サテライトシンポジウム

Genotype1b高ウイルス量の高齢C型慢性肝炎患者に対するPeg-IFN α-2b＋リバビリン併用療法適応の検討

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【目　的】

本邦におけるC型慢性肝炎患者は欧米に比べ高齢化が先行しているために高齢者に対する抗ウイルス療法が課題となっている。しかし，治療効果が最も期待できるペグインターフェロン（Peg-IFN）α-2b＋リバビリン併用療法は副作用の観点から高齢者には慎重に適応を決めることが望まれている。

今回，我々は60歳以上のGenotype 1bかつ高ウイルス量患者に対するPeg-IFN α-2b＋リバビリン併用療法を行い，60歳未満の若年患者と比較してその有用性と問題点について検討した。

【対象と方法】

当科および関連病院において2002年2月から2005年11月までにPeg-IFN α-2b，リバビリン併用療法投与を開始したGenotype 1bでHCV RNA量が100KIU/ml以上のC型慢性肝炎42例（平均年齢52.6±11.6歳（範囲18－71歳），男女比26：16）を対象とし，60歳未満の若年者群（A群）と60歳以上の高齢者群（B群）の2群に分け，各群のウイルス学的治療効果および副作用による減量・中止，ウイルス学的効果について検討を行った。

統計学的解析：成績は平均値±SDおよび比率で示し，平均値の差はStudent's t test,

表1．背景因子の比較

	A群	B群	p
症例数	30	12	
年齢（歳） （範囲）	48.2±10.3 (18-58)	64.4±4.0 (60-71)	
男女比（男：女）	20：10	6：6	0.255
治療歴（初：再）	16：14	11：1	0.019
投与前検査値			
ヘモグロビン（g/dl）	14.5±1.3	14.0±1.1	0.214
血小板数（x10⁴/μl）	16.5±4.2	15.8±2.8	0.582
アルブミン（g/dl）	4.1±0.3	3.9±0.3	0.071
ALT（IU/l）	85.9±52.2	64.8±35.4	0.301
HCV-RNA量（KIU/ml）	1900±1329	2386±1776	0.433

表 2. 治療前の合併症の比較

	A群(n=30)	B群(n=12)	p
合併症のある症例	23.3% (7/30)	58.3% (7/12)	0.037
合併症(重複あり)			
糖尿病	10.0% (3/30)	8.3% (1/12)	0.680
高血圧症	10.0% (3/30)	33.3% (4/12)	0.088
不整脈	0% (0/30)	8.3% (1/12)	0.113
甲状腺機能障害	3.3% (1/30)	16.6% (2/12)	0.134
自律神経失調症	3.3% (1/30)	0% (0/12)	0.527
胃潰瘍	0% (0/30)	8.3% (1/12)	0.113
脳外傷後後遺症	0% (0/30)	8.3% (1/12)	0.113
血小板低下で脾摘後	3.3% (1/30)	8.3% (1/12)	0.497
心内膜床欠損症術後	3.3% (1/30)	0% (0/12)	0.527

表 3. 薬剤の減量と中止

	A群(n=30)	B群(n=12)	P
●Peg-IFNの減量	6.6% (2/30)	8.3% (1/12)	0.646
理由：	体重減少 1例	食欲不振、体重減少 1例	
●リバビリンの減量	26.6% (8/30)	50.0% (6/12)	0.139
理由：	Hbの低下 8例 息切れ 2例	Hbの低下 6例 息切れ 2例	
●両薬剤の減量	3.3% (1/30)	8.3% (1/12)	0.495
理由：	体重減少・Hbの低下	食欲不振、体重減少 Hbの低下	
●両薬剤の中止	6.6% (2/30)	16.6% (2/12)	0.320
理由：	血小板3万以下の低下 1例 間質性肺炎 1例	肝癌の発生 摂食障害	

Mann-Whitney's *U* test，比率の差は chi-square test，Fisher's exact testにて検定し，$p<0.05$を有意差とした。

【成　績】

1. 背景因子の比較

患者背景因子の比較を表1に示した。症例数はA群30例（平均年齢48.2 ± 10.3（範囲18－58歳），男女比20：10），B群12例（平均年齢B群64.4 ± 4.0歳（範囲60－71歳），男女比6：6）であったが，初回治療は，B群で有意に多かった。ヘモグロビン値，血小板数，血清アルブミン値，血清ALT値，HCV-RNA量は両群で有意差を認めなかった。

2. 治療前合併症の比較

治療前合併症を比較検討した（表2）。合併症を有す例はA群，B群ともに7例であったが，合併症を有する率はA群23.3%，B群58.3%で，有意にB群で高かった（P=0.037）。主な合併症の内訳は糖尿病がA群10.0%，B群8.3%，高血圧がA群10.0%，B群33.3%であった。

3. 薬剤の減量と中止

Peg-IFNとリバビリンの減量と中止について表3に示した。Peg-IFNの減量はA群6.6％（2例），B群8.3％（1例）であった。Peg-IFNの減量理由は両群とも体重減少であった。リバビリンの減量はA群26.6％（6例），B群50.0％（8例）であった。リバビリンの減量は両群ともリバビリンの副作用であるヘモグロビンの低下であった。Peg-IFN・リバビリンの両剤減量はA群3.3％，B群8.3％で，減量理由はPeg-IFNおよびリバビリンの減量理由を合わせたものであった。Peg-IFN・リバビリンの両剤中止はA

表 4. 薬剤の減量と中止の時期

		A群(n=30)	B群(n=12)
●Peg-IFNの減量	0～4週	-	-
	5～12週	1例	-
	13～24週	1例	-
	25～48週	-	1例
●リバビリンの減量	0～4週	-	2例
	5～12週	6例	3例
	13～24週	2例	1例
	25～48週	-	-
●両薬剤の減量	0～4週	-	-
	5～12週	1例 (リバビリン減量)	1例 (リバビリン減量)
	13～24週	1例 (Peg-IFN減量)	-
	25～48週	-	1例 (Peg-IFN減量)
●両薬剤の中止	0～4週	-	-
	5～12週	-	1例
	13～24週	1例	-
	25～48週	1例	1例

同一症例

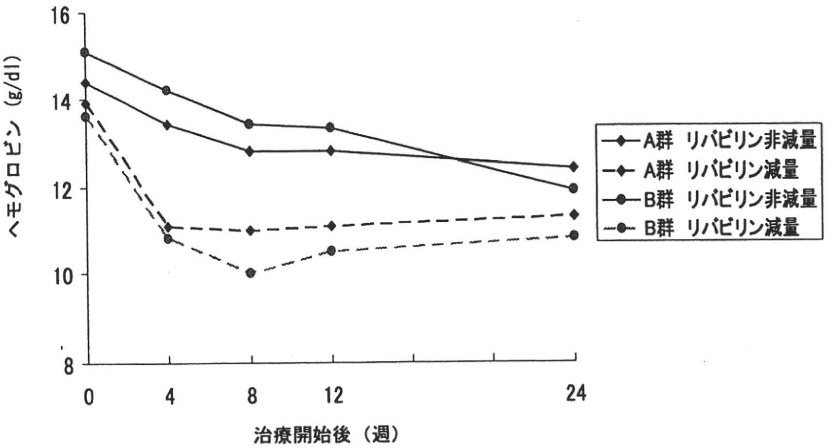

図 1. ヘモグロビンの推移

群，B群ともに2例で，A群6.6％，B群16.6％であった。中止率はB群で高かったが，有意差は認めなかった。中止理由は，A群で血小板3万以下の低下および間質性肺炎の出現，B群で摂食障害および肝癌の発生であった。Peg-IFNの減量，リバビリンの減量，Peg-IFN・リバビリンの両剤減量，Peg-IFN・リバビリンの両剤中止はB群で高い傾向にあったが，有意差は認めなかった。

4. 薬剤の減量と中止の時期

Peg-IFNとリバビリンの減量と中止の時期を表4に示した。減量および中止時期を投与開始後0～4週，5～12週，13～24週，25～48週に分けで検討した。Peg-IFNの減量時期はA群で5～12週に1例，13～24週に1例，B群で25～48週に1例であった。リバビリンの減量時期はA群で5～12週に6例，13～24週に2例，B群では0～4週に2例，5～12週に3例，13～24週に1例で，B群で0～4週の早期にリバビリンの減量を要する例が多かった。Peg-IFNとリバビリンの両剤減量は両群で各々1例あり，リバビリンの減量後にPeg-IFNを減量していた。Peg-IFNとリバビリンの両剤中止時期はA群で13～24週に1例，25～48週に1例，B群で5～12週に1例，25～48週に1例であった。

5. ヘモグロビンの推移

リバビリンの減量が両群とも多く治療開始後24週までに減量していることより，治療開始後24週までの両群のヘモグロビンの推移をリバビリン減量例，非減量例に分けて検討を行った（図1）。治療開始時の平均ヘモグロビン値はA群の非減量例で14.4g/dl，減量例13.9g/dl，B群の非減量例で15.1g/dl，減量例13.6g/dlであり，治療開始時の平均ヘモグロビン値が14.0g/dl以上

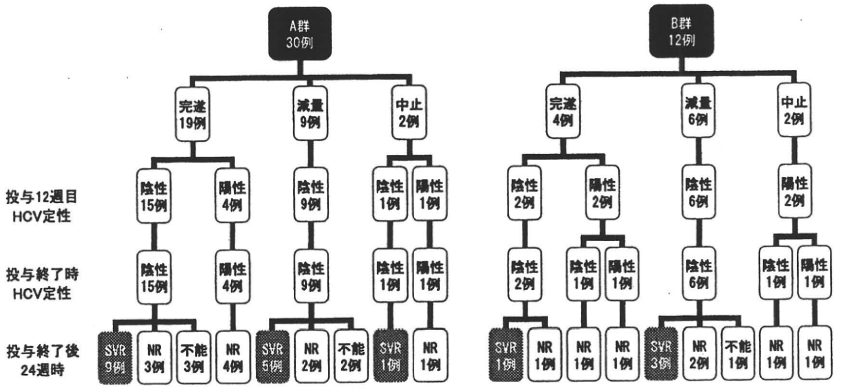

図 2. 治療成績

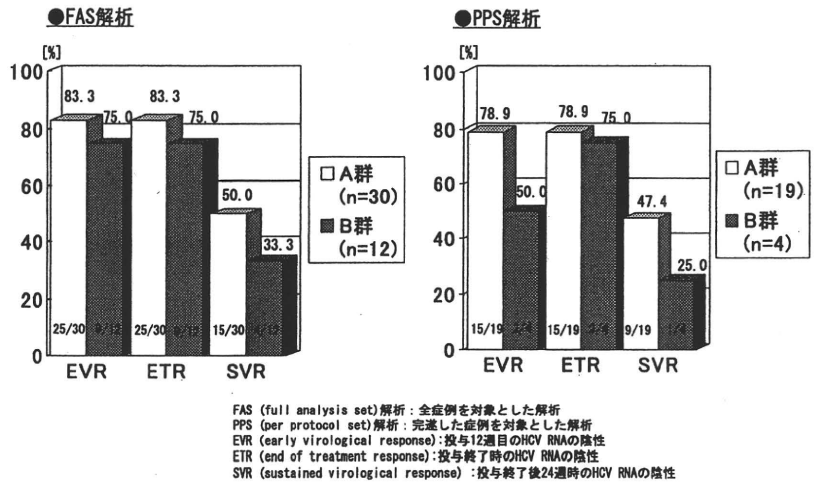

図 3. 治療成績

の症例では減量を要しなかった。また，治療開始後，平均ヘモグロビン値の推移はA群，B群の非減量例では0～8週の間で徐々に減少してゆくのに対して，A群，B群の減量例は，0～4週の間に急激に減少していた。

6. 治療成績

治療成績を図2，図3に示した。全症例を対象としたFull analysis set（FAS）解析では投与12週目までにHCV RNAが陰性化したearly virological response（EVR）はA群83.3％，B群75.0％，投与終了時にHCV RNAが陰性であったEnd of treatment response（ETR）はA群83.3％，B群75.0％であった。投与終了後24週時にHCV RNAが陰性であったsustained virological response（SVR）はA群50.0％，B群33.3％で有意差を認めなかった（P=0.327）。Peg-IFNもしくはリバビリンの減量，Peg-IFNとリバビリンの両剤減量・中止することなく完遂した例はA群19例，B群4例で，完遂率はA群63.3％，B群33.3％あった。完遂した症例を対象としたPer protocol set（PPS）解析ではEVRはA群78.9％，B群50.0％，ETRはA群78.9％，B群75.0％，SVRはA群47.4％，B群25.0％であった。FAS解析，PPS解析において，EVR，ETR，SVRはいずれもB群で低率であったが，有意差を認めなかった。

【考　案】

本邦は米国より20年先にC型慢性肝炎が広がったと推定されており[1]，本邦におけるC型慢性肝炎患者は欧米に比べ高齢化が先行している。また，近年，本邦における新規HCV感染者の減少に伴い，HCV感染者の高齢化が進んできている[2]。それに伴い，高齢者C型慢性肝炎に対する抗ウイルス療法が課題と

なっている。

年齢別にみたGenotype1b，高ウイルス量のC型慢性肝炎に対するIFN治療の有効性については，IFN/リバビリン併用療法で，60歳未満，60歳以上，65歳未満，65歳以上のSVRはFAS解析でそれぞれ32％，13％，11％，PPS解析でそれぞれ34％，17％，16％[3]，Peg-IFN/リバビリン併用療法で，50歳未満，50歳以上65歳未満，65歳以上のSVRはFAS解析でそれぞれ66.0％，36.2％，30.6％，PPS解析でそれぞれ72.2％，44.6％，32.4％[4]と報告され，Peg-IFN/リバビリン併用療法で治療効果が向上してきているが，年齢が高齢になるとSVR率が低下する傾向にある。当科における検討でもSVRは60歳未満の若年者群に比し60歳以上の高齢者群でFAS解析，PPS解析ともに有意差を認めなかったが，低率であった。

また，高齢者に対するPeg-IFN/リバビリン併用治療の問題点は，高齢になるにつれ副作用の出現率が高まり，治療完遂率は低下することである。とくにリバビリン関連の貧血により，高齢者の減量・中止・脱落が増加し，完遂率が低下すると考えられる。当科の検討でも，60歳未満の若年者群に比較して60歳以上の高齢者群でリバビリンの減量を要する症例が多く，治療開始後4週以内の早期にリバビリンを減量する例が多かった。そのため，リバビリンの減量投与[5]，Two by two ruleによるリバビリンの減量[6]やリバビリンの全身クリアランス（CL/F）をベースとしてリバビリン投与量を設定する[7][8]などのリバビリン投与の工夫が必要とされる。

以上より，高齢者に対するPeg-IFN/リバビリン併用治療における問題点は，①Genotype1b，高ウイルス量例では著効になりにくい，②高齢者では副作用の出現率が高まり，治療完遂率は低下することである。しかし，60歳以上のC型慢性肝炎患者にIFN療法を行い，非投与例との間で肝疾患関連死亡率を比較した報告[9][10]では，IFN治療群の標準化死亡比および肝疾患関連による死亡の標準化死亡比は非治療群に比較して有意に低値であったこと，IFN療法を行った例では非治療例より肝癌発生が抑制されたこと[11][12]より，C型慢性肝炎に対するIFN療法は60歳以上の患者においても生命予後改善効果が期待されると考えられる。

「慢性肝炎の治療ガイドライン2008」ではインターフェロン（IFN）治療の適応[13]を「原則として，ALT異常でHCV感染を認めるすべての成人が適応となる」とされているが，一般に高齢者は合併症を有する率が高く，肝臓以外に患者の生命予後に影響する重篤な疾患を有する例は治療適応外と考えられる。しかし，注釈にも述べられているように，「高齢者は実年齢のみでなく，全身的な健康状態（心疾患，腎疾患，糖尿病，癌など），肝病変進展度（発癌のリスク），HCVの種類（genotype）・HCV-RNA量など総合的に判断する」することにより，高齢者でも治療適応例が増加するものと考えられる。

【結　論】

60歳以上のGenotype 1b 高ウイルス量患者に対するPeg-IFN α-2b＋リバビリン併用療法においては，合併症の頻度が高く，治療の減量，中止率も高い。しかし，リバビリン関連の貧血の進行に十分注意し，合併症の対策をうまく行えばSVRが見込める可能性がある。

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内科

B型肝炎キャリアの長期経過

Q 67歳、男性。幼小児期にHBVキャリアであった。20～30歳代にかけてトランスアミナーゼが上昇し、結果的にHBe抗原（－）、HBe抗体（＋）とSC（seroconversion）を確認されている。その後も在職中は定期的に肝機能検査を行っていた。退職後は検査はしていないが、自覚症状はない。最近の検査で表1のようなデータが得られた。

(1)HBV-DNAが陰性であるからDane粒子（その外被がHBs抗原）も小型球形粒子、管状粒子も形成されないと思うが、この患者血中に大量のHBs抗原が存在している理由について。

(2)SCしてからかなりの年数が経っているのにHBs抗原→HBs抗体への転換が起こっていないのはなぜか。

(3)HBe抗原を産生できる野生株からそれを産生できない変異株に変わるとHBe抗体が検出できるようになる（SC）。HBe抗原が産生されている間はHBe抗体は検出されないが、変異株になってHBe抗原量が少なくなると検出できるようになるといわれているが、理由を。

（高知県　F）

A 幼少時からHBウイルス（HBV）感染が確認されているHBVキャリアの自然経過と検査法に関わる質問にお答えする。

まず、一般的なHBVキャリアの自然経過を述べる[1)2)]。ほとんどのHBVキャリアは母児間感染（垂直感染）か、免疫力が未熟な3歳頃までにHBVに感染してキャリア（持続感染）化している。幼少時は免疫力が未熟なために肝炎は起こらない「免疫寛容期（immune tolerance）」で、HBVの増殖は活発でHBV-DNA量は10^8～10^{11}コピー／mlと高値を示し、HBe抗原陽性、肝機能正常である。次に免疫力が成熟する20歳前後に肝機能異常を認めて「肝炎期（immune clearance）」となる。HBV-DNA量が減少し始めてHBV-DNA量は10^6～10^{10}コピー／mlとなり、85%前後はHBe抗原、抗体のSCを認める。HBV-DNA量が10^4コピー／ml以下と著明

表1　最近の肝機能検査結果

RBC	412 × 10^4／μl	
WBC	62 × 10^2／μl	
Hg	13.4g／dl	
Hct	39.9%	
血小板	16.0 × 10^4／μl	
AST	24.0IU／l	
ALT	14.2IU／l	
γ-GTP	22.3IU／l	
HBs抗原定量	× 4,096	[× 8未満]
HBs抗体	× 8未満	[× 8未満]
HBe抗原	(－)	
	測定値 0.27s／co	[／未満]
HBe抗体	陽性	
	抑制率 99.5%	[50.0未満]
HBe抗体	陽性	
	10.9s／co	[<1.00未満]
HBV-DNA TMA	3.7未満 LGE／ml	[<3.7未満]
AFP（精密）	2.0未満 ng／ml	[<10.0以下]
コリンエステラーゼ	227U／l	[185～420]
4型コラーゲン	163ng／ml	[150以下]

[　]内は基準値.

に減少すると肝炎が沈静化して肝機能正常となり、HBe抗原、抗体のSCを認めている「非活動性キャリア期（low replication）」となる。

なお、HBe抗原陽性あるいは陰性でもHBV-DNA量が十分減少せず（10^5コピー／mℓ以上）に肝炎が持続することも一定の割合で認める。また、非活動性キャリア期（low replication）に移行したHBVキャリアの約20～30％で自然経過、あるいは免疫抑制剤投与によりHBVが再増殖してHBV-DNA量は10^3～10^8コピー／mℓとなり、肝炎の再燃が認められる「再燃期（reactivation）」となる。

さらに非活動性キャリア期（low replication）からHBs抗原が消失し、HBs抗体が陽性化する「recovery期」に移行し、血中HBV-DNAは検出限界以下となることがほとんどである。HBVキャリアにおけるHBs抗原の年間消失率は0・5～2・5％で、平均1・0％と報告されている[3]。しかし、この時期であっても免疫抑制剤を投与されるとHBs抗原の再出現が起こり、HBVが再増殖するde novo肝炎が多く経験されている。また、1998年に日本の肝移植においてもHBs抗原陰性、HBc抗体陽性ドナーからの移植でレシピエントにHBVが感染したとの報告[4]がある。つまり、HBVは肝組織中に存在し、recovery期でもHBVは消失していないのである。

(1)については、まだHBVが存在していることが理由である。検査方法の限界を考慮することが大事である。TMA法は1mℓに5000個未満のウイルス量は定量できないので3・7未満LGE／mℓでもHBV-DNA陰性ではなく、0～5000未満のウイルス量を示している。ちなみに、現在（2008年3月31日）は保険認可されている検査方法で最も感度が高いのはTaqMan PCR法で、58コピー／mℓが95％以上の検出率が得られた下限である[5]。この方法では、HBV-DNAが測定できる可能性が十分考えられる。つまり、本例の場合はHBV-DNA測定法の感度の問題である。

(2)はHBe抗原、抗体のSCは肝炎期（immune clearance）に認める。この時期のHBVはコアプロモーター領域やプレコア領域の遺伝子の変異によりHBe抗原を産生できない変異株が優位になってくるが、HBV-DNA量は免疫寛容期（immune tolerance）より減少を認めるものの、まだ10^6～10^{10}コピー／mℓくらい認められる。したがってHBs抗原はまだ産生されており、非活動性キャリア期（low replication）を経てHBV-DNAが測定限界以下となるrecovery期にHBs抗原→HBs抗体への転換、つまりHBs抗原、抗体のSCが認められるため、HBe抗原、抗体のSCとの間には年数の隔たりがあるのが普通である。住民検診では70歳以上でもHBs抗原陽性（HBe抗原、抗体はSC）が認められる。なお、稀であるがHBs抗体エスケープ変異株[6]が存在するとHBe抗原、抗体のSC以前にHBs抗原→HBs抗体への転換が認められることがある。

(3)については、かつては液性免疫（抗体産生能）が不十分のためと考えられていたこともあるが、HBe抗原量が減少してHBe抗体が検出できるようになる理由はHBe抗体の測定方法にある。通常HBe抗体はinhibition assay法により測定されるが、この方法の欠点は、血清中にある程度の量のHBe抗原が含まれていると血清中にHBe抗体が存在しても検出されないことである。丸山ら[7]により開発されたdirect ELISA法では、HBe抗原陽性のHBVキャリアでもHBe抗体が測定できることが報告され、最近では測定キットの改良によりHBe抗原と抗体の共存例が観察されることがある。

以上のように、HBVキャリアの自然経過と検査法について理解を深めていただければ幸いである。

▼▼▼文　献▼▼▼

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◆◆◆回　答◆◆◆

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Original Article

Standardized Prevalence Ratios for Chronic Hepatitis C Virus Infection Among Adult Japanese Hemodialysis Patients

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ABSTRACT

Background: Many studies have estimated the prevalence of anti-hepatitis C virus (HCV) antibody among hemodialysis (HD) patients; however, the prevalence of HCV core antigen—which indicates the presence of chronic HCV infection—is not known.

Methods: Standardized prevalence ratios (SPRs) for anti-HCV antibody and HCV core antigen among HD patients ($n = 1214$) were calculated on the basis of data from the general population ($n = 22\,472$) living in the same area.

Results: The prevalences of anti-HCV antibody and HCV core antigen were 12.5% and 7.8%, respectively, in male hemodialysis patients, and 8.5% and 4.1% in female hemodialysis patients. The SPRs (95% confidence interval) for anti-HCV antibody and HCV core antigen were 8.39 (6.72–10.1) and 12.9 (9.66–16.1), respectively, in males, and 5.42 (3.67–7.17) and 8.77 (4.72–12.8) in females.

Conclusions: The prevalences of chronic HCV infection among male and female HD patients were 13-fold and 9-fold, respectively, those of the population-based controls. Further studies should therefore be conducted to determine the extent of chronic HCV infection among HD patients in other populations and to determine whether chronic HCV infection contributes to increased mortality in HD patients.

Key words: hepatitis C virus infection; hemodialysis; standardized prevalence ratio (SPR); population-based study; cross-sectional analysis

INTRODUCTION

The prevalence of hepatitis C virus (HCV) infection in hemodialysis patients is very high.[1–15] Because hemodialysis patients are vulnerable to HCV infection due to the risk of HCV exposure associated with the dialysis procedure and blood transfusion,[16–18] infection control measures have been established to reduce the risks of HCV infection. Tests for detecting antibodies to HCV were first licensed by the Food and Drug Administration (FDA) in 1990[19] and are now used worldwide. The risk of HCV infection due to dialysis and blood transfusion has therefore dramatically decreased.

The estimated prevalence of HCV infection in hemodialysis patients, although lower than in the past, remains high in developed countries in Europe, despite measures to prevent transmission of HCV.[13,20,21] It has been suggested that HCV infection independently contributes to increased mortality among hemodialysis patients.[14,22–26] In order to reduce mortality associated with HCV infection among hemodialysis patients, the prevalence of HCV infection and the factors that predispose hemodialysis patients to HCV infection require immediate investigation.

The prevalence of anti-HCV antibody among hemodialysis patients has been estimated in many studies, but the prevalence of chronic HCV infection is not known. In

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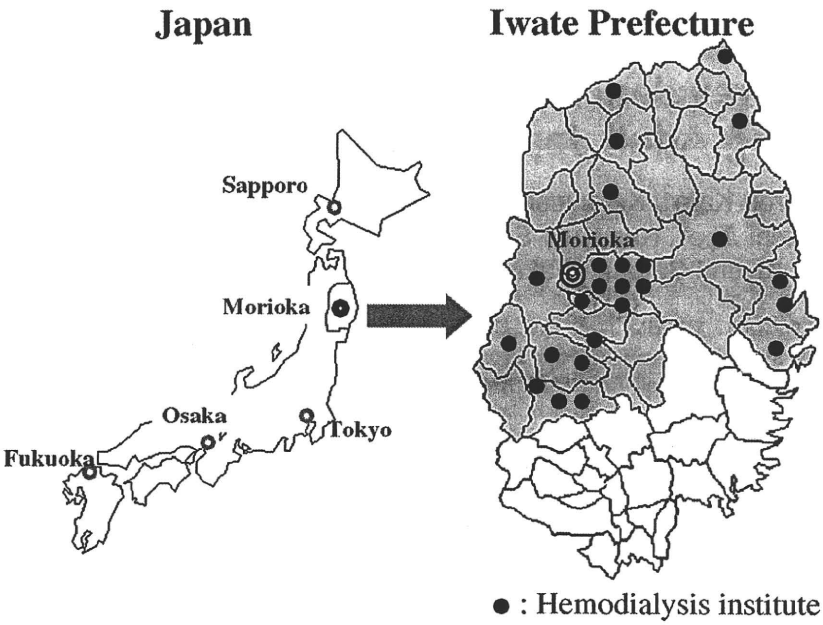

Figure 1. Maps of the KAREN Study area.
The maps show the location of Morioka (the capital of Iwate Prefecture), in northeastern Honshu island. The KAREN Study area (shaded area) covers approximately two-thirds of Iwate Prefecture, and includes 26 hemodialysis facilities; only 1 facility (in which 7 patients were treated) was not included in the study. Closed circles indicate the sites of the hemodialysis facilities.

general, patients who are anti-HCV antibody-positive include those who are chronically infected and those who have recovered from infection. However, all patients who are HCV core antigen-positive are considered chronically infected. Therefore, it is necessary to test for both anti-HCV antibody and HCV core antigen to accurately assess the extent of chronic HCV infection in hemodialysis patients.

We investigated the prevalences of anti-HCV antibody and HCV core antigen in hemodialysis patients. We then compared these prevalences with those of the general population and examined associations between the prevalences and hemodialysis vintage.

SUBJECTS AND METHODS

Subjects

We have conducted the "Kaleidoscopic Approaches to patients with end-stage RENal disease Study" (the KAREN Study) since 2003 in northern Japan (Figure 1). The KAREN Study is a population-based prospective study designed to determine the effects of risk factors on cardiovascular morbidity and mortality in end-stage renal disease (ESRD) patients.[27] A total of 1214 adult hemodialysis patients (80.6% of the total number of hemodialysis patients in the study area; age 22 to 95 years; 779 males and 435 females) are included in the KAREN Study. Figure 2 shows a flow chart of the procedure for selecting subjects participating in the KAREN Study.

Control subjects were recruited from the general population living in the same area, and comprised 22 474 participants (7650 men and 14 824 women) who underwent annual health check-ups in Iwate Prefecture and HCV screening tests in 2005.

This study was approved by the Medical Ethics Committee of Iwate Medical University and was conducted in accordance with the guidelines of the Declaration of Helsinki.

Measurements

The initial investigations in the KAREN Study were conducted from June 2003 through March 2004. These consisted of a questionnaire, review of medical records, measurements of blood pressure and anthropometric data, and blood tests. Anthropometrical examinations and blood pressure measurements were performed in a consistent manner. Self-administered questionnaires were used to collect individual information on demographic characteristics, history of cardiovascular disease, use of medication, alcohol consumption, and smoking status.[27]

Two medical doctors and 8 nurses visited 25 medical facilities and reviewed patients' medical records and treatment regimens. They recorded patient characteristics, such as age, sex, past history, family history, date when hemodialysis was initiated, length of hemodialysis sessions, number of hemodialysis sessions per week, prescribed dry weight, interdialysis weight gain at the beginning of the week, cause of ESRD, diabetes status, comorbid conditions, current medications, and history of other hemodialysis treatment.[27]

In the present study, information on anti-HCV antibody serology testing was collected by reviewing medical charts. All anti-HCV antibody serology tests at the 25 medical

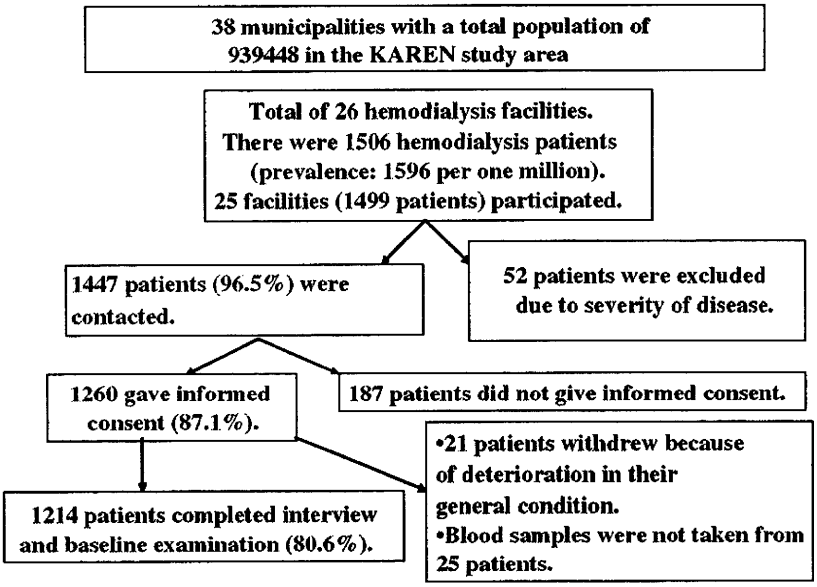

Figure 2. Flow chart for selecting subjects participating in the KAREN Study.
A total of 1506 adult patients were undergoing hemodialysis in 26 institutes in the study area. We were able to contact 1447 patients (96.5%); an additional 52 patients were excluded because of the severity of their condition. A total of 1260 patients (87.1%) gave written informed consent for participation in the study. Of these, 1214 (80.6%) completed the baseline examination.

facilities were performed by using a second- or third-generation assay.

Predialysis blood sampling was performed by dialysis nursing staff immediately before beginning hemodialysis sessions. Blood samples were drawn from arteriovenous fistulae or grafts through hemodialysis cannulae into vacuum tubes. The blood samples were transported to a laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Morioka branch office), and biochemical measurements and combined blood counts were performed on the same day. Residual sera of each sample were collected and stored at −80 °C in our laboratory.

Results of anti-HCV antibody tests could not be obtained from 50 patients upon reviewing their medical charts. Frozen serum samples from those patients were unfrozen and anti-HCV antibody tests were performed using a second-generation assay (Architect HCV, Abbott, Japan). Frozen samples from patients who were positive for anti-HCV antibody (as confirmed by chart review or by HCV antibody determination using frozen samples) were unfrozen and HCV core antigen tests were performed using the Chemiluminescent Enzyme Immunoassay (CLEIA). Quantitative determination of HCV-RNA by reverse transcription polymerase chain reaction (RT-PCR) was not performed in hemodialysis patients who were positive for anti-HCV antibody and negative for HCV core antigen (Figure 3).

The HCV screening survey of the general population was conducted in Iwate Prefecture in 2005. All samples were transported to a laboratory (Iwate Health Service Association), and HCV antibody serology tests were performed by using an enzyme immunoassay (AxSYM HCV Dynapack II, Abbott Japan). Additional HCV core antigen tests were also performed using CLEIA in subjects who were positive for HCV antibody. A total of 236 samples from participants who were positive for anti-HCV antibody and negative for HCV core antigen were then used for qualitative determination of HCV-RNA by RT-PCR (AMPLICOR TM HCV test, Roche, Figure 4).

Statistical analysis

Hemodialysis patients and population-based control subjects were divided into sex- and age-specific groups (20–39, 40–49, 50–59, 60–69, and ≥70 years). Sex- and age-specific prevalences of anti-HCV antibody and HCV core antigen were determined both in hemodialysis patients and controls.

Among hemodialysis patients, the expected number of patients positive for anti-HCV antibody (or HCV core antigen) in each sex- and age-specific group was calculated by using the prevalence of each sex- and age-specific group from the population-based controls. The total number of expected patients positive for anti-HCV antibody (or HCV core antigen) among hemodialysis patients was calculated by summing the numbers of positive individuals expected in all age-specific groups. The ratio of the observed number of hemodialysis patients with anti-HCV antibody (or HCV core antigen) to the expected number was defined as the standardized prevalence ratio (SPR). We assumed that the data would have a Poisson distribution; therefore, the confidence intervals for the SPRs were estimated using standard errors.[28]

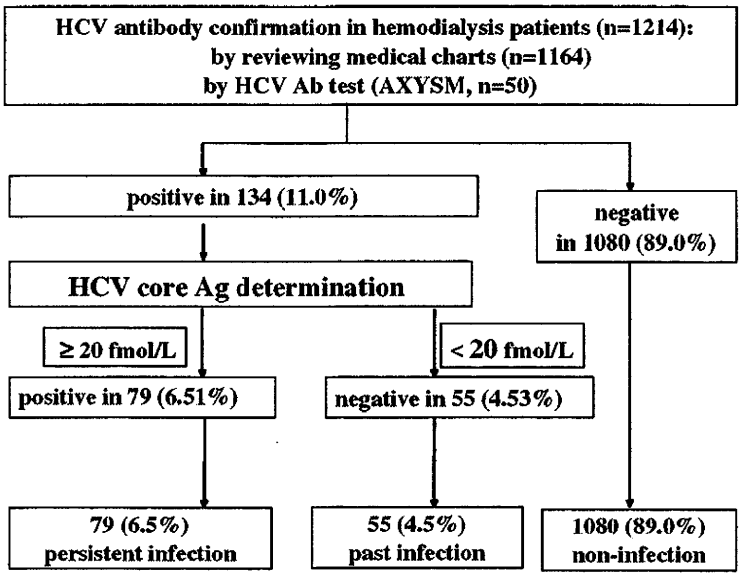

Figure 3. Flow chart of HCV antibody and HCV core antigen screening in hemodialysis patients in the KAREN Study. Information on HCV serology tests was not collected from 50 subjects in the KAREN Study. For those 50 subjects, we defrosted frozen serum samples and performed HCV antibody tests using Architect HCV (Abbott, Japan). A total of 134 subjects (11.0%) were positive for HCV antibody. HCV core antigen tests were then performed for those subjects. A total of 79 were positive for HCV core antigen and were classified with persistent HCV infection (6.0%).

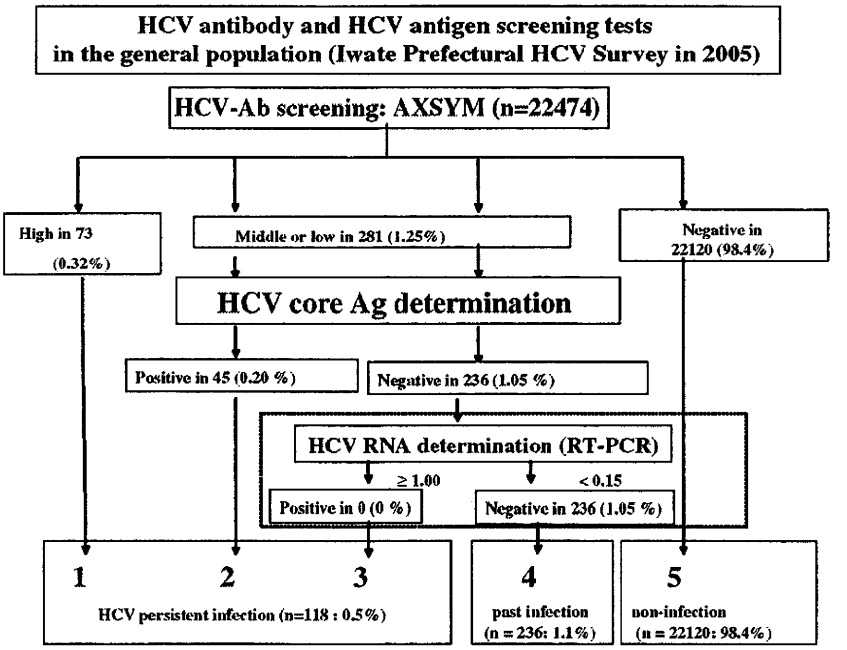

Figure 4. Flow chart of HCV antibody and HCV core antigen screening in population-based controls (Iwate Prefectural HCV survey in 2005).
There were 22474 participants who underwent annual health check-ups and HCV screening. A total of 354 subjects were positive for HCV antibody (1.57%). HCV core antigen tests were performed in subjects with low- or middle-range positivity for HCV antibody. A total of 45 were positive for HCV core antigen. HCV-RNA determination using the RT-PCR method was performed in 236 subjects, but none were positive. Ultimately, 118 subjects were classified with persistent HCV infection (0.53%).

Hemodialysis patients were also divided into 6 groups according to dialysis "vintage" (length of time on dialysis): <6 months, 6 to 23 months, 2 to 4 years, 5 to 9 years, 10 to 14 years, or 15 years or longer. Prevalences of anti-HCV antibody and HCV core antigen in each group were estimated. Differences in prevalences by sex or dialysis vintage (vintage ≥10 years vs <10 years) were tested using the chi-square test. To examine whether each risk factor was

Table 1. Sex- and age-specific prevalences of anti-HCV antibody in hemodialysis patients and a general population

Age group	General population		HD patients	
	Total No.	HCV Ab-positive (%)	Total No.	HCV Ab-positive (%)
Men				
20–39	36	0 (0.0%)	52	4 (7.7%)
40–49	890	16 (1.8%)	96	13 (13.5%)
50–59	1564	14 (0.9%)	191	38 (19.9%)
60–69	3001	43 (1.4%)	233	27 (11.6%)
≥70	2159	50 (2.3%)	207	15 (7.2%)
total	7650	123 (1.6%)	779	97 (12.5%)
Women				
20–39	62	0 (0.0%)	22	0 (0.0%)
40–49	2662	22 (0.8%)	55	5 (9.1%)
50–59	3980	40 (1.0%)	121	5 (4.1%)
60–69	4927	87 (1.8%)	116	1 (0.9%)
≥70	3193	82 (2.6%)	121	11 (9.1%)
total	14824	231 (1.6%)	435	37 (8.5%)

Abbreviations: HCV, hepatitis C virus; HD, hemodialysis; No., number; Ab, antibody.

Table 2. Sex- and age-specific prevalences of HCV core antigen in hemodialysis patients and normal controls

Age group	General population		HD patients	
	Total No.	HCV core Ag-positive (%)	Total No.	HCV core Ag-positive (%)
Men				
20–39	36	0 (0.0%)	52	3 (5.8%)
40–49	890	8 (0.9%)	96	8 (8.3%)
50–59	1564	5 (0.3%)	191	32 (16.8%)
60–69	3001	16 (0.5%)	233	12 (5.2%)
≥70	2159	21 (1.0%)	207	6 (2.9%)
total	7650	50 (0.7%)	779	61 (7.8%)
Women				
20–39	62	0 (0.0%)	22	0 (0.0%)
40–49	2662	5 (0.2%)	55	2 (3.6%)
50–59	3980	5 (0.1%)	121	5 (4.1%)
60–69	4927	28 (0.6%)	116	4 (3.4%)
≥70	3193	30 (0.9%)	121	7 (5.8%)
total	14824	68 (0.5%)	435	18 (4.1%)

Abbreviations: HCV, hepatitis C virus; HD, hemodialysis; No., number; Ag, antigen.

independently associated with chronic HCV infection or past HCV infection, logistic regression analysis was performed using presence of chronic HCV infection or history of HCV infection as the dependent variable and age, sex, and dialysis vintage as explanatory variables. A P value less than 0.05 was considered statistically significant. All statistical analyses were performed using the SPSS software package (SPSS, Japan Inc., Version 14.0).

RESULTS

Table 1 shows sex- and age-specific prevalences of anti-HCV antibody in hemodialysis patients and population-based controls. Among population-based controls, the prevalence of anti-HCV antibody increased with advancing age; however, no such association was observed among hemodialysis patients. A sex difference in the prevalence of anti-HCV antibody was not found in the population-based controls; however, among the hemodialysis patients, the prevalence of anti-HCV antibody was higher in men than in women (12.5% vs 8.5%, $P < 0.05$).

The prevalence of anti-HCV antibody was considerably higher in hemodialysis patients than in controls. The SPR (95% CI) for anti-HCV antibody was 8.39 (6.72–10.1) in male hemodialysis patients and 5.42 (3.67–7.17) in female hemodialysis patients.

Table 2 shows sex- and age-specific prevalences of HCV core antigen in hemodialysis patients and population-based controls. A positive association between the prevalence of HCV core antigen and age was found in controls but not in hemodialysis patients. The prevalence of HCV core antigen was also higher in male hemodialysis patients than in female hemodialysis patients (7.8% vs 4.1%, $P < 0.05$). The SPR (95% CI) for HCV core antigen was 12.9 (9.66–16.1) in male hemodialysis patients and 8.77 (4.72–12.8) in female hemodialysis patients.

Table 3. Prevalences of anti-HCV antibody and HCV core antigen among hemodialysis patients, stratified by hemodialysis vintage

HD vintage	No.	HCV Ab-positive (%)	HCV core Ag-positive (%)
Men			
<6 months	44	4 (9.1%)	3 (6.8%)
6–23 months	158	14 (8.9%)	8 (5.1%)
2–4 yrs	218	18 (8.3%)	10 (4.6%)
5–9 yrs	176	15 (8.5%)	7 (4.0%)
10–14 yrs	75	10 (13.3%)	8 (10.7%)
≥15 yrs	108	36 (33.3%)	25 (23.1%)
total	779	97 (12.5%)	61 (4.6%)
Women			
<6 months	18	1 (5.6%)	1 (5.6%)
6–23 months	74	4 (5.4%)	3 (4.1%)
2–4 yrs	129	8 (6.2%)	4 (3.1%)
5–9 yrs	109	8 (7.3%)	4 (3.7%)
10–14 yrs	49	3 (6.1%)	3 (6.1%)
≥15 yrs	56	13 (23.2%)	3 (5.4%)
total	435	37 (8.5%)	18 (4.1%)

Abbreviations: HCV, hepatitis C virus; HD, hemodialysis; No., number; Ab, antibody; Ag, antigen.

Table 3 shows prevalences of anti-HCV antibody and HCV core antigen by dialysis vintage. Male and female patients with longer hemodialysis vintages (10–14 years or ≥15 years) had high prevalences of anti-HCV antibody than did male and female patients with a dialysis vintage less than 10 years ($P < 0.05$). Male and female patients with a dialysis vintage of 15 years or more had extremely high prevalences of anti-HCV antibody. However, among the dialysis vintage subgroups,

Table 4. Odds ratios for each risk factor for past or chronic HCV infection

Risk factor	Chronic HCV infection			Past HCV infection		
	OR	95%CI	*P*	OR	95%CI	*P*
Age (per 1 year increase)	0.99	(0.97–1.01)	0.484	1.02	(0.99–1.05)	0.107
Male sex	1.99	(1.14–3.44)	0.014	1.06	(0.60–1.89)	0.843
Dialysis vintage (per 1 year increase)	1.09	(1.06–1.12)	<0.001	1.09	(1.06–1.13)	0.006

Odds ratios and their 95% confidence intervals were estimated by logistic regression analysis.
Abbreviations: OR, odds ratio; CI, confidence interval.

Table 5. Prevalences of anti-HCV antibody and HCV core antigen (or RNA) among hemodialysis patients from various countries

Country	Author or name of study	Sample size	HCV Ab-positive (%)	Positive for HCV Ag or RNA (%)	Years tested
Japan	Washio[15]	540	24.3	—	1990
	Nakayama[24]	1470	18.8	—	1993
	DOPPS[8]	not obtained	19.9	—	1997–2001
	Kumagai[6]	1882	—	12.9[a]	1999–2003
	KAREN	1214	11.0	6.5[b]	2003–2004
United States	DOPPS[8]	not obtained	14.4	—	1997–2001
	Da Vita[14]	13664	11.6	—	2001–2004
Belgium	Jadoul[13]	629	6.8	—	2000
France	DOPPS[8]	not obtained	14.7	—	1997–2001
Germany	Hinrichsen[9]	2796	7.0	—	1996–1997
United Kingdom	DOPPS[8]	not obtained	2.7	—	1997–2001
Italy	DOPPS[8]	not obtained	22.2	—	1997–2001
Iran	Shamshirsaz[10]	593	—	8.6[a]	2004[c]
Tunisia	Hmaied[11]	395	20	14[a]	2001–2003
Thailand	Luengrojanakul[12]	221	—	19.9[a]	1994

Abbreviations are the same as those used in Tables 1, 2, and 3. Italics indicate the present study.
Superscript numbers correspond to the reference used in the present study.
[a], determined by HCV-RNA test by the PCR method; [b], determined by HCV core antigen test.
[c], Not clearly described when blood sampling was performed (published in 2004).

male patients with a dialysis vintage of 15 years or more had the highest prevalence of HCV core antigen.

Both male and female patients in the 4 groups with the shortest dialysis vintage (ie, <10 years) had similar prevalences of HCV antibody, regardless of dialysis vintage (approximately 9% in male hemodialysis patients and 5% in female hemodialysis patients in each of the 4 groups).

Table 4 shows the odds ratios attributable to each factor for having chronic HCV infection or past HCV infection. Male sex and dialysis vintage were independently associated with a higher prevalence of chronic HCV infection. The prevalence of chronic HCV infection among male hemodialysis patients was double that of female patients. However, only hemodialysis vintage was independently associated with an increased prevalence of past HCV infection.

DISCUSSION

In this study, we analyzed the prevalences of HCV antibody and HCV core antigen in adult hemodialysis patients. We estimated SPRs for both anti-HCV antibody and HCV core antigen among hemodialysis patients, and compared these estimates to those of the general population living in the same area.

Patients who are positive for HCV core antigen all have chronic HCV infection, whereas patients with anti-HCV antibody include those who have recovered from HCV infection, as well as those with chronic HCV infection. In a general population, patients who have recovered from HCV infection never develop liver cirrhosis or hepatocellular carcinoma (HCC) due to HCV, whereas patients with chronic HCV infection will develop liver cirrhosis or HCC 20 to 30 years after initial infection.[29] Therefore, in a general population, information regarding chronic HCV infection is more important than information on anti-HCV antibody.

In their study of Tunisian hemodialysis patients, Bouzgarrou et al reported that an HCV core antigen assay based on the HCV-RNA test had high sensitivity and high specificity; however, they were unable to provide an accurate estimate of the prevalence of chronic HCV infection and past HCV infection because of the large number of missing cases.[30]

Table 5 shows prevalences of anti-HCV antibody and chronic HCV infection (positivity for HCV core antigen or HCV RNA) in several studies with large sample sizes.[6,8–15,24] Hmaied reported the prevalences of both anti-HCV antibody and HCV-RNA.[11] The proportion of patients with HCV-RNA

among patients with anti-HCV antibody was 70% in their study, and this proportion is similar to that of patients with HCV core antigen among patients with anti-HCV antibody in our study; it is also similar to the proportion of patients with chronic infection among all patients with HCV infection in the general population.[31]

We determined the prevalences of anti-HCV antibody and HCV core antigen in hemodialysis patients who were divided into 6 groups according to hemodialysis vintage. Patients with a hemodialysis vintage of 10 years or more had significantly higher prevalences of anti-HCV antibody and HCV core antigen than did patients with shorter hemodialysis vintages. Furthermore, patients with a hemodialysis vintage of 15 years or more had significantly higher prevalences of anti-HCV antibody than did other groups.

Since 1981, the Japanese Red Cross Blood Transfusion Service has excluded blood samples from donors with high serum ALT levels (≥36 KU/mL) in order to prevent transfusion of blood with non-A non-B hepatitis virus. Erythropoietin has been used clinically for treatment of anemia since 1986. In 1989, the Japanese Red Cross Blood Transfusion Service began using a first generation assay to screen blood donors for anti-HCV antibody.[32] The timing of the introduction of these programs explains the relatively low prevalence of HCV infection among patients with a dialysis vintage less than 10 years and the extremely high prevalence of HCV infection among patients with a dialysis vintage of 15 years or more.

Choo and Kuo first developed a specific assay for HCV in 1989,[33,34] and a second-generation ELISA, which was more sensitive than the first-generation ELISA, was developed in 1992 and became widely used as a clinical diagnostic tool and for epidemiological and other investigative purposes. As a result, the risk of nosocomial HCV infection has dramatically decreased among hemodialysis patients who started hemodialysis treatment after 1992. Our results showing a high prevalence of HCV infection among patients with a hemodialysis vintage of 10 years or more are consistent with the fact that risks for HCV infection have been reduced by the development and widespread use of HCV assays.

However, as compared to the general population, patients with a hemodialysis vintage of less than 10 years had a significantly higher prevalence of HCV infection, even though they would be expected to be at low risk of HCV infection due to blood transfusion and dialysis. This cross-sectional analysis also showed that prevalences were similar among the groups of patients with a dialysis vintage less than 10 years (ie, <6 months, 6–23 months, 2–4 years, 5–9 years), which suggests that most hemodialysis patients with HCV infection became infected before initiation of hemodialysis treatment, and that only a few patients with HCV infection developed the infection after initiation of hemodialysis treatment.

The incidence rate of HCV infection among hemodialysis patients is reported to be lower than 0.5 percent per year,[6,35] indicating that the very high prevalence of HCV infection among hemodialysis patients is not entirely due to the elevated risk of nosocomial infection associated with dialysis therapy. There are several possible pathways for HCV transmission before initiation of hemodialysis. Patients with renal failure may have a high prevalence of HCV infection, regardless of the severity of renal failure, or, alternatively, patients with HCV infection may have a high prevalence of renal failure. It has been shown that HCV is associated with an increased prevalence of renal insufficiency.[36] Renal diseases associated with HCV infection may also contribute to the high prevalence of HCV infection among patients with kidney disease.[37]

Another possible explanation is that patients with mild-to-moderate renal failure (ie, patients with chronic kidney disease) tend to develop ESRD after HCV infection, which may contribute to the high prevalence of HCV among patients with ESRD. Two studies have shown that HCV infection contributed to an increased risk of developing ESRD.[38,39] If HCV infection does indeed contribute greatly to the development of ESRD, better prevention and treatment strategies for HCV infection should not only decrease liver disease-related mortality, they should also decrease the development of ESRD and its related mortality in patients with CKD and in the general population.

Although there was no sex-based difference in the prevalence of HCV infection in the general population, the prevalences of anti-HCV antibody and HCV core antigen were higher in male hemodialysis patients than in female hemodialysis patients. This suggests that male hemodialysis patients are at greater risk for HCV infection, perhaps due to the presence of predisposing factors for HCV infection.

Male hemodialysis patients with a long hemodialysis vintage (≥10 years) had a high rate of chronic HCV infection (70%: the percentage of patients who were positive for HCV core antigen among those were positive for anti-HCV antibody); however, female patients with a similarly long hemodialysis vintage had a lower rate of chronic HCV infection (37.5%). Male sex was independently associated with a high prevalence of HCV core antigen in logistic regression analysis. These data suggest that male hemodialysis patients have a greater risk of HCV infection, and a greater risk of persistent HCV infection, than do female hemodialysis patients.

Thomas et al reported that the spontaneous clearance rate of HCV among female patients was 1.58 times that of male subjects; however, the finding was of only marginal statistical significance.[40] Women are less likely to be regular alcohol drinkers.[27,31] In addition, they have higher levels of serum HDL cholesterol[27,41] and perhaps other unknown protective factors. This may attenuate their risks of initial and chronic HCV infection, and may explain the observed sex-based differences.

Another possible explanation is that women who had recovered from HCV were selectively registered in the study

because of a very high mortality rate for women with chronic HCV infection. However, to our knowledge, no studies have shown that female patients with chronic HCV infection have a higher mortality rate than that of patients who have recovered from HCV infection.

One major feature of this study is the long dialysis vintage of the participants. Mean dialysis vintage of the study participants exceeded 7 years; mean dialysis vintage was only approximately 3 years in reports from the United States and Europe.[42] The generous medical insurance reimbursement system for Japanese dialysis patients and the high quality of hemodialysis treatment, which includes legal controls that strictly restrict re-use of a dialyzer, may have contributed to the longevity of hemodialysis patients. More than 20% of patients in the present study had long dialysis vintage (≥10 years), and long dialysis vintage was associated with a high prevalence of HCV infection in our study.

Since hemodialysis patients have a short life expectancy, there are few cases in which liver cirrhosis or HCC develops long after initiation of hemodialysis. Nakayama and Fabrizi found that hemodialysis patients who were anti-HCV antibody-positive had higher rates of liver disease-related deaths.[24,26] However, the authors did not reveal whether an elevated mortality rate among hemodialysis patients with anti-HCV antibody was totally attributable to the increase in liver disease-related deaths. It is necessary to determine which cause of death contributes to the increase in mortality among hemodialysis patients with HCV infection.

This study was based on data from a population-based study and the sample size was sufficient to satisfy our objectives. Indeed, the large sample size of population-based controls living in the same area is one of the strengths of the study. However, several limitations to our study should be noted. The cross-sectional design of the present study cannot prove causal relationships. In addition, the lack of HCV-RNA data on the hemodialysis subjects who were positive for HCV antibody and negative for HCV core antigen is a major limitation in our study. It is possible that hemodialysis patients who are negative for HCV core antigen nevertheless have very low levels of HCV-RNA; however, the possibility of missing such cases in the present study is very low because, among the population-based controls, none were simultaneously positive for both HCV-RNA and HCV antibody and negative for HCV core antigen (Figure 4). Therefore, we believe that the results of the study were not distorted by lack of data regarding HCV-RNA. A history of blood transfusion is a strong predisposing factor for HCV infection. Thus, lack of information about past history of blood transfusion is also a major limitation. In addition, people who did not participate in the annual health check-ups may have been in poor health and might have had liver disease. This would have resulted in an underestimation of HCV infection in the general population and overestimation of the SPR for HCV among hemodialysis patients.

In conclusion, the prevalences of chronic HCV infection in male and female hemodialysis patients are 13 times and 9 times those of men and women in the general population. Further studies should therefore be carried out to determine the extent of chronic HCV infection in hemodialysis patients in other populations and to determine whether chronic HCV infection contributes to increased mortality in hemodialysis patients.

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Long-Term Presence of HBV in the Sera of Chronic Hepatitis B Patients with HBsAg Seroclearance

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Key Words
Chronic hepatitis B · Hepatitis B virus DNA · Seroclearance, hepatitis B surface antigen

Abstract
Objects: The aim of this study was to elucidate the presence of serum hepatitis B virus (HBV) DNA at a prolonged time after seroclearance of hepatitis B surface antigen (HBsAg). ***Methods:*** Seventy Japanese patients who had been observed for >5 years after HBsAg seroclearance were included in this study. Anti-HBs, anti-HBe and anti-HBc antibodies were measured 0, 5 and 10 years after HBsAg seroclearance. Serum HBV DNA was measured using nested polymerase chain reaction (PCR) at 0, 5 and 10 years after HBsAg seroclearance. The PCR detection of serum HBV DNA using the X gene and core gene primers was done. The HBV DNA was regarded as positive when PCR detection of HBV DNA using either or both the X gene and core gene primers was positive. A multivariate regression analysis was used to assess the factors contributing to the positivity of serum HBV DNA 5 years after HBsAg seroclearance: the factors examined included age, gender, histological findings, HBV genotype, aminotransferase, total protein and interferon administration. ***Results:*** The titers of 200-fold diluted serum anti-HBc were 6.5 ± 4.0 at 0 year after HBsAg seroclearance, 1.8 ± 1.4 at 5 years and 0.9 ± 0.7 at 10 years. The titers of 200-fold diluted serum anti-HBc decreased 5 and 10 years after HBsAg seroclearance with statistical significance. The positive rate of HBV DNA by the nested PCR was 71.4% (50/70) at 0 year after HBsAg seroclearance, 21.4% (15/70) at 5 years and 14.3% (3/21) at 10 years. However, there were no significant factors contributing to the positivity of serum HBV DNA 5 years after HBsAg seroclearance. ***Conclusion:*** Our results suggest that serum HBV DNA disappears with an incidence of 10–20% 5 and 10 years after HBsAg seroclearance.

Introduction

Chronic hepatitis B is a serious liver disease with significant mortality. In patients with chronic hepatitis B virus (HBV) infection, persistent viral replication is associated with ongoing necroinflammation in the liver and progressive liver damage [1–3]. However, in patients with seroclearance of hepatitis B envelope antigen (HBeAg) and marked reduction of HBV DNA, the prognosis of the disease is generally improved [4–6]. Moreover, hepatitis B surface antigen (HBsAg) seroclearance has probably been associated with a good prognosis [7–12].

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An explosion of papers argue that some patients with seroclearance of HBsAg showed positive HBV DNA at the time of HBsAg seroclearance or within 1 year of HBsAg seroclearance [13–17]. However, it is not clear how long serum HBV DNA could be detected after prolonged observation after HBsAg seroclearance. Moreover, it is still a question whether the patients with seroclearance of HBsAg could be really cleared of serum HBV DNA or not. To further investigate these issues, we performed the present study on the long-term virological outcome after HBsAg seroclearance in Japanese patients.

Materials and Methods

Patients

From 1972 to 2002, a total of 5,055 chronic HBsAg carriers, who were known to be seropositive for HBsAg for at least 6 months, were studied in Toranomon Hospital in Tokyo, Japan. After a mean follow-up period of 4 years (range 0.5–30 years), 231 patients were noted to have delayed HBsAg seroclearance, which is defined as persistent absence of HBsAg antigenemia by radioimmunoassay for at least 1 year until the last examination. Of these 70 patients had the following criteria: (1) laparoscopy and liver biopsy taken before HBsAg seroclearance showed histological features of chronic active hepatitis or liver cirrhosis; (2) the follow-up period was more than 5 years after seroclearance of HBsAg.

We excluded from the study all the patients: (1) with concurrent hepatitis C virus and hepatitis D virus; (2) with a history of alcohol abuse or autoimmune liver disease; (3) with clinical evidence of hepatocellular carcinoma at entry into the study on the basis of ultrasonography, α-fetoprotein levels (<200 ng/ml) and/or histology; (4) with a history or clinical evidence of complications of decompensated cirrhosis at enrollment (that is ascites, encephalopathy or icterus).

Thirty-seven of 70 patients had spontaneous seroclearance of HBsAg, 20 patients had been given interferon (IFN) therapy for 1–16 months, 9 had been given steroid withdrawal monotherapy and 4 had been treated with combination therapy of steroid + IFN. The total median dose of IFN therapy was 336 mega units (range, 168–624 mega units). The patients treated with steroids were generally given prednisolone for 4 weeks, in a single dose of 40 mg/day for 1 week, 30 mg/day for 1 week, 20 mg/day for 1 week and then 10 mg/day for 1 week until it was abruptly withdrawn (total dose 700 mg).

Mthods

The serums were stored at −80° until enzyme assays and measurement of HBV DNA level by the nested PCR method could be performed on all the samples for 70 patients at one time. Serum samples had been conserved at 0, 5 and 10 years after seroclearance of serum HBsAg. Serum HBV DNA was determined using the nested PCR independently by an experienced technician (J.S.), who had no clinical information or knowledge of each patient. The sensitivity of HBV DNA according to the manufacturer is about 50–100 copies/ml in the nested PCR method. Two kinds of primers in the core and X gene of HBV were used in the nested PCR method. First of all, primers used for the detection of HBV were Cof1 (sense, 5′-CTGCCTTACTTTTGGAGAGA-3′) and Cer1 (antisense, 5′-ACTTTACTGGGCTTTATTA-3′) for the first PCR and core sense (sense, 5′-GAGTGTGGATTCGCACTCC-TC-3′) and anticore (5′-GATTGAGATCTTCTGCGACGC-3′) for the second PCR in the core gene. Second, primers used for detection of HBV were P2 (sense, 5′-GTCCCGTCGGCGCTGAAT-CCC-3′) and Br102 (antisense, 5′-GCAGATGAGAAGGCACA-GAC-3′) for the first PCR and X sense (sense, 5′-CTGGATCCT-GCGCGG GACGTCCTT-3′) and anti-X (5′-GTTCACGGTGGT-CTCCAT-3′) for the second PCR in the X gene. In the first PCR and the second PCR, amplification was performed over 35 cycles (94 for 1 s; 55° for 1 s; 72° for 1 s) after initial denaturing at 94° for 4 min and a final extension at 72° for 7 min. Negative and positive controls confirmed the HBV DNA band in parallel. Ten healthy volunteers without HBsAg and anti-HCV were selected for negative HBV DNA controls. Ten patients with chronic hepatitis B and with HBsAg were selected for positive controls. The HBV DNA was considered positive when PCR detection of HBV DNA using either or both the X gene and core gene primers showed positivity. On the other hand, the HBV DNA was considered negative when PCR detection of HBV DNA using both the X gene and core primers showed negativity.

When serum samples showed positive HBV DNA by the nested PCR, we also examined the serum HBV DNA level. It was measured by a transcription-mediated amplification and hybridization-protection assay (Chugai Diagnostics, Tokyo, Japan), and the results were expressed as log genome equivalents (LGE) per milliliter. The lower detection limit of this assay is 3.7 LGE/ml, which is equivalent to 5,000 copies/ml.

HBsAg, anti-HBs, HBeAg, anti-HBe and antibody to HDV were all assayed using commercially available radioimmunoassay kits. Anti-HBc was assayed by chemiluminescent enzyme immunoassay. Antibody against HCV was detected with a third-generation enzyme-linked immunoassay (Ortho Diagnostic Japan, Tokyo). The HBV genotype was determined using a previously reported method [18]. Biochemical tests were made using routine automated techniques and carried out in the laboratories of Toranomon Hospital. This study was approved by the institutional review board of our hospital. The physicians in charge explained the purpose and method of this clinical trial to each patient, who gave their informed consent for participation.

Liver Histology

Liver biopsy specimens were obtained percutaneously under the observation by laparoscopy using a modified Vim Silverman needle with an internal diameter of 2 mm (Tohoku University style, Kakinuma Factory, Tokyo, Japan).

Statistical Analysis

We used Fisher's exact test (two-tailed) or the Wilcoxon rank sum test to compare differences between groups. Moreover, we used univariate analysis and multivariate analysis (multiple logistic regression analysis) to establish which factors contributed to the positivity of HBV DNA 5 years after HBsAg seroclearance. Results for each variable were transformed into categorical data consisting of two simple original numbers for univariate and multivariate analyses. Variables that achieved statistical significance