は、解析手法は違うが我々の研究結果との比較をしても問題はないと考えられる。

先行研究結果と我々の研究結果を比較すると、解析対象となった患者の平均年齢と男女比に大きな違いはなかった。二つの先行研究報告によると、透析患者、健常対照ともに血清中ヒ素濃度は10μg/L程度であり、透析患者の血清中ヒ素濃度は決して高いものではないことが示されている[23,24]。一方先行研究の10倍以上の透析患者数と健常対照者を集めた我々の研究では、日本人透析患者の血清ヒ素濃度が明らかに高かったことが示されている。

日本人を対象とした透析患者ならびに多数の一般住民で血清ヒ素濃度を測定した検討は見当たらない。我々の検討結果では、一般住民の血清中ヒ素濃度は10μg/Lと欧米で行われた先行研究とほぼ同じ結果であったが、透析患者の平均血清ヒ素濃度は40μg/Lと明らかに高く、最も高い血清ヒ素濃度の値は573μg/Lであった。何故我々が解析対象とした日本人透析患者の血清中ヒ素濃度が高かったのかを明らかにする必要がある。

ヒ素による健康被害は、主に飲料水に含まれる無機ヒ素が大きく関ってきたとされる。無機ヒ素の摂取源とされる水道水のヒ素濃度はWHOでは10μg/L以下に制限することが求められ[25]、日本においては厳格に基準が守られており、特殊な理由でヒ素の汚染が生じた場合を除いてヒ素による健康被害が生じる可能性はない。研究対象地域の水道水のヒ素濃度は（2町の提供する複数回の測定結果はいずれもヒ素及びその化合物濃度が0.001ｍｇ／Ｌ未満であった）決して高くはなく、透析患者で観察された血清ヒ素濃度の高さに飲料水中のヒ素が影響したとは考えにくい。

一方日本を含む東アジア人は食品として海産物を好んで摂取し、有機ヒ素の摂取が多いことが指摘されている[16]。我々の検討では、健常対象者の平均の血清ヒ素濃度は10μg/Lと決して高くはなかったが、50μg/Lを超えていた健常対照者が2例存在していた。高値を示した2例では、たまたま測定日の直前に食物摂取によって有機ヒ素が体内に取り込まれ、ヒ素が尿中排泄されるまでの期間に採血されたことで血清ヒ素濃度が高かったことが推測された。

日本人は平均して1日あたりおよそ180μｇのヒ素を摂取し、その多くがアルセノベタインを始めとする有機ヒ素と考えられる[16]。例外的にヒジキには比較的多量の無機ヒ素が含まれ、含有ヒ素の60％が無機ヒ素であることが示されている。2001年にはカナダ、2004年には英国でヒジキ摂取によるヒ素の健康被害の可能性を考慮して摂食を控えるべきとの勧告をだした[16]。しかし、日本人を対象とした研究では通常の食事でヒジキから摂取される無機ヒ素と有機ヒ素は健康に影響を与えないとしている[26]。

経口摂取された有機ヒ素の人体への直接の急性毒性は一般にはないと言われている[25]。Buchetらの検討では経口摂取された有機ヒ素（モノメチルヒ酸（monomethylarsonic acid：MMA）とジメチルヒ酸（dimethylarsinic acid：DMA）の75-85％が1日以内に尿中に排泄されている[27]。Francesconiらの検討ではアルセノシュガーを摂取した後4日以内に80％が尿中に排泄されたとしている[28]。また食品から最もよく検出される有機ヒ素の多くを占めるアルセノベタインは、1980年代に行われた動物実験によると摂取後速やかに吸収され全く代謝を受けずに速やかに尿中に排泄されたとされる[29]。

日本人透析患者は魚介類摂取により有機ヒ素を比較的多量に摂取している可能性があるが、従来の勧告は健常人を対象としたヒ素の体内動態に基づいたものであり、腎機能が廃絶している透析患者での検討は筆者が渉猟しえた限り報告はない。一般人を対象とした多くの疫学研究では、魚介摂取が多いほど動脈硬化性疾患発症リスクや死亡リスクが下がることが示されている。そしてその理由として、魚介に多く含まれるω3多価不飽和脂肪酸が動脈硬化進展を抑制することが指摘されている[30～32]。しかし、本研究結果から類推すると、もしヒ素の体内過剰が魚介摂取によってもたらされているとしたら、透析患者においては魚介摂取によるメリットは発揮されずに、むしろ魚介に含まれるヒ素が体内に蓄積して結果として動脈硬化性疾患罹患リスクを高めてしまう可能性を示唆する。

表6　ヒ素が循環器疾患有病・発症・死亡と関係があったと報告した研究の概要

研究時期とデザイン	ヒ素曝露による循環器疾患への影響 研究結果
1900年初頭：症例報告。ヒ素曝露と心不全の関係	Raynolds E：Manchester近郊で起きたヒ素に汚染されたビールによる6000人以上の健康被害を報告。70人以上が死亡。典型的なヒ素中毒（皮膚病変・神経病変・消化器病変）とともに心不全で死亡する例が多いことを報告。
1980年代：症例報告。ヒ素曝露と心筋梗塞罹患	Zaldivar, R：Chileの飲料水の高濃度ヒ素地域のヒ素皮膚病変を有する40歳未満の急性心筋梗塞（AMI）死亡14例とヒ素皮膚病変のない若年AMI14例とを比較。ヒ素のAMI発症への関連性について報告。
1980年代：ケースコントロール研究。 ヒ素曝露と黒肢病との有病関係。	Chen CJ:台湾で241人の高濃度ヒ素曝露地域の黒肢病患者とヒ素曝露のない健常対照と比較検討。高濃度ヒ素曝露が黒肢病と有意に関係。また予後追跡では、黒肢病患者で循環器疾患死亡、がん死亡のリスクが高いことを示した。
1990年代：生態学的研究とケースコントロール研究	Chen CJ, : 台湾高濃度ヒ素曝露地域60村で生態学的研究とBFD患者263人と非患者2293人の5年間追跡調査。生態学研究ではヒ素曝露量と冠動脈疾患死亡との関係性あり。BFD者は2.5倍冠動脈疾患で死亡。
1990年代：横断解析	Chiou, HY .:12003人の台湾住民を対象として井戸水のヒ素濃度と脳卒中罹患リスクとの関係性をみた。井戸水のヒ素濃度が高くなるほど脳卒中有病リスクが高くなる線形の関係性が認められた。
1990年代：横断解析、SMR算出	Tsai, SM :186-93.台湾の高濃度ヒ素曝露地域で死因別のSMRを算出。各種癌疾患死亡とともに、血管疾患死亡・冠動脈疾患死亡・脳血管疾患死亡率が高いことが示された。
2000年代：横断解析	Tseng CH, :高濃度ヒ素曝露地域の台湾住民462人で井戸水のヒ素曝露量（濃度×飲水期間）と冠動脈疾患の有病率との関連を検討した。曝露量が大きいほど冠動脈疾患有病率が高かった。
2000年代：生態学的研究	Yuan, Y. .:チリの高濃度ヒ素曝露地域（477000人）と対照地域で死亡診断書閲覧による心筋梗塞死亡の相対危険度を算出。高濃度曝露地域では男性で1.45倍、女性で1.26倍心筋梗塞死亡リスクが高いことが示された。

魚介摂取が透析患者の動脈硬化性疾患罹患リスクに悪影響を与えるのかは栄養調査を実施して検証することが必要である。また、健常人では何ら健康被害を起こすことなく速やかに尿中排泄されると考えられてきたこれらのヒ素化合物は腎機能の廃絶した透析患者の体内でどのような動態を示すのかは全く検討されていない。もし摂取された有機ヒ素が血液中から除去されずに長く体内に残留し、週3回行われる血液透析治療でも十分に除去されず、一部は血中から組織へ移行し、長期的には動脈硬化症進展や発ガンに寄与する可能性があるのであれば、事実関係を明らかにして予防対策を実施する必要がある。その関連性について早急な解明が待たれる。

我々は本研究によって1,041名の透析患者の血清ヒ素濃度の測定を行って血清中ヒ素濃度が高くなるほど心筋梗塞罹患リスクが高いことを明らかにした。体内のヒ素濃度が長期的な予後に影響したと報告をした前向き研究は、現時点では一つも存在しない。従来行われた研究は、ヒ素高濃度曝露者において高率に観察された疾患発症の症例報告や[30]、ヒ素の高濃度曝露地域においてヒ素の非曝露地域との比較を通して疾患有病率・死亡率が高くなることを報告した生態学的研究が主であった[7~13]。表6は高濃度のヒ素が有病リスクを上げているとする研究の概要を示したものである。今回の研究テーマに因んで、ヒ素が循環器疾患有病や死亡に影響している可能性を示した研究に着目して列挙した。

1900年代初頭からヒ素による循環器疾患発症との関連性については言及されてきたが[33]、ヒ素と循環器疾患とのかかわりについて大きな知見をもたらしたのは環境中のヒ素が高濃度に存在したチリと台湾での観察研究である。1980年代チリでは飲料水のヒ素濃度が高い地域で若年の心筋梗塞患者が多いことが報告され[7]、同じころ台湾の風土病とも言われた鶏肢病（黒肢病）には井戸水から摂取する高濃度の無機ヒ素が関連しているとす

る論文がいくつか出された[12]。本研究では、血清ヒ素濃度が高いほど透析患者の末梢動脈疾患有病率が高くなる正の線形関係が認められ、台湾で観察された井戸水のヒ素濃度と鶏肢病有病率との関連性と類似した所見と考えられる。更に台湾では高濃度ヒ素曝露により鶏肢病のみならず冠動脈疾患死亡[8]や脳卒中の有病リスクが[13]高くなる可能性も指摘している。一方最近の死亡診断書による死因と飲料水のヒ素との関係を検討したチリの研究では、高濃度ヒ素曝露は心筋梗塞による死亡とは関係していたものの脳卒中死亡とは関係していなかったと報告しており[11]、今後の検討課題である。上記研究はいずれも生態学的研究・後ろ向きの研究または横断的解析の手法をとった研究であり、直接の因果関係を示したものではない。

ヒ素が動脈硬化症を進展させるメカニズムについて、ヒ素はIL8（好中球の主要なケモカイン）などの炎症性サイトカインの遺伝子発現に関することや[34]、ヒ素そのものがNFκBやAP-1などの転写因子の強力な活性化作用を有することが動物実験で示され[35～37]、ヒ素が動脈硬化症進展に関る炎症反応を更新させることが示唆されている。またヒ素が血小板凝集を増強させることや[38]、ヒ素が薬物による血管拡張反応を抑制することが動物実験で示されている[39]。以上よりヒ素は複数の要因に働きかけて動脈硬化症の進展に関っていることが推測される。

心筋梗塞とアテローム性脳梗塞は、ともに粥状動脈硬化症の進展によって生じるが、ヒ素はこの粥状動脈硬化症を加速進展させることで、両者の罹患リスクを上げている可能性がある。しかし、我々の研究結果では血清ヒ素濃度が高いほど心筋梗塞罹患リスクが上がっていたが、虚血性脳卒中罹患リスクは上がってはいなかった。その理由として、虚血性脳卒中には、アテローム性脳梗塞のみならず、より高血圧の影響を強く受ける細動脈硬化症（穿通枝硬化症）由来の脳梗塞も多く含まれることから、患者全体の8割が高血圧症を有する透析患者集団では[18]、虚血性脳卒中発症にはヒ素に比べて高血圧がより強く関与し、血清ヒ素濃度の虚血性脳卒中発症リスクへの関与が明らかにできなかった可能性がある。

我々は多数の日本人透析患者の血清ヒ素濃度を測定して、一般住民との比較を通した横断研究により透析患者で血清ヒ素濃度が高いことを、前向き研究によって血清ヒ素濃度が透析患者の心筋梗塞罹患に影響していることを明らかにした。同様の研究結果を示した報告は全くなく、現時点では我々の検討が唯一のものである。

しかし、我々の検討結果には補足すべき事項が幾つか存在する。まず本研究では男女を一緒に合わせて解析を行ったが、性別に分けた解析を行うとイベント数が不足して統計的解析パワーが不足し、Cox回帰分析による検定ができなかった。血清ヒ素濃度と性との交互作用が存在した可能性を考慮し、交互作用モデルによる検討を行ったが、血清ヒ素と性別との間に明らかな交互作用は存在しなかった（交互作用モデル $p = 0.414$）。従って、ヒ素濃度が透析患者の予後に与える影響を男女一緒にあわせて検討したが、その解析結果は大きく歪められてはいないと考えられる。

次に、血清ヒ素濃度が透析患者で高かった理由として日本人特有の魚介類の多量摂取を推測したが、本研究では栄養調査を実施しておらず、透析患者や一部の一般健常人で観察された血清ヒ素濃度の高値が、本当に食品中に含まれるヒ素によって影響を受けているのかを栄養調査を実施することで確認する必要がある。

透析患者や一般住民で測定された血清中のヒ素の成分分析が必要である。従来の研究は、尿中に排泄されるヒ素を化学形態別に測定した報告が多いが、本研究では末期透析患者が対象であるために尿中の測定は不可能であった。このため保存血清を用いてヒ素総量を測定したが、一般住民と透析患者で血清中のヒ素の化学形態について、魚介類に多いとされるアルセノベタインがそのほとんどを占めていたのか、それとも違う化合物の割合が高かったのか、等について明らかにする必要がある。化学形態別の測定を実施した上で、アルセノベタインに代表されるヒ素含有食品を摂取した場合に血清中ヒ素濃度の一般住民や透析患者における時間的推移を明らかにする必要がある。

更に透析患者では透析治療でヒ素が化学形態によってどのように除去されていくのかを明らかに

することによって、透析患者で高濃度の血清ヒ素濃度が持続する原因を明らかにできると考えられる。これらの結果を踏まえて、改めてヒ素が体内に残存して長期的には動脈硬化症を促進させるのか、発ガンに影響するのかについて検討する必要がある。これらの解決すべき課題に対しての答えを用意する必要があり、その上で日本人透析患者で認められた高い血清中ヒ素濃度と高い血清中ヒ素濃度が将来の心筋梗塞発症確率を上げていたことの意味を再考し、その機序を明らかにするとともに予防施策を構築する必要がある。

（本論文の要旨は第46回日本循環器管理研究協議会総会・日本循環器病予防学会（平成22年5月29日）：一般口演において発表された）

研究助成

本研究の登録調査は、岩手県保健医療研究補助金助成を受けて実施した。透析患者コホート研究追跡調査は、科学研究費補助金事業「透析患者の循環器疾患発症・死亡のリスク要因に関する大規模コホート研究（課題番号18590568（H18年～H19年）、主任研究者　大澤正樹）」を受け実施した。また、透析患者のヒ素濃度測定に関しては、上原記念生命科学財団補助金事業平成19年度研究助成「成人血液透析患者の血清微量元素濃度と循環器疾患罹患率・死亡率との関連（研究代表者　大澤正樹、研究協力者　藤岡知昭、板井一好、丹野高三）」を受けて実施した。

謝　　辞

今回の研究を実施するにあたり、研究に快く参加していただいた岩手県内在住の透析患者さんならびに各透析施設の関係各位に深く感謝申し上げます。また追跡調査で多くの患者診療記録を閲覧して直接の情報収集に当たった、研究看護師古沢智子さん、畠山雅子さん、星川綾子さん、データ整理を担当していただいた栗林純子さんとメリケ・アブリズさん、事務の吉田美貴子さん、佐々木弓枝さん、新里朋子さん、鈴木優子さん、山田静香さん、膨大な検体の微量元素測定を手伝っていただいた菊池鉄弥君の献身的なご助力に改めて感謝を申し上げます。

カレン研究グループ

研究代表者：藤岡知昭（岩手医科大学泌尿器科学講座）

主任研究者：加藤香廉（三愛病院泌尿器科）；大澤正樹、丹野高三、板井一好、小野田敏行、坂田清美（岩手医科大学医学部衛生学公衆衛生学講座）；藤島洋介（岩手医科大学泌尿器科学講座）；岡山明（結核予防会第一健康相談所）；中村元行（岩手医科大学内科学講座）

研究協力者：藤島幹彦、中村祐子（矢巾クリニック）；山内文俊、清野耕治、那須雅孝、上林多代、前田信子（三愛病院）；佐藤倫郎（三島内科）；長澤　茂、鈴木　明（友愛病院）；山田行夫（山田クリニック）；岩動　孝（いするぎ医院）；沼里　進（盛岡赤十字病院）；大日向　充（大日向医院）；松坂純一（沼宮内クリニック）；青木　光（二戸クリニック）；高田　耕、戸田忠夫（岩手県立一戸病院）；阿部　正、杉村　淳（岩手県立久慈病院）；漆久保潔、笠月瑞子（国保種市病院）；小原紀彰（小原クリニック）；石原敬夫（宝陽病院）；北村道彦、梶川恒雄、藤澤宏光（岩手県立中部病院）；斎藤和好、鈴木　薫（北上済生会病院）；金澤重俊（日高見中央クリニック）；小池博之（きたかみ腎クリニック）；菅野千治、尾張幸久（岩手県立宮古病院）；後藤康文、後藤康樹（後藤泌尿器科医院）；後藤　尚（後藤医院）；柴野良博（岩泉済生会病院）

文　　献

1) Foley R, Parfrey P, Sarnak M. Clinical epidemiology of cardiovascular disease in chronic renal failure. Am J Kidney Dis 1998;32 (supple 5) S112-S19.
2) Kalantar-Zadeh K, Block G, Humphreys MH, et al. Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. Kidney Int 2003;63(3):793-808.
3) Stenvinkel P, Heimburger O, Paultre F, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int 1999;55(5):1899-911.
4) Silverberg DS, Wexler D, Blum M, et al. The cardio renal anemia syndrome: correcting anemia in

patients with resistant congestive heart failure can improve both cardiac and renal function and reduce hospitalizations. Clin Nephrol 2003;60 Suppl 1:S93-102.

5) Tonelli M, Wiebe N, Hemmelgarn B, et al. Trace elements in hemodialysis patients: a systematic review and meta-analysis. BMC Med 2009;7:25.

6) Nordberg G, Fowler B, Nordberg M, Friberg L, editors. Handbook on the Toxicology of Metals. Third Edition ed. Burington, MA: Academic Press, 2007.

7) Zaldivar R. A morbid condition involving cardio-vascular, broncho-pulmonary, digestive and neural lesions in children and young adults after dietary arsenic exposure. Zentralbl Bakteriol B 1980;170(1-2):44-56.

8) Chen CJ, Chiou HY, Chiang MH, et al. Dose-response relationship between ischemic heart disease mortality and long-term arsenic exposure. Arterioscler Thromb Vasc Biol 1996;16(4):504-10.

9) Tsai SM, Wang TN, Ko YC. Mortality for certain diseases in areas with high levels of arsenic in drinking water. Arch Environ Health 1999;54(3):186-93.

10) Tseng CH, Chong CK, Tseng CP, et al. Long-term arsenic exposure and ischemic heart disease in arseniasis-hyperendemic villages in Taiwan. Toxicol Lett 2003;137(1-2):15-21.

11) Yuan Y, Marshall G, Ferreccio C, et al. Acute myocardial infarction mortality in comparison with lung and bladder cancer mortality in arsenic-exposed region II of Chile from 1950 to 2000. Am J Epidemiol 2007;166(12):1381-91.

12) Chen CJ, Wu MM, Lee SS, et al. Atherogenicity and carcinogenicity of high-arsenic artesian well water. Multiple risk factors and related malignant neoplasms of blackfoot disease. Arteriosclerosis 1988;8(5):452-60.

13) Chiou HY, Huang WI, Su CL, et al. Dose-response relationship between prevalence of cerebrovascular disease and ingested inorganic arsenic. Stroke 1997;28(9):1717-23.

14) Tonelli M, Wiebe N, Hemmelgarn B, et al. Trace elements in hemodialysis patients: a systematic review and meta-analysis. BMC Med 2009;7:25.

15) 食品安全に関するリスクプロファイルシート（検討会用）　更新日平成21年11月30日　ハザードの名称　ヒ素. 東京：農林水産省, 2009:1-7.

16) 平成20年度食品中に含まれるヒ素の食品影響評価に関する調査　報告書. 東京：内閣府食品安全委員会食品安全情報システム, 2009.

17) Mohri T, Hisanaga A, Ishinishi N. Arsenic intake and excretion by Japanese adults: a 7-day duplicate diet study. Food Chem Toxicol 1990;28(7):521-9.

18) Ohsawa M, Kato K, Itai K, et al. Cardiovascular risk factors in hemodialysis patients: results from baseline data of kaleidoscopic approaches to patients with end-stage renal disease study. J Epidemiol 2005;15(3):96-105.

19) 大澤正樹、加藤香廉、藤島洋介、他　岩手県透析患者の悉皆的コホート研究：２年間の追跡調査結果報告. 日循予防誌 2007;42:86-96.

20) Itai K, Onoda T, Nohara M, et al. Serum ionic fluoride concentrations are related to renal function and menopause status but not to age in a Japanese general population. Clin Chim Acta 2010;411(3-4):263-6.

21) Ohsawa M, Kato K, Itai K, et al. Standardized prevalence ratios for chronic hepatitis C virus infection among adult Japanese hemodialysis patients. J Epidemiol 2010;20(1):30-9.

22) Kennedy J, Carey R, Coolen R, et al. Evaluation of precision performance of clinical chemistry devices; approved guideline. . Wayne, PA: National Committee for Clinical Laboratory Standards., 1999.

23) Mayer DR, Kosmus W, Pogglitsch H, et al. Essential trace elements in humans. Serum arsenic concentrations in hemodialysis patients in comparison to healthy controls. Biol Trace Elem Res 1993;37(1):27-38.

24) Zhang X, Cornelis R, Mees L, et al. Chemical

speciation of arsenic in serum of uraemic patients. Analyst 1998;123(1):13-7.

25) Environmental Health Criteria 224 Arsenic and arsenic compounds 2nd edition. Geneva: International Programme on Chemical Safety WHO, 2001.

26) Nakajima Y, Endo Y, Inoue Y, et al. Ingestion of Hijiki seaweed and risk of arsenic poisoning. Appl Organomet Chem 2006;20:557-64.

27) Buchet JP, Lauwerys R, Roels H. Comparison of the urinary excretion of arsenic metabolites after a single oral dose of sodium arsenite, monomethylarsonate, or dimethylarsinate in man. Int Arch Occup Environ Health 1981;48(1):71-9.

28) Francesconi KA, Tanggaar R, McKenzie CJ, et al. Arsenic metabolites in human urine after ingestion of an arsenosugar. Clin Chem 2002;48(1):92-101.

29) Vahter M, Marafante E, Dencker L. Metabolism of arsenobetaine in mice, rats and rabbits. Sci Total Environ 1983;30:197-211.

30) Marckmann P, Gronbaek M. Fish consumption and coronary heart disease mortality. A systematic review of prospective cohort studies. Eur J Clin Nutr 1999;53(8):585-90.

31) He K, Song Y, Daviglus M, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. Circulation 2004;109(22):2705-11.

32) He K, Song Y, Daviglus M, et al. Fish consumption and incidence of stroke: a meta-analysis of cohort studies. Stroke 2004;35(7):1538-42.

33) Anonymous. Royal Commision Appointed to Inquire into Arsenical Poisoning form the consumption of beer and other articles of food or drink. Final report. Part I. London, 1903.

34) Simeonova PP, Hulderman T, Harki D, et al. Arsenic exposure accelerates atherogenesis in apolipoprotein E(-/-) mice. Environ Health Perspect 2003;111(14):1744-8.

35) Barchowsky A, Dudek EJ, Treadwell MD, et al. Arsenic induces oxidant stress and NF-kappa B activation in cultured aortic endothelial cells. Free Radic Biol Med 1996;21(6):783-90.

36) Burleson FG, Simeonova PP, Germolec DR, et al. Dermatotoxic chemical stimulate of c-jun and c-fos transcription and AP-1 DNA binding in human keratinocytes. Res Commun Mol Pathol Pharmacol 1996;93(2):131-48.

37) Simeonova PP, Wang S, Toriuma W, et al. Arsenic mediates cell proliferation and gene expression in the bladder epithelium: association with activating protein-1 transactivation. Cancer Res 2000;60(13):3445-53.

38) Lee MY, Bae ON, Chung SM, et al. Enhancement of platelet aggregation and thrombus formation by arsenic in drinking water: a contributing factor to cardiovascular disease. Toxicol Appl Pharmacol 2002;179(2):83-8.

39) Lee MY, Jung BI, Chung SM, et al. Arsenic-induced dysfunction in relaxation of blood vessels. Environ Health Perspect 2003;111(4):513-7.

ABSTRACT

A study of serum arsenic concentrations among hemodialysis patients: comparison with controls and its effect on risk for death and the development of myocardial infarction and ischemic stroke

Masaki Ohsawa[*1], Kazuyoshi Itai[*1], Kozo Tanno[*1], Yosuke Fujishima[*1,2], Karen Kato[*3], Akira Okayama[*4], Shigeatsu Endo[*5], Toshiyuki Onoda[*1], Kiyomi Sakata[*1], Motoyuki Nakamura[*6], Toru Kuribayashi[*7], Tomoaki Fujioka[*2], and the KAREN Study Group

[*1] Department of Hygiene and Preventive Medicine, School of Medicine, Iwate Medical University
[*2] Department of Urology, School of Medicine, Iwate Medical University
[*3] Division of Urology, San-ai Hospital
[*4] The First Institute of Health Service, Japan Anti-tuberculosis Association
[*5] Department of Critical Care Medicine, School of Medicine, Iwate Medical University
[*6] Division of Cardiovascular Medicine, Nephrology and Endocrinology, Department of Internal Medicine, School of Medicine, Iwate Medical University
[*7] Department of Health and Physical Education, Faculty of Education, Iwate University

A population-based cohort study of hemodialysis patients (The KAREN Study, n=1041) has been carried out in Iwate Prefecture, Japan since 2003. Serum arsenic concentrations (μg/L) were determined by ICP-MS and compared in hemodialysis patients and randomly recruited healthy controls (n=385) using analysis of covariance □ANCOVA□ after adjustment for age and sex. Adjusted mean (95% confidence intervals) of serum arsenic levels in hemodialysis patients was higher than that in the controls (42.4 (40.1-44.6) vs 11.6 (7.82-15.4), $p<0.05$ by ANCOVA). Follow-up studies were completed in all 1,041 patients a 5-year after initial registration. The total observation period was 4152 patient-years. There were 382 deaths, 48 cases of acute myocardial infarction and 112 cases of ischemic stroke. Patients were divided into quartile groups according to their serum arsenic levels and the crude mortality rates and crude incident rates of acute myocardial infarction and ischemic stroke in these groups were estimated. A linear trend test between the serum arsenic level and the risk of death, the incidence of myocardial infarction and ischemic stroke was performed after adjustment for following risk factors: age, sex, blood pressure, body mass index, high CRP level, low albumin level and co-morbid conditions (diabetes mellitus, dyslipidemia, a history of myocardial infarction, a history of stroke and a history of malignant diseases). A positive association between the serum arsenic level and a risk for the incidence of myocardial infarction was observed even after multivariate adjustment (trend $p=0.014$). The association become clearer after excluding patients with an old myocardial infarction (trend $p=0.009$). Associations between the serum arsenic level and death risk and between the arsenic level and risk for ischemic stroke were not observed.
Japanese hemodialysis patients have high serum arsenic levels and the higher serum arsenic level contributes to a higher risk for a myocardial infarction to occur.

Key Words : *Hemodialysis, arsenic, population-based cohort study, myocardial infarction, ischemic stroke, The KAREN Study*

Received ●.●, 2011. • Accepted ●.●, 2011.　　(JJCDP 46: ●-●, 2011)

NDT Advance Access published March 3, 2011

Nephrol Dial Transplant (2011) 0: 1–7
doi: 10.1093/ndt/gfq859

Original Article

NDT
Nephrology Dialysis Transplantation

Serum selenium levels are inversely associated with death risk among hemodialysis patients

Yosuke Fujishima[1,2], Masaki Ohsawa[1], Kazuyoshi Itai[1], Karen Kato[3], Kozo Tanno[1], Tanvir Chowdhury Turin[4], Toshiyuki Onoda[1], Shigeatsu Endo[5], Akira Okayama[6] and Tomoaki Fujioka[2]

[1]Department of Hygiene and Preventive Medicine, Iwate Medical University, Morioka, Japan, [2]Department of Urology, Iwate Medical University, Morioka, Japan, [3]Division of Urology, San-ai Hospital, Morioka, Japan, [4]Department of Medicine, University of Calgary, Calgary, Canada, [5]Department of Critical Care, Iwate Medical University, Morioka, Japan and [6]The First Institute of Health Service, Japan Anti-Tuberculosis Association, Tokyo, Japan

Correspondence and offprint requests to: Yosuke Fujishima; E-mail: fyosuke@iwate-med.ac.jp

Abstract

Background. Previous studies have indicated that serum selenium levels are decreased in hemodialysis patients. Selenium deficiency may contribute to an increased risk for death among hemodialysis patients.

Methods. A population-based prospective cohort study in adult hemodialysis patients was conducted. A total of 1041 patients were enrolled. Patients were divided into quartile groups according to serum selenium levels. Mortality rates between the groups were compared by the log-rank test. Associations between serum selenium levels and cause-specific mortality risks in hemodialysis patients were examined by Cox's regression model.

Results. A total of 382 patients died during the 5-year follow-up period (median follow-up period, 4.9 years). Crude mortality rates in quartile groups according to serum selenium levels were 134.5, 99.9, 85.9 and 55.2 (per 1000 patient-years), respectively. The lowest quartile group had significantly higher mortality rates from all-cause and infectious disease-related death than the rates in the other three groups ($P < 0.001$, by log-rank test). Mortality rates from cardiovascular and malignant disease-related death were similar between the groups. A strong inverse relationship between selenium levels and infectious disease-related death was observed even after multivariate adjustment (trend $P = 0.024$).

Conclusions. Serum selenium levels were inversely associated with death risk, especially death risk due to infectious disease, among hemodialysis patients. Decreased serum selenium level may contribute to immunity dysfunction and may increase the risk of death from infectious disease in hemodialysis patients.

Keywords: hemodialysis; mortality; prospective study; risk factors; selenium

Introduction

Selenium is an essential component of the antioxidant enzyme and is needed for proper functioning of the immune system [1]. In the general population, selenium deficiency has been observed in patients with cardiovascular disease (CVD) [2], patients with cancer [3] and patients with viral infection [4], and the effects of selenium supplementation on prevention of several diseases such as cancer [5, 6] and CVD [2] have been reported on the basis of results of clinical trials and epidemiological studies.

Several studies have shown that serum levels of selenium in hemodialysis patients were lower than those in normal controls [7–12]. Insufficient dietary intake of selenium and/or loss of selenium through hemodialysis membranes may contribute to the low serum levels of selenium in hemodialysis patients [7]. These findings suggest that hemodialysis patients have low levels of serum selenium and that a considerable number of hemodialysis patients suffer from significant selenium deficiency.

Hemodialysis is associated with considerable morbidity and mortality due to accelerated CVD and general infectious disease [13, 14]. Hemodialysis-related risk factors for CVD mortality, including low body mass index (BMI), low serum cholesterol and low blood pressure, have been reported [15]. Moreover, the presence of 'malnutrition-inflammation complex syndrome' (MICS) [16] and 'malnutrition-inflammation-atherosclerosis syndrome' (MIAS) [17] in hemodialysis patients strongly contribute to poor prognosis.

However, much remains to be learned about other factors that contribute to the risk for death in hemodialysis patients. Selenium deficiency in hemodialysis patients may contribute to an increased risk for death from various diseases, and selenium deficiency may be one of the unknown strong risk factors for death in hemodialysis patients.

The aim of this study was to determine whether low serum selenium levels contribute to increased mortality in hemodialysis patients.

Materials and methods

Subjects

We have been conducting the 'Kaleidoscopic Approaches to patients with end-stage RENal disease Study' (the KAREN Study) since 2003 in the northern part of Japan. The KAREN Study is a population-based prospective study designed to determine the effects of risk factors on cardiovascular morbidity and mortality in hemodialysis patients. The study subjects were prevalent cases of adult hemodialysis patients.

Initial registration was completed in 1214 patients. Serum selenium tests were not done in 173 patients. Therefore, data for 1041 participants (663 men aged 22–91 years with a mean age of 61.2 ± 13.4 years and 378 women aged 25–88 years with a mean age of 61.1 ± 12.7 years) were analyzed in this study (see Figure 1) [18, 19].

Written informed consent for participation in the study was obtained from all subjects. The 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

Initial investigations in the KAREN Study were conducted from June 2003 to March 2004 and the examinations consisted of a questionnaire, review of medical records, measurements of blood pressure and anthropometric data and blood tests. The data gathering methodology was previously described [18]. The blood samples were transported to a laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Inc., Morioka branch office) and analyzed the same day. Residual sera were stored at −80°C in our laboratory until determination of selenium.

Measurements of serum selenium concentrations

Frozen serum samples were unfrozen and each serum specimen (1 mL) was pipetted into a Teflon tube and then 3.0 mL of high-purity nitric acid was added. The solution was allowed to sit for 2 h at room temperature. Then, the tube was heated to 120°C for 12 h to completely degrade the organic matter in the serum sample. The resultant solution was cooled to room temperature and then transferred into a Teflon beaker. The beaker was heated to 100°C until desiccated. Dried samples were dissolved with 5 mL of 10% nitric acid and used for measurements. Selenium levels in sample solutions were determined using inductively coupled plasma-mass spectrometry (Elan 6000; PerkinElmer Co Ltd.). The with-run and total imprecision were determined according to the National Committee for Clinical Laboratory Standards Approved Guideline [20]. Two replicates of selenium measurements in mixed sera per day were carried out. The method produced a within-run standard deviation of 3.9 μg/L to 139.8 μg/L. Total precision gave a standard deviation of 6.2 μg/L to 139.8 μg/L.

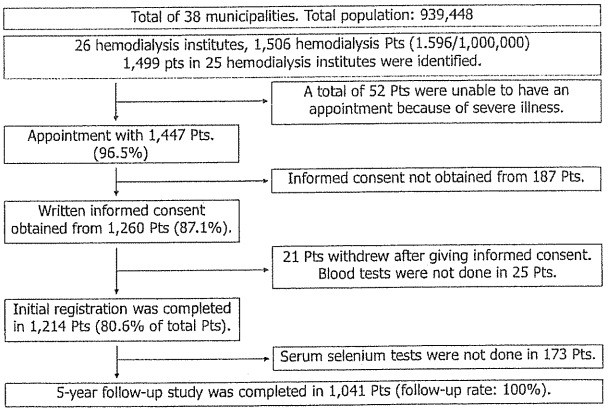

Fig. 1. Flow chart of the procedure used to select patients for participation in the KAREN Study. There were 1506 adults undergoing hemodialysis in the 26 centers in the study area. We were able to make contact with 1447 patients (96.5%). Fifty-two patients were then excluded because of their serious clinical condition. A total of 1260 patients (87.1%) provided written informed consent for participation in the study. A total of 1214 patients (80.6%) completed baseline examinations. Serum selenium tests were not done in 173 patients. We ascertained the vital status of all participants after completion of a 5-year follow-up survey for 1041 patients.

Definitions of comorbid conditions and risk factors

We determined the causes of renal failure and comorbid conditions based on medical records according to the KAREN study criteria [18]. Hypertension was defined as systolic blood pressure of 140 mmHg or higher and diastolic blood pressure of 90 mmHg or higher or use of antihypertension medication. Diabetes mellitus was defined as casual plasma glucose of 200 mg/dL or higher and Hb A_{1C} of 6.5% or higher or past or current use of hyperglycemic agents. Dyslipidemia was defined as serum total cholesterol (TC) of 220 mg/dL or higher or high-density lipoprotein (HDLC) of <40 mg/dL or use of anti-dyslipidemia agents. Smoking status was classified into non-smoker, current smoker and past smoker. Regular alcohol drinking was defined as drinking ≥5 days/week.

Follow-up surveys and determination of causes of deaths

Follow-up studies were performed annually at each center. Members of the KAREN Study team reviewed all the medical records of study participants. The medical records of deceased patients were summarized. The cause of death was independently determined, based on the summaries, by KAREN Outcome Review Committee physicians who were blinded to the patient's characteristics including serum selenium levels. Discordant cases were discussed and final determination was reached by consensus.

In this study, we determined three major causes of death (CVD death), infectious disease-related death and malignant disease-related death) after coding according to the ICD10th revision (see Table 1). CVD includes cardiac death (I20–I25), death from pulmonary embolism (I26), stroke death (I60–I69), vascular death (I70–I77) and sudden cardiac death (I46, I49 and R96). Infectious disease-related death includes death from certain infectious and parasitic diseases (A00-B99), death from infectious diseases in the nervous system (G00 and G04.2), death from infectious diseases in the respiratory tract (J10-J18, J20, J69 and J86), death from infectious diseases in the gastrointestinal tract and digestive organs (K65, K80.3, K81) and death from infectious diseases in skin and subcutaneous tissue (L03 and L89). Malignant disease-related death is death from neoplasms (C02, C15, C16, C18, C20, C22, C34, C45, C55, C61, C64, C67, C72, C76 and D43).

Statistical analysis

Hemodialysis patients were divided into quartile groups according to serum selenium levels. Continuous variables are expressed as means (standard deviations) or sex- and age-adjusted means [95% confidence intervals (CIs)] estimated by analysis of covariance (ANCOVA) in quartile groups. Multiple comparisons were performed using the Bonferroni method. Sex- and age-adjusted proportions were determined by logistic regression analysis.

Cumulative probability of death was estimated by the Kaplan–Meier method, and mortalities were compared between the groups by the log-rank test. Crude mortality rates and direct age-adjusted mortality rates (per 1000 patient-years) stratified by quartile groups were calculated. Age-adjusted mortality rate was calculated by the direct method using the WHO standard population of 2000–25. Sex- and age-adjusted hazard ratios (HRs) and multivariate-adjusted HRs and their 95% CIs for total death, infection disease death, CVD death and malignant disease death were estimated in the upper three quartile groups compared with those for patients in the Q1 group serving as a reference after adjustment for risk factors [age, male gender, underweight (BMI < 18.5), overweight (BMI ≥ 27.5), hypertension (systolic blood pressure ≥ 140 or diastolic blood pressure ≥ 90 or medication), dyslipidemia (TC ≥ 220 mg/dL or HDLC < 40 mg/dL), diabetes mellitus, serum albumin levels, high-sensitivity CRP (hsCRP) levels, history of myocardial infarction, history of stroke, history of malignant disease, smoking status and regular drinking habit] using Cox's regression model. All P-values were based on two-sided tests and P-values <0.05 were considered statistically significant. The Statistical Package for Social Sciences (SPSS version 15.0; SPSS Inc., Tokyo, Japan) was used for the analysis.

Results

Table 2 shows baseline characteristics of patients stratified by quartile groups according to serum selenium levels. Ranges of selenium levels of the quartile groups were 18.4–85.3 (μg/L) in the Q1 group, 85.4–99.9 in the Q2 group, 100.0–114.0

Table 1. Criteria for determining causes of death in the KAREN Study (based on ICD-10)

Cardiovascular death: I01–I99	
Cardiac death: I20–I25, I29, I27, I30–I52	
I20–I25	Coronary artery disease
I33	Acute and subacute endocarditis
I50	Heart failure
Pulmonary embolism death: I26	
Stroke death: I60–I69	
I60	Subarachnoidal hemorrhage
I61, I62	Intracerebral hemorrhage
I63	Cerebral infarction
I64, I67	Other type of stroke
Vascular death: I70–I77	
I70	Atherosclerosis
I71	Aortic anuerysm and dissection
I72, I73	Other peripheral artery disease
I74	Arterial embolism and thrombosis
I77	Other arterial disease
Sudden cardiac death: I46, I49, R96	
Cardiac arrest: I46	
I46.0	Cardiac arrest with successful resuscitation
I46.1	Sudden cardiac death, so described
I46.9	Cardiac arrest, unspecified
Ventricular fibrillation and flutter: I49	
I49.0	Ventricular fibrillation and flutter
Other sudden death, cause unknown R96	
R96.0	Instantaneous death
R96.1	Death occurring less than 24 h from onset of symptoms, not otherwise explained
Infectious disease-related death	
A: bacterial infection: A00-A09, A15-A19, A40-A41	
B: viral infection, fungal and other microorganism infection	
G: infectious diseases in nervous system	
G00	Bacterial meningitis, not elsewhere classified
G04.2	Bacterial meningoencephalitis and meningomyelitis, not elsewhere classified
J: infectious diseases in respiratory tract	
J10-J11	Influenza
J12-J18	Pneumonia
J20	Acute bronchitis
J69	Pneumonitis due to solids and liquids
J86	Pyothorax
K: infectious diseases in gastrointestinal tract and digestive organ	
K65	Peritonitis
K80.3	Calculus of bile duct with cholangitis
K81	Cholecystitis
L: infectious diseases in skin and subcutaneous tissue	
L03	Cellulites
L89	Decubitus ulcer
Malignant neoplasm: (C02, C15, C16, C18, C20, C22, C34, C45, C55, C61, C64, C67, C72, C76, D43)	
C02	Malignant neoplasm of tongue
C15	Malignant neoplasm of esophagus
C16	Malignant neoplasm of stomach
C18	Malignant neoplasm of colon
C20	Malignant neoplasm of rectum
C22	Liver cell carcinoma
C34	Malignant neoplasm of bronchus or lung
C45	Methothelioma of pleura
C55	Malignant neoplasm of uterus
C61	Malignant neoplasm of prostate
C64	Malignant neoplasm of kidney
C67	Malignant neoplasm of bladder
C72	Malignant neoplasm of spinal cord
C76.2	Malignant neoplasm of abdomen (origin unknown)
D43.1	Neoplasm of brain (infratentorial)
Other causes of death	

in the Q3 group and 114.2–226.2 in the Q4 group. Mean age in the Q1 group was significantly older than mean ages in the other groups (Q1 versus Q2, $P = 0.006$; Q1 versus Q3, $P < 0.001$; Q1 versus Q4, $P < 0.001$, by analysis of variance), and an inverse relationship between serum selenium levels and age was observed (trend $P < 0.05$). Proportions of male patients and mean vintage of hemodialysis were not different between the groups.

Both sex- and age-adjusted means of BMI in the Q3 and Q4 groups were significantly higher than that in the Q1 group (Q1 versus Q2, $P = 0.450$; Q1 versus Q3, $P = 0.020$; Q1 versus Q4, $P = 0.043$, by ANCOVA). Adjusted means of serum albumin in the Q3 and Q4 groups were significantly higher than that in the Q1 group (Q1 versus Q2, $P = 0.292$; Q1 versus Q3, $P = 0.004$; Q1 versus Q4, $P < 0.001$, by ANCOVA). Adjusted geometric means of hsCRP levels in the Q2, Q3 and Q4 groups were significantly lower than that in the Q1 group (Q1 versus Q2, $P = 0.001$; Q1 versus Q3, $P = 0.002$; Q1 versus Q4, $P < 0.001$, by ANCOVA). The proportion of patients with hypertension in the Q4 group was significantly higher than that in the Q1 group (Q1 versus Q2, $P = 0.819$; Q1 versus Q3, $P = 0.777$; Q1 versus Q4, $P = 0.024$, by logistic regression). The proportion of patients having a current smoking habit in the Q2 group was significantly higher than that in the Q1 group (Q1 versus Q2, $P = 0.029$; Q1 versus Q3, $P = 0.901$; Q1 versus Q4, $P = 0.113$, by logistic regression). Proportions of other comorbid conditions were not different between the groups after sex and age adjustment.

After completion of 5-year follow-up studies, the observed patient-years were 4152. Mean and median follow-up periods were 4.0 and 4.9 years, respectively. A total of 382 patients died during the 5-year observation period. Figure 2 shows Kaplan–Meier estimated cumulative probability of death for the groups. The Q1 group had a significantly higher mortality rate from all causes of death than the rates in the other three groups (Q1 versus Q2, $P = 0.024$; Q1 versus Q3, $P < 0.001$; Q1 versus Q4, $P < 0.001$, by log-rank test, Figure A). The Q1 group also had a significantly higher mortality rate from infectious disease than the rates in the other three groups (Q1 versus Q2, $P < 0.001$; Q1 versus Q3, $P < 0.001$; Q1 versus Q4, $P < 0.001$, Figure C). No significant differences in mortality rate between the groups were observed for CVD-related death and malignant disease-related death.

Table 3 shows numbers of deaths and crude or age-adjusted mortality rates (per 1000 patient-years) by the quartile groups according to serum selenium levels in the hemodialysis patients. Crude (age-adjusted) mortality rates in the quartile groups (Q1, Q2, Q3 and Q4) were 134.5 (49.6), 99.9 (37.7), 85.9 (31.6) and 55.2 (25.2), respectively, for all-cause death and 52.7 (14.4), 20.2 (6.6), 17.9 (4.8) and 8.9 (3.6), respectively, for infectious disease-related death. These data suggested that lower selenium levels in hemodialysis patients contributed to increased risks for all-cause and infectious disease-related deaths. On the other hand, relationships of serum selenium levels with CVD-related death and malignant disease-related death were not observed. Results were similar when the general Japanese population was used for age-standardization (data not shown).

Figure 3 shows adjusted HRs for all-cause death, CVD-related death, infectious disease-related death and malignant

Table 2. Baseline characteristics stratified by quartile groups according to serum selenium levels[a]

Groups (*n*) Se range (ug/L)	Q1 (260) 18.4 to 85.3	Q2 (261) 85.4 to 99.9	Q3 (259) 100.0 to 114.0	Q4 (261) 114.2 to 226.2	Excluded subjects (173)
Men, *n* (%)	160 (63.0)	165 (65.6)	160 (63.6)	159 (63.0)	116 (67.1)
Means (SD)					
Age (years)	65.8 (12.7)	62.0 (12.4)*	61.3 (12.4)*	55.6 (13.0)*	61.8 (12.3)
Vintage of dialysis	6.8 (6.7)	6.2 (6.0)	6.2 (5.9)	6.7 (6.4)	10.0 (8.8)§
Sex-and age-adjusted means (95% CI)					
BMI (Kg/m^2)	20.4 (20.0 to 20.7)	20.8 (20.5 to 21.2)	21.1 (20.8 to 21.5)†	21.1 (20.7 to 21.5)†	20.6 (20.2 to 21.0)
SBP (mmHg)	155.0 (152 to 158)	156.0 (153 to 159)	156.0(153 to 159)	154.0 (151 to 158)	152.9 (149 to 156)
Serum albumin (g/dL)	3.7 (3.6 to 3.7)	3.7 (3.7 to 3.8)	3.8 (3.7 to 3.8)†	3.9 (3.8 to 3.9)†	3.7 (3.6 to 3.7)
Hb (g/dL)	10.1 (10.0 to 10.3)	10.2 (10.1 to 10.4)	9.9 (9.8 to 10.1)	10.2 (10.0 to 10.4)	10.5 (10.3 to 10.8)§
Ht (%)	31.1 (30.6 to 31.6)	31.6 (31.1 to 32.1)	30.6 (30.1 to 31.1)	31.4 (30.9 to 31.9)	32.5 (31.9 to 33.1)§
hsCRP (mg/dL)	1.81 (1.52 to 2.16)	1.13 (0.95 to 1.35)†	1.14 (0.95 to 1.35)†	0.94 (0.78 to 1.12)†	1.38 (0.95 to 1.68)
Sex- and age-adjusted proportions of comorbid conditions, cause of renal failure and habits (%)					
Hypertension	87.7	88.3	88.4	93.4‡	80.9§
DM	29.1	33.9	32.2	29.0	31.9
CGN	28.3	28.9	29.4	27.5	29.3
DMN	24.4	28.2	28.9	27.2	28.8
History of stroke	19.3	15.8	14.0	14.0	14.3
History of MI	3.9	5.5	5.8	3.4	3.1
Current smoker	31.7	43.0‡	31.9	40.9	48.7§
Past smoker	36.0	31.5	43.2	33.8	31.6
Regular drinker	9.0	5.5	10.3	13.4	7.5

[a]Continuous variables are expressed as means (standard deviations) or adjusted means (95% CIs) estimated by ANCOVA. Multiple comparisons were performed using the Bonferroni method. Proportions are expressed as percentages. Proportions were estimated by logistic regression analysis after adjusting for sex and mean age (61.2 years). Abbreviations: Se, selenium; *n*, number; SBP, systolic blood pressure; Hb, hemoglobin; Ht, hematocrit; DM, diabetes mellitus; CGN, chronic glomerulonephritis; DMN, diabetetic nephropathy; MI, myocardial infarction.
*$P < 0.05$ by one-way ANOVA. †$P < 0.05$ by ANCOVA. ‡$P < 0.05$ by logistic regression. (Q1 versus each group), §$P < 0.05$ by Student's *t*-test or chi-square test (subjects versus excluded subjects).

disease-related death in the upper three groups compared with those for patients in the Q1 group. An inverse relationship between serum selenium levels and all-cause mortality was observed after adjustment for sex and age (trend $P = 0.007$), and an inverse relationship between serum selenium and infectious disease-related mortality was observed after adjustment for sex and age (trend $P < 0.001$). Risks for infectious disease-related mortality in the Q2, Q3 and Q4 groups were significantly lower than that in the Q1 group (each $P < 0.001$) after adjustment for sex and age.

Risks for infectious disease-related death in the Q2 and Q4 groups were significantly lower than that in the Q1 group (Q1 versus Q2, $P = 0.038$; Q1 versus Q4, $P = 0.032$), and the risk for infectious disease-related death in the Q3 group was lower but not significantly lower ($P = 0.121$) after multivariate adjustment. An evident inverse relationship between serum selenium levels and infectious disease-related mortality was observed even after multivariate adjustment (trend $P = 0.024$). On the other hand, relationships between serum selenium levels and all-cause, cardiovascular and malignant disease-related mortality rates were not observed after multivariate adjustment.

Discussion

We showed that low levels of serum selenium increased the risk for death, especially infectious disease-related death, in hemodialysis patients. Previous studies showed that lower levels of serum selenium were associated with the development of cardiovascular diseases [2, 21, 22], malignant neoplasms [5, 6, 22] and viral infectious diseases [4, 23] in the general population. However, to our knowledge, there has been no investigation of whether low serum selenium levels contribute to an increase in mortality either in the general population or hemodialysis patients.

It is known that hemodialysis patients have disturbances of the immune system and subsequent susceptibility to infections. Rate of mortality caused by sepsis was shown to be 50 times higher in hemodialysis patients than in the general population after adjustments for risk factors [24]. Current data suggest that acquired immunity disturbances in hemodialysis patients are attributed mainly to dysfunction of T-lymphocytes and activation of antigen-presenting cells (APCs). Since T-lymphocyte-dependent immune response is impaired in hemodialysis patients, hemodialysis patients tend to develop infectious diseases. In addition, MIAS activates APCs and also exacerbates T-lymphocyte function [25].

The influence of selenium on immunity function has been shown [23]. An appropriate selenium intake is necessary for maintenance of cell-mediated immunity and humoral immunity [26, 27]. Selenium-deficient lymphocytes have less capability for proliferation in response to mitogen [28]. Selenium inhibits activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated

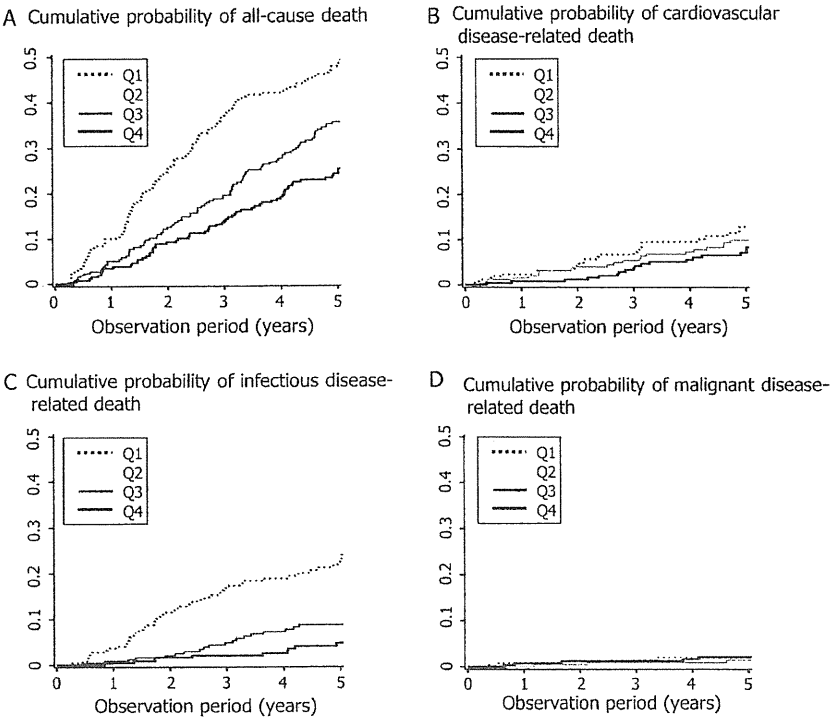

Fig. 2. Kaplan–Meier estimated cumulative cause-specific mortality curves. The upper left schema (A) shows Kaplan–Meier estimated cumulative probability of all-cause death by quartile groups according to serum selenium levels. The Q1 group had a significantly higher rate of mortality from all-cause death than the mortality rates in the other three groups (Q1 versus Q2 P = 0.024; Q1 versus Q3 P < 0.001; Q1 versus Q4 P < 0.001, by log-rank test). The lower left schema (C) shows cumulative probability of infectious disease-related mortality in the quartile groups. The Q1 group had a significantly higher rate of mortality from infectious disease-related death than the mortality rates in the other three groups (Q1 versus Q2 P < 0.001; Q1 versus Q3 P < 0.001; Q1 versus Q4 P < 0.001, by log-rank test). No significant differences in mortality rates between groups were observed in CVD-related death (B) and malignant disease-related death (D).

Table 3. Number of deaths and crude or age-adjusted mortality rates (per 1000 patient-years) by quartile groups according to serum selenium levels in hemodialysis patients[a]

Quartile groups (*n*) Range of Se (pg/L)	Q1 (*n* = 260) 18.4–85.3	Q2 (*n* = 261) 85.4–99.9	Q3 (*n* = 259) 100.0–114.0	Q4 (*n* = 261) 114.2–226.2	P for trend*
All-cause death	*n* = 125 134.5 (49.6)	*n* = 104 99.9 (37.7)	*n* = 91 85.9 (31.6)	*n* = 62 55.2 (25.2)	0.008
CVD-related death	*n* = 41 44.1 (15.5)	*n* = 60 57.6 (22.2)	*n* = 52 49.1 (20.6)	*n* = 30 26.7 (12.3)	0.289
Infectious disease-related death	*n* = 49 52.7 (14.4)	*n* = 21 20.2 (6.6)	*n* = 19 17.9 (4.8)	*n* = 10 8.9 (3.6)	<0.001
Malignant disease-related death	*n* = 4 4.3 (0.7)	*n* = 5 4.8 (2.9)	*n* = 3 2.8 (2.4)	*n* = 5 4.5 (1.6)	0.615

[a]Variables indicate numbers of deaths and crude (age-adjusted) mortality rates (per 1000 patient-years). Age-adjusted mortality rates were calculated by the direct method based on WHO standard population. Abbreviations are the same as those used in Table 2*, trend test after adjustment for age.

B cells, which regulates genes that encode inflammatory cytokines. Reduction of selenium contributes to activated inflammation [29, 30]. These studies suggested that selenium deficiency exacerbates immune function and activates inflammation. Selenium deficiency also exacerbates hemodialysis-related immune dysfunction and may contribute to an increase in risks for severe infection and infectious disease-related death.

It was not definitely determined in this observational study whether decreased selenium directly contributes to increased risks for death due to infection and there remains the possibility that selenium deficiency is one of the common phenomena just secondarily derived from a systemic deconditioning status. In our previous study, cross-sectional analysis indicated that decreased selenium levels were associated with malnutrition and activated inflammation status [31]. However, we also revealed that low levels of serum selenium increased the risk for infectious disease-related death independently of elevated hsCRP and low albumin level in hemodialysis patients. This suggested that low levels of serum selenium increased the risk for infectious disease-related death independently of MIAS and that decreased selenium level is not only a subsequent event secondary to systemic deconditioning but also an

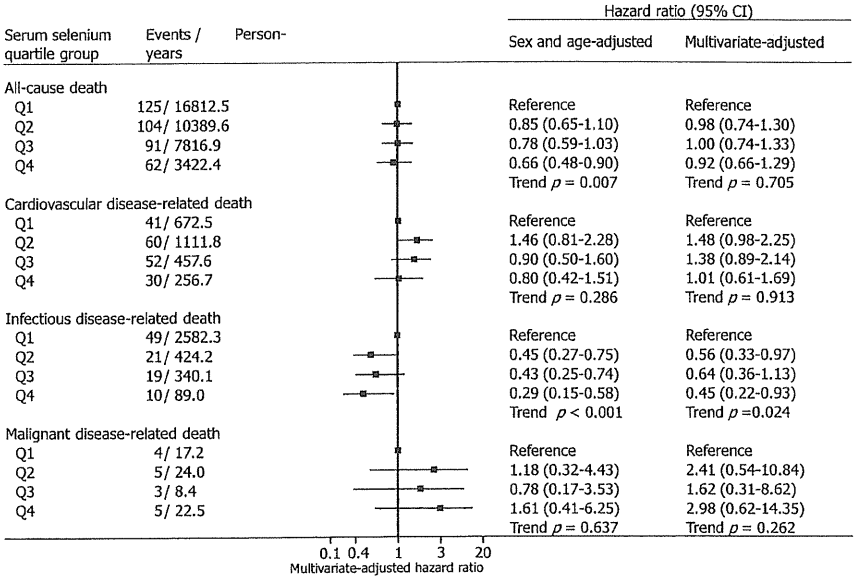

Fig. 3. Adjusted HRs for death (all-cause, CVD, infectious disease and malignant disease-related death) in the upper three quartile groups with patients in the Q1 group serving as a reference. Risk for all-cause death in the Q4 group was significantly lower than that in the Q1 group ($P < 0.001$, by Cox's regression), and risks for infectious disease-related death in the upper three groups were lower than that in the Q1 group after sex and age adjustment (each $P < 0.001$). Inverse relationships between serum selenium levels and all-cause mortality and between serum selenium levels and infectious disease-related mortality were observed even after sex and age adjustment (each trend $P = 0.007$, $P < 0.001$). An evident inverse relationship between serum selenium levels and infectious disease-related mortality was observed even after multivariate adjustment (trend $P = 0.003$). Relationships between serum selenium levels and all-cause, cardiovascular and malignant disease-related mortalities were not observed after multivariate adjustment.

independent predictive risk factor for infectious disease-related death.

Since it was shown that severe selenium deficiency caused endemic cardiomyopathy [32], relationships between selenium deficiency and cardiac diseases have been investigated in several studies. Salonen *et al.* [21] showed that the adjusted relative risk for cardiovascular death due to low serum selenium level (Se < 45 μg/L) was 2.2 (95% CI: 1.20–4.00, P < 0.01) in a case–control study in Finland, where selenium level in the soil is very low. A meta-analysis (including 14 prospective cohort studies and 11 case–control studies) indicated that the pooled relative risk for coronary events in a comparison of the highest to lowest categories of selenium level was 0.85 in cohort studies (95% CI: 0.74–0.99) and 0.43 in case–control studies (95% CI: 0.29–0.66) [2]. This meta-analysis showed that high levels of serum selenium contribute to decreased risk of coronary events.

An inverse relationship was not observed between serum selenium levels and cardiovascular mortality among hemodialysis patients in our study. There are several possible reasons for this. Although serum selenium levels in hemodialysis patients in our study were lower than those in the Japanese general population [33], the levels of serum selenium in our study samples were not greatly decreased compared to those in the study in Finland [21]. The small number of subjects with severe selenium deficiency in our study may have attenuated the inverse relationship between selenium levels and cardiovascular death. In addition, dialysis itself is a strong risk factor for cardiovascular disease [34], and a relatively weak inverse relationship between selenium levels and cardiovascular mortality may be negated by a strong effect of dialysis on cardiovascular risks.

An inverse relationship was also not observed between serum selenium levels and malignant disease-related mortality among hemodialysis patients in our study. Previous studies suggested associations between selenium deficiency and risks for development of several cancers [5, 6]. It was hypothesized that selenium deficiency contributes to increased risks for death due to several cancers. However, only 17 patients died of malignant disease-related death and we were not able to perform accurate risk assessment for malignant disease-related death due to the small number of subjects.

This study firstly showed that lower selenium levels in hemodialysis patients independently contributed to increased risks for infectious disease-related death. However, greatly reduced levels of selenium (<70 μg/L) [35] were found in only 80 patients, and the rather small sample size of patients with selenium deficiency probably contributed to weak statistical power for estimating risks for death after multivariate adjustment. There were 173 patients who did not provide additional serum samples to determine serum selenium levels in this study. They had rather unfavorable characteristics. Since we excluded data for these 173 patients from analysis, the results of this study were for rather healthy hemodialysis patients and the relationship between serum selenium levels and risk for death may be attenuated.

Selenium deficiency is a serious problem that commonly occurs in a very restricted area where environmental selenium is greatly depleted. However, our data indicated that selenium deficiency might occur in hemodialysis patients in non-specific areas where environmental selenium

is sufficient such as in Japan [33]. Nonetheless, a strong inverse relationship between serum selenium and infectious disease-related death was clearly indicated; therefore, we should pay more attention to selenium deficiency in hemodialysis patients and should examine why serum selenium levels in hemodialysis patients are decreased.

Acknowledgements. This study was supported by a grant from the Uehara Memorial Foundation, a grant from the Bureau of Medical Affairs of the Iwate Prefectural Government, a grant from the Japan Arteriosclerosis Prevention Fund, two grants from the Japanese Ministry of Education, Science, Sports and Culture, a Grant-in-Aid for Scientific Research (C), No. 018590568, a Grant-in-Aid for Scientific Research (C), No. 21590660 and a Grant-in-Aid from the Ministry of Health and Welfare, H18-Kan-en-ippan-002, in Japan. We are grateful for the collaboration of medical doctors and staff of the institutes who took part in the KAREN study.

Conflict of interest statement. None declared.

References

1. Rayman MP. The importance of selenium to human health. *Lancet* 2000; 356: 233–241
2. Flores-Mateo G, Navas-Acien A, Pastor-Barriuso R *et al.* Selenium and coronary heart disease: a meta-analysis. *Am J Clin Nutr* 2006; 84: 762–773
3. Schrauzer GN. Selenium and selenium-antagonistic elements in nutritional cancer prevention. *Crit Rev Biotechnol* 2009; 29: 10–17
4. Beck MA, Levander OA, Handy J. Selenium deficiency and viral infection. *J Nutr* 2003; 133: 1463S–1467S
5. Clark LC, Combs GF Jr, Turnbull BW *et al.* Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin. A randomized controlled trial. Nutritional Prevention of Cancer Study Group. *JAMA* 1996; 276: 1957–1963
6. Lippman SM, Klein EA, Goodman PJ *et al.* Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2009; 301: 39–51
7. Saint-Georges MD, Bonnefont DJ, Bourely BA *et al.* Correction of selenium deficiency in hemodialyzed patients. *Kidney Int Suppl* 1989; 27: S274–S277
8. Kallistratos G, Evangelou A, Seferiadis K *et al.* Selenium and haemodialysis: serum selenium levels in healthy persons, non-cancer and cancer patients with chronic renal failure. *Nephron* 1985; 41: 217–222
9. Dworkin B, Weseley S, Rosenthal WS *et al.* Diminished blood selenium levels in renal failure patients on dialysis: correlations with nutritional status. *Am J Med Sci* 1987; 293: 6–12
10. Hampel G, Schaller KH, Rosenmuller M *et al.* Selenium-deficiency as contributing factor to anemia and thrombocytopenia in dialysis patients. *Life Support Syst* 1985; 3: 36–40
11. Foote JW, Hinks LJ, Lloyd B. Reduced plasma and white blood cell selenium levels in haemodialysis patients. *Clin Chim Acta* 1987; 164: 323–328
12. Tonelli M, Wiebe N, Hemmelgarn B *et al.* Trace elements in hemodialysis patients: a systematic review and meta-analysis. *BMC Med* 2009; 7: 25
13. Foley RN, Collins AJ. End-stage renal disease in the United States: an update from the United States Renal Data System. *J Am Soc Nephrol* 2007; 18: 2644–2648
14. United States Renal Data System. *USRD Annual Data Report 2009.* http://www.usrds.org/ (December 2010, date last accessed)
15. Kalantar-Zadeh K, Block G, Humphreys MH *et al.* Reverse epidemiology of cardiovascular risk factors in maintenance dialysis patients. *Kidney Int* 2003; 63: 793–808
16. Kalantar-Zadeh K, Kopple JD, Block G *et al.* A malnutrition-inflammation score is correlated with morbidity and mortality in maintenance hemodialysis patients. *Am J Kidney Dis* 2001; 38: 1251–1263
17. Stenvinkel P, Heimburger O, Paultre F. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. *Kidney Int* 1999; 55: 1899–1911
18. Ohsawa M, Kato K, Itai K *et al.* Cardiovascular risk factors in hemodialysis patients: results from baseline data of kaleidoscopic approaches to patients with end-stage renal disease study. *J Epidemiol* 2005; 15: 96–105
19. Ohsawa M, Kato K, Itai K. Standardized prevalence ratios for chronic hepatitis C virus infection among adult Japanese hemodialysis patients. *J Epidemiol* 2010; 20: 30–39
20. National Committee for Clinical Laboratory Standards. *Evaluation of Precision Performance of Clinical Chemistry Devices.* Villanova, PA: NCCLS; 1999. (NCCLS approved Guideline EP5-A)
21. Salonen JT, Alfthan G, Huttunen JK *et al.* Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. *Lancet* 1982; 2: 175–179
22. Bleys J, Navas-Acien A, Guallar E. Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults. *Arch Intern Med* 2008; 168: 404–410
23. Hoffmann PR, Berry MJ. The influence of selenium on immune responses. *Mol Nutr Food Res* 2008; 52: 1273–1280
24. Sarnak MJ, Jaber BL. Mortality caused by sepsis in patients with end-stage renal disease compared with the general population. *Kidney Int* 2000; 58: 1758–1764
25. Eleftheriadis T, Antoniadi G, Liakopoulos V *et al.* Disturbances of acquired immunity in hemodialysis patients. *Semin Dial* 2007; 20: 440–451
26. Broome CS, McArdle F, Kyle JA *et al.* An increase in selenium intake improves immune function and poliovirus handling in adults with marginal selenium status. *Am J Clin Nutr* 2004; 80: 154–162
27. Bauersachs S, Kirchgessner M, Paulicks BR. Effects of different levels of dietary selenium and vitamin E on the humoral immunity of rats. *J Trace Elem Electrolytes Health Dis* 1993; 7: 147–152
28. Arthur JR, McKenzie RC, Beckett GJ. Selenium in the immune system. *J Nutr* 2003; 133: 1457S–1459S
29. Maehira F, Miyagi I, Eguchi Y. Selenium regulates transcription factor NF-kappaB activation during the acute phase reaction. *Clin Chim Acta* 2003; 334: 163–171
30. Zheng HT, Zhou LN, Huang CJ. Selenium inhibits high glucose- and high insulin-induced adhesion molecule expression in vascular endothelial cells. *Arch Med Res* 2008; 39: 373–379
31. Fujishima Y, Ohsawa M, Itai K *et al.* Serum selenium levels in hemodialysis patients are significantly lower than those in healthy controls. *Blood Purif;* In press (DOI: 10.1159/000323538)
32. Ge K, Xue A, Bai J *et al.* Keshan disease-an endemic cardiomyopathy in China. *Virchows Arch A Pathol Anat Histopathol* 1983; 401: 1–15
33. Combs GF Jr. Selenium in global food systems. *Br J Nutr* 2001; 85: 517–547
34. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. *Am J Kidney Dis* 1998; 32: S112–129
35. Neve J. Human selenium supplementation as assessed by changes in blood selenium concentration and glutathione peroxidase activity. *J Trace Elem Med Biol* 1995; 9: 65–73

Received for publication: 12.10.10; Accepted in revised form: 29.12.10

J Epidemiol 2011;21(6):491-499
doi:10.2188/jea.JE20100187

Original Article

Seropositivity for Anti-HCV Core Antigen is Independently Associated With Increased All-Cause, Cardiovascular, and Liver Disease-Related Mortality in Hemodialysis Patients

Masaki Ohsawa[1], Karen Kato[2], Kozo Tanno[1], Kazuyoshi Itai[1], Yosuke Fujishima[1,3], Akira Okayama[4], Tanvir Chowdhury Turin[5], Toshiyuki Onoda[1], Kazuyuki Suzuki[6], Motoyuki Nakamura[7], Kazuko Kawamura[8], Takashi Akiba[9], Kiyomi Sakata[1], and Tomoaki Fujioka[3]

[1]Department of Hygiene and Preventive Medicine, Iwate Medical University, Iwate, Japan
[2]Division of Urology, San-ai Hospital, Iwate, Japan
[3]Department of Urology, Iwate Medical University, Morioka, Japan
[4]The First Institute of Health Service, Japan Anti-Tuberculosis Association, Tokyo, Japan
[5]Department of Medicine, University of Calgary, Calgary, Alberta, Canada
[6]Division of Gastroenterology and Hepatology, Department of Internal Medicine, Iwate Medical University, Morioka, Japan
[7]Division of Cardiovascular Medicine, Nephrology and Endocrinology, Department of Internal Medicine, Iwate Medical University, Morioka, Japan
[8]Iwate Health Service Association, Morioka, Japan
[9]Division of Blood Purification, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan

Received December 24, 2010; accepted July 27, 2011; released online October 15, 2011

ABSTRACT

Background: It is not known whether chronic or past hepatitis C virus (HCV) infection contributes to the high mortality rate in hemodialysis patients.

Methods: This prospective study of 1077 adult hemodialysis patients without hepatitis B virus infection used Poisson regression analysis to estimate crude and sex- and age-adjusted rates (per 1000 patient-years) of all-cause, cardiovascular, infectious disease-related and liver disease-related mortality in patients negative for HCV antibody (group A), patients positive for HCV antibody and negative for anti-HCV core antigen (group B), and patients positive for anti-HCV core antigen (group C). The relative risks (RRs) for each cause of death in group B vs group C as compared with those in group A were also estimated by Poisson regression analysis after multivariate adjustment.

Results: A total of 407 patients died during the 5-year observation period. The sex- and age-adjusted mortality rate was 71.9 in group A, 80.4 in group B, and 156 in group C. The RRs (95% CI) for death in group B vs group C were 1.23 (0.72 to 2.12) vs 1.60 (1.13 to 2.28) for all-cause death, 0.75 (0.28 to 2.02) vs 1.64 (0.98 to 2.73) for cardiovascular death, 1.64 (0.65 to 4.15) vs 1.58 (0.81 to 3.07) for infectious disease-related death, and 15.3 (1.26 to 186) vs 28.8 (3.75 to 221) for liver disease-related death, respectively.

Conclusions: Anti-HCV core antigen seropositivity independently contributes to elevated risks of all-cause and cause-specific death. Chronic HCV infection, but not past HCV infection, is a risk for death among hemodialysis patients.

Key words: hepatitis C virus; hemodialysis; mortality; population-based cohort study

INTRODUCTION

Hepatitis C virus (HCV) infection, currently the most common blood-borne infection, is an emerging public health problem.[1] Only 20% to 30% of patients with acute HCV infection spontaneously recover; the rest develop chronic HCV infection. Most patients who recover from HCV infection do not develop liver cirrhosis or hepatocellular carcinoma (HCC), whereas patients with chronic HCV infection develop liver cirrhosis or HCC within 20 to 30 years of initial infection.[2]

Hemodialysis patients are especially vulnerable to HCV infection, because of exposure associated with dialysis and blood transfusion.[3–5] The prevalence of HCV in hemodialysis patients is very high (2.7%–30.0%).[6–21] Studies suggest that HCV infection independently contributes to increased mortality among hemodialysis patients.[22–27] However, it is not known whether chronic HCV infection or a history of past

Address for correspondence. Masaki Ohsawa, MD, Department of Hygiene and Preventive Medicine, Iwate Medical University, 2-1-1 Nishitokuta, Yahaba-cho, Iwate 028-3694, Japan (e-mail: masakio@iwate-med.ac.jp).

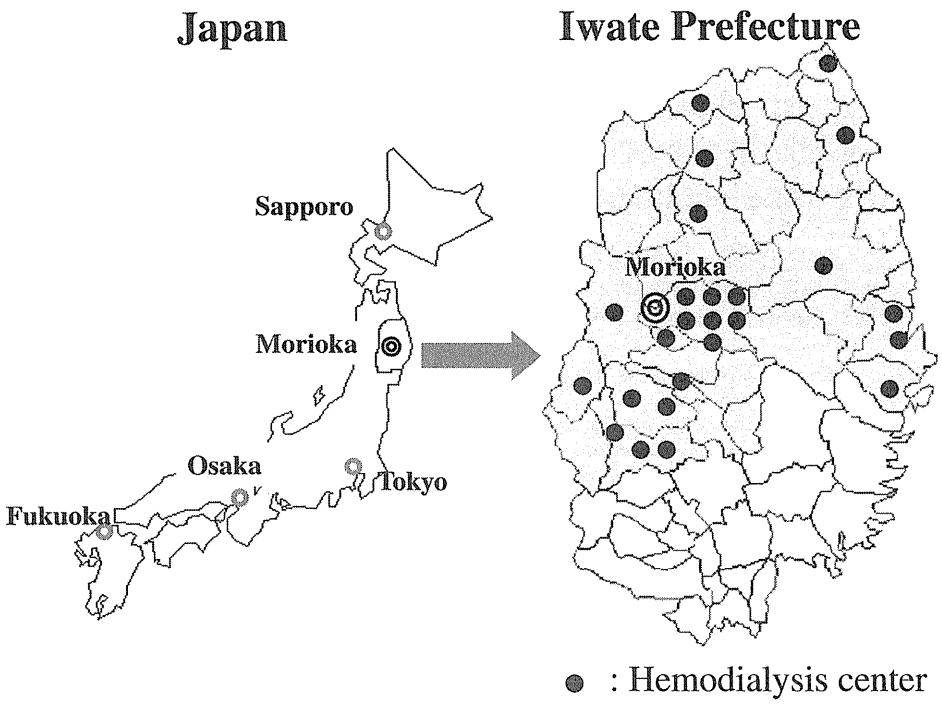

Figure 1. Map of the KAREN Study area. A map of Japan. Morioka, the capital of Iwate Prefecture, is located in northeast Honshu island. The KAREN Study area (the shaded area covering about two-thirds of Iwate Prefecture) has 26 hemodialysis centers. Only 1 center, which treats 7 patients, was not included in the study. Each closed circle represents a hemodialysis center.

HCV infection increases mortality. Moreover, it has not been established whether the elevated mortality risk due to HCV infection is mostly attributable to an increase in liver disease-related deaths.

To assess the contribution of past and chronic HCV infection among hemodialysis patients, we estimated the relative risks of all-cause and cause-specific death attributable to HCV antibody seropositivity and anti-HCV core antigen seropositivity.

METHODS

Participants

The eligible participants were adult hemodialysis patients who participated in the KAREN study, a population-based prospective study that has been conducted since 2003 in northern Japan (Figure 1).[28] A total of 1214 adult hemodialysis patients (80.6% of all hemodialysis patients in the study area; age 22 to 95 years; 779 men and 435 women) are included in the KAREN study. The participants in the KAREN study are patients who were undergoing adult hemodialysis in April 2003. A total of 137 patients who were positive for hepatitis B surface antigen were excluded. Ultimately, data from 1077 patients were analyzed. We ascertained the vital status of all subjects in a 5-year follow-up survey (Figure 2). All participants gave written informed consent to participate. 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.

Data collection

The initial investigations in the KAREN Study consisted of a questionnaire, review of medical records, measurements of blood pressure and anthropometric data, and blood testing. The data gathering methodology was previously described.[21,28] Information on HCV antibody serology testing was collected by reviewing medical charts.[21]

Results of anti-HCV antibody tests could not be obtained from chart review for 50 patients. Frozen serum samples from those patients were thawed and anti-HCV antibody tests were performed using a second-generation assay (Architect HCV, Abbott Laboratories, Japan). Frozen samples from patients who were positive for anti-HCV antibody were thawed, and HCV core antigen tests were performed using a chemiluminescent enzyme immunoassay (Lumispot Eiken HCV antigen, Eiken Chemical Co., LTD, Japan).[21]

Outcomes

Follow-up studies were performed annually at each center. Members of the KAREN Study team reviewed all the medical records of study participants. The medical records of deceased patients were summarized. Cause of death was independently determined by physicians on the KAREN Outcome Review

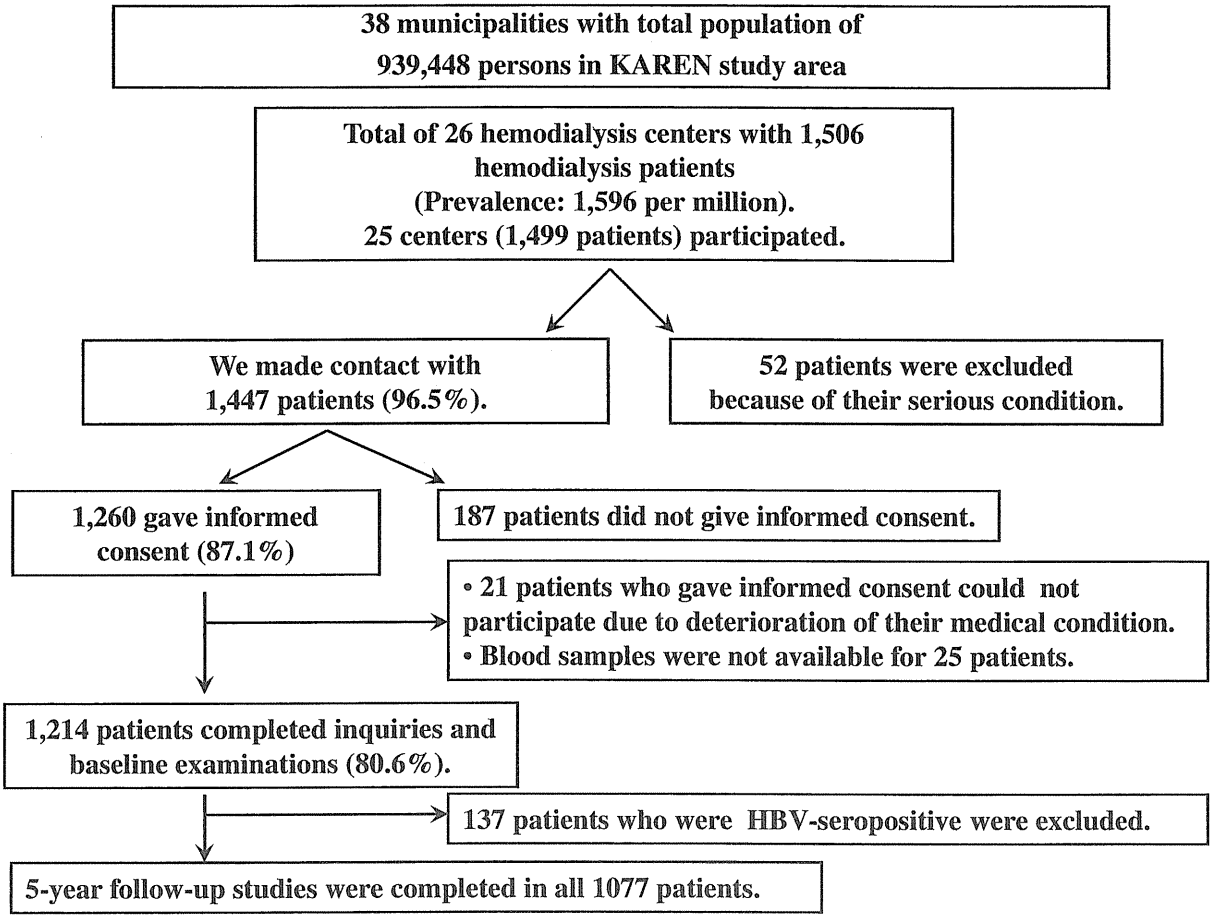

Figure 2. Flow chart of the procedure used to select patients for participation in the KAREN Study. There were 1506 adults receiving hemodialysis in 26 centers in the study area. We were able to contact 1447 patients (96.5%). Fifty-two patients were excluded because of their serious clinical condition. A total of 1260 patients (87.1%) provided written informed consent for participation in the study, and 1214 patients (80.6%) completed the baseline examinations. A total of 137 patients who were positive for hepatitis B surface antigen were excluded. Finally, data from 1077 patients were analyzed. We ascertained the vital status of all participants after completion of a 5-year follow-up survey.

Committee, based on the summaries. Disagreements regarding cause of death were discussed, and the final determination was reached by consensus. We identified the 3 major causes of death (cardiovascular, infectious disease-related, and liver disease-related) using codes form the Tenth Revision of the International Classification of Diseases (ICD-10; Table 1).

Classification and definition

The participants were divided into 3 groups based on the results of HCV antibody testing and anti-HCV core antigen testing at the baseline survey. Group A consisted of patients who were negative for anti-HCV antibodies ($n = 968$). Group B consisted of patients who were positive for anti-HCV antibodies and negative for anti-HCV core antigen antibodies ($n = 55$). Group C consisted of patients who were positive for both anti-HCV antibodies and anti-HCV core antigen antibodies ($n = 79$). These 3 groups were selected because they roughly corresponded to patients without HCV infection (group A), patients with past HCV infection (group B), and patients with chronic HCV infection (group C).[21]

High blood pressure was defined as systolic blood pressure (SBP) in the highest quartile of this study population (SBP ≥169 mm Hg). Low blood pressure was defined as SBP in the lowest quartile (<140 mm Hg). Diabetes was defined as a nonfasting plasma glucose level of 200 mg/dL or higher, a plasma HbA1c of 6.5% or higher, use of antidiabetic medication, or a combination thereof. Dyslipidemia was defined as serum total cholesterol (TC) of 220 mg/dL or higher, serum high-density lipoprotein cholesterol (HDL-C) level less than 40 mg/dL, use of antidyslipidemic medication, or a combination thereof. High body mass index (BMI) was defined as a BMI of 27.5 kg/m^2 or higher. Low BMI was defined as a BMI less than 18.5 kg/m^2. High-sensitivity C reactive protein (hs-CRP) level was considered high if it was in the highest quartile (≥3.6 mg/L). Hypoalbuminemia was

Table 1. Criteria for determining causes of death in the KAREN Study (based on ICD-10)

Cardiovascular death: I01–I99 plus R96		
cardiac death: I20–I25, I27, I29, I30–I52		
	I20–I25	coronary artery disease
	I33	Acute and subacute endocarditis
	I50	heart failure
pulmonary embolism: I26		
stroke death: I60–I69		
	I60	subarachnoidal hemorrhage
	I61, I62	intracerebral hemorrhage
	I63	cerebral infarction
	I64, I67	other type of stroke
vascular death: I70–I77		
	I70	Atherosclerosis
	I71	aortic anuerysm and dissection
	I72, I73	other peripheral artery disease
	I74	arterial embolism and thrombosis
	I77	other arterial disease
sudden cardiac death: I46, I49, R96		
cardiac arrest: I46		
	I46.0	Cardiac arrest with successful resuscitation
	I46.1	Sudden cardiac death, so described
	I46.9	Cardiac arrest, unspecified
ventricular fibrillation and flutter: I49		
	I49.0	Ventricular fibrillation and flutter
other sudden death, cause unknown R96		
	R96.0	Instantaneous death
	R96.1	Death occurring less than 24 hours from onset of symptoms, not otherwise explained
Infectious disease-related death:		
A: bacterial infection: A00–A09, A15–A19, A40–A41 (septicemia)		
B: viral infection, fungal and other microorganism infection		
G: infectious diseases in nervous system		
	G00	Bacterial meningitis, not elsewhere classified
	G04.2	Bacterial meningoencephalitis and meningomyelitis, not elsewhere classified
J: infectious diseases in respiratory tract		
	J10–J11	influenza
	J12–J18	pneumonia
	J20	acute bronchitis
	J69	Pneumonitis due to solids and liquids
	J86	Pyothorax
K: infectious diseases in gastrointestinal tract and digestive organ		
	K65	Peritonitis
	K80.3	Calculus of bile duct with cholangitis
	K81	Cholecystitis
L: infectious diseases in skin and subcutaneous tissue		
	L03	Cellulitis
	L89	Decubitus ulcer
Liver disease-related death		
	K71.2	Toxic liver disease with acute hepatitis
	K72	Acute and subacute hepatic failure
	K73	Chronic hepatitis, not elsewhere classified
	K74	Fibrosis and cirrhosis of liver
	C22.0	Liver cell carcinoma

defined as a serum albumin level less than 3.5 mg/dL. A smoking habit was defined as current smoking. Regular drinking was defined as alcohol consumption on 5 or more days per week.

Statistical analysis

Risk factor-related variables were expressed as sex- and age-adjusted means plus 95% CI and compared across HCV infection status groups using analysis of covariance (ANCOVA). The hs-CRP level was expressed as a sex- and age-adjusted geometric mean plus 95% CI. The χ^2 test was used to compare frequencies.

We defined the follow-up period as the period from the initial survey to the first outcome or the end of observation. Individuals who were free of outcomes in the 5-year follow-up study were administratively censored. The cumulative probability of each cause of death was estimated using the Kaplan-Meier method, and differences in the cumulative probability of death were assessed by the log-rank test. Crude mortality rates and sex- and age-adjusted mortality rates were estimated in the 3 groups (groups A, B, and C) by Poisson regression analysis in which multivariate-adjusted mortality rate ratios and their 95% CIs were calculated in groups B and C, with those of group A serving as reference. The variables used in the multivariate adjustment were traditional risk factors, including age, male sex, high BMI, dyslipidemia, diabetes, high blood pressure, history of myocardial infarction, stroke, or malignant disease, smoking habit, and regular drinking habit (model A). Hemodialysis-related risk factors, including low BMI, low blood pressure, high CRP level, and hypoalbuminemia, were also additionally used as explanatory variables in model B. All P values were 2-tailed, and values less than 0.05 were considered to indicate statistical significance. The statistical package PASW (version 18.0, IBM Japan Inc., Tokyo, Japan) was used for the statistical analysis.

RESULTS

Table 2 shows the baseline characteristics of the patients, stratified by HCV infection status. The proportions of patients in groups A, B, and C were 90.0%, 3.6%, and 6.5%, respectively. As compared with patients in group A, those in group C had significantly lower serum TC, serum low-density lipoprotein cholesterol (LDL-C), serum albumin, and serum creatinine levels, and lower platelet and white blood cell (WBC) counts ($P < 0.05$ for all tests). Patients in group B had significantly lower systolic blood pressure, TC, and LDL-C levels, and lower platelet and WBC counts than did patients in group A ($P < 0.05$ for all tests).

The proportion of current smokers in group C was the highest of the 3 groups ($\chi^2 = 6.47$, $P = 0.03$). There was no significant difference among groups in the proportions of patients with chronic glomerulonephritis ($\chi^2 = 5.66$, $P = 0.06$), diabetic nephropathy ($\chi^2 = 3.06$, $P = 0.22$), diabetes mellitus ($\chi^2 = 5.41$, $P = 0.07$), or past histories of myocardial infarction ($\chi^2 = 1.65$, $P = 0.44$), stroke ($\chi^2 = 0.93$, $P = 0.63$), or malignancy ($\chi^2 = 4.12$, $P = 0.13$). There were 4233 observed patient-years, after 5 years of follow-up. The mean and median follow-up periods were 3.9 and 4.9 years, respectively. A total of 406 patients died during the 5-year observation period.

Figure 3 shows the Kaplan-Meier estimated cumulative probabilities of death for patients in the 3 groups. The cumulative probability of all-cause death (upper left) in group C was significantly higher as compared with group A

Table 2. Baseline characteristics of patients stratified by HCV seropositivity

HCV seropositivity status groups (number of subjects)	group A HCV Ab(−) *n* = 968	group B HCV Ab(+) Ag(−) *n* = 39	group C HCV Ab(+) Ag(+) *n* = 70
male *n*, (%)	605 (62.5%)	25 (64.1%)	53 (75.7%)
mean age (SD) (yrs)	61.2 (13.3)	61.1 (13.6)	58.8 (10.9)
median vintage of HD (25–75%) (yrs)	4.5 (1.9–8.3)	8.9 (3.6–21.2)	8.3 (2.4–21.8)
Sex- and age-adjusted mean levels and their 95% CIs of anthropometrical and blood test measurements			
body mass index (kg/m^2)	21.0 (20.8–21.2)	20.6 (19.6–21.5)	20.2 (19.5–20.9)
SBP (mm Hg)	156 (154–157)	146 (138–153)[b]	155 (149–161)
total cholesterol level (mg/dl)	157 (155–159)	140 (129–150)[c]	136 (128–144)[c]
HDLC (mg/dl)	47.1 (46.1–48.0)	43.4 (38.7–48.1)	44.7 (41.2–48.2)
LDLC (mg/dl)	86.1 (84.4–87.8)	74.2 (65.9–82.5)[b]	71.8 (65.6–78.0)[c]
total protein (g/dl)	6.48 (6.46–6.52)	6.48 (6.33–6.63)	6.66 (6.55–6.78)[c]
serum albumine (g/dl)	3.78 (3.76–3.80)	3.74 (3.63–3.84)	3.50 (3.42–3.58)[c]
serum creatinine (mg/dl)	11.2 (11.1–11.4)	10.7 (9.94–11.5)	10.4 (9.87–11.0)[b]
hemoglobin (g/dl)	10.2 (10.1–10.3)	9.9 (9.50–10.4)	10.2 (9.93–10.5)
platelet count (10^4/μl)	18.7 (18.2–19.1)	15.0 (13.0–17.0)[c]	16.1 (14.5–17.6)[c]
white blood count (/μl)	5814 (5706–5923)	5025 (4487–5562)[b]	5120 (4718–5522)[c]
hsCRP[a] (mg/l)	1.16 (1.06–1.28)	1.40 (0.89–2.20)	1.20 (0.85–1.69)
Causes of renal filure, comorbid conditions and habits expressed as numbers (%)			
CGN	276 (28.5%)	18 (46.2%)	20 (28.6%)
DMN	241 (24.9%)	5 (12.8%)	16 (22.9%)
HTN	97 (10.0%)	4 (10.3%)	9 (12.9%)
PCK	37 (3.8%)	1 (2.6%)	0 (0.0%)
Lupus N	4 (0.4%)	0 (0.0%)	0 (0.0%)
Others	62 (6.4%)	4 (10.3%)	6 (8.6%)
unknown	251 (25.9%)	7 (17.9%)	19 (27.1%)
MI	42 (4.3%)	1 (2.6%)	1 (1.4%)
stroke	159 (16.4%)	5 (12.8%)	9 (12.9%)
malignancy	67 (6.9%)	6 (15.4%)	6 (8.6%)
DM	289 (29.9%)	5 (12.8%)	19 (27.1%)
dyslipidemia	440 (45.5%)	19 (48.7%)	32 (45.7%)
current smoker	254 (26.2%)	12 (30.8%)	28 (40.0%)[d]
past smoker	247 (25.5%)	8 (20.5%)	16 (22.9%)
regular drinker	71 (7.3%)	2 (5.1%)	2 (2.9%)

[a]hsCRP levels are expressed as adjusted geometric means (95% CIs) estimated by ANCOVA.
[b]$P < 0.05$ [c]$P < 0.01$ estimated by multiple comparisons using Bonferroni adjustment.
[d]$P < 0.05$ estimated by chi squared test.
Abbreviations: SD, standard deviation; HD, hemodialysis; CI, confidence interval; SBP, systolic blood pressure; HDLC, high-density lipoprotein cholesterol level; LDLC, low-density lipoprotein cholesterol level; hsCRP, serum high-sensitive C reactive protein; CGN, chronic glommerulonephritis; DMN, diabetic nephropathy; HTN, hypertensive nephrosclerosis; PCK, congenital polycystic kidney disease; Lupus N, lupus nephritis; MI, myocardial infarction; DM, diabetes mellitus; ANCOVA, analysis of covariance.

($P = 0.007$, log rank test), but not as compared with group B ($P = 0.174$). Group C also had higher probabilities of cardiovascular death (upper right, $P = 0.033$) and liver disease-related death (lower right, $P < 0.001$) as compared with group A. Group B did not have significantly higher probabilities of cardiovascular death ($P = 0.118$), infectious disease-related death ($P = 0.775$), or liver disease-related death ($P = 0.457$).

Table 3 shows the number of deaths, crude mortality rates, and sex- and age-adjusted mortality rates per 1000 patient-years (95% CIs), and relative risks for death expressed as sex- and age-adjusted relative mortality rate ratios (95% CIs) in group B and group C as compared with the reference (group A). The crude mortality rates in groups A, B, and C were 92.7, 94.0, and 147, respectively. Sex- and age-adjusted mortality rates (95% CI) in groups A, B, and C were 71.9 (62.6 to 81.3), 80.4 (37.9 to 123), and 156 (104 to 207), respectively. The relative risks (95% CI) for all-cause, cardiovascular, infectious disease-related, and liver disease-related death in group C were 2.16 (1.53 to 3.07), 1.98 (1.19 to 3.28), 2.46 (1.27 to 4.76), and 30.8 (5.34 to 178), respectively. In contrast, group B did not have significantly higher risks for death, with the exception of liver disease-related death (RR, 13.7; 95% CI, 1.24 to 152).

Table 4 shows the relative risks for each cause of death in groups B and C, as compared with the reference (group A), expressed as multivariate-adjusted mortality rate ratios. The RRs for all-cause and cardiovascular death in group C were approximately 2.0, which indicated significantly higher risks for such deaths in group C. The RR for infectious disease-related death in group C was also approximately 2.0, although the result was of marginal significance ($P = 0.051$ after

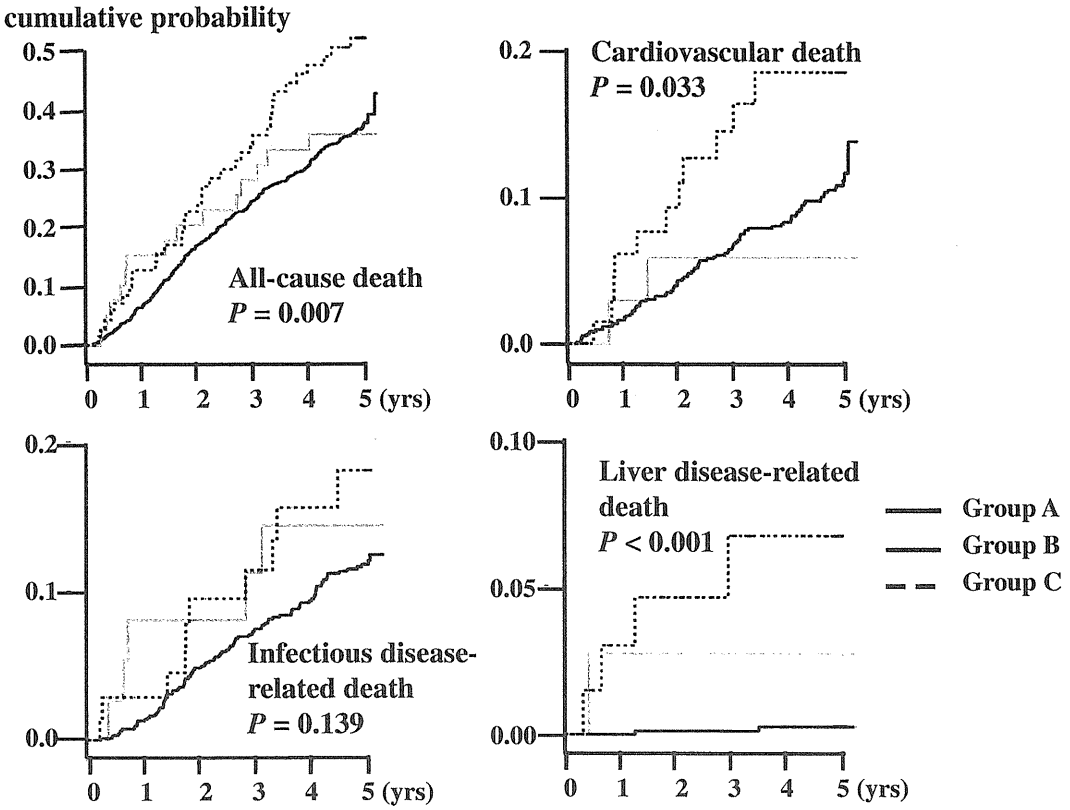

Figure 3. Estimated Kaplan-Meier cumulative probability of death in the 3 groups. The upper left graph shows the cumulative probability of all-cause death. Patients in group C had a significantly higher probability of death than did patients in group A ($P = 0.007$, log rank test), but there was no significant difference in probability of death between groups A and B ($P = 0.174$). The upper right graph shows the cumulative probability of cardiovascular death. Patients in group C had a significantly higher probability of cardiovascular death than did patients in group A ($P = 0.033$); the probability of cardiovascular death did not significantly differ between groups A and B ($P = 0.118$). The lower left graph shows the cumulative probability of infectious disease-related death, which did not significantly differ among the 3 groups. The lower right graph shows the cumulative probability of liver disease-related death. Patients in group C had a significantly higher probability of liver disease-related death as compared with patients without HCV infection ($P < 0.001$). The probability of liver disease-related death in group B did not significantly differ from that of group A ($P = 0.457$).

model A adjustment; $P = 0.14$ after model B adjustment). In contrast, the RRs for all-cause, cardiovascular, and infectious disease-related death in group B ranged from 0.75 to 1.66, and there was no significant increase in the risk of such deaths. The risk of liver disease-related death was 15.3 in group B and 28.8 in group C, which were significantly higher as compared with the reference group.

DISCUSSION

In this study, we estimated crude and sex- and age-adjusted rates for all-cause death and cause-specific death in hemodialysis patients who were negative for HCV antibodies, those who were positive for HCV antibodies, and those who were positive for both HCV antibodies and anti-HCV core antigen antibodies. We also calculated the relative risks of all-cause death and cause-specific death in patients positive for HCV antibodies only and patients positive for both HCV antibodies and anti-HCV core antigen antibodies as compared with patients who were negative for anti-HCV antibodies. These 3 groups roughly correspond to patients without HCV infection (group A), patients with past HCV infection (group B), and patients with chronic HCV infection (group C). Therefore, the results showed higher risks of all-cause, cardiovascular, infectious disease-related, and liver disease-related death among the chronic HCV subgroup, whereas past HCV infection was not associated with increased risk of any cause of death, except liver disease-related death.

Most prior studies investigated only the relative risks of all-cause and/or cause-specific death attributable to HCV infection among hemodialysis patients, without further differentiating between past and chronic HCV infection.[19,22,24,25] In a meta-analysis, Fabrizi et al found that the adjusted RR for all-cause mortality due to HCV infection was 1.34.[27] However, Stehman-Breen et al used quantitative estimation of HCV RNA levels to determine whether chronic