Search terms used were "hepatitis C virus," "alcohol" and "hepatocellular carcinoma." Only English-language papers were considered.

3. Results

There have been several studies which have investigated the correlation between alcohol consumption and the progression of chronic hepatitis C on the development of cirrhosis and HCC. There are a number of risk factors affecting the progression toward HCC development, including; age over 40 to 50 years at the time of infection, type II diabetes mellitus, metabolic syndromes, co-infection with HBV, HCV and HIV, degree of active inflammation, fibrosis on liver biopsy, and the amount of daily alcohol consumed daily. However, HCV infection and alcohol consumption seem to be the most important factors (4). The risk of HCC in alcoholics with a HCV infection, is 100-fold higher compared with other populations (13). In Peters et al. researches, using 50 g or more of alcohol daily, is considered as alcohol abuse (16). Many previous studies have confirmed that there is a significant relationship between alcohol abuse and the progression of chronic hepatitis C, toward cirrhosis and the development of HCC (9, 17, 18). For instance, in a study by Wiley et al. it was shown that consumption of 40g to 60g per day of alcohol, for more than five years, results in an increased risk of liver cirrhosis (10). In an experiment by Harris et al. the risk of cirrhosis has been shown to be 4-fold higher in HCV-infected patients who consume more than 80 g/day of alcohol, compared with HCV-infected patients without a history of alcohol abuse (19). In the case of HCC, a number of studies have demonstrated an increased risk of HCC, and an increased number of anapestic tumors in patients who were heavy drinkers (20, 21). Among the conducted researches, few studies have mentioned the effect of sex differences on alcohol abuse and the progression of chronic hepatitis relating to liver disease. However, there is some evidence which shows that the adverse effects of alcohol on development of liver diseases, are more pronounced in HCV-infected women, compared with HCV-infected men (22, 23). However, Fukushima et al. have reported contrary outcomes. They found that there was no significant association between alcohol abuse and the progression of HCC, in HCV-infected patients (24). On the basis of clinical observations, several mechanisms have been reported to produce synergetic effects on the development of liver injury, in HCV infected patients with alcohol:

1) Hepatic cellular injury directly induced by alcohol consumption. This effect can lead to liver fibrosis and cirrhosis (25).

2) Conversion of procarcinogens to carcinogens, mediated by the metabolism of alcohol by microsomal enzymes in the liver (26).

3) Consumption of ethanol, which is associated with a significant inhibitory effect on liver regeneration (27).

4) Ethanol consumption suppresses the immune response by several mechanisms (28, 29), including reduction of proteasome function, which has an essential role in Ag presentation. Ethanol suppresses proteasome function in the liver, which in turn reduces production of MHC-I antigenic peptides in liver cells (30-33). Recognition of MHC-I antigens on the surface of hepatocytes is a significant factor in the clearance of viral-infected hepatocytes by cytotoxic T-cells (34). Another mechanism of suppressing immune response by alcohol is through its effect on dendritic cells (DCs). As we discuss further below, HCV proteins decrease the number and also the function of DCs. Ethanol significantly compounds the negative effects of HCV proteins on DCs' function. The mechanism of this enhancement may be through a decrease in the expression of co-stimulatory molecule B7 and IL-12 production, and also by increasing IL-10 production (35-37). Consistent with this, neopterin serum level, which is an indicator for the activation of cell–mediated immunity, is shown to be lower in habitual drinkers than in non-habitual drinkers (38).

5) Ethanol inhibits the effects of the interferon alpha antiviral response (13, 39).

6) Alcohol increases hepatic iron stores, and iron overload is associated with HCV disease progression (40-42).

7) Programmed cell death (apoptosis) and hepatocyte function, can both be regulated by active ethanol consumption (43). This can be due to downregulation of the expression of B-cell lymphoma 2 (Bcl-2) protein, which is an inhibitor of apoptosis, and an effect of alcohol. Alcohol consumption in HCV-infected individuals enhances liver fibrosis through apoptotic hepatocyte death (44).

8) HCV quasispecies complexity, in the hyper-variable regions, is enhanced under the influence of alcohol. This result was reported in a study by Takahashi et al. The mean polymerase chain reaction polymorphism, related to hyper-variable regions, was higher in patients consuming alcohol, compared to the ones who abstain (45, 46).

9) Alcohol impairs cell-mediated immunity by the inhibition of DCs, which results in hindering the immune system to overcome HCV infection (44). In addition, it has been reported that under the effects of alcohol, interleukin 2 (IL-2) and IL-12 production is reduced and IL-10 production is increased (36, 47, 48).

10) Alcohol and HCV core proteins affect the lipid peroxidation process synergistically. They also increase the expression of tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) in hepatocytes (13).

11) Ethanol induces mitochondrial injury by raising reactive oxygen species (ROS) production (42). In HCV-infected patients who also consume alcohol, either moderately or heavily, the markers of oxidative stress were 3-fold and 13-24-fold higher, respectively (49).

12) Hepatic iron levels are also increased by alcohol consumption. Activation of nuclear factor-κ and the production of TNF occur in Kupffer cells in the liver (50, 51).

13) Changes in the intestinal mucosal barrier to form lipopolysaccharides (LPS) increase with alcohol consumption, leading to the activation of Toll-like receptors (TLRs) on Kupffer cells in the liver. The consequence of this process is the production of pro-inflammatory cytokines (52).

3.1. How Hepatitis C Virus is Involved in Causing Hepatocellular Carcinoma

1) A proposed role of HCV in causing HCC, is an indirect involvement via hepatic inflammation (53).

2) The activity of HCV proteins may induce neoplasia in hepatocytes. In an experiment conducted on transgenic mice, the mice carrying the core gene developed HCC, whereas the transgenic mice carrying envelope genes and the ones with the entire non-structural genes did not develop HCC. Therefore, the oncogenic potential of the core protein of HCV was indicated (1, 7, 54). In the previously mentioned study, the proposed mechanism for this property of the core protein was through induction of oxidative stress overproduction (13, 43). The oxidative stress overproduction may be the outcome of mitochondrion dysfunction, which is affected by the HCV core protein (55, 56). One of the main causes of oxidative stress in the liver is the metabolism of alcohol by the enzyme CYP2E1 (17).

3) One of the possible pathways of inducing HCC by the HCV core protein, is via alteration of the expression of cellular genes (such as TNF-α and IL-1β), and modulation of intracellular pathways, for instance the mitogen-activated protein kinase (MAPK) pathway, which is involved in cell proliferation, and finally by interaction with cellular proteins like retinoid x receptor alpha (RXR-α), which play a significant role in cell proliferation (57-59).

4) Upregulation of the TLR signaling pathway takes place in many kinds of chronic liver diseases. On the other hand, a number of cell types in the liver express TLRs, HCV core protein and non-structural protein 3 (NS3), and these proteins activate TLRs on monocytes to produce inflammatory cytokines (60). HCV protein induces chronic inflammation, and leads to enhanced NF-kB activation and TNF-α production, by increasing TLRs signaling. As a result of the noted events, tumor growth is promoted (61).

5) The functioning of DCs is affected by HCV infection in several ways. First of all, HCV proteins, including core proteins NS3 and NS5, induce apoptosis in DCs, the consequence of which is the loss of peripheral DCs (62, 63). Secondly, HCV core and E1 genes decrease DCs' capacity to stimulate the allogeneic T-cell response (64). Thirdly, HCV results in downregulation of human leukocyte antigen-DR (HLA-DR) expression and decreases the level of co-stimulatory molecules; therefore, the function of the DCs is also decreased. The outcome of all the aforementioned mechanisms is impairment of the immune system (65).

6) HCV proteins impact on Ag presentation. Mutations of HCV proteins may result in altered processing of cytotoxic T-cell epitopes (66). On the other hand, a non-structural protein of HCV (NS3) interacts with a subunit of proteasome and this has a negative effect on the function of proteasome in producing antigenic peptides (67). Impairment of Ag presentation will lead to a weakened immune system against infections such as HCV. The inability of the immune system to clear the HCV infection results in chronic infection. The long term consequences could be the occurrence of liver diseases, including HCC.

Previously identified information suggests that alcohol intake increases HCV RNA serum levels, at least in the presence of cellular immunity impairment (68). Additionally, it has been confirmed that HCV RNA levels drop when heavy drinkers with HCV infection abstain from alcohol or significantly reduce their alcohol consumption (69). In vitro studies have indicated that in hepatic cell lines infected with HCV replicons, HCV RNA expression increases as a result of alcohol consumption. Alcohol seems to activate the nuclear factor kappa B promoter, which is responsible for increased gene transcription. The mentioned procedure may lead to raising HCV RNA levels in HCV-infected alcoholic patients (70). Moreover, circulating autoantibodies have been reported in cases with chronic alcoholism (71). There is evidence that even a low alcohol intake in HCV carriers increases viremia and hepatic fibrosis (72). Although alcohol intake and HCV infection are independent risk factors for liver cirrhosis, the coexistence of a HCV infection accelerates the alcohol associated risk of cirrhosis and HCC (23, 28, 68, 73-80). Two or three fold greater risk of liver cirrhosis and liver disease has been reported in patients with chronic alcoholism (10).

3.2. Effects of Alcohol on Signaling Pathways

A chromosome abnormality is the most common abnormality in patients with hepatitis C and HCC, which is associated with alcohol consumption. Although the mechanisms of this process are still not completely understood, it is likely that deregulation of some mitotic proteins, such as; cyclin B1 and aurora kinase A, and the phosphorylation of gamma tubulin, controlling centrosome maturation and separation, chromosome alignment and segregation, bipolar spindle assembly and cytokinesis, are involved. Furthermore, it is known that these changes are dependent on PKR, p38MAPK and JNK pathways. Considering the results of this study, both HCV and ethanol interfere with the regular control of mitosis in hepatocarcinoma cell lines. In addition, both HCV and ethanol act through disruption of the cell cycle, causing G2/M arrest in liver cells, which occurs through its effect on p38MAPK, PKR and JNK pathways (9).

3.3. Effects of Alcohol Consumption on Liver Enzymes and Proteins

According to studies by Drumright et al. alcohol consumption increases alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the normal population. Levels of these enzymes have been reported to be higher in HCV-infected intravenous drug users than in normal groups, and it should also be noted that these changes are considerably higher in those who have HCV RNA in their sera. Therefore, this is evidence for the synergistic effects of HCV and alcohol metabolism on liver damage (17). Moreover, in a study by Kazuhiro et al. it was shown that albumin levels are higher in alcoholic cirrhosis, than in patients with cirrhosis caused by HCV. These results indicate that albumin synthesis may be affected by either alcohol metabolism or HCV replication (81).

3.4. Alcohol Consumption and Impact on Anti–Hepatitis C Virus Therapy

Ongoing alcohol consumption has been reported to decrease the effectiveness of anti-HCV treatments. A direct relationship has been shown between alcohol and the response to interferon (IFN) therapy (13). Several studies have indicated that the response to interferon is decreased in patients with alcoholism (82-86). In HCV infected patients, the IFN response is influenced by two factors, ethanol intake and HCV RNA levels. There is a significant difference between drinkers and patients that abstain (82). The accumulated data suggest that lifelong alcohol consumption has a strong negative effect on the long-term response to interferon therapy, especially in heavy drinkers (38, 86). Consequently, interferon IFN therapy for chronic hepatitis C is less effective in heavy drinkers than in non-drinkers (13). Researches have indicated that anti-HCV response rates were inversely proportional to alcohol consumption (44). Moreover, a six month period of abstinence may not be sufficient to resolve this negative effect on treatment outcomes (86). In this study, the most important predictor for nonresponse to interferon-therapy, was genotype I and age, in addition alcohol intake was the next most significant factor (86). As a result, the adverse impact of alcohol consumption on interferon therapy seems to be permanent, even after a six month avoidance period. Despite this, heavy drinkers who drank more than 69 gm/d, with more than six months avoidance before entering therapy programs, had a significant rise in their treatment response, compared to the control group. This suggests that the adverse effects of alcohol consumption on anti-HCV therapy responses may be reversible.

The proposed mechanism, by which alcohol modulates the antiviral effects of interferon, is via inhibition of signal transducers and activators of transcription (STAT1) tyrosine phosphorylation. Moreover, oxidative stress, which is induced by alcohol, was shown to impair interferon signaling, therefore this could be responsible for the resistance to antiviral therapy by INF alpha (87). However, there remains a significant question: Is there any safe amount of alcohol intake in HCV-infected patients? This question was answered in a survey by Leggio et al. who assessed the effect of different amounts of alcohol intake on the development of liver diseases. It was demonstrated that there is no safe amount of alcohol intake at present. Any amount of alcohol leads to an increased risk of liver disease. Therefore, complete avoidance should be the goal in HCV-infected patients (18).

4. Conclusions

There appears to be sufficient evidence to suggest that even low levels of alcohol consumption correlate with both the presence and progression of hepatic fibrosis. Consequently, total abstention ought to be recommended to patients (69, 72, 80). Furthermore, interferon therapy is less effective among patients with alcoholism, than non-alcoholic patients, even after a period of abstinence (69). Consequently, patients with chronic hepatitis C should limit their alcohol intake and if cirrhosis is present or interferon therapy is planned, complete avoidance from alcohol should be encouraged (69). In conclusion chronic alcoholism in patients with chronic hepatitis C appears to cause more severe and rapidly progressive liver disease, leading more frequently to liver cirrhosis and HCC.

Acknowledgements

The authors would like to gratefully thank the staff of Research Center for Molecular Medicine in Hamadan University of Medical Sciences for their cooperation.

Authors' Contribution

All authors read and approved the submission of this manuscript and contributed equally to this research.

Financial Disclosure

There was no potential conflict of interest in this article.

Funding/Support

There was no funding support for this research.

References

1. Seifi SJ, Sabouri Ghannad M. A study of HDV in HBsAg positive patients in Tabriz, Northwestern Iran. *Hepat Mon.* 2010;**10**(2):110–5.
2. Keyvani H, Mohammadi A, Sabouri Ghannad M, Hajabdolbaghi M. The Effect of GBV-C Infection on CD4 Count and Viral Loads in Patients Infected With HIV. *Hepat Mon.* 2012;**12**(1):39–42.
3. Sabouri Ghannad M, Zamani A. The full length hepatitis C virus polyprotein and interactions with the interferon-beta signalling pathways in vitro. *Iran Biomed J.* 2008;**12**(1):23–34.
4. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology.* 2012;**142**(6):1264–73 e1.
5. Taura N, Fukuda S, Ichikawa T, Miyaaki H, Shibata H, Honda T, et al. Relationship of alpha-fetoprotein levels and development of hepatocellular carcinoma in hepatitis C patients with liver cirrhosis. *Exp Ther Med.* 2012;**4**(6):972–6.
6. Bartenschlager R, Lohmann V. Novel cell culture systems for the hepatitis C virus. *Antiviral Res.* 2001;**52**(1):1–17.
7. Sabouri Ghannad M, Hojati SA, Mirzaei M, Sahebkar AH. Prevalence of hepatitis B and hepatitis C in patients referred to health centers in the Hamadan province, Iran:an epidemiologic study of infections between 2004 and 2007. *Asia Biomed.* 2013;**7**(5):619–25.
8. Seeff LB. Natural history of hepatitis C. *Hepatology.* 1997;**26**(3 Suppl 1):21S–8S.
9. Alisi A, Ghidinelli M, Zerbini A, Missale G, Balsano C. Hepatitis C virus and alcohol: same mitotic targets but different signaling pathways. *J Hepatol.* 2011;**54**(5):956–63.
10. Wiley TE, McCarthy M, Breidi L, McCarthy M, Layden TJ. Impact of alcohol on the histological and clinical progression of hepatitis C infection. *Hepatology.* 1998;**28**(3):805–9.
11. Singal AK, Anand BS. Mechanisms of synergy between alcohol and hepatitis C virus. *J Clin Gastroenterol.* 2007;**41**(8):761–72.
12. Kwon SY, Ahn MS, Chang HJ. Clinical significance of hepatitis C virus infection to alcoholics with cirrhosis in Korea. *J Gastroenterol Hepatol.* 2000;**15**(11):1282–6.
13. McCartney EM, Beard MR. Impact of alcohol on hepatitis C virus replication and interferon signaling. *World J Gastroenterol.* 2010;**16**(11):1337–43.
14. Cooper ML. Alcohol and increased behavioral risk for AIDS. *Alcohol Health & Research World.* 1992;**16**(1):67–72.
15. Kaplan AJ, Zone-Smith LK, Hannegan C, Norcross ED. The prevalence of hepatitis C in a regional level I trauma center population. *J Trauma.* 1992;**33**(1):126–8. Discussion 128-9.
16. Peters MG, Terrault NA. Alcohol use and hepatitis C. *Hepatology.* 2002;**36**(5 Suppl 1):S220–5.
17. Drumright LN, Hagan H, Thomas DL, Latka MH, Golub ET, Garfein RS, et al. Predictors and effects of alcohol use on liver function among young HCV-infected injection drug users in a behavioral intervention. *J Hepatol.* 2011;**55**(1):45–52.
18. Leggio L, Ferrulli A, Zambon A, Caputo F, Kenna GA, Swift RM, et al. Baclofen promotes alcohol abstinence in alcohol dependent cirrhotic patients with hepatitis C virus (HCV) infection. *Addict Behav.* 2012;**37**(4):561–4.
19. Harris DR, Gonin R, Alter HJ, Wright EC, Buskell ZJ, Hollinger FB, et al. The relationship of acute transfusion-associated hepatitis to the development of cirrhosis in the presence of alcohol abuse. *Ann Intern Med.* 2001;**134**(2):120–4.
20. Kubo S, Kinoshita H, Hirohashi K, Tanaka H, Tsukamoto T, Shuto T, et al. High malignancy of hepatocellular carcinoma in alcoholic patients with hepatitis C virus. *Surgery.* 1997;**121**(4):425–9.
21. Aizawa Y, Shibamoto Y, Takagi I, Zeniya M, Toda G. Analysis of factors affecting the appearance of hepatocellular carcinoma in patients with chronic hepatitis C. A long term follow-up study after histologic diagnosis. *Cancer.* 2000;**89**(1):53–9.
22. Bellentani S, Pozzato G, Saccoccio G, Crovatto M, Croce LS, Mazzoran L, et al. Clinical course and risk factors of hepatitis C virus related liver disease in the general population: report from the Dionysos study. *Gut.* 1999;**44**(6):874–80.
23. Bellentani S, Saccoccio G, Costa G, Tiribelli C, Manenti F, Sodde M, et al. Drinking habits as cofactors of risk for alcohol induced liver damage. The Dionysos Study Group. *Gut.* 1997;**41**(6):845–50.
24. Fukushima W, Tanaka T, Ohfuji S, Habu D, Tamori A, Kawada N, et al. Does alcohol increase the risk of hepatocellular carcinoma among patients with hepatitis C virus infection? *Hepatol Res.* 2006;**34**(3):141–9.
25. Trichopoulos D, Bamia C, Lagiou P, Fedirko V, Trepo E, Jenab M, et al. Hepatocellular carcinoma risk factors and disease burden in a European cohort: a nested case-control study. *J Natl Cancer Inst.* 2011;**103**(22):1686–95.
26. Lieber CS, Seitz HK, Garro AJ, Worner TM. Alcohol-related Diseases and Carcinogenesis. *Cancer research.* 1979;**39**(7 Part 2):2863–86.
27. Duguay L, Coutu D, Hetu C, Joly JG. Inhibition of liver regeneration by chronic alcohol administration. *Gut.* 1982;**23**(1):8–13.
28. Vento S, Cainelli F. Does hepatitis C virus cause severe liver disease only in people who drink alcohol? *Lancet Infect Dis.* 2002;**2**(5):303–9.
29. Gao B. Interaction of alcohol and hepatitis viral proteins: implication in synergistic effect of alcohol drinking and viral hepatitis on liver injury. *Alcohol.* 2002;**27**(1):69–72.
30. Osna NA, Clemens DL, Donohue TM, Jr. Interferon gamma enhances proteasome activity in recombinant Hep G2 cells that express cytochrome P4502E1: modulation by ethanol. *Biochem Pharmacol.* 2003;**66**(5):697–710.
31. Kessova IG, Cederbaum AI. The effect of CYP2E1-dependent oxidant stress on activity of proteasomes in HepG2 cells. *J Pharmacol Exp Ther.* 2005;**315**(1):304–12.
32. Bardag-Gorce F, French BA, Nan L, Song H, Nguyen SK, Yong H, et al. CYP2E1 induced by ethanol causes oxidative stress, proteasome inhibition and cytokeratin aggresome (Mallory body-like) formation. *Exp Mol Pathol.* 2006;**81**(3):191–201.
33. Bardag-Gorce F, Li J, French BA, French SW. The effect of ethanol-induced CYP2E1 on proteasome activity: the role of 4-hydroxynonenal. *Exp Mol Pathol.* 2005;**78**(2):109–15.
34. Osna NA, White RL, Todero S, McVicker BL, Thiele GM, Clemens DL, et al. Ethanol-induced oxidative stress suppresses generation of peptides for antigen presentation by hepatoma cells. *Hepatology.* 2007;**45**(1):53–61.
35. Szabo G, Dolganiuc A, Mandrekar P, White B. Inhibition of antigen-presenting cell functions by alcohol: implications for hepatitis C virus infection. *Alcohol.* 2004;**33**(3):241–9.
36. Dolganiuc A, Kodys K, Kopasz A, Marshall C, Mandrekar P, Szabo G. Additive inhibition of dendritic cell allostimulatory capacity by alcohol and hepatitis C is not restored by DC maturation and involves abnormal IL-10 and IL-2 induction. *Alcohol Clin Exp Res.* 2003;**27**(6):1023–31.
37. Mandrekar P, Catalano D, Dolganiuc A, Kodys K, Szabo G. Inhibition of myeloid dendritic cell accessory cell function and induction of T cell anergy by alcohol correlates with decreased IL-12 production. *J Immunol.* 2004;**173**(5):3398–407.
38. Oshita M, Hayashi N, Kasahara A, Hagiwara H, Mita E, Naito M, et al. Increased serum hepatitis C virus RNA levels among alcoholic patients with chronic hepatitis C. *Hepatology.* 1994;**20**(5):1115–20.
39. Bruggmann P, Dampz M, Gerlach T, Kravecz L, Falcato L. Treatment outcome in relation to alcohol consumption during hepatitis C therapy: an analysis of the Swiss Hepatitis C Cohort Study. *Drug Alcohol Depend.* 2010;**110**(1-2):167–71.
40. Balbi M, Donadon V, Ghersetti M, Grazioli S, Valentina GD, Gardenal R, et al. Alcohol and HCV chronic infection are risk cofactors of type 2 diabetes mellitus for hepatocellular carcinoma in Italy. *Int J Environ Res Public Health.* 2010;**7**(4):1366–78.
41. Nash KL, Woodall T, Brown AS, Davies SE, Alexander GJ. Hepatocellular carcinoma in patients with chronic hepatitis C virus infection without cirrhosis. *World J Gastroenterol.* 2010;**16**(32):4061–5.
42. Choi J. Oxidative stress, endogenous antioxidants, alcohol, and hepatitis C: pathogenic interactions and therapeutic considerations. *Free Radic Biol Med.* 2012;**52**(7):1135–50.
43. Mas VR, Fassnacht R, Archer KJ, Maluf D. Molecular mechanisms

involved in the interaction effects of alcohol and hepatitis C virus in liver cirrhosis. *Mol Med.* 2010;**16**(7-8):287–97.
44. Siu L, Foont J, Wands JR. Hepatitis C virus and alcohol. *Semin Liver Dis.* 2009;**29**(2):188–99.
45. Sherman KE, Rouster SD, Mendenhall C, Thee D. Hepatitis cRNA quasispecies complexity in patients with alcoholic liver disease. *Hepatology.* 1999;**30**(1):265–70.
46. Takahashi K, Takahashi T, Takahashi S, Watanabe K, Boku S, Matsui S, et al. Difference in quasispecies of the hypervariable region 1 of hepatitis C virus between alcoholic and non-alcoholic patients. *J Gastroenterol Hepatol.* 2001;**16**(4):416–23.
47. Dolganiuc A, Kodys K, Kopasz A, Marshall C, Do T, Romics L, Jr, et al. Hepatitis C virus core and nonstructural protein 3 proteins induce pro- and anti-inflammatory cytokines and inhibit dendritic cell differentiation. *J Immunol.* 2003;**170**(11):5615–24.
48. Pachiadakis I, Pollara G, Chain BM, Naoumov NV. Is hepatitis C virus infection of dendritic cells a mechanism facilitating viral persistence? *Lancet Infect Dis.* 2005;**5**(5):296–304.
49. Rigamonti C, Mottaran E, Reale E, Rolla R, Cipriani V, Capelli F, et al. Moderate alcohol consumption increases oxidative stress in patients with chronic hepatitis C. *Hepatology.* 2003;**38**(1):42–9.
50. Farinati F, Cardin R, De Maria N, Della Libera G, Marafin C, Lecis E, et al. Iron storage, lipid peroxidation and glutathione turnover in chronic anti-HCV positive hepatitis. *J Hepatol.* 1995;**22**(4):449–56.
51. Tsukamoto H, Lin M, Ohata M, Giulivi C, French SW, Brittenham G. Iron primes hepatic macrophages for NF-kappaB activation in alcoholic liver injury. *Am J Physiol.* 1999;**277**(6 Pt 1):G1240–50.
52. Wheeler MD. Endotoxin and Kupffer cell activation in alcoholic liver disease. *Alcohol Res Health.* 2003;**27**(4):300–6.
53. Koike K, Tsutsumi T, Miyoshi H, Shinzawa S, Shintani Y, Fujie H, et al. Molecular basis for the synergy between alcohol and hepatitis C virus in hepatocarcinogenesis. *J Gastroenterol Hepatol.* 2008;**23 Suppl 1**:S87–91.
54. Koike K, Moriya K, Ishibashi K, Matsuura Y, Suzuki T, Saito I, et al. Expression of hepatitis C virus envelope proteins in transgenic mice. *J Gen Virol.* 1995;**76 (Pt 12)**:3031–8.
55. Moriya K, Todoroki T, Tsutsumi T, Fujie H, Shintani Y, Miyoshi H, et al. Increase in the concentration of carbon 18 monounsaturated fatty acids in the liver with hepatitis C: analysis in transgenic mice and humans. *Biochem Biophys Res Commun.* 2001;**281**(5):1207–12.
56. Okuda M, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, et al. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology.* 2002;**122**(2):366–75.
57. Tsutsumi T, Suzuki T, Moriya K, Yotsuyanagi H, Shintani Y, Fujie H, et al. Alteration of intrahepatic cytokine expression and AP-1 activation in transgenic mice expressing hepatitis C virus core protein. *Virology.* 2002;**304**(2):415–24.
58. Tsutsumi T, Suzuki T, Shimoike T, Suzuki R, Moriya K, Shintani Y, et al. Interaction of hepatitis C virus core protein with retinoid X receptor alpha modulates its transcriptional activity. *Hepatology.* 2002;**35**(4):937–46.
59. Tsutsumi T, Suzuki T, Moriya K, Shintani Y, Fujie H, Miyoshi H, et al. Hepatitis C virus core protein activates ERK and p38 MAPK in cooperation with ethanol in transgenic mice. *Hepatology.* 2003;**38**(4):820–8.
60. Szabo G, Wands JR, Eken A, Osna NA, Weinman SA, Machida K, et al. Alcohol and hepatitis C virus--interactions in immune dysfunctions and liver damage. *Alcohol Clin Exp Res.* 2010;**34**(10):1675–86.
61. Karin M, Greten FR. NF-kappaB: linking inflammation and immunity to cancer development and progression. *Nat Rev Immunol.* 2005;**5**(10):749–59.
62. Bain C, Fatmi A, Zoulim F, Zarski JP, Trepo C, Inchauspe G. Impaired allostimulatory function of dendritic cells in chronic hepatitis C infection. *Gastroenterology.* 2001;**120**(2):512–24.
63. Siavoshian S, Abraham JD, Thumann C, Kieny MP, Schuster C. Hepatitis C virus core, NS3, NS5A, NS5B proteins induce apoptosis in mature dendritic cells. *J Med Virol.* 2005;**75**(3):402–11.
64. Crotta S, Stilla A, Wack A, D'Andrea A, Nuti S, D'Oro U, et al. Inhibition of natural killer cells through engagement of CD81 by the major hepatitis C virus envelope protein. *J Exp Med.* 2002;**195**(1):35–41.
65. Averill L, Lee WM, Karandikar NJ. Differential dysfunction in dendritic cell subsets during chronic HCV infection. *Clin Immunol.* 2007;**123**(1):40–9.
66. Seifert U, Liermann H, Racanelli V, Halenius A, Wiese M, Wedemeyer H, et al. Hepatitis C virus mutation affects proteasomal epitope processing. *J Clin Invest.* 2004;**114**(2):250–9.
67. Khu YL, Tan YJ, Lim SG, Hong W, Goh PY. Hepatitis C virus nonstructural protein NS3 interacts with LMP7, a component of the immunoproteasome, and affects its proteasome activity. *Biochem J.* 2004;**384**(Pt 2):401–9.
68. Pianko S, Patella S, Ostapowicz G, Desmond P, Sievert W. Fas-mediated hepatocyte apoptosis is increased by hepatitis C virus infection and alcohol consumption, and may be associated with hepatic fibrosis: mechanisms of liver cell injury in chronic hepatitis C virus infection. *J Viral Hepat.* 2001;**8**(6):406–13.
69. Schiff ER. The alcoholic patient with Hepatitis C Virus infection. *Am J Med.* 1999;**107**(6):95–9.
70. Zhang T, Li Y, Lai JP, Douglas SD, Metzger DS, O'Brien CP, et al. Alcohol potentiates hepatitis C virus replicon expression. *Hepatology.* 2003;**38**(1):57–65.
71. Cook RT. Alcohol abuse, alcoholism, and damage to the immune system--a review. *Alcohol Clin Exp Res.* 1998;**22**(9):1927–42.
72. Pessione F, Degos F, Marcellin P, Duchatelle V, Njapoum C, Martinot-Peignoux M, et al. Effect of alcohol consumption on serum hepatitis C virus RNA and histological lesions in chronic hepatitis C. *Hepatology.* 1998;**27**(6):1717–22.
73. Corrao G, Arico S. Independent and combined action of hepatitis C virus infection and alcohol consumption on the risk of symptomatic liver cirrhosis. *Hepatology.* 1998;**27**(4):914–9.
74. Donato F, Tagger A, Gelatti U, Parrinello G, Boffetta P, Albertini A, et al. Alcohol and hepatocellular carcinoma: the effect of lifetime intake and hepatitis virus infections in men and women. *Am J Epidemiol.* 2002;**155**(4):323–31.
75. Hassan MM, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, et al. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology.* 2002;**36**(5):1206–13.
76. Kovach SJ, Sitzmann JV, McKillop IH. Inhibition of alcohol dehydrogenase blocks enhanced Gi-protein expression following ethanol treatment in experimental hepatocellular carcinoma in vitro. *Eur J Gastroenterol Hepatol.* 2001;**13**(10):1209–16.
77. Noda K, Yoshihara H, Suzuki K, Yamada Y, Kasahara A, Hayashi N, et al. Progression of type C chronic hepatitis to liver cirrhosis and hepatocellular carcinoma--its relationship to alcohol drinking and the age of transfusion. *Alcohol Clin Exp Res.* 1996;**20**(1 Suppl):95A–100A.
78. Ostapowicz G, Watson KJ, Locarnini SA, Desmond PV. Role of alcohol in the progression of liver disease caused by hepatitis C virus infection. *Hepatology.* 1998;**27**(6):1730–5.
79. Schiff ER. Hepatitis C and alcohol. *Hepatology.* 1997;**26**(3 Suppl 1):39S–42S.
80. Westin J, Lagging LM, Spak F, Aires N, Svensson E, Lindh M, et al. Moderate alcohol intake increases fibrosis progression in untreated patients with hepatitis C virus infection. *J Viral Hepat.* 2002;**9**(3):235–41.
81. Kotoh K, Fukushima M, Horikawa Y, Yamashita S, Kohjima M, Nakamuta M, et al. Serum albumin is present at higher levels in alcoholic liver cirrhosis as compared to HCV-related cirrhosis. *Exp Ther Med.* 2012;**3**(1):72–5.
82. Loguercio C, Di Pierro M, Di Marino MP, Federico A, Disalvo D, Crafa E, et al. Drinking habits of subjects with hepatitis C virus-related chronic liver disease: prevalence and effect on clinical, virological and pathological aspects. *Alcohol Alcohol.* 2000;**35**(3):296–301.
83. Anand BS, Currie S, Dieperink E, Bini EJ, Shen H, Ho SB, et al. Alcohol use and treatment of hepatitis C virus: results of a national multicenter study. *Gastroenterology.* 2006;**130**(6):1607–16.
84. Chang A, Skole K, Gautam M, Schmutz J, Black M, Thomas R, et al. The impact of past alcohol use on treatment response rates in patients with chronic hepatitis C. *Aliment Pharmacol Ther.* 2005;**22**(8):701–6.
85. Mochida S, Ohnishi K, Matsuo S, Kakihara K, Fujiwara K. Effect of

alcohol intake on the efficacy of interferon therapy in patients with chronic hepatitis C as evaluated by multivariate logistic regression analysis. *Alcohol Clin Exp Res.* 1996;**20**(9 Suppl):371A–7A.
86. Tabone M, Sidoli L, Laudi C, Pellegrino S, Rocca G, Della Monica P, et al. Alcohol abstinence does not offset the strong negative effect of lifetime alcohol consumption on the outcome of interferon therapy. *J Viral Hepat.* 2002;**9**(4):288–94.
87. Plumlee CR, Lazaro CA, Fausto N, Polyak SJ. Effect of ethanol on innate antiviral pathways and HCV replication in human liver cells. *Virol J.* 2005;**2**:89.