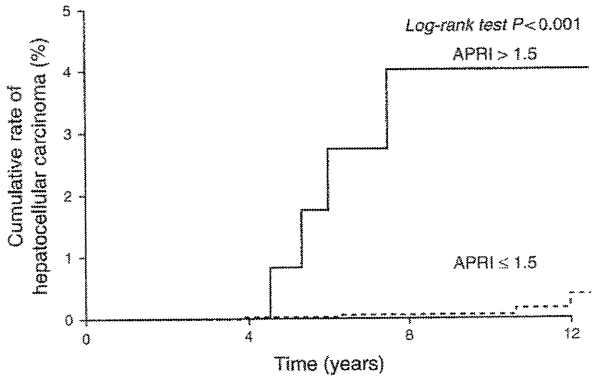

Figure 3. Cumulative rate of development of hepatocellular carcinoma in Japanese patients with non-alcoholic fatty liver disease diagnosed by ultrasonography according to the APRI. APRI, aspartate aminotransferase to platelet ratio index.

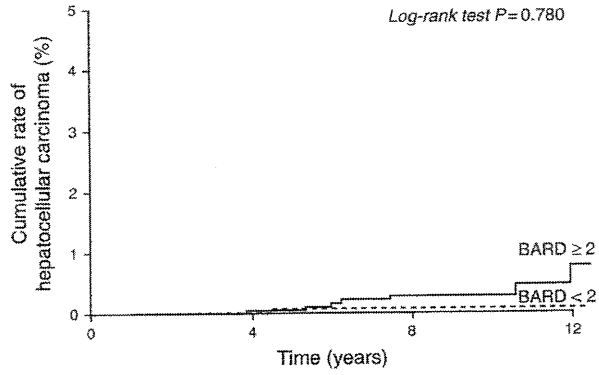

Figure 4. Cumulative rate of development of hepatocellular carcinoma in Japanese patients with non-alcoholic fatty liver disease diagnosed by ultrasonography according to the BARD score. BARD, body mass index, AST/alanine aminotransferase ratio, and diabetes.

15,944 patients were diagnosed as having a non-alcoholic history (past daily alcohol intake of <20 g/day) and without complicated fatty liver by US between January 1997 and December 2010 at the Department of Hepatology and the Health Management Center (Toranomon Hospital, Tokyo, Japan), and in this large population at the same institute, HCC occurred in only 2 of 15,944 (0.013%) patients during the follow-up period. In this study, the incidence of HCC in NAFLD patients was 0.25%, which is higher than that in the non-alcoholic, non-fatty liver population.

In this study, advanced age, high AST level, thrombocytopenia (marker of progression of liver fibrosis), and diabetes were identified as risk factors for the development of HCC in Japanese patients with US-diagnosed NAFLD. These results are in agreement with the previously reported risk factors of NASH-related HCC, namely advanced age, advanced fibrosis, cirrhosis, and diabetes (49). In this regard, a high serum ALT level was reported to be a surrogate for histopathological diagnosis of NAFLD (50). Clinically, most patients with NAFLD are known to have high ALT levels. Our analysis identified elevated AST levels, but not elevated ALT levels, as a risk factor for NAFLD-related HCC. The exact reason for this finding is not clear, but we speculate the following: based on the pathological features of NASH, necroinflammatory changes and perisinusoidal fibrosis usually appear around zone 3, i.e., the pericentral vein area of the liver. Among liver enzymes, the distribution of AST is closer to zone 3 than distributions of other enzymes. Thus, the correlation with high AST levels observed in this study may reflect the significance of AST as a factor related to NASH disease progression, in contrast to serum ALT levels.

Advanced liver fibrosis in NASH is considered to be an important etiological factor for the incidence of HCC. In this study, we identified a ≥10% decrease in platelet counts (relative to baseline) in 9 of the 16 patients whose NAFLD progressed to HCC. Thrombocytopenia has also been previously reported to be a risk factor for the incidence of HCC (39). Thus, it seems that the decrease in platelet count during progression is also an important etiological factor in the incidence of HCC, as it is in viral-induced hepatitis, and may indicate advancing liver fibrosis in NAFLD.

The results of this study revealed that with respect to APRI, the incidence of HCC was significantly higher in patients with an APRI of >1.5; however, no significant associations between the BARD score and the incidence of HCC were observed. **Table 3** shows the change of APRI and BARD scores from the beginning of follow-up to the time of diagnosis of HCC. At the beginning of follow-up, 5 of 16 (31.3%) patients had a >1.5 APRI. However, at the time of diagnosis of HCC, only 2 (12.5%) patients had a >1.5 APRI. Furthermore, in 8 of 16 (50.0%) patients, the APRI had improved at the time of diagnosis of HCC. Of these 8 patients, 1 patient underwent splenectomy due to associated thrombocytopenia, although the platelet count had increased at the time of diagnosis of HCC; however, 2 patients in whom the platelet count had decreased ≥10% since the beginning of follow-up were included. In contrast, with respect to the BARD score, 12 of 16 (75.0%) patients had a BARD score of ≥2, and BARD scores were maintained or increased in all cases. On the basis of this result, the BARD score may be more useful for evaluating disease progression in NAFLD patients than the APRI. Thus, although each of these fibrosis estimation procedures were previously believed to have both strengths and weaknesses, these results demonstrated that both estimations can be clinically applied for early detection of patients at high risk for HCC. Interestingly, two patients in this study with fatty liver but without fibrosis developed HCC. This finding differs from that of another large-scale study of NAFLD patients (25,43–45), which did not report the development of HCC from fatty liver without fibrosis. The above findings emphasize the need for further studies to identify factors that trigger the onset of HCC process in NAFLD patients without fibrosis, including single-nucleotide polymorphisms.

This study has certain limitations. First, this was a retrospective cohort trial. Second, the male:female ratio was strongly biased toward males. This heterogeneity makes it difficult to interpret the study results. Third, this study was not performed as a comparison to the background incidence of HCC in the Japanese general population without NAFLD and alcoholic liver disease.

Table 3. Characteristics of patients with non-alcoholic fatty liver disease who developed hepatocellular carcinoma during follow-up

			At study entry							At diagnosis of hepatocellular carcinoma									
Case	Sex	Diabetes	Age (yrs)	BMI (kg/m²)	AST (IU/L)	ALT (IU/L)	Platelet count (×10³/μL)	APRI	BARD score	Age (yrs)	BMI (kg/m²)	AST (IU/L)	ALT (IU/L)	Platelet count (×10³/μL)	APRI	BARD score	Treatment	Pathological diagnosis	Follow-up (yrs)
1	M	Present	41	35.4	42	50	225	0.57	4	51	40.4	26	22	82	0.96	4	Resection	NASH Stage 4 (cirrhosis)	9.9
2	M	Absent	41	29.7	77	94	146	1.61	3	63	29.0	38	32	116	0.99	3	RFA	Not performed	21.7
3	M	Absent	50	37.7	41	60	111	1.12	1	55	31.8	32	50	111	0.86	1	TACE	NASH Stage 4 (cirrhosis)	4.5
4	M	Absent	55	24.5	72	62	47	4.64	2	61	24.5	31	20	117	0.80	2	RFA	NASH Stage 4 (cirrhosis)	6.0
5	M	Absent	56	27.7	43	64	304	0.43	0	81	24.5	54	43	240	0.68	2	RFA	NASH Stage 1	24.7
6	M	Present	56	28.7	17	24	227	0.23	2	68	23.0	20	14	140	0.43	3	TACE	Not performed	12.0
7	M	Absent	59	25.3	46	60	217	0.64	2	79	23.3	20	17	206	0.29	2	RFA	Fatty liver without fibrosis	19.6
8	M	Absent	60	23.1	14	16	170	0.25	2	79	24.0	26	24	131	0.60	2	Resection	Fatty liver without fibrosis	18.8
9	M	Absent	60	28.6	31	46	161	0.58	1	73	28.3	41	46	105	1.18	3	RFA	NASH Stage 3 (pre-cirrhosis)	13.0
10	M	Present	62	23.7	41	68	222	0.56	1	84	24.7	57	52	119	1.45	3	RFA	NASH Stage 2	21.5
11	M	Present	65	29.4	60	80	138	1.32	2	72	29.1	33	26	67	1.49	4	RFA	Not performed	7.5
12	F	Absent	58	38.1	75	68	106	2.14	3	72	35.3	41	19	69	1.80	3	Resection	Liver cirrhosis	14.3
13	F	Present	63	24.2	40	32	75	1.60	3	67	25.7	36	27	71	1.54	3	TACE	Not performed	3.8
14	F	Present	64	24.4	22	20	271	0.25	3	80	24.0	31	24	225	0.42	3	RFA	NASH Stage 3 (pre-cirrhosis)	16.2
15	F	Present	68	22.1	67	54	159	1.28	3	75	22.1	49	37	162	0.92	3	RFA	Not performed	6.2
16	F	Absent	83	23.1	140	109	163	2.60	2	88	25.3	56	46	178	0.95	2	TACE	NASH Stage 2	5.3

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; BARD, body mass index, AST/ALT ratio, and diabetes; M, male; NASH, non-alcoholic steatohepatitis; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization; APRI, aspartate aminotransferase to platelet ratio index.

Thus, these results do not adequately address whether NAFLD as a whole is associated with a higher risk of HCC. However, the strengths of this study include the long-term follow-up period and the inclusion of a large number of patients.

In conclusion, this retrospective study is the first to describe the cumulative incidence and risk factors of HCC in a large number of Japanese patients with NAFLD. On the basis of these results, we recommend careful monitoring and follow-up of elderly NAFLD patients with high serum AST, thrombocytopenia, and diabetes for early diagnosis and treatment of HCC.

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CONFLICT OF INTEREST

Guarantor of the article: Yusuke Kawamura, MD.

Specific author contributions: Yusuke Kawamura: study concept and design, acquisition of data, statistical analysis, and drafting of manuscript; Yasuji Arase: acquisition of data, statistical analysis, and study supervision; Kenji Ikeda: acquisition of data; Yuya Seko: acquisition of data; Norihiro Imai: acquisition of data; Tetsuya Hosaka: acquisition of data; Masahiro Kobayashi: acquisition of data; Satoshi Saitoh: acquisition of data; Hitomi Sezaki: acquisition of data; Norio Akuta: acquisition of data; Fumitaka Suzuki: acquisition of data; Yoshiyuki Suzuki: acquisition of data; Yuki Ohmoto: acquisition of data; Hiroshi Tsuji: acquisition of data; Kazuhisa Amakawa: acquisition of data; Hiromitsu Kumada: acquisition of data.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- The incidence of hepatocellular carcinoma (HCC) in patients with non-alcoholic fatty liver disease (NAFLD) reported in several longitudinal follow-up studies from non-Asian countries ranged from 0 to 0.5%, whereas that in patients with non-alcoholic steatohepatitis (NASH) ranged from 0 to 2.8%.
- Several previous studies have reported that advanced age, advanced fibrosis, cirrhosis, and diabetes are risk factors for NASH-related HCC.

WHAT IS NEW HERE

- The prevalence of HCC over a long follow-up period in Japanese patients with NAFLD diagnosed by ultrasonography was 0.25%, with an annual rate of 0.043%.
- In addition to high aspartate aminotransferase (AST) level, thrombocytopenia (suggested to be associated with advanced liver fibrosis), advanced age, and diabetes were independent risk factors for HCC in Japanese NAFLD patients.
- Non-invasive procedures used to predict liver fibrosis, such as the AST to platelet ratio index (APRI), may be useful to predict which Japanese NAFLD patients are at high risk of developing HCC.

REFERENCES

1. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999;340:745–50.
2. Bosch X, Ribes J, Borras J. Epidemiology of primary liver cancer. Semin Liver Dis 1999;19:271–85.
3. Okuda K, Fujimoto I, Hanai A *et al.* Changing incidence of hepatocellular carcinoma in Japan. Cancer Res 1987;47:4967–72.
4. Johnson PJ, Williams R. Cirrhosis and the aetiology of hepatocellular carcinoma. J Hepatol 1987;4:140–7.
5. Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. Hepatology 1993;18:47–53.
6. Yamanaka N, Tanaka T, Tanaka W *et al.* Correlation of hepatitis virus serologic status with clinicopathologic features in patients undergoing hepatectomy for hepatocellular carcinoma. Cancer 1997;79:1509–15.
7. Koike Y, Shiratori Y, Sato S *et al.* Risk factors for recurring hepatocellular carcinoma differ according to infected hepatitis virus-an analysis of 236 consecutive patients with a single lesion. Hepatology 2000;32:1216–23.
8. Yotsuyanagi H, Shintani Y, Moriya K *et al.* Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. J Infect Dis 2000;181:1920–8.
9. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–31.
10. Williams R. Global changes in liver disease. Hepatology 2006;44:521–6.
11. Torres DM, Harrison SA. Diagnosis and therapy of nonalcoholic steatohepatitis. Gastroenterology 2008;134:1682–98.
12. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and non-alcoholic steatohepatitis: selected practical issues in their evaluation and management. Hepatology 2009;49:306–17.
13. Fan JG, Farrell GC. Epidemiology of non-alcoholic fatty liver disease in China. J Hepatol 2009;50:204–10.
14. Watanabe S, Yaginuma R, Ikejima K *et al.* Liver diseases and metabolic syndrome. J Gastroenterol 2008;43:509–18.
15. Vega GL, Chandalia M, Szczepaniak LS *et al.* Metabolic correlates of nonalcoholic fatty liver in women and men. Hepatology 2007;46:716–22.
16. van der Poorten D, Milner KL, Hui J *et al.* Visceral fat a key mediator of steatohepatitis in metabolic liver disease. Hepatology 2008;48:449–57.
17. Fan JG, Li F, Cai XB *et al.* Effects of nonalcoholic fatty liver disease on the development of metabolic disorders. J Gastroenterol Hepatol 2007;22:1086–91.
18. Angulo P, Keach JC, Batts KP *et al.* Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30:1356–62.
19. Stern SE, Williams K, Ferrannini E *et al.* Identification of individuals with insulin resistance using routine clinical measurements. Diabetes 2005;54:333–9.
20. Muniyappa R, Lee S, Chen H *et al.* Current approaches for assessing insulin sensitivity and resistance *in vivo*: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008;294:E15–26.
21. Adams LA, Feldstein A, Lindor KD *et al.* Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. Hepatology 2004;39:909–14.
22. Tanné F, Gagnadoux F, Chazouillères O *et al.* Chronic liver injury during obstructive sleep apnea. Hepatology 2005;41:1290–6.
23. Kheirandish-Gozal L, Sans Capdevila O, Kheirandish E *et al.* Elevated serum aminotransferase levels in children at risk for obstructive sleep apnea. Chest 2008;133:92–9.
24. Arase Y, Suzuki F, Ikeda K *et al.* Multivariate analysis of risk factors for the development of type 2 diabetes in nonalcoholic fatty liver disease. J Gastroenterol 2009;44:1064–70.
25. Adams LA, Lymp JF, St Sauver J *et al.* The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–21.
26. Lee RG. Nonalcoholic steatohepatitis: a study of 49 patients. Hum Pathol 1989;20:594–8.
27. Powell EE, Cooksley WG, Hanson R *et al.* The natural history of non-alcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. Hepatology 1990;11:74–80.
28. Teli MR, James OF, Burt AD *et al.* The natural history of nonalcoholic fatty liver: a follow-up study. Hepatology 1995;22:1714–9.
29. Matteoni CA, Younossi ZM, Gramlich T *et al.* Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–9.
30. Dam-Larsen S, Franzmann M, Andersen IB *et al.* Long term prognosis of fatty liver: risk of chronic liver disease and death. Gut 2004;53:750–5.

31. Evans CD, Oien KA, MacSween RN *et al.* Non-alcoholic steatohepatitis: a common cause of progressive chronic liver injury? J Clin Pathol 2002;55:689–92.
32. Wai CT, Greenson JK, Fontana RJ *et al.* A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 2003;38:518–26.
33. Loaeza-del-Castillo A, Paz-Pineda F, Oviedo-Cárdenas E *et al.* AST to platelet ratio index (APRI) for the noninvasive evaluation of liver fibrosis. Ann Hepatol 2008;7:350–7.
34. Harrison SA, Oliver D, Arnold HL *et al.* Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut 2008;57:1441–7.
35. Lonardo A, Bellini M, Tartoni P *et al.* The bright liver syndrome. Prevalence and determinants of a "bright" liver echopattern. Ital J Gastroenterol Hepatol 1997;29:351–6.
36. Mancia G, De Backer G, Dominiczak A *et al.* Management of Arterial Hypertension of the European Society of Hypertension; European Society of Cardiology. 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). J Hypertens 2007;25:1105–87.
37. Genuth S, Alberti KG, Bennett P *et al.* Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Follow-up report on the diagnosis of diabetes mellitus. Diabetes Care 2003;26:3160–7.
38. Fleming TR, Harrington DP, O'Brien PC. Designs for group sequential tests. Control Clin Trials 1984;5:348–61.
39. Brunt EM, Janney CG, Di Bisceglie AM *et al.* Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–74.
40. Ishak K, Baptista A, Bianchi L *et al.* Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–9.
41. Ratziu V, Bonyhay L, Di Martino V *et al.* Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. Hepatology 2002;35:1485–93.
42. Siegel AB, Zhu AX. Metabolic syndrome and hepatocellular carcinoma: two growing epidemics with a potential link. Cancer 2009;115:5651–61.
43. Ekstedt M, Franzén LE, Mathiesen UL *et al.* Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44: 865–73.
44. Ong JP, Pitts A, Younossi ZM. Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. J Hepatol 2008;49:608–12.
45. Rafiq N, Bai C, Fang Y *et al.* Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol 2009;7:234–8.
46. Amarapurkar DN, Hashimoto E, Lesmana LA *et al.* How common is non-alcoholic fatty liver disease in the Asia-Pacific region and are there local differences? J Gastroenterol Hepatol 2007;22:788–93.
47. Kojima S, Watanabe N, Numata M *et al.* Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. J Gastroenterol 2003;38:954–61.
48. Hamaguchi M, Kojima T, Takeda N *et al.* The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med 2005;143: 722–8.
49. Starley BQ, Calcagno CJ, Harrison SA. Nonalcoholic fatty liver disease and hepatocellular carcinoma: a weighty connection. Hepatology 2010;51: 1820–32.
50. Ruhl CE, Everhart JE. Determinants of the association of overweight with elevated serum alanine aminotransferase activity in the United States. Gastroenterology 2003;124:71–9.

＜短　報＞

NS5A阻害剤とNS3プロテアーゼ阻害剤併用投与における早期の抗ウイルス効果

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緒言：C型慢性肝炎に対する現在の標準治療はペグインターフェロン（PEG-IFN）製剤とリバビリン（Riba）の併用投与が基本であるが，最近では，効果の向上と治療期間の短縮を目的に新たな蛋白合成阻害剤の併用試験が行われ良好な結果が得られることが報告されている．芥田らの報告によればPEG-IFN＋Ribaに蛋白合成阻害剤であるtelaprevirを加えた三剤併用投与では，24週間の投与で60％以上の完全著効がえられており，今後の治療の主流をなしていくと思われる[1]．今回我々は更なる治療効果の向上を目指してNS5A阻害剤とNS3阻害剤の併用投与を行い，治療早期の抗ウイルス効果につき検討を行ったので報告する．

対象と方法：標準治療であるPEG＋Riba併用療法を24週以上行いながらも，開始前のウイルス量から2 log IU/ml以上の低下が認められなかったHCV-1b高ウイルス量のnull-responderの5例を対象とした．2種類のNS5AとNS3に対する阻害剤を経口で連日24週間投与するという治療計画であり，NS5A阻害剤は60 mgを1日1回，NS3阻害剤は600 mgを1日2回いずれも食後に併用投与した．投与初日は，1，2，4，8，12時間後に，また，24，48時間後とさらに7，15日目にHCV-RNA量を経時的に測定し，投与早期の抗ウイルス効果を解析した．HCV-RNAの測定はTaqman PCR法を用いて行い，1.2 log IU/ml未満でかつシグナルが検出されなくなった時点で陰性化したと判定した．

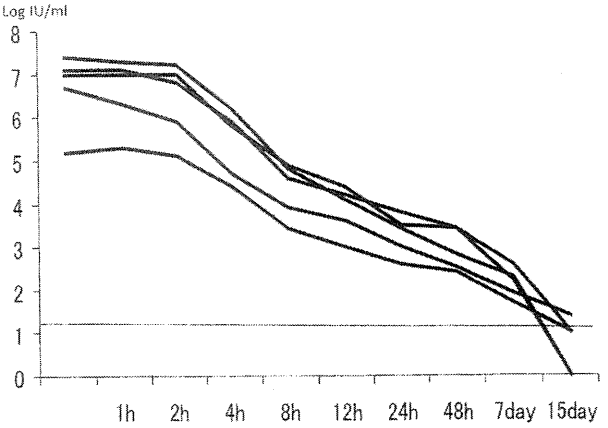

Fig. 1　Changes in hepatitis C (HCV) RNA concentration over duration of study treatment

結果：投与5症例の背景を示すと，男性2例（40％），年齢の中央値は60歳（53-69歳），IL-28BのSNP（rs8099917）は，TT2例，TG3例であった．また，ウイルス側の要因であるcoreの変異は70, 91においては，1例がdouble wild，1例が70 mutant，91 wildで，3例がdouble mutantであり，ISDR変異は，0が1例，1が4例であった．開始前のALT値は中央値で70 IU/l（範囲13～114），HCV-RNA量は中央値7.0 log IU/mL（範囲5.2～7.4）であった．投与後の経時的ウイルス量の変化をFig. 1に示すが，2 log IU/mL低下までにかかった時間はそれぞれ，4，8，8，8，12時間と短時間で急激なウイルス量の減少が認められた．ウイルス低下速度とIL-28B等の予測因子との関係の詳細を示すと，4時間で2 log IU/mL低下した症例は，TTでcoreは70 mutant，91 wildであり，同様に8時間の3例はTTかつdouble wildが1例，TGかつdouble mutantが2例であった．12時間かかった症例はTGかつdouble mutantであった．また，15日までに2例が陰性化．2

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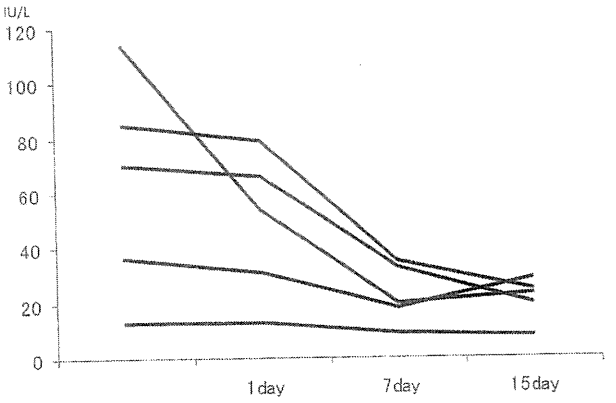

Fig. 2 Changes in ALT over duration of study treatment

例が測定感度以下に低下している．次にFig. 2に示すようにALT値は7日で全例正常化し，途中中止の1例を除きその後も正常域を維持している．

投与中止例となった症例は60歳の女性で，開始7日目にはAST/ALTとも正常化し，10日目までは副反応もなく，ウイルス量も5.2から1.7と良好な減少を示した．開始10日目に高脂肪食を摂取後より軟便，下痢をきたし発熱と共に炎症所見の上昇が認められた．これに対して抗生剤の投与を開始ししだいに解熱傾向となった．肝胆道系酵素の上昇は当初認められず，ビリルビン値のみが上昇，16日目に6.5 mg/dlまで上昇したため服用を中止した．20日目よりAST優位の肝酵素の上昇が認められ投与30日目に432/315 IU/lとピークを迎えその後低下した．この間，胆道系酵素の上昇は一度も認められておらず，ビリルビン値も薬剤中止後速やかに低下している．ウイルスは15日目に1.2 log IU/mL＞陽性であったが，中止2週後に陰性化し，その後投与終了24週まで陰性を持続している．本症例のウイルス消失については，肝炎の再燃に伴いウイルス排除が起こった可能性も否定できないが肝酵素上昇のピークよりも前にすでにウイルスは消失しており，本治療薬による効果の可能性が高いと判断している．

考察：新たなC型慢性肝炎治療薬であるNS5A阻害剤とNS3阻害剤の併用投与における早期の抗ウイルス効果につき報告した．NS5Aは多機能性蛋白質であり，in vitroおよびin vivoにおけるHCVの複製に必要であり，ヒトでの相同体が知られていないことからHCV治療の標的として期待されている．また，非構造蛋白質(NS)3のN末端はセリンプロテアーゼ(NS3プロテアーゼ)であり，NS4Aと協力して蛋白質分解活性を有する複合体を形成する．NS3/4Aプロテアーゼ複合体の活性は，in vitroでのウイルス複製に非常に重要な役割を果たしており，今回の薬剤はNS3プロテアーゼに特異的な阻害活性を有している．

少数例の検討ではあるものの早期の抗ウイルス効果はこれまでに類を見ないくらい良好であり，15日目には40％の症例に陰性化が得られたということは対象がPEG＋Ribaのnull responderということを勘案すれば十分すぎる効果といえる．特にIL-28BがTGであり，coreがdouble mutantで，前回のPEG＋Riba治療がnull responderというような最難治例において，現状の治療ではSVRの望みがほとんどないような症例が3例とも投与開始後12時間以内にウイルスの十分な低下が得られていることは特筆すべきものがある．これまで1b型高ウイルス量症例に対する標準的治療では，約50％のSVRがえられるものの，その治療効果の向上のためには投与期間の延長や他の薬剤の併用などといった更なる負担が課せられてきた．今回の経口剤投与のみの治療においては，IFNに伴うような感冒様症状，食欲不振，貧血などの副反応は認められていない．我々はこれまでにテラプレビル単剤投与にてSVRを獲得した症例の報告をしてきた[2]．本症例は1b型で低ウイルス量であるものの副反応の出現もなく24週間の経口剤のみの投与で完全著効がえられた．また，最近ではpolymerase inhibitor（RG7128）とdanoprevirの組み合わせで早期に抗ウイルス効果が認められるという報告や[3]，danoprevir単独投与は早期に抗ウイルス効果を発揮すると共にHOMA-IRを改善するといった報告[4]もあり，IFNを使用しない治療法が盛んに試されまた治療効果に期待がもたれている．今回の症例が今後どのような経過をとるのかは投与予定期間の24週が終了してみなければ断定できないが，現時点ではこれまでの中で最も抗ウイルス効果の高い治療に無反応であった症例全てにおいてウイルスが陰性化したということは評価できることと考える．今後さらに経過を観察すると共に，副反応の出現にも注意を怠らないことが肝要であると思われる．

索引用語：C型肝炎ウイルス，NS5A阻害剤，プロテアーゼ阻害剤

文献：1) Akuta N, Suzuki F, Hirakawa M, et al. Hepatology 2010; 52 (2): 421—429　2) Suzuki F,

Suzuki Y, Akuta N, et al. J Clin Virol 2010; 47 (1): 76—78　3) Gane EJ, Roberts SK, Stedman CA, et al. Lancet 2010; 376 (9751): 1467—1475　4) Moucari R, Forestier N, Larrey D, et al. Gut 2010; 59 (12): 1694—1698

英文要旨

Effect of early antiviral agent therapy (NS3 and NS5A inhibitors) in chronic hepatitis C null responders

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To further improve therapeutic effect on chronic hepatitis C, we have administered NS3 inhibitor and NS5A inhibitor together, and examined effects of early antiviral agent therapy. The subjects were five cases where interferon is ineffective (null responders). The NS5A and NS3 inhibitors are oral drugs and were daily administered for 24 weeks. Figure 1 shows time-dependent change of the number of viruses after the therapy started, and rapid decrease of viruses is recognized. Within 12 hours, HCV-RNA decreased by more than 2 log IU/m*l* in every patient. Two patients became negative for the virus by the 15th day after the therapy started. Furthermore, 80% of cases by the 28th day and all the cases by the 56th day became negative. The new therapy has manifested excellent early antiviral effect.

Key words: hepatitis C virus, NS5A inhibitor, protease inhibitor

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＜速　報＞

B型慢性肝疾患に対する核酸アナログ療法によるHBs抗原消失とその関連因子の検討

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緒言：B型肝疾患に対する核酸アナログ療法の有効性は広く知られており，経過観察期間が長くなるにつれ，B型肝炎治療の最終目標であるHBs抗原（HBsAg）消失を得られる症例も散見されている．本邦及び海外からいくつかの報告もあるが[1)~4)]，いまだ長期に渡る核酸アナログ使用例での報告はない．今回我々は長期間の核酸アナログ治療によるHBsAg消失とその関連因子について検討した．

対象と方法：1995年～2006年までに当院でB型慢性肝疾患に対して，ラミブジン単独投与を開始した769例を対象とした．これら全ての症例で6カ月以上のHBV持続感染を確認した．核酸アナログ投与内容の内訳はラミブジン単独投与継続306例，ラミブジン投与開始後耐性ウイルス出現に対してラミブジン＋アデフォビル併用を行った症例297例，ラミブジン→エンテカビルへの切り替え症例166例であった．これらの症例のうち，何らかの理由で投与中止した症例は46例存在し，それ以外の症例はすべて継続投与を行った．HBsAg測定はCLIA法（ARCHITECT® HBsAg QT）を用いた．

Table　Factors associated with HBsAg clearance by univariate and multivariate analysis.

	Univariate		Multivariate	
factors	Hazard Ratio (95%CI)	P	Hazard Ratio (95%CI)	P
Age (≥50yr)	0.94 (0.48-1.89)	0.865		
Gender (F)	0.59 (0.21-1.68)	0.323		
Family history of HBV infection	**0.43 (0.22-0.84)**	**0.014**		
Presence of cirrhosis	0.79 (0.56-1.12)	0.192		
Previous IFN therapy	**2.70 (1.31-5.59)**	**0.007**	**2.96 (1.34-6.54)**	**0.008**
HBV genotype (A)	**3.39 (2.27-5.08)**	**<0.0001**	**3.64 (2.40-5.52)**	**<0.0001**
HBeAg (positive)	1.23 (0.61-2.48)	0.563		
HBV DNA (≥6.0 logcopies/mL)	1.20 (0.52-2.78)	0.674		
HBsAg (<2000 IU/mL)	1.40 (0.70-2.80)	0.346		
ALT (≥300 IU/L)	**1.47 (1.02-2.11)**	**0.040**		
Platelets count ($<1.2\times10^5$/mm^3)	0.91 (0.34-2.43)	0.123		
Treatment response at 6 months				
HBeAg positive → clearance	**3.15 (1.49-6.66)**	**0.003**	**2.22 (1.01-4.88)**	**0.046**
HBV DNA (<2.6 logcopies/mL)	**3.56 (1.22-10.4)**	**0.021**	**4.07 (1.36-12.2)**	**0.012**

The bolded numbers: statically significant.
Abbreviation: HBsAg, Hepatitis B surface antigen; IFN, interferon; HBeAg: Hepatitis B envelope antigen

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ラミブジン開始後のHBsAg消失に寄与する因子についてCox比例ハザードモデルを用いて，単変量及び多変量解析を行い検討した．

結果：ラミブジン投与開始からの観察期間の中央値は6.3年（0.7–13.5年）であった．ラミブジン投与前にIFN治療歴を有する症例が297例（39%）存在した（投与期間の中央値は27週（2–575週））．HBV感染の家族歴を有する症例が538例（70%）存在した．ラミブジン投与開始後のHBsAg消失は33例で認められた（内訳は投与中消失31例，投与終了後消失2例）．全体での累積HBsAg消失率は5年：1.8%，10年：7.3%であった．HBsAg消失に寄与する因子について単変量解析を行ったところ，抽出された因子は，家族歴あり（48% vs. 74%），IFN治療歴あり（64% vs. 37%），genotype A（25% vs. 2.6%），開始時ALT高値（300 IU/L以上）（33% vs. 20%），治療開始6カ月以内のHBe抗原消失（30% vs. 12%：HBeAg持続陽性例や持続陰性例に比して），治療開始後6カ月時点でのHBVDNA陰性化（<2.6 log copies/ml）（85% vs. 67%）の6因子が抽出された（Table）．また治療法別で検討すると，ラミブジン単独またはエンテカビル切り替え症例では，ラミブジン＋アデフォビル併用療法症例に比してHBsAg消失率が高率であった（P＝0.014）．

上記の因子を用いて，HBsAg消失に寄与する因子について多変量解析を行ったところ，独立因子としてgenotype A，IFN治療歴，治療開始6カ月時点でHBeAg陽性→陰性化，治療開始後6カ月時点でのHBVDNA陰性化の4因子が抽出された（Table）．

考察：今回の検討では核酸アナログ投与後のHBsAg消失にはHBV genotypeが強く関わっている事が分かった．これまでテルビブジンやPegIFNでの報告のように[4][5]，genotype AではHBsAg量の低下が，他のgenotypeより起こりやすいため，HBsAg消失が起こりやすいと考えられる．またIFN治療歴や核酸アナログ治療早期の反応性などがHBsAg消失に寄与し，治療開始時ALTの上昇が強い症例でもHBsAgが消失しやすい傾向にあったことから，核酸アナログ治療によりHBsAgを消失させるためには，核酸アナログ自体の抗ウイルス作用だけでなく，宿主の免疫反応が必要と推察される．今後HBsAg消失を目指した，核酸アナログ治療法の工夫が望まれる．この研究はラミブジン投与症例での検討であるが，今後は現在の標準治療であり，薬剤耐性出現が極めて低率のエンテカビル投与症例での検討も必要と思われる．

索引用語：HBsAg，核酸アナログ，IFN

文献：1）Kobayashi M, Suzuki F, Akuta N, et al. J Med Virol 2007; 79: 1472—1477　2）Manesis EK, Hadziyannis ES, Angelopoulou OP, et al. Antiviral Ther 2007; 12: 73—82　3）Gish RG, Chang TT, Lai CL, et al. J Viral Hepat 2010; 17: 16—22　4）Wursthorn K, Jung M, Riva A, et al. Hepatology 2010；52：1612—1620　5）Moucari R, Martinot-Peignoux M, Mackiewicz V, et al. Antivir Ther 2009; 14: 1183—1188

英文要旨

Clearance of hepatitis B surface antigen during long-term nucleot(s)ide analogues treatment in chronic hepatitis B

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Clearance of HBsAg is considered the ultimate goal in the treatment for chronic hepatitis B. We analyzed clinical factors associated with HBsAg clearance during long-term nucleot(s)ide analogue treatment. By univariate analysis, HBV genotype, family history of HBV infection, previous IFN therapy, HBeAg clearance at 6 months, and undetectable HBV DNA at 6 months were significant predictive factors. By multivariate analysis, HBV genotype, previous IFN therapy, HBeAg clearance at 6 months, and undetectable HBV DNA at 6 months were independent and significant predictive factors of HBsAg clearance. We conclude that patients with genotype A have high probability of HBsAg clearance, and it seems that not only the antiviral potential of nucleot(s)ide analogue but host immune response is needed to achieve HBsAg clearance.

Key words: hepatitis B surface antigen, nucleot(s)ide analogues, interferon

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＜短　報＞

コバス TaqMan HBV「オート」v2.0における同一時の血清検体と血漿検体のHBV DNA検出率の検討

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緒言：HBV DNAの測定は，1996年に分岐HBV DNAプローブ法が臨床応用されてから，検査技術の進歩に伴いTMA（transcription-mediated amplification）法やPCR法などの高感度な測定法の開発が進んできた．現在，日常の臨床で使用されているreal-time PCR法は，HBV DNA量が1.5～2.0 Log copies/mL程度まで検出可能となった．今回我々は，TaqMan HBV v2.0法（コバスTaqMan HBV「オート」v2.0[1)]：ロシュ・ダイアグノスティックス，東京）を用い，血清と血漿の同時採血を行い，各検体の有用性について検討を行ったので報告する．

対象と方法：対象は，B型慢性肝炎および肝硬変の成人でEntecavir投与1年以上経過しALT（alanine aminotransferase）値が30 IU/l以下を持続している52症例（104検体）とした．内訳は，男性29例（55.8%），年齢52歳：中央値（27～81歳）であった．HBV genotypeはgenotype A：2例，genotype B：5例，genotype C：44例，typing不能：1例であった．52症例に対し治療効果の均一化を計るため同一検体で2回の採血を実施しHBV DNAを測定した．2回目のポイントの採血は，1回目の採血後，8週±2週の間に実施した．血清用採血管で全血5 mLと血漿用採血管（EDTA-2K）で全血8 mLを採血，速やかに遠心分離後，TaqMan HBV v2.0法（最小検出感度は，血清検体：2.0 Log copies/mL，血漿検体：1.7 Log copies/mL）にて測定を行った．統計解析は，統計解析ソフトウェアSTAT Flex ver. 5.0を用い，$P<0.05$で有意とした．本試験は，当院の倫理審査委員会の承認を受け，実施についてのインフォームド・コンセントを行った．

結果：血清・血漿ペア検体104例のうち，血清と血漿の両者でHBV DNAを検出したのは，25例（24.0%），両者ともに検出不能は，41例（39.4%）であったが，血清で検出したが血漿では検出不能であったのは，6例（5.8%）であり，血漿で検出したが血清では検出不能であったのは，32例（30.8%）で，血漿での検出率は，血清より有意（$P<0.001$［McNemar検定］）に高率であった（Table 1）．

考察：核酸アナログ製剤を長期に投与することによりその耐性株の出現および肝炎の悪化が認められることから，特に若年者においては核酸アナログ製剤を中止することも考え，HBV DNA量をはじめ，HBs抗原，HBコア関連抗原などの種々のHBVマーカーについて検討が行われている[2)]．Drug freeが可能な症例選定の必要条件の一つはHBV DNAの持続陰性化であり[3)]，投与中止後ALT値の再上昇による重症化・劇症化が懸念されることより，高感度にHBV DNAを検出することが重要である可能性がある．

そこで今回，我々は臨床検体を用いTaqMan HBV

Table 1　Detail correlation between plasma specimen (EDTA-2K) and serum specimen

		Serum	
		detected	not detected
plasma (EDTA-2K)	detected	25 (24.0%)	32* (30.8%)
	not detected	6* (5.8%)	41 (39.4%)

*: $P<0.001$［McNemar検定］

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v2.0 の血清検体と血漿検体の有用性の検討を行った．対象の 104 検体のうち血清または血漿のいずれかで HBV DNA を検出したのは，血清は 5.8％に対し血漿では 30.8％と血漿での HBV DNA の検出率は統計学的有意差（P＜0.001）をもって高率であった．一方，血清で HBV DNA を検出したが血漿では検出不能であった検体も 5.7％存在したが，年齢，性別，genotype などに一定の偏りは無く，この現象は，最小検出感度未満の極めて低濃度の検体で発生するバラツキに起因する確率論的な現象と考えられた．

以上から，血漿検体を用いることにより血清検体より高感度に HBV DNA を測定することが可能となった．今後より高感度な測定が必要な分野での臨床応用が期待される．

索引用語：B 型肝炎ウイルス，
TaqMan PCR 法，高感度

文献：1）Goedel S, Rullkoetter M, Weisshaar S, et al. Journal of Clinical Virology 2009; 45: 232—236　2）Matsumoto A, Tanaka E, Minami N, et al. Hepatology Research 2007; 37: 661—666　3）田中榮司．厚生科学研究費補助金（肝炎等克服緊急対策研究事業）総括研究報告書，2010，H21-肝炎—一般-001

英文要旨

The evaluation of the sensitivity between serum and plasma specimen for COBAS TaqMan HBV v2.0

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The sensitivity in serum and plasma for HBV DNA was evaluated by using 104 clinical specimens from 52 patients who were treated with entecavir for ≥1 year and continued ALT levels ≤30 IU/*l*. The measurement employed the COBAS TaqMan HBV v2.0. Twenty-five specimens (24.0%) were detected from both serum and plasma, and 41 specimens (39.4%) were not detected from both. On the other hand, there were 32 specimens (30.8%) with detectable from plasma but undetectable from serum, and only 6 specimens (5.8%) with detectable from serum but undetectable from plasma. This result suggested the sensitivity of HBV DNA using plasma specimen is more sensitive than that of serum specimen with statistical significance ($p<0.001$).

Key words: hepatitis B virus, TaqMan, high sensitivity

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BRIEF ARTICLE

Serum manganese superoxide dismutase and thioredoxin are potential prognostic markers for hepatitis C virus-related hepatocellular carcinoma

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Abstract

AIM: To evaluate the clinical significance of oxidative stress markers in patients with hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC).

METHODS: Sixty-four consecutive patients who were admitted to Kagoshima University Medical and Dental Hospital were enrolled in this retrospective study. All patients had chronic liver disease (CLD) due to infection with HCV. Thirty patients with HCV-related HCC, 34 with HCV-related CLD without HCC (non-HCC), and 20 healthy volunteers (HVs) were enrolled. Possible associations between serum manganese superoxide dismutase (MnSOD) and thioredoxin (TRX) levels and clinical parameters or patient prognosis were analyzed over a mean follow-up period of 31.7 mo.

RESULTS: The serum MnSOD levels were significantly higher in patients with HCV-related HCC than in patients without HCC ($P = 0.03$) or HVs ($P < 0.001$). Similarly, serum TRX levels were also significantly higher in patients with HCV-related HCC than in patients without HCC ($P = 0.04$) or HVs ($P < 0.01$). However, serum levels of MnSOD and TRX were not correlated in patients with HCC. Among patients with HCC, the overall survival rate (OSR) was lower in patients with MnSOD levels $\geq$ 110 ng/mL than in patients with levels < 110 ng/mL ($P = 0.01$), and the OSR tended to be lower in patients with TRX levels < 80 ng/mL ($P = 0.05$). In addition, patient prognosis with HCC was poorest with serum MnSOD levels $\geq$ 110 ng/mL and serum TRX levels < 80 ng/mL. Furthermore, a multivariate analysis using a Cox proportional hazard model and serum levels of five factors (MnSOD, prothrombin time, serum albumin, serum α-fetoprotein (AFP), and serum des-γ-carboxy prothrombin) revealed that MnSOD levels $\geq$ 110 ng/mL (risk ratio: 4.12, 95% confidential interval: 1.22-13.88, $P = 0.02$) and AFP levels $\geq$ 40 ng/mL (risk ratio: 6.75; 95% confidential interval: 1.70-26.85, $P < 0.01$) were independent risk factors associated with a poor patient prognosis.

CONCLUSION: Serum MnSOD and TRX levels are potential clinical biomarkers that predict patient prognosis in HCV-related HCC.

Key words: Oxidative stress; Manganese superoxide dismutase; Thioredoxin; Hepatitis C virus; Hepatocellular carcinoma

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INTRODUCTION

As a significant cause of global cancer morbidity and mortality, hepatocellular carcinoma (HCC) is the fifth- and seventh-most frequently diagnosed cancer worldwide in men and women, respectively, and is the second- and sixth-most frequent cause of cancer deaths in men and women, respectively[1]. HCC is most frequently caused by persistent infection with hepatitis C or B virus. Early HCC diagnosis and better treatments have helped to improve the prognosis for patients with HCC. Also, interferon (IFN)-based treatments not only eliminate hepatitis C virus (HCV) infection, but also prevent HCC in patients with chronic hepatitis C (CHC)[2]. However, IFN-based therapies do not always effectively eliminate HCV infection or prevent HCC. Thus, biomarkers that are indicative of HCC pathological condition would have many clinical benefits, including aiding in the selection of the most appropriate treatment for a patient's disease.

Oxidative stress results from an imbalance in the production of reactive oxygen species (ROS) and the antioxidative defenses that maintain a cellular redox state. ROS include superoxide anions, hydrogen peroxide, hydroxyl radicals and nitric oxide, all of which are indispensable elements in many biochemical processes[3]. ROS are mainly derived from Kupffer and inflammatory cells in the liver[4], and upon exposure to other cells are thought to induce apoptosis, necrosis, inflammation, immune responses, fibrosis and tissue regeneration[5]. In liver disease, there is an overproduction of ROS from endogenous sources such as the mitochondria, peroxisomes, and activated inflammatory cells. In particular, ROS of mitochondrial origin were recently reported to be elevated in patients with alcoholic liver disease, non-alcoholic steatohepatitis (NASH)[6,7] and HCV-related chronic liver disease (CLD)[8]. Conversely, cells are protected from oxidative stress by intracellular antioxidants such as glutathione (GSH) and thioredoxin (TRX) and by various antioxidant enzymes such as superoxide dismutase (SOD), GSH peroxidase, catalase, and heme oxygenase-1[9-11]. Collectively, the relative expression levels of these molecules may serve as biomarkers for various liver diseases, including HCV-related HCC.

Manganese SOD (MnSOD) is an antioxidant enzyme that catalyzes the dismutation of the highly reactive superoxide anion to O_2 and to the less reactive species H_2O_2. We have previously demonstrated that MnSOD expression was induced in primary cultured hepatocytes that were loaded with hydrogen peroxide *in vitro* and that serum MnSOD levels can be used to distinguish between NASH and simple steatosis in patients with nonalcoholic fatty liver disease[7]. However, the clinical significance of serum MnSOD levels in HCV-related CLD has not been fully investigated.

TRX was originally discovered in *Escherichia coli* as a proton donor for ribonucleotide reductase[12]. Subsequently, the human *TRX* gene was cloned as an adult T-cell leukemia-derived factor and was originally described as an interleukin-2 receptor inducer present in the cell culture supernatant of human T-lymphotropic virus type-1 -transformed cells[13]. TRX expression is induced by various oxidative stressors in patients with acquired immunodeficiency syndrome[14], Sjögren's syndrome[15], rheumatoid arthritis[16], and malignant neoplasms[17,18]. Previous studies have reported that serum TRX is an oxidative stress marker and that serum TRX levels increase in patients with HCV-related CLD during liver fibrosis progression[19]. In addition, serum TRX levels are reported to be elevated in patients with NASH compared to patients with simple steatosis[20]. However, the clinical significance of elevated TRX levels among patients infected with HCV in relation to HCC diagnosis and prognosis has not been elucidated.

In this study, we aimed to clarify the clinical significance of serum levels of MnSOD and TRX in patients with HCV-related CLD, and in particular among patients with HCC.

MATERIALS AND METHODS

Patients

Sixty-four consecutive patients who were admitted to Kagoshima University Medical and Dental Hospital between December 2006 and November 2008 were enrolled in this retrospective study. All patients had CLD due to an HCV infection and were diagnosed with HCC (30 patients; HCC group) or without HCC (34 patients; non-HCC group). Twenty healthy volunteers (HVs) were also enrolled in this study.

In this study, HCC was diagnosed based on findings from abdominal ultrasound, abdominal computed tomography, and serum levels of α-fetoprotein (AFP) and des-γ-carboxy prothrombin (DCP, also known as PIVKA-II). Patients were excluded from this study if they were positive for hepatitis B surface antigen; other types of hepatitis, including autoimmune hepatitis and alcoholic liver disease; or other malignancies.

The study endpoint was patient death, the available follow-up date, or December 31, 2010. Patient follow-up

periods ranged from 5.1 to 44.6 mo, with a mean observation time of 31.7 mo. Informed consent was obtained from all study patients and healthy controls. This study was approved by the ethical committees of Kagoshima University Graduate School of Medical and Dental Sciences and Kagoshima University Medical and Dental Hospital.

Laboratory markers

The clinical laboratory parameters assessed included platelet count (Plt), prothrombin time (PT), albumin (Alb), total bilirubin (T-Bil), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), AFP and DCP. The serologically defined HCV genotype (HCV serotype) was determined using a serological genotyping assay kit (Immunocheck F-HCV Grouping; International Reagents Co., Tokyo, Japan). If the HCV serotype could not be determined, the HCV genotype was evaluated using the HCV Core Genotype assay (SRL, Tokyo, Japan). HCV genotype 1b was included with serotype I, while genotypes 2a and 2b were included with serotype II. No other HCV genotype was detected in this study population. HCV RNA titers were quantified using either quantitative RT-PCR (Amplicor monitor version 2, Roche, Tokyo, Japan) or the Cobas TaqMan PCR assay (Roche, Tokyo, Japan). Patients were categorized as having a high viral load if their values were 100 KIU/mL or greater based on quantitative RT-PCR analysis, or 5 log IU/mL or more based on the Cobas TaqMan PCR assay.

Evaluation of clinical stage

Hepatic function was assessed in the HCC group using Child-Pugh staging based on both clinical (ascites and encephalopathy) and laboratory (Alb, T-Bil, and PT) parameters. HCC clinical stage was assessed based on a patient's Cancer of the Liver Italian Program (CLIP) score, which was calculated by adding points for the following four variables: Child-Pugh stage, tumor morphology, AFP value, and portal venous invasion[21,22]. The Japan Integrated Staging (JIS) system[23,24], developed by the Liver Cancer Study Group of Japan and based on a combination of Child-Pugh stage and HCC TNM classification, was used to clinically stage HCC.

Serum MnSOD and TRX levels

Serum was obtained from peripheral blood samples by centrifugation at 4000 *g* for 5 min at room temperature. Serum samples were frozen at -80 ℃ until further use. Serum MnSOD or TRX levels were measured using the Human Superoxide Dismutase 2 (AbFRONTIER, Seoul, Korea) and human thioredoxin (Redox Bio Science, Kyoto, Japan) ELISAs, respectively.

Statistical analysis

Results are expressed as the mean and standard deviation. *P* values less than 0.05 were regarded as statistically significant. Statistical analyses were performed using the Fischer's exact test or the Mann-Whitney *U* test, as appropriate. The area under the curve (AUC) was calculated for the receiver operating characteristic (ROC) curve in order to measure the overall accuracy of the test. The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of diagnostic test were additionally determined according to the protocol described previously[25]. Differences among the three groups were evaluated using the Kruskal-Wallis test followed by Dunn's multiple comparison tests. Correlation coefficients were calculated using Spearman's rank correlation analysis. The Kaplan-Meier method was used to estimate death for each parameter that had been identified at enrollment, and the death distribution curves were compared using the log-rank test. Univariate and multivariate analyses of patient outcome risk ratios were performed using Cox's proportional hazards regression analyses. All statistical analyses were conducted using PASW Statistics v. 18 (SPSS Inc., Chicago, IL).

Table 1 Patient clinical characteristics

Characteristics	Non-HCC group (*n* = 34)	HCC group (*n* = 30)	*P* value[1]
Age (yr)	62.3 ± 11.0	72.2 ± 7.5	< 0.001
Sex (male/female)	10/24	21/9	< 0.01
Plt (× 10^4/μL)	17.0 ± 5.5	10.3 ± 5.2	< 0.001
PT (%)	99.7 ± 13.3	77.6 ± 11.8	< 0.001
Alb (g/dL)	4.3 ± 0.4	3.6 ± 0.6	< 0.001
T-Bil (mg/dL)	0.8 ± 0.3	1.5 ± 0.8	< 0.001
ALT (IU/L)	44.8 ± 30.2	52.0 ± 28.2	0.12
γ-GTP (IU/L)	31.3 ± 16.1	56.2 ± 44.3	< 0.01
AFP (ng/mL)	7.2 ± 22.8	85.9 ± 197.6	< 0.001
DCP (mAU/mL)	22.8 ± 14.7	485.5 ± 1982.6	0.001
HCV serotype group (1/2)	18/10 (*n* = 28)	21/3 (*n* = 24)	0.06
HCV RNA level (high/low)	28/5 (*n* = 33)	21/4 (*n* = 25)	0.99

Data are shown as the mean ± SD. *n*: Number of patients or the number of samples analyzed. [1]Differences between mean values were evaluated using either the Fischer's exact test or the Mann-Whitney *U* test, as appropriate. Plt: Platelet count; PT: Prothrombin time; Alb: Albumin; T-Bil: Total bilirubin; ALT: Alanine aminotransferase; γ-GTP: γ-glutamyl transpeptidase; AFP: alpha-fetoprotein; DCP: des-γ-carboxy prothrombin; HCV: Hepatitis C virus; RNA: Ribonucleic acid.

RESULTS

Patient characteristics and classification according to the presence of hepatocellular carcinoma

Table 1 summarizes the baseline clinical characteristics of the 64 patients who were classified based on the presence or absence of HCC. Age, sex, and clinical laboratory parameters, including Plt, PT, Alb, T-Bil, γ-GTP, AFP and DCP, were significantly different between these two groups.

Serum MnSOD and TRX levels in hepatocellular carcinoma patients

Serum MnSOD levels were significantly higher in patients with HCC compared to patients without HCC ($P = 0.03$) and HVs ($P < 0.001$) (Figure 1A). The serum TRX levels were also significantly higher in the HCC group compared to the non-HCC group ($P = 0.04$) and HV group ($P < 0.01$) (Figure 1B). However, there was no correlation between these two markers in the HCC group ($P = 0.28$, $r = 0.20$) (Figure 1C).

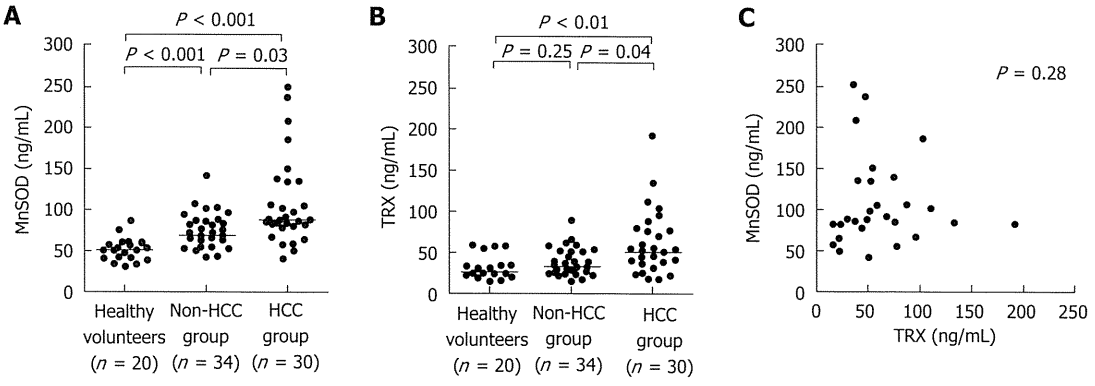

Figure 1 Serum levels of manganese superoxide dismutase and thioredoxin in the hepatocellular carcinoma, non-hepatocellular carcinoma and healthy volunteer groups. A: Serum manganese superoxide dismutase (MnSOD) levels were significantly higher in the hepatocellular carcinoma (HCC) group than in either the non-HCC group ($P = 0.03$) or the healthy volunteers (HV) group ($P < 0.001$); B: Serum thioredoxin (TRX) levels were also significantly higher in the HCC group than in either the non-HCC group ($P = 0.04$) or the HV group ($P < 0.01$); C: No significant correlation was detected between serum MnSOD and TRX levels in the HCC group.

Table 2 Sensitivity, specificity, positive predictive value, negative predictive value and accuracy of manganese superoxide dismutase and α-fetoprotein serum levels for diagnosis of hepatocellular carcinoma in all patients (%)

Factors	Sensitivity	Specificity	PPV	NPV	Accuracy
MnSOD (≥ 110 ng/mL)	26.7	97.1	88.9	60.0	64.1
AFP (≥ 40 ng/mL)	33.3	97.1	90.9	62.3	67.2
Combination[1]	46.7	94.1	87.5	66.7	71.9

[1]MnSOD ≥ 110 ng/mL and/or AFP ≥ 40 ng/mL. PPV: Positive predictive value; NPV: Negative predictive value; MnSOD: Manganese superoxide dismutase; AFP: α-fetoprotein.

Table 3 Correlation between serum manganese superoxide dismutase or thioredoxin levels and laboratory data in the hepatocellular carcinoma group

Factors	HCC group (n = 30)			
	Serum MnSOD levels		Serum TRX levels	
	Correlation coefficient	P value	Correlation coefficient	P value
Age (yr)	-0.97	0.61	0.11	0.55
Plt (× 10^4/μL)	0.03	0.89	0.66	< 0.001
PT (%)	-0.36	0.05	0.12	0.53
Alb (g/dL)	-0.63	< 0.001	0.19	0.33
T-Bil (mg/dL)	0.25	0.18	0.05	0.79
ALT (IU/L)	0.12	0.52	0.15	0.42
γ-GTP (IU/L)	0.30	0.11	0.28	0.13
AFP (ng/mL)	0.38	0.04	0.11	0.57
DCP (mAU/mL)	0.57	0.001	0.12	0.52

P values were assessed by Spearman's rank correlation analysis. MnSOD: Manganese superoxide dismutase; TRX: Thioredoxin; HCC: Hepatocellular carcinoma; Plt: Platelet count; PT: Prothrombin time; Alb: Albumin; T-Bil: Total bilirubin; ALT: Alanine aminotransferase; γ-GTP: γ-glutamyl transpeptidase; AFP: α-fetoprotein; DCP: des-γ-carboxy prothrombin.

Diagnostic value of serum MnSOD and TRX levels for patients with hepatocellular carcinoma and hepatitis C virus infection

Serum AFP and DCP concentrations are established diagnostic markers for HCC. To evaluate the utility of MnSOD and TRX for the diagnosis of HCC, we measured AFP and DCP expression in addition to MnSOD and TRX expression. In an AUC-ROC analysis, AFP was the strongest diagnostic marker for HCC (AUC-ROC, 0.90). AUC-ROCs for MnSOD, TRX and DCP were 0.73, 0.77 and 0.77, respectively. Additional analyses showed that the accuracy of AFP (≥ 40 ng/mL) for diagnosis of HCC was higher than that of MnSOD (≥ 110 ng/mL) (Table 2), while the combination of AFP and MnSOD was a more accurate marker of HCC than either marker alone.

Association of serum MnSOD or TRX levels with laboratory data in the HCC group

Serum MnSOD levels for the 30 patients in the HCC group were positively correlated with serum AFP and DCP levels and were negatively correlated with serum Alb levels (Table 3). Serum MnSOD levels were also significantly higher in patients with two or more HCC tumors than in patients with a single HCC tumor [average ± SD (ng/mL), 125.4 ± 50.9 *vs* 87.4 ± 48.8, $P = 0.008$], although HCC tumor size was not associated with serum MnSOD levels. In addition, HCC patient serum MnSOD levels increased in parallel with the Child-Pugh stage, CLIP score and JIS score (Figure 2A-C). In contrast, serum TRX levels were only associated with platelet counts (Table 3). Serum TRX levels were not associated with HCC tumor number or size. Furthermore, there were no significant correlations between serum TRX levels for various scores (Figure 2D-F).

Overall survival rate based on serum MnSOD or TRX levels in the HCC group

In the HCC group, the overall patient survival rate was significantly lower ($P = 0.01$) in patients with MnSOD levels ≥ 110 ng/mL compared to patients with levels < 110 ng/mL (Figure 3A). In addition, the overall survival rate tended to be lower ($P = 0.05$) in patients with TRX levels < 80 ng/mL compared to those with levels ≥ 80 ng/mL (Figure 3B). Furthermore, among all HCC groups, patients who had both serum MnSOD levels ≥ 110

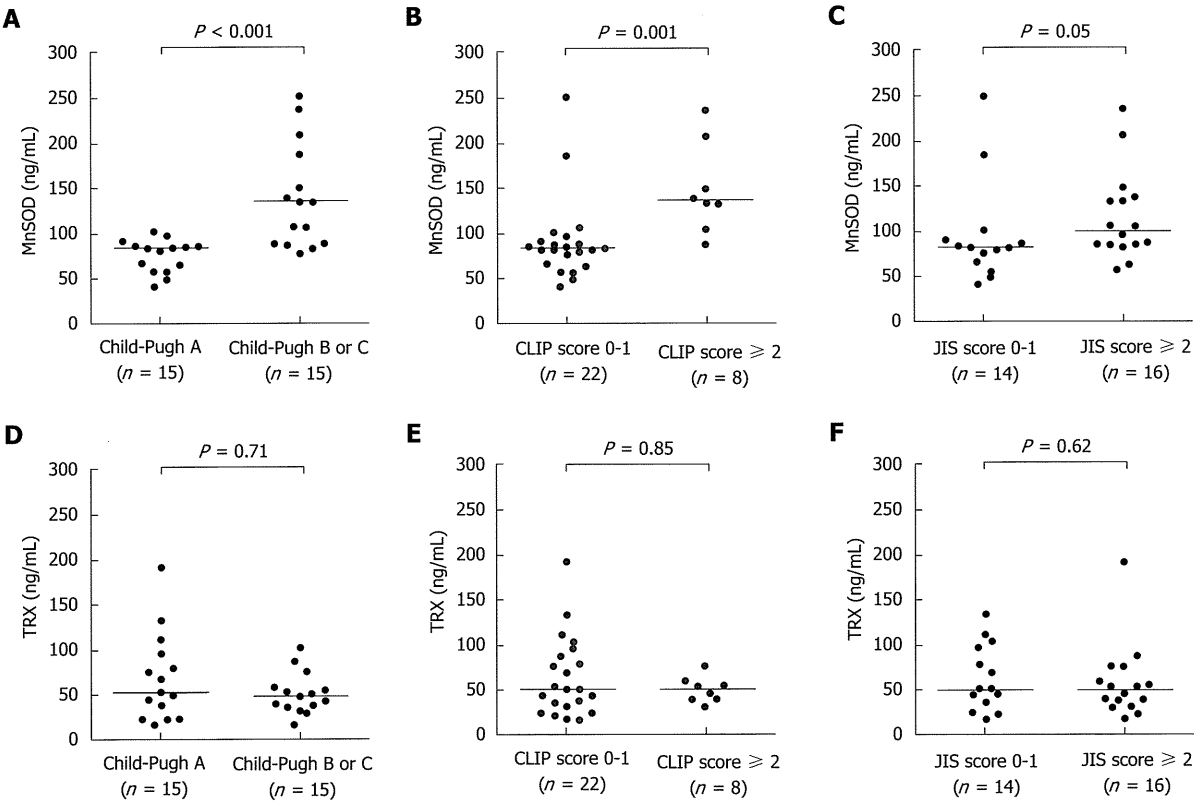

Figure 2 Clinical significance of serum manganese superoxide dismutase and thioredoxin levels in hepatocellular carcinoma. In the hepatocellular carcinoma (HCC) group, differences in serum manganese superoxide dismutase (MnSOD) and thioredoxin (TRX) levels were evaluated based on Child-Pugh stage, cancer of the liver italian program (CLIP) score and Japan integrated staging (JIS) score. A: Serum MnSOD levels were significantly higher in patients with Child-Pugh B or C compared to those with Child-Pugh A ($P < 0.001$); B: Serum MnSOD levels in patients with a CLIP score of 2 or greater were significantly higher compared to levels in patients with a CLIP score of 0 or 1 ($P = 0.001$); C: In addition, serum MnSOD levels tended to be higher in patients with a JIS score of 2 or greater compared to patients with a JIS score of 0 or 1 ($P = 0.05$); D-F: In contrast, serum TRX levels were not significantly different based on Child-Pugh stage, CLIP score or JIS score.

ng/mL and TRX levels < 80 ng/mL had a significantly poorer prognosis. Conversely, patients with a serum TRX level ≥ 80 ng/mL had a favorable prognosis, regardless of their serum MnSOD level (Figure 3C).

In addition to serum MnSOD and TRX levels, other possible prognostic factors were also investigated in the HCC group. A univariate analysis (log-rank test) revealed that the survival rate was significantly different between patients with high and low levels of MnSOD, PT, Alb, AFP and DCP, but not other factors such as TRX (Table 4). A multivariate analysis using a Cox proportional hazard model and five markers (MnSOD, PT, Alb, AFP and DCP) selected based on the results of the univariate analysis revealed that MnSOD levels ≥ 110 ng/mL and AFP levels ≥ 40 ng/mL were independent risk factors that were associated with a poor patient prognosis (Table 5). In addition, similar results were obtained from a similar multivariate analysis using the same five factors and TRX, supporting the finding that TRX is not an independent risk factor associated with HCC prognosis. Furthermore, patient Child-Pugh stage, CLIP score and JIS score, which were calculated based on several factors including clinical symptoms and laboratory data, were also prognostic factors for patients with HCC (Table 4). A multivariate analysis using the three markers of MnSOD, Child-Pugh stage and CLIP score indicated that Child-Pugh stage was also a significant prognostic factor (risk ratio: 6.19, 95% confidential interval: 1.33-28.95, $P = 0.02$).

DISCUSSION

HCV infection is the most important known contributor to the etiology of HCC. An increasing incidence of HCC has been largely attributed to a rise in HCV infections in the general population during the last 50 to 60 years[26]. During HCV infection, ROS production increases and persists throughout the infection. In addition, ROS are thought to play a major role in the pathogenesis of chronic inflammatory changes in the liver, leading to increased hepatic fibrosis and decreased hepatic function. In this study, we have shown that both serum MnSOD and TRX levels are elevated in patients with HCV-related HCC, with no correlation between these two markers. In addition, serum MnSOD and TRX levels were a useful predictor of overall patient survival. Serum MnSOD and TRX levels are reported to be biomarkers of oxidative stress in several diseases, including liver disease[7,14,17,19,27-29]. There were a small number of enrolled patients in this study and other contributors to liver diseases such as chronic hepatitis B infection should be further evaluated. However, our

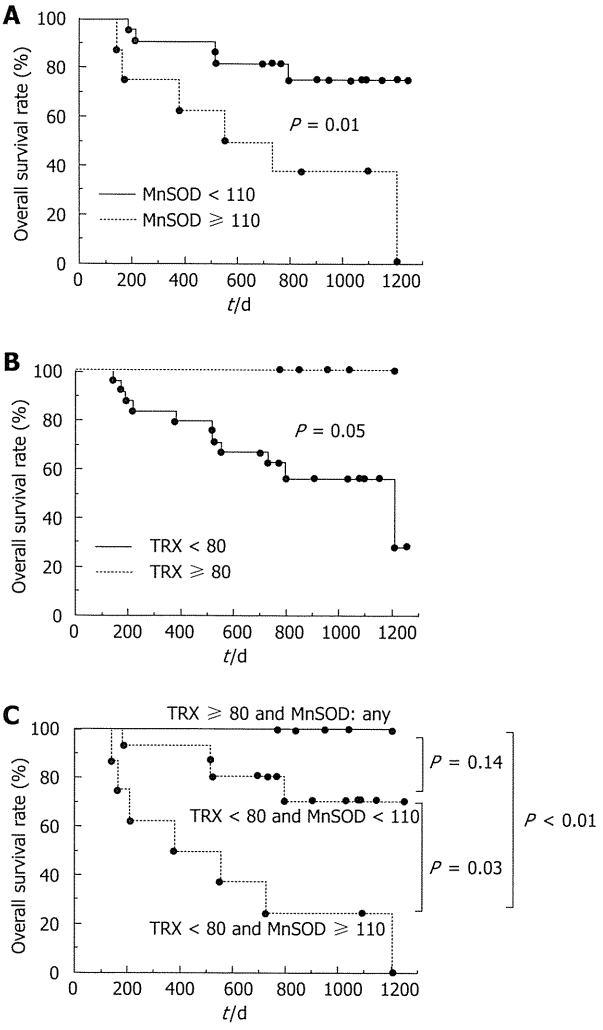

Figure 3 Overall hepatocellular carcinoma patient survival based on serum levels of manganese superoxide dismutase or thioredoxin. Overall survival was plotted using the Kaplan-Meier method after separation into two or three groups defined as follows: A: Manganese superoxide dismutase (MnSOD) < 110 ng/mL or ≥ 110 ng/mL; B: Thioredoxin (TRX) < 80 ng/mL or ≥ 80 ng/mL; TRX ≥ 80 ng/mL, TRX < 80 ng/mL; C: MnSOD < 110 ng/mL, or TRX < 80 ng/mL and MnSOD ≥ 110 ng/mL. The overall survival rate was lower in patients with MnSOD levels ≥ 110 ng/mL (P = 0.01) (A). Also, cumulative patient survival rate tended to be lower in patients with TRX levels < 80 ng/mL (P = 0.05) (B). Among these groups, patients with serum TRX levels < 80 ng/mL and serum MnSOD levels ≥ 110 ng/mL had the poorest prognosis (C).

Table 4 Univariate analysis of prognostic factors in the hepatocellular carcinoma group

Factors	Category	Number	*P* value[1]
Single marker			
MnSOD (ng/mL)	< 110/≥ 110	22/8	0.01
TRX (ng/mL)	< 80/≥ 80	24/6	0.05
Age (yr)	< 70/≥ 70	12/18	0.23
Plt (× 10^4/μL)	< 10/≥ 10	19/11	0.38
PT (%)	< 80/≥ 80	15/15	0.02
Alb (g/dL)	< 3.5/≥ 3.5	15/15	0.02
T-Bil (mg/dL)	< 1.5/≥ 1.5	18/12	0.34
ALT (IU/L)	< 40/≥ 40	11/19	0.58
γ-GTP (IU/L)	< 50/≥ 50	17/13	0.98
AFP (ng/mL)	< 40/≥ 40	20/10	< 0.01
DCP (mAU/mL)	< 40/≥ 40	16/14	0.02
Staging system			
Child-Pugh stage	A/≥ B	16/14	< 0.01
CLIP score	0-1/≥ 2	22/8	0.01
JIS score	0-1/≥ 2	14/16	0.41

[1]*P* values were assessed using the log-rank test. MnSOD: Manganese superoxide dismutase; TRX: Thioredoxin; Plt: Platelet count; PT: Prothrombin time; Alb: Albumin; T-Bil: Total bilirubin; ALT: Alanine aminotransferase; γ-GTP: γ-glutamyl transpeptidase; AFP: Alpha-fetoprotein; DCP: Serum des-γ-carboxy prothrombin; CLIP: Cancer of the Liver Italian Program; JIS: Japan Integrated Staging.

Table 5 Multivariate analysis of prognostic factors in the hepatocellular carcinoma group

Factors	Risk ratio	95% CI	*P* value
MnSOD (≥ 110 ng/mL)	4.12	1.22-13.88	0.02
AFP(≥ 40 ng/mL)	6.75	1.70-26.85	< 0.01

95% CI: 95% confidence interval; MnSOD: Manganese superoxide dismutase; AFP: α-fetoprotein.

study has clearly demonstrated the clinical significance of these markers in patients with HCV-related HCC.

Serum MnSOD and TRX levels should both reflect hepatic oxidative stress. The results of the current study showed that both of these markers were increased in the HCC group relative to levels in the non-HCC group and the HV group (Figure 1A and B). However, there was no correlation between these two markers in the HCC group (Figure 1C). MnSOD is primarily localized to the mitochondrial matrix[3] and abnormal mitochondrial morphologies are frequently observed in CHC[8]. Therefore, MnSOD may be an indicator of mitochondrial disorders that are induced by oxidative stress. On the other hand, there are two TRX proteins, cytoplasmic TRX1 and mitochondrial TRX2[30]. TRX1 negatively regulates the apoptosis signal-regulating kinase 1 (ASK1)-c-Jun N-terminal kinase/P38 apoptotic pathway by binding to and inhibiting the kinase activity of ASK1, which plays an important role in ROS-induced cellular responses[31]. TRX2 is an essential regulator of mitochondrial ROS levels that has been associated with mitochondrial outer membrane permeability[32]. In the present study, we examined the serum levels of TRX1, but not TRX2, using a sandwich ELISA. Thus, the MnSOD and TRX proteins that were examined in this study have different origins in the mitochondria and cytoplasm, respectively, which could contribute to the lack of correlation between these two markers.

Several studies have shown that the HCV core protein directly inhibits the electron transport system and modulates apoptosis, transcription, and cell signaling[33]. Abdalla *et al*[34] reported that expression of not only the HCV core protein but also the HCV NS proteins increases ROS and further showed that the presence of these proteins can increase endogenous expression levels of antioxidant enzymes and prooxidants such as MnSOD. Several reports have shown that serum MnSOD levels in patients with HCV-related CLD[35-37] are associated with

various clinical findings, such as fibrosis and hepatic oxidative stress. However, the significance of serum MnSOD levels has not been fully examined in patients with HCC. We previously reported that serum MnSOD levels may be correlated with fibrosis in patients with NAFLD[7]. In addition, serum MnSOD levels decreased in patients with CHC after administration of an interferon-based treatment (data not shown). These results indicate that serum MnSOD levels are likely associated with hepatic fibrosis or oxidative stress in patients with CHC. In the present study, however, MnSOD levels were not associated with platelet counts, which is a simple predictor of hepatic fibrosis in this patient population[38]. Thus, advanced hepatic fibrosis or oxidative stress may be one reason why serum MnSOD levels have diagnostic and prognostic utility with HCC, but other mechanisms should also be considered.

The present study revealed that serum MnSOD levels were significantly higher in the HCC group than in the non-HCC group (Figure 1A). In the HCC group, serum MnSOD levels were negatively correlated with serum Alb and tended to negatively correlate with PT (Table 3); these results showed an association between MnSOD and Child-Pugh stage (Figure 2A). It is known that in humans, MnSOD activity is comparatively higher in the liver compared to other tissues[39]. In addition, although a previous immunohistochemical study showed that MnSOD expression was higher in both cancerous and non-cancerous liver tissues from patients with HCC, this positive immunoreactivity was strongly observed in non-cancerous liver tissues, especially in normal hepatocytes surrounding HCC, regenerative small hepatocytes in the tumor boundary, and mononuclear inflammatory cells in necroinflammatory lesions[40]. Furthermore, ROS are overproduced by Kupffer cells and inflammatory cells in liver disease[5,41]. In the present study, serum MnSOD levels were also positively correlated with the serum tumor markers AFP and DCP (Table 3) and with Child-Pugh stage and CLIP score (Figure 2). These results indicate that increased MnSOD expression reflects hepatocyte oxidative stress and correlates with decreased hepatic function, increased hepatic fibrosis and ROS production by inflammatory cells in liver cirrhosis. These features comprise the main background characteristics leading to HCC and may be associated with the indirect effects of liver cancer progression. These associations may also explain why serum MnSOD levels predicted the overall survival of patients with HCC.

It was previously reported that serum levels of TRX, which is a stress-induced protein, increase relative to the degree of hepatic fibrosis, and that high serum concentrations of TRX may indicate advanced hepatic fibrosis[19,20]. In contrast, it has also been reported that a higher degree of hepatic fibrosis is associated with lower platelet counts[38]. Therefore, the present study may present a conflict, since results indicated that serum TRX level was positively correlated with platelet count. A previous report showed that the survival rate following LPS plus GalN-induced hepatitis was much higher in transgenic mice overexpressing TRX than in wild-type mice, and that thioacetamide-induced hepatic fibrosis was suppressed in TRX transgenic mice compared to wild-type mice[42]. Although it is still unclear why TRX and platelet counts are positively correlated, we speculate that elevated serum TRX in patients with HCC and advanced hepatic fibrosis potentially improves overall survival by suppressing oxidative stress[43]. In addition, patients with HCC, low levels of TRX, and high levels of MnSOD, which may be indicative of excessive oxidative stress without TRX attenuation, have the poorest prognosis. This result supports the hypotheses presented above. In order to better assess these findings, future studies are needed that incorporate sequential observations of serum TRX and MnSOD levels over time in patients with chronic hepatitis, cirrhosis and HCC.

Serum MnSOD and TRX may be useful biomarkers for HCC diagnosis (Figure 1). AFP is also a diagnostic marker for HCC, and the present results indicate that AFP can be used to distinguish between patients with and without HCC (Table 2). However, AFP is not a sufficiently sensitive marker for identification of the majority of patients with small HCCs[44,45], and AFP testing is not currently included in the recommendations for HCC surveillance in the updated HCC guidelines published by the American Association for the Study of Liver Disease[46]. Therefore, clinicians and clinical researchers should consider using MnSOD and TRX as diagnostic biomarkers for early HCC or as additional markers in a HCC surveillance program using ultrasonography or AFP. In addition, it is highly important to know whether these markers decrease in response to HCC therapy and reductions in tumor burden. These markers also may have utility in patients on a transplant waiting list who are treated with neo-adjuvant therapy for tumor downstaging.

Our study demonstrated that elevated serum AFP level is indicative of a poor prognosis for patients with HCC (Table 4), as was previously reported[47]. The CLIP score, which is calculated based on four factors such as the AFP value, was also useful to predict the prognosis of HCC patients in this study as well as in a previous report[48]. Other markers such as the protein survivin have been reported as poor prognostic factors for HCC[49]. Similarly, MnSOD was an independent predictive factor for overall survival in the HCC group (Figure 3A, Table 5). Although TRX was not an independent predictor of overall survival in patients with HCC (Table 4), we speculate that a combination assay using both MnSOD and TRX could be used to predict overall patient survival. It will be important to conduct further prospective evaluations of each individual marker as well as a combination of these markers using a large number of patients.

In conclusion, serum MnSOD and TRX levels increased as HCV-related chronic liver disease progressed, especially among patients with HCC. Although there was no correlation between serum levels of MnSOD and TRX, higher serum MnSOD levels and lower TRX levels in patients with HCC trended towards an indication of poor

patient prognosis. These results suggest that serum Mn-SOD and TRX levels are not only a potential biomarker for HCV-related progressed liver disease, but may also serve as prognostic markers in HCC.

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COMMENTS

Background

During hepatitis C virus (HCV) infection, production of reactive oxygen species (ROS) is persistently increased throughout HCV infection. ROS are thought to play an important role in the pathogenesis of chronic inflammatory changes in the liver, which may lead to the development of hepatic fibrosis, decreased hepatic function or hepatocellular carcinoma (HCC). However, there is little information currently available regarding serum oxidative stress markers in patients with HCV-related HCC.

Research frontiers

Cells are protected from oxidative stress by antioxidant enzymes such as superoxide dismutase (SOD) and by intracellular antioxidants such as thioredoxin (TRX). Serum manganese SOD (MnSOD) and TRX are thought to be biomarkers for various liver diseases, including HCV-related liver disease, but these possibilities have not been fully investigated. In this study, the authors demonstrated the clinical significance of serum levels of MnSOD and TRX in patients with HCV-related HCC.

Innovations and breakthroughs

Although there was no correlation between serum levels of MnSOD and TRX, serum levels of both markers increased as HCV-related chronic liver disease progressed, and in particular among patients with HCC. In addition, higher serum MnSOD levels and lower TRX levels tended to indicate a poor prognosis among patients with HCC.

Applications

Serum MnSOD and TRX levels are not only potential biomarkers for progression of HCV-related liver disease, but they may also serve as prognostic markers for patients with HCC. Therefore, clinicians should consider using serum levels of MnSOD and TRX as diagnostic biomarkers for early HCC or as additional markers in HCC surveillance programs. In addition, it will be important to know whether these markers change after therapy for liver disease, including HCC.

Peer review

Oxidative stress is closely associated with carcinogenesis. If oxidative stress markers could be useful in predicting clinical outcome in chronic hepatitis C and HCV-related HCC, they would provide us with a practical and informative tool. However, there are some limitations of this investigation, including a relatively small number of patients studied. Thus, the overall assessment is "good".

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2001; **61**: 69-90
2 Zhang CH, Xu GL, Jia WD, Li JS, Ma JL, Ge YS. Effects of interferon treatment on development and progression of hepatocellular carcinoma in patients with chronic virus infection: A meta-analysis of randomized controlled trials. *Int J Cancer* 2010; Epub ahead of print
3 **Matés JM**, Pérez-Gómez C, Núñez de Castro I. Antioxidant enzymes and human diseases. *Clin Biochem* 1999; **32**: 595-603
4 **Jaeschke H**, Farhood A. Neutrophil and Kupffer cell-induced oxidant stress and ischemia-reperfusion injury in rat liver. *Am J Physiol* 1991; **260**: G355-G362
5 **Loguercio C**, Federico A. Oxidative stress in viral and alcoholic hepatitis. *Free Radic Biol Med* 2003; **34**: 1-10
6 **Niemelä O**, Parkkila S, Juvonen RO, Viitala K, Gelboin HV, Pasanen M. Cytochromes P450 2A6, 2E1, and 3A and production of protein-aldehyde adducts in the liver of patients with alcoholic and non-alcoholic liver diseases. *J Hepatol* 2000; **33**: 893-901
7 **Takami Y**, Uto H, Tamai T, Sato Y, Ishida Y, Morinaga H, Sakakibara Y, Moriuchi A, Oketani M, Ido A, Nakajima T, Okanoue T, Tsubouchi H. Identification of a novel biomarker for oxidative stress induced by hydrogen peroxide in primary human hepatocytes using the 2-nitrobenzenesulfenyl chloride isotope labeling method. *Hepatol Res* 2010; **40**: 438-445
8 **Mottola G**, Cardinali G, Ceccacci A, Trozzi C, Bartholomew L, Torrisi MR, Pedrazzini E, Bonatti S, Migliaccio G. Hepatitis C virus nonstructural proteins are localized in a modified endoplasmic reticulum of cells expressing viral subgenomic replicons. *Virology* 2002; **293**: 31-43
9 **Tsan MF**. Superoxide dismutase and pulmonary oxygen toxicity: lessons from transgenic and knockout mice (Review). *Int J Mol Med* 2001; **7**: 13-19
10 **Immenschuh S**, Ramadori G. Gene regulation of heme oxygenase-1 as a therapeutic target. *Biochem Pharmacol* 2000; **60**: 1121-1128
11 **Guo X**, Shin VY, Cho CH. Modulation of heme oxygenase in tissue injury and its implication in protection against gastrointestinal diseases. *Life Sci* 2001; **69**: 3113-3119
12 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438
13 **Teshigawara K**, Maeda M, Nishino K, Nikaido T, Uchiyama T, Tsudo M, Wano Y, Yodoi J. Adult T leukemia cells produce a lymphokine that augments interleukin 2 receptor expression. *J Mol Cell Immunol* 1985; **2**: 17-26
14 **Nakamura H**, De Rosa SC, Yodoi J, Holmgren A, Ghezzi P, Herzenberg LA, Herzenberg LA. Chronic elevation of plasma thioredoxin: inhibition of chemotaxis and curtailment of life expectancy in AIDS. *Proc Natl Acad Sci USA* 2001; **98**: 2688-2693
15 **Kurimoto C**, Kawano S, Tsuji G, Hatachi S, Jikimoto T, Sugiyama D, Kasagi S, Komori T, Nakamura H, Yodoi J, Kumagai S. Thioredoxin may exert a protective effect against tissue damage caused by oxidative stress in salivary glands of patients with Sjögren's syndrome. *J Rheumatol* 2007; **34**: 2035-2043
16 **Lemarechal H**, Allanore Y, Chenevier-Gobeaux C, Ekindjian OG, Kahan A, Borderie D. High redox thioredoxin but low thioredoxin reductase activities in the serum of patients with rheumatoid arthritis. *Clin Chim Acta* 2006; **367**: 156-161
17 **Deng ZH**, Cao HQ, Hu YB, Wen JF, Zhou JH. TRX is upregulated by fibroblast growth factor-2 in lung carcinoma. *APMIS* 2011; **119**: 57-65
18 **Cha MK**, Suh KH, Kim IH. Overexpression of peroxiredoxin I and thioredoxin1 in human breast carcinoma. *J Exp Clin Cancer Res* 2009; **28**: 93
19 **Sumida Y**, Nakashima T, Yoh T, Nakajima Y, Ishikawa H, Mitsuyoshi H, Sakamoto Y, Okanoue T, Kashima K, Nakamura H, Yodoi J. Serum thioredoxin levels as an indicator of oxidative stress in patients with hepatitis C virus infection. *J Hepatol* 2000; **33**: 616-622
20 **Sumida Y**, Nakashima T, Yoh T, Furutani M, Hirohama A, Kakisaka Y, Nakajima Y, Ishikawa H, Mitsuyoshi H, Okanoue T, Kashima K, Nakamura H, Yodoi J. Serum thioredoxin levels as a predictor of steatohepatitis in patients with nonalcoholic fatty liver disease. *J Hepatol* 2003; **38**: 32-38
21 A new prognostic system for hepatocellular carcinoma: a retrospective study of 435 patients: the Cancer of the Liver Italian Program (CLIP) investigators. *Hepatology* 1998; **28**: 751-755
22 Prospective validation of the CLIP score: a new prognostic system for patients with cirrhosis and hepatocellular carcinoma. The Cancer of the Liver Italian Program (CLIP) Investigators. *Hepatology* 2000; **31**: 840-845
23 The general rules for the clinical and pathological study of primary liver cancer. Liver Cancer Study Group of Japan. *Jpn J Surg* 1989; **19**: 98-129