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＜症例報告＞

フィアンセから感染したと推測されペグインターフェロンα2aで軽快したC型急性肝炎の女性症例

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要旨：症例は26才女性．2007年11月21日，AST235 U/L，ALT636 U/L，T-Bil.3.5 mg/dlで紹介を受けた．HBs抗原・IgM-HA抗体・HCV抗体陰性であったがHCV RNA定性（アンプリコア法）は陽性であった．ALTは正常化せず，08年2月8日HCVグループ2，RNA定量3.0 Log IU/ml（リアルタイム法），3月21日肝生検F1A1であったため，3月25日からペグインターフェロンα2aを12週投与してHCV RNAは陰性化した．尚，07年8月頃から付き合い始めたフィアンセは刺青を有し07年11月C型急性肝炎を発生，11月28日HCVグループ2，RNA定量430 KIU/ml（ハイレンジ法）であった．保存血清を用いて分子系統樹解析を名古屋市立大学臨床分子情報医学教室において行ったところ患者とフィアンセはおなじ感染ルートであることが推測された．日本のC型慢性肝炎は高齢化し治療に難渋しているが，若い世代を中心に麻薬や刺青によるC型肝炎感染が散見され性交渉によってさらに拡大しているとも言われている．C型肝炎は感染早期にインターフェロンを投与した方が治療効果は高く早期治療が望ましい．若い世代に対する積極的HCV対策も今後は必要である．

索引用語：　C型急性肝炎　　ペグインターフェロンα2　　性行為感染
分子系統樹解析

はじめに

HCVスクリーニング法の確立により輸血後肝炎はほとんど認められなくなった[1)]が，若い世代を中心に麻薬注射による経静脈的感染や刺青によるC型肝炎感染は散見されている[2)3)]．また，性交渉による経粘膜的感染でHCVがさらに拡大しているとも言われている[4)~6)]．性行為感染を証明するためにはウイルスの同一性を証明する必要があるが，近年ではMizokamiらの方法[7)]によりウイルス遺伝子学的手法で塩基配列の相同性を比較することで遺伝子系統樹解析により証明できるようになり，本症例もその方法に拠った．

C型急性肝炎の自然治癒は稀で約70％は慢性化すると考えられている[8)]．しかし，急性肝炎の約30％は自然治癒するとも言え，C型急性肝炎の適切な治療開始時期や治療法については必ずしも明らかにされていない．日本においてC型急性肝炎の発生が少ないため，臨床研究が行いにくいのも理由の一つと思われる．一方，欧米では薬物乱用によるC型急性肝炎発生例が多く，臨床研究も散見されるため，欧米の論文も参考にして考察を行った．

症　例

患者：26才，女性．

既往歴：特記事項なし．飲酒・喫煙なし．

現病歴：2007年11月12日，全身倦怠感と皮膚掻痒感で近医受診．同日の採血にてAST 948 U/L，ALT 1394 U/L，T-Bil. 4.5 mg/dlと肝障害を指摘され，IgM-HA抗体陰性，HBs抗原陰性，HCV抗体2.84よりC型急性肝炎を疑って保存的に経過観察されていた．2007年11月21日，当院紹介となったが，同日の採血では

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Table 1 Laboratory data on admission

Peripheral Blood		Blood chemistry	
RBC	461×10^4 /mm^3	AST	30 IU/l
Hb	14.5 g/dl	ALT	50 IU/l
Ht	43.4 %	LDH	148 IU/l
WBC	6400 /mm^3	γ-GTP	14 U/l
Neut	50.0 %	ALP	115 IU/l
Eosino	5.4 %	Total biririubin	1.07 mg/dl
Baso	1.0 %	Direct bilirubin	0.11 mg/dl
Lymph	38.4 %	ChE	217 IU/l
Mono	5.2 %	T-Cho	201 mg/dl
Plt	32.2×10^4 /mm^3	Total protein	7.6 g/dl
Coagulofibrinolysis		Alb	4.4 g/dl
PT	129 %	α1-glb	0.21 g/dl
HPT	122 %	α2-glb	0.56 g/dl
Urine Analysis		β-glb	0.79 g/dl
Prptein	(－)	γ-glb	1.47 g/dl
Birilubin	(－)	CRE	0.56 mg/dl
Urobilinogen	(－)	BUN	10 mg/dl
Occult blood	(－)	AFP	3 ng/ml
		Hepatitis virus markers	
		HBs Ag	(－)
		Anti-HCV (III)	14.16 (＋)
		HCV serotype	Group2
		HCV RNA	3.0 Log IU/ml

AST 235 U/L，ALT 636 U/L，T-Bil. 3.5 mg/dl と肝機能は改善し，症状も消失しており PT 113% と凝固能も良好につき外来にて経過観察とした．当院の検査ではHBs 抗原陰性・IgM-HA 抗体陰性・HCV コア蛋白<20 fmol/L であったが HCV RNA 定性（アンプリコア法）は陽性であった．2008 年 2 月 18 日 AST 34 U/L，ALT 52 U/L と正常化せず，HCV グループ 2，HCV RNA 定量 3.0 Log IU/ml（リアルタイム法）と判明したためC型肝炎の慢性化を疑い入院となった．尚，07 年 8 月頃から付き合い始めたフィアンセは刺青歴(5 年前と 1 年前)を有し 07 年 11 月他院にC型急性肝炎で入院し，11 月 22 日 HCV 抗体陰性，HCV RNA 定量 430 KIU/ml（ハイレンジ法）であったが，12 月 28 日 HCV 抗体 4.03 S/Co と陽性化したためC型急性肝炎と診断された．HCV グループは 2 であった．12 月末には肝機能は正常化し退院した．HIV は両者とも陰性であった．

現症：皮膚・眼瞼結膜に黄染を認めず．皮膚に湿疹を認めず．胸腹部の理学的所見に異常を認めず．

入院時検査成績：末梢血・尿に異常を認めず，AST と ALT の上昇を認めた．プロトロンビン時間は正常であった．HBs 抗原陰性，第 3 世代 HCV 抗体陽性，HCV 血清型は 2 型，HCV RNA は 3.0 Log IU/ml であった（Table 1）．

臨床経過：入院後の 3 月 21 日，肝生検を行ったところ慢性肝炎（F1A1）と診断された（Fig. 1）．腹部 CT では軽度の脾腫を認めるのみであった(Fig. 2)．3 月 25 日からペグインターフェロン α2a(ペガシス)を投与開始した．4 月 30 日には HCV RNA は陰性化し 6 月 10 日まで週一回合計 12 回投与を行ったが，初期の発熱以外に副作用・合併症は認められなかった．投与終了後，2009 年 2 月 20 日まで HCV RNA 陰性を確認した(Fig. 3)．

名古屋市立大学臨床分子情報医学教室において本症例の 2007 年 11 月 21 日とフィアンセの 2007 年 11 月 26 日の保存血清を用いて検討を行ったところいずれも Genotype2b で，NS5B 領域の 338 塩基長の配列を比較したところ 100% の相同性を得た．分子系統樹解析に置いて，患者とフィアンセから分離された HCV 株は単

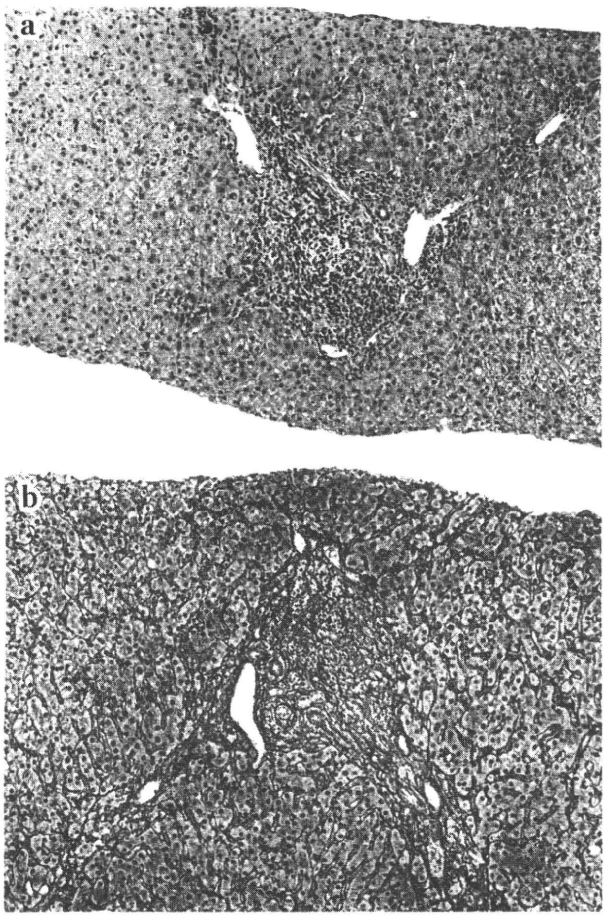

Fig. 1 Histopathological findings of liver needle biopsy. Liver biopsy revealed chronic hepatitis corresponding to F1A1. (a) H.E. stain, ×100 (b) silver stain, ×100

独のclusterを形成し，同一のHCV株と結論された[9]（Fig. 4）．尚，本症例2007年11月21日保存血清のHCV RNAは2.68 Log IU/mlであった．

考　察

HCVは主に輸血などの経血液感染による[1]が，刺青[2][3]・性交渉[4][5]も可能性があるとされているが，調査方法によりその頻度は異なりHCV発見以前の非A非B型肝炎と扱われていた時代では感染経路不明が最も多いと

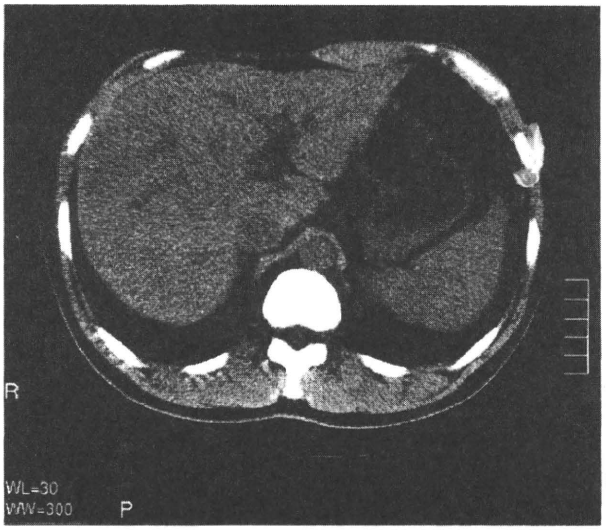

Fig. 2 Abdominal plain CT on admission Plain CT showed slight splenomegaly.

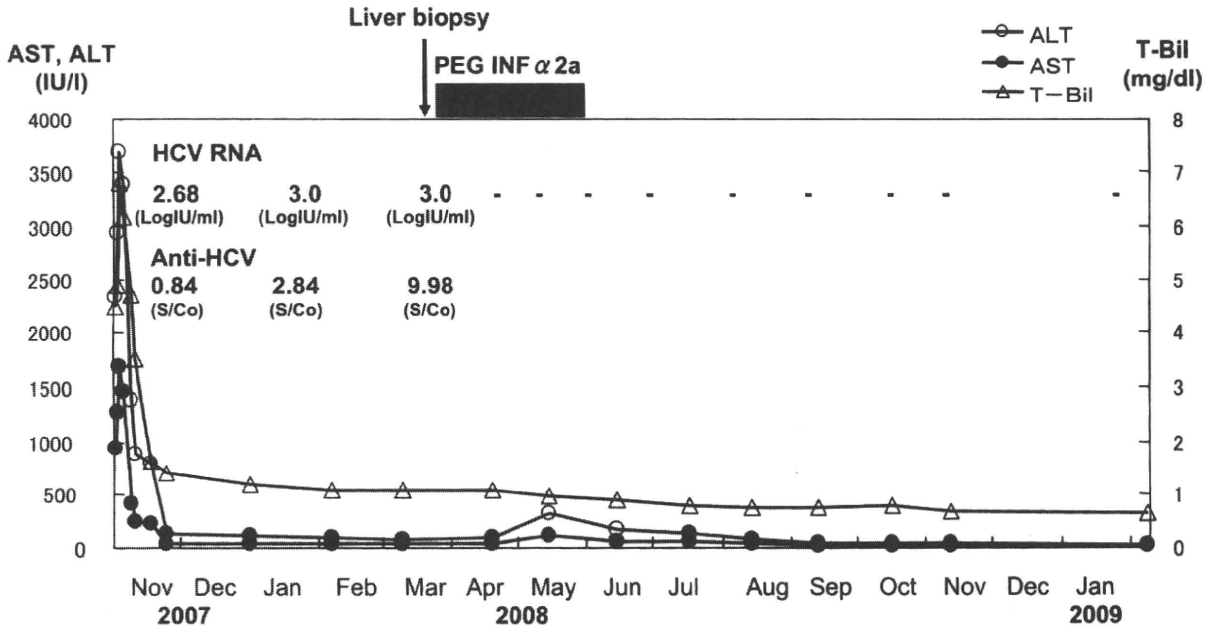

Fig. 3 Clinical course

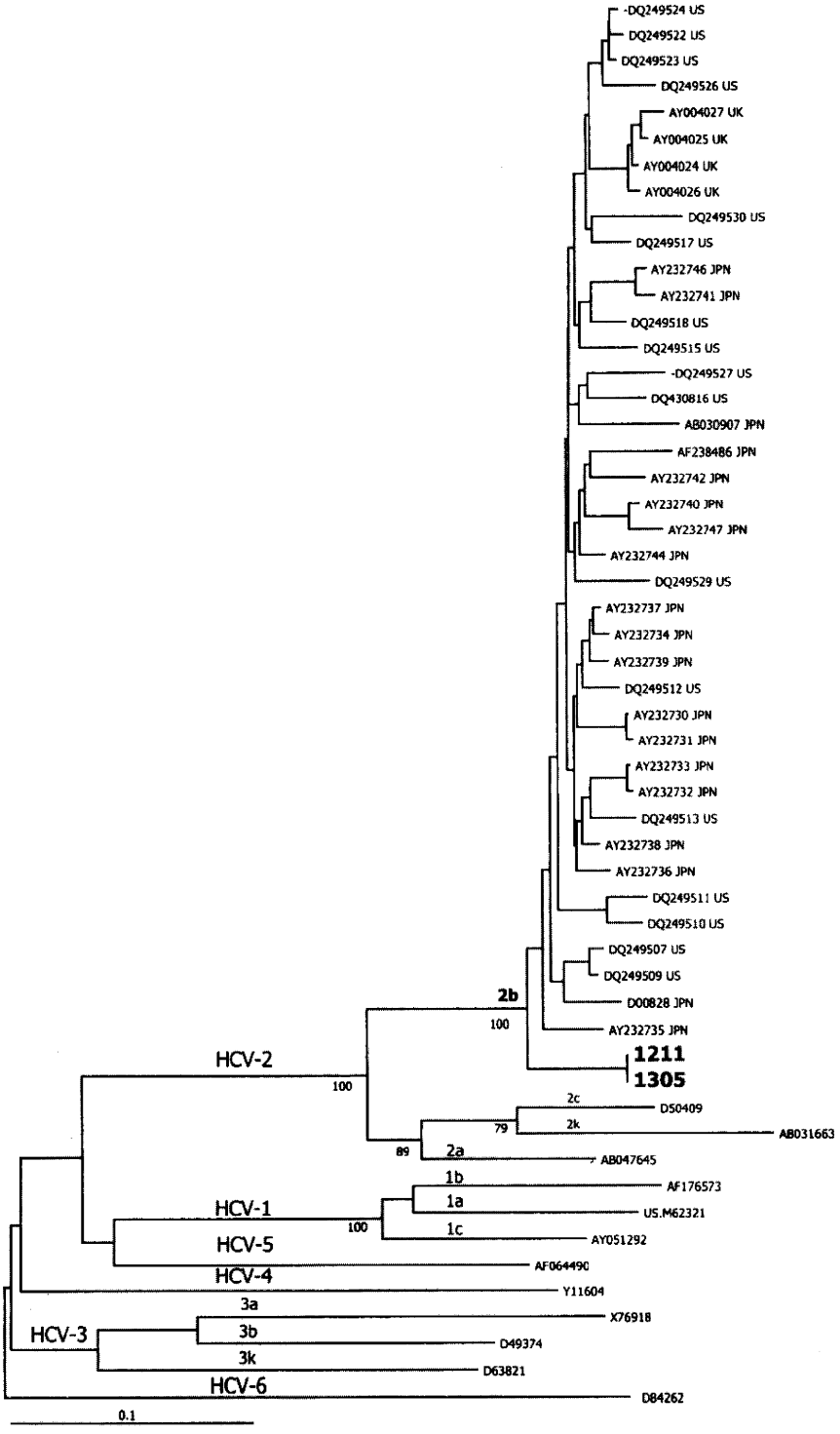

Fig. 4 Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the NS5B (338 nucleotide sequences) of 39 HCV isolates of genotype 2b and 12 reference sequences (genotype 1 ～ 6). 1211: Patient on admission (November 21, 2007), 1305: her fiancé at November 26, 2007

報告した論文もある[10]．HCVの発見後は献血・輸血時のHCVスクリーニング法が確立したために，輸血後C型肝炎の発症は極めて稀となった．日本の報告では，Nakashimaら[5]はHCVの感染経路として輸血の次に性行為をあげている．

本報告では，患者本人とフィアンセのHCV遺伝子型が同じ2型であった．両者の血清を検索しHCV RNAの塩基配列が高率な相同性を示し分子系統樹で同じclusterを形成したことと，病歴聴取より感染危険因子である歯ブラシ・カミソリなどの日用品や注射針を共有したことが無いことを確認できたことから性行為以外の共通行為は考えにくいためフィアンセ間の性感染である可能性が高いと推測した．2005年，Nakayamaらも同様の方法でC型急性肝炎を発症した配偶者の報告を行っている[11]．本患者はフィアンセと07年8月頃から付き合い始め，急性肝炎発生3カ月前頃から性交渉があったと話している．2007年11月頃ほぼ同時に急性肝炎で発症しているが，確定は出来ないがフィアンセから本患者にHCV感染が起こった可能性が考えられる．

一方，C型急性肝炎は約70％の確率で慢性化することが知られている[8]．いったん慢性化すると自然治癒は稀で徐々に線維化が進行し肝硬変から肝細胞癌の合併が高頻度となる．HCV消失が認められる例が約30％存在するが，消失する場合は発生3カ月以内に認められ，それ以降の消失は稀である．発生3カ月を過ぎても，HCV陽性の症例は慢性化している可能性が高く，インターフェロン治療が勧められるとしている．急性肝炎発生早期でのインターフェロン治療は慢性肝炎に比し高いウイルス駆除が得られることが知られている[12][13]．しかし，報告症例数が少なく治療開始時期や治療法の明確な基準は示されていない．Kamalら[14]はC型急性肝炎に対するペグインターフェロンα2b治療開始時期の検討をしている．このなかで1型は8週以内に投与開始した成績が最も良好であったが，2型は治療開始時期によらず90％以上のウイルス駆除が得られたとしている．治療にリバビリンの併用は必須ではなく，ペグインターフェロン単独で十分と結論している[15]が，投与期間について1型は24週投与が必要だが2型は8から12週投与で良いと報告している[16]．今回の症例は2型であり，ペグインターフェロンα2a単独投与を12週行ってHCV RNAは陰性化した．2型はインターフェロンの反応が良好であるので，2型のC型急性肝炎の治療を何時行うべきであるかは異論があるが，女性は年齢が進むほど治癒率が低下すると言われている[17]．本症例では早期のインターフェロンが望ましいと考え，発生5カ月後の肝生検で慢性化を確認の上でペグインターフェロンα2a投与を行いHCVの消失を認めた．

結　語

分子系統樹解析からフィアンセから感染したと推測されたC型急性肝炎の女性症例を経験した．急性肝炎後に慢性化したため早期のペグインターフェロン治療を行いC型肝炎は治癒したと思われた．若い世代を中心に麻薬や刺青によるC型肝炎感染が散見され，性交渉によってHCVがさらに拡大しているとも言われている．日本のHCV感染者は高齢化し治療に難渋している．治療反応性の良い若い世代に対する積極的HCV対策も今後は必要である．

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A case of acute hepatitis C, most likely by interlovers sexual transmission, cured by peginterferon α2a therapy

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A 29-year-old woman with acute hepatitis C visited our hospital at November 21, 2007. ALT did not become normal. HCV serotype revealed Group 2 and HCV RNA was 3.0 Log IU/ml (Real time method) at March 21, 2008. Liver Biopsy showed chronic hepatitis F1A1 at March 21, so she was treated with pegylated interferon α 2a for 12 weeks and attained sustained virological response. On the other hands, her fiancé treated for acute hepatitis C at another hospital revealed HCV Group 2 and HCV RNA was 430 KIU/ml (High ranged method). The HCV isolates from the patients and her fiancé shared 100% identity in the 338 nucleotide sequences of the NS5B region. Phylogenitic analysis of the 338 sequences revealed that the two isolates segregated into a cluster. Thus the patient has acquired HCV infection from her fiancé, most likely by sexual transmission.

Key words: acute hepatitis C　pegylated interferon α 2a　sexual transmission　phylogenitic analysis

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＜速　報＞

前インターフェロン不応C型慢性肝炎に対する二重濾過血漿交換併用ペグインターフェロン・リバビリン療法の初期効果
—第1報—

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緒言：ペグインターフェロン・リバビリン（PEG・Rib）療法により，Genotype 1b・高ウイルス量の難治性C型慢性肝炎の著効率は改善したが約50％は未だにC型肝炎ウイルス（HCV）の駆除が得られない．この治療抵抗例にはISDR[1)]やCoreの変異と脂質代謝が関与する[2)]ことが最近判明してきた．一方，2007年4月からGenotype 1b・高ウイルス量のC型慢性肝炎に対し二重濾過血漿交換[3)]（Double Filtration Plasmapheresis；DFPP）が保険適応になった．今回我々は，以前インターフェロン治療（IFN）を行ったが一度もHCV陰性化が得られなかった無効例に対し，DFPPとPEG・Riboα2bを併用して治療を行ったので，安全性と初期効果，脂質の変化につき検討した．

対象と方法：対象は，前治療IFN無効3例と前治療PEG・Rib無効6例であり，ISDR変異0が7例・1が1例・3が1例，Core 70番変異なし（wild）3例・変異あり（mutant）6例，Core 91番wild 4例・mutant 5例の全9例である（図中1～9）．DFPPを第1週目に3回，2週目に2回行った．DFPPは一次膜に旭化成クラレメディカル社プラズマフローOP，二次膜にカスケードフローEC-50Wを使用し血漿処理50 mL/kgを目標とした．初回DFPP直後にPEGを注射しRib内服を開始，4回目DFPP直後にPEG 2回目注射を行った．PEGα2a・Rib投与中HCV RNA再上昇の1症例（症例3）のみ途中22週でDFPPを併用した．DFPP前，DFPP開始後2週目，以後1カ月ごとにPEG注射日の早朝空腹でHCV RNA（リアルタイム法）・TG・T-Chol・LDL-Chol・HDL-Cholの測定を行った．

成績：9例ともにDFPP中の副作用は認められず，DFPP直後にPEGを注射することによる副作用の増強も認められなかった．2009年8月末現在，DFPP開始後のPEG・Rib継続期間は症例1から9の順に各々50週，42週，36週，26週，24週，23週，14週，10週，6週である．このうち，症例1，3，4，5，7はDFPP後，各々21週，20週，5週，4週，7週でHCV RNAが陰性化し以後維持している．一方，脂質の変化をみてみると，HCV RNAが消失した症例1，3，4，5，7の5例中TGは5例全例，T-Cholは4例，LDL-Cholは3例でDFPP開始前値より上昇する傾向にあったが，HCV RNAが陰性化していない症例2，6，8，9の4例では上昇していなかった（Fig. 1）．

考案：まずDFPPとPEG・Rib併用に伴う副作用は認められず併用は安全性に施行できると思われた．一方，ISDR変異0または1，Core 70番mutant，Core 91番mutantの症例はPEG・Ribを行っても非常に難治とされ[4)]，今回の9症例は前治療IFN無効および前治療PEG・Rib無効を反映した難治例の集団であった．しかし，9例中5例で24週以内にHCV RNAが陰性化した．特に，症例3，7はISDR変異0・1かつCore double mutantの極めて難治症例とされているにも関わらずHCV RNAの陰性化が認められた．一方，PEG・Rib終了後HCV RNA陰性継続例のみがTG，T-Chol，LDL-Cholが治療終了後に前値より上昇する[5)]と報告がある．DFPPを加えた今回の検討では，DFPPで機械的に除去されて2週間後は一旦低下するものの，HCV RNA消失例ではPEG・Rib終了を待たずにTG，T-Chol，LDL-Cholが上昇していた．DFPP2次膜の穴は約30 nmで物理的にHCVを捕らえるが，HCVはLDLに結合していることから両者はDFPPで同時に除去されていると推測される．DFPPによりHCVの機械的除去と共に，治療早期に脂質代謝が改善されてPEG・Ribが利きやすい環境に変化しているとも推測された．

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HCV RNA negative cases

Case	Sex	Age	Prior IFN (effect)	ISDR mutation	Core70 mutation	Core91 mutation
1	F	69	IFN α 2b (NR)	3	wild	mutant
3※	F	44	PEG α 2a・Rib (NR)	0	mutant	mutant
4	F	70	PEG α 2b・Rib (NR)	0	wild	wild
5	F	37	IFN α (NR)	0	mutant	wild
7	F	61	IFN α 2b (NR)	1	mutant	mutant

※DFPP was done at 22 week
(HCV RNA was 6.7 at the start of PEG・Rib)

HCV RNA positive cases

Case	Sex	Age	Prior IFN (effect)	ISDR mutation	Core70 mutation	Core91 mutation
2	F	52	PEG α 2b・Rib (NR)	0	mutant	mutant
6	M	57	PEG α 2b・Rib (NR)	0	mutant	wild
8	F	60	PEG α 2b・Rib (NR)	0	wild	mutant
9	M	74	PEG α 2b・Rib (NR)	0	mutant	wild

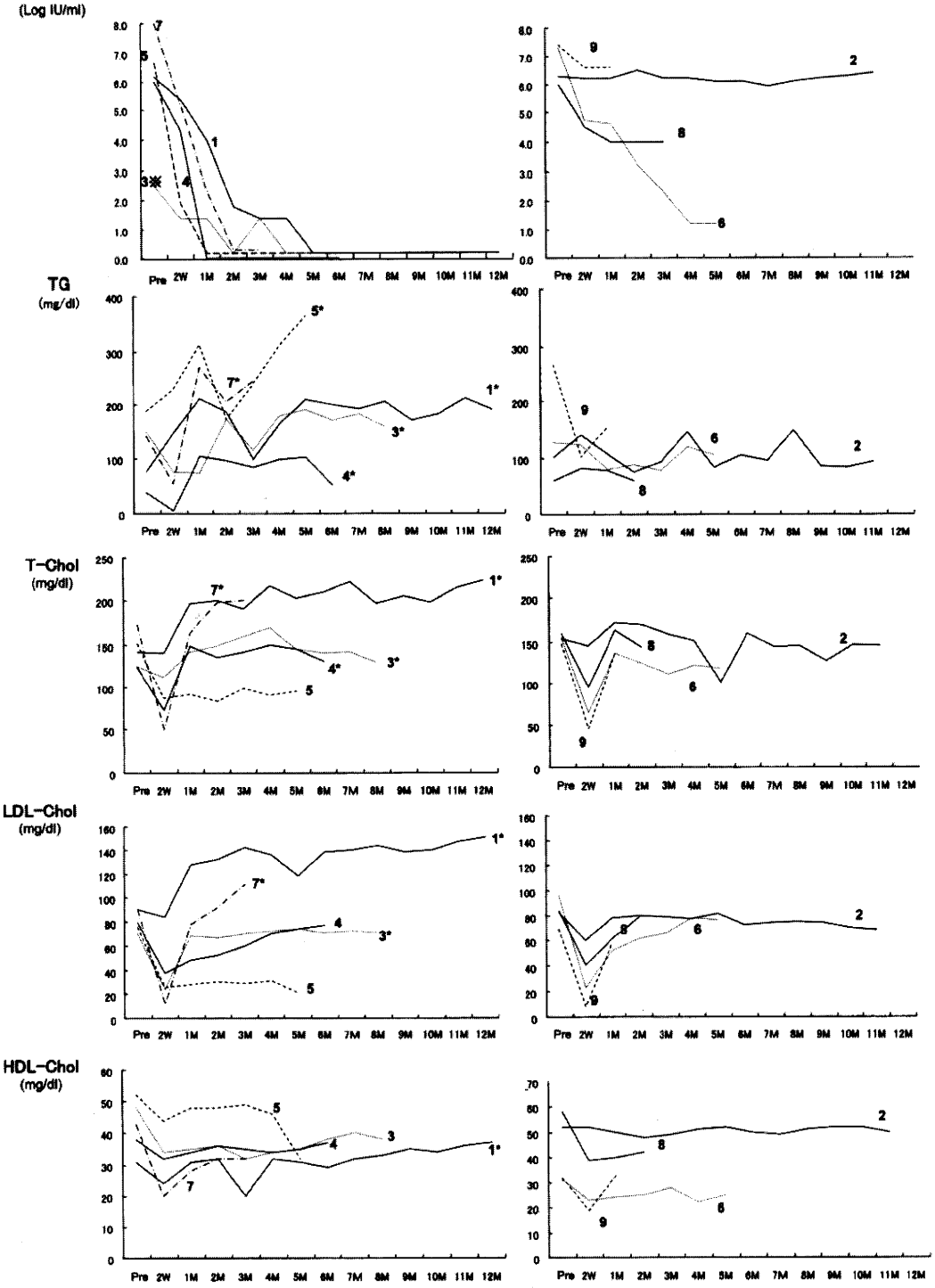

Fig. 1 Change in triglyceride (TG), total cholesterol (T-Chol), LDL-Chol and HDL-Chol levels during double filtration plasmapheresis (DFPP) and peginterferon plus ribavirin (Peg・Rib) combination therapy. *Levels increased after DFPP and higher than those before treatment

索引用語：C型慢性肝炎，二重濾過血漿交換，
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英文要旨

Double filtration plasmapheresis and peginterferon plus ribavirin combination therapy for chronic hepatitis C patients non-responded by previous interferon therapy

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We investigated lipid metabolism in nine patients with chronic hepatitis C virus (HCV), not responded by previous interferon therapy (IFN), undergoing double filtration plasmapheresis (DFPP) and peginterferon plus ribavirin (PEG・Rib) combination therapy. Three patients were non-responder of previous IFN monotherapy and 6 were PEG・Rib. HCV RNA became negative within 24 weeks in 5 out of 9. In the HCV RNA negative group, Triglyceride (TG) and Total-Cholesterol (T-Chol) or LDL-Chol levels increased gradiently after DFPP and were higher than those before treatment, but not in HCV positive group. DFPP plus PEG・Rib combination therapy might not only produce a reduction of HCV but also improve the environment of lipid metabolism effective for PEG・Rib during the early stage of treatment.

Key words: chronic hepatitis C,
double filtration plasmapheresis,
peginterferon plus ribavirin therapy

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Differences in prognostic factors according to viral status in patients with hepatocellular carcinoma

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Abstract. The number and ratio of both HBsAg- and HCV Ab-negative hepatocellular carcinoma (HCC-nonBC) cases have been steadily increasing in Japan. The aim of this study was to examine the frequency of detection of HCC-nonBC by screening methods and to elucidate the clinical characteristics of HCC-nonBC compared with those of hepatitis C and/or B virus-associated HCC (HCC-virus). We recruited 624 patients with HCC who were diagnosed between 1982 and 2007 at the Department of Gastroenterology and Hepatology, Nagasaki University Hospital. They were categorized into 2 groups as follows: i) 550 were included in the HCC-virus group: positive for HBsAg and/or positive for HCV Ab, and ii) 74 were included in the HCC-nonBC group: negative for both HBsAg and HCV Ab. The follow-up patterns until the initial detection of HCC and the survival rates were analyzed and compared between the 2 groups. Multivariate analysis identified follow-up, alcohol consumption, albumin level, total bilirubin level, α-fetoprotein (AFP) level, and tumor-node-metastasis (TNM) stage as independent and significant risk factors for prognosis. Among the 397 patients with HCC in TNM stage I or II, multivariate analysis identified the cause of liver disease, gender, Child-Pugh score, serum albumin level and TNM stage as independent and significant risk factors for prognosis. We reported that the poor prognoses of patients with HCC-nonBC were attributable to its late detection in an advanced condition due to the absence of a surveillance system for the early detection of HCC. However, in early-stage patients, patients with HCC-nonBC showed significantly better prognosis than those in the HCC-virus group.

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Key words: hepatocellular carcinoma, viral hepatitis

Introduction

Primary liver cancer is the most common cancer of the liver, accounting for approximately 6% of all human cancers. It is estimated that half a million cases of this disease occur worldwide each year, making primary liver cancer the fifth most common malignancy in men and the ninth in women (1-6). Hepatocellular carcinoma (HCC) accounts for 85 to 90% of primary liver cancers, (7) and the age-adjusted HCC mortality rate has increased over the past few decades in Japan (8). Similarly, a trend in increasing incidence rates of HCC has been reported for several developed countries in North America, Europe and Asia (9,10). HCC often develops in patients with liver cirrhosis caused by hepatitis B virus (HBV), hepatitis C virus (HCV), excessive alcohol consumption or nonalcoholic fatty liver disease. HCV is the predominant causative agent of HCC in Japan (11-14). However, it has been reported that the number and ratio of both HBsAg- and HCV Ab-negative HCC (HCC-nonBC) have been steadily increasing in Japan (15,16).

The prognosis for patients with HCC is still poor. Surgical resection and liver transplantation are the standard treatment methods available. Radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) have recently been recognized as effective methods of achieving complete tumor necrosis in small HCCs (17); however, the chances of curative treatment are often limited by several features of HCC. HCCs usually grow to a large size before symptom manifestation. Bilobar or multifocal tumors are common, and the incidence of associated cirrhosis is high, being over 80% in most cases (18-20). Transcatheter arterial chemoembolization (TACE), which is considered to be an ineffective method of achieving complete necrosis of HCCs, also depends on the above factors (21). Early detection of HCC by α-fetoprotein (AFP) and/or imaging screening has been implemented in many countries to increase the chances of successful intervention and to improve survival (22-26).

The aim of this study was to examine the frequency of detection of HCC-nonBC by screening methods and elucidate the differences in the clinical characteristics between non-B, non-C HCC and hepatitis C and/or B virus-associated HCC (HCC-virus).

Patients and methods

Patients and study groups. We recruited 624 patients with HCC who were diagnosed between January, 1982 and December, 2007 at the Department of Gastroenterology and Hepatology, Nagasaki University Hospital. Informed consent was obtained from all patients. The diagnosis of HCC was based on AFP levels; results of imaging techniques such as ultrasonography (USG), computerized tomography (CT), magnetic resonance imaging (MRI) and hepatic angiography (HAG); and/or liver biopsy. The diagnostic criteria included characteristic liver biopsy findings, elevated AFP (≥20 ng/ml) and neovascularization on HAG and/or CT.

Sera were stored at -80˚C until they were used for the following assays. The diagnosis of chronic hepatitis C virus (HCV) infection was based on the presence of HCV Ab (microparticle enzyme immunoassay; Abbott Laboratories) and HCV RNA as detected by polymerase chain reaction. The diagnosis of chronic HBV infection was based on the presence of HBsAg (enzyme-linked immunosorbent assay; Abbott Laboratories); the serum AFP level was measured by radioimmunoassay (Abbott Laboratories). The history of alcohol intake was noted from medical records; habitual drinking was defined as an average daily consumption of an amount equivalent to 80 g of pure ethanol for a period of more than 10 years.

The patients were categorized into 2 groups as follows: i) HCC-virus group (550) comprising patients positive for HBsAg and/or positive for HCV Ab and ii) HCC-nonBC group (74) comprising patients negative for both HBsAg and HCV Ab. We analyzed and compared the 2 groups for age distribution, gender ratio, body-mass index, alcohol intake, serum AFP level, tumor-node metastasis (TNM) stage of hepatocellular carcinoma tumors at the time of initial detection, Child-Pugh score, follow-up pattern until the initial detection of HCC and the survival rates.

Follow-up. All patients were categorized into 2 groups: the follow-up group included 365 (58%) patients with subclinical HCC diagnosed by screening; the non-follow-up group consisted of 259 (42%) patients who were diagnosed at our hospital owing to the appearance of symptoms indicative of HCC. AFP levels and liver function were assessed every 3 to 6 months, and USG or CT imaging was performed every 3 to 12 months over a period of at least 12 months prior to the diagnosis of HCC in patients of the follow-up group. The non-follow-up group patients presented with clinical symptoms such as abdominal pain, discomfort, nausea or weight loss which led to the evaluation and diagnosis of HCC.

Treatment modalities. Patients diagnosed with HCC were assessed for surgery on the basis of the extent of lobar involvement and liver function status. The extent of lobar involvement was evaluated by a combination of USG, CT, MRI and HAG. Patients were considered unfit for resection when they met the following criteria: i) bilobar involvement, ii) evidence of tumor infiltration into the main portal vein or thrombosis of the vein, iii) evidence of extrahepatic metastases, iv) Child's grade C cirrhosis or v) poor cardiac and respiratory statuses. If the patients were deemed unfit for operation or refused to undergo operation, PEI therapy was the second choice of treatment offered to such patients with HCCs <3 cm in diameter. The remaining patients without main portal vein thrombosis or extrahepatic metastasis were advised to undergo TACE irrespective of the size and number of tumors.

After initial treatment, AFP levels and liver function of the patients were assessed every 1 to 3 months, and USG imaging was performed every 3 to 6 months during the follow-up period. Patients suspected to have HCC recurrence were further evaluated by CT and/or MRI. The assessment of treatment for recurrent HCC was based on lobar involvement and liver function status as described for the initial treatment. RFA or liver transplantation to treat HCC was started at our institution in 2002; none of the patients were treated by these methods between 1982 and 2001. Furthermore, none of the subjects in our study received either of these treatments for recurrent HCC during the follow-up period.

Statistical analysis. The time of survival was measured from the time of the diagnosis of HCC to the time of death or until the time of preparation of the manuscript. The data were analyzed by the Mann-Whitney test for continuous ordinal data, and the Chi-square test with Yates' correction and Fisher's exact test were performed for intergroup comparisons to determine the association between 2 qualitative variables. The survival rate was analyzed using the Kaplan-Meier method, and the differences between the survival probability curves were tested using the log-rank test. The independent risk factors associated with the rate of survival were estimated by the non-time-dependent stepwise Cox regression analysis. The standard error was calculated based on the binomial model to estimate the response rate. A value $P<0.05$ was considered statistically significant. Data analysis was performed with SPSS version 16.0 software for Windows.

Results

Patient characteristics at enrollment. We diagnosed 624 patients with HCC during the study period. Patient characteristics at the time of diagnosis of HCC are presented in Table I. The underlying causes of HCC were determined to be as follows: 120 (19%) patients were positive for HBsAg, 411 (66%) were positive for HCV Ab, 19 (3%) were positive for both HBsAg and HCV Ab and 74 (12%) were negative for HBsAg and anti-HCV.

Comparison of clinical characteristics and survival between patients with and without hepatitis virus infection. The patients were divided into 2 groups: the HCC-nonBC group (74 patients) and the HCC-virus group (550 patients); the characteristics of each group were compared (Table I). There were no significant differences in gender, BMI, Child-Pugh score, prothrombin time, or albumin and total bilirubin levels. However, there were significant differences between the 2 groups in terms of median age ($P=0.001$), habitual drinkers ($P=0.015$), TNM stage ($P=0.030$), AFP ($P=0.002$) and follow-up group ($P=0.010$). The HCC-nonBC group had a lower proportion of patients who were followed up when compared to those of the HCC-virus group.

Table II indicates the results of univariate and multivariate analyses of the prognosis factors for HCC using the

Table I. Comparison between HCC patients with and without virus infection.

	All patients		HCC-nonBC		HCC-virus		P-value
Total	624		74		550		
Median age, years	65	(13)	70	(6)	64	(12)	0.001
Gender (%)							
Male	478	(77)	54	(73)	424	(77)	
Female	146	(23)	20	(27)	126	(23)	NS
BMI	22.4	(4.2)	23.1	(6.0)	22.3	(4.8)	NS
Alcohol consumption (%)							
Not excessive	497	(80)	51	(69)	446	(81)	
Excessive	127	(20)	23	(31)	104	(19)	0.015
Follow-up (%)							
Follow-up group	365	(58)	33	(45)	332	(60)	
Non-follow-up group	259	(42)	41	(55)	218	(40)	0.010
Child-Pugh score	6	(1)	5	(2)	6	(2)	NS
Hepatitis virus							
HBsAg (+)/HCV Ab (-)	120	(19)	0	(0)	120	(22)	
HBsAg (-)/HCV Ab (+)	411	(66)	0	(0)	411	(75)	
HBsAg (+)/HCV Ab (+)	19	(3)	0	(0)	19	(3)	
HBsAg (-)/HCV Ab (-)	74	(12)	74	(100)	0	(0)	-
TNM stage (%)							
I	158	(25)	11	(15)	147	(27)	
II	239	(38)	30	(40)	209	(39)	
III	142	(23)	20	(27)	122	(22)	
IV	85	(14)	13	(18)	72	(12)	0.030
Laboratory data							
Albumin (g/dl)	3.7	(0.8)	3.8	(0.9)	3.7	(0.8)	NS
Prothrombin time (%)	80	(22)	85	(22)	80	(22)	NS
Total bilirubin (mg/dl)	1.0	(0.8)	0.9	(0.7)	1.0	(0.8)	NS
AFP (ng/ml)	51	(446)	16	(290)	59	(452)	0.002

Data are median (IQR) or frequency (%). NS, not significant.

Cox proportional hazards model. Univariate analysis revealed that 9 of 12 factors (male, excessive alcohol intake, Child-Pugh score ≥7, albumin <3.7 g/dl, prothrombin time <80%, total bilirubin ≥1.1 mg/dl, AFP ≥52 ng/ml, TNM stage III or IV, and the follow-up group) significantly affected the survival rate in patients with HCC. Multivariate analysis identified follow-up (follow-up group, relative risk 0.71), alcohol consumption (excessive drinker, relative risk 1.32), albumin (<3.7 g/dl, relative risk 1.37), total bilirubin (≥1.1 mg/dl, relative risk 1.53), AFP (≥52 ng/ml, relative risk 1.44), and TNM stage (III or IV, relative risk 2.50), as independent and significant risk factors (P=0.002, 0.043, 0.046, <0.001, 0.001 and <0.001, respectively) for prognosis.

Comparison of clinical characteristics and survival between patients with and without hepatitis virus infection in those patients with TNM stage I or II. Characteristics of patients with TNM stage I or II at the time of HCC diagnosis are presented in Table III. No significant differences were observed in gender, habitual drinkers, BMI, TNM stage, prothrombin time, or total bilirubin level. However, there were significant differences in the median age (P<0.001), Child-Pugh score (P=0.012), albumin level (P=0.009), AFP (P<0.001) and follow-up group (P=0.010).

Table IV indicates the results of univariate and multivariate analyses of the prognosis factors for HCC using the Cox proportional hazards model. Univariate analysis revealed that 6 of 12 factors (male, Child-Pugh score ≥7, albumin <3.7 g/dl, AFP ≥52 ng/ml, TNM stage II and HCC-nonBC) significantly affected the survival rate in HCC patients. Multivariate analysis identified HCC-nonBC (HCC-nonBC, relative risk 0.55), gender (male, relative risk 1.58), Child-Pugh score (≥7, relative risk 1.47), albumin (<3.8 g/dl, relative risk 1.62) and TNM stage (stage II, relative risk

Table II. Univariate and multivariate analyses of prognostic factors for HCC in the 624 patients.

Variable		Univariate analysis		Multivariate analysis	
		P-value	Relative risk (95% CI)	P-value	Relative risk (95% CI)
Age (years)	≥65	0.058	0.82 (0.67-1.01)		
Gender	Male	0.003[a]	1.46 (1.14-1.88)	0.800	1.28 (0.97-1.68)
BMI	≥25	0.177	0.84 (0.65-1.08)		
Alcohol consumption	Excessive	0.011[a]	1.37 (1.08-1.75)	0.043[a]	1.32 (1.01-1.72)
Follow-up	Followed up	<0.001[a]	0.63 (0.52-0.77)	0.002[a]	0.71 (0.56-0.89)
Child-Pugh score	≥7	<0.001[a]	2.10 (1.70-2.59)	0.134	1.30 (0.92-1.82)
Albumin (g/dl)	<3.7	<0.001[a]	1.98 (1.62-2.43)	0.046[a]	1.37 (1.01-1.85)
Prothrombin time (%)	<80	0.002[a]	1.37 (1.12-1.68)	0.959	0.99 (0.78-1.27)
Total bilirubin (mg/dl)	≥1.1	<0.001[a]	1.67 (1.36-2.05)	<0.001[a]	1.53 (1.22-1.92)
AFP (ng/ml)	≥52	<0.001[a]	1.83 (1.49-2.24)	0.001[a]	1.44 (1.16-1.79)
TNM stage	III or IV	<0.001[a]	3.02 (2.45-3.72)	<0.001[a]	2.50 (2.00-3.13)
Etiology of liver disease	HCC-nonBC	0.139	0.77 (0.54-1.09)		

CI, confidence interval.

Table III. Comparison between HCC in TNM stage I or II patients with and without virus infection.

	All patients		HCC-nonBC		HCC-virus		P-value
Total	397		41		356		
Median age, years	65	(13)	72	(13)	65	(13)	<0.001
Gender (%)							
Male	288	(73)	27	(66)	261	(73)	
Female	109	(27)	14	(34)	95	(27)	NS
BMI	22.3	(4.0)	23.7	(5.2)	22.3	(3.9)	NS
Alcohol consumption (%)							
Not excessive	328	(83)	31	(76)	297	(83)	
Excessive	69	(17)	10	(24)	59	(17)	NS
Follow-up (%)							
Follow-up group	268	(68)	21	(51)	247	(60)	
Non-follow-up group	129	(32)	20	(49)	109	(40)	0.019
Child-Pugh score	6	(2)	5	(1)	6	(2)	0.012
Hepatitis virus							
HBsAg (+)/HCV Ab (-)	70	(18)	0	(0)	70	(20)	
HBsAg (-)/HCV Ab (+)	274	(69)	0	(0)	274	(77)	
HBsAg (+)/HCV Ab (+)	12	(3)	0	(0)	12	(3)	
HBsAg (-)/HCV Ab (-)	40	(10)	40	(100)	0	(0)	-
TNM stage (%)							
I	158	(40)	11	(15)	147	(27)	
II	239	(60)	30	(40)	209	(39)	NS
Laboratory data							
Albumin (g/dl)	3.8	(0.7)	4.0	(0.6)	3.8	(0.8)	0.009
Prothrombin time (%)	82	(22)	87	(20)	80	(21)	NS
Total bilirubin (mg/dl)	0.9	(0.6)	0.8	(0.4)	1.0	(0.7)	NS
AFP (ng/ml)	32	(222)	9	(32)	36	(254)	<0.001

Data are median (IQR) or frequency (%). NS, not significant.

Table IV. Univariate and multivariate analyses of prognostic factors for HCC in patients with TNM stage I or II.

Variable		Univariate analysis		Multivariate analysis	
		P-value	Relative risk (95% CI)	P-value	Relative risk (95% CI)
Age (years)	≥65	0.514	0.91 (0.69-1.20)		
Gender	Male	0.039[a]	1.40 (1.02-1.94)	0.008[a]	1.58 (1.13-2.21)
BMI	≥25	0.062	0.71 (0.50-1.02)		
Alcohol consumption	Excessive	0.083	1.36 (1.96-1.93)		
Follow-up	Followed up	0.270	0.85 (0.64-1.13)		
Child-Pugh score	≥7	<0.001[a]	2.04 (1.52-2.73)	0.041[a]	1.47 (1.02-2.11)
Albumin (g/dl)	<3.8	<0.001[a]	2.04 (1.56-2.68)	0.007[a]	1.62 (1.15-2.30)
Prothrombin time (%)	<82	0.083	1.27 (0.97-1.67)		
Total bilirubin (mg/dl)	≥0.9	0.067	1.30 (0.98-1.72)		
AFP (ng/ml)	≥32	<0.001[a]	1.64 (1.26-2.16)	0.065	1.31 (0.98-1.74)
TNM stage	II	0.004[a]	1.52 (1.14-2.01)	0.004[a]	1.53 (1.14-2.04)
Etiology of liver disease	HCC-nonBC	0.020[a]	0.51 (0.29-0.90)	0.048[a]	0.55 (0.30-0.99)

CI, confidence interval.

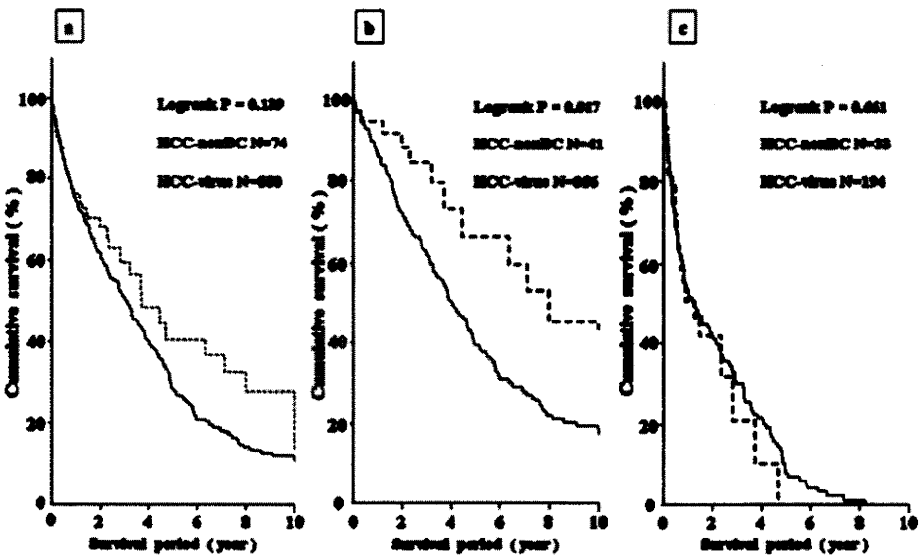

Figure 1. The cumulative survival rate in HCC patients without hepatitis virus infection (HCC-nonBC, dashed-line) and in HCC patients infected with hepatitis virus (HCC-virus, thin line) according to the TNM staging system.

1.53), as independent and significant risk factors (P=0.048, 0.008, 0.041, 0.007 and 0.004, respectively) for prognosis.

Patient survival. Overall, the median survival of all 624 patients was 1.84 years. No significant difference was detected in the survival rate between patients with and without hepatitis virus infection (Fig. 1a). When patients were classified according to the TNM stage, patients in the HCC-nonBC group with TNM stage I or II had a higher cumulative survival rate than those in the HCC-virus group (Fig. 1b; P=0.017). Patients who had TNM stage III or IV and HCC-nonBC and HCC-virus patients did not show significant differences in survival rates (Fig. 1c).

Discussion

The age-adjusted mortality rate for HCC has increased over the past few decades in Japan (27). However, the majority of patients are still diagnosed at an advanced stage and so have a short survival time after diagnosis. Patients with chronic HBV and/or HCV infection complicated by cirrhosis should be monitored with ultrasonography, CT or MRI of the liver to detect tumors at an early stage. In 58% of our patients, the tumors were detected on follow-up. Patients in the follow-up group had smaller tumors at the time of diagnosis and were more likely to be eligible for treatment. In addition, there was a significant improvement in survival rates among the

follow-up group (24-26,28-32). We recognized that the 2 groups of patients could not be evaluated in a prospective study, and improved survival in the follow-up group patients may be owing to the effect of lead-time bias. Nevertheless, our data corroborate those of previous studies indicating that follow-up may have increased rates of early detection and eligibility for curative treatment, which may in turn translate to improved survival.

In the TNM stages I and II, patients with HCC-nonBC had a better prognosis than those with HCC-virus. This difference may be explained as follows. HCC secondary to liver cirrhosis is less frequent in patients with HCC-nonBC than in those with HCC-virus (12). Patients with HCC-nonBC are less likely to progress to liver cirrhosis (33). However, in the TNM stage III and IV, the patients with HCC-nonBC had a similar prognosis to those with HCC-virus. The percentages of advanced stage HCC and non-follow-up patients were significantly higher in the HCC-nonBC group than in the HCC-virus group. Taken together, these results indicate that the prognosis of patients with HCC-nonBC is linked to the follow-up studies for detecting HCC.

A large proportion of people infected with HCV, HBV or both have latent cancer. Therefore, it is essential that HCC is detected at an early stage in individuals who harbor chronic HCV or HBV infections. In this study, more than 80% of patients had HCC associated with HBV and/or HCV; therefore, the target population for the surveillance of HCC must be easily identifiable. However, the incidence of hepatitis virus associated with HCC will decrease in Japan (15,34,35) because of the following reasons. In Japan, the population of individuals infected with chronic HCV is rapidly aging (36,37), and chronic HBV infection has been preventable since the licensing of the hepatitis B vaccine in 1982. In fact, primary tumors in 12% of our patients with HCC were negative for both HBsAg and HCV Ab. Of these, non-alcoholic fatty liver disease (NAFLD) may be a cause of HCC. Bugianesi *et al* suggested that liver disease was caused by NAFLD in 23/641 (4%) patients with HCC (38). However, it will be difficult to select patients for the screening of HCC, who are negative for both HBsAg and HCV Ab.

HCC surveillance for patients eligible for imaging tests is usually performed at 6-month intervals. Additionally, a combined imaging test and a serological test such as AFP or des-γ carboxy prothrombin is a sensitive method to detect HCC (29,39). The target population for the surveillance of HCC may not be easily identified in Japan. It has been reported previously that more than 60% of patients in the follow-up group had HCCs measuring less than 3 cm in diameter (26). It is possible that 12-month intervals for the imaging test were reasonable to ensure the detection of treatable tumors in patients with HCC.

In summary, the poorer prognosis of patients with HCC-nonBC was attributable to its late detection in an advanced condition, owing to the lack of a surveillance system for early detection of HCC. However, among early-stage patients, those with HCC-nonBC showed a significantly better prognosis than those with HCC-virus. To conclude, we suggest that the entire population of Japan should be tested using imaging techniques at least every 12 months along with an abdominal examination.

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The level of fasting serum insulin, but not adiponectin, is associated with the prognosis of early stage hepatocellular carcinoma

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Abstract. Impaired glucose tolerance influences the prognosis of hepatocellular carcinoma (HCC), but this mechanism is still not fully understood. We investigated the impact of the fasting serum levels of insulin and adiponectin on the prognosis of HCC and its recurrence. One hundred and forty patients with newly diagnosed HCC were enrolled in the prognosis study. Their fasting serum levels of insulin and adiponectin were determined. Of 140 patients, 59 patients who underwent curative treatment were subjected to analysis of the recurrence-free survival. The 140 patients were divided into two groups by the 50th percentile value of insulin (7.73 μIU/ml) or total adiponectin (6.95 μg/ml). Kaplan-Meier analysis indicated that high insulin group (>7.73 μIU/ml) exhibited a significantly poorer prognosis than low insulin group (<7.73 μIU/ml) in early stage HCC (P=0.018). In contrast, the level of total adiponectin had no impact on the prognosis of HCC. Multivariate analysis indicated that fasting hyperinsulinemia was an independent risk factor for a poorer prognosis in early stage HCC (P=0.044). Likewise, Kaplan-Meier analysis indicated that the recurrence-free survival of high insulin group was significantly lower than that of low insulin group (P=0.017). The level of total adiponectin had no impact on the recurrence-free survival of HCC. Multivariate analysis indicated that fasting hyperinsulinemia was an independent risk factor for the lower recurrence-free survival of HCC (P=0.049). In conclusion, our study suggests that the fasting insulin level affects the clinical course of early stage HCC.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most frequent malignant neoplasm in the world (1). Extensive evidence of the rising incidence of HCC has been reported in the United States, Japan, and several other countries (2,3). In addition to chronic infection by the hepatitis C virus (HCV), diabetes mellitus (DM) is thought to be a rising risk factor of note, because it is associated with nonalcoholic fatty liver disease (NAFLD) including its severe form, nonalcoholic steatohepatitis (NASH) (4). NASH is a chronic necroinflammatory condition that can lead to liver fibrosis, cirrhosis, and subsequently to HCC (5,6). In earlier studies it was suggested that there was no evidence linking DM to HCC, whereas more recent studies have indicated the association between DM and HCC (6-9). Moreover, several studies have reported that the coexistence of DM in chronic liver disease caused by chronic infection of HBV and HCV is closely related to the risk of not only the development of HCC, but also a poor prognosis (10,11). However, it is not clear why coexisting DM influences the development and progression of HCC in chronic liver disease.

The abnormality of the glucose metabolism found in chronic liver disease is due to the existence of a decline of insulin degradation, and peripheral insulin resistance (12). We have also reported that the developing of liver fibrosis is closely associated with insulin resistance in HCV infected patients (13). Taken together, it is likely that insulin resistance in chronic liver disease triggers hyperinsulinemia, and it may modulate the biological characteristics of HCC cells. It has been reported that insulin displays growth promoting and anti-apoptotic effects on human hepatoma cells *in vitro* and in animal models (10,14,15). Moreover, Saito *et al* have

previously reported that postprandial hyperinsulinemia is associated with the accelerated growth of HCC (16).

Recently, adiponectin, a physiologically active polypeptide secreted exclusively by adipose tissue, has been the focus of research interest as a factor involved in glucose metabolism. This hormone has a potent insulin-sensitizing effect (17-19), and a low level of circulating adiponectin is found in several types of metabolic syndrome including insulin resistance and type 2 diabetes (20). Several studies have reported the association between the values of circulating adiponectin and liver disease. Xu *et al* have reported that adiponectin administration alleviates hepatomegaly and steatosis and also significantly attenuates the inflammation and the elevated levels of serum alanine aminotransferase in alcoholic and nonalcoholic fatty liver murine models (21). In humans, the level of circulating adiponectin has been found to increase in patients with advanced cirrhosis (22-24). In addition, Hui *et al* reported that the level of serum adiponectin increases in advancing liver fibrosis and declines with a reduction in fibrosis in chronic hepatitis B (25).

The aim of the present study was to clarify whether the levels of fasting serum insulin and adiponectin are relevant to the prognosis of HCC in patients newly diagnosed to have HCC and the recurrence of HCC in those who underwent curative therapy.

Patients and methods

Patients. A total of 140 patients, who were newly diagnosed to have HCC between January 1995 and December 2004 at the First Department of Internal Medicine in Nagasaki University Hospital and fulfilled the criteria specified below, were enrolled in the current cohort. The inclusion criteria were: 1) not diagnosed as having DM on admission, in other words, no dietary intervention and no regular use of medication to affect insulin sensitivity or insulin secretion, 2) on admission a fasting serum sample was drawn and stored, and 3) no life-threatening illness other than liver disease.

The diagnosis of HCC was based on the typical findings detected by ultrasonography (US), dynamic computerized tomography (CT), magnetic resonance imaging (MRI), abdominal angiography, and/or histological manifestation of liver tumor. Underlying liver diseases, such as chronic hepatitis (CH) and liver cirrhosis (LC) were diagnosed by liver histologic examination following a liver biopsy and/or by the findings of US, dynamic CT, and MRI. LC was graded according to the Child-Pugh classification (26). The body mass index (BMI) was calculated as body weight in kilograms divided by the square of the height in meters (kg/m^2). The alcohol intake was assessed by interview and recorded in grams per day. The patients were divided into two groups according to the mean alcohol consumption per day; not excessive drinkers (<80 g/day) and excessive drinkers (≥80 g/day). Fasting blood samples were obtained in the early morning for an analysis of biochemical and hematological data or fasting blood glucose, and serum samples were stored at -80°C until further assay. Hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (HCVAb) were tested by commercial immunoassays (Fuji Rebio, Tokyo). The serum AFP level was measured by enzyme immunoassay (AxSYMAFP, Abbott Japan, Tokyo).

Table I. Clinical and laboratory characteristics of the study subjects.

Variable	Number or mean (SD)
Patients	140
Onset age, y.o.	65.1 (9.5)
Gender	
Male	110
Female	30
BMI, kg/m^2	23.1 (3.1)
Alcohol intake	
<80 g/day	108
≥80 g/day	32
Etiology	
HBsAg(+)	29
HCVAb(+)	99
non-B, non-C	12
Underling liver diseases and Child-Pugh grade	
CH	30
LC grade A	72
LC grade B	31
LC grade C	7
Total bilirubin, mg/dl	1.4 (2.2)
Ferritin, ng/ml	304.2 (345.2)
Serum iron, μg/ml	151 (75)
Fasting insulin, μIU/ml	10.0 (9.6)
Fasting blood glucose, mg/dl (n=62)	102.7 (51.5)
HOMA-R, % (n=28)	3.0 (2.6)
Total adiponectin, μg/m	8.1 (4.8)
HMW, μg/ml	3.9 (3.0)
MMW, μg/ml	1.8 (1.2)
LMW, μg/ml	2.4 (1.3

Measurement of insulin and adiponectin. Fasting serum samples stored at -80°C were used for the assay. Fasting serum insulin was measured by enzyme immunoassay (Fuji Rebio). Fasting total adiponectin was measured by an enzyme immunoassay (Daiichi Pure Chemicals Co., Ltd., Tokyo). Serum adiponectin exists in a complex form and is classified according to its molecular weight. Therefore, high molecular weight (HMW), middle molecular weight (MMW), and low molecular weight (LMW) adiponectins were also measured separately by enzyme immunoassay (Daiichi Pure Chemicals Co.).

HCC assessment. The size and number of HCCs were confirmed by US, CT, MRI, or angiography. We used the

Table II. HCC characteristics of the study subjects.

Variable	Number or mean (SD)
Tumor size, cm	3.4 (2.8)
<2 cm	57
2 - <5 cm	62
≥5 cm	21
Number of tumor lesions	
1	75
2	29
3 - and diffuse	36
TNM stage	
I	39
II	53
III	37
IV	11
AFP, ng/ml	7792.9 (62230.8)
Therapy	
Surgical resection	7
PEIT and/or RFA	53
TACE or TAI	73
Others	7

tumor-node-metastasis (TNM) classification system of the Liver Cancer Study Group (LCSG) of Japan in 2000 (27). The T category is determined by the 3 factors of number, size, and vascular or bile duct invasion. The N category is the presence of lymph node metastasis, and the M category is the presence of distant metastasis. TNM staging has four stages according to the T, N, and M categories. The therapy for HCC was divided into four groups; the surgical resection group, percutaneous ethanol injection therapy (PEIT) and/or radiofrequency ablation (RFA) group, transcatheter arterial chemoembolization (TACE) or transarterial infusion (TAI) group, and other therapy or palliative therapy group. In this study, the curative therapy was defined as a condition characterized by the no findings of recurrence over six months after the initial therapy for HCC, including surgical resection, PEIT, RFA, and TACE or TAI.

Statistical analysis. The data were analyzed by the Mann-Whitney test for continuous ordinal data, χ^2 test with Yates' correction and Fischer exact test for the association between 2 qualitative variables, and Kaplan-Meier survival analysis. Parametric comparisons were assessed by analyses of variance. The significance of individual differences was evaluated by use of Scheffe's test. $P<0.05$ was considered to be statistically significant.

Results

Clinical features of studied patients. A total of 140 HCC patients were enrolled in this study. Patient characteristics are presented in Table I. There were 110 men (78.6%) and 30 women (21.4%). The mean age was 65.1 years. The mean BMI was 23.1 kg/m^2. Excessive drinkers comprised 22.9% (32 of 140) and not excessive was 77.1% (108 of 140). Patients who were HBsAg positive was 20.7% (29 of 140), whereas 70.7% (99 of 140) were HCVAb positive, and 8.6% (12 of 140) were negative for both (non-B, non-C). Chronic hepatitis (CH) was 21.4% (30 of 140). Liver cirrhosis (LC) was 78.6% (110 of 140). The Child-Pugh grade of LC patients was: grade A: 51.4% (72 of 140), grade B: 22.1% (31 of 140), and grade C: 5.0% (7 of 140). The mean level of total bilirubin was 1.4 mg/dl. The mean levels of ferritin and serum iron were 304.2 ng/ml and 151 μg/ml, respectively. The mean level of fasting insulin was 10.0 μIU/ml. The mean level of fasting blood sugar in 62 patients measured during the hospital stay was 102.7 mg/dl. In a similar fashion, the level of HOMA-R in the 28 patients calculated was 3.0%. The mean values of total, HMW, MMW, and LMW adiponectins were 8.1, 3.9, 1.8 and 2.4 μg/ml, respectively.

The characteristics of newly diagnosed HCC on admission are presented in Table II. The mean size of HCC was 3.4 cm and its distribution was: <2 cm: 57 (40.7%), ≥2 cm and <5 cm: 62 (44.3%), and ≥5 cm: 21 (15.0%). The number of HCCs in the subjects was: 1: 75 (53.6%), 2: 29 (20.7%), and 3 or more and diffuse: 36 (25.7%). The TNM stage of the HCC was: stage I: 39 (27.9%), stage II: 53 (37.9%), stage III: 37 (26.4%), and stage IV: 11 (7.9%). The mean level of AFP was 7792.9 ng/ml. Of the studied patients, 5.0% (7 of 140) underwent surgical resection, 37.9% (53 of 140) underwent PEIT and/or RFA, 52.1% (73 of 140) underwent TACE or TAI, and 5.0% (7 of 140) received other therapy or palliative care only.

The values of fasting insulin and adiponectin in subjects. We evaluated the values of fasting insulin and total adiponectin in underlying liver diseases or in the TNM stage of HCC. The mean values of insulin of CH, LC with Child-Pugh grade A, B, and C were 6.9, 10.3, 11.9, and 12.7 μIU/ml, respectively (Fig. 1A). The mean value of insulin seemed to increase in LC (Child-Pugh grade A, B, C) compared to CH, but no significant differences were observed between them. The mean values of insulin of TNM stage I, II, III, and IV were 8.1, 11.1, 11.5, and 7.1 μIU/ml, respectively. No significant differences were observed in these groups.

The mean values of total adiponectin of CH, LC with Child-Pugh grade A, B, and C were 6.4, 7.7, 9.5, and 13.4 μg/ml, respectively (Fig. 1B). In parallel with the decline of liver function, the mean value of total adiponectin increased obviously, and the mean value of total adiponectin of LC with Child-Pugh grade C showed a significantly higher level than that of CH and LC with Child-Pugh grade A. In contrast, the mean values of total adiponectin of TNM stage I, II, III, and IV were 8.5, 8.0, 8.3, and 6.6 μg/ml, and there were no significant differences.

Association of fasting insulin level with prognosis of HCC. To evaluate the association of fasting insulin level with the prognosis of HCC, the 140 patients were divided into two groups in terms of the 50th percentile of the value of insulin (7.73 μIU/ml). The mean level of insulin in the low insulin

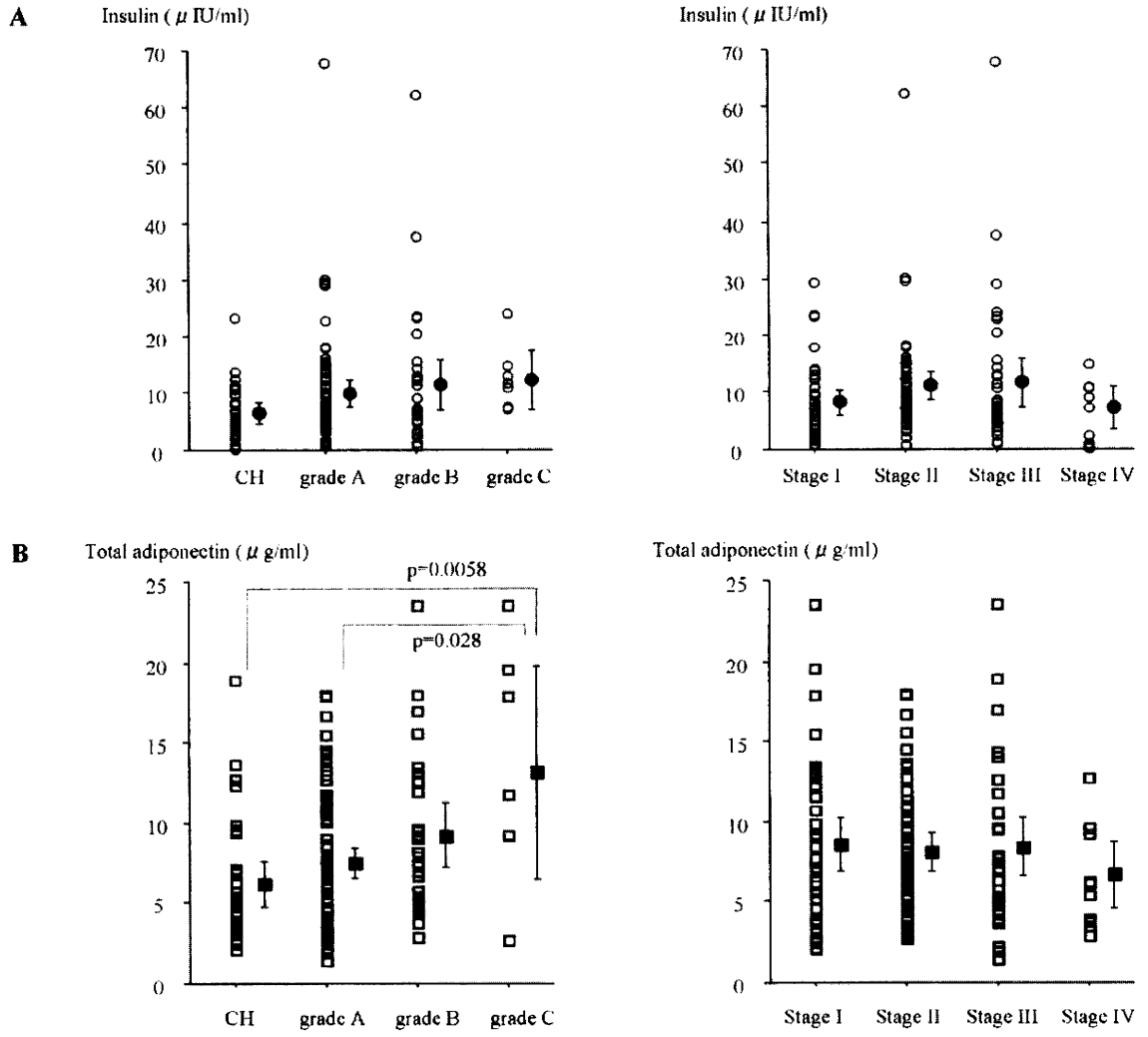

Figure 1. The mean ± SD values of fasting insulin in each underlying liver disease (CH and Child-Pugh grade) (A) and TNM stage (B), the mean ± SD values of total adiponectin in each underlying liver disease (CH and Child-Pugh grade) (C) and TNM stage (D).

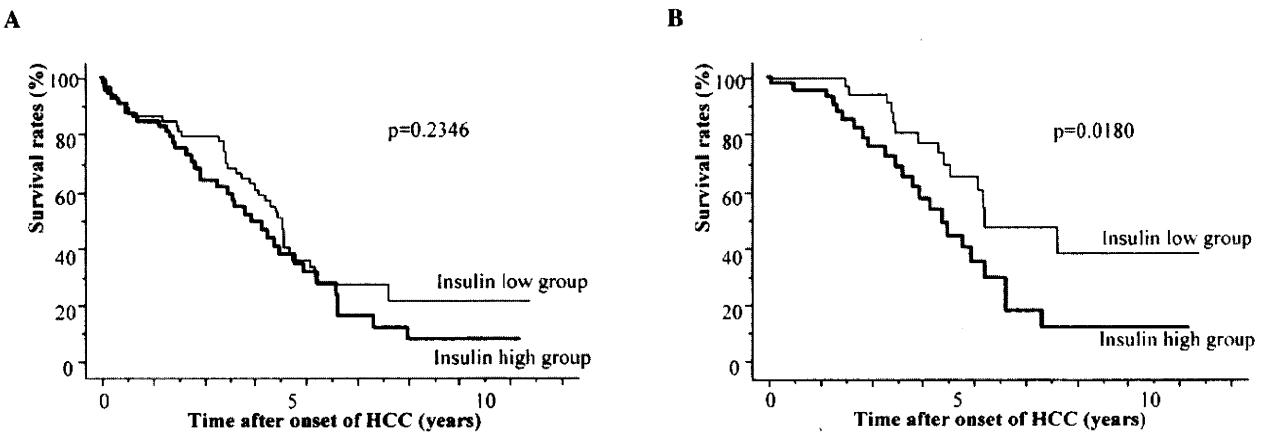

Figure 2. Kaplan-Meier curves for survival between low insulin group (thin line) and high insulin group (heavy line) in all stages of HCC patients (n=140) (A) and in TNM stage I + II HCC patients (n=92) (B).

group (<7.73 μIU/ml, n=70) was 4.3 μIU/ml. On the other hand, that in the high insulin group (>7.73 μIU/ml, n=70) was 15.8 μIU/ml. Table III shows the comparison of patient characteristics between the low and high insulin groups. The BMI in the high insulin group was significantly higher than it was in the low insulin group. The HOMA-R level was