表 RIFLE および AKIN 分類

RIFLE Stage	血清 Cr 上昇，その他（RIFLE）	尿量減少（RIFLE，AKIN 共通）	血清 Cr 上昇，その他（AKIN）	AKIN Stage
Risk	≧150～200%もしくは GFR 低下>25%	6 時間を超える乏尿（<0.5 mg/kg/時）	≧0.3 mg/dL 上昇もしくは≧150～200%	1
Injury	>200～300%もしくは GFR 低下>50%	12 時間を超える乏尿（<0.5 mg/kg/時）	>200～300%	2
Failure	>300%もしくは GFR 低下>75%もしくは≧4 mg/dL かつ≧0.5 mg/dL 上昇	24 時間を超える乏尿（<0.3 mg/kg/時）もしくは 12 時間を超える無尿	>300%もしくは≧4 mg/dL かつ≧0.5 mg/dL 上昇もしくは透析導入	3
Loss	4 週を超える腎不全			
ESRD	3 カ月を超える腎不全			

療法開始のタイミングをとらえる評価法も臨床的価値が高い。

ここでは，1）に関しては GFR の変化を評価するにあたっての血清 Cr の意義と問題点，シスタチン C の意義を記述する。2）はバイオマーカーとしての考え方であり他稿に譲る。また，3）では腎前性急性腎不全と腎性急性腎不全の鑑別について述べる。4）に関して，血清尿素窒素の意義を述べる。

血清 Cr

GFR を推測する目的で最もよく用いられる血清検査項目は，いうまでもなく血清 Cr である。AKI ではわずかな血清 Cr の上昇が予後との関連を有することが認識されており，前述したように RIFLE 分類，AKIN 分類でも血清 Cr 上昇の程度をもって重症度判定に用いている。GFR の急激な低下時においてみられる血清 Cr の挙動についての注意点を記す。

1．急激な GFR 低下が発症した際に，血清 Cr 上昇は 1～2 日遅れる

血清 Cr は，急激に腎機能が低下する場合には「蓄積した老廃物」の指標であるため，腎機能低下発症より Cr 上昇は数日遅れる。たとえば，成人の一日 Cr 産生は約 1 g であり，全く排泄がなくとも，体重 60 kg（体液量 36 L）では血清 Cr は 3 mg/dL/日程度の上昇にとどまる。すなわち，AKI において，血清 Cr は採血時点の GFR を反映するものではなく，したがって CKD 診療で広く用いられる GFR 推算式（日本人用，MDRD 式，CKD-EPI 等）や，クレアチニンクリアランスを推算する Cockcroft-Gault 式などは腎機能が急速に変化する状況では腎機能推算には適さない。

2．AKI では血清 Cr のわずかな上昇が GFR の高度低下の現れであることがある

血清 Cr の増加率は GFR の定常状態，産生率，尿細管分泌，分布容積などによって規定される。Cr は筋肉由来のクレアチンが肝臓で非酵素的に脱水されることで形成されるため，重度の肝機能障害や，筋肉量低下が存在すると，Cr 産生が減少する。AKI を発症する重症患者では肝機能障害がしばしば合併し，また高齢者も多いため筋肉量低下も著明であることはまれでなく，Cr 産生低下の可能性が高い。また，敗血症でも Cr 産生が低下することが動物実験で示されている。その機序の一つに，クレアチン→ Cr の変換が敗血症に伴う低体温下で減少することが想定されている。また，心疾患術後などの循環動態不安定で急速な体液貯留が発生する病態では Cr の分布容積が増加し，腎機能低下に伴う血清 Cr 濃度増加が相殺される可能性もある。

以上のことから，AKI が発症する重症患者にお

いては，血清 Cr は定常状態で想定される GFR と比較して，過小評価となっている可能性が高く，またその変動率も患者の全身状態により一定せず，わずかな血清 Cr 上昇の背後に大きな GFR 低下が存在する可能性を常に念頭に置く必要がある。また，0.3〜0.4 mg/dL 程度の血清 Cr 増加が存在すると，Cr変化のない場合に比べて死亡の危険度が 1.7 倍となるとも報告されている[1]。

3. AKI でのより高い血清 Cr 値はパラドキシカルに良好な予後と関連する可能性がある

一方，AKI における高い血清 Cr 値がよい生存率と関連する，との報告もみられる[2]。その背景因子として最も受け入れられている考え方は，1) CKD がすでに存在する患者に AKI が発症した場合，ベースラインの血清 Cr が高いため，Cr の絶対値は上昇しやすいが，腎代替療法を開始した時点で，今回のエピソードで新たに生じた腎障害は相対的に軽い可能性がある，という説である。それ以外に，今後検証が必要と考えられる仮説を記す。2) 高い血清 Cr は筋肉量が多いことを反映しており，そういった患者では全身状態がよいことが期待される，3) 腎代替療法を必要とする時点で血清 Cr が低いケースは，体液量過剰を伴っている可能性があり，そのことが予後を悪化させる要因となっているのではないか，また，4) 上述した血清 Cr 上昇速度が鈍いと想定される筋肉量の少ない，体液量過剰，肝障害，敗血症などを伴った重症患者で腎代替療法開始が遅れる可能性があり，これらの患者の予後をさらに悪化させている。

シスタチン C

シスタチン C は内因性 cysteine proteinase inihibitor の一種で，あらゆる有核細胞からコンスタントに産生される，分子量約 13 kDa の蛋白質で，血中では単独で存在し，糸球体から自由に濾過されほぼすべてが近位尿細管で再吸収され分解・代謝される。この特性から，血清シスタチン C は優れた GFR マーカーとして認識され，保険適用となっている。

血清シスタチン C は腎機能以外にも，急激な体液量の変化や産生速度により影響を受ける。血清シスタチン C は高濃度の副腎皮質ステロイドや甲状腺機能亢進状態で増加し，甲状腺機能低下で減少する。炎症が血清シスタチン C 濃度に影響するとの意見もあるが，それほどの変化はないとされている[1]。なお，尿中に排泄されたシスタチン C は近位尿細管障害を反映するもので，GFR とは無関係である。

いくつかの報告から，血清シスタチン C は AKI において GFR の低下を検出する特性が血清 Cr より優れると考えられるが，一方血清 Cr や血清尿素窒素と同様の診断的価値とする報告もあり，評価は一定しない[3]。また，現時点ではシスタチン C 測定は各種キットによる測定の標準化が未整備であり，この点は今後の普及にとって課題である。

腎前性と腎性の鑑別

この目的のためにはナトリウム分画排泄率（fractional excretion of sodium：FENa）が最も重要である。腎前性では Na は in-out のバランスが保たれる方向であるが，尿細管壊死をきたした腎性 AKI では Na 再吸収が低下し，排泄率が上昇する。尿 Na 濃度や尿浸透圧が用いられることもあるが，乏尿状態では腎前性 AKI でもこれらが高くなり得るので，可能な限り FENa を用いる。

なお，FENa も食塩摂取量，体液量や GFR に応じて 0.1〜1% まで変動し得る点に注意する。たとえば，GFR 正常（たとえば 140 L/day）であれば，100 mEq 程度の一日 Na 排泄量（＝摂取量）は FENa では約 0.5% に相当するし，GFR がその 50% 程度まで低下すれば FENa は 1% となる。これらを勘案して，FENa が 0.1〜1% では腎前性 AKI を，1% を超える場合には腎性 AKI を考える。

一方，AKI でよく用いられるループ利尿薬使用下では Na が強制的に排泄されるため，FENa が高値となる。この状況下では尿素窒素分画排泄率（FEur）が参考になるとされる。近年反論も発表されているが，それでもその使用は考慮に値すると思われる[6]。FEur＜35% で腎前性，FEur＞35% で腎性と判断する。

血清尿素窒素

尿素窒素は1773年にヒトの尿中で見出された物質で，腎臓病分野で最も古いバイオマーカーといってよい。しかし，腎機能（GFR）を推測するには適しているとはいいがたく，血清尿素窒素値はGFR以外にも蛋白質摂取量，異化状態，体液量，上部消化管出血，副腎皮質ステロイド使用などによって影響される。一方，尿素窒素それ自体は最も無害な窒素化合物といってもよく，尿毒症の原因物質ではないこともよく知られている。しかし，血清尿素窒素の上記の特性は，AKIなどの全身性の重症患者の状態を総合的に反映するものとして有用と考えられており，予後推測にしばしば用いられる[4]。

AKIにおいて腎代替療法（RRT）を開始するタイミングは予後を左右するものとして重要と考えられる。RRT開始の基準は確立されておらず，実際の臨床現場では担当医の経験によるところが大きい。緊急透析が必要な状況，すなわち利尿薬抵抗性の肺水腫・心不全，高度の高K血症（>6 mEq/Lなど），コントロール困難な酸-塩基平衡異常（動脈血pH<7.15など），症候性/高度の尿毒症では速やかなRRT開始が必要である。血清尿素窒素をRRT開始基準の目安として用いる場合，近年のAKIデータベース（PICARD）の二次解析から，ベースラインの血清尿素窒素値が76 mg/dLを超えてからのRRT開始で有意に死亡率が高いことが示されており[5]，基準を考えるうえで参考になるものといえる。

おわりに

本稿ではAKIの腎機能評価について，現在保険診療で用いられる検査項目の用い方について述べたが，これら指標の問題点，解釈にあたっての留意点に重点をおいた記述となった。AKIへの早期介入にあたっては新たなバイオマーカーの創出・導入が不可欠であり，この分野の発展を期待したい。

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14）コレステロール塞栓症と急性腎傷害（AKI）

Cholesterol crystal embolization and acute kidney injury

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Key words | cholesterol crystal embolization, acute kidney injury

はじめに

コレステロール塞栓症は，動脈壁のアテローム粥腫が何らかの原因で崩壊することにより，その構成成分であるコレステロール結晶が飛散して全身の末梢小血管を塞栓する全身性微小塞栓症である。腎臓は主要な障害臓器であり，虚血性腎症の一つと位置付けられている。近年の動脈硬化性疾患の増加に伴い，その重要性が認識されている。

本稿ではコレステロール塞栓症と，それに伴う急性腎傷害（AKI）について概説する。

コレステロール塞栓症の成因と症候

疫学的には60歳以上の男性に多く，危険因子としては高血圧，糖尿病，脂質異常症，喫煙，および冠動脈疾患や末梢動脈疾患，腹部大動脈瘤の既往歴・治療歴などがあげられる。

発症には医原性の要因が関与することが多く，その大部分は血管内カテーテル操作や心血管手術などの心血管手技であり，心臓カテーテル検査や経皮的冠動脈形成術後の約1.4～2%に発症するとされる。しかし，誘因となる既往歴がない状態で本症と診断される例も20%前後にみられる[1]。

臨床症状としては，障害される臓器，コレステロール結晶の大きさや量により多様な病状を呈し，また誘因となる検査や治療から数週間～数カ月後に突然もしくは段階的に発症するため診断は困難である。障害を受けやすい臓器としては腎臓，皮膚，脾臓，消化管，心筋，骨格筋などがあげられ，そのうち腎臓は50%と頻度が高い[2]。理由として，解剖学的に腎動脈は大動脈から大量の血流（体循環の1/5）が直接流入し，粥状硬化巣の崩壊の影響を直接的に受けやすいためである。

腎臓においては，腎動脈分岐部より上流からのコレステロール結晶が弓状動脈，小葉間動脈，細動脈，糸球体毛細血管に塞栓することで発症する。病態としては，コレステロール結晶による細動脈の閉塞による循環障害だけではなく，塞栓によって生じる局所の炎症反応や，免疫学的機序を介した血管炎に類似する障害が主たるものである。

そのため，臨床症状の発現までに時間的なばらつきが生じ，腎機能障害については発症形式により3種類に分類される。1つ目は誘因となる血管内操作から1週間程度で急激に腎機能障害を生じるAKIのタイプ，2つ目は数週間にかけて段階的に腎機能障害を生じる亜急性のタイプ，3つ目は緩徐な経過で腎機能障害を生じるタイプである。臨床的には亜急性のタイプが半数程度を占めて最も多く，次いでAKIのタイプが多い。

皮膚障害は34%に認められ，四肢末端の血色不良，足趾のチアノーゼ（blue toes），網状皮斑（livedo reticularis）などがあり，コレステロール塞栓症の初期症状である。網状皮斑はたとえ軽微であっても，後に虚血性の疼痛や間欠性跛行，腓腹筋痛などの重い随伴症状を伴うことが多い。また進行例では，潰瘍化や壊死など高度な動脈閉塞

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性疾患を思わせる皮膚症状があるにも関わらず，足背動脈など比較的太い動脈の拍動が保たれていることが疾患特異的である[2]。

消化管の障害は腸管虚血の程度により，腹痛や下痢が惹起され，およそ10%程度では消化管出血も認められる[3]。内視鏡などの所見は非特異的であり，診断は困難である。

その他，虚血に伴う壊死性膵炎や巣状の肝細胞壊死，胆嚢炎，胆管炎なども認められることがある。

診　断

患者背景，心血管手技，症状（腎，腎外）などから疑うことができるが，多彩な症状を呈するため診断が困難な場合も多く，生前の診断率は12%という剖検例の報告もある[4]。

検査所見としては，白血球増多やCRPの上昇，血沈亢進などの炎症所見や，血清クレアチニンの上昇などの腎機能障害，免疫学的機序を示唆する好酸球増多[5]，補体低下などが認められる。好酸球の増加は高率に出現し，腎機能障害と好酸球の増加から本症が疑われることもある。

尿所見は血尿，白血球尿，好酸球尿，顆粒円柱などを認めることもあるが比較的乏しい。また60%程度の症例で蛋白尿が認められ，大量となることはまれであるが，ときとしてネフローゼレベルの蛋白尿を認める場合もある。

組織所見では，コレステロール結晶は55～200 μm の直径で，主に末梢の100～300 μm 程度の動脈を塞栓する。腎血管では小葉間動脈レベルに相当し，血管内のコレステロール結晶は両端を血管壁に接して配列する針状の空隙として観察され，周囲への炎症細胞浸潤が認められる。

確定診断には皮膚生検や腎生検などで組織学的に動脈内にコレステロール結晶を確認することが必要であるが，臨床経過から急性腎不全，四肢末梢の塞栓症状などの臨床症状が揃っていれば，組織所見がなくても診断は可能とされている。

AKIとしてのコレステロール塞栓症：造影剤腎症との鑑別

コレステロール塞栓症によるAKIは，血管内操作後の腎障害として発症することが多いため，特に造影剤腎症との鑑別が重要となる。造影剤腎症は通常，造影剤使用の24～48時間後に血清クレアチニンが上昇し始め，3～7日後にピークとなり，10～14日後に前値に戻る。またほとんどが無症候性，非乏尿性，可逆性であるが，糖尿病，高齢，脱水などの造影剤腎症のリスクが高い患者などでは造影剤投与後に乏尿となり，重篤なAKIを発症する場合もある。このような場合の尿所見は急性尿細管壊死のパターンとなる。

これに対し，コレステロール塞栓症では誘因となる手技の後，1週間以上，ときには数カ月してから発症し，2週間以上腎機能障害が持続する。

その他の鑑別としては腎前性急性腎不全，薬剤性腎障害，多発性血管炎，横紋筋融解症など，さまざまなものがあげられる。

治　療

本症には確立された治療法はなく，発症（再発）回避と対症療法が中心となる。組織の虚血性障害の進行の防止，コレステロール結晶の再流出防止が重要で，具体的には抗凝固療法の中止，血管内カテーテル操作や心血管手術などの回避，血圧，血糖のコントロール，動脈壁在プラークの安定化を狙ってHMG-CoA阻害薬の投与，急性腎不全の治療としての利尿剤や，積極的な血液透析による体液コントロール，経静脈的な栄養管理などを実施する。

全身状態の悪化や炎症反応の上昇，新たな塞栓症状などの病態が進行する症例に対しては，炎症反応や免疫反応の抑制を狙った副腎皮質ステロイドの投与や血漿交換療法が試みられてきた。症例報告レベルでは，ステロイド療法ならびにステロイドと血漿交換の併用療法の有効性を示す報告が散見される[6~9]一方で，最近の症例対照研究ではステロイドによる腎機能の改善効果は示されず，

表　コレステロール塞栓症に対する内科的治療に関する主な報告

報告者	対象	治療	結果
Hasegawa M, et al（文献6）	亜急性の経過で腎不全に至ったコレステロール塞栓症5症例	4症例で血漿交換を併用，3症例ではプレドニゾロン（PSL）30 mg/日（約0.6 mg/kg/日）で加療	血漿交換と積極的なステロイド治療により3症例を救命。そのうち1例は維持透析となったが，2例ではCr 2.1 mg/dL，1.9 mg/dLと安定した。2例は多臓器不全で死亡。
Stabellini N, et al（文献7）	コレステロール塞栓症に伴うAKIと診断された7例	PSL 40 mg/日を静注で4日間治療を開始し，1週間で0.4～0.5 mg/kg/日まで減量。以後は数カ月の単位でPSLは漸減中止	腎機能は速やかに改善，その他の臨床症状も改善を認めた。
Sugimoto T, et al（文献8）	CABG後にAKIと診断された75歳，男性	メチルプレドニゾロン（mPSL）125 mg/日を3日間の後，PSL 30 mg/日で加療	皮膚症状は劇的に改善，腎機能も改善し透析を離脱した。
Nakayama M, et al（文献9）	コレステロール塞栓症によるAKIと診断された7例（平均年齢69歳）	経口PSL 15～20 mg/日の投与を2～4週間，その後2～4週間かけて5 mgずつ漸減し5 mg/日を維持量とした。PSL減量中に再発した場合には初期投与量よりも増量しPSLを投与	全例で血清クレアチニン，好酸球ともに並行して改善。最終的に5例で腎機能の改善，1例が肺癌で死亡，1例は多臓器不全で死亡。
Gutiérrez Solís E, et al（文献10）	1989～2005年の間で3つのスペインの病院でコレステロール塞栓症と診断された45例	45例のうち15例でステロイド治療	ステロイド治療例では死亡率が高く，腎機能の改善も乏しかった（統計的に有意差なし）。またステロイド治療例では腎死までの期間が短かった。
Tamura K, et al（文献11）	冠動脈造影後3カ月でAKI，blue toeからコレステロール塞栓症と診断された68歳，男性	血漿交換後に低用量のステロイド，ARBで加療	血漿交換後に劇的に皮膚症状は改善，腎機能障害も改善を認めた。
Tsunoda S, et al（文献12）	コレステロール塞栓症によりAKI，皮膚障害をきたした2例	LDLアフェレーシスを週1～2回，total 10回の加療。処理量は1回3L程度	皮膚障害はLDLアフェレーシス施行後に速やかに改善したが，腎機能は改善を認めなかった。

生命予後，腎予後は同様に不良であった[10]。

また，積極的な治療としてLDLアフェレーシスの有効例の報告もあるが[11,12]，効果のエビデンスは乏しい。内科的治療に関する主な報告を表にまとめた。

塞栓源が画像上明らかな症例に対し，再発・増悪を予防するためのバイパス術，血管内膜除去術，ステントの留置などの報告が海外からなされている[13]。

予　後

コレステロール塞栓症の患者は，背景に重度の動脈硬化性病変が存在するため，従来の報告では1年死亡率は64～81％程度と予後不良であった[2]。しかし，本症の認知度の増加に伴い早期診断されるようになり，最近の1年死亡率は20～30％程度となってきている。しかし，腎機能障害を生じた場合には30％程度が透析を要し，離脱できるのはこのうちの30％程度と報告されている[1]。

おわりに

近年の日本社会では高齢化や高血圧，糖尿病，脳梗塞，虚血性心疾患などの動脈硬化性疾患を有する患者の増加に加え，多種多様な血管内カテーテル治療・検査が急速に発達しており，このような状況下では今後さらに本症が増加することが予想される。

コレステロール塞栓症はいったん発症すると確立された治療法がないため，発症の回避，再発防止が重要である。腎不全，皮膚病変，好酸球増多が三大臨床兆候であり，本疾患の存在に留意した診療が必要である。

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Therapeutic Apheresis and Dialysis 14(3):334–340
doi: 10.1111/j.1744-9987.2009.00782.x

Relationship of Skin Autofluorescence to Cardiovascular Disease in Japanese Hemodialysis Patients

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Abstract: Advanced glycation end products (AGE) are significantly increased in end-stage renal disease patients and it has been suggested that AGE accumulation is related to the progression of cardiovascular disease. An autofluorescence reader non-invasively assesses AGE accumulation using skin autofluorescence under ultraviolet light. Skin autofluorescence has been reported to be an independent predictor of mortality in Caucasian hemodialysis patients. The aim of this study was to assess whether skin autofluorescence in Japanese hemodialysis patients is related to the presence of cardiovascular disease. In this cross-sectional study, patients on maintenance hemodialysis ($N = 128$; 59 men, 69 women) were included. AGE accumulation was assessed by skin autofluorescence using an autofluorescence reader. Associations between skin autofluorescence, cardiovascular disease, and other parameters were studied. Skin autofluorescence correlated with age ($r = 0.32$, $P < 0.01$), diabetes ($r = 0.21$, $P = 0.02$), carotid intima-media thickness (IMT) ($r = 0.23$, $P = 0.02$), high-sensitivity C-reactive protein (hsCRP) ($r = 0.20$, $P = 0.03$), and plasma pentosidine ($r = 0.20$, $P = 0.03$). Each parameter was compared in patients with and without cardiovascular disease; the gender distribution, age, carotid IMT, high-density lipoprotein cholesterol, hsCRP, and skin autofluorescence were significantly related to the presence of cardiovascular disease. Multiple logistic regression analysis identified carotid IMT (OR 6.76), hsCRP (OR 1.41), and skin autofluorescence (OR 2.29) as significant factors for the presence of cardiovascular disease. Increased skin autofluorescence was related to the presence of cardiovascular disease in Asian (non-Caucasian) hemodialysis patients, and therefore an autofluorescence reader might have the potential to be a useful assessment of cardiovascular risk in these patients. **Key Words:** Advanced glycation end product, Arteriosclerosis, Autofluorescence, Cardiovascular disease, Hemodialysis.

Advanced glycation end products (AGE), synthesized by the non-enzymatic response of glucose to protein (the Maillard reaction), have been implicated as a contributing factor in the progression of chronic, age-related diseases, such as diabetic complications, dialysis-related amyloidosis, Alzheimer's disease, rheumatoid arthritis, and arteriosclerosis (1–3).

Accumulation of AGEs is caused by hyperglycemia, oxidative stress, carbonyl stress, and renal dysfunction. It has been reported that plasma pentosidine levels were correlated with serum creatinine levels (4), and were significantly elevated in uremia, even in the absence of hyperglycemia, because of reactive carbonyl compound accumulation (carbonyl stress) (2). AGEs accumulate in arteriosclerotic lesion sites, and an association between AGEs and arteriosclerosis has been suggested (5–7).

AGE-modified proteins are fluorescent (emission at 440–450 nm upon excitation at 360–370 nm), and fluorospectrometric analysis is used to assess tissue/serum AGEs. Some distinct AGE structures have been identified, and more specific and sensitive biochemical methodologies for assessing AGEs, such as high performance liquid chromatography (HPLC) and gas chromatography, have been developed. Several different AGE structures, for example

Received July 2009; revised September 2009.
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Presented in part at the 40th Annual Meeting of the American Society for Nephrology held 2–5 November 2007 in San Francisco, CA, USA.

TABLE 1. *Characteristics of healthy control subjects and hemodialysis patients*

	Hemodialysis patients			
	All subjects	Diabetic	Non-diabetic	Controls
N	128	44	84	19
Gender (male : female)	59 : 69	21 : 23	38 : 46	11 : 8
Age (years)	65.1 ± 11.6	64.5 ± 10.3	65.4 ± 12.2	64.1 ± 12.4
BMI (kg/m^2)	22.1 ± 3.3	22.8 ± 3.8	21.8 ± 2.9	24.6 ± 3.2**
Dialysis duration (years)	5.8 ± 5.7	4.4 ± 3.1	6.5 ± 6.5	-
Carotid IMT (mm)	0.9 ± 0.4	1.0 ± 0.5	0.9 ± 0.4	-
Albumin (g/dL)	3.7 ± 0.4	3.7 ± 0.4	3.7 ± 0.4	-
Hemoglobin (g/dL)	10.1 ± 1.3	10.3 ± 1.4	10.0 ± 1.3	13.5 ± 1.6***
Creatinine (mg/dL)	10.4 ± 3.4	9.8 ± 3.6	10.7 ± 3.3	0.8 ± 0.1***
LDL cholesterol (mg/dL)	85.6 ± 27.6	80.8 ± 21.7	88.3 ± 30.2	-
HDL cholesterol (mg/dL)	45.5 ± 13.6	41.7 ± 14.2	47.6 ± 12.9*	-
Triglyceride (mg/dL)	111.8 ± 64.7	119.4 ± 73.4	107.6 ± 59.5	-
Pentosidine (pmol/mL)	1156 ± 512	1295 ± 654	1075 ± 390	-
hsCRP (mg/L)	1.4 ± 1.7	1.4 ± 1.8	1.5 ± 1.6	-
Oxidized LDL (U/mL)	6.9 ± 5.8	5.8 ± 3.6	7.5 ± 6.7	-
Skin autofluorescence (×10^{-2})	2.35 ± 0.68	2.52 ± 0.69	2.27 ± 0.67*	1.30 ± 0.37***
ACEi or ARB medication	85 (66.4%)	33 (75.0%)	52 (61.9%)	-

Values are expressed as mean ± SD. *$P < 0.05$ vs. diabetic hemodialysis patients; **$P < 0.05$ vs. hemodialysis patients; ***$P < 0.01$ vs hemodialysis patients. ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; IMT, intima-media thickness.

pentosidine and N-carboxymethyllysine (CML), have been measured and applied clinically. It has been reported that the levels of these AGEs in the arterial wall or skin collagen are correlated with diabetes control, severity of diabetic complications, and creatinine clearance (5). Accumulation of AGEs is thought to be a useful biomarker for the development of diabetic complications such as nephropathy. Monnier et al. reported that tissue autofluorescence is related to AGE accumulation and progression of diabetic complications, in which evaluation of tissue autofluorescence were assessed by skin biopsy specimen (8). However, evaluation of tissue AGE accumulation requires invasive procedures. It has also been reported that serum AGE levels do not reflect tissue AGE levels (9), and they do not predict mortality in dialysis patients (10,11). Meerwaldt et al. described a non-invasive optical tool, the autofluorescence reader, for assessing AGE accumulation in patients based on skin autofluorescence under ultraviolet light. They reported that skin autofluorescence was correlated with collagen-linked fluorescence, pentosidine, and CML accumulation in the skin, and with long-term complications in patients with diabetes, and that it was a strong predictor of mortality in ESRD (12,13). However, skin autofluorescence has not been sufficiently evaluated in non-Caucasian subjects, except for a report that skin autofluorescence is associated with arterial stiffness (pulse wave velocity) (14).

The aim of this study was to investigate the association between cardiovascular disease and skin autofluorescence in Japanese (non-Caucasian) ESRD patients.

PATIENTS AND METHODS

Study population

This cross-sectional study included 128 patients receiving maintenance hemodialysis (HD) in the dialysis unit of Fujita General Hospital and Hohrai East Clinic. All patients were non-Caucasian (Japanese). Patients with acute/chronic inflammatory disease and active malignancy were excluded. The control subjects were outpatients of various diseases who visited Fujita General Hospital between 3–6 July 2006, and were age- and sex-matched to HD patients. In the control subjects, diabetes mellitus and renal disease were excluded by conventional criteria described below and measurement of serum creatinine levels (<1.0 mg/dL), respectively. This study protocol complies with Declaration of Helsinki and was approved by the ethics committees at Fukushima Medical University, Fujita General Hospital, and Hohrai East Clinic. All patients received an explanation of the procedures and possible risks of this study, and gave written informed consent to participate in this study. The characteristics of both the control subjects and HD patients are summarized in Table 1.

Definition of cardiovascular disease and diabetes

The presence of cardiovascular disease was defined if at least one of the following events occurred before the time of skin autofluorescence measurement:

acute myocardial infarction due to clinical and ECG or laboratory changes, angina pectoris based on clinical characteristics, coronary artery disease documented by coronary angiography, cerebral infarction verified by computed tomography (CT), magnetic resonance imaging (MRI) and/or the course of neurological disorders and peripheral artery disease. The definition of peripheral artery disease included patients with intermittent claudication (Fontaine's stage II), ischemic rest pain (stage III) or ulcer, necrosis or a history of amputation (stage IV). Diabetes was defined by glucose values ≥200 mg/dL at any time, fasting glucose values ≥126 mg/dL, or the use of insulin or oral hypoglycemic drugs.

Skin autofluorescence

AGE accumulation was assessed based on skin autofluorescence using the autofluorescence reader (AGE reader; DiagnOptics, Groningen, The Netherlands) as described in detail previously (13). The measure of autofluorescence was defined as the average light intensity per nm in the range between 420 and 600 nm, divided by the average light intensity per nm in the range between 300 and 420 nm. The amount of ultraviolet light exposure is small and the autofluorescence reader has already been tested in several studies without any adverse effects (12,13). All measurements were performed at room temperature with the patients in a seated position, at the volar side of the lower arm, approximately 10–15 cm below the elbow fold. Care was taken to perform the measurement at a normal skin site, thus without visible vessels, scar, lichenification, or other skin abnormalities. The intra-assay coefficient of variation for repeated autofluorescence reader measurement on the same day was 5.0% ($N = 10$). Autofluorescence was calculated offline by automated analysis and was observer independent.

Measurement of carotid intima-media thickness

B-mode ultrasonographic scanning of the carotid artery was performed with a high resolution real-time ultrasonography with 10 MHz linear-probe (SSD-5000; Aloka, Tokyo, Japan). The carotid artery was scanned at the level of the bifurcation of the common carotid artery and was investigated bilaterally. All scans were performed by the same operator. The operator was not blind to the participants' information such as age, medical history, and dialysis duration, but was blinded to the blood sample date and skin autofluorescence data. The common carotid artery intima-media thickness (IMT) was defined as the distance from the leading edge of the first echographic line to the leading edge of the second echographic line on the scans, with the first line representing the collagen-containing upper layer of the tunica adventitia. In each longitudinal projection, the site of the greatest IMT thickness was detected by scanning along the vessel from the common carotid artery to the internal carotid artery. Three measurements of the IMT on both sides were performed at the site of greatest thickness and two other points (1 cm proximal and 1 cm distal to this site) for each patient. We defined the carotid IMT as the average value of six measurements (three from the right side and three from the left side) for each patient in this study.

Data collection

Blood samples were collected prior to the initiation of the HD session, and serum albumin, hemoglobin, creatinine, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and triglycerides were measured according to the automated standardized laboratory technique in the clinical laboratory of each institution. The plasma level of pentosidine was measured by HPLC using a fluorescence detector (Hitachi F-1050; Hitachi, Tokyo, Japan). The plasma level of high-sensitivity C-reactive protein (hsCRP) was measured by the nephelometry method using Behring Nephelometer II (BNII) (Dade Behring, Tokyo, Japan). The plasma level of oxidized LDL was measured by an enzyme immunoassay (EIA) using AP96 (Kyowa Medex, Tokyo, Japan). The plasma levels of pentosidine, hsCRP, and oxidized LDL were measured at Mitsubishi Chemical Medience Corporation (Tokyo, Japan). The mean glycoalbumin level of the previous year was measured in 44 diabetic patients.

Statistical analysis

Statistical analysis was performed using commercially available software, Dr SPSS II (SPSS Japan, Tokyo, Japan). All variables are expressed as the mean ± SD. The Spearman's rank correlation test was used to estimate the relationships between variables. Stepwise multiple regression analysis was performed for determination of the independent relationship of variables with skin autofluorescence. The independent effects of variables on the presence of cardiovascular disease were assessed by forward stepwise logistic regression analysis (0.05 for entry and 0.10 for removal probability). Differences were considered significant at $P < 0.05$. In the stepwise multiple regression analysis, the F-value was set at 4.0 at each step.

RESULTS

Clinical and biochemical characteristics

Table 1 shows the clinical characteristics of the control subjects and the 128 HD patients (59 men, 69 women; mean age 65.1 ± 11.6 years; mean duration of dialysis 5.8 ± 5.7 years; mean weekly dialysis time 11.4 ± 1.5 h; and mean Kt/V 1.27 ± 0.25). Of the 128 patients, 44 were diabetic and 84 were non-diabetic (of the 84 non-diabetic patients, 54 had primary glomerulonephritis, 16 had hypertension, and 14 had other conditions). In 128 HD patients, the mean titer of skin autofluorescence was 2.35 ± 0.68×10^{-2} with a range of $0.59–4.09 \times 10^{-2}$. Skin autofluorescence was 1.8 times higher in HD patients compared with control subjects (2.35 ± 0.68 vs. 1.30 ± 0.37; $P < 0.01$), and significantly increased in diabetic HD patients compared with non-diabetic HD patients (2.52 ± 0.69 vs. 2.30 ± 0.70; $P = 0.02$). Body mass index and hemoglobin were significantly lower in HD patients compared with control subjects.

Correlation between skin autofluorescence and other parameters in HD patients

Skin autofluorescence did not correlate with gender, body mass index, Kt/V, weekly dialysis time, serum albumin, hemoglobin, creatinine, LDL cholesterol, HDL cholesterol, or triglycerides; however, age ($r = 0.32$, $P < 0.01$), diabetes ($r = 0.21$, $P = 0.02$), carotid IMT ($r = 0.23$, $P = 0.02$), plasma pentosidine ($r = 0.20$, $P = 0.03$), hsCRP ($r = 0.20$, $P = 0.03$), and oxidized LDL ($r = 0.22$, $P = 0.02$) had weak but significant correlations with skin autofluorescence (Fig. 1A–C). Dialysis duration ($r = 0.17$, $P = 0.06$) showed a trend ($P < 0.10$) for a correlation with skin autofluorescence. Of 128 HD patients, 85 were treated with angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor blocker (ARB). ACEi and ARB were reported to reduce AGE accumulation (15); however, skin autofluorescence tended to be lower in ACEi and ARB users compared with non-users, the differences of which were not significant ($P = 0.07$). In the multiple regression model, which included age, dialysis duration, diabetes, carotid IMT, plasma pentosidine, hsCRP, oxLDL, and medication with ACEi or ARB (yes = 1, no = 0), the independent determinants of skin autofluorescence were age ($\beta = 0.38$, $P < 0.01$), diabetes ($\beta = 0.24$, $P = 0.01$), and dialysis duration ($\beta = 0.23$, $P = 0.02$) (Table 2). Glycemic control (mean glycoalbumin level of the previous year) was not significantly correlated with skin autofluorescence in 44 diabetic HD patients; however, the plasma pentosidine level was significantly correlated with the mean glycoalbumin level ($r = 0.48$, $P < 0.01$).

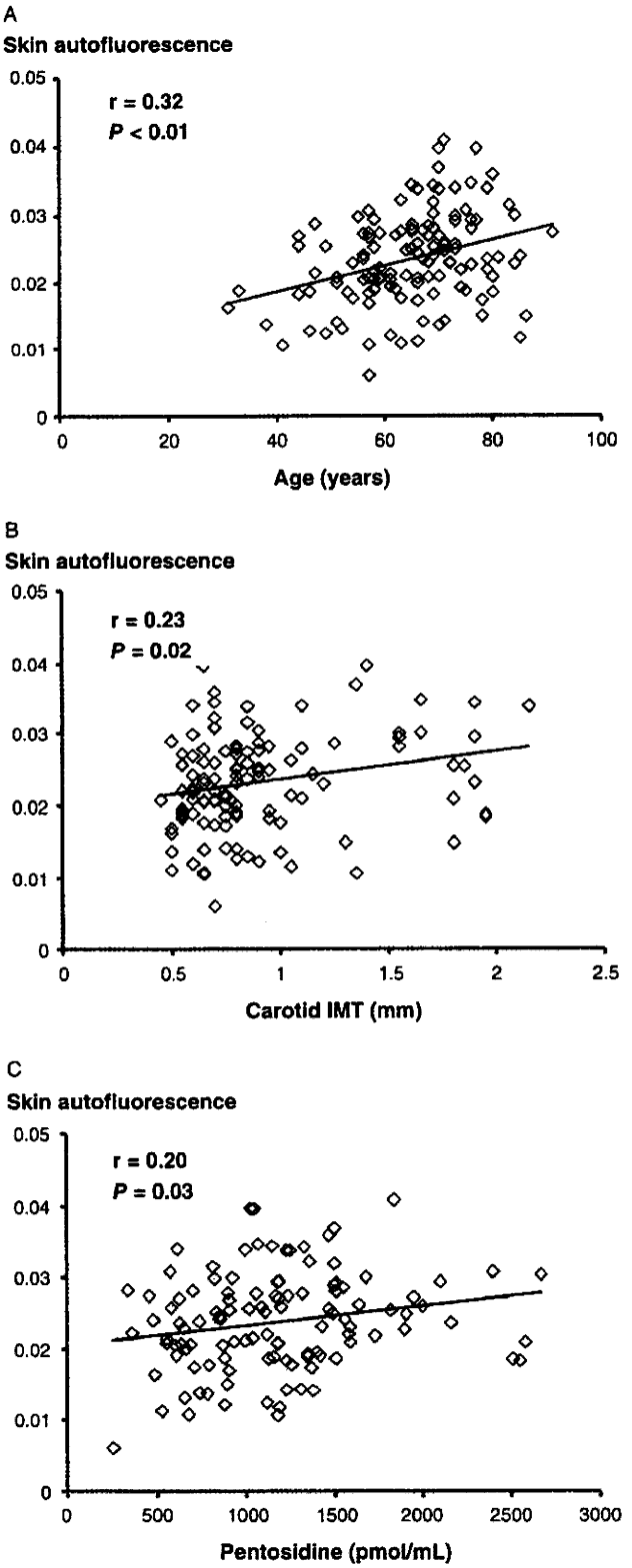

FIG. 1. Correlation between skin autofluorescence and (A) age, (B) carotid intima-media thickness (IMT), and (C) plasma pentosidine in hemodialysis patients.

TABLE 2. *Determinants of skin autofluorescence in multiple regression analysis*

Variable			
Dependent	Independent	β	*P* value
Skin autofluorescence	Age	0.38	<0.01
	Diabetes	0.24	0.01
	Dialysis duration	0.23	0.02

The F value was set at 4.0 at each step. The final result is given in the table. β is the standard regression coefficient; the multiple coefficient of determination (R^2) = 0.18.

Comparison of data between patients with and without cardiovascular disease

Of the 128 HD patients, 39 (30.5%) had cardiovascular disease. Table 3 shows the unadjusted univariable odds ratios for the presence of cardiovascular disease in 128 HD patients. Gender, age, carotid IMT, HDL cholesterol, hsCRP, and skin autofluorescence were significantly related to the presence of cardiovascular disease. Due to limited number of patients, forward stepwise logistic regression analysis was performed using cardiovascular disease as the dependent variable, and six variables that had significant correlation in the univariable analysis were the independent variables. Gender ($P = 0.16$), age ($P = 0.23$), and HDL cholesterol ($P = 0.17$) were not selected; however, carotid IMT (OR 6.76, 95%CI 2.08–21.96, $P < 0.05$), hsCRP (OR 1.41, 95%CI 1.05–1.88, $P < 0.05$), and skin autofluorescence (OR 2.29, 95%CI 1.02–5.12, $P < 0.05$) were independently related to the presence of cardiovascular disease in this model (Table 4).

TABLE 3. *Unadjusted univariable odds ratios (OR) for the presence of cardiovascular disease in hemodialysis patients*

	Unadjusted OR (95% CI)	*P*-value
Gender	2.89 (1.33–6.31)	<0.01
Age (years)	1.08 (1.03–1.12)	<0.01
BMI	1.06 (0.94–1.20)	0.35
Dialysis duration (years)	1.03 (0.96–1.10)	0.40
Diabetes	2.08 (0.95–4.52)	0.07
Carotid IMT (mm)	7.77 (2.75–21.98)	<0.01
Hemoglobin (g/dL)	0.98 (0.74–1.30)	0.89
Albumin (g/dL)	0.55 (0.21–1.43)	0.22
Triglyceride (mg/dL)	1.00 (1.00–1.01)	0.22
LDL cholesterol (mg/dL)	1.01 (1.00–1.03)	0.14
HDL cholesterol (mg/dL)	0.96 (0.92–0.99)	<0.01
Pentosidine (pmol/mL)	1.00 (1.00–1.00)	0.07
hsCRP (mg/L)	1.44 (1.14–1.81)	<0.01
Oxidized LDL (U/mL)	0.98 (0.91–1.06)	0.66
Skin autofluorescence ($\times 10^{-2}$)	2.88 (1.51–5.50)	<0.01
ACEi or ARB medication	0.86 (0.39–1.90)	0.72

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; IMT, intima-media thickness.

TABLE 4. *Variables related to the presence of cardiovascular disease by multivariable logistic regression analysis*

	Adjusted OR (95% CI)	*P*-value
Skin autofluorescence	2.29 (1.02–5.12)	<0.05
Carotid IMT	6.76 (2.08–21.96)	<0.05
hsCRP	1.41 (1.05–1.88)	<0.05

hsCRP, high-sensitivity C-reactive protein; IMT, intima-media thickness.

Receiver operating characteristic analysis

Figure 2 shows receiver operating characteristic (ROC) curve for skin autofluorescence to discriminate cardiovascular disease. The area under the ROC curve was 0.71 (95%CI 0.63–0.79). The best cut-off point for skin autofluorescence was determined to be 2.27×10^{-2}) (sensitivity 84.2%, specificity 56.3%, and likelihood ratio 1.93).

DISCUSSION

Most studies about skin autofluorescence have been performed in Caucasian populations. It has been reported that skin autofluorescence is associated with cardiovascular disease, and it has been found to be an independent predictor of mortality in Caucasian patients with ESRD (12) and diabetes (16). In non-Caucasian patients with ESRD, although skin autofluorescence has been reported to be associated with pulse wave velocity, a marker for arterial stiffness in Japanese people (14), relationships between cardiovascular disease and skin autofluorescence have not been fully investigated. The

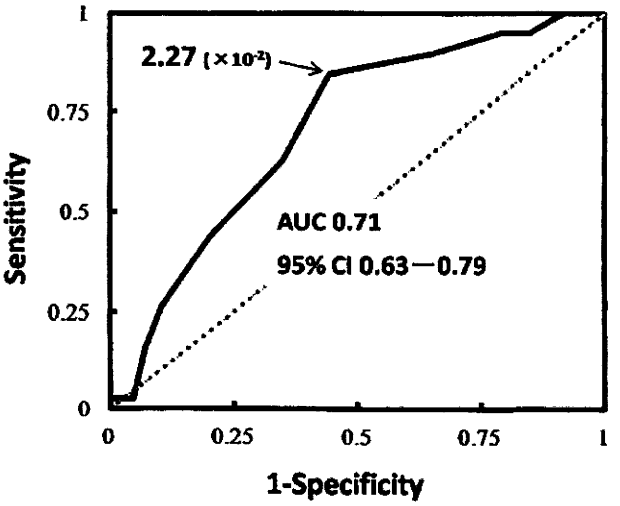

FIG. 2. Receiver operating characteristic curve for skin autofluorescence to discriminate cardiovascular disease.

present data demonstrate that skin autofluorescence was significantly increased in patients with cardiovascular disease compared to those without, and the best cut-off point for skin autofluorescence to discriminate the presence of cardiovascular disease was determined to be 2.27×10^{-2} by ROC analysis. The likelihood ratio of skin autofluorescence for the presence of cardiovascular disease was relatively low in these limited patients; however, the results of logistic regression analysis indicated that increased skin autofluorescence is an independent factor for the presence of cardiovascular disease in Asian (non-Caucasian) chronic HD patients. Thus, this study is the first to show the relationship between skin autofluorescence and cardiovascular disease in non-Caucasian ESRD patients.

Internal carotid artery IMT is a non-invasive marker reflecting the severity of arteriosclerosis, and its usefulness as an independent prognostic factor has already been reported in HD patients (17). It has also been documented that the hsCRP level increases as kidney disease advances, and that, compared to healthy individuals, hsCRP in chronic kidney disease patients, particularly ESRD patients, is markedly higher (18,19). Wanner et al. reported that hsCRP was a good predictor of cardiovascular disease in HD patients (19). In the present multivariate analysis, it is suggested that carotid IMT, hsCRP, and skin autofluorescence are related to the presence of cardiovascular disease in these patients. Age, which was one of the major factors to increase skin autofluorescence value and was significantly related to the presence of cardiovascular disease in the univariable analysis, could be a critical confounding factor; however, multivariable analysis revealed that age was not a significant contributing factor for the presence of cardiovascular disease.

The present data showed that skin autofluorescence correlated with age, diabetes, surrogate markers reflecting the severity of arteriosclerosis, such as carotid IMT, plasma pentosidine, hsCRP, and oxLDL, and the presence of cardiovascular disease in non-Caucasian HD patients, although these correlations were relatively low. The result of multiple regression analysis revealed that independent determinants of skin autofluorescence were age, diabetes, and dialysis duration. Glycemic control (mean glycoalbumin of the previous year) was not correlated with skin autofluorescence in diabetic HD patients. In previous studies performed with Caucasian subjects, skin autofluorescence has been reported to be correlated with age, glycemic control (mean Hb_{A1c} of the previous year), and the severity of micro/macroangiopathy in type 2 diabetic patients (20), and with age, diabetes, dialysis duration, and the presence of cardiovascular disease in dialysis patients (12).

On the whole, our data are similar to those seen in Caucasian subjects, except that glycemic control (mean glycoalbumin of the previous year) was not a significant contributing factor for skin autofluorescence. Hyperglycemia is not essential, but is an important factor for AGE accumulation; indeed, the plasma pentosidine level significantly correlated with the mean glycoalbumin of the previous year in our data. Skin autofluorescence may reflect long-term glycemic control more than the plasma AGE level, therefore the evaluation of glycemic control from the initiation of dialysis therapy or the on-set of diabetes is necessary to solve the relationship between glycemic control and AGE accumulation with skin or other organs.

The present study suggests that skin autofluorescence is related to cardiovascular disease in non-Caucasian HD patients, however, there are several limitations. First, patient skin color or pigmentation might affect autofluorescence measurement, and the autofluorescence reader is not reliable for patients with very dark skin coloring because of the high absorption grade of the excited light (13,21,22). So it has not been established whether skin autofluorescence actually reflects AGE accumulation in Japanese patients, although it has been reported that skin autofluorescence strongly correlates with the accumulation of AGEs, such as pentosidine and CML in Caucasian subjects with diabetes and ESRD, in spite of the fact that hyperpigmentation is a frequent skin alteration in dialysis patients (12,13). Second, this study was only a cross-sectional analysis with insufficient size, therefore a sufficiently-sized prospective investigation and better statistical methods are needed to evaluate whether autofluorescence predicts the progression of cardiovascular disease or mortality in Japanese patients. Once these issues have been addressed, the non-invasive and convenient instrument may become a useful tool for clinical risk assessment; however, more research is still needed in various patient populations in order to examine whether skin autofluorescence is a relevant biomarker reflecting accumulation of AGEs for cardiovascular risk in Japanese patients.

In recent years, interventions to prevent AGE accumulation have been developed. Nathan et al. reported that the AGE inhibitor pyridoxamine inhibits the development of renal and vascular disease in Zucker obese rats (23), while Miyata et al. reported that angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors reduce AGE formation (15). Skin autofluorescence may

have the potential to become an important surrogate marker for the effects of these treatments in the future; therefore, it will be necessary to closely examine the role of skin autofluorescence as a useful biomarker for the prognosis of chronic kidney disease (CKD), cardiovascular risk, and total mortality in each stage of CKD.

CONCLUSION

Skin autofluorescence was significantly higher in patients with cardiovascular disease than in those without, and increased skin autofluorescence is independently associated with the presence of cardiovascular disease in Asian (non-Caucasian) HD patients. The non-invasive autofluorescence reader might be a possible marker for assessing cardiovascular risk in these patients.

Acknowledgments: We thank Yoshio Konno, Koji Shibuya, Hideo Kunishima, Atsuko Hashimoto, and the staff of Hohrai East Clinic for their efforts in collecting and analyzing serum samples and the measurement of carotid IMT.

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Nephrol Dial Transplant (2011) 26: 214–220
doi: 10.1093/ndt/gfq369
Advance Access publication 29 June 2010

Skin autofluorescence is associated with renal function and cardiovascular diseases in pre-dialysis chronic kidney disease patients

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Abstract

Background. Tissue accumulation of advanced glycation end-products (AGE) is thought to be a contributing factor to the progression of cardiovascular disease (CVD). Skin autofluorescence, a non-invasive measure of AGE accumulation using autofluorescence of the skin under ultraviolet light, has shown associations with CVD in haemodialysis patients. The present study aimed to evaluate relationships of skin autofluorescence to renal function as well as CVD in pre-dialysis patients with chronic kidney disease (CKD).

Methods. Subjects in this cross-sectional analysis comprised 304 pre-dialysis CKD patients [median age, 62.0 years; median estimated glomerular filtration rate (eGFR), 54.3 mL/min/1.73 m^2; diabetes, n=81 (26.6%)]. AGE accumulation in skin was assessed by skin autofluorescence using an autofluorescence reader. Relationships between skin autofluorescence, eGFR, CVD history and other parameters were evaluated.

Results. Skin autofluorescence correlated negatively with eGFR (r = −0.42, P<0.01) and increased as CKD stage advanced. Multiple regression analysis revealed significant correlations of skin autofluorescence with age, presence of diabetes, eGFR and CVD history in CKD patients (R^2 = 30%). Age, male gender, smoking history, skin autofluorescence and eGFR were significantly correlated with CVD history, and multiple logistic regression analysis identified age [odds ratio (OR), 1.09; 95% confidence interval (CI), 1.03–1.15; P<0.01], history of smoking (OR, 6.50; 95% CI, 1.94–21.83; P<0.01) and skin autofluorescence (OR, 3.74; 95%CI, 1.54–9.24; P<0.01) as independent factors.

Conclusions. Tissue AGE accumulation measured as skin autofluorescence increased as GFR decreased and was related to CVD history in CKD patients. Non-invasive autofluorescence readers may provide potential markers for clinical risk assessment in pre-dialysis CKD patients.

Keywords: advanced glycation end-products; autofluorescence; cardiovascular disease; chronic kidney disease

Introduction

Cardiovascular mortality is greater in patients with chronic kidney disease (CKD) than in the general population and is associated with CKD stage [1–4]. As cardiovascular disease (CVD) is the main cause of death in these patients and possesses higher incidence than the development of end-stage renal disease (ESRD) in CKD patients [5], early recognition of CVD and risk stratification is crucial. However, traditional risk factors for CVD such as hypertension, smoking and diabetes mellitus cannot fully explain the high prevalence of CVD in CKD patients [6].

Advanced glycation end-products (AGE), synthesized by the non-enzymatic response of glucose to protein (the Maillard reaction), have been implicated as a contributing factor in the progression of chronic, age-related diseases such as diabetic vascular complications, dialysis-related amyloidosis, Alzheimer's disease, rheumatoid arthritis and atherosclerosis [7–9]. AGE have also been recognized as a CKD-related (non-traditional) risk factor for CVD. In addition to hyperglycaemia and increased oxidative stress, decreases in glomerular filtration rate (GFR) are thought to be an important determinant contributing to the accumulation of AGE. Plasma pentosidine levels reportedly correlate with serum creatinine levels [10] and are markedly elevated in dialysis patients, even in non-diabetic patients [8]. AGE accumulation in arteriosclerotic lesion sites is thought to play an important role in the pathogenesis of chronic complications such as CVD in patients with diabetes [11–13]. Monnier *et al.* reported that tissue autofluorescence is related to AGE accumulation and progression of diabetic complications, after evaluating tissue autofluorescence using skin biopsy specimens [14]. However, skin biopsy is an invasive and time-intensive method and is not feasible in daily practice for outpatients. In addition, serum AGE levels do not reflect tissue AGE contents [15] and do not predict mortality in dialysis patients [16,17].

An autofluorescence reader (AGE Reader; Diagnoptics, Groningen, the Netherlands) non-invasively assesses AGE accumulation using skin autofluorescence under ultraviolet light, and skin autofluorescence has been validated against AGE measurements in skin biopsies from the site of skin autofluorescence measurement, performed in patients with ESRD, diabetes and healthy controls [18–20]. Skin autofluorescence is reportedly an independent predictor of cardiovascular mortality in dialysis patients [20] and diabetic patients [21] in Caucasian populations. We have recently reported that skin autofluorescence is independently associated with CVD history in Japanese (non-Caucasian) haemodialysis patients [22]. However, the relationship between skin autofluorescence, renal function and CVD in pre-dialysis CKD patients has not been reported. Therefore, in order to assess the validity of skin autofluorescence in pre-dialysis CKD patients, we investigated the association between skin autofluorescence, CKD stage, CVD history and other clinical risk factors in this cross-sectional analysis.

Materials and methods

Study population

This cross-sectional study included 304 pre-dialysis CKD patients who visited Fukushima University Hospital or Tani Hospital between December 2008 and August 2009. Patients receiving dialysis therapy were excluded from this study. The study protocol complied with the Declaration of Helsinki and was approved by the ethics committees at Fukushima Medical University. All patients received an explanation of the procedures and possible risks of this study and provided written informed consent to participate. All patients were Japanese (non-Caucasian). Patients with acute/chronic inflammatory disease and active malignancy were excluded.

Data collection

Blood pressure was taken as a seated single measurement using an aneroid device, obtained after 5 min of rest. Blood samples were collected at the clinic by venipuncture from every patient in a non-fasting state. Serum creatinine was measured using an enzyme-based method, and serum albumin, haemoglobin, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol were measured according to the automated standardized laboratory techniques in the clinical laboratories of each participating institution. Diabetic retinopathy was determined by independent ophthalmologists based on retinal photography, and mean haemoglobin A1c level of the previous year was measured in 81 diabetic patients.

Definition of chronic kidney disease, diabetes and cardiovascular disease

The estimation equation for Japanese patients with CKD was applied for estimation of GFR. This equation calculates GFR from serum creatinine, age and gender using the following formula: [estimated glomerular filtration rate (eGFR) (mL/min./1.73 m^2) = $194 \times \text{Serum creatinine}^{-1.094} \times \text{Age}^{-0.287}$ (×0.739 for women)]. This formula has been validated against the GFR measured by using inulin clearance, which is the gold standard for measuring GFR, in Japanese patients [23]. Since this equation estimates GFR more accurately for the Japanese population than the previously reported equations such as the Modification of Diet in Renal Disease Study equation with Japanese coefficient and Cockcroft–Gault equation, the Japanese Society of Nephrology recommends using this equation for GFR estimation for Japanese in clinical practice and for epidemiological study. CKD was defined as eGFR <60 mL/min/1.73 m^2 or positive dipstick results for proteinuria (≥1+) [24]. Diabetes was defined by glucose values ≥200 mg/dL at any time, fasting glucose values ≥126 mg/dL or the use of insulin or oral hypoglycaemic drugs. A history of CVD was defined if at least one of the following events occurred before the time of skin-autofluorescence measurement: acute myocardial infarction due to clinical and electrocardiographic or laboratory changes; angina pectoris based on clinical characteristics; coronary artery disease documented by coronary angiography; cerebral infarction verified by computed tomography (CT), magnetic resonance imaging (MRI) and/or the course of neurological disorders; aortic disease including dissection and aneurysm verified by CT and/or MRI; and peripheral artery disease. The definition of peripheral artery disease included patients with intermittent claudication (Fontaine's stage II), ischaemic rest pain (stage III) or ulcer, necrosis or a history of amputation (stage IV).

Skin autofluorescence

AGE accumulation was assessed based on skin autofluorescence using the AGE Reader, as described in detail previously [18,19]. The measure of autofluorescence was defined as the average light intensity per nanometer in the range between 420 and 600 nm, divided by the average light intensity per nanometer in the range between 300 and 420 nm. Autofluorescence was expressed in arbitrary units (AU). The amount of ultraviolet light exposure is small, and the autofluorescence reader has already been tested in several studies without any adverse effects [18–22]. All measurements were performed at room temperature with the patient in a seated position, at the volar side of the lower arm, approximately 10–15 cm below the elbow fold. Care was taken to perform the measurement at a normal skin site, thus without visible vessels, scar, lichenification or other skin abnormalities. The intra- and inter-day assay precision expressed as coefficients of variation for autofluorescence reader measurements were 2.5% (n=10) and 4.6% (n=12), respectively. Autofluorescence was calculated offline by automated analysis and was observer-independent.

Statistical analysis

Statistical analysis was performed using SPSS Statistics version 17.0 software (SPSS Japan, Tokyo, Japan). All variables are expressed as median [interquartile range (IQR)]. Spearman's rank correlation test was used to estimate relationships between variables. Multiple linear regression analysis was performed to determine the independent relationship of variables with skin autofluorescence. Independent effects of variables on CVD were assessed by forward stepwise logistic regression analysis ($P<0.05$ for entry and $P\geq0.10$ for removal). Differences were considered significant at the $P<0.05$ level.

Results

Clinical and biochemical characteristics

Table 1 shows the clinical characteristics of the 304 CKD patients. Median age was 62.0 years (IQR, 49.3–73.0 years), and 51.3% of subjects were male. Median titre of skin autofluorescence was 2.07 AU (IQR, 1.75–2.43 AU; range, 0.91–3.90 AU). Angiotensin-converting enzyme inhibitors (ACEi) or angiotensin II receptor blockers (ARB) were being administered to 216 patients (71.1%). History included: CVD in 21 patients (6.9%), ischaemic heart disease in six patients (2.0%), cerebral infarction in seven patients (2.3%), peripheral artery disease in five patients (1.6%) and aortic disease in six patients (2.0%).

Correlations between skin autofluorescence and other parameters in chronic kidney disease patients

Skin autofluorescence was increased as CKD stage advanced [median skin autofluorescence for: stage 1, 1.60 AU (IQR, 1.25–1.95); stage 2, 1.90 AU (IQR, 1.59–2.14); stage 3, 2.23 AU (IQR, 1.89–2.49); stage 4 or above, 2.37 AU (IQR, 2.00–2.78)]. These differences were significant in stage 1 vs stage 2 and stage 2 vs stage 3 ($P<0.01$) and non-significant in stage 3 vs stage 4 or above ($P=0.08$).

Table 1. Clinical characteristics of patients with chronic kidney disease

Variable	CKD patients
N	304
Age (years)	62.0 (49.3–73.0)
Gender (male)	156 (51.3%)
History of smoking	128 (42.1%)
Body mass index (kg/m^2)	23.6 (21.3–26.5)
Diabetes	81 (26.6%)
Systolic BP (mmHg)	132.0 (119.0–147.8)
Diastolic BP (mmHg)	76.0 (68.0–84.0)
Skin autofluorescence (AU)	2.07 (1.75–2.43)
eGFR (mL/min/1.73 m^2)	54.3 (42.7–70.1)
Albumin (g/dL)	3.90 (3.63–4.20)
Haemoglobin (g/dL)	13.1 (11.9–14.2)
LDL cholesterol (mg/dL)	107.0 (89.0–134.0)
HDL cholesterol (mg/dL)	54.0 (46.0–62.0)
CVD history	21 (6.9%)
ACEi or ARB	216 (71.1%)

Values are expressed as medians (interquartile range). CKD, chronic kidney disease; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2. Determinants of skin autofluorescence in multiple regression analysis

Variable			
Dependent	Independent	β	P
Skin autofluorescence	Age	0.22	<0.01
	Diabetes	0.16	<0.01
	eGFR	−0.18	<0.01
	CVD history	0.14	<0.01

The final result is given in the table. β is the standard coefficient; the multiple coefficient of determination (R^2)=0.30.

Skin autofluorescence did not correlate with gender distribution, history of smoking, body mass index, systolic blood pressure, LDL cholesterol or medication with ACEi or ARB. However, age (r=0.42, P<0.01), diabetes (r= 0.33, P<0.01), eGFR (r = −0.42, P<0.01), serum albumin (r = −0.19, P<0.01), haemoglobin (r = −0.34, P<0.01), HDL cholesterol (r = −0.12, P=0.04) and CVD history (r=0.26, P<0.01) were significantly correlated with skin autofluorescence in CKD patients. Multiple linear regression analysis showed that 30% (R^2) of the variance of skin autofluorescence could be predicted by age, diabetes, eGFR and CVD history (Table 2). Serum albumin, haemoglobin and HDL cholesterol were not significant contributors in this model.

The presence of diabetes was independently and positively associated with skin autofluorescence in CKD patients. We compared skin autofluorescence in patients with and without diabetes. Figure 1 shows skin-autofluorescence value in each category of age and eGFR. Skin-autofluorescence titre was elevated in diabetic patients compared with non-diabetic patients in each age and eGFR category, with significant differences (P<0.05) in the age categories 51–61, 62–72 and >72 years, but not in the age category <51 years (P=0.06), and significant differences (P<0.01) in the eGFR categories 60–89 and 30–59 mL/min/1.73 m^2 but not in the ≥90- and <30-mL/min/1.73 m^2 categories. The age category <51 years included only four patients with diabetes, and the eGFR ≥90 mL/min/1.73 m^2 category included only two patients with diabetes.

In patients with diabetes, skin autofluorescence correlated with serum albumin (r = −0.43, P<0.01), haemoglobin (r = −0.38, P<0.01), presence of diabetic retinopathy (r=0.35, P<0.01), and CVD history (r=0.25, P=0.02); eGFR showed a trend toward a correlation with skin autofluorescence, but this was not significant (P=0.06). Twenty-five per cent of the variance in skin autofluorescence in diabetic CKD patients could be explained by the independent effects of haemoglobin (β = −0.48, P<0.01), CVD history (β = 0.27, P=0.01) and the mean haemoglobin A1c level of the previous year (β = 0.24, P=0.04). eAge, GFR and duration of diabetes did not show any independent effects on skin autofluorescence in this sub-group analysis. In patients without diabetes, skin autofluorescence correlated with age (r=0.40, P<0.01), systolic blood pressure (r=0.15, P=0.02), eGFR (r = −0.42, P<0.01), serum albumin (r = −0.14, P=0.04), haemoglobin (r = −0.18, P<0.01), HDL cholesterol (r = −0.14, P=0.03) and CVD history (r=0.14, P=0.04). Twenty-seven per cent of the variance in skin autofluorescence among non-diabetic patients could be explained by the independent effects of eGFR (β = −0.29, P<0.01), age (β = 0.24, P<0.01), and CVD history (β = 0.14, P=0.03). CVD history had independent and positive effects on skin autofluorescence in both diabetic and non-diabetic patients.

Comparison of data between patients with and without cardiovascular disease

Skin autofluorescence was 30% higher in patients with CVD history [median, 2.66 AU (IQR, 2.12–3.19)] than in those without [median, 2.05 AU (IQR, 1.71–2.39); P< 0.01]. Skin autofluorescence had significant effects on CVD in both the diabetic group [odds ratio (OR), 4.26; 95% confidence interval (CI), 1.21–15.04; P=0.02] and the non-diabetic group (OR, 5.46; 95%CI, 1.95–15.33; P<0.01) (Figure 2), and these effects remained significant after adjustment by age (diabetic group: OR, 3.76; 95% CI, 1.07–13.28; P=0.03; non-diabetic group: OR, 3.28; 95%CI, 1.10–9.82; P=0.03).

Table 3 shows unadjusted and adjusted ORs for the presence of CVD in CKD patients. Age, male gender, history of smoking, skin autofluorescence and eGFR were significantly related to CVD. Due to the limited sample size, we performed forward stepwise logistic regression analysis using CVD as the dependent variable and identified age, smoking history and skin autofluorescence as independently related to CVD. Male gender and eGFR were still significant factors for CVD after adjustment by age (male: OR, 5.94; 95%CI, 1.87–18.88; P<0.01; eGFR: OR, 0.96; 95%CI, 0.94–0.99; P<0.01) but were not selected in this multivariable model.

Discussion

This cross-sectional study found that skin autofluorescence increased as CKD stage advanced. CVD history showed

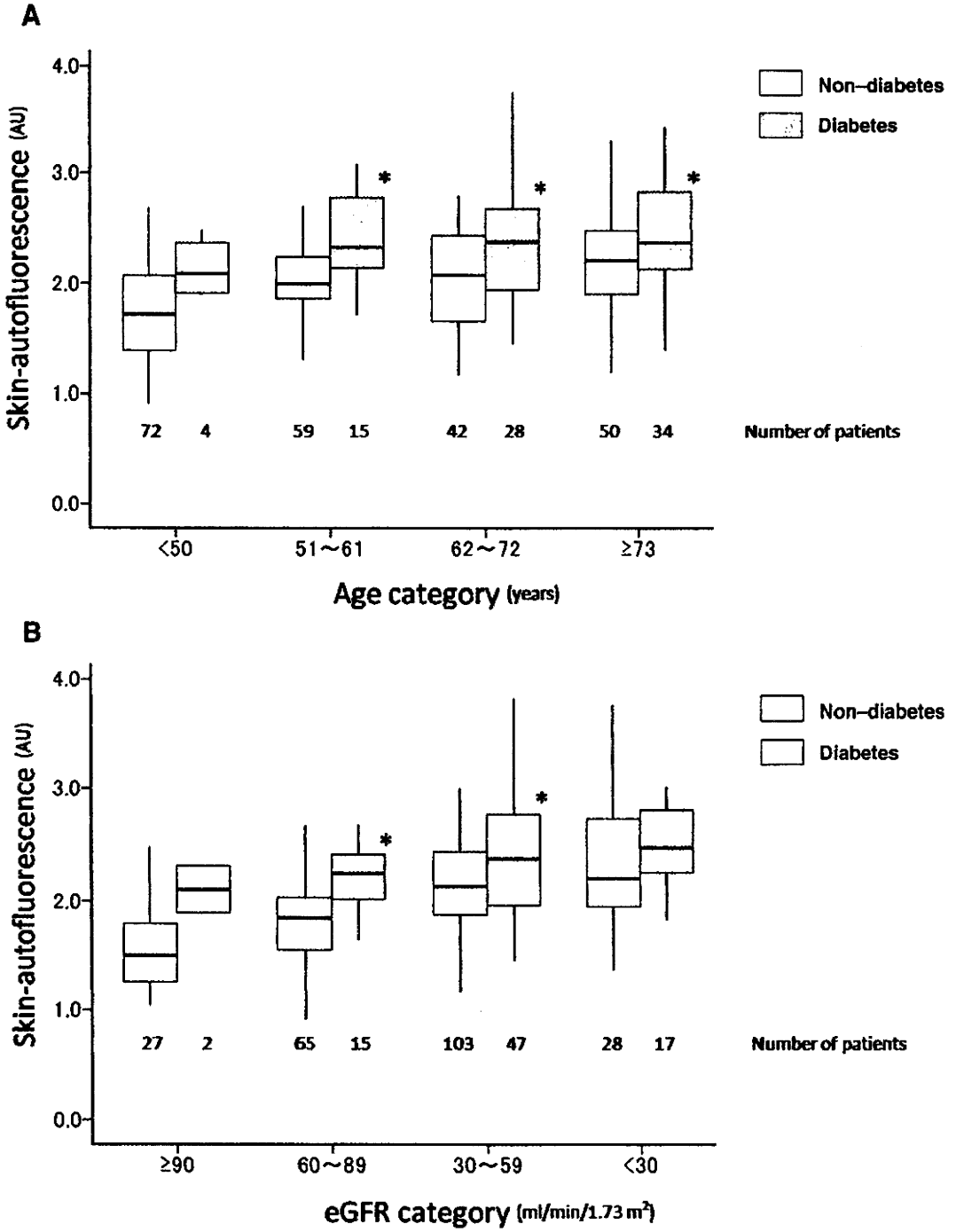

Fig. 1. These boxplots show the distribution of skin autofluorescence in each category of age (A) and eGFR (B) among chronic kidney disease patients with or without diabetes. Age category was divided by quartile of age. *P<0.05 vs non-diabetic patients.

independent effects on skin autofluorescence in both the diabetic and non-diabetic groups. Moreover, skin autofluorescence was higher in patients with CVD than in those without and still showed a significant contribution to CVD in the multivariable logistic regression model that included traditional risk factors for CVD such as age, smoking, blood pressure and diabetes. This study is thus the first to show the independent relationship of skin autofluorescence to renal function and CVD in pre-dialysis CKD patients.

As progression of CVD and AGE accumulation are time-dependent processes, the present results could be biased by age. We always included age as a dependent variable in multivariate analysis to reduce the potential effects of such biases, and our data still showed a significant correlation between skin autofluorescence and CVD.

Reduced GFR is a recognized risk factor for progression of CVD, the prevalence of which increases with decreased GFR [3]. In the present study, eGFR was one of the independent determinants for skin autofluorescence and dis-

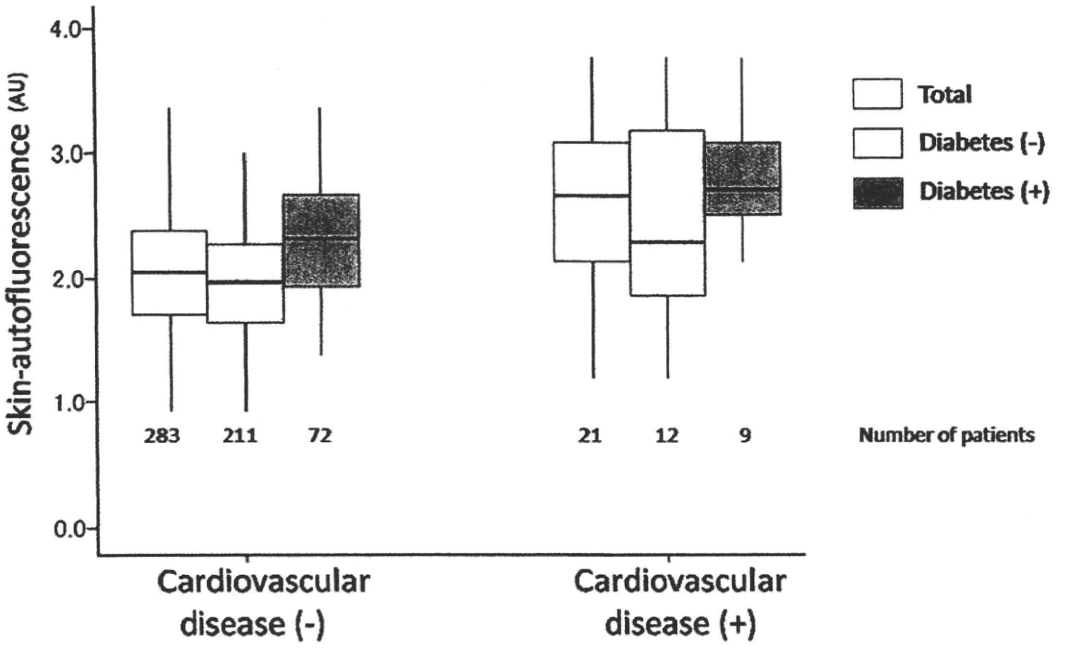

Fig. 2. Skin autofluorescence in patients with or without cardiovascular disease. Skin autofluorescence was significantly higher in patients with cardiovascular disease than in those without, for all chronic kidney disease patients ($P<0.01$), non-diabetic chronic kidney disease patients ($P<0.05$) and diabetic patients ($P<0.05$). Skin autofluorescence was elevated in diabetic patients compared with non-diabetic patients for patients both with and without cardiovascular disease ($P<0.05$).

played a significant relationship to CVD even after age adjustment. However, eGFR was not selected as an independent factor for CVD in the multivariable logistic regression model. As this study included only a cross-sectional analysis with limited patients, prospective investigation is necessary to evaluate the relationship between eGFR, skin autofluorescence and cardiovascular risk in CKD patients.

Hyperglycaemia is one of the major contributors to AGE accumulation. Previous studies have shown that the presence of diabetes has an independent effect on skin-autofluorescence values in dialysis patients [20,22], and skin autofluorescence was indeed higher in diabetic patients compared to non-diabetic patients in each category of age and eGFR, showing an independent relationship to the presence of diabetes and glycaemic control in diabetic patients in the present study. Skin autofluorescence is reportedly positively correlated to the severity of diabetic vascular complications and predicts progression of CVD and mortality in diabetic patients [21,25,26]. However, few studies have evaluated skin autofluorescence in non-diabetic CKD patients. We performed sub-analysis in patients with or without diabetes and found that skin autofluorescence exhibited a significant relationship to CVD in both the diabetic and non-diabetic groups even after adjusting for age. Independent determinants of skin autofluorescence were eGFR, age, body mass index and CVD in non-diabetic patients. However, eGFR did not show a significant effect on skin autofluorescence in diabetic

Table 3. Variables related to cardiovascular disease in chronic kidney disease patients by logistic regression analysis

	Univariate			Multivariate		
Variables	OR	95%CI	P	OR	95%CI	P
Age (years)	1.08	1.03–1.13	<0.01	1.09	1.03–1.15	<0.01
Gender (male)	4.40	1.45–13.41	<0.01			NS
History of smoking	6.39	2.10–19.49	<0.01	6.50	1.94–21.83	<0.01
Body mass index (kg/m^2)	1.00	0.90–1.12	0.95			NS
Diabetes	2.20	0.89–5.43	0.09			NS
Systolic BP (mmHg)	1.02	1.00–1.04	0.07			NS
Diastolic BP (mmHg)	1.01	0.98–1.04	0.62			NS
Skin autofluorescence (AU)	5.14	2.40–11.03	<0.01	3.74	1.54–9.14	<0.01
eGFR (mL/min/1.73 m^2)	0.96	0.94–0.98	<0.01			NS
Albumin (g/dL)	0.70	0.41–1.21	0.21			NS
Haemoglobin (g/dL)	0.83	0.67–1.03	0.09			NS
LDL cholesterol (mg/dL)	1.00	0.99–1.01	0.84			NS
HDL cholesterol (mg/dL)	0.97	0.93–1.00	0.06			NS

NS, not significant.

patients. This may reflect that decreased GFR has a greater contribution to AGE accumulation in non-diabetic patients than in diabetic patients.

ACEi and ARB reportedly reduce AGE formation [27], but medication with these agents had no significant correlation with skin autofluorescence in the present cross-sectional analysis. Evaluation of whether these drugs reduce AGE accumulation and whether skin autofluorescence represents a possible surrogate marker for the effects of these treatments in prospective investigations is both necessary and interesting.

Several limitations to the present study must be considered. First, skin-autofluorescence measurements are affected by skin colour and pigmentation and are not reliable for patients with very dark skin due to the high absorption grade of excited light [18,28,29]. The autofluorescence reader has not been sufficiently validated for non-Caucasian (Japanese) patients at present, but skin autofluorescence has been reported to be strongly correlated with AGE accumulation in evaluations assessed by skin biopsy specimens among Caucasian patients with diabetes and ESRD, despite the fact that hyperpigmentation is one of the frequent skin alterations in ESRD. Several recent studies have presented skin-autofluorescence results in Japanese patients with ESRD [22,30], rheumatoid arthritis, osteoarthritis and dialysis-related spondyloarthropathy [31] and cerebral infarction [32] and suggested that skin autofluorescence has potential as a useful marker in both Caucasian and non-Caucasian subjects. Second, the present study was only a cross-sectional analysis with insufficient size. A prospective investigation with sufficient sample size and better statistical methods is still needed to clarify whether skin autofluorescence is a relevant predictor for the progression of CVD and mortality in patients with CKD.

The importance of assessment for CKD-related (non-traditional) risk factors such as anaemia, malnutrition, inflammation, oxidative stress and AGE accumulation as well as traditional risk factors for CVD is higher in CKD patients. Early detection and intervention for these risks is necessary to prevent CVD. As a non-invasive, convenient instrument, the autofluorescence reader may have a potentially important role to play as a useful tool for assessing cardiovascular risk in daily practice among CKD patients. Early and close screening for CVD in patients with increased skin-autofluorescence value may have a potential to prevent CVD or improve mortality; however, further investigation is still necessary to closely examine whether skin autofluorescence is a relevant marker reflecting AGE accumulation for cardiovascular risk. Recently, some AGE breakers have been reported to inhibit the development of renal and vascular disease on experimental animals. Skin autofluorescence might offer a tool to monitor the effects of treatment as well, when these drugs apply in clinical practice in the future.

Tissue AGE measured as skin autofluorescence is independently related to renal function and CVD history in pre-dialysis CKD patients. Thus, non-invasive autofluorescence readers may have potential for providing useful biomarkers of cardiovascular risk in CKD patients, although prospective investigations are needed to evaluate whether skin autofluorescence predicts progression of cardiovascular disease or mortality and the therapeutic effectiveness.

Acknowledgements. The authors wish to thank the staff of Tani Hospital for their efforts in collecting and analysing serum samples and measuring skin autofluorescence.

Conflict of interest statement. None declared.

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