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Part 5

透析スタッフの感染を防ぐために

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はじめに

透析患者は，かつてエリスロポエチンの導入以前輸血を受ける機会が多かったことや免疫能が低下していることより，感染症を合併する率が高い．これらの患者と接し，観血的処置をする透析スタッフも，感染のリスクが高い環境にいるといえる．

1970年代はじめ，一般の医療機関の医療従事者におけるB型肝炎の感染率が年間5％だったころ，透析施設においては感染率が年間25～44％と，異常に高かった時代とは比べようもないほど，透析スタッフの院内感染は減少したが，未だ根絶されたとはいえない．透析スタッフの感染は本人にとっても大問題であるとともに，透析患者に対する感染源となる可能性もあり，その施設全体の医療レベルが問われる事態であるといっても過言ではない．

さらに，「自分を守ることが患者を守ることに通じる」という意味でも，透析施設の院内感染対策において，透析スタッフの感染予防対策は重要な柱である．

このために，必要な方策として，

①標準的な透析基本操作の徹底
②感染症患者の透析時の対策
③定期健康診断および予防注射
④スタッフ教育
⑤感染事故時の対応

が挙げられる．

標準的な透析基本操作の徹底

院内感染対策の基本中の基本であり，他の項目と重複する点が多々あり，詳しくは2000年3月に各透析施設に配布された，厚生省厚生科学特別研究事業の「透析医療における標準的な透析操作と院内感染予防に関するマニュアル」を参照されたい[1]．

その要点は，すべての患者の血液，体液，分泌物，排泄物（汗を除く），障害のある皮膚，粘膜は感染源となりうるので，これらに接触したら，手洗いをすること，直接接触する可能性がある場

合は手袋を着用し，使用後手洗いをすること，顔面に飛散することが予想されるときにはマスク，眼鏡を，身体に飛散することが予想されるときにはガウンを着用することなど，いわゆるstandard precautionにつきる[2]．

これらの順守は，スタッフ個人の問題というより，施設全体でこのように仕向ける必要がある．たとえば，血液感染事故で最も問題になっている針刺し事故を防ぐには，リキャップ禁止が有効であるが，安全な廃棄容器が十分な数，適切な場所に用意されていなければ，個人の努力のみでは，100%の達成は不可能である．手洗いや手袋にしても同様に施設全体として取り組まなければならない問題である．

感染症患者の透析時の対策

透析スタッフへの感染源および感染対策の周知徹底が基本である．誰が感染症を有しているのかは，透析スタッフ全員にわかるようにしなければならない．ただし，患者のプライバシーにも配慮する必要があり，あからさまな目印を付けたりすることは避ける．スタッフの感染予防の観点からの各感染症対策の要点を挙げる．

1）肝炎ウイルス，HIV

いずれも，血液媒介感染であり，手袋の着用，手洗い，血液飛散が予想される際のゴーグル着用で予防可能である．

2）結核

空気感染するので，排菌のある結核患者を透析する際は，患者には通常のマスクをしてもらい，スタッフはN95規格の微粒子用のマスク（薄い紙マスクは無効である）および予防衣を着用する．また，換気を頻回に行う（1時間に6回程度）．

3）MRSA

健康な人間には病原性を示さないが，スタッフの手などを通して，患者間の感染を媒介する可能性があること，スタッフが無症候性キャリアーとなって，感染源となることがあるので，手袋の着用，手洗いの励行が必要である．

定期健康診断および予防注射

透析施設における感染予防上必要な定期健康診断項目は，胸部X線，肝機能検査，HBs抗原・抗体，HCV抗体である．肝機能検査，HBs抗原・抗体，HCV抗体は年2～3回施行する．新規採用時には，できるだけ早期に前記の検査を施行するが，HBs抗原やHCV抗体の検査結果を採用の条件としてはならない．

スタッフがHBs抗原陽性の無症候性キャリアーであった場合は，出血時の注意や排尿・排便時の処置など感染予防のための注意事項を守る限り，通常の透析業務に支障はない．肝機能異常をともなうHBs抗原陽性者やHCV抗体陽性者では，専門医を受診させ，指示に従う．

定期検診で，新たにHBs抗原やHCV抗体が陽性化したスタッフについては，肝機能を含めた再検査を施行し，その結果，肝機能の異常を伴い，HBs抗原やHCV抗体陽性が確認されたら，急性肝炎の可能性があるので，専門医を受診させるとともに感染経路を調査する．肝機能に異常がなく，HBs抗原やHCV抗体陽性が新たに確認されたら，急性肝炎の潜伏期，不顕性感染，従来の検査が抗原価あるいは抗体価が低かったため陰性と判定されていた可能性があり，慎重

な経過観察を要する[3].

ツベルクリン検査も，39歳以下の透析スタッフの新規採用時には施行しておくことが望ましい．その際，最初のツベルクリン検査で強陽性でない場合，以前のBCG接種によるツベルクリン反応の減弱の可能性があるため，2週間後に再度ツベルクリン検査を行って判定する2段階法が推奨されている．BCG接種歴がない，30歳未満のツベルクリン陰性者では，BCG注射を行い，翌年再度ツベルクリン検査を行うことが強く勧められている．一方，ツベルクリン陰性でも，BCG接種歴がある場合の，BCGの再接種の効果については，意見の統一がみられていない．

B型肝炎は針刺し事故後の感染率が6～30%と血液感染症のなかでは高い．HBs抗体陰性のスタッフでは，B型肝炎ワクチンの接種が勧められる．ワクチンは0，1，6ヵ月の3回施行する．HBワクチンでHBs抗体ができたスタッフでは，その抗体価を上回る抗原量（ウイルス量）の暴露があれば，肝炎を発症するが，抗体価より少なければ，発症しない．針刺し事故の場合，抗体価がPHA法で24以上であれば，通常発症は予防できる[4].

また，スタッフの精神的・肉体的疲労が感染事故にもつながりうるので，日常の健康管理のみでなく，勤務体制に無理がないか，などをつねに管理者は心掛ける必要がある．

スタッフ教育

前出のstandard precautionについては，完全に理解するように繰り返し教育が必要である．また，傷のある荒れた手や，炎症のある手で直接感染源に触れないことや，眼，口腔（こうくう）粘膜などからも感染する危険があることなどを理解すれば，おのずと透析室や検査室での飲食，喫煙が禁止されることは納得されるであろう．

新人スタッフに対しては，とくに感染対策教育が重要である．教育の内容については，透析マニュアルや日常業務上の注意をはじめ，各種感染症の感染経路を含めた病態，感染事故時の対応（後述）や，患者の人権保護なども含め，具体的に説明する．

たとえ事故につながらなかったとしても，ふだんの小さなミスを報告するようにしておく．管理者はこれらのミスに対して，短絡的に当事者をしかったり，罰したりするのでなく，マニュアル違反や思い違いがなかったかなどのミスの原因を明らかにすることが大事である．小さなミスを教訓として，当事者だけでなくスタッフ全体が注意を喚起し，再教育などの対策をとることが結局は大きなミスを防ぐことになる．

感染事故時の対応

1）針刺し事故

針刺し事故は肝炎ウイルスやHIVウイルスなどの血液感染症の透析スタッフへの感染で最も危険で注意すべき事故である．全国のエイズ拠点病院を対象にした「HIV（エイズウイルス）感染症に関する臨床研究班」の3年間の調査によると，100床あたり年間4件という高率にみられることが明らかにされた．針刺し事故を予防する方策としては，①リキャップをしない（使った針はリキャップをせずに，固い容器に廃棄する），②穿刺および抜針時には，針を廃棄するまで介助をしない，などが挙げられる．

針刺し事故における感染率は，B型肝炎ウイルスで6～30%，C型肝炎ウイルスで1～2%，HIVで0.5%以下といわれている．針刺し事故に共通の処置の第一は，受傷部位を流水で洗浄しながら，穿刺部位よりできるだけ，血液を絞り出すことである．そして，ただちに，医師か婦長などの第3者へ報告する．

B型肝炎ウイルスの針刺し事故では，HBs抗体がないか，低い（PHA法で24未満，あるいはEIA法で50mU/ℓ未満）場合，48時間以内にB型肝炎免疫グロブリンを注射する（ヘプスブリン-I 1000単位〔体重70kg以上であれば2000単位〕十生食100mℓ）．さらに，HBワクチンを同時期に投与して能動免疫も追加しておくほうが感染予防効果が高いので，併用する．HBワクチンは1ヵ月後と3ヵ月後あるいは6ヵ月後にも追加投与し，最低6ヵ月間は肝機能を観察する（図1）．受傷したスタッフがHBs抗原あるいは抗体が陽性の場合は前記の処置は必要ない．

C型肝炎ウイルスの場合は，未だ針刺し事故直後の治療法で確立されたものはない．インターフェロンの予防投与の効果は証明されておらず，原則として予防投与はしないというのが大勢である．

当院では，非常に大量の血液が注入された場合，副作用（発熱，感冒症状，白血球減少，血小板減少など）の可能性を説明したうえで，消化器内科の主治医の判断でインターフェロン（600万単位/日を4日間）を投与する．検査は事故直後の血清を保存し，事故直後のHCV抗体が陰性であるものについては，その後1ヵ月ごとにHCV抗体（第3世代），HCV-RNA，肝機能検査を1年間行う．C型肝炎は感染後時間が経つほどウイルス量が増加し，インターフェロン治療の有効性が低下するが，早期に発見してインターフェロン治療を行えば，慢性化を防ぐことができる可能性が高い．

HIV抗体陽性もしくは非常に強く陽性が疑われる患者の針刺し事故あるいは粘膜や皮膚が血液や体液に暴露されたときには，HIV抗体検査，HBs抗原検査（HIV陽性者はHBs抗原陽性である確率が高い）をただちに行い，同時に血清を1ml保存する．できるだけ早期に，予防薬（AZT，3TC，Indinavir）を服用する（図2）[5]．

ただし，これらの予防薬は妊婦に投与した場合の安全性，とくに妊娠早期の胎児への安全性が確認されていないので，HIV感染の危険性（針刺し事故で0.3～0.5%，飛沫（ひまつ）による粘膜への暴露で

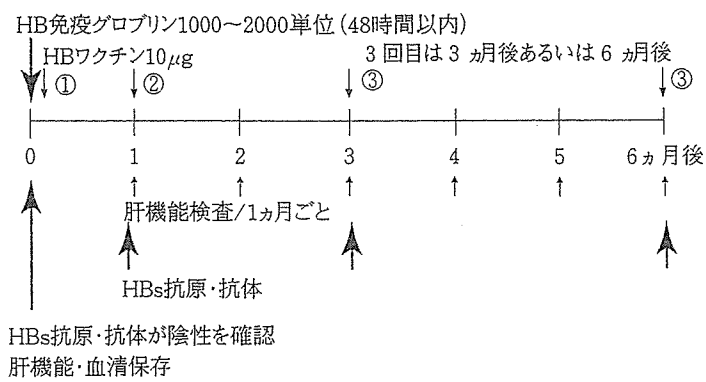
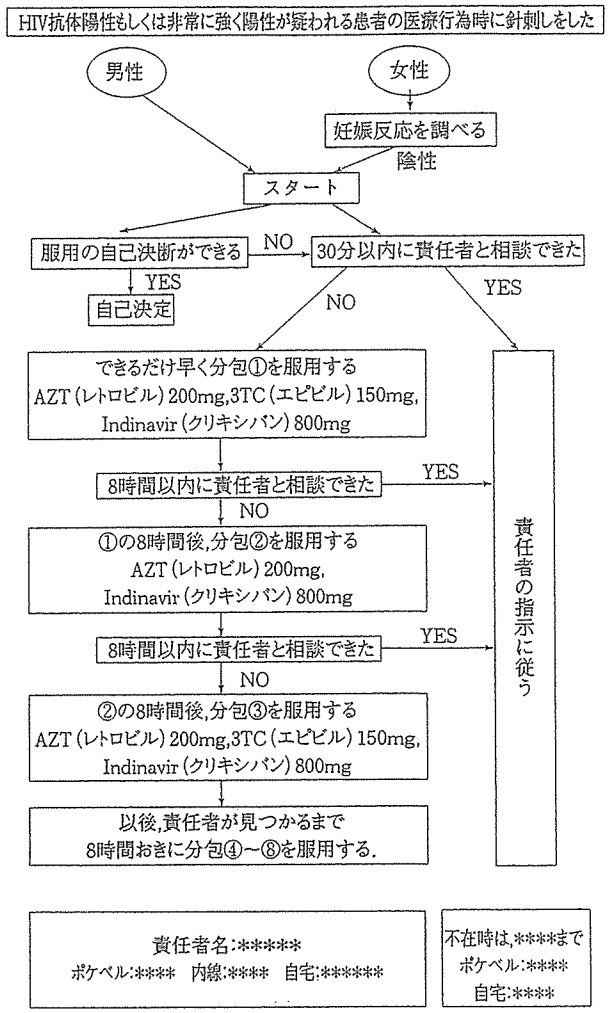


図1　B型肝炎ウイルス陽性血針刺し事故時の対応



(針刺し後のHIV感染防止のための予防服用マニュアル,国立国際医療センター病院エイズ治療・研究開発センター(Home Page版):http://www.acc.go.jp/accpage/より引用)

図2　HIV抗体陽性血液針刺し事故後のフローチャート

0.1%，皮膚で0.1%以下）と母子への薬の危険性をはかりにかけて，最後には自己決定してもらうことになる．

予防薬の内服は第1回目がもっとも重要で，少なくとも1～2時間以内に服用する必要がある．その後，1ヵ月間予防薬を内服する．これらの予防薬はエイズ拠点病院には常備してあり，24時間対応することになっているので，連絡先などをわかるようにしておく．

2）結核患者発生時

排菌のある結核患者が発生したら，原則として，まず，隔離施設のある透析センターへの転院を考える．これは，免疫能の低下した，他の透析患者への院内感染を防ぐのみでなく，比較的結核への免疫が少ない若い透析スタッフの感染を防ぐことにもつながる．

透析スタッフには，胸部X線やツベルクリンなどの臨時の健康診断を施行する．ツベルクリンは結核の発生が判明して2ヵ月後に通常は29歳以下のスタッフのみ行うが，感染の可能性が高い場合は年齢を広げて施行する．その場合でも40歳以上では，ツベルクリンの診断価値は少ない．ベースのツベルクリンよりも10mm以上大きく，30mm以上であれば，感染と診断し，29歳以下では，公費で化学予防（INHを6ヵ月間内服する）の対象となる．しかし，二次感染患者発生時には，40歳以上でも，ツベルクリンや化学予防を考慮する．感染者に化学予防を行えば，発病を約50%減少させる効果がある．

しかし，ツベルクリンによる感染の診断は，とくにベースのツベルクリンが実施されていなければ，かなり，不確実であり，自覚症状（2週以上続く咳や微熱）がある場合は，受診を勧める．胸部X線検査は年齢を問わず行うが，1～2年の長期的な観察が必要である．

表は感染危険度指数（治療前3回の喀痰(かくたん)検査のうち最大ガフキー号数X咳の持続月数）により，10以上を「最重要」，0.1～9.9を「重要」，0を「その他」と分類した患者に接触したスタッフの検診について示したものである[6]．

3）MRSA保菌

健康な透析スタッフがMRSA感染症を起こすこ

表　結核患者発生時のスタッフ検診

	スタッフの年齢	29歳以下	30歳以上
時期	初発患者の感染危険度カテゴリー		
患者発生より2ヵ月以内	最重要	直後に胸部X線 ツ反応は2ヵ月後	直後に胸部X線 ツ反応は特別の場合を除いて不要
	重要	2ヵ月以内に胸部X線 ツ反応は原則として不要	2ヵ月以内に胸部X線 ツ反応は不要
	その他	2ヵ月以内に胸部X線 ツ反応は不要	2ヵ月以内に胸部X線 ツ反応は不要
8～14ヵ月後	最重要	胸部X線	胸部X線
	重要	胸部X線	胸部X線
	その他	不要	不要
8～14ヵ月後	最重要	胸部X線	胸部X線
	重要	できれば胸部X線	できれば胸部X線
	その他	不要	不要

文献6）より改変

とはないが，保菌者になることは少なくない．透析患者と接する透析スタッフの保菌者に対しては除菌が有効である．鼻腔内保菌者では，ムピロシン軟膏を1日3回，3日間使用すれば，ほとんど除菌できる[7]．咽頭保菌者では，1日3回のポビドンヨードによるうがいを，皮膚保菌者では，ポビドンヨードか消毒用エタノールによる清拭を行う．除菌の判定は処置中止後1～2週間の間に3回以上MRSA培養検査が陰性になることにより行われる．

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Original Article

Cardiovascular Risk Factors in Hemodialysis Patients: Results from Baseline Data of Kaleidoscopic Approaches to Patients with End-stage Renal Disease Study

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BACKGROUND: The prevalence of cardiovascular risk factors and the prevalence of comorbidities in adult hemodialysis patients in Japan are not fully understood.
METHODS: In "Kaleidoscopic Approaches to Patients with End-stage Renal Disease Study" (The KAREN Study, 2003), trained research stuff examined 1,214 adult hemodialysis patients (mean age, 61.2 years; 779 males and 435 females) of 1,506 patients in northern areas of Iwate Prefecture. Cardiovascular risk factors and the prevalence of comorbidities in hemodialysis patients were compared with those in the general population using direct age-adjustment methodology and standardized morbidity ratios (SMRs).
RESULTS: In hemodialysis patients, common causes of end-stage renal disease were chronic glomerulonephritis (29.8%), diabetic nephropathy (24.5%), and other diseases. Prevalence and SMR of myocardial infarction were 5% and 9.6, respectively, and those of stroke were 13% and 5.7. The prevalences of hypertension and diabetes mellitus were 87% and 29%, respectively. Mean systolic blood pressure and mean diastolic blood pressure were 155 mmHg and 85 mmHg, respectively. Mean levels of total serum cholesterol, high-density lipoprotein cholesterol, and albumin in patients with end-stage renal disease were lower than those of the general population (160.6 vs. 203.3 mg/dL, 48.5 vs. 59.7 mg/dL, and 3.7 vs. 4.4 g/dL, respectively). Mean levels of C-reactive protein were higher than those of the general population (3.80 vs. 1.16 mg/L).
CONCLUSION: Hemodialysis patients have a high prevalence of cardiovascular risk factors and comorbidities. Levels of nutrition-related markers were lower, and C-reactive protein levels were higher, in hemodialysis patients than in the general population.
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Key words: the KAREN Study, Renal Dialysis, Risk Factors, Population, Cross-Sectional Studies.

More than 200,000 patients (1,722 per million) with end-stage renal disease (ESRD) underwent maintenance renal replacement therapy in Japan in 2002.1 In the United States, the prevalence of patients with ESRD was 1,403 per million in 2001.[2] The incidence and prevalence of ESRD, especially in diabetic and elderly patients, have been increasing over the past two decades in both countries.[1,2]

ESRD patients have a high mortality rate. The crude annual mortality rate of patients with ESRD has remained unchanged for the last ten years in Japan, at around 9%.[1] The high mortality rate of patients with ESRD is partly attributable to their high incidence of cardiovascular disease (CVD).[3]

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Little is known, however, about CVD risk factors in Japanese ESRD patients. One population-based study in ESRD patients was carried out more than ten years ago and reported that the prevalence of coronary artery disease in patients with ESRD was 1.4% in the 1970's and 2.5% in the 1980's.[4,5] Prevalences of CVD risk factors and cardiovascular comorbidities in recent years, however, have not been determined despite an increase in the numbers of diabetic and elderly patients.

The aim of this study was to reveal the prevalence of CVD risk factors in hemodialysis patients using a population-based study. We also compared the prevalence of CVD risk factors in hemodialysis patients with those of the general population.

METHODS

Setting of the Study

We have conducted the "Kaleidoscopic Approaches to patients with end-stage RENal disease Study" (the KAREN Study). The KAREN Study is a population-based prospective study designed to determine the effects of risk factors on CVD morbidity and mortality in ESRD patients. The study region is a section of northern Iwate Prefecture located in the northern part of the main island of Japan. The study area consists of 38 municipalities with a total population of 939,448 in 2002. There are 26 dialysis institutes in this region.

A preliminary survey to determine the number of dialysis patients in this area was carried out by sending facsimiles or letters to 26 dialysis institutes in April 2003. All the 26 institutes informed us of their numbers of ESRD patients, which totaled 1,506 adult hemodialysis patients. The prevalence of hemodialysis was 1,596 per million, and 6% of ESRD patients were undergoing peritoneal dialysis. Directors of 25 institutes, in which 1,499 hemodialysis patients were undergoing hemodialysis therapy, agreed to participate in the study.

Initial investigations for the KAREN study began in June 2003 and finished in March 2004. Annual checks of patients' medical records were scheduled to ascertain interim cardiovascular and cerebrovascular events, and will be continued for at least five years. This study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

Subjects

We used baseline data from the KAREN Study for cross-sectional analysis. There were 1,499 adult hemodialysis patients in the KAREN Study, though we were not able to contact 52 of these patients because of serious physical conditions or mental disorders. We obtained written informed consent for participation in the study from 1,260 patients (acceptance rate: 87.1%). Baseline examinations were not conducted in 21 of the 1,260 patients because of deterioration in their general conditions. In the end, we enrolled 1,239 patients in our study.

In the cross-sectional analysis, we excluded data from 25 patients because blood samples were not obtained. Data from 1,214 patients (80.6% of the total patients, aged 22 to 95 years, 779 males and 435 females) were used for analysis.

Research Staff and Data Collection

The KAREN staff includes two physicians (an urologist and a cardiologist), eight nurses, and 22 assistants. Assistants were recruited on an area-by-area basis, and they were involved in obtaining informed consents, checking questionnaires, and measuring blood pressure and body height. All the research staff were trained and approved before conducting the survey. A coordinating center was set up in the Department of Hygiene and Preventive Medicine, School of Medicine, Iwate Medical University, Morioka City, Iwate Prefecture, Japan.

Paper forms were brought to the coordinating center by the KAREN staff. Blood test data were sent to the coordinating center electronically, and only staff with permission was able to enter the room and edit data.

Initial Examinations

The baseline examination consisted of a questionnaire, measurements of blood pressure and anthropometric data, medical information reviews, and blood tests.

(1) Questionnaire

Each participant was asked to complete a questionnaire during a hemodialysis session. The questionnaire consisted of 24 questions regarding past history, family history, medication history, alcohol drinking habits, smoking habits, sleeping time per day, occupational status, the number of housemates, food preferences, and self-assessment of personality. The KAREN staff helped disabled patients fill out questionnaires, without manipulation of responses.

(2) Blood Pressure and Anthropometric Data

KAREN research staff took all measurements of body height and blood pressure. Body weight was measured using an automated scale at each institute before dialysis. Body height was measured as the length from between the heels to the centriciput point, in the supine position, using a metallic tape measure. Blood pressure was measured in the contralateral arm in patients with patent arteriovenous fistulae or grafts. Pre-dialysis blood pressure was measured twice in the supine position using an automatic device (BP-103i II Model 513000, Nippon Colin, Komaki, Japan) after a five-minute bed rest prior to cannulation. Post-dialysis blood pressure was measured in the supine position in a similar manner after a five-minute bed rest immediately following removal of the cannulae. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were each calculated as the mean of two measurements. Body mass index (BMI) was calculated as dry weight (kg) divided by the square of body height (m).

(3) Reviews of Medical Records

The two physicians and eight nurses visited the 25 institutes and reviewed patients' medical records and treatment regimens. They recorded patients' characteristics such as age, sex, past history,

family history, date hemodialysis was initiated, length of hemodialysis sessions, number of hemodialysis sessions per week, prescribed dry weight, inter-dialysis weight gain at the beginning of the week, cause of ESRD, diabetic status (based on past or current use of hypoglycemic agents), previous extremity amputation, comorbid conditions, current medications, falls in blood pressure (both falls in SBP of 30 mmHg or more and falls in SBP to below 90 mmHg) during hemodialysis sessions or the use of a vasopressor agent during hemodialysis sessions, blood pressure elevation (elevation in SBP of 30 mmHg or more) during hemodialysis sessions, use of erythropoietin, and other hemodialysis regimens.

(4) Blood Test Data

Pre-dialysis blood sampling was carried out at the beginning of hemodialysis sessions by the dialysis nursing staff. Blood samples were drawn from arteriovenous fistulae or grafts through dialysis cannulae into vacuum tubes containing EDTA or a serum separator gel or citrate. The blood samples were transported to a laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Morioka branch office) and analyzed the same day.

Levels of total cholesterol, triglyceride, uric acid, and creatinine were measured by enzymatic assays. The urease GLC method was used to determine levels of Blood urea nitrogen (BUN). High-density lipoprotein (HDL) cholesterol levels were determined by a direct quantitative assay, while concentrations of sodium ion, chloride ion, and potassium ion were determined using electrodes. Serum levels of calcium were determined by the o-cresolphthalein complexone method. Total protein levels were determined by the biuret method, and serum albumin levels were determined by the bromcresol green method. All of the above biochemical data were analyzed using an automated analyzer (AU5232, Olympus Corp., Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol levels were determined by a direct quantitative assay, and serum phosphate levels were determined by an enzymatic assay. These biochemical data were analyzed using an automated analyzer (AU800, Olympus Corp., Tokyo, Japan). Plasma glucose levels were determined by an enzymatic assay using an automated analyzer (H-7150 Hitachi High-Technology Corp., Tokyo, Japan). Glycosylated hemoglobin (HbA_{1c}) levels were determined by a latex agglutination turbidimetric immunoassay using an automated analyzer (JCA-BM9030, JEOL Ltd, Tokyo, Japan). Serum levels of C-reactive protein (CRP) were determined by the latex-enhanced immunonephelometric method (Dade Behring Diagnostic, Germany). Combined blood cell counts were determined using automated blood cell counters (Sysmex XE-2100 and Sysmex SE-9000, Sysmex, Kobe, Japan). Determinations of total cholesterol levels and HDL cholesterol levels were performed under the quality control program of the Centers for Disease Control and Prevention in the United States through the Osaka Medical Center for Health Science and Promotion, Japan.[6]

Data Handling and Classification

We determined causes of ESRD and comorbid conditions primarily according to diagnostic criteria (Table 1).[7,8] To compare characteristics of patients with similar causes of ESRD, patients were divided into three groups: a chronic glomerulonephritis group, a diabetic nephropathy group, and an other renal diseases group.

Habitual smoking was defined as currently smoking. Regular alcohol drinking was defined as drinking five or more days per week. Pulse pressure (PP) was defined as the difference between SBP and DBP, and delta SBP was defined as pre-dialysis SBP minus post-dialysis SBP. We defined persons with HDL cholesterol levels of less than 40 mg/dL as persons with low HDL cholesterol levels. We defined persons with CRP levels of more than 10 mg/L as persons with high CRP levels.

The Iwate KENCO Study is a population-based study that has been carried out in the general population in the same area as the KAREN Study.[9,10] We compared CVD risk factors and cardiovascular comorbid conditions in hemodialysis patients in the KAREN Study to those in the general population, as determined in the Iwate KENCO Study.

Statistical Analysis

Continuous variables are expressed as means ± standard deviation, and the Student's t test or the chi square test was used to compare two groups. The Mann-Whitney U test was used for skewed data (TG levels and CRP levels). One-way analysis of variance (ANOVA) or the Kruskal-Wallis test (TG levels and CRP levels) was used to compare three or more groups. Multiple comparisons were performed using Bonferroni's method, and age-adjusted values were calculated by the direct method based on data from the Iwate KENCO Study. Standardized morbidity ratios (SMRs) of myocardial infarction, stroke, hypertension, and diabetes mellitus in hemodialysis patients were also calculated based on data from the Iwate KENCO Study.

A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS(r) software (SPSS, Japan Inc., Version 11.0).

RESULTS

Table 2 shows patient characteristics. The mean age of the 1,214 patients (779 males and 435 females) was 61.2 ± 13.0 years (range, 22 to 95 years). The mean age at the start of hemodialysis was 54.2 ± 15.8 years, and the mean duration of hemodialysis was 7.0 ± 6.7 years. These numbers are similar for both male and female patients. Mean BMIs were 21.2 ± 2.9 in the male patients and 20.2±3.1 in the female patients.

The most common cause of ESRD was chronic glomerulonephritis (29.8%), and the second-most common cause was diabetic nephropathy (24.5%). The etiology was unknown in 24.9% of the patients. The proportions of patients with myocardial infarction, stroke, and peripheral arterial disease were 5%, 13%, and 16%, respectively. The proportions of smokers were 39.5% of

Table 1. Criteria for determining the causes of end-stage renal disease and comorbid conditions in hemodialysis patients.

Causes of end-stage renal disease		Comorbid conditions	
Chronic glomerulonephritis (CGN)	1 Hematuria 2 Proteinuria (2+, 3+) 3 Sustained renal insufficiency The diagnosis of CGN required that all three above-mentioned criteria or pathology be diagnosed by biopsy.	Myocardial infarction	1 Evolving Q-wave (at least 2 lead) myocardial infarction 2 Cardiac enzymes elevation: more than twice the normal range 3 Sustained chest pain lasting at least 30 minutes The diagnosis of myocardial infarction required two of the above-mentioned criteria.
Diabetic nephropathy (DMN)	1 Clinically diagnosed as diabetes mellitus 2 Proteinuria (≥ 300 mg/day) or edema or hypertension or renal insufficiency The diagnosis of DMN required that both above-mentioned criteria or pathological diagnosis be confirmed by biopsy.	Peripheral arterial disease	1 History of bypass surgery or angioplasty 2 Ankle-arm systolic blood pressure ratio of ≤ 0.8. 3 Exertional leg pain relieved by rest plus claudication diagnosed by physician. The diagnosis of peripheral artery disease required one of the above-mentioned criteria or image modality identification.
Hypertensive nephrosclerosis	1 Proteinuria (±,+) 2 Hypertension 3 Sustained renal insufficiency The diagnosis of hypertensive nephrosclerosis required that all above-mentioned 3 criteria or pathological diagnosis by biopsy.	Stroke	1 Abrupt onset of new neurologic deficit lasting at least 24 hours, with specific localizing findings comfirmed by physician 2 Without evidence for underlying nonvascular cause. The diagnosis of stroke required both 1 and 2 criteria or image modality identification (CT or MRI).
Polycystic kidney disease	The diagnosis of polycystic kidney disease required that image modalities (CT, US or MRI) identify multiple cysts in both kidneys.	Hypertension (HTN)	1 Anti-hypertension medication 2 Systolic blood pressure ≥ 140 mmHg 3 Diastolic blood pressure ≤ 90mm Hg The diagnosis of HTN required one of the above-mentioned criteria
Lupus nephritis	1 Clinically diagnosed as systemic lupus erythematosus Sustained renal insufficiency 2 The diagnosis of lupus nephritis required that both above-mentioned criteria and pathological diagnosis be confirmed by biopsy.	Diabetes mellitus (DM)	1 Past or current use of hypoglycemic agents 2 Casual plasma glucose ≥ 200 mg/dL 3 HbA_{1c} ≥ 6.5% The diagnosis of DM required one of the above-mentioned criteria.
		Dyslipidemia	1 Past or current use of anti-hyperlipidemia agents 2 Serum total cholesterol level ≥ 220 mg/dL 3 Serum low-density lipoprotein cholesterol level ≥ 140 mg/dL 4 Serum high-density lipoprotein level ≤ 40 mg/dL The diagnosis of dyslipidemia required at least one of the above-mentioned criteria.

CT: computed tomography
US: ultrasonography
MRI: magnetic resonance imaging

Table 2. Characteristics of patients in the KAREN Study.

		Total	Male	Female	p-value
Number		1214	779	435	
Age	(year)	61.2 ± 13.0	61.1 ± 13.1	61.4 ± 12.7	NS
Age at starting hemodialysis	(year)	54.2 ± 15.8	54.1 ± 16.0	54.3 ± 15.3	NS
Body Mass Index	(kg/m^2)	20.8 ± 3.0	21.2 ± 2.9	20.2 ± 3.1	< 0.001
Duration of hemodialysis	(year)	7.0 ± 6.7	6.9 ± 6.9	7.1 ± 6.5	NS
Sessions of hemodialysis	(/week)	2.88 ± 0.35	2.89 ± 0.33	2.85 ± 0.39	NS
Length of a hemodialysis session	(hour)	3.73 ± 0.64	3.80 ± 0.65	3.62 ± 0.54	< 0.001
Cause of end-stage renal disease	(%)				
Glomerulonephritis		29.8	29.1	31.0	NS
Diabetic nephropathy		24.5	27.5	19.3	0.002
Hypertensive nephrosclerosis		9.8	9.9	9.7	NS
Polycystic kidney		3.5	3.2	4.1	NS
Other minor diseases		7.4	6.4	9.2	0.036
Unknown		24.9	23.9	26.7	NS
Comorbid condition					
Myocardial infarction		5.2	5.4	4.8	NS
Stroke		13.1	13.1	13.1	NS
Peripheral artery disease		16.1	16.2	16.1	NS
Habits	(%)				
Currently smoking		28.2	39.5	7.8	< 0.001
Regular drinking		6.9	9.1	3.0	< 0.001
Hypertension	(%)	87.1	88.2	85.3	NS
Anti-hypertension medication	(%)	68.5	70.6	64.6	0.036
Number of prescribed drugs		1.36	1.41	1.28	0.070
Pre-systolic blood presxure	(mmHg)	155 ± 24	155 ± 23	155 ± 25	NS
Pre-diastolic blood pressure	(mmHg)	85 ± 13	85 ± 14	85 ± 134	NS
Post-systolic blood pressure	(mmHg)	142 ± 26	143 ± 25	140 ± 28	0.041
Post-diastolic blood pressure	(mmHg)	80 ± 14	80 ± 14	79 ± 14	NS
Delta-systolic blood pressure	(mmHg)	13 ± 23	11 ± 22	15 ± 23	0.012
Diabetes mellitus	(%)	29.1	32.1	23.7	< 0.001
HbA_{1c}	(%)	4.68 ± 0.95	4.69 ± 0.93	4.65 ± 0.98	NS
Plasma glucose	(mg/dL)	128.3 ± 54.8	129.6 ± 57.6	126.0 ± 49.4	NS
Dyslipidemia	(%)	43.1	46.1	37.2	< 0.001
Total cholesterol	(mg/dL)	154.9 ± 35.6	148.1 ± 33.6	166.9 ± 36.0	< 0.001
Triglyceride	(mg/dL)	108.6 ± 67.7	106.6 ± 72.3	112.3 ± 58.3	< 0.001*
High-density lipoprotein (HDL) cholesterol	(mg/dL)	47.0 ± 15.3	45.1 ± 14.9	50.4 ± 15.4	< 0.001
Low-density lipoprotein (LDL) cholesterol	(mg/dL)	84.9 ± 27.0	81.0 ± 26.2	91.8 ± 26.9	< 0.001
% of low HDL cholesterol (< 40mg/dL)	(%)	35.9	42.0	25.1	< 0.001
Nutrition-related data					
Total protein	(g/dL)	6.5 ± 0.5	6.5 ± 0.5	6.4 ± 0.5	0.001
Serum albumin	(g/dL)	3.7 ± 0.4	3.8 ± 0.4	3.7 ± 0.4	0.011
Blood urea nitrogen	(mg/dL)	71.2 ± 15.7	70.9 ± 15.4	71.8 ± 16.1	NS
Serum creatinine	(mg/dL)	11.0 ± 2.8	11.5 ± 3.0	10.1 ± 2.2	< 0.001
Inflammatory markers					
White blood cell count	(/μL)	5732 ± 1739	5891 ± 1765	5446 ± 1654	< 0.001
C-reactive protein (CRP)	(mg/L)	4.01 ± 9.26	4.27 ± 8.40	3.54 ± 10.62	NS*
% of high CRP (> 10 mg/L)		9.4%	10.7%	7.0%	0.025

Data are expressed as means ± standard deviation, or percentages.

P-values were obtained by a Student's t test, the chi square test, or the Mann-Whitney U test (triglyceride levels and CRP levels).

*: p-values by the Mann-Whitney U test.

Table 3. Characteristics of patients in groups according to cause of end-stage renal disease.

		Chronic glomerulonephritis (a)	Diabetic nephropathy (b)	Others ©	p-value	multiple comparisons or χ^2 test a vs b	a vs c	b vs c
Number		362	298	554				
Male/Female		227 / 135	214 / 84	338 / 216	0.006	*		*
Age	(year)	57.7 ± 12.9	62.8 ± 11.0	62.5 ± 13.6	< 0.001	**	**	
Age at starting hemodialysis	(year)	48.1 ± 15.9	59.2 ± 11.3	55.5 ± 16.6	< 0.001	**	**	**
Body Mass Index	(kg/m^2)	20.5 ± 2.8	21.3 ± 3.0	20.8 ± 3.1	0.002	**		**
Duration of hemodialysis	(year)	9.6 ± 7.7	3.7 ± 3.3	7.1 ± 6.7	< 0.001	**	**	**
Sessions of hemodialysis	(/week)	2.91 ± 0.33	2.84 ± 0.37	2.87 ± 0.36	NS			
Length of a hemodialysis session	(hour)	3.80 ± 0.61	3.69 ± 0.63	3.71 ± 0.62	0.039	*		
Comorbid condition	(%)							
Myocardial infarction		5.5	4.4	5.4	NS			
Stroke		10.8	14.1	14.1	NS			
Peripheral artery disease		19.1	15.1	14.8	NS			
Hypertension	(%)	83.4	95.3	85.2	< 0.001	*		*
Anti-hypertension medications	(%)	63.8	82.9	63.7	< 0.001	*		*
Number of prescribed drugs		1.23 ± 1.18	1.78 ± 1.29	1.23 ± 1.18	< 0.001	**		**
Pre-systolic blood presxure	(mmHg)	150 ± 23	166 ± 25	152 ± 22	< 0.001	**		**
Pre-diastolic blood pressure	(mmHg)	86 ± 13	85 ± 13	84 ± 14	0.048		**	
Pre-pulse pressure	(mmHg)	64 ± 16	81 ± 18	68 ± 16	< 0.001	**		**
Post-systolic blood pressure	(mmHg)	137 ± 26	153 ± 27	140 ± 24	< 0.001	**		**
Post-diastolic blood pressure	(mmHg)	80 ± 15	80 ± 13	79 ± 14	NS			
Post-pulse pressure	(mmHg)	56 ± 16	73 ± 19	60 ± 13	< 0.001	**		**
Delta-systolic blood pressure	(mmHg)	13 ± 20	13 ± 25	12 ± 22	NS			
Diabetes mellitus	(%)	5.2	100	6.5	< 0.001	*		*
HbA_{1c}	(%)	4.34 ± 0.61	5.60 ± 1.13	4.41 ± 0.66	< 0.001	**		**
Plasma glucose	(mg/dL)	111.8 ± 35.5	169.6 ± 69.8	116.9 ± 43.8	< 0.001	**		**
Dyslipidemia	(%)	43.1	56.4	41.3	< 0.001	*		*
Total cholesterol	(mg/dL)	155.1 ± 32.1	152.9 ± 37.9	155.8 ± 36.5	NS			
Triglyceride	(mg/dL)	109.3 ± 59.2	116.7 ± 81.2	103.8 ± 64.4	0.024†	‡		‡
High-density lipoprotein (HDL) cholesterol	(mg/dL)	47.4 ± 16.2	44.5 ± 14.4	48.0 ± 15.0	0.005	**		**
Low-density lipoprotein (LDL) cholesterol	(mg/dL)	84.7 ± 25.0	83.7 ± 27.5	85.6 ± 27.9	NS			
% of low HDL cholesterol (< 40mg/dL)	(%)	36.7	44.0	31.0	< 0.001			*
Habits								
Currently smoking	(%)	28.4	29.2	27.5	NS			
Regular drinking	(%)	9.1	7.0	5.4	NS			
Nutrition-related data								
Total protein	(g/dL)	6.5 ± 0.5	6.5 ± 0.5	6.5 ± 0.5	NS			
Serum albumin	(g/dL)	3.8 ± 0.4	3.7 ± 0.4	3.8 ± 0.4	0.001	**		**
Blood urea nitrogen	(mg/dL)	72.1 ± 14.8	68.7 ± 15.4	71.9 ± 16.2	0.007	**		**
Serum creatinine	(mg/dL)	11.8 ± 2.8	9.8 ± 2.5	11.2 ± 2.7	< 0.001	**		**
Inflammatory markers								
White blood cell count	(/μL)	5682 ± 1783	6087 ± 1658	5572 ± 1728	< 0.001	**		**
C-reactive protein (CRP)	(mg/L)	3.87 ± 9.56	4.43 ± 9.36	3.87 ± 900	NS†			
% of high CRP (> 10 mg/L)	(%)	9.1	11.1	8.5	NS			

Data are expressed as means ± standard deviations, or as percentages.

P-values were obtained by ANOVA, the chi square test, or the Kruskal-Wallis test (†).

*: $p < 0.05$, **: $p < 0.01$, by the multiple comparison test (Bonferroni method) or the chi square test.

Table 4. Comparison of prevalence, comorbidity, anthropometrical data, and blood sampling data in end-stage renal disease (ESRD) patients in the KAREN Study with those of the general population, using the Iwate KENCO Study.

	Male				Female				Total			
	General population	ESRD patients			General population	ESRD patients			General population	ESRD patients		
Number	4029	779			7338	435			11367	1214		
Comorbidity												
	prevalence	prevalence	SMR	(95% CI)	prevalence	prevalence	SMR	(95% CI)	prevalence	prevalence	SMR	(95% CI)
Myocardial infarction	0.81%	5.5%	8.0	(5.6, 10.4)	0.18%	4.9%	28.2	(16.2, 40.3)	0.40%	5.2%	9.6	(6.7, 12.3)
Stroke	4.05%	13.1%	3.6	(2.9, 4.4)	1.66%	13.1%	8.3	(6.1, 10.5)	2.50%	13.1%	5.7	(4.8, 6.6)
Hypertension	23.0%	88.2%	4.3	(4.0, 4.6)	23.2%	85.3%	3.8	(3.4, 4.2)	23.2%	87.1%	4.0	(3.8, 4.3)
Diabetes mellitus	6.92%	32.1%	5.1	(4.5, 5.7)	3.65%	23.7%	6.8	(5.5, 8.1)	4.81%	29.5%	6.5	(5.8, 7.2)
% high C-reeactive protein (> 10mg/L)	1.44%	10.9%	8.4	(6.6, 10.2)	1.17%	6.99%	6.1	(3.9, 8.3)	1.27%	9.47%	7.8	(6.4, 9.3)
Anthopometrical and blood sampling data (mean value)												
		Age-adjusted mean			Age-adjusted mean				Age- and sex-adjusted mean			
Body Mass Index	(kg/m^2)	23.7	21.2		24.0	20.1			23.9	20.5		
Systolic blood pressure	(mmHg)	130	155		126	155			127	155		
Diastolic blood pressure	(mmHg)	77	85		74	85			75	85		
Total cholesterol	(mg/dL)	193.5	149.1		208.1	166.9			203.3	160.6		
HDL cholesterol	(mg/dL)	56.2	45.1		61.5	50.4			59.7	48.5		
Serum albumin	(g/dL)	4.4	3.8		4.4	3.7			4.4	3.7		
C-reactive protein	(mg/L)	1.39	4.27		1.04	3.54			1.16	3.80		

Data are expressed as means or percentages or standardized morbidity ratios (SMRs).

SMR: standardized morbidity ratios

CI: confidence interval

male patients and 7.8% of female patients, while 9.1% of the male patients and 3.0% of the female patients were regular alcohol drinkers.

The majority (87.1%) of patients had hypertension, and 78.6% of the patients with hypertension took anti-hypertension medication. Pre-dialysis SBP and DBP were similar in the male patients and female patients. Post-dialysis SBP in the female patients was significantly lower than that of the male patients. About one-third (29.1%) of the patients had diabetes mellitus; the percentage of male patients with diabetes mellitus was higher than that of female patients with diabetes mellitus, but the mean levels of plasma glucose and HbA1c were similar in both male and female patients. The proportion of patients with dyslipidemia was 43.1%, and 83.1% of the patients with dyslipidemia had low HDL cholesterol levels. Mean levels of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides in the female patients were higher than the corresponding levels in male patients. Mean levels of CRP were 4.27 mg/L in the male patients and 3.54 mg/L in the female patients. CRP levels were higher than 10 mg/L in 10.7% of the male patients and in 7.0% of the female patients.

Table 3 shows characteristics of patients in the three renal disease groups. The mean age at the beginning of hemodialysis in the diabetic nephropathy group was higher than the mean ages of the other two groups, and the mean BMI and percentage of male patients in the diabetic nephropathy group were higher than those of the other two groups. Mean duration of hemodialysis differed between the three groups, with the shortest in the diabetic nephropathy group and the longest in the chronic glomerulonephritis group.

In the diabetic nephropathy group, all blood pressure parameters except for DBP remained high regardless of intensive anti-hypertension medication. The percentage of patients with dyslipidemia and the mean levels of triglycerides were higher, and the mean levels of HDL cholesterol were lower, in the diabetic nephropathy group relative to the other two groups. Nutrition-related parameters such as mean levels of serum albumin, BUN, and creatinine were lower in the diabetic nephropathy group than in the other groups. The mean white blood cell count in the diabetic nephropathy group was higher than in the other two groups.

Table 4 compares comorbid conditions, BMI, blood pressure, lipid levels, albumin levels, and CRP levels in hemodialysis patients to those of the general population. SMRs of myocardial infarction and stroke were 9.6 (8.0 in males and 28.2 in females) and 5.7 (3.6 in males and 8.3 in females), respectively. Mean levels of total serum cholesterol, HDL cholesterol, albumin, and BMI in the ESRD patients were lower than in the general population. Both the mean levels of CRP and the percentage of patients with high CRP levels were higher than in the general population.

DISCUSSION

The KAREN Study was designed as a population-based prospective study to assess the effects of risk factors on CVD morbidity and mortality in ESRD patients under a quality control program, and the study covered more than 80% of hemodialysis patients in the area of interest. Analysis of baseline data from the KAREN Study revealed CVD risk factors and cardiovascular comorbidities in hemodialysis patients.

About 90% of the hemodialysis patients in the KAREN Study had hypertension, and 78.6% of the hypertensive patients took anti-hypertension medications. More than 40% of the patients in the KAREN Study had dyslipidemia. More than 80% of patients with dyslipidemia had low HDL cholesterol levels, a similar percentage to that found in a previous study.[11]

Diabetic nephropathy accounted for 25% of all causes of ESRD and 29% of the KAREN Study patients had diabetes mellitus. The proportion of patients with hypertension and mean WBC count were higher, and levels of nutrition-related markers were lower, in the diabetic nephropathy group than in the other two groups. These conditions may be related to the poor prognoses for patients with diabetic nephropathy.[12,13]

The Okinawa Dialysis Study (OKIDS) revealed cardiovascular comorbid conditions in ESRD patients more than ten years ago,[4,5] but the prevalence of cardiovascular comorbidity for each renal disease subgroup was not shown. One benefit of our study was that it revealed comorbid conditions of hemodialysis patients for each renal disease group. The prevalences of myocardial infarction and stroke were similar between the renal disease groups. The cardiovascular comorbidities in the diabetic nephropathy patients were not different from those in patients with other renal diseases, a finding that disagrees with the results of an ESRD study in the United States.[13] The percentage of smokers in the KAREN Study (28%) is reflective of the general population of Japan.[9,14] Further studies are needed to determine whether smoking contributes to the high risk of CVD in ESRD patients in Japan, and efforts should be increased to encourage ESRD patients to stop smoking.[15]

The percentage of patients with diabetic nephropathy in the KAREN Study was similar to that of the Japanese Society for Dialysis Therapy (JSDT) Survey.[1] However, the proportion of patients with chronic glomerulonephritis in the KAREN Study was lower, and the percentage of patients with hypertensive nephrosclerosis in the KAREN Study was higher, than those in the JSDT Survey.[1]

In the current study, we identified causes of ESRD using information from medical records. The most common reason for classifying patients as unknown etiology was insufficient information regarding whether onset of proteinuria preceded that of hypertension. The low rate of diagnostic renal biopsy (10.4%) also made differential diagnosis difficult. Thus, it is possible that some patients with chronic glomerulonephritis or hypertensive nephrosclerosis should have been classified as patients with unknown etiology.

The percentage of patients with chronic glomerulonephritis was higher, and the percentage of patients with hypertensive nephrosclerosis was lower in the KAREN Study than those

reported by United States Renal Data System (USRDS).[2] The prevalence of hypertension was similar, the prevalence of myocardial infarction was lower, and the prevalence of stroke was higher in the KAREN Study. These results seem to reflect a high prevalence of stroke and a low prevalence of myocardial infarction in the Japanese general population relative to the general American population.[16,17]

In this study, the prevalence of CVD comorbidities was higher, albumin levels were lower, and CRP levels were higher in hemodialysis patients than in the general population. The lower albumin levels in hemodialysis patients may contribute to the high incidence of CVD.[18] Serum CRP levels in hemodialysis patients were significantly higher than those in the general population (1.80 mg/L vs. 0.84 mg/L) even after removal of subjects with apparently elevated CRP levels (10+ mg/L), and the high risk for CVD in hemodialysis patients might be partly explained by the large percentage of subjects with low-grade inflammation.[19-22]

It has been shown that traditional risk factors were not associated with the development of CVD in hemodialysis patients.[23] Some authors reported that malnutrition, inflammation, and atherosclerosis were closely linked in ESRD patients, and suggested that malnutrition and inflammation are stronger predictors than are traditional risk factors for hemodialysis patients.[23,24]

Instead of collecting isolated cases, we collected prevalent cases of hemodialysis in our study. This approach may fail to detect cases that are more serious. We were unable to make appointments with 52 patients because of serious physical conditions, and initial investigations were not conducted for 21 patients because of deteriorated health. Patients who would not give informed consent were probably in poorer condition. These factors might have reduced the number of serious cases of ESRD in our study; thus, the results obtained of our study might represent results for ESRD patients in relatively good condition. The prevalence of risk factors and comorbidities might therefore be underestimated.

We compared comorbid conditions in ESRD patients with the general population. In the Iwate KENCO Study, comorbid conditions were assessed using self-reported questionnaires, while in the KAREN Study, they were assessed using patients' medical records. This difference may artificially exaggerate differences in the prevalences of comorbidities between hemodialysis patients and the general population.

In conclusion, hemodialysis patients have a high prevalence of cardiovascular risk factors and comorbidities. Levels of nutrition-related markers were lower, and CRP levels were higher, in hemodialysis patients relative to the general population.

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APPENDIX

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Dynamics of immature subsets of dendritic cells during antiviral therapy in HLA-A24–positive chronic hepatitis C patients

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Background. The cellular immune response is important in chronic hepatitis C (CHC). To better understand its mechanism, we examined dendritic cells (DCs) and hepatitis C virus (HCV)-specific cytotoxic T cells (CTLs), which are thought to contribute to liver injury and viral clearance. ***Methods.*** CHC patients received 24 weeks of interferon-α-based antiviral therapy. We analyzed time-sequential frequencies of peripheral DCs, classified as myeloid DCs (mDCs) or plasmacytoid DCs (pDCs), together with peptide major histocompatibility class I tetramers, epitope specific for HCV core 129–137 (t*24/c129) or HCV NS3 1296–1304 (t*24/ns1294), directly ex vivo. ***Results.*** The mDC and pDC populations changed in parallel ($P < 0.05$), showing a significant transient decrease at weeks 12 and 16 during the therapy, and then recovering. However, neither of the tetramer results showed a direct correlation with the kinetics of peripheral DCs. ***Conclusions.*** There is an apparent effect of antiviral therapy or a subsequent reduction of HCV on host immunity, but the effect may not include the induction of CTLs in CHC.

Key words: MHC-tetramer, dendritic cells, hepatitis C virus, cytotoxic T lymphocytes, anti-viral therapy

Introduction

Hepatitis C virus (HCV) is one of the leading causes of chronic liver disease and hepatocellular carcinoma worldwide. However, causes of the high persistent infection rate with HCV remain to be clarified.[1–5] Some evidence suggests an important role of the T-cell response in HCV elimination.[6,7] Particularly, the activity of cytotoxic T lymphocytes (CTLs) in patients with self-limited infection has been shown to be vigorous and to be directed against multiple epitopes of HCV.[8] On the other hand, in chronic hepatitis C (CHC), CTLs are found at low frequencies in peripheral blood,[9] whereas they are found at higher frequencies in the peripheral blood of patients with a low viral level.[10] In addition, the loss of a virus-specific $CD4^+$ T-cell response is observed in patients who develop persistent HCV infection.[11] Thus, induction of both CTLs and the surrounding immune system may positively control HCV replication not only in the natural disease course but also during antiviral therapy for CHC.

Dendritic cells (DCs) are important for antigen presentation and T-cell differentiation.[12] Among the enormous functional potencies and controversies regarding DC classification, two distinct types of DCs have recently been described: myeloid DCs (mDCs) and plasmacytoid DCs (pDCs). mDCs express CD11c on the cell surface and have strong antigen presentation and interleukin (IL)-12 production abilities, while pDCs have CD123 (IL-3 receptor α) and are known to be a potent source of type 1 interferon (IFN). Their precursor phenotypes can also be detected in human peripheral blood.[13]

In this study, we analyzed the population of DCs in peripheral blood by a phenotypic analysis with flow cytometry and the frequencies of HCV-specific CTLs, using two recently developed HLA-A24 tetramers, in order to investigate whether antiviral therapy alters cellular immunity and its relation to HCV control.

Materials and methods

Subjects

Seventeen CHC patients and 12 healthy subjects were enrolled in this study. Among the CHC patients, only

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HLA-A24-positive patients ($n = 7$, summarized in Table 1) were followed up for serial changes in peripheral DC populations and cytotoxic T lymphocytes; because of the privacy policy for blood donation, we could not specify the ages or sex of the healthy donors. CHC was diagnosed by elevated alanine aminotransferase (ALT) levels with a duration of more than 6 months along with the presence of anti-HCV antibody (Third Generation Test, Ortho Diagnostics, Chicago, IL, USA) in serum. We confirmed that all patients were positive for HCV-RNA by qualitative reverse transcriptase-polymerase chain reaction (RT-PCR; Amplicor HCV, Roche Diagnostics, Tokyo, Japan). Liver biopsy, which was obtained from all patients, showed active necroinflammatory changes in the liver. Patients infected by hepatitis B virus and/or human immunodeficiency virus were excluded from the study. Clinical outcomes were evaluated at the end of therapy and 24 weeks after the completion of therapy. Each patient gave us written informed consent in advance. The study protocol was approved by the Ethical Committee of the Tohoku University School of Medicine. Peripheral blood was obtained at the start of therapy and then at 4-week intervals until the end of the therapy period. Immediately after the sampling, peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation.

Human leukocyte antigen typing

First, we checked human leukocyte antigen (HLA) class I phenotypes using a conventional microlymphocytotoxicity test, and then we determined HLA genotypes by amplification refractory mutation system (ARMS)-PCR using sequence-specific oligonucleotide primers.[14]

Monoclonal antibodies

Antihuman monoclonal antibodies used in this study were CD8-FITC, Lin 1-FITC, CD11c-PE, CD123-PE, HLA-DR-APC, and CD38-APC. All of them were purchased from BD Biosciences (San Jose, CA, USA).

Synthetic peptides

Two 9-mer peptides (HCV core 129–137, GFADLMGYI; HCV NS3 1296–1304, TYSTYGKFL) were commercially synthesized by Research Genetics (Huntsville, AL, USA). They were first selected as epitope candidates by a database search for a shared HLA-A*2402-binding motif. Subsequently, we confirmed that these two sequences were recognized by CTLs in an HLA-A24-restricted manner.[15–17]

Preparation of tetrameric HLA/β2-microglobulin/peptide complex

Monomeric complexes were synthesized as previously described.[18] Briefly, the transmembrane and cytosolic region of HLA-A*2402 was substituted for a 15-amino acid substrate peptide (BSP) of a BirA-dependent biotinylation enzyme, and the HLA-A*2402 BSP fusion protein and β2-microglobulin (β2m) were expressed in *Escherichia coli*. The correctly refolded HLA/β2m/peptide complex was purified by gel filtration chromatography and biotinylated on a single lysine within the BSP with the BirA enzyme (Avidity, LLC, Denver, CO, USA).

Tetramers were prepared just before use by mixing the biotinylated HLA/β2m/peptide complex with streptavidin-phycoerythrin conjugate (Molecular Probes, Eugene, OR, USA) at a 4:1 molar ratio.

Flow cytometry to evaluate peripheral DCs

Cells were incubated with monoclonal antibodies according to the manufacturer's instructions in a staining buffer, phosphate-buffered saline (–), containing 2% fetal calf serum, for 30 min at 4°C. Then, they were washed twice with the buffer and fixed with 1% paraformaldehyde prior to the analysis. We used CellQuest (BD Biosciences) and FACSCalibur (BD Biosciences) software for all flow cytometric experiments in this study. DCs were identified as surface-labeled Lin 1-FITC$^-$/HLA-DR$^-$APC$^+$ events, and DC subsets were identified with CD11c-PE and CD123-PE.[19,20] Briefly, nuclear cells were obtained by gate R1 (Fig. 1a). Thereafter, Lin1$^-$ cells were selected from nuclear cells by gate R2 (Fig. 1b). DC subpopulations were finally defined as Lin1$^-$CD11c^{high}DR$^+$ cells (mDC, Fig. 1c) and Lin1$^-$CD123$^+$DR$^+$ cells (pDC, Fig. 1d).

Tetramer staining of peptide-specific CD8$^+$ T cells

Isolated PBMCs were first stained with HLA A*2402/core 129–137 (t*24/c129) or HLA A*2402/NS3 1296–1304 (t*24/ns1294) tetramer for 30 min at 37°C in 100 μl of tetramer staining buffer (staining buffer supplemented with 0.02% sodium azide), followed by staining with CD8-FITC, CD38-APC, and Viaprobe (7-amino-actinomycin D solution; BD Biosciences). Isotype controls for each fluorochrome were also used to determine the cut-off line. Dead cells were excluded by negative gating of the Viaprobe-positive fraction to minimize the effect of nonspecific tetramer binding. Frequencies of tetramer-positive cells were considered reliable when they were above 0.02% in CD8+ cells, in accordance with the results previously obtained with HLA-A24-positive healthy subjects.

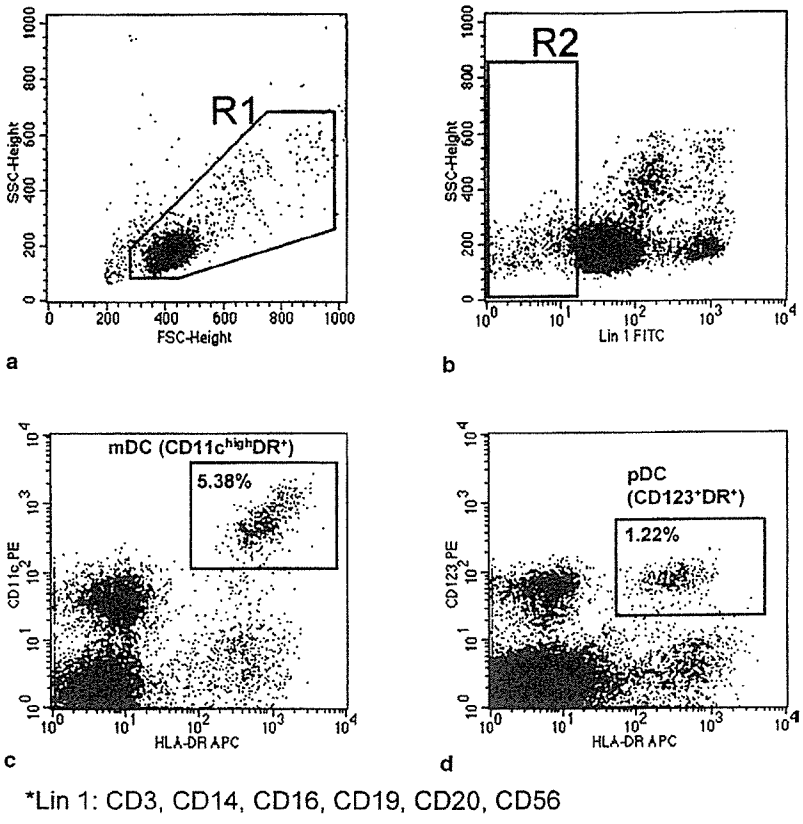

Fig. 1a–d. Detection of peripheral dendritic cell (DC) subsets. Figures show the usual gating strategy for peripheral DCs on flow cytometric analysis. Lin1$^-$ cells were obtained by serial gating, that is, by gate R1 for nuclear cells (**a**) and subsequently by gate R2 (**b**). Thereafter, cells of the combined fractions from gates R1 and R2 were analyzed in other windows for HLA-DR$^+$ CD11c^{high+} cells (myeloid DCs, *mDC*, **c**) and HLA-DR$^+$ CD123$^+$ (plasmacytoid DCs, *pDC*, **d**). A representative result for a healthy donor is shown

Statistics

Data analysis was performed either by Student's *t* test or one-way analysis of variance. *P* values of <0.05 were considered statistically significant.

Results

Clinical features

Major profiles of the patients are shown in Table 1. Four patients completed 24 weeks of IFN-α (6 MU/day, thrice a week) and ribavirin (600 or 800 mg/day according to body weight) combination therapy, while the others received 24 weeks IFN-α (6 MU/day, thrice a week) monotherapy. All patients showed an early viral response, namely, they were HCV-RNA-negative by week 12, except for patient 7, who was HCV-RNA-negative by week 20.

Kinetics of mDC and pDC during antiviral therapy

Populations of peripheral mDCs and pDCs were identified (Fig. 1). Untreated CHC patients ($n = 17$) had a smaller mDC population than healthy subjects ($n = 12$, $6.89 \pm 2.39\%$ vs. $14.37 \pm 2.32\%$, mean ± SEM, $P < 0.05$), whereas there was no difference in the pDC populations ($5.51 \pm 1.48\%$ vs. $5.21 \pm 1.45\%$). Analysis among seven followed-up patients showed unique kinetics of the DC subsets. They declined from the baseline level ($9.34 \pm 3.48\%$ for mDC and $7.92 \pm 1.92\%$ for pDC), reaching a minimum at week 16 ($3.98 \pm 1.83\%$ for mDC, $P < 0.05$, and $4.22 \pm 1.91\%$ for pDC, $P < 0.05$; Fig. 2). Subsequently, both DC subsets recovered ($7.39 \pm 2.85\%$ for mDC and $7.87 \pm 2.24\%$ for pDC at week 24). Among all patients, populations of mDC and pDC changed simultaneously with a similar pattern ($P < 0.05$).

Difference in kinetics of peptide-specific CD8$^+$ cells

Kinetics of tetramer-positive cells in peripheral blood differed between the two epitopes during antiviral