ると、壊死、炎症を表すgrading、線維化を表すstagingはともに、1年の投与で有意(それぞれp<0.0001, p<0.01)に改善した。変異株検出率(全対象)は投与1年後7.2%、1.5年後23.3%、2年後38.2%と、2年後に高率に出現した。HBe抗原陽性例と陰性例の比較では、投与1年後、1.5年後および2年後の変異株出現率は、HBe抗原陽性例でそれぞれ6.7%、36.7%および53.3%、HBe抗原陰性例でそれぞれ7.9%、10.0%および20.0%と、HBe抗原陽性例で有意(1.5年後、2年後それぞれp<0.05)に高率であった。

■エンテカビル

エンテカビルはラミブジンと同様に核酸アナログ剤で、2006年9月に保険適用となった。核酸アナログ未治療例には0.5mg/day、ラミブジン不応例には1mg/dayが常用量とされ、吸収率の点より空腹時に服用することが必要とされている。

国内治験(3試験)の報告[9](図2、3)によると、用量相関試験(24週)では、エンテカビル0.5mg投与群はラミブジン100mg投与群に比しHBV DNA変化量(log copies/mL)が有意に大(−5.16 vs −4.29)であった。未治療例対象試験では、エンテカビル0.5mg群において48週投与でHBV DNA平均変化量は−4.84、ALT正常化率は93.8%、HBe抗原陰性化率は29.6%であり、組織学的にも、壊死・炎症、線維化ともに有意な改善が認められた。また、ラミブジン不応例に対する1mg投与群では、HBV DNA平均変化量は−3.75、ALT正常化率は78.4%、HBe抗原陰性化率は15.2%であった。有害事象はほとんどが軽微で一過性であった。

海外では、Changら[10]が、核酸アナログ未治療のHBe抗原陽性例に対するラミブジンとのrandomised double-blind trial(二重盲検化試験)において、ラミブジンに比し組織学的改善度(72% vs 62%; p<0.01)、HBV DNA陰性化率(67% vs 36%; p<0.001)(図4)およびALT正常化率(68% vs 60%; p<0.05)が有意に良好であったことを報告している(48週)。また、Shermanら[11]はラミブジン不応のHBe抗原陽性例に対するラミブジンとの二重盲検化試験(エンテカビルは1mg/day)においても同様の報告(組織学的改善度、HBV DNA陰性化率およびALT正常化率いずれもp<0.0001 vsラミブジン)をしている。

エンテカビル変異株に関しては、2006年の米国肝臓学会での発表によると、核酸アナログ未治療例では48週服用後0.1%、ラミブジン抵抗例では1%にエンテカビル遺伝子型耐性を伴うウイルス量のリバウンドがみられている。

■アデホビル

アデホビル単独治療は、現在治験中で保険適用にはなっていない。しかし、欧米ではHBe抗原陽性例に対する有効性が報告されている。Marcellinら[12]はアデホビル10mg、48週投与で組織学的改善、HBV DNA、ALT値の低下およびHBe抗原抗体seroconversion率の上昇を、また、3年(144週)投与[13]にて53%でHBe抗原消失、46%でHBe抗原抗体seroconversion、48%でHBV DNA陰性化を認めている。アデホビル変異株(N236T、A181V/T)検出率についてHadziyannisら[14]は、4〜5年投与例において、2年3%、3年11%、4年18%および5年29%と報告している。アデホビル変異株にはラミブジンやエンテカビルが有効と考えられている[15]。ラミブジン、アデホビル併用治療無効例も存在するが、それらに対するテノホビルの有効性が報告[16]された。テノホビルは現在、HIV感染症に承認されている薬剤であるが、B型慢性肝炎にも緊急避難的には使用を考慮すべきと考える。

図2 エンテカビル3用量およびラミブジン100mgによる用量相関試験におけるHBV DNA変化量

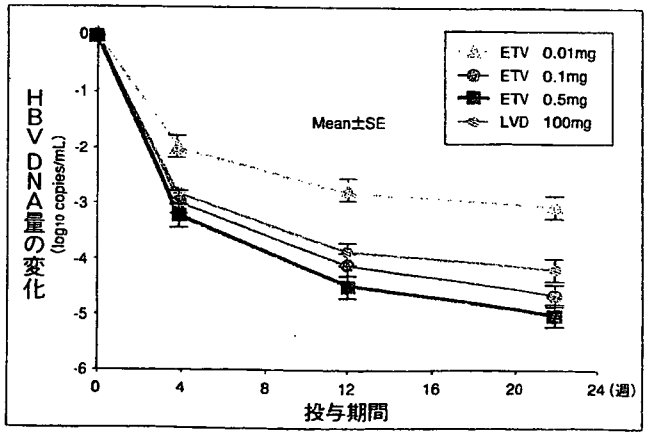


図3　未治療例およびラミブジン不応例に対するエンテカビルに投与によるHBV DNA変化量

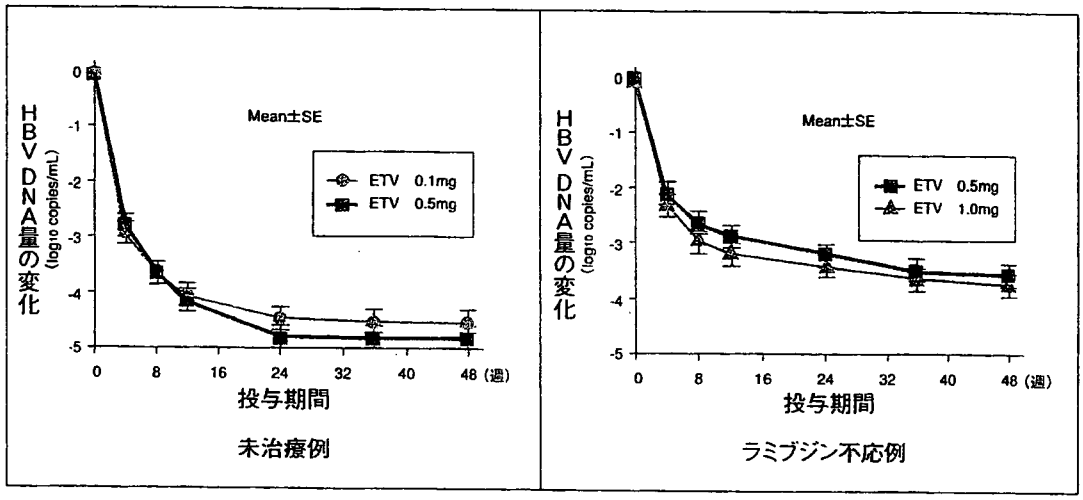
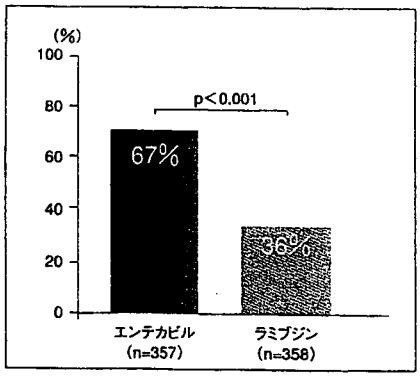


図4　HBe抗原陽性例に対する
エンテカビル、ラミブジン比較試験に
おけるHBV DNA陰性化率



おわりに

これまでIFN、ラミブジンおよびアデホビルが保険適用剤であったが、2006年9月よりエンテカビルが保険適用となり、B型慢性肝炎に対する抗ウイルス治療も新しい局面を迎えた。B型はC型に比し治療対象の選択がより重要で、それぞれの対象に対する適切な治療方法の選択と的確な治療の遂行が肝不全や発癌を防止し、予後の改善に寄与すると考えられる。

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＜原　著＞

肝細胞癌の年代別発生傾向

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要旨：1986から2005年までに当院で経験した初発肝細胞癌655例を対象として成因，発癌年齢，背景肝組織の推移を1期（1986-90年），2期（1991-95年），3期（1996-2000年），4期（2001-05年）の各時期に分けて検討した．成因別ではB型が減り，C型はやや増加，アルコール，原因不明は横ばいである．発癌年齢は全体で年々高齢化し，B型はほぼ同じだがC型，アルコール，原因不明は高齢化している．女性の占める割合は徐々に増えている．非癌部の組織では慢性肝炎の占める割合が年々高くなっている．糖尿病は発癌年齢を有意に低め，高血圧は有意に高める．以上より従来の肝発癌の危険因子が変化し，ますます高齢化して慢性肝炎と女性の占める率が高くなり発癌に高齢化という因子が強く関わるようになってきたと推測された．

索引用語：　肝細胞癌　HBV　HCV　慢性肝炎　疫学

はじめに

わが国の肝細胞癌（HCC）の原因の大部分はC型肝炎ウイルス（HCV），B型肝炎ウイルス（HBV）の持続感染によるものであり，その発生は依然として増加傾向にある[1)~9)]．また最近，非B非Cの肝細胞癌も増え糖尿病，肥満などの生活習慣病との関連が示唆されている[10)]．これまで発癌は肝炎ウイルスの持続感染による慢性炎症もしくは慢性炎症の結果としての肝硬変の存在が重要な働きをしていると考えられていたが[11)]最近，肝疾患患者全体の高齢化に伴い発癌の様相にも変化が生じているのではないかと印象をもったので今回，年次推移の特徴を検討した．

対象と方法

1986～2005年に当院で経験した初発HCC655例を対象として成因，発癌年齢，背景肝組織の推移を1期（1986～90年，152例），2期（1991～95年，143例），3期（1996～2000年，169例），4期（2001～05年，191例）の各時期に分けて検討した．HBs抗原陽性をB型，HCV抗体陽性をC型，日本酒換算一日平均3合10年以上の飲酒歴を有しHBs抗原，HCV抗体陰性をAL，その他をUNとした．背景肝組織はHCC診断前5年以内の生検またはHCC診断後5年以内の剖検により317例が検討可能であった．高血圧，糖尿病の診断はそれぞれの治療薬を内服している例とした．

統計解析：すべてのデータはmean±SDで表した．比率の比較はχ^2-testで，その他の群間の比較はWilcoxon signed-rank testで行い$p<0.05$を統計学的有意とした．

結　果

(1) 成因別頻度の推移（Table 1）

B型は年々減少し，C型は増加している．AL，UNは横ばいである．

(2) 成因別発癌年齢の推移（Table 2）

B型の発癌年齢はほぼ同じだがC型，UN型は高齢化している．

(3) 男女別発癌年齢の推移（Table 3）

男女とも発癌は年々高齢化している．

(4) B型，C型肝癌の年齢分布の推移（Table 4）

B型肝癌では年齢分布はほぼ横ばいで50～60歳代が多い．C型肝癌は高齢化し71歳以上が増加している．

(5) 男女比の推移（Table 5）

男女比は4.8，3.0，2.4，1.4と年々女性の占める割合が高くなっている．

(6) 背景肝組織（Table 6，7）

肝癌診断前5年以内の生検または肝癌診断後5年以内の剖検により背景肝組織を確認できた317例を検討した．

①背景肝組織が慢性肝炎の割合は20.3%，23%，33%，33.3%と年々増え，しかも男女比は6.5，4.7，4.3，2

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Table 1 Distributions of etiologic factors in each term

	B (%)	C (%)	AL (%)	UN (%)
1st term	26.3	48.7	6.6	2.6
2nd term	12.6	76.9	4.2	2.1
3rd term	10.1	75.7	7.7	1.2
4th term	8.4†	82.7†	5.2	2.6

†$p < 0.01$, compared to 1st term

Abbreviations: B, HBV; C, HCV; AL, alcohol; UN, unknown

Table 2 Changes of the average age of hepatocelullar carcinoma development in each etiologic factor

	B	C	AL	UN
1st term	59±10	63±8	60±5	69±6
2nd term	64±10	67±8	55±1	57±5
3rd term	57±8	69±7	65±4	69±8
4th term	58±10	71±8*	68±9	72±4§

age: yrs.±SD

*$p < 0.001$, compared to 1st term, 2nd term, 3rd term

§$p < 0.05$, compared to 2nd term

Table 3 Changes of the average age of hepatocelullar carcinoma development in male and female

	all cases	male	female	p
1st term	62±9	63±8	60±10	N.P
2nd term	65±9	64±8	68±8†	< 0.05
3rd term	67±8	65±8	69±6	< 0.01
4th term	69±9*	68±9*§	72±8*	< 0.01

age: yrs. ±SD

*$p < 0.001$, compared to 1st term, 2nd term, 3rd term in all cases, to 1st term, 2nd term in male, to 1st term in female

§$p < 0.05$, compared to 3rd term

†$p < 0.01$, compared to 3rd term, 4th term

Table 4 Changes of age distribution in cases with HBV and HCV related HCC in each term

	< 41y		41-50y		51-60y		61-70y		71y <	
	B (%)	C (%)	B (%)	C (%)	B (%)	C (%)	B (%)	C (%)	B (%)	C (%)
1st term	1 (2.5)	0	6 (15)	2 (2.6)	12 (30)	30 (39.5)*. **	16 (40)	27 (35.5)*	5 (12.5)	17 (22.3)
2nd term	1 (5.6)	1 (0.1)	0	3 (2.6)	3 (16.7)	24 (20.9)§	9 (50)	61 (53)**	5 (27.8)	26 (22.6)
3rd term	1 (6.3)	0	2 (12.5)	1 (0.1)	6 (37.5)	21 (15.8)	4 (12.5)	71 (53.4)§	3 (18.8)	40 (30)†
4th term	1 (5.9)	0	2 (11.8)	5 (2.7)	5 (29.4)	16 (8.6)	7 (41.2)	62 (33.3)	2 (11.8)	103 (55.3)**

C: *$p < 0.001$, **$p < 0.0001$, §$p < 0.05$, compared to 4th term in 51-60y. *$p < 0.001$, compared to 2nd, 3rd term, **$p < 0.0001$, §$p < 0.05$, compared to 4th term, in 61-70y. **$p < 0.001$, compared to 1st term, 2nd term, †$p < 0.01$, compared to 4th term in 71y <

Table 5 Changes of gender distribution of hepatocelullar carcinoma development in each term

	male	female	male/female
1st term	126	26	4.8*
2nd term	107	36	3.0†
3rd term	119	50	2.4§
4th term	113	78	1.4

*$p < 0.001$, †$p < 0.01$, §$p < 0.05$, compared to 4th term in male/female

と年々女性の割合が多くなっているが有意差はなかった．背景肝組織が慢性肝炎からの発癌も年々高齢化している．

②組織診断＋臨床的慢性肝炎

上記の組織を確認できたのは655例中317例と全体の過半数にも満たないので症例にbiasがかかっている可能性がありICG20%以下，PLT15万以上，PT>90%，食道静脈瘤F0のうち3項目以上を有する例を臨床的に慢性肝炎とした．組織診断に臨床的診断を加えた619

Table 6 Changes of frequency and gender distribution in cases with histologically chronic hepatitis in the noncarcinomatous part of the liver

	CH/all cases	male/female (ratio)	age
1st term	15/74 (20.3%)	13/2 (6.5)	62.5±9.4**
2nd term	17/74 (23%)	14/3 (4.7)	64.0±9.7*
3rd term	32/97 (33%)	26/6 (4.3)	68.7±8.9§
4th term	24/72 (33.3%)	16/8 (2)	68.8±8.7

CH, chronic hepatitis

**p < 0.0001, *p < 0.001, §p < 0.05, compared to 4th term

Table 7 Changes of frequency and gender distribution in cases with histologically and clinically chronic hepatitis

	CH/all cases	male/female (ratio)	age
1st term	27/142 (19%)	24/3 (8)§	61±10*
2nd term	28/132 (21.2%)	22/6 (3.7)	65±9†
3rd term	41/159 (25.7%)	31/10 (3.1)	68±8
4th term	53/186 (28.5%)	37/16 (2.3)	69±8

CH, chronic hepatitis,

§p < 0.05, compared to 4th term

*p < 0.001, †p < 0.01, compared to 4th term

例で検討した結果は組織的診断例のみと同じで慢性肝炎の占める割合は19%，21.2%，25.7%，28.5%と年々高くなっているが有意差はなかった．男女比は8，3.7，3.1，2.3と年々女性の占める割合が多くなっている．発癌年齢も年々高齢化している．

(7) 糖尿病合併の有無別発癌年齢の比較（Table 8）

①全例の比較：糖尿病合併106例の平均年齢は64±8歳で糖尿病を合併しない549例の平均年齢66±9歳より有意に発癌年齢が低かった．

②各時期別に比較すると3期のみ有意差が認められた（糖尿病合併有り64±7歳　vs.　無し67±8歳）．

(8) 高血圧合併の有無別発癌年齢の比較（Table 9）

①全例の比較：高血圧合併100例の平均年齢は69±7歳で合併しない555例の平均年齢66±9歳より有意に発癌年齢が高かった．

②各時期別に比較するといずれの時期でも高血圧合併例のほうが発癌年齢は高かったが有意差はみられなかった．

(9) UN群について

UN群14例の特徴をまとめると年齢は50～78歳（平均67±8歳，男7例女7例），輸血歴は1例に認めた．7例がDM，高血圧，高脂血症のいずれかを合併（DM 5例，高血圧5例，高脂血1例）し，6例では2つを合併症していた．BMI15～32.2（平均28±9），その分布は18.5以下の低体重が1例，18.5～25の普通体重が4例，25～30のやや肥満が5例，30～35の軽度肥満が1例でUN群全体では明らかな肥満傾向は認めない．背景肝は慢性肝炎4例，肝硬変8例，正常肝1例．肝癌発見のきっかけは肝機能異常の経過観察中7例，他疾患で通院中4例，食思不振，黒色便，黄疸，腹部膨満感などの自覚症状で紹介が3例である．

考　察

HCCの発癌リスクはこれまでいくつか報告されていてC型に関しては肝硬変，男性，高齢者，アルコール摂取量が1日50g以上，感染時年齢が高齢，HIVとの重複感染，糖尿病ないし肥満の合併，HCV1b型，AFP高値が[12)～15)]，HBV関連肝硬変では診断時年齢，ICGが独立した因子である[15)]．いずれも肝硬変が共通した発癌のリスクである．最近，発癌年齢の高齢化とともに慢性肝炎や肝機能が軽度異常例から発癌する例が増えている印象をもったので今回，他の要因も合わせて発

Table 8 Comparison of the average age of hepatocelullar carcinoma development between the cases with and without diabetes mellitus in each term

	With diabetes mellitus		Without diabetes mellitus		*P*
	n (%)	age (yrs)	n (%)	age (yrs)	
Total	106 (16.2)	64±8	549 (83.8)	66±9	< 0.05
1st term	20 (13.2)	62±8	132 (86.8)	62±9	N.S
2nd term	24 (16.8)	62±7	119 (83.2)	65±9	N.S
3rd term	31 (18.3)	64±7	138 (81.7)	67±8	< 0.05
4th term	31 (16.2)	66±10	160 (83.4)	70±9	N.S

Abbreviation: DM, diabetes mellitus

Table 9 Comparison of the average age of hepatocelullar carcinoma development between the cases with and without hypertension

	With hypertension		Without hypertension		*P*
	n (%)	age (yrs)	n (%)	age (yrs)	
Total	100 (15.3)	69±7	555 (84.7)	66±9	< 0.01
1st term	13 (8.6)	66±6	139 (91.4)	62±9	N.S
2nd term	25 (17.5)	66±7	118 (82.5)	65±9	N.S
3rd term	27 (16.0)	68±7	142 (84.0)	67±8	N.S
4th term	35 (18.3)	71±5	156 (81.7)	70±9	N.S

Abbreviation: HT, hypertension

癌における年代別推移を検討した.

成因別頻度ではB型が減り，C型は増加，AL，UNは横ばいである.B型，C型では輸血のスクリーニングや使い捨て医療器具の普及により，またB型ではワクチンによる母子間感染予防により新たな感染者は激減しているが，C型では高齢化により慢性肝炎からの発癌が増えてきたためと考えられる.近年，UNが増加しているとの報告が多くみられるようになり，そのリスクとして肥満，高脂血症，糖尿病などのメタボリック症候群との関係が示唆されている[10].糖尿病それ自体，C型肝炎やアルコール性肝硬変のような慢性肝疾患がなくとも肝発癌のリスクを優位に高めるという報告がある[16].本研究のUN群14例の検討では年齢はCとほぼ同じでBやALに比べて高齢である.糖尿病，高血圧，高脂血症のいずれかの合併は半数のみでBMIもやや肥満が5例，軽度肥満が1例にすぎず，高齢以外に明らかな特徴は見出せなかった.

男女比は1期が4.8であったのが4期では1.4と女性の占める割合は年々高くなっている.肝発癌は男性の方が2～4倍リスクが高いことが報告されている[17]~[19].これは飲酒，タバコなどの発癌に関係するライフスタイルもあるが[20][21]，性ホルモンの差も関係している[22]~[25].にもかかわらず近年，女性の割合が増加しているのは女性患者の高齢化も関係していると思われる.

一般に高齢者は若年者に比べて免疫能の低下がみられ，癌細胞のapoptosisによる発癌抑制が低下するために発癌しやすいと推測されるが，HCCでも全体で発癌年齢は年々高齢化している.B型はほぼ同じだがC型，AL，UNが高齢化している.インターフェロン（IFN）投与による肝線維化の抑制やUDCAやSNMCなどの肝庇護剤によるALT値コントロールにより線維化の進行が抑制されたためなどの可能性が考えられるが，本研究のIFN投与例は1期が3例（2%），男1例女2例，2期が12例(8.4%)，男7例女5例，3期が34例(20.1%)，男24例女10例，4期が25例(13.1%)，男13例女12例と必ずしも多いわけではなく，しかも投与例は男性の方が多い.またAL，UNでも高齢化していることから，わが国の平均寿命が延びたことと関係しているかもしれない.

組織的に慢性肝炎例の占める割合は1期20.3%から4期33.3%へと高くなっている.日本肝癌研究会追跡調査報告書の統計でも86年から2年ごとの集計では切除

標本の非癌部が肝硬変の割合は53.5%，68.3%，65.8%，61.7%，53.3%，53.8%，53.8%，49%，44%と徐々に減少傾向にあることがわかる[1)~9)]．発癌の高齢化と合わせて考えると慢性肝炎例でも高齢という要素が加わって発癌しやすくなったものと推測される．HCV感染者では組織的に肝硬変でなくとも発癌することが報告されている[26)~28)]．

糖尿病と肝発癌の関係では，C型肝炎では糖尿病を有する例は有さない例よりも有意に発癌しやすく約1.5～4倍の相対危険率を有する[29)]．今回の検討では高血圧は発癌年齢を有意に上げていたが，これは内服している降圧剤の抗線維化作用によるものかもしれない．これまで高血圧と肝発癌の関係についての報告はみられない．今後，さらに追跡調査が必要である．

以上より，肝発癌の年次推移の特徴を検討した結果，発癌は年々高齢化しており，組織的にも肝硬変からの発癌は減少し慢性肝炎の状態から発癌する傾向が増えつつある．これはこれまでの発癌因子に高齢という要素が強く加わってきたものと推測される．今後もこの傾向は強まるものと思われ，高齢者では慢性肝炎や肝機能が良好でも発癌する可能性があり注意深い経過観察が必要である．

結　語

1986～2005年に当院で経験した初発HCC 655例を対象として5年ごとの4期に分けて検討した結果，

(1) 成因別ではB型が減り，C型は増加，AL，UNは横ばいである．

(2) 全体で発癌年齢は年々高齢化している．B型はほぼ同じだがC型，UNが高齢化している．

(3) 女性の占める割合は徐々に増えている．

(4) 慢性肝炎の占める割合は増加傾向にある．

(5) 糖尿病は発癌年齢を有意に低め，高血圧は有意に高めている．

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Chronological analysis for the hepatocellular carcinoma development

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We chronologically analyzed the hepatocellular carcinoma cases during 1885 to 2005 every five years, i.e. 1st. term (1986～90), 2nd. term (1991～95), 3rd. term (1996～2000), 4th. Term (2001～05), respectively. HBV group has slightly decreased, HCV group has slightly increased and alcoholic group whose ethanol consumption is over 80g per day for more than 10 years and UN group in which HBV, HCV and AL group were excluded has remained unchanged in frequency,respectively. Female's percentage of HCC development has increased. The age of HCC development has been getting older year by year, except for HBV group which has remained unchanged. The percentage of chronic hepatitis in noncarcinomatous part of the liver has increased. The age of HCC development is significantly lower in patients with DM, on the other hand significantly higher in patients with hypertension. The above results suggest that we must pay attention to HCC development even in case of histologically or clinically chronic hepatitis in the elderly patients.

Key words: hepatocellular carcinoma HBV HCV chronic hepatitis epidemiology

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Serum HBV RNA is a Predictor of Early Emergence of the YMDD Mutant in Patients Treated with Lamivudine

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Lamivudine (LAM) is a nucleoside analogue widely used for the treatment of chronic hepatitis B virus (HBV) infection. Emergence of resistant strains with amino acid substitutions in the tyrosine-methionine-aspartate-aspartate (YMDD) motif of reverse transcriptase is a serious problem in patients on LAM therapy. The amount of covalently closed circular DNA in the serum is reported to be higher in patients who develop YMDD mutants than in those without mutants. However, there is no useful serum marker that can predict early emergence of mutants during LAM therapy. Analysis of patients who were treated with entecavir (n = 7) and LAM (n = 36) showed some patients had high serum levels of HBV RNA. Median serum levels of HBV RNA were significantly higher in patients in whom the YMDD mutant had emerged within 1 year (n = 6, 1.688 log copies/ml) than in those in whom the YMDD mutant emerged more than 1 year after treatment (n = 12, 0.456 log copies/ml, $P = 0.0125$) or in whom the YMDD mutant never emerged (n = 18, 0.688 log copies/ml, $P = 0.039$). Our results suggest that HBV RNA is a valuable predictor of early occurrence of viral mutation during LAM therapy. (HEPATOLOGY 2007;45:1179-1186.)

The hepatitis B virus (HBV) is a member of the hepadnaviridae family. Worldwide, approximately 350 million people are estimated to be chronically infected with HBV.[1] Patients with chronic HBV infection develop chronic hepatitis, cirrhosis, and hepatocellular carcinoma, accounting for approximately 1 million deaths per year.[2] Recently, inhibitors of reverse transcriptase have been developed and widely used for patients with chronic HBV infection. Lamivudine (LAM), a cytosine nucleoside analogue, was first developed as an antiviral agent against HIV and later was used effectively against HBV because HBV also uses reverse transcriptase for replication.[3,4] Because LAM suppresses HBV replication, patients who are treated with LAM show a decreased level or disappearance of HBV DNA in serum and hepatitis B e antigen, normalization of serum alanine aminotransferase (ALT) level, and histological improvement.[5-12] However, discontinuation of therapy often leads to reactivation of HBV.[6,8,13,14] Therefore, long-term therapy is necessary for many patients with chronic HBV infection. During long-term LAM therapy, drug-resistant mutants with amino acid substitutions in the tyrosine-methionine-aspartate-aspartate (YMDD) motif emerge, resulting in expression of HBV DNA increasing again and in worsening of hepatitis.[6,10,15-18] Moreover, some patients develop a severe flare-up of hepatitis that could lead to fatal hepatic failure. Therefore, prediction of the emergence of YMDD mutants is an important issue.

In our hunt for useful serum markers to detect the early emergence of YMDD mutants, we noticed some patients who showed a discrepancy in the expression of HBV DNA measured by the transcription-mediated amplifica-

Abbreviations: cccDNA, covalently closed circular DNA; ETV, entecavir; HBV, hepatitis B virus; LAM, lamivudine; PCR, polymerase chain reaction; RT, reverse transcription; TMA-HPA, transcription-mediated amplification and hybridization protection assay; YMDD, tyrosine-methionine-aspartate-aspartate.

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Potential conflict of interest: Nothing to report.

Table 1. Clinical Characteristics of the 3 Groups

	Group A	Group B	Group C
Number	6	12	18
Age, median (range)	50 (37-67)	49 (31-66)	49 (27-68)
Sex (M:F)	3:3	9:3	13:5
Observation period (months)	34.5 (13-58)	38 (16-64)	34 (13-58)
Time before emergence of mutants (months)	8.5 (4-11)	19 (13-36)	
HBV DNA (LGE/ml)	7.8 ± 0.95	6.13 ± 0.84	6.64 ± 1.63
Hbe-antigen-positive	4 (66.7%)	6 (50%)	10 (55.6%)
Hbe-antibody-positive	1 (16.7%)	6 (50%)	9 (50%)
ALT (U/l)	136.1 ± 122.8	114.5 ± 104.1	129.8 ± 206.4

Group A: patients who showed early emergence of the mutants (within 1 year).
Group B: patients who developed resistance after 1 year of LAM therapy.
Group C: patients in whom mutants did not develop.

tion and hybridization protection assay (TMA-HPA) and that measured by the Amplicor HBV Monitor test. Because the former method detects both HBV DNA and HBV RNA, we thought that the difference in measurement by the 2 methods was a result of the presence of a large amount of HBV RNA.[19-21] We thus studied patients with chronic HBV infection who were being treated with LAM or entecavir (ETV) for the presence of HBV RNA. We also assumed that the presence of a large amount of HBV RNA would indicate that transcription and virus particle formation were still active in such patients. We thus assessed the value of this indicator in the prediction of the emergence of YMDD mutants during LAM therapy.

Patients and Methods

Patients. We studied 36 patients with chronic hepatitis B who were being treated with LAM from 2001 to 2006 at Hiroshima University Hospital, Kawakami Clinic, and Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital. We also analyzed 7 patients who were being treated with ETV from 2004 to 2006 at Hiroshima University Hospital. No patients showed clinical signs of cirrhosis or hepatocellular carcinoma. They were not treated with other antiviral agents, corticosteroids, or immunosuppressant drugs during LAM/ETV therapy. The LAM-treated patients were 25 men and 11 women whose median age was 52 years (range 27-68 years; Table 1). They were divided into 3 groups (groups A, B, and C) according to how long it took for YMDD mutants to appear. Group A (n = 6) was composed of patients who showed early emergence of the mutants (within 1 year); group B (n = 12) had patients who developed resistance after 1 year of LAM therapy; and group C (n = 18) was composed of patients who did not show resistance to LAM therapy. Each of the 36 patients received 100 mg of LAM daily for 4-58 months (median, 21.5 months). All patients continued LAM therapy throughout the course of the study. Patients in the ETV group were 6 men and 1 woman whose median age was 37 years (32-50 years). They received 0.01-0.5 mg of ETV daily for 21-28 months (median, 25 months), and all patients continued ETV therapy throughout the course of the study. Blood samples were obtained from patients of both groups just before commencement of antiviral therapy and every 4 weeks during therapy. Informed consent was obtained from each patient.

Quantification of HBV DNA. HBV DNA serum level was determined by using the TMA-HPA (Fujirebio Inc., Tokyo, Japan) and the Amplicor HBV monitor test (Roche Diagnostics, Tokyo, Japan). The measurement range of the former assay is $10^{3.7}$-$10^{8.7}$ genome equivalents (GE)/ml (3.7-8.7 LGE/ml),[22] whereas the range of the latter test was $10^{2.6}$-$10^{7.6}$ copies/ml (2.6-7.6 log copies/ml).[23] These quantitative assays of HBV DNA were performed at the Special Reference Laboratory (Tokyo, Japan).

Extraction of Nucleic Acid of HBV and Reverse Transcription. Nucleic acid was extracted from 100 μL of serum by the SMITEST (Genome Science Laboratories, Tokyo, Japan) and dissolved in 20 μL of H_2O for DNA analysis or 8.8 μL of ribonuclease-free H_2O for RNA analysis. The latter solution was reverse-transcribed by using random primer (Takara Bio Inc., Shiga, Japan) and M-MLV reverse transcriptase (ReverTra Ace, TOYOBO Co., Osaka, Japan). In the next step, 25 pM of random primer was added to 8.8 μL of nucleic acid extract and heated at 65°C for 5 minutes. The samples were set on ice for 5 minutes. Then 4 μL of 5× reverse transcription (RT) buffer, 2 μL of 10 mM dNTPs, 2 μL of 0.1 M dithiothreitol (DTT), 8 units of ribonuclease inhibitor, and 100 units of M-MLV reverse transcriptase were added to each sample. The reaction mixture was incubated at 30°C for 10 minutes and 42°C for 60 minutes, followed by inactivation at 99°C for 5 minutes.

Quantitative Analysis of HBV DNA by Real-Time Polymerase Chain Reaction. One microliter of DNA solution or cDNA solution was amplified by real-time polymerase chain reaction (PCR) with an ABI Prism 7300 Sequence Detection System (Applied Biosystems, Foster City, CA) according to the instructions provided by the manufacturer. Amplification was performed in a 25-μL reaction mixture containing SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA), 200 nM of forward primer (5′-TTTGGGGCATGGACATTGAC-3′, nucleotides 1893-1912), 200 nM of reverse primer (5′-GGTGAACAATGGTCCGGAGAC-3′, nucleotides 2029-2049), and 1 μL of DNA or cDNA solution. After incubation for 2 minutes at 50°C, the sample was heated for 10 minutes at 95°C for denaturing, followed by a PCR cycling program consisting of 40 2-step cycles of 15 seconds at 95°C and 60 seconds at 60°C. The lower detection limit of this assay was 10^3 copies/ml.

Confirmation of Presence of HBV RNA in Serum by RNase Digestion. To confirm the presence of HBV RNA, nucleic acid extracted from the serum samples by SMITEST (Genome Science Laboratories, Tokyo) was digested with 1 μg/μL of RNase A (Wako Pure Chemical Industries, Osaka, Japan) at 37°C for 60 minutes, digested with proteinase K (New England Biolabs Inc., Ipswich, MA) at 37°C for 60 minutes, extracted with phenol/chloroform, precipitated with ethanol, and dissolved in water. Treated nucleic acid with or without RNase was analyzed by real-time PCR after reverse transcription with a random primer and reverse transcriptase, as already described.

Detection of YMDD Mutant. Mutations in the YMDD motif of reverse transcriptase of HBV were examined by PCR with peptide nucleic acid clamping, as described previously.[24]

Statistical Analysis. Differences between groups were examined for statistical significance using the Student t test, and correlations of parameters were examined by the Spearman's rank correlation. A difference with a P value less than 0.05 was considered statistically significant. All statistical analyses were performed with StatView version 5.0 (SAS Institute, Cary, NC).

Results

HBV DNA Levels Determined by TMA-HPA and Amplicor HBV Monitor Test During ETV Therapy. High expression of HBV RNA was initially observed by measuring HBV nucleic acid with the TMA-HPA and HBV DNA with the Amplicor HBV monitor test. As shown in Fig. 1, expression of HBV nucleic acid was higher than HBV DNA during the initial 6 months of ETV therapy. We assumed that the discrepancy in the measurements by these 2 methods was a result of the large amount of HBV RNA in the serum because the TMA-HPA measures both HBV DNA and HBV RNA, whereas the Amplicor HBV monitor test detects only HBV DNA. We measured the HBV nucleic acid levels in the 7 patients who received ETV therapy 3 and 6 months after the start of therapy. The HBV nucleic acid levels of all 7 patients determined by the TMA-HPA were 10-100 times higher than those determined by the Amplicor HBV Monitor test except for 2 patients who received a small amount (0.01 mg) of ETV (Fig. 2). The small dif-

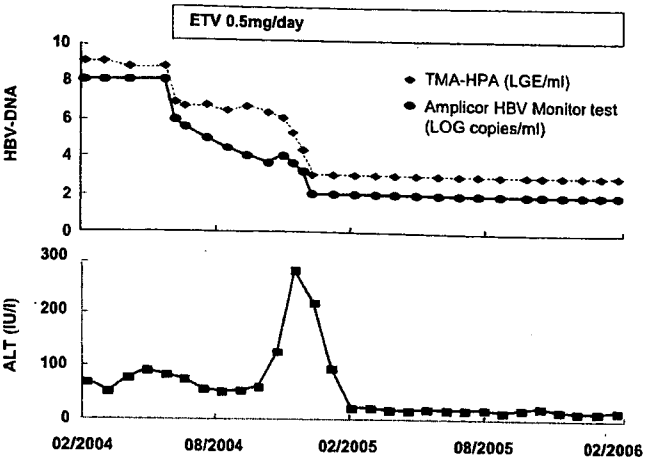

Fig. 1. Time courses of serum HBV DNA and ALT levels of patients treated with ETV. Expression of HBV nucleic acids determined by the TMA-HPA was higher than that determined by the Amplicor HBV Monitor test soon after beginning administration of ETV. The discrepancy was less marked when both measurements were low and when both were negative.

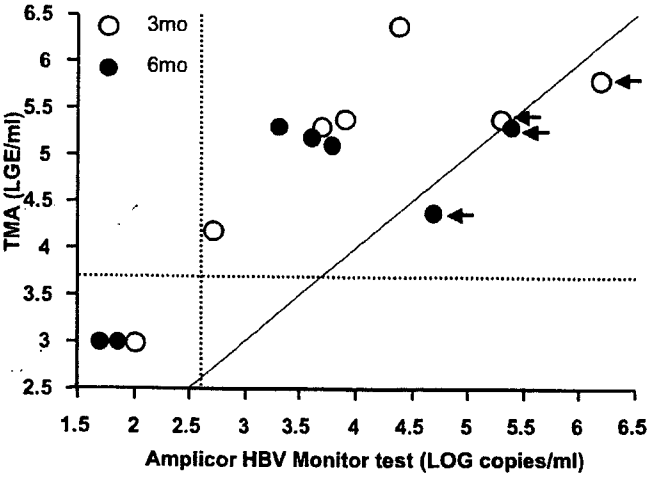

Fig. 2. Correlation of HBV nucleic acid levels determined by the TMA-HPA with HBV DNA levels determined by the Amplicor HBV Monitor test during ETV therapy. Serum samples obtained from the 2 patients who received low-dose ETV (0.01 mg) are indicated by arrows. The vertical and horizontal dotted lines indicate the lower detection limits of the Amplicor HBV Monitor test and the TMA-HPA, respectively.

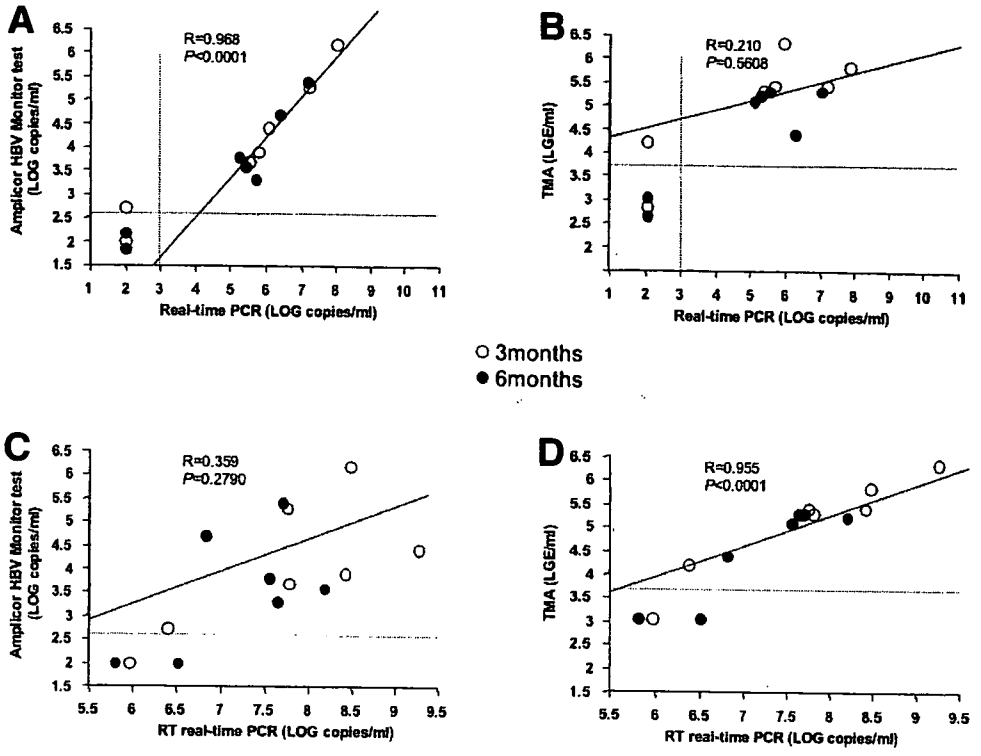

Fig. 3. Correlation between HBV nucleic acid and HBV DNA measurements after 3 and 6 months of ETV therapy. (A) Correlation between HBV DNA level determined by Amplicor HBV Monitor test and that determined by in-house real-time PCR. (B) Correlation of HBV nucleic acid level determined by the TMA-HPA and of HBV DNA determined by real-time PCR. (C) Correlation of HBV DNA level determined by the Amplicor HBV Monitor test with HBV nucleic acid level determined by real-time RT-PCR. (D) Correlation of HBV nucleic acid level determined by the TMA-HPA with that determined by real-time RT-PCR. The vertical and horizontal dotted lines represent the lower detection limits of the Amplicor HBV Monitor test or TMA-HPA and in-house real-time PCR, respectively.

ference in nucleic acid level of these patients is probably a result of the small effect of the small amount of ETV.

Comparisons of HBV Nucleic Acid and DNA Values Determined by 4 Measurement Methods—TMA-HPA, Amplicor Monitor Test, In-House Real-Time PCR Assay, and Real-Time RT-PCR—in Patients Treated with ETV. We measured HBV DNA by in-house real-time PCR and HBV nucleic acid by real-time RT-PCR using serum samples obtained from the patients after 3 and 6 months of ETV therapy and compared these values with those obtained by the TMA-HPA and the Amplicor monitor test. HBV DNA determined by real-time PCR correlated well with that obtained by the Amplicor HBV Monitor test ($r = 0.968$, $P < 0.0001$; Fig. 3A), but not with HBV nucleic acid determined by the TMA-HPA ($r = 0.210$, $P = 0.5608$; Fig. 3B). Expression of HBV DNA determined by the in-house real-time PCR assay was $10^{1.5}$-10^{2} higher than that determined by the Amplicor HBV Monitor test. We confirmed the accuracy of our assay using limiting dilution and detection with nested PCR assay. When we diluted the standard samples used in our in-house assay to 1 copy/μL, we detected them by nested PCR using 1 μL of such samples. Three of the 10 (30%) samples tested positive by nested PCR. We thus conclude that our assay accurately measure the amount of HBV DNA in serum.

To examine if measurement by the TMA-HPA reflected the total amount of HBV RNA and HBV DNA in serum samples, we performed real-time RT-PCR using serum samples obtained from patients after 3 and 6 months of ETV therapy. In contrast to the values determined by real-time PCR without RT, the measurement of HBV nucleic acid determined by RT-PCR did not correlate well with that obtained by the Amplicor HBV Monitor test ($r = 0.359$, $P = 0.2790$; Fig. 3C), but did correlate well with that obtained with the TMA-HPA ($r = 0.955$, $P < 0.0001$; Fig. 3D). These results show that the TMA-HPA measures both HBV DNA and HBV RNA in serum. To further confirm the presence of HBV RNA, we digested 3 nucleic acid samples arbitrarily picked from serum samples obtained from patients treated by lamivudine for 3 months, by RNase A. As shown in Fig. 4, RNase treatment reduced the amount of HBV DNA detected by real-time RT-PCR to about 1% of that originally detected.

HBV DNA Levels Determined by TMA-HPA and Amplicor HBV Monitor Test during LAM Therapy. We then investigated the levels of HBV DNA in serum samples obtained from 36 patients after 3 and 6 months of LAM therapy. In some patients, HBV DNA was already negative after 3 and 6 months of therapy (Fig. 5). Similar to the results obtained from patients treated with ETV, comparisons of values obtained from patients who showed measurable HBV DNA levels revealed that HBV nucleic acid levels determined by the TMA-HPA tended to be higher than those determined by the Amplicor HBV Monitor test (Fig. 4).

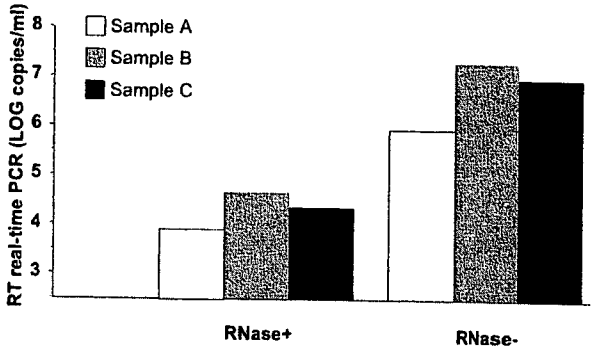

Fig. 4. Presence of HBV RNA confirmed by RNA treatment of 3 nucleic acid samples (samples A-C) obtained from patients after 3 months of LAM therapy. Extracted nucleic acid samples with or without RNase digestion were further digested by proteinase K and ethanol-precipitated after phenol/chloroform extraction. The amount of HBV DNA in each sample was then measured by real-time RT-PCR.

Comparisons of HBV Nucleic Acid Values and HBV DNA Determined by 4 Measurement Methods—TMA-HPA, Amplicor Monitor Test, In-House Real-Time PCR Assay, and Real-Time RT-PCR—in Patients Treated with LAM. We measured HBV nucleic acid and DNA levels by the same 4 methods and investigated the correlations between them after 3 and 6 months of LAM therapy (Fig. 6). HBV DNA levels determined by real-time PCR correlated better with those determined by the Amplicor HBV Monitor test (r = 0.653, P = 0.0083; Fig. 6A) than with those determined by the TMA-HPA (r = 0.456, P = 0.1173; Fig. 6B). Similarly, measurement of HBV nucleic acid by RT-PCR did not correlate well with that obtained by the Amplicor HBV Monitor test (Fig. 6C), but showed better correlation with that obtained by the TMA-HPA (r = 0.452, P = 0.0907, and r = 0.675, P = 0.0114, respectively; Fig. 6D). These results also showed that the TMA-HPA detects both HBV RNA and HBV DNA.

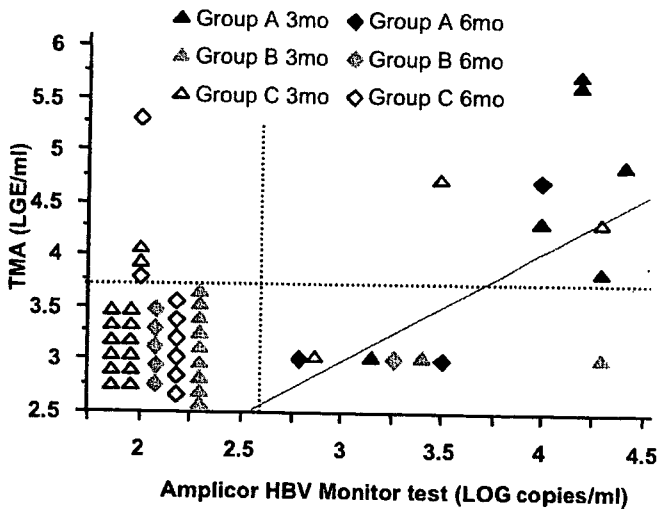

Fig. 5. Correlation of HBV nucleic acid levels determined by the TMA-HPA with HBV DNA levels determined by the Amplicor HBV Monitor test during LAM therapy. During ETV therapy the TMA-HPA showed higher expression of HBV DNA in patients regardless of the presence of the mutation than did the Amplicor HBV Monitor test. The vertical and horizontal dotted lines indicate the lower detection limits of the Amplicor HBV Monitor test and the TMA-HPA, respectively.

HBV RNA in Serum after 3 Months of LAM Therapy Is Higher in Patients Who Showed Early Emergence of YMDD Mutants. In LAM-treated patients, it was assumed that a high serum level of HBV RNA was a marker of the active transcription form of covalently closed circular DNA (cccDNA) and packaging of HBV RNA in the liver. We assumed that YMDD mutants easily emerged under such condition. We compared HBV RNA values (HBV nucleic acid determined by real-time RT-PCR minus HBV DNA determined by real-time PCR) in patients who showed early emergence of mutants (within 12 months) with those who showed late emergence of mutants (more than 12 months) and those who did not show emergence of mutants (Table 1). As shown in Fig. 7, HBV RNA levels were significantly higher in patients who showed early emergence of mutants than the other 2 groups after 3 months of LAM therapy. There was no significant difference in the amount of HBV RNA between group A (patients who showed emergence of mutants within 12 months) and the other 2 groups at the beginning of LAM therapy (data not shown).

Discussion

In this study, we addressed the discrepant measurements of HBV nucleic acid by the TMA-HPA and the Amplicor Monitor test. The presence of HBV RNA in serum samples of patients with HBV infection has been previously reported.[19-21] Because the TMA-HPA uses RNA transcription and amplification of transcripts by T7 RNA polymerase,[22] we assumed that the discrepancy was a result of the presence of HBV RNA in the serum of LAM- and ETV-treated patients. The presence of HBV RNA in a patient treated with LAM was reported previously.[21] In that report, the authors mainly analyzed truncated HBV RNA, which they assumed was transcribed from the integrated genome.[20, 21] They showed a large difference between HBV DNA and truncated HBV RNA, which did not decrease during LAM therapy. We also detected HBV DNA and HBV nucleic acid by real-time PCR and real-time RT-PCR. The values determined by these 2 methods showed less than a 1 log difference (data not shown); we assume that the effect of truncated HBV RNA in serum was only minimal in our study. As we demonstrated in this study, HBV nucleic acid measured by real-time RT-PCR correlated with that determined by the TMA-HPA. This finding suggests that the

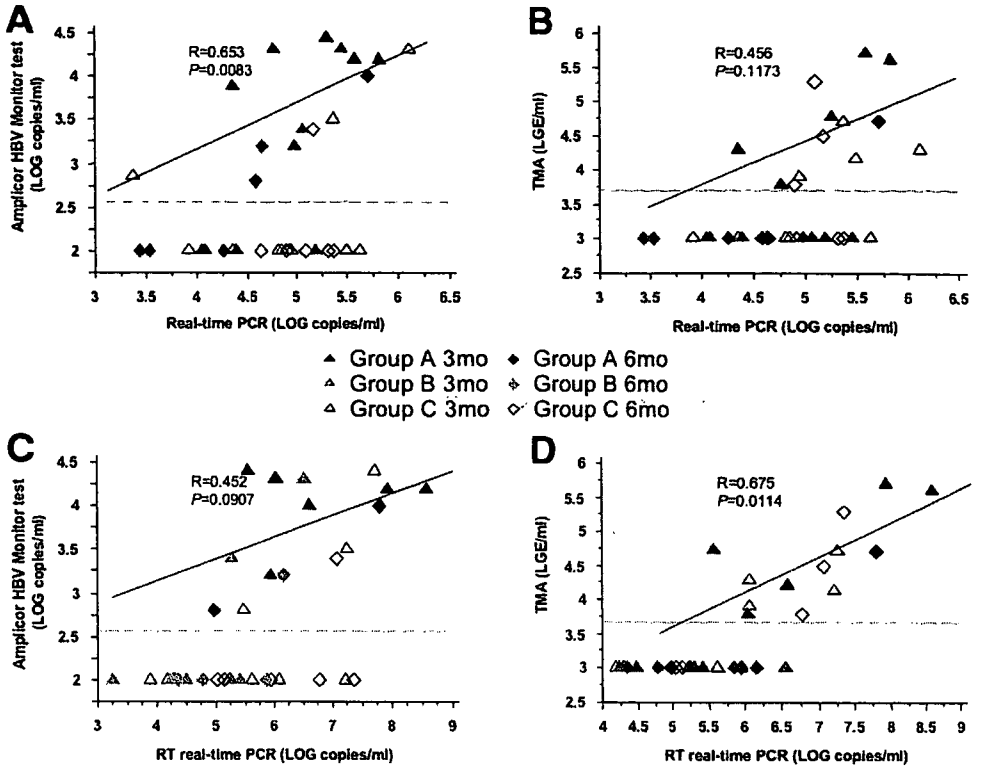

Fig. 6. Correlations between HBV nucleic acid and HBV DNA measurements after 3 and 6 months of LAM therapy. (A) Correlation of HBV DNA level determined by the Amplicor HBV Monitor test with that determined by in-house real-time PCR. (B) Correlation of HBV nucleic acid level determined by the TMA-HPA with HBV DNA by real-time PCR. (C) Correlation of HBV DNA level determined by the Amplicor HBV Monitor test with HBV nucleic acid level determined real-time RT-PCR. (D) Correlations of HBV nucleic acid level determined by the TMA-HPA with that determined by real-time RT-PCR. The vertical and horizontal dotted lines represent the lower detection limits of the Amplicor HBV Monitor test or TMA-HPA and in-house real-time PCR, respectively.

discrepancy in the values measured by the TMA-HPA and the Amplicor Monitor test is a result of the presence of HBV RNA in the serum.

We showed that a large amount of HBV RNA in the serum was produced during the early stage of ETV (Fig. 1) and LAM treatments (within 6 months). Because ETV and LAM work only on reverse transcription, it is difficult to conceive that the level of transcription from the cccDNA was altered by these drugs. Thus, the slow decrease in HBV RNA seems to reflect that a certain amount of cccDNA still existed in the liver and that the virus replication machinery was still actively operational. This is consistent with previous reports that showed that the amount of cccDNA in the liver tissues[25, 26] and in serum,[26] which correlated well with intrahepatic cccDNA,[27] reflected the effect of LAM and is a marker for cessation of therapy without viral level increasing again after stopping the therapy.

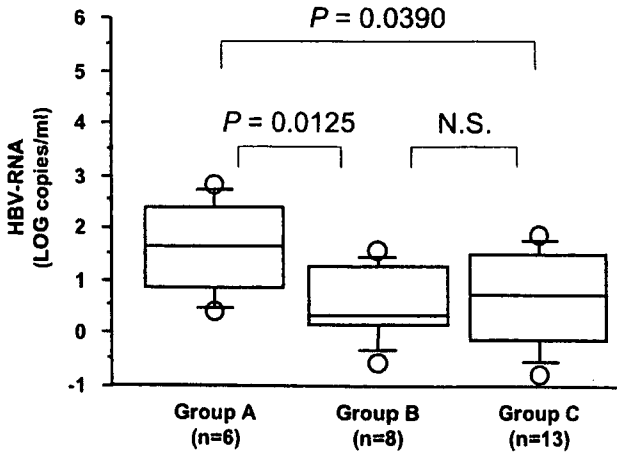

Fig. 7. Box plots of HBV RNA levels of patients in group A (patients who showed emergence of the mutants within 1 year, group B (those who developed resistance after 1 year of LAM therapy), and group C (patients who did not show resistance to LAM therapy). HBV RNA level represents the difference between HBV nucleic acid level determined by real-time RT-PCR minus HBV DNA level determined by in-house real-time PCR. Nine samples that tested negative for in-house real-time PCR were omitted from the analysis (4 samples of group B and 5 samples of group C).

Whether a large amount of HBV RNA originates from a large amount of cccDNA template in hepatocytes or from active transcription (or both) is actually unknown. However, it is assumed that the probability of developing mutants is high in patients who have large amounts of HBV RNA. We thus analyzed the amount of HBV RNA in patients treated with LAM and compared it in patients who showed early emergence of mutants and those who did not. As expected, the amount of HBV RNA in the serum was significantly higher in patients who showed early emergence of mutants than in those who showed late emergence and those who did not show emergence of mutants.

Using complex analysis, previous studies identified several factors predictive of emergence of YMDD mutants such as HBV genotype,[28] ALT level,[29, 30] HBV DNA level

before therapy,[28,30-32] degree of decline of HBV DNA level during therapy,[33,34] presence of hepatitis B e antigen,[17,29,31,32,35] presence of core promoter mutations,[36] deletion of pre-S region,[37] and HBV core–related antigen.[38] We also showed that a slow decrease in HBV nucleic acid measured by the TMA-HPA is a marker of early emergence of mutants. Our finding is important because this assay is routinely used in daily clinical practice. However, the results did not reach statistical significance, probably because of the small number of patients analyzed in our study and the low sensitivity of the assay (detection limit 3.7 log copies/ml). We assume that a sensitive measurement of HBV RNA is useful for predicting the emergence of mutants. Development of such an assay is needed for the proper treatment of patients using different nucleotide and nucleoside analogues. Mechanisms that control transcription of HBV from cccDNA deserve further investigation in order to develop more effective therapies for HBV infection.

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Short Communication

Accordion Index: A new tool for the prediction of the efficacy of peg-interferon-α-2b and ribavirin combination therapy for chronic hepatitis C

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Aim: An optimal treatment regimen based on individual virological response is essential to maximize the efficiency of interferon (IFN) therapy for chronic hepatitis C.

Methods: Using indicators of the virological response and the treatment intensity, we developed the Accordion Index as a new tool for the efficacy prediction of peg-IFN and ribavirin (RBV) combination therapy. For the Accordion Index, the IFN-AC ratio and RBV-AC ratio were defined as follows: IFN-AC ratio = (total IFN dose given during the entire treatment period)/(total IFN dose required to achieve hepatitis C virus [HCV]–RNA negativity), RBV-AC ratio = (total RBV dose given during the entire treatment period)/(total RBV dose required to achieve HCV–RNA negativity).

Results: The analysis of the association between the Accordion Index and the sustained virological response (SVR) revealed that of 25 patients who had HCV–RNA negativity during treatment, all 10 patients with an IFN-AC ratio and RBV-AC ratio of at least 4.0 achieved SVR, while only four of 15 patients with an IFN-AC ratio or RBV-AC ratio of less than 4.0 achieved SVR. With the cut-off value for both the IFN-AC ratio and RBV-AC ratio at 4.0 or higher, the quality of SVR prediction was as follows: the positive predictive value was 100%, the negative predictive value was 73.3%, and accuracy was 84.0%.

Conclusion: The Accordion Index will thus be a useful tool for planning optimal treatment regimens for individual patients.

Key words: Accordion Index, interferon-AC ratio, peg-interferon, ribavirin-AC ratio, ribavirin, sustained virological response

INTRODUCTION

COMBINATION THERAPY WITH peg-interferon (PEG-IFN) and ribavirin (RBV) is the current first-line therapy used to eliminate hepatitis C virus (HCV) in patients with chronic hepatitis C.[1] The duration of treatment is determined based on viral genotype, with treatment administered for 48 weeks in patients with genotype 1 and 24 weeks in patients with genotypes 2 or 3. Approximately 30% of patients with HCV genotype 1 who are HCV-RNA-negative at the end of treatment relapse after the discontinuation of treatment, and the sustained virological response (SVR) rate is 42–52%.[2–4] The SVR rate in patients with HCV genotype 2 is 81–84%,[4,5] and some of these patients may be overdosed.[6]

Several studies have shown that the SVR rate correlates with the duration of treatment required to achieve HCV–RNA negativity.[7,8] Current treatment regimens are designed in accordance with expected sensitivity of individual patients to treatments to reduce potential adverse drug reactions and to increase the possibility of SVR. Specifically, the treatment duration might be shortened in patients who achieve HCV–RNA negativity at an early stage of treatment,[9] while the treatment duration should be extended in patients with slow virological response.[10] Franciscus introduced this concept of the treatment design as an "accordion" effect at the Digestive Disease Week Conference Highlights held in Los Angeles in 2006 (Franciscus A., 2006, unpublished data). By further advancing the concept, we developed a new tool for SVR prediction, the Accordion Index (the IFN-AC

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ratio and the RBV-AC ratio). In the present study, we analyzed the association between the Accordion Index and treatment efficacy, and evaluated the usefulness of this index in determining doses and treatment duration for individual patients.

METHODS

Patients and methods

BETWEEN JANUARY 2005 and December 2006 at the National Hospital Organization Kokura Hospital, 82 patients received combination therapy with PEG-IFN-α-2b and RBV for the treatment of chronic hepatitis C, and efficacy evaluation was possible in 44 patients. Of these 44 patients, the 25 patients who achieved HCV-RNA negativity during treatment (11 males and 14 females; mean age: 56.0 ± 12.8 years) were included in the study. Pre-treatment serum HCV-RNA levels were quantified using the Amplicor HCV monitor test, version 2.0 (Roche Diagnostics, Tokyo, Japan). A serum HCV qualitative assay using the Amplicor HCV test, version 2.0 (Roche Diagnostics, Tokyo, Japan) was conducted at least every 4 weeks after the start of treatment to determine the time of HCV-RNA negativity. Patients who remained HCV-RNA negative at 6 months after the end of treatment were judged to be SVR, and patients who achieved HCV-RNA negativity during treatment, but had recurrence after the end of treatment, were considered to have a transient response (TR).

For the Accordion Index, the IFN-AC ratio and RBV-AC ratio were defined as follows: IFN-AC ratio = (total IFN dose given during the entire treatment period)/(total IFN dose required to achieve HCV-RNA negativity), RBV-AC ratio = (total RBV dose given during the entire treatment period)/(total RBV dose required to achieve HCV-RNA negativity).

The study protocol was approved by the institutional ethics committee of the National Hospital Organization Kokura Hospital, and all of the patients gave their informed consent to participate in this study. The study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice.

RESULTS

Study patients

OF THE 25 patients in the study, 14 (seven males and seven females; mean age: 51.1 ± 12.7 years) were in the SVR group. Ten had HCV serogroup 1, and four had serogroup 2. The pretreatment HCV-RNA load was 1.4 ± 2.6 × 10^6 IU/mL. The TR group consisted of 11 patients (four males and seven females; mean age: 61.5 ± 10.1 years) of whom seven had HCV serogroup 1, and four had serogroup 2. The pretreatment HCV-RNA load was 2.3 ± 2.7 × 10^6 IU/mL.

Association between the Accordion Index and treatment efficacy

The IFN-AC ratio and RBV-AC ratio for individual patients in the SVR and TR groups are shown in Figure 1. All 10 patients whose IFN-AC ratio and RBV-AC ratio were 4.0 or higher achieved SVR, while only four of 15 patients whose IFN-AC ratio or RBV-AC ratio was less than 4.0 achieved SVR. With the cut-off value for both the IFN-AC ratio and RBV-AC ratio at 4.0 or higher, the quality of the SVR prediction was as follows: the positive predictive value was 100%, the negative predictive value was 73.3%, and accuracy was 84.0%.

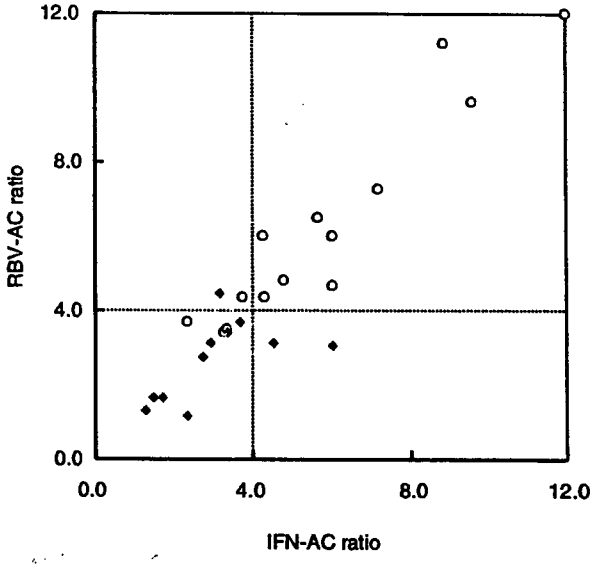

Figure 1 Interferon (IFN)-AC ratio and ribavirin (RBV)-AC ratio in sustained virological response (○ SVR) and transient response (◆ TR) patients. IFN-AC ratio and RBV-AC ratio were calculated by the following formulas: IFN-AC ratio = (total IFN dose given during the entire treatment period)/(total IFN dose required to achieve hepatitis C virus [HCV]-RNA negativity), RBV-AC ratio = (total RBV dose given during the entire treatment period)/(total RBV dose required to achieve HCV-RNA negativity).

DISCUSSION

THE PRESENT STUDY shows that the Accordion Index is closely associated with SVR and that the Index is a useful tool for determining optimal doses and treatment duration for individual patients scheduled to receive combination therapy with PEG-IFN and RBV.

There is no established theory referring to how long treatment should be continued following HCV-RNA negativity to achieve SVR. Drusano *et al.* developed a hypothesis that the longer the absence of HCV-RNA, the more likely SVR is achieved. Their study showed that the SVR rate was 80% with a 32-week absence of HCV-RNA and 90% with a 36-week absence of HCV-RNA.[11] The model developed by Drusano *et al.*, however, was not consistent with the subsequent study outcome. The SVR rate among rapid virological responders (HCV-RNA negative at week 4 of treatment) with HCV genotype 1 was approximately 90% after 24 weeks of treatment,[9] while approximately 40% of slow virological responders (HCV-RNA positive at week 12 and HCV-RNA negative at week 24) had recurrence after 72 weeks of treatment.[10] These studies suggest that rapid virological responders can achieve SVR with short-term treatment after achieving HCV-RNA negativity, while slow virological responders require a much longer treatment period to achieve SVR.

The original "accordion" theory is a hypothesis describing the correlation between the treatment period required to achieve HCV-RNA negativity and the subsequent treatment duration required to achieve SVR. We believe, however, that the doses given to the patient must be taken into consideration, together with the treatment duration. Increasing the doses of PEG-IFN and RBV is known to promote viral reduction at an early stage of treatment and to improve the SVR rate.[12,13] Doses are often reduced in combination treatment with PEG-IFN and RBV due to various adverse drug reactions, such as decreased platelets, decreased neutrophils, anemia, and malaise. It is inappropriate to analyze the treatment period after dose reduction with the same weighting as the treatment period before dose reduction in such cases. We therefore integrated the drug doses and treatment duration to develop the Accordion Index. In the formulas of the Accordion Index, the total drug dose required to achieve HCV-RNA negativity is representative of the individual virological response, while the total dose given during the entire treatment period is an indicator of the treatment intensity. Specifically, IFN-AC is the ratio of the total IFN dose given during the entire treatment period to the total IFN dose required to achieve HCV-RNA negativity, and RBV-AC is the ratio of the total RBV dose given during the entire treatment period to the total RBV dose required to achieve HCV-RNA negativity.

Our analysis identifies the close association of the IFN-AC ratio and RBV-AC ratio with SVR, indicating a sufficient positive predictive value, negative predictive value, and accuracy for SVR prediction with the cut-off value for both the IFN-AC ratio and RBV-AC ratio at 4.0 or higher. The study outcomes suggest that individualized treatment regimens may be planned based on the virological response of patients by calculating the total doses of PEG-IFN and RBV so far given at the time HCV-RNA negativity is achieved and determining the subsequent doses and treatment duration to ensure that the total doses at the end of treatment will be more than four times the doses required to achieve HCV-RNA negativity.

The number of elderly patients with chronic hepatitis C is increasing in Japan. Treating high-risk patients who have underlying heart disease or anemia with fixed doses of PEG-IFN or RBV frequently results in treatment discontinuation due to adverse drug reactions.[14] The virological responses of patients who receive combination therapy with PEG-IFN and RBV vary greatly. While some patients achieve HCV-RNA negativity with short-term treatment and low doses, others do not respond even with long-term treatment and high doses. Physicians therefore need to plan optimal treatment regimens for individual patients by finely adjusting drug doses and treatment periods in accordance with their response and tolerability.

The Accordion Index is expected to help physicians plan optimal individual regimens of PEG-IFN and RBV combination therapy. Further analyses in a larger sample size and a prospective study should be conducted following the current pilot study.

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