表2　尿所見から見た糸球体疾患の分類

	主な臨床病型（症候群）	検尿のパターン		
		蛋白尿のみ	蛋白尿＋血尿	血尿のみ
一次性糸球体疾患				
微小変化型ネフローゼ	ネフローゼ	◎		
膜性腎症	ネフローゼ，慢性腎炎	◎		
IgA腎症	慢性腎炎，ネフローゼ，持続性血尿	△	◎	○
膜性増殖性腎炎	ネフローゼ，慢性腎炎		◎	
巣状糸球体硬化症	ネフローゼ，慢性腎炎	◎	○	
半月体形成性腎炎	急速進行性腎炎，慢性腎炎，ネフローゼ		◎	○
二次性糸球体疾患				
糖尿病性腎症	ネフローゼ	◎		
ループス腎炎	慢性腎炎，ネフローゼ，持続性血尿	◎	◎	○
腎硬化症	慢性腎炎（1g／日未満）	◎		
腎アミロイドーシス	ネフローゼ，慢性腎炎	◎	○	

◎：最も高頻度，○：中頻度，△：低頻度，なし：稀

表3　経皮的腎生検の禁忌と合併症

禁　忌	合併症
出血傾向のある場合，片腎，嚢胞腎，水腎症，腎膿瘍，腎周囲炎，腎動脈瘤，コントロール不可能な高血圧，高度の心不全，呼吸停止不可能な患者，非協力者	腎周囲出血，血腫形成，腹腔内出血，肉眼的血尿，他臓器損傷，動静脈瘻，ショック，発熱，感染

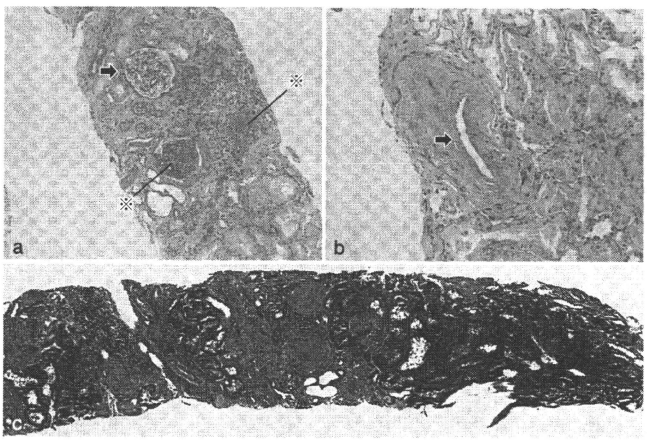


図2　腎生検

a：球状硬化に陥った糸球体（※）と，ほぼ正常な糸球体（矢印）が観察される．糸球体の増殖変化はない．尿細管の萎縮，間質の細胞浸潤を認める（PAS染色）．

b：動脈の中膜の肥厚を認める（矢印，PAS染色）．

c：間質の約50%に線維化を認める．一般に腎機能と間質病変の広がりは並行すると言われる（マッソン・トリクローム染色）．

治療方針

以上の結果から、**表4**に示す治療プランを立てた。

すなわち、腎硬化症を原疾患とするCKDステージ3で、尿蛋白1g／日以上、高血圧、肥満、脂質代謝異常を伴う患者の治療方針である。

表4　腎硬化症を原疾患とするCKDステージ3で，尿蛋白1g／日以上，高血圧，肥満，脂質代謝異常を伴う患者の治療方針

治療項目	治療目標	具体的指導・治療方針
生活習慣改善	禁煙，BMI 25未満	禁煙指導，適度の運動
食事指導	BMI 25未満	減量，減塩（6g／日未満），蛋白制限（50g／日未満）
血圧管理	血圧125／75mmHg未満	減塩，RAS系阻害薬を中心とした降圧治療
脂質管理	LDL-C120mg／dℓ未満	生活習慣改善後，必要に応じスタチン投与

◆初回の指導

本例のような肥満を伴う場合には、3カ月間で現体重の5％減を目標に体重調整を行い、最終的にはBMIが25未満を目標とする。そのために管理栄養士・看護師等のコメディカルと連携を取り、**表5**に示す問題がないのかの確認を行い、以下の指導を行った。

表5　生活習慣改善上の問題点

①1日1～2回の欠食がある（朝食・昼食は少量または欠食で，夕食にまとめ食いをする）
②食物繊維・ビタミン・ミネラルの摂取が不足している
③菓子類・清涼飲料水・アルコール類の摂取が過剰である
④同じような調理法のメニューが多い
⑤噛む回数が少なく，早食い
⑥運動習慣がない

①血圧測定、体重測定記録

自己管理の基本として、毎日の体重測定ならびに起床直後・就寝前の血圧測定の記録を習慣づけてもらうこととした。さらに今後の食事指導、腎機能の経過観察を考慮し、外来前日には自宅でも蓄尿を行い、24時間での尿量の確認と、その一部を外来に持参するように指導を行った。蓄尿検査の結果より、**表6**に示す各項目の結果を得ることが可能となる。

②運動・生活指導

禁煙指導を行い、禁煙の必要性について説明した。運動については、急に激しい運動を行うことの問題点も指摘されており、当初は毎日30分程度の早歩き（有酸素運動）と筋トレを行い、外来での腎機能、蛋白尿の程度、血圧を確認しながら実施するなどの生活指導を行った。

③食事指導

食事指導については、これまでの生活習慣改善を主体とし、あまりに急激な食事内容の変更は避け、適正なカロリー摂取30～35kcal／kg／日での指導をまずは行い、併せて、塩分制限（6g／日未満）の指導のみを開始した。また腎機能低下があることから、投薬もまずはこのような生活・食事指導の効果を確認した後に行うこととし、本人・家族の了承の下、次回外来での指導へと移行した。

◆2回目以降の指導

外来での血圧は145／90mmHgで、蓄尿検査の結果では、24時間尿量1800mℓ、尿中Na 62mEq／ℓ，尿中BUN 298mg／dℓ、尿蛋白定量68mg／dℓであった。

以上から尿蛋白量1・2g／日、尿中NaCl排泄量6・6g／日、蛋白摂取量48・5g／日であり、塩分制限と適切なカロリーの摂取指導のみで、蛋白摂取量の目標である0・6～0・8g／kg／日は達成されていた。

したがって、蛋白摂取については、これまでの塩分制限、生活指導のみで目標程度の摂取量に到達していることが確認できたため、特に制限を加えず、経過観察することとした。

◆降圧療法について

腎硬化症による腎機能障害で、中等度の蛋白尿を伴うこのような症例に対しては、腎機能保護のためには、十分な降圧を図ることが最も重要である。本例では生活習慣の改善、運動、減塩、減量により、血圧は145／90mmHg程度まで低下したものの、さらに尿

蛋白を減らす効果と腎機能の保護効果を期待し、RAS系阻害薬を使用することとした。

薬剤の選択に当たっては、RAS系阻害薬の中でアンジオテンシンⅡ受容体拮抗薬（ARB）とアンジオテンシン変換酵素（ACE）阻害薬については、腎保護効果は同等と考えられており、本例は若干の腎機能低下があることから、腎排泄ではないARBを用いることとし、オルメサルタン10mg朝1回で開始した。ARB追加後2カ月で外来での血圧は130／75mmHgに低下したものの、自宅での起床時血圧は140／90mmHg前後が持続するため、シルニジピン10mg／日を追加した。

表6　24時間蓄尿結果からの各種指標の計算法

項　目	計算法
Ccr（mℓ／分）	{尿中Cr濃度（mg／dℓ）×尿量（mℓ）}／{血清Cr濃度（mg／dℓ）×1440（分）}
蛋白摂取量（g／日）	{尿中BUN排泄量（g／日）＋0.031×体重（kg）}×6.25または尿中BUN排泄量（g／日）×6.25＋15
食塩摂取量（g／日）	尿中Na排泄量（mEq／ℓ）×尿量（ℓ）／17

◆脂質異常症について

外来加療3カ月目で、自宅早朝血圧130／70mmHg前後、外来随時血圧120／70mmHg、血清Cr1・52mg／dℓ、LDL-C130mg／dℓ、体重74・0kg、蓄尿検査では24時間尿量1500mℓ、尿中Na74mEq／ℓ、尿中BUN324mg／dℓ、尿中Cr59・7mg／dℓ、尿蛋白定量43mg／dℓであった。

以上の結果から、Ccr47・7mℓ／分（体表面積補正なし）、尿蛋白量0・65g／日、尿中NaCl排泄量6・5g／日、蛋白摂取量44・7g／日であった。血圧管理の改善、尿蛋白の減少を認めたが、LDL-C高値は生活習慣改善後も持続するため、腎機能悪化抑制効果も期待できるスタチンの追加を行うこととし、ピタバスタチン1mg／日を開始した。

本症例では、生活習慣の改善、自宅血圧を含めた厳格な血圧管理、RAS系阻害薬の併用により、尿蛋白量の減少が認められており、腎機能悪化抑制が期待できる。今後も長期の管理加療を要するため、コメディカルとも協力して、管理加療に当たる予定である。

ポイント

①生活・食事指導：CKDの管理は長期間に及ぶ。生活習慣の改善には、適切な運動、食事指導が重要であり、管理栄養士、看護師などコメディカルとも連携し、家族の理解を得た上で、適切な医療連携を実施していくことが必須である。
・食事指導については、これまでの食習慣を見極め、無理のない指導を行う。
・肥満の改善のため、適切な運動習慣、エネルギー摂取量調節を行う。
・塩分制限は、血圧コントロールに有効であると同時に尿蛋白減少効果も期待できる。
・加齢、腎機能低下に伴い、蛋白摂取量は減じてくることが多く、塩分制限、減量の指導を行うと、蛋白摂取量0・8g／kg／日程度であれば、無理なく達成されることが多い。

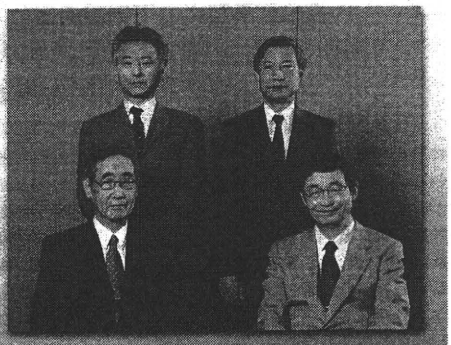
②血圧コントロール
・厳格な血圧のコントロールが重要である。
・血圧値は日中の外来随時血圧だけでなく、家庭血圧も参考にする。
・血圧コントロールには禁煙、減塩、肥満の改善、適度な運動指導を行う。
・降圧薬は尿蛋白減少効果、腎機能悪化抑制効果をも期待し、RAS系阻害薬を第一選択とする。

③脂質代謝異常
・LDL-C高値などの脂質代謝異常には、食事、生活習慣の改善指導を優先する。
・生活習慣の改善指導後、3～6カ月後も脂質代謝異常が持続する場合には薬物治療を考慮する。

座談会

わが国のエビデンスに基づくCKD治療とは

CAP-KD試験から見た経口吸着薬の役割



慢性腎臓病(CKD)の治療目標は，末期腎不全(ESRD)および心血管疾患(CVD)の発症・進展抑制にあり，CKD対策は必須である。しかし，CKD患者の増加傾向に歯止めがかかっておらず，かかりつけ医と腎臓専門医の連携を通じた集学的CKD治療が求められている。

このほど経口吸着薬(クレメジン®)による尿毒素吸着除去療法が，早期～中期CKD患者の腎不全進行抑制に寄与するというエビデンス(CAP-KD試験)がわが国から発信され，注目されている。

そこで今回は，自治医科大学名誉教授，古河赤十字病院長の浅野泰氏のご司会のもと，CKD治療のエキスパートである3名の先生方にお集まりいただき，CKD治療における経口吸着薬の役割や，CKD治療の今後の展望についてご討議いただいた。

司会
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尿毒素蓄積・腎不全進行の悪循環を断ち切る経口吸着薬


浅野氏

浅野　近年，CKDという概念は腎臓専門医にとどまらず，かかりつけ医の先生方にも浸透してきています。その一方で，わが国における慢性透析患者は2008年には28万人を突破し，医療経済の面においても問題視されています(**図1**)。

そこで今回は，日本を代表する腎臓専門医の先生方にお集まりいただき，このほど結果が発表された経口吸着薬のエビデンスをご紹介いただきながら，CKD治療の今後の展望について討議したいと思います。まずはCKD治療の現状と，経口吸着薬の位置付けについてご紹介ください。

秋澤　CKDに対しては，レニン-アンジオテンシン(RA)系阻害薬であるアンジオテンシン変換酵素(ACE)阻害薬やAT1受容体拮抗薬(ARB)を用いた降圧療法，スタチンを用いた脂質低下療法，エリスロポエチン製剤(ESAs)による貧血の管理，栄養指導といった治療が行われています。加えて，わが国独自の治療法として，経口吸着薬による尿毒素吸着除去療法があります。

CKDでは，腎不全の進行によってインドキシル硫酸をはじめとする尿毒素が蓄積され，蓄積された尿毒素がさらなる腎不全の進行につながるという悪循環を形成すると言われています。経口吸着薬は，インドキシル硫酸の前駆物質インドールを腸内で吸着，便中に排泄することにより，血中インドキシル硫酸濃度を用量依存的に低下させます(**図2**)。このような機序のもと，経口吸着薬は尿毒素の蓄積と腎不全の進行の悪循環を断ち切ることで，腎不全の進行抑制に寄与していると考えられています。

早期～中期のCKD患者に対する経口吸着薬の有用性が示されたCAP-KD試験

浅野　経口吸着薬による腎不全の進行抑制効果を検討したわが国の臨床試験が実施された当時は，現在の標準的治療であるACE阻害薬やARBなどのRA系阻害薬による降圧療法は行われていませんでした。それに加え，従来の臨床試験では，対象が血清クレアチニン(SCr) 5.0mg/dL以上の保存期腎不全患者に限られており，早期～中期CKD患者に対する経口吸着薬投与の有用性は明らかにされていませんでした。

そこで，早期～中期のCKDに対する経口吸着薬の有用性を検討した医師主導型臨床試験であるCarbonaceous oral Adsorbent's effects on Progression of chronic Kidney Disease(CAP-KD)試験が本邦で行われ，先ごろ結果が発表されました。その概要と結果についてご紹介ください。


秋澤氏

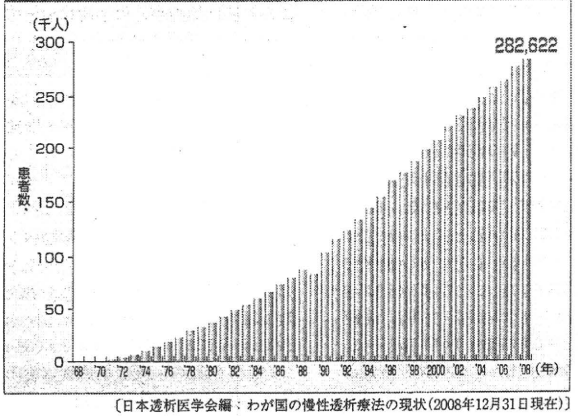
秋澤　CAP-KD試験は，ACE阻害薬，ARBを単独あるいは併用投与されているSCr 5.0mg/dL以下で20歳以上の保存期腎不全患者460例を対象にした多施設共同無作為化並行群間比較試験です。対象患者をACE阻害薬および/またはARBの投与，および食事療法(蛋白制限食：蛋白<0.8g/kg/日)による既存治療を継続する群(既存治療群)と，既存治療に経口吸着薬(クレメジン®)6 g/日を併用する経口吸着薬群に無作為に割り付けし，56週間追跡しました。主要評価項目は，①イベント(透析導入，腎移植)の発生，②死亡，③SCrの2倍化またはSCr 6.0mg/dLへの到達のいずれかの複合エンドポイントとし，副次評価項目としてクレアチニンクリアランス(CCr)の経時的変化を検討しました。

その結果，主要評価項目である複合エンドポイントに有意差は認められませんでしたが(**図3a**)，副次評価項目であるCCrのベースラインからの低下度は，既存治療群に比し，経口吸着薬群で有意に緩徐であることが示されました。また，推算糸球体濾過値(eGFR)による腎機能の経時的変化を見たところ，経口吸着薬群が既存治療群に比し，eGFRの低下を有意に抑制することが示されました(**図3b**)。

今井　SCr 5.0mg/dL以下の早期～中期CKDで，なおかつ十分に治療されている患者に対しても，経口吸着薬が腎不全の進行を抑制したことは，きわめて意義深いですね。

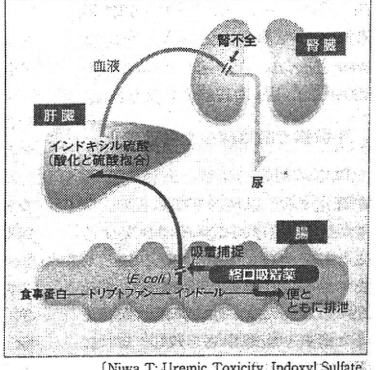
山縣　既存治療群，経口吸着薬群ともに腎機能低下の速度が遅い印象を受けるのですが，CAP-KD試験にはどのような先生方が参加されたのですか。

図1　慢性透析患者数の推移



〔日本透析医学会編：わが国の慢性透析療法の現状(2008年12月31日現在)〕

図2　インドキシル硫酸の代謝経路と経口吸着薬の作用点



〔Niwa T: Uremic Toxicity. Indoxyl Sulfate. Textbook of Nephrology. 2001, pp1269-1272〕
〔Niwa T, et al. *Kidney Int* 1997; 52(Suppl 62): S-23-S-28〕

秋澤　降圧薬の使い方や血圧管理に長けておられる，基幹病院の腎臓専門医の先生方が治療を担われたので，両群の患者は十分に治療されていると言えます。そのため，腎不全の進行速度が想定より遅かったことが，主要評価項目において有意な差を見出せなかった要因の1つと考えられます。


山縣氏

山縣　しかし一方で，血圧管理，栄養管理を厳格に行うことにより，腎不全の進行が抑制できることを改めて示したとも言えるのではないでしょうか。それとともに，経口吸着薬の併用が腎機能低下のさらなる抑制に寄与するという結果は，かかりつけ医の先生方に対する強力なメッセージになったと思います。

腎臓専門医-かかりつけ医の協力を促進する診療システム構築へ―FROM-Jの意義

浅野　われわれ腎臓専門医が約1,300万人に及ぶCKD患者を診療することは不可能であるため，かかりつけ医の先生方に広くCKD治療に参加していただくことが求められています。そこで，厚生労働省の戦略研究の1つであるThe Frontier of Renal Outcome Modifications in Japan(FROM-J)が2008年4月から始められました。研究の目的と概要をご説明いただけますか。

山縣　FROM-Jは，かかりつけ医および非腎臓専門医の先生方と腎臓専門医との協力を促進する診療システムの構築と，そのシステムの有用性を検討することが目的です。

本研究の対象患者2,494例は，クラスターランダム化により医師会ごとに無作為に介入A群(1,211例)，介入B群(1,202例)に割り付けられました。介入A群には「CKD診療ガイド2009」(以下，CKD診療ガイド)に沿った治療を行い，介入B群にはCKD診療ガイドに沿った治療に加え，管理栄養士による食事指導と，受診促進支援センターによる診療支援を実施します(**図4**)。主要評価項目は受診継続率，かかりつけ医および非腎臓専門医と専門医の連携達成，CKDのステージ進行率であり，副次評価項目はCKD診療目標の実施率などとしています。FROM-Jの成果目標は，CKD診療ガイドの遵守率，達成目標の達成度を上げることにより，5年後の透析導入患者を予測値から15%減少させることにあります。

秋澤　両群とも医学的にはほぼ同様の治療を行うわけですから，かかりつけ医および非腎臓専門医の先生方と腎臓専門医との連携や，コメディカルとの連携が，どの程度の成果につながるのか注目しています。

山縣　Devins G.M.らによるカナダ人重症慢性腎不全患者を対象にした同様の検討では，2日間のワークショップで90分程度の個人指導を行い，その後は3週間に1度の電話指導を18か月継続することにより，透析導入が17か月延長されることが報告されています。FROM-Jはより早期のCKD患者が対象であり，研究期間も4年という違いがありますが，よい結果が出ることを期待しています。

浅野　FROM-Jにおいて，経口吸着薬はどのような基準で投与されるのですか。

山縣　CKD診療ガイドでは，ステージ4以降のCKD患者に対して経口吸着薬の投与が推奨されています。したがって，介入B群でステージ4以降の患者に経口吸着薬の投与がなされていない症例では，担当医の先生に投与を推奨するという対応になります。

心腎連関の観点から見た経口吸着薬の新たな可能性

浅野　経口吸着薬による治療について，今後に期待されていることはございますか。


今井氏

今井　これまでの経口吸着薬を用いた検討は，腎臓に与える影響を見たものが大半を占めており，心臓に対していかなる影響を与えるか，十分な検討がされていなかったように思います。

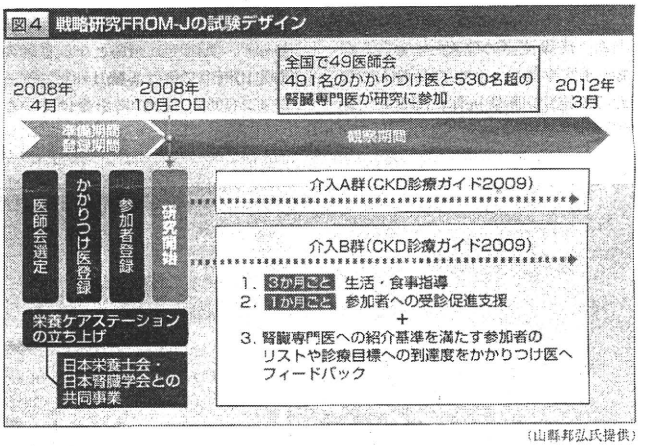
しかしながら，このほど神戸大学の藤井氏らが，経口吸着薬の心肥大に与える影響について興味深い報告をしています。進行性腎障害モデルラットを用いたこの検討では，経口吸着薬が血圧や腎機能，尿蛋白の変化とは非依存性に，酸化ストレスの亢進を抑制し，さらに左心室重量の増加(**図5**)，心筋線維化の進行を抑制することが示されました。

山縣　インドキシル硫酸は血管平滑筋の増殖を促進するとの報告もありますので，経口吸着薬が毛細血管における血流障害を改善し，このことが心機能に好ましい影響を与えている可能性も考えられますね。

秋澤　経口吸着薬は，インドキシル硫酸のみならず，終末糖化産物(AGEs)を吸着する可能性も指摘されています。今後はこうした機序にかかわる詳細な解明とともに，経口吸着薬がヒトにおけるCVD発症・進展に与える影響を検討する長期の臨床試験が行われることにも期待しています。

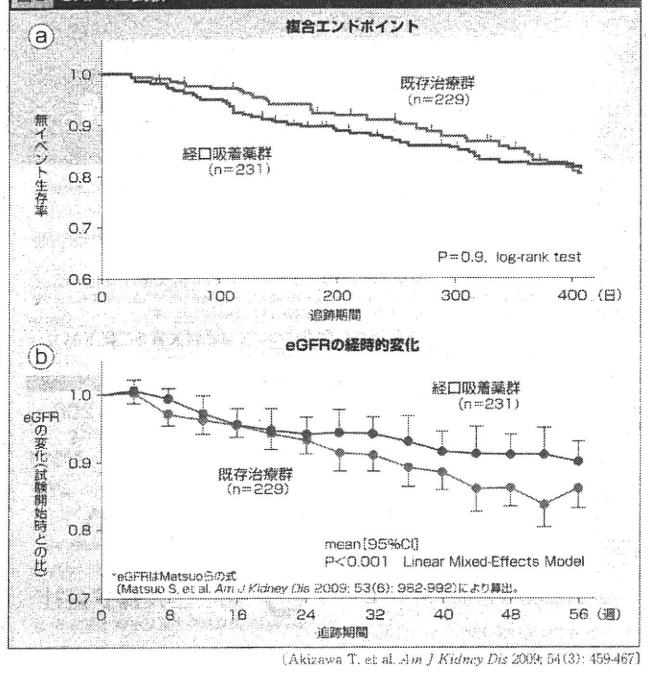
浅野　本日はCAP-KD試験を中心に，CKDに対する治療戦略について，さまざまな角度からご意見をいただきました。今回の討議内容を，かかりつけ医の先生方のCKD治療に広く活用していただければ幸いです。先生方，誠にありがとうございました。

図4　戦略研究FROM-Jの試験デザイン



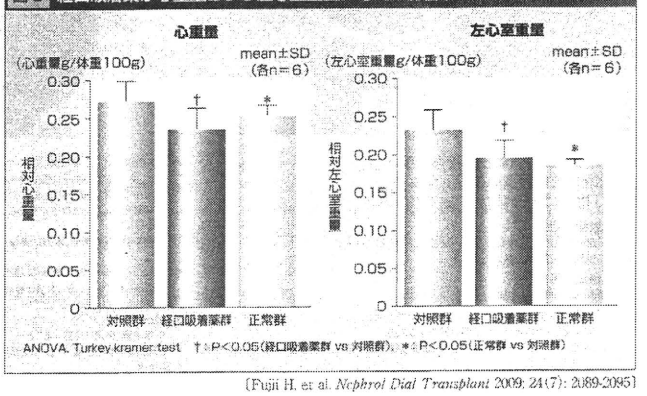
(山縣邦弘氏提供)

図3　CAP-KD試験



(Akizawa T, et al. *Am J Kidney Dis* 2009; 54(3): 459-467)

図5　経口吸着薬が心重量および左心室重量に与える影響(ラット)



(Fujii H, et al. *Nephrol Dial Transplant* 2009; 24(7): 2089-2095)

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Serum 25-hydroxyvitamin D as an independent determinant of 1-84 PTH and bone mineral density in non-diabetic predialysis CKD patients

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ABSTRACT

The role of 25-hydroxyvitamin D [25(OH)D] and fibroblast growth factor-23 (FGF-23) in chronic kidney disease–mineral and bone disorder (CKD-MBD) remains elusive in predialysis CKD patients. From the fact that FGF-23 suppresses bone mineralization *in vitro* and that 1α-hydroxylase is present in parathyroid cells and osteoblasts, they may be associated with bone mass or serum parathyroid hormone (PTH) level. In this cross-sectional observational study, we investigated the potential associations of 25(OH)D or FGF-23 with 1-84 PTH and bone mineral density (BMD) in the femoral neck (FN) and lumbar spine (LS) of 325 non-diabetic patients. All patients had stages 3–5 CKD and had never been treated with bisphosphonate, estrogen, or vitamin D. We measured bone-specific alkaline phosphatase (bone ALP), intact FGF-23 and 1-84 PTH in a third generation assay, and performed a multiple regression analysis for 1-84 PTH and BMD Z-score. In our cohort, 80.1% had 25(OH)D levels less than 30 ng/mL, and 4.1% had levels less than 15 ng/mL. A univariate analysis indicated a negative association for 25(OH)D with 1-84 PTH and bone ALP. A multivariate analysis showed that the significant determinants for 1-84 PTH were 25(OH)D, estimated glomerular filtration rate (eGFR), corrected calcium, serum calcitriol and phosphate. Intriguingly, the three former parameters had negative associations with 1-84 PTH while calcitriol had a positive association. While further adjustment of FGF-23 extinguished the positive association of phosphate and 1-84 PTH, there was absolutely no increase in the R^2. With regard to the BMD Z-score, 25(OH)D and the body mass index were the significant common independent positive determinants for both FN and LS, whereas bone ALP was the negative determinant even though there was no correlation noted for 1-84 PTH, calcitriol, or FGF-23 with BMD. In addition, eGFR positively contributed to the Z-score only in FN. Therefore, despite a positive correlation between 25(OH)D and calcitriol, their contribution to the CKD-MBD appears to be different. Since the significant associations for 25(OH)D with 1-84 PTH and BMD were independent of serum calcitriol and bone ALP, this might imply that 25(OH)D has a direct effect on the parathyroid gland and bone.

Introduction

It is generally accepted that low serum levels of calcium and calcitriol combined with high phosphate levels contribute to the pathogenesis of secondary hyperparathyroidism (II HPT) in chronic kidney disease (CKD) patients [1–4]. However, even after adjusting for the many confounding factors, a clear correlation between 25-hydroxyvitamin D [25(OH)D] with predialysis II HPT and bone mineral density (BMD) has yet to be demonstrated.

For a long time, 25(OH)D was believed to be just a precursor of calcitriol that had no inherent biological activity, and thus, was simply used as an indicator of the nutritional state. However, recent studies have demonstrated that parathyroid cells and osteoblasts contain 1α-hydroxylase [5] and megalin, which is the main endocytic receptor in the proximal tubules for the re-uptake of proteins, including the vitamin D-binding protein (DBP)/25(OH)D complex [6]. These data suggest a possible clinical relevance of 25(OH)D in bone metabolism and in parathyroid function.

An insufficiency of 25(OH)D is a common condition seen in both the general population and in CKD patients [7,8]. In order to manage II HPT in predialysis CKD patients, the Kidney Disease Outcome Quality Initiative (K/DOQI) guidelines have recommended ergocalciferol administration to stages 3 or 4 CKD patients if they have intact parathyroid hormone (PTH) levels greater than the target range and 25(OH)D levels less than 30 ng/mL (75 nmol/L) [9]. In fact, serum 25(OH)D has been shown to be inversely correlated with PTH in individuals with or without CKD [10–13], as well as in renal transplantation patients [14]. However, PTH levels in these studies were measured using a second-generation (intact PTH) assay that also

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detects 7-84 PTH as well as the bioactive 1-84 PTH. Kazama et al. [15] reported that the ratio of 1-84 PTH to intact PTH changes remarkably as the renal function declines. In addition, vitamin D status itself has also been shown to affect this ratio [16]. Therefore, in cases of renal failure, it is unclear if the vitamin D status actually contributes to the serum 1-84 PTH level (not the intact PTH level).

When studying the contribution of 25(OH)D to 1-84 PTH, one of the important confounders is the novel phosphaturic hormone, fibroblast growth factor 23 (FGF-23). FGF-23 affects vitamin D metabolism through the inhibition of 1α-hydroxylase [17]. This hormone is also upregulated under the conditions of excessive PTH [18]. Bone cells express fibroblast growth receptor 1, suggesting that FGF-23 could alter bone mineralization through a direct effect on the skeleton. A recent study has shown that overexpression of FGF-23 suppresses osteoblast differentiation and matrix mineralization *in vitro* [19]. Therefore, the potential exists for this hormone to affect the BMD in predialysis CKD patients.

In the present study, after adjusting for confounders including FGF-23, we attempted to determine the difference between serum 25(OH) D and calcitriol with regard to the contribution to CKD-mineral and bone disorders (CKD-MBD). To achieve this, we examined which laboratory data were associated with BMD and serum 1-84 PTH (not intact PTH) in a group of non-diabetic predialysis patients.

Patients and methods

Study design and subjects

In this cross-sectional observational study, we enrolled 325 predialysis patients who were 18 years or older and who had stages 3–5 CKD (210 men and 115 women). Patients were excluded if they had a history of treatment with glucocorticoids, calcium, vitamin D analogues, ergocalciferol, cholecalciferol, bisphosphonates, sodium bicarbonate, estrogens, or selective estrogen receptor modulators. Patients with primary hyperparathyroidism or liver cirrhosis were also excluded. Because diabetes mellitus affects bone turnover [20] due to impaired osteoblast function [21], we studied non-diabetic predialysis patients with stages 3 to 5 CKD.

Laboratory measurements

Blood samples were drawn from ambulatory patients at the outpatient clinic of Osaka University Hospital and Osaka General Medical Center. We centrifuged the blood at room temperature at 3000 rpm for 5 min, with the serum then stored at −80 °C until analysis. Blood chemistry [serum creatinine, albumin (Alb), calcium (Ca), and phosphate] was measured using standard automated techniques. Full length 1-84 PTH was measured using a third-generation assay (whole PTH, Scantibodies, Santee, CA, USA). The biologically active form of FGF-23 was measured using a sandwich enzyme-linked immunosorbent assay system (Kainos Laboratories, Inc., Tokyo, Japan). Bone-specific alkaline phosphatase (bone ALP) was assayed by using the Osteolinks-Bone ALP high-sensitivity diagnostic enzyme immunoassay (EIA) kit (Sumitomo Pharmaceuticals, Co., Osaka, Japan). The levels of serum calcitriol and 25-hydroxyvitamin D were measured by using a 1,25-hydroxyvitamin D RIA kit 'TFB' (Immunodiagnostic Systems Ltd., Boldon, UK) and a ^{125}I RIA kit (DiaSorin Inc., Stillwater, MN, USA), respectively. The serum Ca level was corrected for Alb by the formula (S-Ca; serum corrected Ca = Ca + B (4 − Alb), if Alb < 4.0 g/dL) [22]. We measured the BMD of the second to fourth lumbar vertebrae (L2–4) in addition to the femoral neck with a dual-energy X-ray absorptiometer (Discovery A, Hologic Inc., Bedford, MA, USA) in the posterior-anterior projection. Values were expressed by the age- and sex-adjusted *Z*-score.

Table 1
The characteristics of enrolled patients

CKD stage	3	4	5	*P*
Numbers of the patients (Female)	143 (52)	133 (47)	49 (16)	n.s.
Age (years)	62.4±13.8	63.0±14.5	61.6±14.8	n.s.
BMI (kg/m^2)	24.0±3.3	22.4±3.1	22.5±4.0	0.0118
Corrected Ca (mg/dL)	9.39±0.49	9.30±0.40	9.00±0.61	<.0001
Phosphate (mg/dL)	3.26±0.50	3.47±0.59	4.10±0.75	<.0001

CKD, chronic kidney disease; BMI, body mass index.

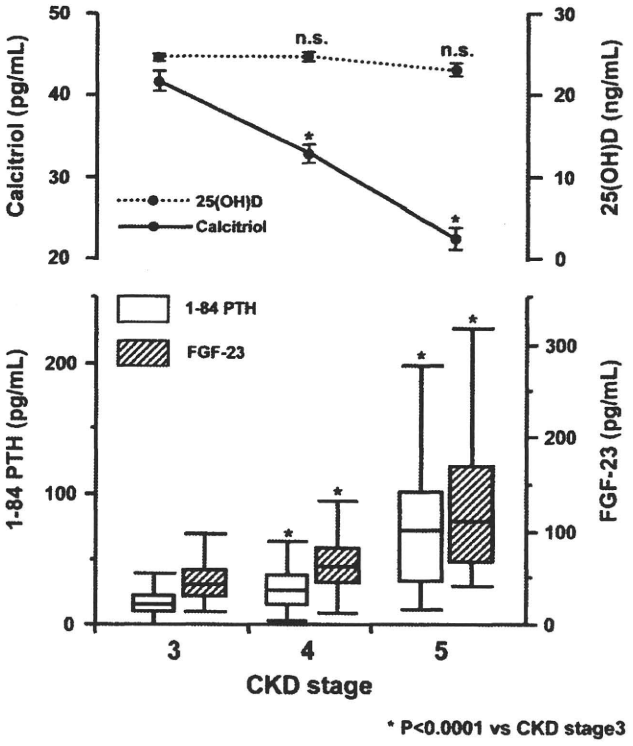

Fig. 1. Changes of phosphaturic hormones, calcitriol and vitamin D status across CKD stages. Calcitriol and 25(OH)D are shown as the mean (±SEM). The boxes for 1-84 PTH and FGF-23 represent the interquartile range, with the upper and lower edges representing the 75th and 25th percentiles, respectively. The central horizoxntal lines represent the median levels. The vertical lines above and below the boxes represent the range of outliers up to 1.5 times the interquartile range. *P<0.05 vs. CKD stage 3.

Statistical analysis

Data are reported as the mean±SD or the median and range. The estimated GFR (eGFR) was calculated by the MDRD formula optimized for Japanese patients [(0.741 × 175 × Cr$^{-1.154}$ × Age$^{-0.203}$ × 0.742 (if female))] [23]. Patients were categorized and placed into three groups according to their eGFR (CKD stage 3: eGFR 30 to 60 mL/min/1.73 m^2; CKD stage 4: eGFR 15 to 30 mL/min/1.73 m^2; or CKD stage 5: eGFR less than 15 mL/min/1.73 m^2). We investigated the independent determinants of 1-84 PTH and the age- and sex-adjusted BMD *Z*-score by using a multiple linear regression analysis. Variables that were significantly affected in the bivariate analysis (P<0.2) were included in a forward and backward stepwise multiple regression analysis. To take into account the contribution of the seasons with regard to the vitamin D status, we converted the seasons to dummy variables in the multiple regression analyses for 1-84 PTH and bone ALP. Because their distributions were skewed, bone ALP, 1-84 PTH and FGF-23 were log transformed before the regression analysis. Statistical tests were two-sided, and *P*-values less than 0.05 were considered statistically significant. All analyses were

performed with JMP ver. 7.0.1J for Windows (SAS Institute Inc., Cary, NC, USA).

Results

The characteristics of the study population are summarized in Table 1. In accordance with the K/DOQI guideline recommendations, we found that 234 out of 291 patients (80.1%) had 25(OH)D levels less than 30 ng/mL (vitamin D insufficient), while 12 patients (4.1%) had levels less than 15 ng/mL (vitamin D deficient). However, if we used a cut-off value of 20 ng/mL to indicate a vitamin D deficiency [13], then 54 patients (16.6%) were considered to be vitamin D deficient. Serum 25(OH)D levels did not differ across the CKD stages. The variations of 25(OH)D, calcitriol, FGF-23 and 1-84 PTH across the CKD stages are illustrated in Fig. 1. There was a significantly positive association between the serum calcitriol levels and the serum 25(OH)D levels in CKD stage 3 (r=0.14, P=0.045) and stage 4 (r=0.39, P=0.0008) patients. No significance was noted for the CKD stage 5 patients.

Determinants of 1-84 PTH

In a univariate analysis, serum 25(OH)D had a significant negative association not only with 1-84 PTH but also with the bone formation marker, bone ALP (Fig. 2). S-Ca, phosphate, calcitriol, eGFR and FGF-23 were also significantly associated with 1-84 PTH. By using these parameters along with age and gender as independent variables, we constructed two models and performed a multiple regression analysis

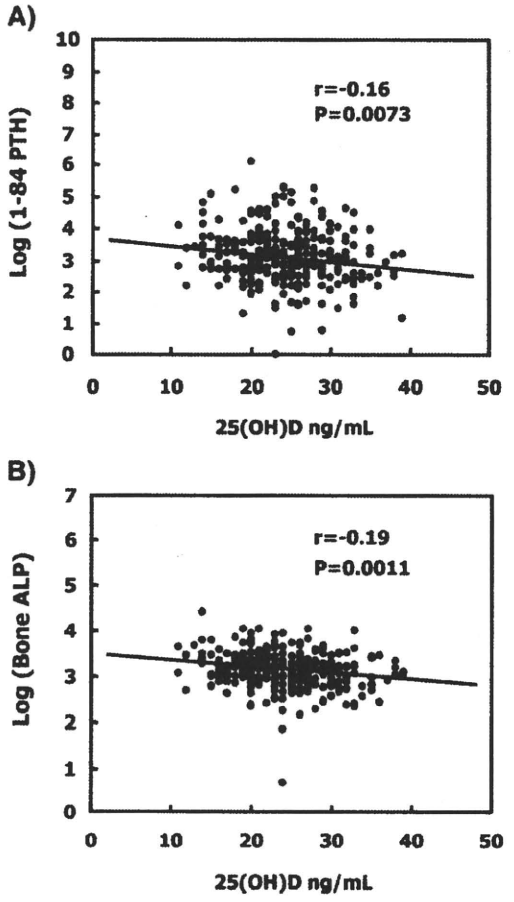

Fig. 2. Relationships between 25(OH)D and (A) Log(1-84 PTH), and (B) Log(Bone ALP). Serum 25-hydroxyvitamin D was negatively associated with not only with 1-84 PTH, but also with the bone turnover marker bone ALP.

Table 2
Multiple regression analysis for log (1-84 PTH)

	Model 1:FGF-23 (−)			Model 2:FGF-23 (+)		
	β	S.E.	*P* value	β	S.E.	*P* value
25(OH)D	−0.0250	0.0073	0.0008	−0.0258	0.0077	0.0010
Calcitriol	0.0107	0.0033	0.0012	0.0119	0.0034	0.0005
Corrected Ca	−0.2985	0.0837	0.0004	−0.2999	0.0860	0.0006
eGFR	−0.0496	0.0048	<.0001	−0.0442	0.0054	<.0001
Phosphate	0.1421	0.0692	0.0408	0.0522	0.0782	0.50 (n.s.)
Log (FGF-23)				0.2233	0.0905	0.0143
			R^2=0.45*			R^2=0.46*

SE, Standard error; 25(OH)D, 25-hydroxyvitamin D; eGFR, estimated glomerular filtration rate.; FGF-23, fibroblast growth factor-23.
* Corrected R^2 for degree of freedom.

for 1-84 PTH in order to investigate the factors promoting II HPT. FGF-23 was excluded in model 1 and included in model 2. In both models, 25(OH)D, corrected Ca, and eGFR were negatively associated with 1-84 PTH. Intriguingly, calcitriol was found to be positively associated with 1-84 PTH. As for phosphate, the positive association seen in model 1 disappeared in model 2, in which FGF-23, in stead of phosphate, showed positive association with 1-84 PTH (Table 2). Moreover, when we made adjustments for the seasons, there were no changes noted in the results.

Multiple regression analysis showed that only 1-84 PTH was a determinant of bone ALP in males, whereas in females, in addition to 1-84 PTH, younger age and post-menopausal status were also associated with a higher bone ALP (Table 3). The ratio for the bone ALP to 1-84 PTH (bone ALP/1-84 PTH) was demonstrated to be positively associated with eGFR [Log (bone ALP/1-84 PTH)= 0.03*eGFR-0.93, R^2=0.29, P<0.0001], which suggests there is a low skeletal sensitivity to PTH in advanced renal failure.

Determinants of age- and sex-adjusted BMD Z-scores in the femoral neck and lumbar spine

The associations between the CKD stages and the age- and sex-adjusted *Z*-scores of BMD are shown in Fig. 3A. A significant trend towards decreased *Z*-scores with the higher CKD stages was observed for the femoral neck (FN) but not for the lumbar spine (LS). We investigated the association of the *Z*-scores with the number of achieved K/DOQI guideline target ranges for S-Ca, phosphate, and intact PTH (recommended PTH target ranges were: 35–70, 70–110 and 150–300 pg/mL for CKD stages 3, 4 and 5, respectively). With higher BMDs for both the FN and LS, there was an associated increase in the number of target achievements, although this trend was not statistically significant (Fig. 3B).

To elucidate the significant determinants of the BMD *Z*-score, we performed a multiple regression analysis that used the S-Ca, phosphate, 25(OH)D, calcitriol, bone ALP, FGF-23, 1-84 PTH, eGFR and BMI as the potential explanatory variables. Common significant

Table 3
Multiple linear regression analysis for bone specific alkaline phosphatase

	β	S.E.	*P* value
Male			
Log (1-84 PTH)	0.1395	0.0347	<.0001
			R^2=0.15*
Female			
Age	−0.0121	0.0046	0.0104
Log (1-84 PTH)	0.2277	0.0499	<.0001
Postmenopause	0.2346	0.0725	0.0017
			R^2=0.3*

PTH, parathyroid hormone.
* R^2 for degree of freedom.

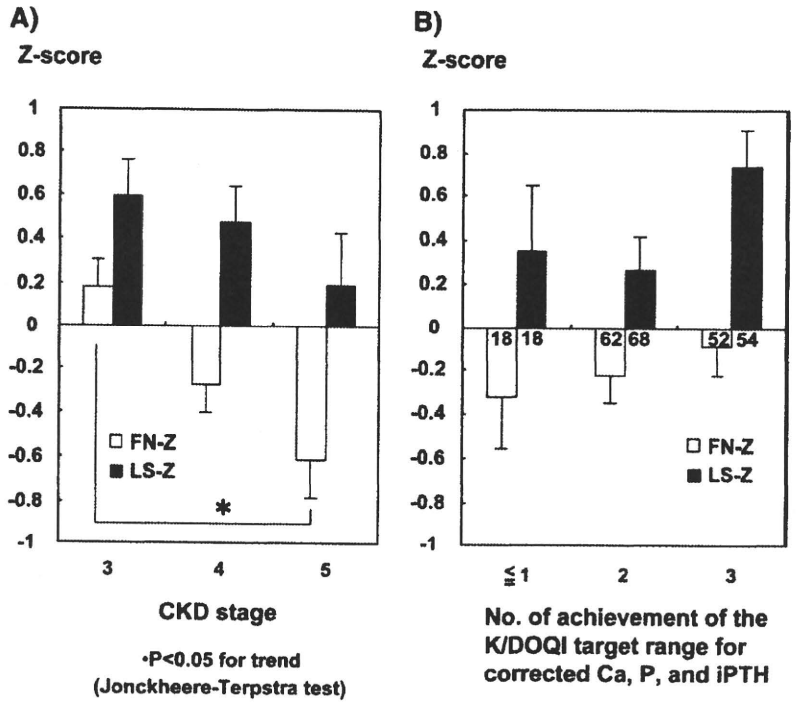

Fig. 3. Changes of age- and sex-adjusted Z-scores at the femoral neck (FN) and lumbar spine (LS) across the CKD stages (A), and across the achieved K/DOQI guideline recommended target ranges for calcium, phosphate and intact PTH(B). In (A) and (B), we observed a tendency for negative and positive associations. However, this significant trend was only observed between the FN-Z and CKD stages. P: serum phosphate, K/DOQI: Kidney Disease Outcome Quality Initiative, FN-Z: BMD Z-score at the femoral neck, LS-Z: BMD Z-score at the lumbar spine, CKD: chronic kidney disease.

positive determinants of BMD at both sites were 25(OH)D and BMI, whereas bone ALP was the common negative determinant. The estimated GFR was a significant positive determinant of BMD only at the FN, which is rich in cortical bone (Table 4). However, there was no significant correlation found between the BMD Z-score and calcitriol, 1-84 PTH, or FGF-23.

To further investigate the correlation between the BMD Z-score and these independent BMD determinants, we divided the subjects into tertiles based on 25(OH)D, bone ALP, and BMI. We then classified the subjects into nine categories based on the tertile combinations that consisted of 25(OH)D and bone ALP or the tertiles of 25(OH)D and BMI (Fig. 4). A gradual increase in the BMD Z-scores was observed when there was higher 25(OH)D and lower bone ALP or higher BMI. Unexpectedly, when compared to the other groups that had the lowest 25(OH)D tertile, the BMDs at both sites were lower in the group that had the lowest tertiles for bone ALP and 25(OH)D (the LL group) (Fig. 4). Moreover, in the LL group, the 1-84 PTH level was the lowest among all of the nine groups (data not shown).

Discussion

In our study, after adjusting for S-Ca, eGFR, and FGF-23, a multivariate analysis indicated there was a negative association for 25(OH)D with 1-84 PTH and a positive association for calcitriol with 1-84 PTH [24]. We also found that 25(OH)D was a positive determinant of the BMD Z-score in addition to BMI and bone ALP for both the LS and FN, whereas neither serum calcitriol nor FGF-23 exhibited any association with BMD.

In addition to the negative association noted for 25(OH)D with 1-84 PTH, our univariate analysis showed that 25(OH)D but not calcitriol exhibited a negative correlation with bone ALP, which is a marker of bone turnover in CKD [25]. These data might imply that serum 25(OH)D, but not calcitriol, is a surrogate marker for bone turnover and bone mass in predialysis patients. Our data are reminiscent of a bone biopsy study by Coen et al. in hemodialysis patients [26]. This previous study revealed that serum 25(OH)D was a significant determinant of bone turnover independently of serum calcitriol. Therefore, the direct

Table 4
Multiple linear regression analysis for age- and sex-adjusted BMD Z-score

	FN-Z			LS-Z		
	β	S.E.	P value	β	S.E.	P value
25(OH)D	0.0454	0.0148	0.0026	0.0506	0.0205	0.0147
Log(bone ALP)	−0.5010	0.2056	0.0162	−0.5763	0.2885	0.0477
BMI	0.0744	0.0221	0.0010	0.0667	0.0294	0.0250
eGFR	0.0296	0.0103	0.0049	0.0120	0.0139	0.39 (n.s.)
			R^2=0.26*			R^2=0.12*

FN-Z, age- and sex-adjusted BMD Z-score at the femoral neck; LS-Z, Age- and Sex- Adjusted BMD Z-score at the lumbar spine; S.E., standard error; 25(OH)D, 25-hydroxyvitamin D; bone ALP, bone specific alkaline phosphatase; BMI, body mass index; eGFR, estimated glomerular filtration rate.
* R^2 for degree of freedom.

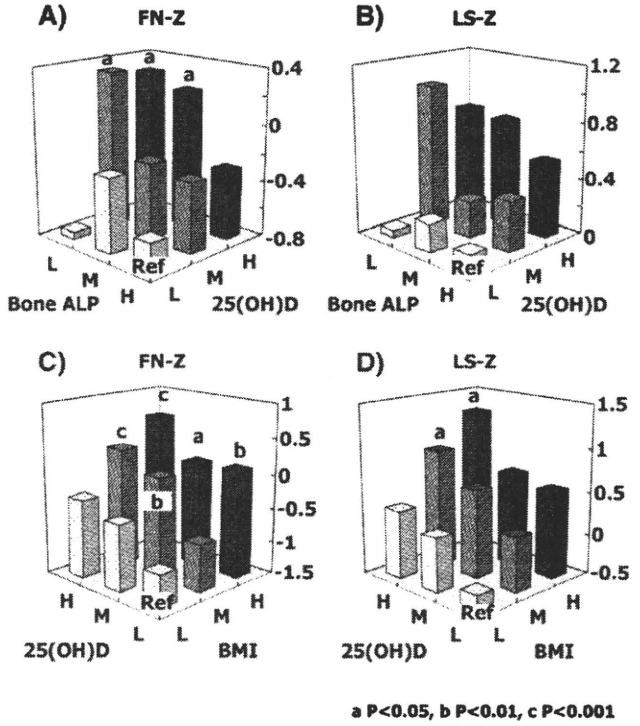

Fig. 4. BMD *Z*-scores for the nine different tertile combinations for (A, B) 25(OH)D and bone ALP, and (C, D) 25(OH)D and BMI. Patients were categorized and then placed into nine groups based on the tertiles of 25(OH)D, bone ALP, and BMI. The group with the lowest 25(OH)D and highest bone ALP, and the group with lowest 25(OH)D and lowest BMI were used as the reference groups in each figure. H, high; M, middle; L, low. a: $P<0.05$, b: $P<0.01$, c: $P<0.001$ as compared with the reference group (Dunnett's test).

action of 25(OH)D on bone metabolism through auto/paracrine actions in osteoblast might explain the mechanism for the association of 25(OH)D and bone turnover [27].

One of the limitations of our study was that it was a cross-sectional observational one, and thus, it was not possible to elucidate the causal relationship. To truly clarify the contribution of 25(OH)D to CKD-MBD, a further longitudinal interventional study with 25(OH)D supplementation is required. While Ziyad Al-aly et al. performed a retrospective study that followed this format [28], they did not have data with regard to changes in BMD or in bone turnover markers when administering ergocalciferol to predialysis patients. Hernandez et al. reported that administration of ergocalciferol completely improved the clinical symptoms associated with osteomalacia in a young hemodialysis patient, despite discontinuation of paricalcitol. In this patient, reduction of PTH and ALP, an increase in BMD, and a remarkable improvement in the overall bone histology were observed [29]. The findings of their case report are in agreement with our current cross-sectional study.

Intriguingly, in our study, a multivariate analysis showed that 1-84 PTH was positively associated with calcitriol even in the late stages of CKD. This result is compatible with the physiological role of PTH, which upregulates 1α-hydroxylase in the proximal tubules [30]. In fact, in primary hyperparathyroidism, serum calcitriol levels are usually high because of this unregulated, elevated hormone [31]. However, in CKD patients, 1-84 PTH fails to upregulate 1α-hydroxylase enough, because of the reduced number of viable nephrons. In hemodialysis patients, the phosphate load is roughly reflected in the serum phosphate levels, and the serum phosphate levels are positively correlated with 1-84 PTH [32]. As in the multivariate model that did not include FGF-23, this positive association also applies to the predialysis patients. However, after adjustment for FGF-23, the positive association of phosphate with 1-84 PTH was extinguished. Given that FGF-23 is also upregulated by the oral phosphate load [2,32,33], our results imply that 1-84 PTH can be upregulated not only by serum phosphate but also by the phosphate load. Measurement of urinary phosphate or phosphate intake would likely have revealed this, but we did not measure these levels, which is another limitation of our study. Acute injections of recombinant FGF-23 was shown to reduce PTH in rats without renal failure [34]. However, we did not observe a negative association between PTH and FGF-23. The main reason for this might be that PTH, in turn, can upregulate FGF-23 [18]. High PTH even under the condition of extremely high levels of FGF-23 could be attributed to some FGF-23 resistance of the parathyroid cells due to reduced Klotho expression, which was previously reported in human primary HPT [35].

Diverse difference of the skeletal sensitivity to PTH across the CKD stages might explain the reason for the lack of association between 1-84 PTH and BMD. This difference is possibly due to the down-regulation of the PTH-1 receptor caused by the uremic toxins [36], and is reflected in our finding of a positive association of bone ALP/1-84 PTH with eGFR in the current study. In contrast to osteocalcin, renal function did not interfere with the levels of bone ALP [37]. Moreover, our data showed that the postmenopausal high bone turnover state was also reflected in bone ALP [25]. These should be the main reason why bone ALP not 1-84 PTH is a determinant of BMD *Z*-score. A recent study has found that in hemodialysis patients, full-length biologically active FGF-23 had no significant correlation with bone mass or bone turnover markers [38]. This observation was also noted in the predialysis CKD patients in the present study.

Among the nine groups based on the 25(OH)D and bone ALP, the mean BMD *Z*-score was unexpectedly low in the group that exhibited a low bone ALP and low 25(OH)D. In this group, 1-84 PTH was the lowest among all of the nine groups. Thus, the poor response of this anabolic hormone to the low vitamin D status might lead to impaired bone formation, which in turn could lead to the low BMD in this group.

In summary, this study demonstrated the importance of the vitamin D status in non-diabetic CKD-MBD with regard to II HPT and BMD. Since the serum calcitriol level itself was the consequence of the regulations by endogenous 1-84 PTH and FGF-23, we propose that it is not the serum calcitriol but its precursor 25(OH)D that should be monitored in predialysis patients.

Conflict of interest statement
None declared.

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RESEARCH ARTICLE

A method for assessment of *Helicobacter pylori* genotype using stool specimens

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Keywords
Helicobacter pylori; stool specimen; noninvasive genotyping; virulence factor; healthy individual.

Abstract

Helicobacter pylori infection has been regarded as a major factor associated with the development of gastric diseases. The characterization of infected *H. pylori* in asymptomatic individuals is important for the prediction of the onset of such diseases. However, because of the difficulty in obtaining gastric biopsy samples, *H. pylori* in healthy subjects have not been studied sufficiently. Therefore, we tested a noninvasive method for the characterization of *H. pylori* using stool specimens. This method involved *H. pylori* antigen detection in stool specimens by immunochromatography; confirmation of *H. pylori* DNA by real-time PCR that involved the detection of its 16S rRNA gene in the DNA extracted from stool specimens; and nested PCR with genotype-specific primer pairs. A total of 80 samples obtained from asymptomatic subjects were assessed using this method. The results showed that the prevalence of *H. pylori* in asymptomatic Japanese individuals was 37.5%. The detection rate of the virulence factor gene *cagA* was 18.8%. Furthermore, all the detected *cagA* belonged to the highly virulent East-Asian type. These data suggest that the method used in this study is valuable for studying the molecular epidemiology of *H. pylori* infection in asymptomatic people.

Introduction

Helicobacter pylori has been recognized as a group I carcinogen by the International Agency for Research on Cancer, because infection with this pathogen causes persistent gastritis and is directly linked to the development of peptic ulcer, gastric cancer, and gastric lymphoma of the mucosa-associated lymphoid tissue (Fox & Wang, 2007; Amieva & El-Omar, 2008). Various virulence factors of *H. pylori*, such as VacA, CagA, and urease, have been implicated in the pathology of *H. pylori* infection (Maeda & Mentis, 2007). Among these factors, *cagA*, which is located in the pathogenicity island (*cag*PAI) region, has been identified as a critical virulence factor with regard to the initiation of cancer. There are many genotypes of the *cagA* gene, including the East-Asian and Western types. The East-Asian type is more virulent than the Western type because of its strong potency in stimulating signal transduction pathways in the host (Higashi *et al.*, 2002; Hatakeyama, 2006). Therefore, genotyping of the *cagA* gene of clinical *H. pylori* isolates obtained from patients with a gastric disease has been widely conducted. In contrast, the genotype of the virulence factors of *H. pylori* isolates from asymptomatic individuals has rarely been qualitatively and quantitatively assessed due to the difficulty in specimen collection. There are two possible types of specimens for isolating and characterizing the infected bacteria in asymptomatic individuals: one is an endoscopic biopsy specimen and the other is a stool specimen. Although using an endoscopic biopsy specimen is more reliable for isolating the infected *H. pylori*, this method of collection has several disadvantages, including unnecessary invasion into healthy individuals. Consequently, such disadvantages render the carrying out of an epidemiological study difficult, especially with large cohorts. On the other hand, studies using stool specimens are noninvasive and the specimens are easy to collect. These advantages facilitate epidemiological analysis, such as a case–control study, a factor control study, or a prospective study. However, stool has not been considered as a good material for the isolation of *H. pylori*, because the bacteria might be in a 'viable but

nonculturable' state. In fact, only a few reports have described the direct isolation of the bacteria from stool specimens (Kabir, 2001), although many attempts have been made to isolate the bacteria. In addition, the presence of inhibitors and the considerable complexity of the bacteria in stool specimens may also hinder detailed analysis of the *H. pylori* genes using molecular biological techniques such as PCR amplification (Abu Al-Soud & Radstrom, 2000). Molecular characterization of *H. pylori* in asymptomatic individuals is considered as one of the most valuable factors for predicting the onset of diseases associated with silent *H. pylori* infection. Therefore, in this study, in order to assess the virulence of *H. pylori*, we used a noninvasive genotyping method for genotyping the *cagA* gene from stool specimens of asymptomatic individuals.

Table 1. Oligonucleotide primers and probe used for PCR analysis of *Helicobacter pylori* 16S rRNA gene and *cagA*

Gene	Primer	Primer sequence
16S rRNA gene (Yamazaki *et al.*, 2005)	Forward	5′-TGC GAA GTG GAG CCA ATC TT-3′
	Reverse	5′-GGA ACG TAT TCA CCG CAA CA-3′
	Probe	5′-(FAM) CCT CTC AGT TCG GAT TGT AGG CTG CAA C (TAMRA)-3′
cagA detection (This study)	Forward (common)	5′-GGA ACC CTA GTC AGT AAT GGG TT-3′
	Reverse (JR)	5′-AAT TCT TGT TCC CTT GAA AGC CC-3′
	Reverse (WR)	5′-GCT TTA GCT TCT GAT ACC GCT TGA -3′
cagA-Western (Yamazaki *et al.*, 2005)	Forward	5′-AGG CAT GAT AAA GTT GAT GAT-3′
	Reverse	5′-AAA GGT CCG CCG AGA TCA T-3′
cagA-East-Asian (Yamazaki *et al.*, 2005)	Forward	5′-AAA GGA GTG GGC GGT TTC A-3′
	Reverse	5′-CCT GCT TGA TTT GCC TCA TCA-3′

Materials and methods

Clinical samples

The study was conducted from July to August 2007 in Osaka, Japan. Informed consent was given by all participants. Stool specimens were collected from 80 asymptomatic volunteers aged 40 years and above. The research protocols were approved by the ethics committee of the Division of Health Sciences at the Osaka University Graduate School of Medicine (Osaka, Japan).

Detection of *H. pylori* antigen in stool specimens and extraction of the bacterial DNA

A commercially available rapid-test kit (Testmate Rapid Pylori Antigen, BD, Tokyo, Japan) was used for the detection of the *H. pylori* antigen catalase (Suzuki *et al.*, 2002) in stool specimens. The detection was performed by following the manufacturer's instruction manual.

Bacterial DNA was extracted from all stool specimens using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions, with minor modifications. In brief, bacterial DNA was extracted from *c.* 1 g of stool specimen and was obtained in a volume of 200 μL with DNA concentrations of up to 150 ng μL^{-1}. The extracted DNA was stored at −20 °C until further use.

PCR analysis

A solution with an extracted DNA concentration of 50 ng μL^{-1} or a 10-fold diluted solution of the extracted DNA was used as a template for all PCR analyses. In order to detect 16S rRNA gene of *H. pylori*, real-time PCR was performed with gene-specific primers, a probe (Table 1), and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster, CA), according to the manufacturer's instruction, whereby the existence of *H. pylori* DNA in the extracted DNA could be confirmed.

Nested PCR was performed for *cagA* genotyping. The first round of PCR was performed with a common forward primer and any one of the two reverse primers (Table 1). The PCR conditions were as follows: 95 °C for 2 min, followed by 40 cycles consisting of 94 °C for 15 s, 55 °C for 30 s, and 68 °C for 1 min. The second round of PCR was then performed with 1 μL of the first-round PCR products as the template. In second-round PCR, primers specific to these two types were used in separate reactions. The conditions in the second round of PCR were as follows: 94 °C for 2 min, followed by 50 cycles of 98 °C for 10 s and 65 °C for 2 s. The PCR products were visualized by 2% agarose gel electrophoresis and staining with ethidium bromide.

Detection limit of PCR for *H. pylori* DNA in stool samples

The detection limit of PCR was determined by a spike test. The *cagA* genotype-defined clinical isolates of *H. pylori* were used for the spike test. A series of 10-fold dilutions of *H. pylori* culture in phosphate-buffered saline, ranging from 0 to 10^5 CFU g^{-1} of stool, were added to the antigen-negative stool samples. The bacterial DNA was extracted from the stool sample, and the extracted DNA was applied to PCR.

Results and discussion

The prevalence of *H. pylori* in an asymptomatic Japanese population has been studied by a serological test (Asaka *et al.*, 1992; Osawa *et al.*, 1996; Fujisawa *et al.*, 1999; Yamagata *et al.*, 2000). However, the serological test utilized in these studies has several disadvantages, for example, it

Table 2. Summary of the detection of *Helicobacter pylori* and genotyping of *cagA*

	Tested #	Positive # (%)
H. pylori antigen test	80	30 (37.5)
Real-time PCR		
16S rRNA gene	80	26 (32.5)
Conventional PCR		
Detection of *cagA*	80	15 (18.8)
Genotyping of *cagA*	15	
East-Asian type		15 (100.0)

also detects past and cured infections. In this regard, a novel detection method that could directly detect the bacterial antigen in stool specimens using a monoclonal antibody against *H. pylori* catalase has been recently developed, and its sensitivity and specificity are comparable to those of the breath test (Cardenas *et al.*, 2008). Therefore, we applied this detection method to assess the prevalence of *H. pylori* in asymptomatic Japanese individuals.

It is known that the prevalence of *H. pylori* infection increases with age, and a higher prevalence can be found in a population aged over 40 years (Asaka *et al.*, 1992). Therefore, asymptomatic Japanese individuals aged 40 years and above were selected as a source of stool specimens in this study. As is evident in Table 2, the *H. pylori* antigen was detected in 30 (37.5%) of the 80 specimens collected from asymptomatic adult individuals. This rate was lower than that in previous reports, wherein the antigens were determined using the above-mentioned serological method (Asaka *et al.*, 1992; Fujisawa *et al.*, 1999; Yamagata *et al.*, 2000). The reasons for the lower prevalence of *H. pylori* in healthy people examined in this study are not clear. However, it seems that the difference in the present results and previous reports are probably due to the different detection methods used; that is, the antigen detection method in the present study could reveal the active and current *H. pylori* infection, while the serological method used in the previous studies also detected past and cured infection.

In order to determine the genotype of *H. pylori* in asymptomatic individuals, a PCR analysis of the virulence factor gene *cagA* was performed. *Helicobacter pylori* DNA was extracted from all stool specimens. The existence of *H. pylori* DNA in the extracted DNA was confirmed by real-time PCR specific for *H. pylori* 16S rRNA gene (Yamazaki *et al.*, 2005). *cagA* genotyping was achieved by nested PCR that was specific for either East-Asian or Western type. In general, it can be expected that the contents of *H. pylori* DNA may not be high in the DNA extracted from stool specimens. In addition, the DNA extracted from stool samples may not be a good template for PCR because of the existence of PCR inhibitors and its higher level of complexity (Cavallini *et al.*, 2000). Therefore, before the analysis of the DNA extracted from stool specimens, the sensitivity of the PCR used in this study was evaluated using the spike test. The results showed that the detection limit of the real-time PCR specific for 16S rRNA gene and nested PCR for *cagA* genotype was $2.1 \pm 0.1 \times 10^2$ and 1.0×10^4 CFU g^{-1}, respectively. As shown in Table 2, *H. pylori* 16S rRNA gene was detected in 26 of the 80 extracted DNA samples from stool specimens. The low detection rate (32.5%) of *H. pylori* DNA by PCR was probably due to the different targets (antigen vs. DNA) used for the detection and the presence of PCR inhibitors in the extracted DNA samples (Kabir, 2004). Nevertheless, the results of *H. pylori* DNA detection were consistent with a previous report on *H. pylori* DNA detection in stool specimens (Monteiro *et al.*, 2001).

After confirmation of the existence of *H. pylori* DNA, the virulence factor gene *cagA* was detected and genotyped. The *cagA* gene was detected in 15 (18.8%) of the 80 samples. The *cagA* detection rate obtained in this study was relatively lower than that in the previous studies, which showed detection rates ranging from 53.8% to 70.8% (Russo *et al.*, 1999; MacKay *et al.*, 2003; Sicinschi *et al.*, 2003a, b). The reasons for the low detection rate observed in this study may be due to the different target population utilized such as healthy people.

The analysis of *cagA* showed that all of the detected *cagA* belonged to the highly virulent East-Asian genotype. That is, 18.8% of Japanese individuals tested were infected with highly virulent *H. pylori*. In this regard, our recent study of *cagA* genotype in asymptomatic Thai people showed that the East-Asian genotype was present in $< 2\%$ of the samples tested (unpublished data). Therefore, it seems likely that the prevalence of the highly virulent East-Asian *cagA* genotype of *H. pylori* in asymptomatic people may differ across countries. Such a different prevalence, if any, may be related to the incidence of gastric diseases, including stomach cancer. Nevertheless, it can be conjectured from the results obtained in this study that a significant number of Japanese healthy people may be infected with the highly virulent *H. pylori*.

Thus, the results obtained in this study indicate that the method used in this study is useful for the assessment of *H. pylori* infections in healthy people.

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エビデンスに基づくCKD診療ガイドライン2009

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要　旨

・本年3月，日本腎臓学会より「エビデンスに基づくCKD診療ガイドライン」が発刊された。2007年9月に日本腎臓学会より上梓され，現在広く普及している「CKD診療ガイド」との違いは何か？　という質問をよくいただく。
・本ガイドラインは，日本腎臓学会学術委員会が主体となり，上記「CKD診療ガイド」が発刊される前，その形をほぼ整えた2007年7月頃より構想を開始し，意欲的な若手医師をワーキンググループとして公募し，以後約1年半の期間をかけて集中的に論文を読み込み，議論を重ねて作成されたものである。
・診療ガイドと診療ガイドラインは対象の読者や目的とするところが異なり，同時に存在して差し支えないものである。

本稿ではガイドラインについての一般的基本事項，「CKD診療ガイド2009」との位置づけの違いについて述べ，本ガイドライン作成にあたっての考え方，ステートメント作成プロセス，また利用にあたって留意すべき点について解説する。また，本ガイドラインの概要を紹介し，ポイントとして「CKDと生活習慣病」を例示し，最後に今後の展望についても触れる。

はじめに

米国腎臓財団(NKF)により2002年にKidney Disease Outcome Quality Initiative(K/DOQI)からガイドラインが提示され[1]，米国循環器学会は心血管疾患(CVD)のリスクとしての慢性腎臓病(CKD)の重要性を踏まえて，2002年にはCVDリスクとしてのCKDに関するScientific Statement[2]を，また2006年にはCVD患者におけるCKDの早期発見の重要性についてのScience Advisory[3]を発表している。

このような国際的動向を背景として，日本腎臓学会においてもCKDの総合的対策を担う組織として，2005年に慢性腎臓病対策委員会が発足し活動を開始した。その成果の一つとして2007年9月には，「CKD診療ガイド」が上梓され，診療現場で広く活用されている。さらに本年3月，「エビデンスに基づくCKD診療ガイドライン」[4]が発刊され，CKD対策の一層の展開が期待される。

本稿では，本ガイドライン作成のねらい，経緯について述べる。また「CKD診療ガイド2009」[5]との位置づけの違いについて言及する。そして，本ガイドラインの項立てを紹介しポイントについて述べる。

CKDの定義と診断基準

CKDは以下のように診断される。

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CKD の診断基準

(1) GFR の値にかかわらず，腎障害を示唆する所見(検尿異常，画像異常，血液異常など)が 3 カ月以上存在すること

(2) GFR 60 m*l*/min/1.73 m^2未満が 3 カ月以上持続すること

この片方または両方を満たす場合に CKD と診断される。

わが国における CKD の日常診療において，血清クレアチニン，年齢，性別の三つのデータから eGFR を算出する下記の推算式を日本腎臓学会として作成し推奨している[6]。

$$GFR(ml/min/1.73\,m^2) = 194 \times Cr^{-1.094} \times Age^{-0.287} \times 0.739(\text{女性の場合})$$

EBM に基づいた診療ガイドラインの役割

近年，臨床の現場でエビデンスに基づいた医療(EBM)が重要視されるようになり，診療ガイドラインも EBM に立脚した記述で構成されることが一般化した。この EBM に基づいた診療ガイドラインの考え方は欧米で発達してきたもので，その定義は，「医療者と患者が特定の臨床現場で適切な決断を下せるよう支援する目的で，体系的な方法に則って作成された文書。EBM の手順で作成することに最大の特徴がある」(Institute of Medicine, National Academy 1990，米国)とされている。

EBM は重要であり，診療方針選択にあたって一定の科学的根拠を提供することの意義に疑いの余地はない。しかし，EBM は個々の医師の専門的技量に取って代わるものではなく，あくまで治療の主体は医師であり，EBM で得られた情報をどのように実地診療の場で個々の患者に適応していくかは医師の専門家としての能力に依存している。

すなわち，診療ガイドラインとは，決して診療行為を直接規定するものではなく，あくまでも医師の診療の裁量の中でよりよい診療実践を支援するものである。さらに，ガイドラインは医事紛争や医療訴訟における判断基準を示すものと受け取られがちであるが，本来の目的に照らせばガイドラインとはそのようなものではなく，この点について理解が広まることを期待したい。

CKD 診療ガイドと CKD 診療ガイドライン

日本腎臓学会 CKD 対策委員会より 2007 年 9 月に刊行された「CKD 診療ガイド」は，CKD 対策の重要性と治療・管理の包括的方針について，一般医を含む医療従事者全般に啓発するために作成されたものであり，現在広く普及し CKD 診療のレベルアップに貢献している。なお，「CKD 診療ガイド」はその後の学問的進展を盛り込むため，2009 年 3 月に改訂版として「CKD 診療ガイド 2009」が刊行された。

一方，「CKD 診療ガイドライン」は，その読者として専門医レベルを想定したものである。一般に，診療ガイドは作成委員のコンセンサスを土台として記述されるが，診療ガイドラインは EBM (根拠に基づいた医療)を拠り所として作成される。近年の臨床医学領域における EBM の集積に伴い，EBM に準拠したガイドライン作成が可能となり，それを作成し公表するのは臨床医学系の学会の責務とされるようになった。CKD 診療ガイドラインは CKD 診療にかかわるエビデンスを広く収集し，それらを吟味，解釈し，それに基づいた診療指針を提示したものであり，日本腎臓学会が公表するガイドラインとしては初めての EBM に立脚したガイドラインである。

CKD 診療ガイドラインにおけるエビデンスとステートメント

CKD 診療ガイドラインでは，引用した文献のエビデンスレベルを研究遂行のデザインで分け，水準の高いものから，次のレベル 1〜5 に分類した。

1．システマティックレビュー/メタアナリシスによる，

2．一つ以上のランダム化比較試験による，

3．非ランダム化比較試験による，
4．分析疫学的研究(コホート研究や症例対照研究)による，
5．記述研究(症例報告やケース・シリーズ)による，コンセンサスとなっている。

さらに，エビデンスの質やわが国における診療現場に応じた判断も含め，各項目にステートメント(推奨)のグレードを記した。ステートメントは，ある事項についてのエビデンスの集大成であり，その推奨の強さをグレードAからグレードDまでに分けた。

グレードA：行うよう強く勧められる
グレードB：行うよう勧められる
グレードC：行うよう勧めるだけの根拠が明確でない
グレードD：行わないよう勧められる

本ガイドラインではグレードDは該当なしであった。

なお，本診療ガイドラインを参考にする場合は，エビデンスレベルの高さよりもステートメントのグレードを重視していただきたい。エビデンスは欧米において得られたものが少なからずあり，わが国の医療の実情とそぐわないものも散見される。

エビデンスレベルは上述の基準に則って決められるが，推奨レベルは日本の医療の実情を勘案しながら執筆者，グループリーダー，学術委員会の各レベルにおける確認，合意を経て決定した。エビデンスが乏しいが日本の医療現場では，標準となっているような治療法(IgA腎症，ネフローゼ症候群においてみられる)の取り扱いについては，学術委員会での議論を経て，なるべくステートメントに取り上げるが，解説においてエビデンスがないことを明記することで，記載が実臨床と大きく乖離しないこと，また読者がevidence-practice gapを意識し，今後の実地診療に役立つエビデンスの集積へのモチベーションが高まる一助となることを期した。

CKD診療ガイドラインの概要

表1に本ガイドラインの項立てを示す。CKDという語で包含される病態，原因疾患は多岐にわたり，またCKD自体が原因疾患を問わない，機能異常のみを拠り所とする概念である。これらにより，CKDについてのガイドライン作成は一本道のプロセスとはなり難い。

表1 項 目

1．CKDの診断
2．CKDの意義
3．CKDと生活習慣
4．CKDと栄養
5．CKDと高血圧・心血管合併症
6．腎性貧血
7．CKDに伴う骨ミネラル代謝異常
8．糖尿病性腎症
9．IgA腎症
10．ネフローゼ症候群
11．腎硬化症
12．動脈硬化性腎動脈狭窄症
13．常染色体優性多発性嚢胞腎(ADPKD)
14．CKDと脂質代謝異常
15．肥満・メタボリックシンドローム
16．小児CKDの診断
17．小児CKDの治療
18．透析療法
19．腎移植
20．高齢者のCKD診療
21．薬物投与

本ガイドライン作成にあたっては，「CKD」の趣旨に照らして腎機能悪化とCVDのリスク増加に関係する共通メカニズムについての病態解明と治療法選択に主眼を置くべき，という議論もあった。しかし，本書が専門医を主な読者と想定していることより，従来腎臓病学が対象としてきた専門医が診療するべき疾患を含めることには意義があるとの観点から，代表的な腎疾患として糖尿病性腎症，IgA腎症，ネフローゼ症候群(特発性膜性腎症，一次性巣状分節性糸球体硬化症)，常染色体優性多発性嚢胞腎が取り上げられた。これに加え，腎硬化症，腎動脈硬化性腎動脈狭窄症について別項を立てた。

CKDに随伴する異常(腎性貧血，骨ミネラル代謝異常)についても記している。特筆すべき点として，小児のCKDについて，診断，治療に分けて章を設けた。今回の項立ては一つの考え方であり，今後の改訂において新たな視点からの方向性が打

表2　ステートメント

1 喫　煙　グレードA　レベル④

喫煙はCKDの発症および進行に関連する独立した危険因子であり[1~10]，CVDの発症リスクを増加させることから[11~13]，CKD患者は禁煙すべきである。

2 飲　酒　グレードB　レベル④

中等量の飲酒(エタノール20~40 g/日)はCKDのリスクとはならず，むしろ進行を抑制し[2]，CVDの発症も抑制する[13]。一方，大量飲酒(エタノール60 g/日以上)はCKDのリスクとなり，CVDの発症も増加させるため，避けるべきである[14]。

3 運動・身体活動度

① 身体活動度の維持　グレードA　コンセンサス

CKD患者に安静・運動制限を一律に行うべきではなく，肥満の是正，糖尿病新規発症の予防，高血圧の治療，CVD予防のために身体活動度を維持すべきである。

② 運動強度　グレードB　レベル③

運動疲労を起こさない程度の運動(5 METs前後)が安定したCKDを悪化させるという根拠はなく，合併症などの身体状況が許す限り，定期的施行が推奨される[15,16]。

4 ワクチン接種　グレードB　レベル④

CKD患者には，インフルエンザワクチンの接種が推奨される[17]。

5 癌スクリーニング　グレードB　コンセンサス

CKD患者の癌スクリーニングは，一般人と同様の対応が推奨される。腫瘍マーカーの評価に際しては，偽陽性などに注意が必要である。

ち出されることも十分想定される。

本ガイドラインは新規であり，ポイントの紹介は恣意的にならざるを得ないが，個人的には第3章「CKDと生活習慣」は，これまであまり注目されず，エビデンスの有無すらあまり知られていなかった領域について，現時点でのエビデンスの集大成がなされた点に大きな意義を感じている。参考までに，ステートメントをそのまま引用させていただく(**表2**)。

CKD診療ガイドラインの今後の展望

EBMに基づいたガイドライン作成には，良質なエビデンスが不可欠である。また，良質なガイドラインは，最新のEBMを取り入れ定期的に改訂されるべきである。今回，個人的にはCKD診療ガイドラインの作成にかかわる機会に恵まれ大変よい経験をさせていただいたが，同時に，CKD領域のエビデンス，とりわけ，日本人を対象としたデータが不足していることを痛感する機会ともなった。腎臓領域での初の本格的ガイドラインとなった本ガイドラインが嚆矢となり，CKD領域で「日本人の，日本人による，日本人のための」オリジナリティーの高いエビデンスが構築され，それに基づきCKD診療ガイドラインが進化していくことを期待したい。

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ORIGINAL ARTICLE

Negative effects of anemia on quality of life and its improvement by complete correction of anemia by administration of recombinant human erythropoietin in posttransplant patients

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Abstract

Background Anemia is a common complication in posttransplant patients (posttransplant anemia: PTA). We tested the hypothesis that targeting hemoglobin (Hb) over 13.3 g/dl by administration of recombinant human erythropoietin (rHuEPO-ad) has positive impact on quality of life (QOL).

Methods Twenty-four patients, whose initial Hb and estimated glomerular filtration rate (eGFR) were 10.5 ± 0.2 g/dl and 48.5 ± 2.7 ml/(min 1.73 m^2), respectively, were enrolled in the present study. Physical and mental QOL in these patients before and after rHuEPO-ad were acquired and summarized as physical summary sore (PSC) and mental summary sore (MSC), respectively, by the 36-item Short Form (SF-36), an international questionnaire for analysis of QOL.

Results Before rHuEPO-ad, posttransplant patients had preserved MSC (54.1 ± 2.3) but impaired PSC (32.6 ± 3.2). rHuEPO-ad for 6 months increased their Hb to 13.7 ± 0.3 g/dl. This was accompanied by improvement of PSC (49.1 ± 2.1: $P < 0.01$ versus before rHuEPO-ad). MSC was preserved during rHuEPO-ad (54.4 ± 1.6: NS versus before rHuEPO-ad). There was inverse correlation between initial PSC or MSC and responses of these parameters to rHuEPO-ad (PSC, $P = 0.007$; MSC, $P = 0.009$). Patients whose initial PSC was lower than 39.6 or whose initial MSC was lower than 39.4 were expected to improve their PSC or MSC by more than 10 by rHuEPO-ad.

Conclusions Anemia in posttransplant patients has negative impacts on their QOL. Scoring mental and physical QOL by SF-36 in posttransplant patients is useful to identify groups of patients whose QOL could be improved by rHuEPO-ad.

Keywords SF-36 · QOL · EPO · Posttransplant

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Introduction

Anemia is a common complication in patients with chronic kidney diseases, including posttransplant patients (posttransplant anemia: PTA). Prevalence of PTA during the first 5-year posttransplant period has been reported to be 30–40% [1]. Lower GFR has been identified as the major risk factor among the various factors for PTA, including low serum erythropoietin, younger age, female gender, iron deficiency, systemic illnesses, acute and chronic infections, immunosuppressive regimens, and use of angiotensin I converting