現実があります。日本ではB型肝炎のキャリアが110～140万人，C型肝炎は190～230万人，合計350万人との推定があり，その半分はまだ自身の感染を知らないでいます。

広島大学の田中純子先生の推計では，B型肝炎は90万人，C型肝炎は80万人で，合計170万人のキャリアが自身の感染を知らないでいるとのことです。そのような方を発掘して，適切な治療に向けていかなければいけません。

ホスト側の因子，特にB型肝炎のホスト側の因子としてHLA-DPのA1，B1の組み合わせによってはプロテクティブ，あるいはリスクとなるハプロタイプがわかってきましたので，それを調べることで，全員治療をする必要があるのか，あるいはその一部で良いのかある程度の見極めができる時代が来るかもしれません。

そのような知見をどんどん投入すれば，十把ひとからげにやろうとするとクリアできない問題も，何とかなるという期待が持てると思います。

朝比奈　スクリーニング検査を受けないから自分が感染状態であるということがわかっていない，あるいは陽性とわかって抗ウイルス療法が必要なのに，適切な医療を受けていない方がたくさんいらっしゃいます。そのような方をきちんと診断し，治療方針を決めて，適切な治療介入をしていくことが大事です。

C型肝炎についていえば，治療法が劇的に変わり治療成績が飛躍的に向上していますし，副作用も少なくなっています。以前治療を受けたが効かなかった，あるいは専門医を受診して治療適用がないといわれた人も，新たに治療適用になったり，改めて治療したりすると，今まで治療を受けていなかった人より効くこともあります。患者さんの拾い上げとともに，感染がすでにわかっている人も今一度専門医に行って，治療方針の検討がなされるべきだと思います。

齋藤　今後，医療機関にかかっていない方の発掘とともに，さらに新たな治療が望まれているのがB型肝炎，C型肝炎の現状だと思います。

まだまだ話は尽きませんが，時間がまいりました。本日はお忙しいところ，ありがとうございました。

〔2013年9月20日(金)収録〕

報 告

原因不明のHBV再活性化を疑う高齢2症例

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要旨 症例1は80歳，女性．2008年から肺アスペルギルス症にて呼吸器内科で治療中，初めて肝障害を指摘され紹介となった．2011年10月2X日 AST193U/l，ALT180U/l，HBs抗原陽性，HCV抗体陰性，HBe抗原1579 S/CO，HBe抗体陰性，HBV DNA7.9Logコピー/ml，HBc抗体10.18 S/CO，IgM-HBc抗体0.76 S/COであった．11月X日よりエンテカビル0.5mgを投与開始した．Genotype Bであった．症例2は74歳，男性．パーキンソン病にて神経内科で治療中初めてHBs抗原陽性の肝障害を指摘され紹介となった．2012年1月2X日 AST134U/l，ALT188U/l，HCV抗体陰性，HBe抗原1.82 S/CO，HBe抗体陰性，HBV DNA>9.0logコピー/ml，HBc抗体9.38 S/CO，IgM-HBc抗体0.19 S/COであった．1月3X日よりエンテカビル0.5 mgを投与開始した．Genotype Cであった．2症例ともに，①過去の採血でHBc抗体・HBs抗体は測定されていないがHBs抗原陰性であったこと②肝障害発生時にHBs抗原・HBV DNA陽性，HBc抗体陽性，IgM-HBc抗体陰性よりHBV再活性化を起こしたと判断した．2症例ともに免疫療法や化学療法は行っておらず，HIV抗体は陰性，CD4/8は正常であった．高齢者で誘因がはっきりしないがHBV再活性化を起こした疑いが強いと考えられる2症例を経験し注意が必要と考えられた．

キーワード HBV再活性化，原因不明，高齢，エンテカビル

はじめに

最近，免疫療法や化学療法の進歩によってその使用頻度が増え，肝機能正常の非活動性B型肝炎ウイルス（HBV）キャリアのみならずHBs抗原が消失した既感染者からHBVの再増殖が起こり肝炎が惹起される病態はHBV再活性化（reactivation）と呼ばれて注目されている．HBV再活性化の多くは血液悪性腫瘍にステロイドを含む抗癌剤やリツキシマブを使用した場合[1)2)]や自己免疫疾患にステロイドやリツキシマブを使用する[3)4)]など宿主免疫能が低下した場合に発症する．今回われわれは，免疫療法や化学療法といった明らかな原因が無いにも関わらずHBV再活性化を起こした疑いが強いと思われる高齢2症例を経験したので報告する．

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（平成24年8月22日受付，平成24年11月7日受理）
Re'activation of Hepatitis B Virus of Unknown Reason was Suspected in Two Elder Cases
Noboru Hirashima, Yoshiko Kobayashi, Hironao Takahashi, Yuichi Kida*, Tuyoshi Kuno*, Misaki Yoko*, Masayuki Saitoh*, Yosimitsu Rhuge*, Tomoyuki Tsuzuki*, Masaaki Shimada* and Hiroaki lwase*, National Hospital Organization, Higashi Nagoya hospital, ＊National Hospital Organization, Nagoya Medical Center
Key Words: HBV reactivation, unknown reason, elder cases, entecavir

表1　症例1消化器内科初診時検査成績（2011年10月2X日）
HBV再活性化を起こした疑いが強いと考えられた

Hematology	
WBC	6700/μl
Neutro	74.2%
Lympho	16.4%
CD 4/8	1.1
RBC	378×10⁴/μl
Hb	10.1 g/dl
PLT	29.5/μl
Coagulation	
PT	116 %
Viral markers	
IgM-HA Ab	0.28 S/CO (陰性)
HBsAg	2670 IU/ml (陽性)
HBsAb	0.8 mIU/ml (陰性)
HBcAb	10.18 S/CO (陽性)
IgM-HBcAb	0.76 S/CO (陰性)
HBeAg	1579 S/CO (陽性)
HBeAb	0% (陰性)
HBV DNA	7.9 logコピー/ml (陽性)
HBV Genotype	B
HBV pre-core	wild
HBV core promoter	wild
CM IgM	0.35 (陰性)
CM IgG	9.6
EB EA-VCA IgM	<10 (陰性)
EB EA-VC IgG	80 (陽性)
HCV Ab	0.1 C.O.I. (陰性)
HIV Ab	(-)
Blood chemistry	
TP	7.6 g/dl
Alb	3.1 g/dl
T.Bil	0.94mg/dl
AST	193 U/l
ALT	180 U/l
ALP	614 U/l
γGTP	64 U/l
BUN	16 mg/dl
Cr	0.51 mg/dl
Amy	106 IU/l
T.chol	187 mg/dl
BS	108 mg/dl
IgG	2227mg/dl
IgA	501mg/dl
IgM	114mg/dl
抗ミトコンドリア抗体	<20倍
抗核抗体	<40倍
AFP	3.7 ng/dl

症　　例

症例1：80歳，女性

主訴：肝障害

既往歴：2008年から肺アスペルギルス症にて東名古屋病院呼吸器内科で治療中

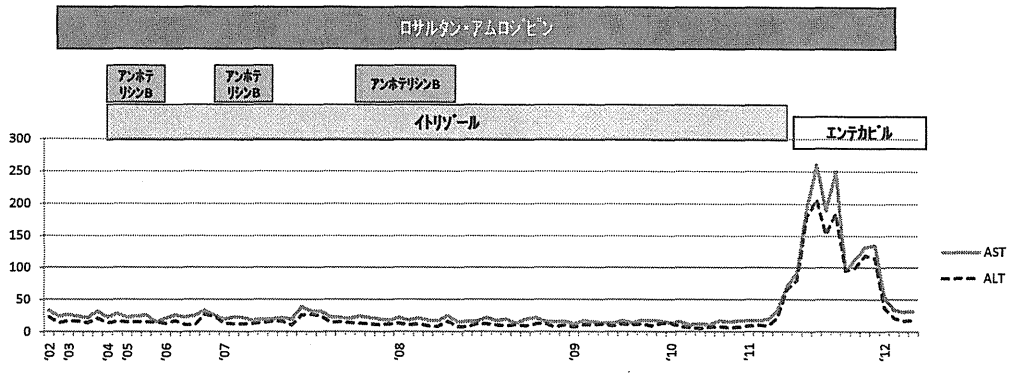
家族歴：特記なし

生活歴：喫煙・飲酒せず

現病歴：肺アスペルギルス症に対し，抗真菌剤アンホテリシンB・イトリゾールを適宜使用して日常生活に支障はない状態であった．高血圧に対しロサルタン・バルサルタン・アムロジピンを内服していた．2007年9月の採血ではHBs抗原陰性であった．2002年10月以降ASTおよびALTは正常であったが，2011年9月1X日AST71U/l，ALT66U/lと初めて肝障害を指摘され消化器科紹介となる．

2011年9月1X日現症：身長145cm，体重40kg，血圧112/76mmHg，脈拍60/min・整，意識清明，貧血・黄疸なし，腹水・浮腫なし．

経過：10月2X日消化器科初診時検査成績は，AST 193U/l（正常範囲：13-33U/D，ALT180U/l（6-27U/l）と肝機能は9月1X日より上昇し，HBs抗原は2670IU/ml（<0.05IU/ml）と陽性化していた．HBe抗原1579 S/CO（<1.0 S/CO）陽性，HBe抗体陰性，HBV DNA7.9 logコピー/ml（<2.1logコピー/ml）と高値を示した．HBc抗体は10.18 S/CO（<1.0 S/CO）と強陽性であった



	07/9/1X	11/10/2X	11/X	12/X	12/2X	12/1/1X	2/X	3/X
HBsAg (IU/ml)	(-)定性	2670			5571			1839
HBsAb (mIU/ml)		0.8						
HBcAb (S/CO)			10.18					
IgM-HBcAb (S/CO)			0.76	0.12				
HBeAg (S/CO)			1579.97	753.79		536.66	219.15	
HBeAb (%)			0	0		0	0	
HBV DNA (logコピー/ml)			7.9	5.8		4.1	3.3	

図1　症例1経過

肺アスペルギルス症に対し抗真菌剤アンホテリシンB・イトリゾールを間欠的に使用していた．高血圧に対しロサルタン・バルサルタン・アムロジピンを内服していた．エンテカビル0.5mgを投与開始しHBV DNAは減少しAST，ALTは正常化した．IgM・HBc抗体は2011年11月と12月の2回測定したが陰性であった．

が，IgM-HBc抗体は0.76 S/CO（<1.0 S/CO）と陰性であった．GenotypeはB，HBV pre-coreはwild，HBV core promoterはwildであった．HCV抗体は陰性，サイトメガロウイルスCMIgMおよびEB EA-VCAlgMは陰性であった．HIV抗体は陰性，CD4/8は正常であった（表1）．IgM-HBc抗体を再検したが0.12 S/COと陰性であったため急性B型肝炎は考えにくく，過去の採血でHBc抗体-HBs抗体は測定されていないもののHBs抗原陰性であったこと，発生時のHBc抗体は強陽性であったことから，HBV再活性化を起こした可能性が強いと判断して11月2日よりエンテカビル0.5mgを投与開始した．HBV DNAは漸減し，2012年3月2日AST31 U/l, ALT17 U/lと正常化した（図1）．2011年12月X日の腹部CT検査では肝硬変・肝細胞癌の所見を認めなかった（図2c）．しかし，2012年3月1X日肺炎・心不全で他界した．

症例2：74歳，男性
既往歴：パーキンソン病にて東名古屋病院神経内科で治療中，
家族歴：特記なし
生活歴：喫煙・飲酒せず
現病歴：パーキンソン病に対し，2007年11月1X日から抗パーキンソン病剤

レボドーパ・ドロキシドーパ・アマンタジン・プラミペキソルで治療開始され，2010年12月X日からソミサシドを追加され車椅子使用し要介護の状態であった．2010年10月1X日から前立腺肥大治療にタムスロシン，過活動性膀胱にソリフェナシンが開始された．2009年9月と2011年5月の採血ではHBs抗原陰性であった．2007年9月以降ASTおよびALTは正常であったが，2012年1月X日，初めて肝障害AST34U/l，ALT31U/lとHBs抗原陽性を指摘され当科紹介となった．

2012年1月X日現症：身長150cm，体重43kg，血圧110/70mmHg，脈拍62/min・整，意識清明，貧血・黄疸なし，腹水・浮腫なし．

経過：2012年1月2X日消化器科初診時検査成績は，AST134U/l，ALT188U/lと肝機能は1月X日より上昇し，HBs抗原は36970 IU/mlと陽性，HBe抗原は1.82 S/CO弱陽性，HBe抗体は27.4%と陰性，HBV DNA>9.0logコピー/mlと高値を示

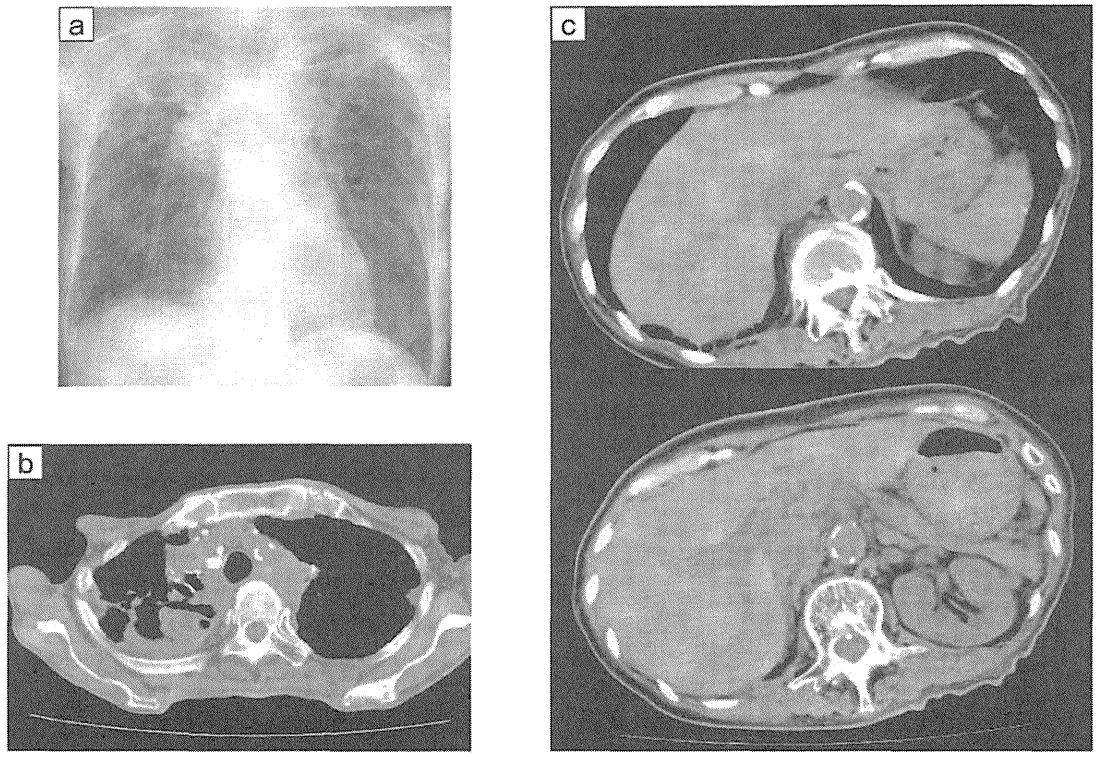


図2
a．症例12010/9/2X 胸部X線写真　右上肺野に浸潤影を認めるが変化を認めない
b．症例12010/9/2X 胸部CT　右上肺野に浸潤影を認める
c．症例12012/9/2X 腹部CT　肝硬変・肝細胞癌の所見を認めなかった

した．HBc抗体は9.38 S/COと強陽性，IgM－HBc抗体は0.19 S/COと陰性であった．Genotypeは C，HBV pre-coreは mutant，HBV core promoterはwildであった．HCV抗体は陰性，サイトメガロウイルス CMIgM および EBEA－VCAIgMは陰性であった．HIV抗体は陰性，CD 4/8は正常であった（表2）．

IgM-HBc抗体を再検したが0.10 S/COと陰性であったため急性B型肝炎は考えにくく，過去の採血でHBc抗体・HBs抗体は測定されていないもののHBs抗原陰性であったこと，発生時のHBc抗体は強陽性であったことから，HBV再活性化を起こした可能性が強いと判断して1月3X日よりエンテカビル0.5mgを投与開始した．HBV DNAは漸減し，2012年4月2X日 AST20U/l，ALT26U/lと正常化した（図3）．2012年1月2X日の腹部CT検査では肝硬変・肝細胞癌の所見を認めなかった（図4a）．2012年5月，原因不明の間質性肺炎を合併したが軽快した（図4b，c）．

考　　案

HBs抗原が陽性の急性肝障害は急性B型肝炎の初感染とHBVキャリアの急性増悪を鑑別する必要があるが，IgM-HBc抗体の測定が有用であるとされている[5)6)]．無症候性HBVキャリアの急性増悪時でもIgM-HBc抗体価は軽度上昇することが多く，従来のEIA法では両者の鑑別は困難な場合も報告されていた[7)]．

最近，化学発光免疫測定法（CLIA法）が開発され，両者の鑑別の正診率が改善された[8)]．2症例ともにCLIA法で測定したIgM-HBc抗体が陰性であり，また約1カ月置いて再検しても陰性であったことより，急性B型肝炎は否定的と考えられた．

成人でHBVに始めて感染すると急性肝炎が起き，通常は1-3カ月で軽快する．

HBs抗原は陰性化しHBs抗体が陽性となる．HBc抗体は感染1カ月後から陽性となり長期にわたり陽性が続く．このようにHBs抗原陰性かつ，

表2　症例2消化器内科初診時検査成績（2012年1月2X日）
HBV再活性化を起こした疑いが強いと考えられた

Hematology	
WBC	5200/μl
Neutro	74.1%
Lympho	16.4%
CD 4/8	1.2
RBC	403×10⁴/μl
Hb	13.2 g/dl
PLT	28.8×10⁴/μl
Coagulation	
PT	122 %
Viral markers	
HBsAg	36970 IU/ml (陽性)
HBsAb	1.1 mIU/ml (陰性)
HBcAb	9.38 S/CO (陽性)
IgM-HBcAb	0.19 S/CO (陰性)
HBeAg	1.82S/CO (陽性)
HBeAb	27.4% (陰性)
HBV DNA	>9.0 logコピー/ml (陽性)
HBV Genotype	C
HBV pre-core	mutant
HBV core promoter	wild
CM IgM	0.35 (陰性)
CM IgG	11.9 (陽性)
EB EA-VCA IgM	<10 (陰性)
EB EA-VC IgG	80 (陽性)
HCV Ab	0.1 C.O.I. (陰性)
HIV Ab	(-)
Blood chemistry	
TP	6.7 g/dl
Alb	3.9 g/dl
T.Bil	1.08mg/dl
AST	134 U/l
ALT	188 U/l
ALP	496 U/l
γGTP	42 U/l
BUN	12 mg/dl
Cr	0.62 mg/dl
Amy	168 IU/l
T.chol	161 mg/dl
BS	95 mg/dl
IgG	1340mg/dl
IgA	234mg/dl
IgM	94mg/dl
AFP	1.2 ng/dl

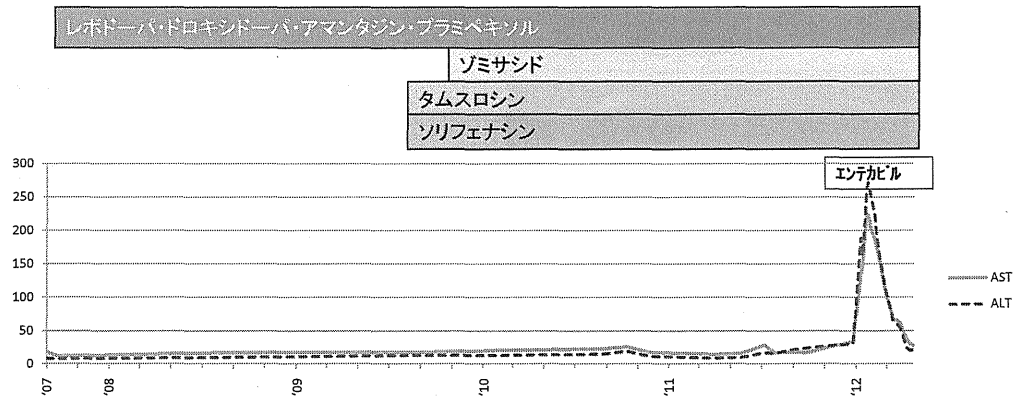
HBc抗体陽性またはHBs抗体陽性の状態をHBV既感染と呼ぶ．既感染でもHBVはcovalently closed circular DNA（cccDNA）として肝細胞核内に残ることが最近判ってきた[9)10)]．HBc抗体陽性またはHBs抗体陽性の既感染者が免疫低下によりHBV再活性化を起こすことはdenovoB型肝炎とも呼ばれている[11)12)]．HBs抗原陰性であるが，血液または肝臓内にHBVが検出される状態はOccult HBV infectionと呼ばれるが，HBc抗体またはHBs抗体が陽性とされている[11)]．

2症例ともに過去の採血でHBc抗体およびHBs抗体は測定されていないため，HBVの過去の感染があったかは不明である．症例1は2007年9月の採血ではHBs抗原陰性であったが測定法はEIAであった．症例2は2009年9月と2011年5月の採血ではHBs抗原陰性であったがいずれもCLIA法での測定であった．

EIA法はCLIA法よる感度が劣るため，症例1では非常に低力価のHBs抗原陽性状態は否定できないものの，2症例ともに以前のHBs抗原は陰性であった．

肝炎発症時2症例共にHBs抗体は陰性であったが，HBc抗体は症例1 10.18 S/CO，症例2 9.38 S/COと高力価陽性であり，HBV既感染状態にあったと推測される．B型肝炎ウイルスに初感染し回復した場合，HBs抗体価は徐々に低下し感度以下になることもあるが，IgG型HBc抗体価は生涯陽性のことが多いとされる[13)14)]．IgM-HBc抗体陰性より急性B型肝炎は否定的と考えられるので，HBs抗原が消失した既感染状態からHBV再活性化を起こしde novoB型肝炎も発症した疑いが強いと考えた．

HBV再活性化による肝炎対策はガイドライン[15)]



	07/9/1X	08/8/1X	09/9/X	11/5/3X	12/1/X	1/2X	2/X	3/X	4/X	4/1X	6/1X
HBsAg (IU/ml)	0.2	0.2	0.4	0	23041			2670			
HBcAb (mIU/ml)					9.38						
HBsAb (S/CO)				1.1	9.38						
IgM-HBcAb (S/CO)					0.19	0.10					
HBeAg (S/CO)					1.82						
HBeAb (%)					27.4						
HBV DNA (logコピー/ml)					>9			5.0	3.8	2.9	2.6

図3　症例2経過

パーキンソン病に対し抗パーキンソン病剤レボドーパ・ドロキシドーパ・アマンタジン・プラミペキソル・ソミサシド投与されていた．前立腺肥大治療にタムスロシン，過活動性膀胱にソリフェナシンが投与されていた．エンテカビル0.5mgを投与開始しHBV DNAは減少しAST，ALTは正常化した．IgM-HBc抗体は2012年1月と2月の2回測定したが陰性であった．

が作成されており，定期的に血中のHBV　DNAを測定し，HBV再活性化認められた時点でエンテカビルを投与する方法が提案されている．HBV再活性化を起こした場合重症化することが多いと報告されているが，われわれの2症例ともにエンテカビル0.5mgを投与開始しHBV DNAは減少し肝機能は正常化した．症例1はGenotypeBで5 pre-core・core promoter共にwildであったが，症例2はGenotypeCでpromoterがmutantであった．pre-coreまたはcore promoterがmutantの場合，reactivationにより肝炎が発症すると劇症化しやすいため注意が必要とされる[16)17)]が肝機能は正常化した．

HBV再活性化の多くは血液悪性腫瘍にステロイドを含む抗癌剤やリツキシマブを使用した場合[1)2)]や自己免疫疾患にステロイドやリツキシマブを使用する[3)4)]など宿主免疫能が低下した場合に発症するとされている．症例1は肺アスペルギルス症に対し抗真菌剤と降圧剤を内服していた．症例2はパーキンソン病に対し抗パーキンソン病剤と前立腺肥大治療剤クムスロシンと過活動性膀胱治療剤ソリフェナシンが使用されていた．いずれの薬剤も免疫に影響するという報告は認められず薬剤によるHBV再活性化は考えにくかった．

症例1は肺アスペルギルス症の基礎疾患が存在した．肺アスペルギルス症は500/μl以下の好中球減少症や免疫抑制剤内服がハイリスク患者とされる[18)]が，いずれにも該当しない．肺アスペルギルス症が存在すること自体が何らかの免疫低下状態にあることを推測させる．症例2はパーキンソン病の基礎疾患が存在した．パーキンソン病に明らかな免疫異常の指摘はないが，無動や栄養摂取低下などにより免疫能低下状態に陥りやすいことは容易に推測される[19)]．また，軽快したもの原因不明の間質性肺炎を合併したことも何らかの免疫低下状態にあったことを推察させる．2症例ともにエンテカビル0.5mgを投与開始しHBV DNAは減少し肝機能は正常化しているので肝予備能低下にともなう免疫低下も考えにくい．また，HIV抗体は陰性，免疫低下の指標とされるCD4/8は正常であった．症例1は肺アスペルギルス症を合併した80歳，症例2はノドーキンソン病を合併した74歳という免疫能低下をきたしやすい基礎疾患を合併した高齢者である以外に，HBV再活性化を起こす誘因は不明であった．

今回我々は，免疫療法や化学療法といった明らか

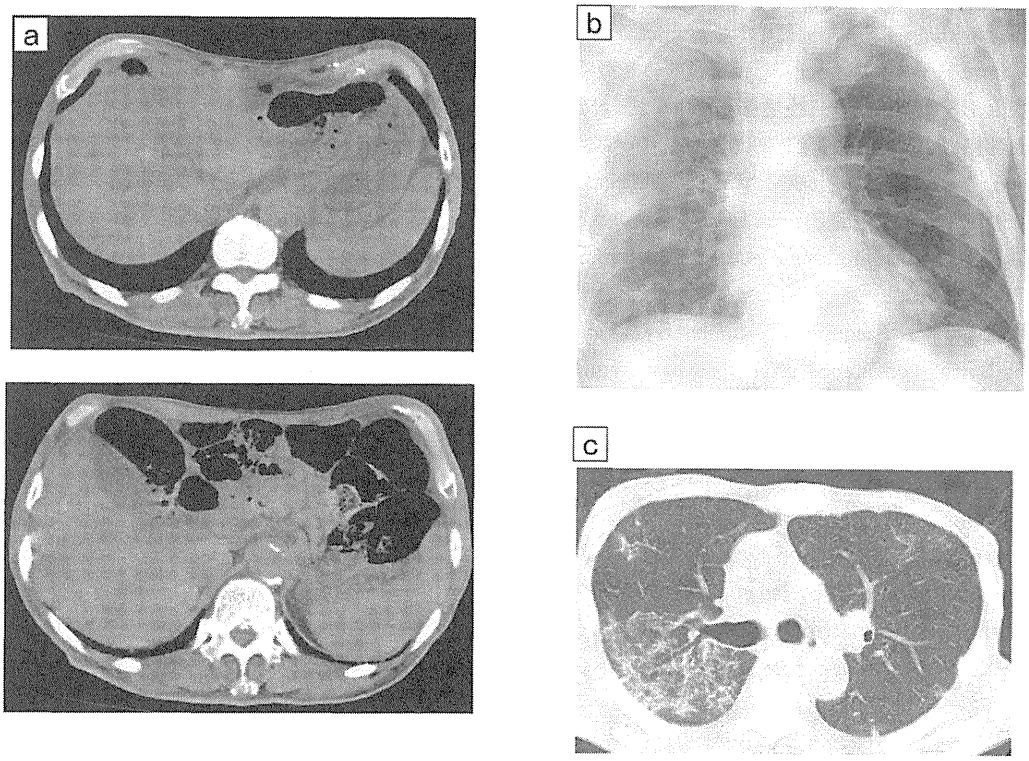


図4　症例2

a．2012/1/2X腹部CT　肝硬変・肝細胞癌の所見を認めない
b．2012/5/X胸部X線写真　右肺野にスリガラス様陰影を認める
c．2012/5/X胸部CT　右肺野に間質性肺炎像を認める

な原因が無いにも関わらずHBV再活性化を起こした疑いが強いと思われる高齢2症例を経験した．このような症例報告は過去に認められていない．日本では2600万人がHBVに感染したことかあると推測されている[20]．日本は高齢化が進行中であり，高齢化を基礎にしたHBV再活性化を起こすことがあるとすれば今後大きな問題になると思われ，同様の症例の積み重ねがきわめて重要と思われた．

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Re'activation of Hepatitis B Virus of Unknown Reason was Suspected in Two

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TuyoshiKuno, MisakiYoko, MasayukiSaitoh, YosimitsuRhuge,
TomoyukiTsuzuki, Masaaki Shimada and Hiroaki lwase

Abstract

Case l was 80-year-old female, treated with pulmonary aspergillosis. She was admitted the treatment of HBs antigen-positive hep atop athy. On October 16, 2011, laboratory test showed following : AST 193 U/l, ALT 180 U/L HBe antigen 1579 S/CO, HBe antibody 0, HBV DNA 7.9 log copies/ml, HBc antibody 10.18 S/CO, IgM-HBc antibody 0.76 S/CO and HBV genotype B. Case 2 was 74-year-old male, treated with Parkinson's disease. He was admitted f ; :) r the treatment of HBs antigen-positive hep atop athy. On January 20, 2012, laboratory test showed following : AST 134 U/l, ALT 188 U/ L HBe antigen 1.82 S/CO, HBe antibody 0, HBV DNA>9.0 log copies/ml, HBc antibody 9.38 S/CO, IgM-HBc antibody 0.19 S/CO and Genotype C. In both two cases HBc antibody and HBs antibody were not tested for the past years. But, we considered that HBV reactivation occurred and administered Entecavir. The reason of HBV reactivation was not unknown.

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ORIGINAL ARTICLE

Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease

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Keywords
fibrosis – micro RNA-122 – NAFLD – steatosis

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Abstract

Background & Aims: Non-alcoholic fatty liver disease (NAFLD) is believed to be a type of metabolic syndrome. MicroRNA-122 (miR-122) is the most abundant microRNA in the liver and is an important factor for the metabolism of glucose and lipids. In the present study, we examined the correlation between the hepatic and serum miR-122 expression levels and the clinicopathological factors of patients with NAFLD. *Methods:* We extracted the total RNA, along with preserved miRNAs, from liver biopsy samples of 67 patients with NAFLD. In 52 of these 67 patients, the total RNA was extracted from serum. The miR-122 that was obtained by quantitative reverse transcription-polymerase chain reaction was quantified using TaqMan MicroRNA assays. *Results:* A significant correlation was detected between serum and hepatic miR-122 expression (correlation coefficient, 0.461; $P = 0.005$). Patients with mild steatosis (<33%) showed significantly lower levels of hepatic miR-122 compared with patients with severe steatosis (>33%) (hepatic miR-122: mild/severe = 2.158 ± 1.786/4.836 ± 7.506, $P = 0.0473$; serum miR-122: mild/severe = 0.002 ± 0.005/0.007 ± 0.001, $P = 0.0491$). Moreover, hepatic and serum miR-122 levels were significantly higher in patients with mild fibrosis than in those with severe fibrosis (hepatic miR-122: mild/severe = 5.201 ± 7.275/2.394 ± 1.547, $P = 0.0087$; serum miR-122: mild/severe = 0.008 ± 0.011/0.002 ± 0.004, $P = 0.0191$). *Conclusions:* We found that the hepatic and serum miR-122 levels were associated with hepatic steatosis and fibrosis. The serum miR-122 level can be a useful predictive marker of liver fibrosis in patients with NAFLD.

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease worldwide (1–8). NAFLD is considered to represent the hepatic manifestation of metabolic syndrome. In Japan, an increase in the incidence of metabolic syndrome has led to an increase in the prevalence of NAFLD (5). NAFLD was traditionally considered as a relatively benign liver disease. However, some patients with NAFLD progress to liver fibrosis, cirrhosis and hepatocellular carcinoma (8–13). Therefore, the precise diagnosis and staging of NAFLD patients is clinically important. Liver biopsy is the gold standard for the evaluation of NAFLD patients in terms of staging. However, liver biopsy is an invasive technique, and the identification of non-invasive biomarkers is required.

Micro-RNAs (miRNAs) are endogenous, small, non-coding RNAs of approximately 21–22 nucleotides that have important gene regulatory functions in animals and plants. miRNAs bind to the messenger RNAs of protein coding genes to direct their post-transcriptional repression (14–16). miRNAs have been reported to play important roles in cell proliferation (17) and apoptosis (18), lymphocyte development (19), and adipocyte differentiation (20). Several recent studies have indicated that miRNAs play important roles in metabolism and metabolic diseases (21–23). MicroRNA-122 (miR-122) is the most abundant miRNA in the liver, and it regulates metabolic pathways, including cholesterol biosynthesis, fatty acid synthesis and oxidation (22, 23).

Recently, extracellular miRNAs were detected in serum, plasma and other body fluids. These circulating miRNAs have been reported to be predictive biomarkers for various cancers and in liver diseases (24, 25). However, the significance of miR-122 expression in the serum and liver of NAFLD patients has not been studied in detail.

In the present study, we analysed the relationship between the clinicopathological features and the expression of miR-122 in the serum and liver of NAFLD patients.

Patients and methods

Patient groups

In this study, we examined consecutive NAFLD patients who visited the Department of Gastroenterology and Hepatology at Nagasaki University Hospital. The patients who exhibited positive results for hepatitis B virus surface antigen or hepatitis C virus antibody, or those showing evidence of inherited, autoimmune, cholestatic or drug-induced liver disease were excluded using clinical, laboratory, imaging and histological criteria. In addition, patients with a history of current or past excessive alcohol intake, as defined by an average daily consumption of more than 20 g of alcohol, were excluded from the study.

Non-alcoholic fatty liver disease was diagnosed by percutaneous liver biopsy and ultrasonography. Liver biopsy specimens were fixed in 10% formalin, cut to a thickness of 4 μm and subjected to haematoxylin–eosin and Azan-Mallory staining. Steatosis was classified as mild (>30%) or severe (30%). Inflammation was scored on a scale of 0–9 according to the standards proposed by the Non-alcoholic Steatohepatitis Clinical Research Network (26). Fibrosis staging was performed using a five-grade scale as follows: F0, no fibrosis; F1, pericellular fibrosis in zone 3; F2, pericellular fibrosis in zone 3 with periportal fibrosis; F3, bridging fibrosis; and F4, cirrhosis defined as mild fibrosis (F0 or F1) and severe fibrosis (>F1).

miRNA extraction and quantification

RNA was extracted from a total of 67 liver biopsy specimens. Total RNA, including the miRNA, was isolated from formalin-fixed paraffin-embedded (FFPE) liver biopsy specimens using the Recover All Total Nucleic Acid Isolation Kit for FFPE (Ambion, Carlsbad, CA, USA) according to the manufacturer's protocol. In 52 of 67 patients, total RNA, along with preserved miRNAs, was extracted from 400 μL of serum using the Trizol reagent (Invitrogen, Carlsbad, CA, USA). Synthetic miR-39 was added to serum samples prior to RNA extraction as an internal control.

The miR-122 obtained by quantitative reverse transcription-polymerase chain reaction was quantified using TaqMan MicroRNA assays (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's protocol. miR-122 expression was calculated by the relative standard curve method and normalized to RNU6 expression in the liver and cell-miR39 expression in the serum.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Data were analysed by the Student's *t*-test for comparison of paired data. Correlations were analysed using the Spearman rank correlation coefficient. A P value of <0.05 was considered statistically significant.

Results

The characteristic of this study population are show in Table 1.

Correlation between hepatic and serum miR-122 expression and clinical factors

No significant correlations were observed between clinical factors and the expression of hepatic (Table 2) or serum (Table 3) miR-122. However, a significant correlation was observed between the serum and hepatic miR-122 expression levels (Fig. 1).

Correlation between hepatic miR-122 level and the pathological findings of NAFLD patients

Patients with mild steatosis (<33%) showed significantly lower levels of hepatic miR-122 than patients with severe steatosis (>33%) (mild/severe = 2.158 ± 1.786/4.836 ± 7.506; $P = 0.0473$). No significant correlation between serum miR-122 level and the NAFLD activity score (NAS) was observed. In contrast, hepatic miR-122 level showed a significant negative correlation with the fibrosis stage [correlation coefficient: −0.292 (−0.497 to −0.056); $P = 0.0161$] (Table 2). Moreover, hepatic miR-122 expression was significantly higher in patients with no or mild fibrosis than in those with severe fibrosis (mild/severe = 5.201 ± 7.275/2.394 ± 1.547; $P = 0.0087$) (Fig. 2).

Correlation between serum miR-122 level and the pathological findings of NAFLD patients

Patients with mild steatosis (<33%) showed significantly lower levels of serum miR-122 than patients with severe steatosis (>33%) (mild/severe = 0.002 ± 0.005/0.007 ± 0.001; $P = 0.0491$). No significant correlation was

Table 1. Clinical characteristics of liver samples (67 cases)

Patient age (years)	51.8 ± 17.4
Male:female	27:40
BMI	28.5 ± 4.2
Type 2 diabetes	46 cases
AST (IU/L)	71.7 ± 42.4
ALT (IU/L)	102.7 ± 64.1
ALP (IU/L)	286.3 ± 117.3
γ-GTP (IU/L)	103.6 ± 121.6
T-cho (mg/dl)	195.1 ± 45.4
TG (mg/dl)	144.7 ± 60.1
Plt (10^4/mm^3)	21.7 ± 7.4
FBS (mg/dl)	115.7 ± 41.4
HbA1c (%)	6.7 ± 2.0

γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, free blood sugar; HbA1c, glyco haemoglobin A1c; Plt, platelet; T-cho, total cholesterol; TG, triglyceride.

Table 2. Relation between hepatic microRNA-122 level and clinical factors

	Correlation coefficient	*P*-value
Age	0.025 (−0.216 to 0.264)	0.8385
BMI	−0.107 (−0.342 to 0.141)	0.3984
AST	−0.142 (−0.369 to 0.102)	0.2541
ALT	−0.042 (−0.279 to 0.201)	0.7390
ALP	−0.072 (−0.307 to 0.142)	0.5657
γ-GTP	−0.082 (−0.318 to 0.163)	0.5125
T-cho	0.054 (−0.199 to 0.300)	0.6785
TG	0.125 (−0.119 to 0.354)	0.3152
Plt	0.123 (−0.121 to 0.352)	0.3422
FBS	0.224 (−0.034 to 0.454)	0.0878
HbA1c	0.250 (−0.017 to 0.483)	0.0660
NAS	0.053 (−0.190 to 0.289)	0.6732
Fibrosis	−0.292 (−0.497 to −0.056)	0.0161

γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, free blood sugar; HbA1c, glyco haemoglobin A1c; NAS, NAFLD activity score; Plt, platelet; T-cho, total cholesterol; TG, triglyceride.

Table 3. Relation between serum microRNA-122 level and clinical factors

	Correlation coefficient	*P*-value
Age	−0.183 (−0.434 to 0.095)	0.1959
BMI	−0.042 (−0.314 to 0.236)	0.7708
AST (IU/L)	−0.049 (−0.317 to 0.386)	0.7340
ALT (IU/L)	0.126 (−0.152 to 0.136)	0.3750
ALP (IU/L)	−0.143 (−0.400 to 0.136)	0.3146
γ-GTP (IU/L)	−0.125 (−0.387 to 0.156)	0.3849
T-cho	0.089 (−0.194 to 0.358)	0.5420
TG	−0.061 (−0.329 to 0.215)	0.6667
Plt	−0.035 (−0.305 to 0.240)	0.8044
FBS	0.212 (−0.087 to 0.476)	0.1626
HbA1c	0.114 (−0.193 to 0.401)	0.4695
NAS	0.138 (−0.140 to 0.396)	0.3312
Fibrosis	−0.316 (−0.543 to 0.048)	0.0218

γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; FBS, free blood sugar; HbA1c, glyco haemoglobin A1c; NAS, NAFLD activity score; Plt, platelet; T-cho, total cholesterol; TG, triglyceride.

detected between serum miR-122 levels and the NAS. Serum miR-122 expression in the liver showed a significant inverse correlation with fibrosis stage [correlation coefficient: −0.316 (−0.543 to 0.048); $P = 0.0218$] (Table 3). Moreover, serum miR-122 levels were significantly higher in patients with mild fibrosis than in those with severe fibrosis (mild/severe = 0.008 ± 0.011/0.002 ± 0.004; $P = 0.0191$) (Fig. 3).

To compare the ability of the blood tests to predict the fibrotic stage, we constructed receiver operating characteristics (ROC) curves for serum miR-122, hyaluronic acid and type IV collagen; the area under the ROC curves for miR-122, hyaluronic acid and type IV collagen were 0.82, 0.74 and 0.72, respectively (Fig. 4).

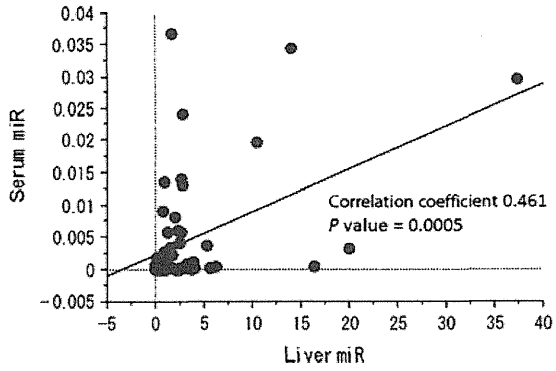

Fig. 1. Correlation between liver and serum miR-122 levels. The serum miR-122 levels were significantly correlated with hepatic miR-122 levels (Spearman correlation coefficient: 0.461; $P = 0.0005$).

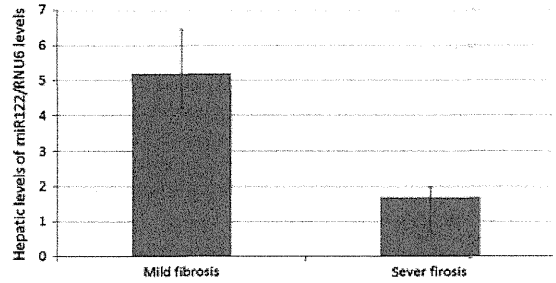

Fig. 2. Correlation between hepatic miR-122 level and the fibrosis stage. Comparisons between groups were performed using the Student's *t*-test ($P = 0.0087$).

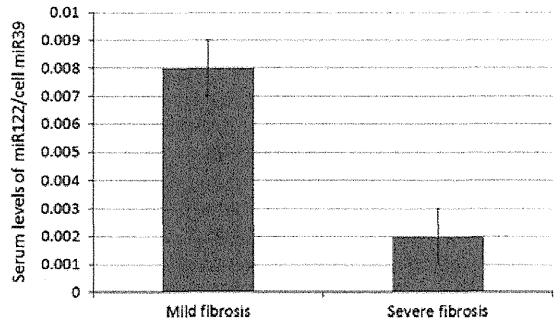

Fig. 3. Correlation between serum miR-122 level and the fibrosis stage. Comparisons between groups were performed using the Student's *t*-test ($P = 0.0191$).

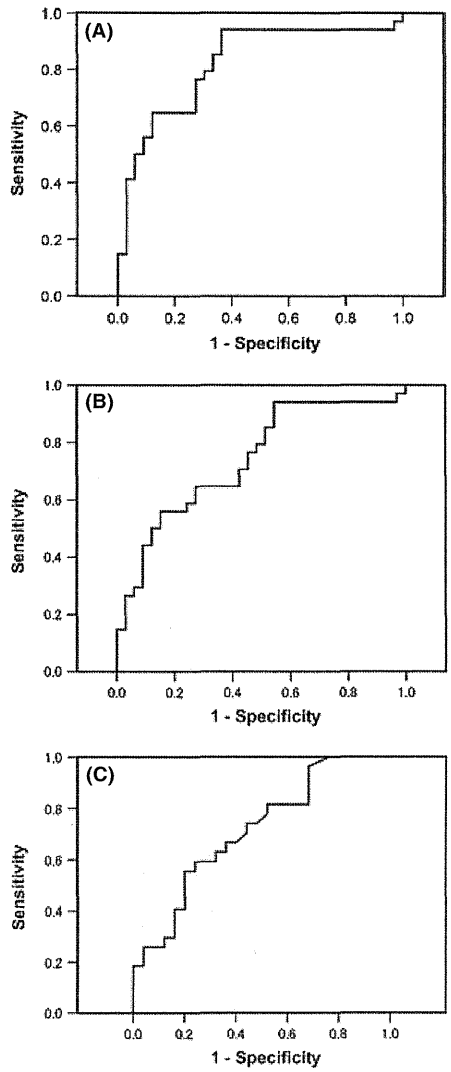

Fig. 4. Receiver operating characteristic (ROC) curve for serum miR-122, hyaluronic acid and Type IV collagen. The area under the ROC curve for serum miR-122 (A), hyaluronic acid (B) and type IV collagen (C) are 0.82, 0.74 and 0.72, respectively.

Discussion

Recent studies have indicated the value of the miR-122 level as a predictive factor of liver disease (27–30). The progression of NAFLD is associated with visceral fat deposition and insulin resistance. miR-122 is a key factor of lipid metabolism (23, 24). In the present study, patients with severe fat deposition showed high miR-122 expression levels in the liver. The role of miR-122 in lipid metabolism has been demonstrated in vitro and in vivo. In in vitro studies using HEP G2 cells, silencing of miR-122 led to the upregulation of the expression of lipid metabolism genes such as fatty acid synthase (FAS), 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and sterol binding element binding protein (SREBP), whereas overexpression of miR-122 led to a significant decrease in the levels of these genes (31). In *in vivo* studies, inhibition of miR-122 expression in mice led to the promotion of hepatic fatty acid (FA) oxidation, decreased FA levels, and decreased liver steatosis (23). Thus, these results support our finding that the expression of miR-122 is correlated with liver steatosis.

However, the liver and serum miR-122 levels did not correlate with the NAS and alanine aminotransferase levels. Several recent studies showed that the miR-122 level is associated with liver inflammation (27–29), which was not observed in the present study. However, the previous studies included patients with other liver diseases such as viral hepatitis. In the present study, most of patients had mild inflammation, which may contribute to the lack of a significant difference in miR-122 expression. Moreover, the NAS—established as a scoring system for NAFLD—evaluates not only inflammation but also steatosis. Thus, this discrepancy could be attributed to the different categories of liver disease included in each study.

In the present study, liver miR-122 levels significantly correlated with the liver fibrosis stage. This result is in agreement with those of previous studies, which reported a decrease in liver miR-122 levels at the later stage of fibrosis in patients with liver disease (27–29). Persistent liver injury results in liver cell death, loss of hepatic cells and the accumulation of extracellular matrix. Moreover, the liver miR-122 levels did not correlate with the NAS, which was reflected the inflammation grade of the NAFLD patients. However, hepatocytes are the main source of miR-122. Thus, the progression of liver fibrosis results in the replacement of hepatocytes by extracellular matrix, and thus leads to a decrease in the levels of hepatic miR-122.

Recently, Li *et al.* reported that miR-122 suppressed collagen maturation in hepatic stellate cells and inhibited the proliferation of activated hepatic stellate cells (32). Therefore, decreased miR-122 expression appears to lead to increased collagen maturation and extracellular matrix production, which is consistent with the present results.

In the present study, decreased serum miR-122 levels were detected in association with mild steatosis and advanced fibrosis stage. These results are similar to those noted for hepatic miR-122 expression. Moreover, serum miR-122 expression was well-correlated with hepatic miR-122 expression, which suggests that the miR-122 released from hepatic cells enters into the bloodstream.

The evaluation of liver fibrosis is important to predict the prognosis of patients with NAFLD. Follow-up liver biopsies or repeat liver stiffness assessment is currently necessary to assess liver fibrosis. However, these methods have some limitations. Liver biopsy is an invasive technique and is associated with certain complications

(33, 34). In addition, the utility of liver stiffness measurement is low in obese patients and in those with ascites and hepatic inflammation (35, 36). In the present study, serum miR-122 levels inversely correlated with liver fibrosis, and decreased miR-122 expression was associated with advanced fibrosis stage. Moreover, the ROC curves showed that the ability of the serum miR-122 to predict fibrosis was superior to that of hyaluronic acid and type IV collagen. Therefore, serum miR-122 may be a valuable tool to predict liver fibrosis.

In conclusion, hepatic and serum miR-122 levels are associated with hepatic steatosis and fibrosis, and the serum miR-122 level can serve as a useful predictive marker of liver fibrosis in patients with NAFLD.

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Frequency of elevated biomarkers in patients with cryptogenic hepatocellular carcinoma

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Background: The incidence of hepatocellular carcinoma (HCC) continues to increase in Japan, but the clinical characteristics of Japanese patients with HCC have not been well described. The aim of this study was to determine the frequencies and utilities of elevated α-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) levels as biomarkers in cryptogenic HCC.

Material/Methods: A total of 2638 patients with HCC diagnosed between 1999 and 2010 in the Nagasaki Association Study of Liver (NASLD) were recruited for this study. The cause of HCC was categorized into 4 groups; HCC-B, HCC-C, HCC-BC, and HCC-nonBC. The significance of factors was examined for HCC-nonBC using logistic regression analysis in all patients.

Results: Multivariate analysis identified age, sex, BMI, alcohol consumption, platelet count, AST, ALT, AFP, DCP, and TNM stage as independent and significant risk factors for HCC-nonBC. According to TNM stage, the median AFP levels in HCC-nonBC with TNM stages I, II, and III were significantly lower than in either HCC-B or HCC-C. In TNM stage IV, the median AFP level in HCC-nonBC was significantly lower than in either HCC-B or HCC-BC. The median DCP levels in HCC-nonBC with TNM stages I and II were significantly higher than those in either HCC-B or HCC-C. In TNM stage III, the median DCP level in HCC-nonBC was significantly higher than that in HCC-C.

Conclusions: DCP was more sensitive than AFP for the diagnosis of early stage cryptogenic HCC. DCP should be used as the main serum test for cryptogenic HCC detection.

Key words: HCC • DCP • AFP

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Background

Primary liver cancer accounts for approximately 6% of all human malignancies. It is estimated that half a million cases occur worldwide annually, 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 in recent decades in Japan [8]. Similarly, a rising HCC trend has been reported in 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. Of the hepatitis viruses that cause HCC, HCV is predominant in Japan [11–14]. However, it has been reported that the absolute numbers and proportion of HBsAg and HCVab negative HCC (HCC-nonBC) have both been steadily increasing in Japan [15,16].

The prognosis for patients with HCC is still poor. Surgical resection and liver transplantation are the standard curative treatments available. Recently, radio-frequency ablation (RFA) and percutaneous ethanol injection (PEI) have also been recognized as effective methods of achieving complete tumor necrosis for small HCCs [17]. However, the chance of curative treatment is often limited by several features of HCC itself. HCCs are usually large before they produce symptoms. Bilobar or multifocal tumors are common. The incidence of associated cirrhosis is also high, exceeding 80% in most series [18–20]. Transcatheter intra-arterial chemoembolization (TACE), with which complete necrosis of HCCs is thought to be difficult to achieve, is also impacted by the above factors [21]. To increase opportunities for meaningful intervention and to improve survival, early detection of HCC by measuring alpha-fetoprotein (AFP) and/or imaging screening is implemented in many countries [15,22–25]. However, the poorer prognosis of patients with HCC-nonBC is reportedly attributable to its late detection in an advanced stage, owing to the lack of a surveillance system for early detection of HCC [26].

In this retrospective cohort study, our aim was to characterize consecutive patients who had been diagnosed with HCC-nonBC during an 11-year period (1999–2010) at the centers comprising the Nagasaki Association Study of Liver Disease (NASLD) group. We evaluated the clinical characteristics of patients with HCC-nonBC, their tumor stages, treatment, AFP and DPC as potential biomarkers, and survival.

Material and Methods

Patients

In total, 2638 patients with HCC diagnosed between 1999 and 2010 in the NASLD were recruited for this study. The diagnosis of HCC was based on AFP and/or DCP levels, as well as the 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/or DCP (≥40 ng/mL), and neovascularization on HAG, CT, and/or MRI.

The diagnosis of chronic HCV infection was positive for both anti-HCV, by a third-generation enzyme-linked immunosorbent assay (ELISA), and for HCV RNA by polymerase chain reaction (PCR). The diagnosis of chronic HBV infection was based on the presence of HBsAg (enzyme-linked immunosorbent assay; Abbott Laboratories); serum AFP was measured by radioimmunoassay (Abbott Laboratories). The history of alcohol intake was obtained 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.

HCC etiologies were categorized into 4 groups: (1) HCC-B, HBsAg positive, and HCVAb negative; (2) HCC-C, HCVAb positive, and HBsAg negative; (3) HCC-BC, both HBsAg and HCVAb positive; and (4) HCC-nonBC, both HbsAg, and HCVAb negative. The significance of age, sex, body mass index (BMI), alcohol intake, diabetes mellitus, underlying liver disease Child-Pugh score, platelet count, prothrombin time (PT), albumin (ALB), total bilirubin (Bil), aspartate aminotransferase (AST), alanine aminotransferase (ALT), AFP, DCP, and Tumor-Node-Metastasis (TNM) stage were examined to identify possible relationships with HCC-nonBC using logistic regression analysis.

Treatment modalities

Patients diagnosed with HCC were assessed for surgery based on the extent of lobar involvement and liver function status. The extent of lobar involvement was evaluated based on a combination of USG, CT, MRI, and HAG findings. Patients were considered to be poor candidates for resection if they met any of the following criteria: (1) bilobar involvement, (2) evidence of tumor infiltration into the main portal vein or thrombosis of the vein, (3) evidence of extrahepatic metastases, (4) Child's grade C cirrhosis, or (5) poor cardiac and/or respiratory status. If surgery was contraindicated or the patient refused to undergo an operation, RFA or PEI therapy was the second treatment choice, offered to those with HCCs less than 3 cm in diameter. The remaining patients without main portal vein thrombosis or extrahepatic metastasis were advised to undergo TACE, regardless of tumor size or number.

After initial treatment, the AFP levels and liver functions 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

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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. Furthermore, none of the subjects in our study received either of these treatments for recurrent HCC during the follow-up period.

Statistical analysis

The survival duration was the time from the diagnosis of HCC until the time of death or the time of preparation of this manuscript. The survival rate was analyzed using the Kaplan-Meier method, and differences between the survival probability curves were tested using the log-rank test. Descriptive summaries of study groups are reported as the median value (SD: standard deviation) and number (%). Data were analyzed using the Mann-Whitney *U* test for continuous ordinal data, and the chi-square test with Yates' correction and Fisher's exact test were used for intergroup comparisons to determine the association between 2 qualitative variables. *P* values <0.05 were considered to indicate a statistically significant difference. Variables achieving statistical significance according to univariate analysis were subsequently included in the multivariate analysis using a logistic regression model and were described as hazard ratios (HR) with 95% confidence intervals (CI). Coefficients were calculated from the linear discriminating function of the variables. Data analysis was performed using SPSS version 16.0 for Windows.

Results

Patient characteristics at enrollment

We diagnosed 2638 patients with HCC during the study period. Patient characteristics at the time of HCC diagnosis are presented in Table 1. The underlying causes of HCC were as follows: 474 (18%) patients were positive for HBsAg, 1533 (58%) were positive for HCVAb, 40 (2%) were positive for both HBsAg and HCVAb, and 591 (22%) were negative for HBsAg and anti-HCV.

Overall, the median survival of all 2638 patients was 1.8 years. The cumulative 5-year survival rates of the patients with HCC-B, HCC-C, HCC-BC, and HCC-nonBC were 43%, 52%, 49%, and 47%, respectively (Figure 1). Patients in the HCC-C group had a higher cumulative survival rate than those in the HCC-B and HCC-nonBC groups.

Univariate and multivariate analyses of the factors associated with HCC-nonBC

Univariate and multivariate analyses were performed to identify factors independently related to HCC-nonBC. In the univariate analysis, the following 13 factors significantly influenced HCC-nonBC: age, sex, BMI, alcohol consumption, diabetes mellitus, underlying liver disease, platelet count, Bil, AST, ALT, AFP, DCP, and TNM stage (Table 2). Multivariate analysis identified age (≥70 years, HR 1.63), sex (female, HR 1.73), BMI (≥25, HR 2.12), alcohol consumption (not excessive, HR 3.41; excessive, HR 14.73), diabetes mellitus (HR 2.42), underlying liver disease (chronic hepatitis, HR 0.46; cirrhosis, HR 0.52), platelet count (<116,000/μL, HR 1.88), AST (<56 IU/L, HR 1.47), ALT (<46 IU/L, HR 2.48), AFP (20–199 ng/mL, HR 0.60; ≥200 ng/mL, HR 0.63), DCP (20–199 mAU/mL, HR 1.64; ≥200 mAU/mL, HR 2.08), and TNM stage (II, HR1.67; III, HR1.88; IV, HR 2.40), as independent and significant factors associated with HCC-nonBC (Table 3).

Comparison of biomarkers according to liver disease cause

The positive rate of AFP (≥20 ng/ml) in HCC-B, HCC-C, HCC-BC, and HCC-nonBC were 62%, 55%, 61%, and 44%, respectively; whereas the positive rate of DCP (≥40 mAU/ml) were 67%, 55%, 75% and 77%, respectively (Figure 2). The positive rate of AFP in HCC-nonBC was significantly lower than those in the HCC-B and HCC-C groups, whereas the positive rate of DCP was significantly higher than that in the HCC-B and HCC-C groups.

The median AFP and DCP levels in HCC-B, HCC-C, HCC-BC, and HCC-nonBC according to TNM stage are presented in Table 4. The median AFP levels in HCC-B, HCC-C, HCC-BC, and HCC-nonBC were 60 ng/mL, 25 ng/mL, 29 ng/mL, and 13 ng/mL, respectively; whereas the DCP levels were 4990 mAU/mL, 418 mAU/mL, 612 mAU/mL, and 3077 mAU/mL, respectively. The median AFP level in HCC-nonBC was significantly lower than those in the other groups; whereas the median DCP level was significantly higher than that in the HCC-C group.

According to TNM stage, the median AFP levels in HCC-nonBC with TNM stages I, II, and III were significantly lower than that in either HCC-B or HCC-C. In TNM stage IV, the median AFP level in HCC-nonBC was significantly lower than that in either HCC-B or HCC-BC. The median DCP levels in HCC-nonBC with TNM stages I and II were significantly higher than that in either HCC-B or HCC-C. In TNM stage III, the median DCP level in HCC-nonBC was significantly higher than that in HCC-C. However, there were no significant differences in the median DCP level among TNM stage IV cases.

The survival rate of patients in the high DCP group (≥200 mAU/mL) was significantly lower than that of patients classified into the low DCP (40–199 mAU/mL) and DCP negative (<40 mAU/mL) groups among those with HCC-B, HCC-C, and HCC-nonBC ($p \leq 0.001$; log-rank test) (Figure 3).

Discussion

The age-adjusted mortality rate for HCC has increased over the past few decades in Japan [27]. However, most patients are

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Table 1. Characteristics of 2,638 HCC patients.

	(SD)	(%)		(SD)	(%)
All	2,638		Treatment		
Age (years)	70.0	10.3	Surgical resection	391	15
Sex			PEIT or RFA	740	28
Male	1,786	68	TACE or TAI	1,221	46
Female	852	32	Chemotherapy	51	2
Alcohol consumption (unknown 454)			Transplantation	10	1
Excessive	236	9	Only palliative care	225	9
Not excessive	511	13	Platelets (104/mL)	11.6	8.0
None	1,437	54	AST (IU/L)	56	101
DM (unknown 326)			ALT (IU/L)	45	402
(+)	651	25	Bil (mg/dL)	0.9	0.5
(–)	1,661	63	Alb (g/dL)	3.7	0.6
BMI (unknown 594)	22.7	0.5	PT (%)	83	18
Etiology of liver disease			AFP (ng/mL)	25	89,580
HBV	474	18	<20	1,209	46
HCV	1,533	58	20–199	761	29
HBV+HCV	40	2	≥200	668	25
NBNC	591	22	DCP (mAU/mL)	89	75,076
Underlying liver disease (unknown, 110)			<40	991	38
Normal	42	2	40–199	583	22
Chronic hepatitis	686	26	≥200	1,064	40
Cirrhosis	1,800	68	Observation period (years)	1.8	2.3
Child-Pugh Grade (unknown, 88)					
A	1796	68			
B	606	23			
C	148	6			
TNM stage					
I	682	26			
II	1,039	39			
III	579	22			
IV	338	12			

Alcohol consumption: excessive; average daily consumption of an amount equivalent to 80 g of pure ethanol for a period of more than 10 years. Not excessive; Alcohol consumption: excessive; average daily consumption of an amount equivalent to 1–79 g of pure ethanol for a period of more than 10 years. Data are presented as median value (SD: standard deviation) or frequency (%).

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