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 (β = 0.38, $P < 0.01$), diabetes (β = 0.24, $P = 0.01$), and dialysis duration (β = 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, $P < 0.01$).

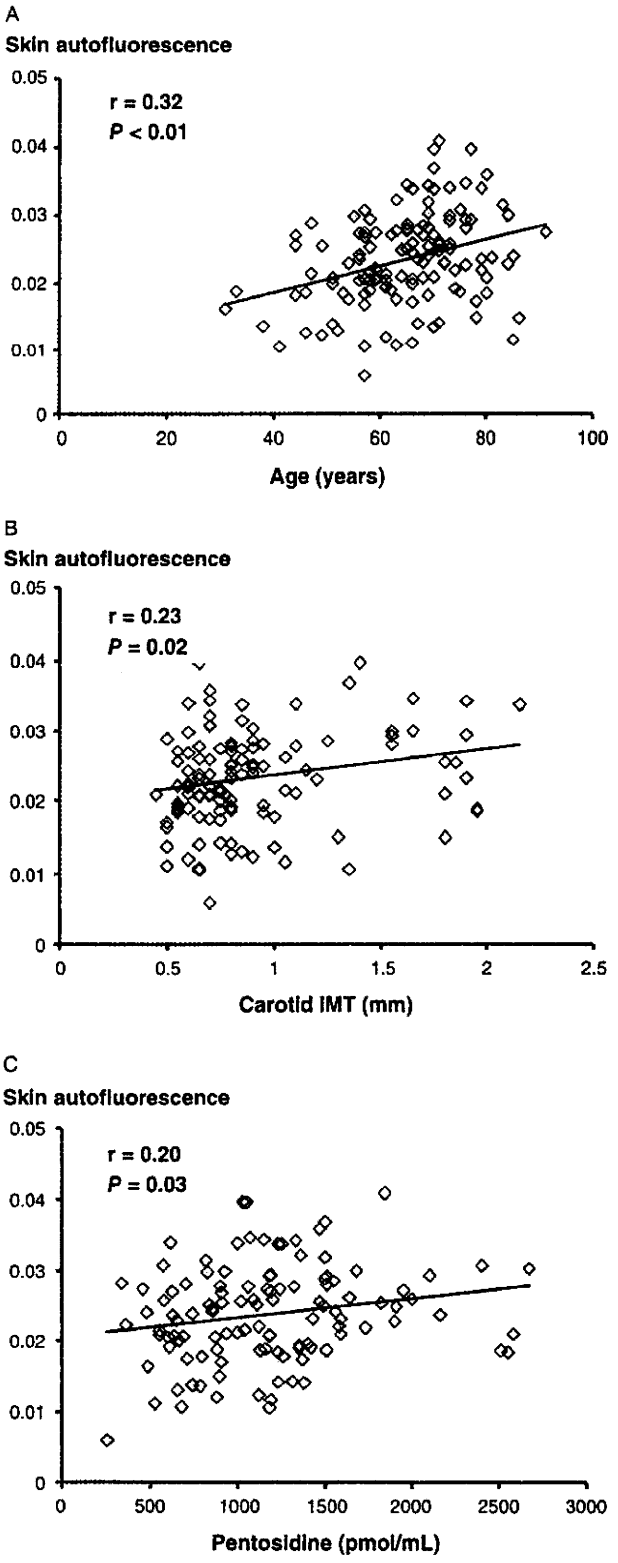

FIG. 1. Correlation between skin autofluorescence and (A) age, (B) carotid intima-media thickness (IMT), and (C) plasma pentosidine in hemodialysis patients.

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

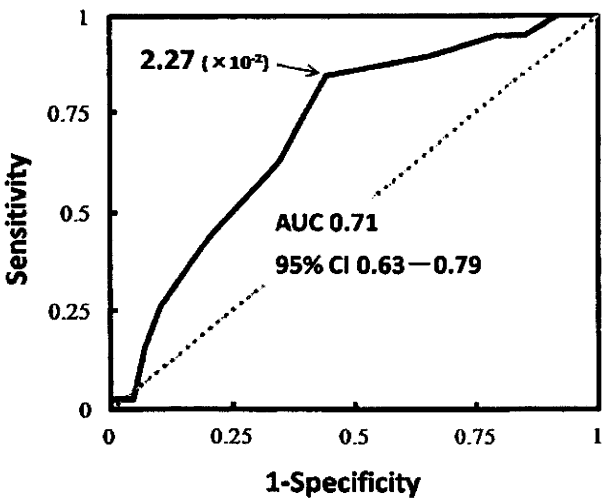

FIG. 2. Receiver operating characteristic curve for skin autofluorescence to discriminate cardiovascular disease.

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.

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REFERENCES

1. Miyata T, Ueda Y, Shinzato T et al. Accumulation of albumin-linked and free-form pentosidine in the circulation of uremic patients with end-stage renal failure: renal implications in the pathophysiology of pentosidine. *J Am Soc Nephrol* 1996;7: 1198–206.
2. Miyata T, Fu MX, Kurokawa K, van Ypersele de Strihou C, Thorpe SR, Baynes JW. Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: is there oxidative stress in uremia? *Kidney Int* 1998;54:1290–5.
3. Miyata T, Kurokawa K. Carbonyl stress: increased carbonyl modification of proteins by autoxidation products of carbohydrates and lipids in uremia. *Int J Artif Organs* 1999;22:195–8.
4. Sugiyama S, Miyata T, Ueda Y et al. Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. *J Am Soc Nephrol* 1998;9:1681–8.
5. Makita Z, Radoff S, Rayfield EJ et al. Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 1991;325:836–42.
6. Mathur S, Devaraj S, Jialal I. Accelerated atherosclerosis, dyslipidemia, and oxidative stress in end-stage renal disease. *Curr Opin Nephrol Hypertens* 2002;11:141–7.
7. Kanauchi M, Tsujimoto N, Hashimoto T. Advanced glycation end products in nondiabetic patients with coronary artery disease. *Diabetes Care* 2001;24:1620–3.
8. Monnier VM, Vishwanath V, Frank KE, Elmets CA, Dauchot P, Kohn RR. Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. *N Engl J Med* 1986;314:403–8.
9. Hricik DE, Wu YC, Schulak A, Fiedlander MA. Disparate changes in plasma and tissue pentosidine levels after kidney and kidney-pancreas transplantation. *Clin Transplant* 1996;10: 568–73.
10. Schwedler SB, Metzger T, Schinzel R, Wanner C. Advanced glycation end products and mortality in hemodialysis patients. *Kidney Int* 2002;62:301–10.
11. Busch M, Franke S, Muller A et al. Potential cardiovascular risk factors in chronic kidney disease: AGEs, total homocysteine and metabolites, and the C-reactive protein. *Kidney Int* 2004;66:338–47.
12. Meerwaldt R, Hartog J, Graaff R et al. Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005;16:3687–93.
13. Meerwaldt R, Graaff R, Oomen PH et al. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004;47:1324–30.
14. Ueno H, Koyama H, Tanaka S et al. Skin autofluorescence, a marker for advanced glycation end product accumulation, is associated with arterial stiffness in patients with end-stage renal disease. *Metabolism* 2008;57:1452–7.
15. Miyata T, van Ypersele de Strihou C, Ueda Y et al. Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in vitro the formation of advanced glycation end products: biochemical mechanisms. *J Am Soc Nephrol* 2002;13:2478–87.
16. Meerwaldt R, Lutgers H, Links T et al. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007;30:107–12.
17. Nishizawa Y, Shoji T, Maekawa K et al. Intima-media thickness of carotid artery predicts cardiovascular mortality in hemodialysis patients. *Am J Kidney Dis* 2003;41:S76–9.
18. Muntner P, Hamm LL, Kusek JW, Chen J, Whelton PK, He J. The prevalence of nontraditional risk factors for coronary heart disease in patients with chronic kidney disease. *Ann Intern Med* 2004;140:9–17.
19. Wanner C, Zimmermann J, Schwedler S, Metzger T. Inflammation and cardiovascular risk in dialysis patients. *Kidney Int* 2002;61:S99–102.
20. Lutgers HL, Graaff R, Links TP et al. Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. *Diabetes Care* 2006;29:2654–9.
21. Na R, Stender IM, Henriksen M, Wulf HC. Autofluorescence of human skin is age-related after correction for skin pigmentation and redness. *J Invert Dermatol* 2001;116:536–40.
22. Gerrits EG, Smit AJ, Bilo HJ. AGEs, autofluorescence and renal function. *Nephrol Dial Transplant* 2009;24:710–13.
23. Alderson NL, Chachich ME, Youssef NN et al. The AGE inhibitor pyridoxamine inhibits lipemia and development of renal and vascular disease in Zucker obese rats. *Kidney Int* 2003;63:2123–33.

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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×Serum creatinine$^{-1.094}$× 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

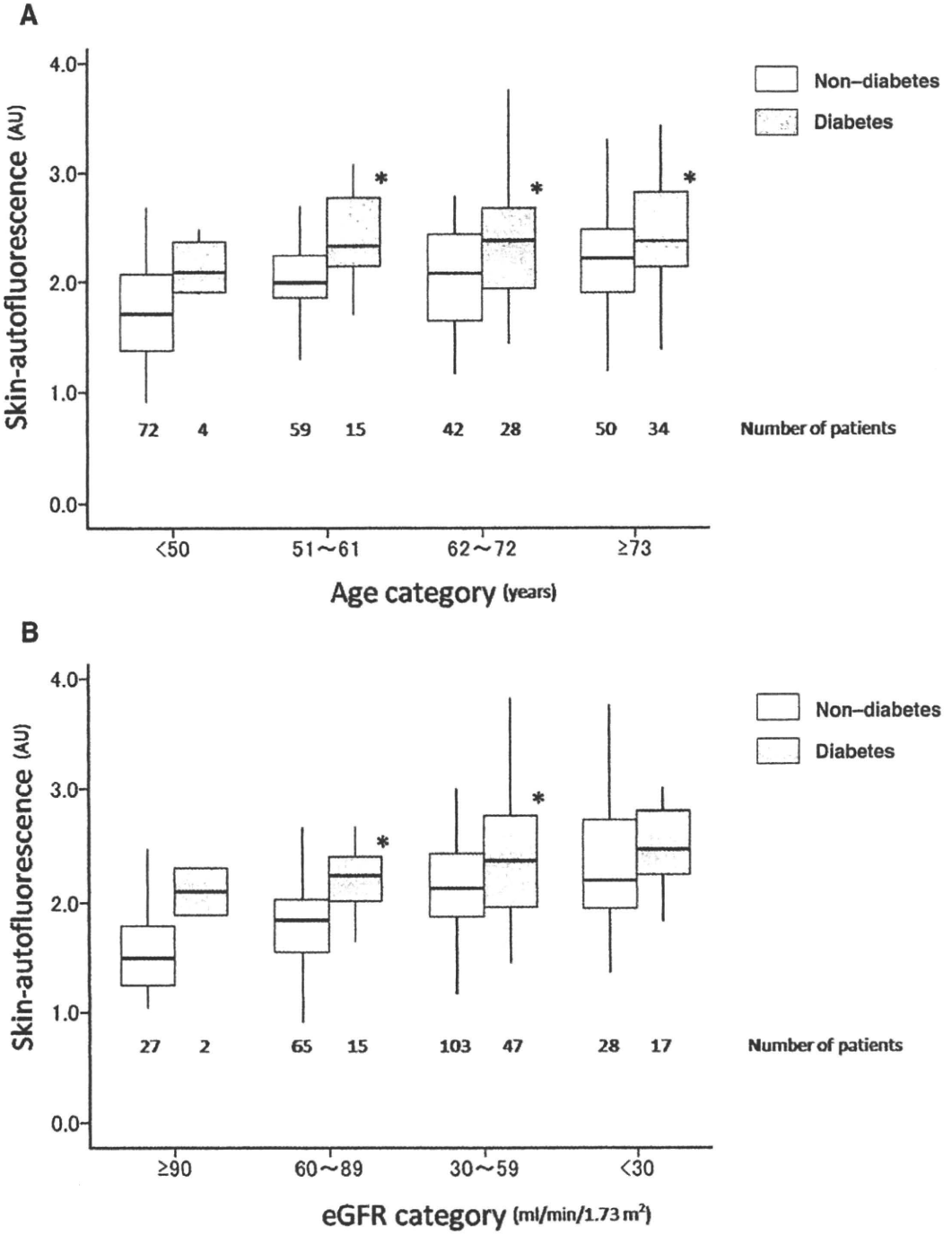

Fig. 1. These boxplots show the distribution of skin autofluorescence in each category of age (**A**) and eGFR (**B**) among chronic kidney disease patients with or without diabetes. Age category was divided by quartile of age. *P<0.05 vs non-diabetic patients.

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-

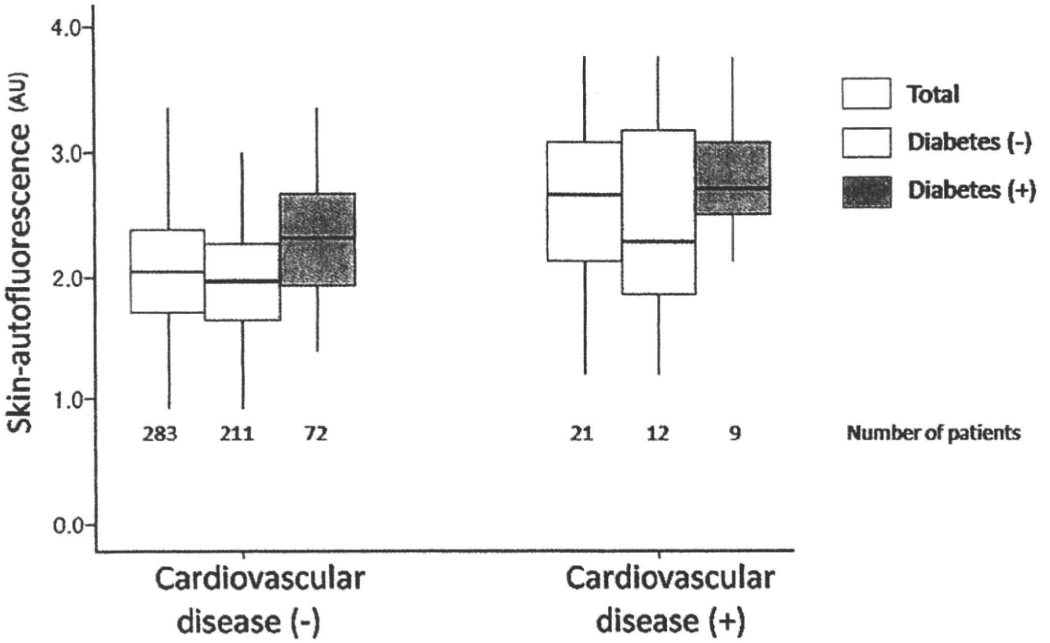

Fig. 2. Skin autofluorescence in patients with or without cardiovascular disease. Skin autofluorescence was significantly higher in patients with cardiovascular disease than in those without, for all chronic kidney disease patients ($P<0.01$), non-diabetic chronic kidney disease patients ($P<0.05$) and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

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Conflict of interest statement. None declared.

References

1. Foley RN, Parfrey PS, Sarnak MJ. Epidemiology of cardiovascular disease in chronic renal disease. *J Am Soc Nephrol* 1998; 9: S16–S23
2. Culleton BF, Larson MG, Wilson PWF *et al.* Cardiovascular disease and mortality in a community-based cohort with mild renal insufficiency. *Kidney Int* 1999; 56: 2214–2219
3. Go AS, Chertow GM, Fan D *et al.* Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; 351: 1296–1305
4. Sarnak MJ, Levey AS, Schoolwerth AC *et al.* Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. *Circulation* 2003; 108: 2154–2169
5. Keith DS, Nichols GA, Gullion CM *et al.* Longitudinal follow-up and outcomes among a population with chronic kidney disease in a large managed care organization. *Arch Intern Med* 2004; 164: 659–663
6. Longenecker JC, Coresh J, Powe NR *et al.* Traditional cardiovascular risk factors in dialysis patients compared with the general population; the CHOICE study. *J Am Soc Nephrol* 2002; 13: 1918–1927
7. Miyata T, Ueda Y, Shinzato T *et al.* Accumulation of albumin-linked and free-form pentosidine in the circulation of uremic patients with end-stage renal failure: renal implications in the pathophysiology of pentosidine. *J Am Soc Nephrol* 1996; 7: 1198–1206
8. Miyata T, Fu MX, Kurokawa K *et al.* Autoxidation products of both carbohydrates and lipids are increased in uremic plasma: is there oxidative stress in uremia? *Kidney Int* 1998; 54: 1290–1295
9. Miyata T, Kurokawa K. Carbonyl stress: increased carbonyl modification of proteins by autoxidation products of carbohydrates and lipids in uremia. *Int J Artif Organs* 1999; 22: 195–198
10. Sugiyama S, Miyata T, Ueda Y *et al.* Plasma levels of pentosidine in diabetic patients: an advanced glycation end product. *J Am Soc Nephrol* 1998; 9: 1681–1688
11. Makita Z, Radoff S, Rayfield EJ *et al.* Advanced glycosylation end products in patients with diabetic nephropathy. *N Engl J Med* 1991; 325: 836–842
12. Mathur S, Devaraj S, Jialal I. Accelerated atherosclerosis, dyslipidemia, and oxidative stress in end-stage renal disease. *Curr Opin Nephrol Hypertens* 2002; 11: 141–147
13. Kanauchi M, Tsujimoto N, Hashimoto T. Advanced glycation end products in nondiabetic patients with coronary artery disease. *Diab Care* 2001; 24: 1620–1623
14. Monnier VM, Vishwanath V, Frank KE *et al.* Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. *N Engl J Med* 1986; 314: 403–408
15. Hricik DE, Wu YC, Schulak A *et al.* Disparate changes in plasma and tissue pentosidine levels after kidney and kidney–pancreas transplantation. *Clin Transpl* 1996; 10: 568–573
16. Schwedler SB, Metzger T, Schinzel R *et al.* Advanced glycation end products and mortality in hemodialysis patients. *Kidney Int* 2002; 62: 301–310
17. Busch M, Franke S, Muller A *et al.* Potential cardiovascular risk factors in chronic kidney disease: AGEs, total homocysteine and metabolites, and the C-reactive protein. *Kidney Int* 2004; 66: 338–347
18. Meerwaldt R, Graaff R, Oomen PH *et al.* Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004; 47: 1324–1330

19. Meerwaldt R, Links T, Graaff R *et al.* Simple noninvasive measurement of skin autofluorescence. *Ann N Y Acad Sci* 2005; 1043: 290–298
20. Meerwaldt R, Hartog J, Graaff R *et al.* Skin autofluorescence, a measure of cumulative metabolic stress and advanced glycation end products, predicts mortality in hemodialysis patients. *J Am Soc Nephrol* 2005; 16: 3687–3693
21. Meerwaldt R, Lutgers HL, Links TP *et al.* Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diab Care* 2007; 30: 107–112
22. Tanaka K, Katoh T, Asai J *et al.* Relationship of skin-autofluorescence to cardiovascular disease in Japanese hemodialysis patients. *Ther Apher Dial* 2010; 14: 334–340
23. Matsuo S, Imai E, Horio M *et al.* Revised equations for e GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; 53: 982–992
24. National Kidney Foundation. K/DOQI clinical practice guideline for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; 39: S1–S266
25. Lutgers HL, Graaff R, Links TP *et al.* Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes. *Diab Care* 2006; 29: 2654–2659
26. Gerrits EG, Lutgers HL, Kleefstra N *et al.* Skin autofluorescence: a tool to identify type 2 diabetic patients at risk for developing microvascular complications. *Diab Care* 2008; 31: 517–521
27. Miyata T, van Ypersele de Strihou C, Ueda Y *et al.* Angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors lower in vitro the formation of advanced glycation end products: biochemical mechanisms. *J Am Soc Nephrol* 2002; 13: 2478–2487
28. Gerrits EG, Smit AJ, Bilo HJ. AGEs, autofluorescence and renal function. *Nephrol Dial Transplant* 2009; 24: 710–713
29. Na R, Stender IM, Henriksen M *et al.* Autofluorescence of human skin is age-related after correction for skin pigmentation and redness. *J Invert Dermatol* 2001; 116: 536–540
30. Ueno H, Koyama H, Tanaka S *et al.* Skin autofluorescence, a marker for advanced glycation end product accumulation, is associated with arterial stiffness in patients with end-stage renal disease. *Metabolism* 2008; 57: 1452–1457
31. Matsumoto T, Tsurumoto T, Baba H *et al.* Measurement of advanced glycation endproducts in skin of patients with rheumatoid arthritis, osteoarthritis, and dialysis-related spondyloarthropathy using non-invasive methods. *Rheumatol Int* 2007; 28: 157–160
32. Ohnuki Y, Nagano R, Takizawa S *et al.* Advanced glycation end products in patients with cerebral infarction. *Inter Med* 2009; 48: 587–501

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Preparing renal replacement therapy in stage 4 CKD patients referred to nephrologists: a difficult balance between futility and insufficiency. A cohort study of 386 patients followed in Brussels

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Abstract

Background. KDOQI guidelines recommend preparation for renal replacement therapy (RRT) once stage 4 chronic kidney disease (CKD) is reached. Recent studies conducted in the general population and in patients referred to nephrologists have shown that CKD patients, especially the elderly, are much more likely to die than to reach RRT. We investigated whether futile preparation for RRT was performed in CKD patients referred to our nephrology department.

Methods. We included all patients (n = 386) with stage 4 CKD and without prior RRT, seen at our outpatient clinic between 1 November 2004 and 30 April 2007. Demographics, clinical and laboratory data at inclusion were collected. Follow-up continued until 1 November 2007 or later (last appointment or study outcome). The primary outcome was death without requiring RRT, and secondary outcomes were RRT, going through our pre-dialysis education programme (PDEP) and undergoing the creation of an arterio-venous fistula (AVF). Factors predicting these outcomes were analysed.

Results. During complete follow-up (average 23.4 months), 47 patients (12.1%) died without requiring RRT and 59 patients (15.3%) started RRT. The rate of death without requiring RRT in the overall cohort increased from 50 years onwards and exceeded that of RRT in incident patients aged ≥80 years. A structured PDEP was offered to 66.1% of patients starting RRT vs 14.9% of patients dying without requiring RRT and 13.9% of patients surviving without requiring RRT ($P < 0.001$). In addition, 53.3% of patients starting haemodialysis had a prior AVF creation vs 6.4% of patients

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ORIGINAL ARTICLE

Genetical, histological, and clinical characteristics of IgA-negative mesangioproliferative glomerulopathy

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Abstract
Background Mesangioproliferative glomerulopathy (MesPGN) is a well-defined pathohistological entity. However, the clinical characteristics and prognosis have not been fully established in patients without immunoglobulin (Ig)A (N-IgAN) in contrast to patients with IgA nephropathy (IgAN).
Methods A total of 837 consecutive patients underwent renal biopsies. Among them, 465 patients were diagnosed with MesPGN by light microscopy. With immunofluorescent study and electron microscopy (EM), 344 were diagnosed as having IgAN. Among the rest, 84 patients who had no immunofluorescence evidence of IgA and no deposits in EM were defined as N-IgAN. We compared the clinical characteristics, histological findings, and genotypes of the angiotensin-converting enzyme (ACE) gene and plasminogen activator inhibitor-1 gene between IgAN and N-IgAN patients.
Results Urinary protein excretion and the degree of hematuria were significantly lower in N-IgAN than IgAN patients (0.50 vs. 0.82 g/day; $P = 0.01$), (1.33 vs. 2.50; $P < 0.001$, respectively). Creatinine clearance was higher in N-IgAN than IgAN patients (89.4 vs. 74.4 ml/min; $P < 0.001$). Histopathologically, N-IgAN patients had significantly less advanced glomerular and tubulointerstitial lesions than IgAN patients. Pathological grades in patients with untreated IgAN were more advanced in a time-dependent manner, whereas there was no relationship between histological grades and time of illness in N-IgAN patients. Frequency of the DD genotype of the ACE gene was significantly lower in N-IgAN (DD/ID+II = 8/76) than IgAN (24/90) patients.
Conclusions IgA-negative MesPGN is a distinct type of glomerulopathy with a benign renal prognosis. Insertion/deletion polymorphisms of the ACE gene may play some role in the genesis and progression of MesPGN.

Keywords ACE polymorphism · IgA nephropathy · Mesangioproliferative glomerulopathy

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Introduction

Mesangioproliferative glomerulopathy (MesPGN) is a well-defined pathohistological entity with increased cell number and extracellular matrix in the glomerular mesangium [1–3]. Patients with immunodeposits containing immunoglobulin (Ig)A in the mesangium are denoted as having IgA nephropathy (IgAN), which is recognized as the most common form of primary glomerulopathy worldwide, particularly in southern Europe and eastern Asia. IgAN is a heterogeneous disease, with 30–40% of IgAN patients developing end-stage renal disease (ESRD) in a 20-year observation period [3–5]. In contrast, for patients with MesPGN and without IgA deposition (N-IgAN), there are few reports describing its clinical characteristics and prognosis [1]. It is still uncertain whether it may be a unique disease entity or whether it is a histological "trash box" for diverse pathologic conditions, with only clinically present asymptomatic proteinuria and/or hematuria.

It has been suggested that genetic factors may influence the pathogenesis and prognosis of renal glomerular

diseases [6–12]. Insertion/deletion (I/D) polymorphisms of angiotensin-converting enzymes (ACE) have been demonstrated to be significantly associated with the incidence and prognosis of various cardiovascular disorders [13–15] and progression of glomerular disease. An I/D polymorphism of the ACE gene plays an important role in the progression of IgAN [9–11]; however, its exact role is still controversial [16–19]. It also remains to be elucidated whether I/D polymorphisms of the ACE gene may affect the pathogenesis and progression of N-IgAN. We previously reported that the 4G/5G polymorphism of the plasminogen activator inhibitor-1 (PAI-1) gene is associated with IgAN progression [12], whereas its role in N-IgAN has not been reported. In this study, we investigated the clinical characteristics, renal pathological findings, and genotypes of the ACE and PAI-1 genes patients with N-IgAN in comparison with those with IgAN.

Methods

Patients and definition of non-IgA glomerulopathy (N-IgAN)

Renal biopsies were performed at the Third Department of Medicine, Fukushima Medical University, for patients with proteinuria ≥2+ by dipstick urine test or with both proteinuria (≥1+) and hematuria or with hematuria (≥1+) for more than 1 year without urological abnormalities. We defined MesPGN as being four or more cells per mesangial area. At least 80% of glomeruli should be involved [20, 21], with a mesangial matrix index (MMI) >7% of glomeruli. We regard an MMI of <7% as minor glomerular abnormality and distinguished such cases from N-IgAN. Among 961 consecutive renal biopsy specimens acquired between January 1999 and October 2005, 465 patients were diagnosed with mesangioproliferative glomerulitis by light microscopy according to the criteria. Based on immunofluorescent and electron microscopy (EM) analyses, 344 of these patients were diagnosed with IgAN and 23 with purpura nephritis. In addition, 84 patients whose specimens showed no IgA under immunofluorescence examination and no dense deposits detected by EM were diagnosed with N-IgAN [1–3]. A total of 14 patients whose specimens showed dense deposits by EM but not upon immunofluorescence were excluded from the study. Of the 344 IgAN patients, 114 patients who were consecutively diagnosed with N-IgAN through January 1999 to December 2000 were examined to make the number of each group comparable.

The grade of microscopic hematuria was defined with a high power field (HPF) and was rated as 0 [1–4 red blood cells (RBC)/HPF), 1 (5–9 RBC/HPF), 2 (10–29 RBC/HPF), 3 (30–50 RBC/HPF), 4 (50–100 RBC/HPF), or 5 (>100 RBC/HPF). Blood pressure level was defined as the mean level of three consecutive measurements on different days during admission at our hospital. Mean urinary protein excretion was determined for 3 days.

Genetic analysis of ACE polymorphism

DNA was isolated from peripheral blood leukocytes using a commercial kit (BDtract™, Maxim Biotech, Inc., USA) and was used for polymerase chain reaction (PCR). Two primers were designed to flank the polymorphic region of the ACE gene. The sense oligonucleotide primer was 5′-CTGGAGACCACTCCCATCCTTTCT-3′ and the antisense primer was 5′-GATGTGGCCATCACATTCGAC AGAT-3′. For the amplification reaction, 80 ng genomic DNA was used in a final volume 50 μl containing 3 mM magnesium chloride ($MgCl_2$), 50 mM potassium chloride (KCl), 10 mM Tris–HCl (pH 8.4), 10 pmol of each primer, 0.2 mM of each deoxynucleotide triphosphate (dNTP), and 1 U Taq polymerase (Takara, Tokyo, Japan). DNA was amplified using a DNA thermal cycler (Takara, Tokyo, Japan) with 1-min denaturation at 94°C, 1-min annealing at 55°C, and 2-min extension at 72°C for 30 cycles. In the last cycle, the extension step was carried out for 10 min. PRC products were separated on 1.5% agarose gels and visualized by ethidium bromide staining.

Genetic analysis of PAI-1 4G/5G polymorphism

We also analyzed the genotype of the 4G/5G polymorphism with a method combining rapid-cycle PCR with real-time monitoring of the amplification process and the generation of allele-specific fluorescent probe melting profiles on a LightCycler™ (Roche, Basel, Switzerland) [22]. The primers 5′-AGCCAGACAAGGTTGTTGACA C-3′ and 5′-CAGAGGACTCTTGGTCTTTCCC-3′ were used to amplify, respectively, a 134- or 135-bp fragment of the PAI-1 gene (GenBank accession no. X13323). The detection probe was an 18-mer oligonucleotide labeled at the 3′-end with fluorescein. The sequence 5′-TGACT CCCCCACGTGTCCT-3′ is complementary to the leading strand of the 5G allele. The anchor probe (5′-AC TCTCTCTGTGCCCCTGAGGGCTCT-3′) was a 26-mer labeled with LightCycler Red 640 at its 5′-end and modified at the 3′-end by phosphorylation to block extension. PCR was performed by rapid cycling in a reaction volume of 10 μl with 0.3 μM of each primer, 0.2-μM anchor and detection probes, and 50 ng genomic DNA. After an initial denaturation step at 94°C for 45 s, amplification was performed using 50 cycles of denaturation (94°C for 0 s), annealing (57°C for 5 s), and extension (72°C for 2 s).

Histopathological analysis

Lesions detected by light microscopy in MesPGN patients were classified into four glomerular grades and three tubulointerstitial grades described below.

G0: Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule is not observed.

G1: Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule seen in <10% of all biopsied glomeruli.

G2: Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule seen in 10–30% of all biopsied glomeruli.

G3: Glomerulosclerosis, crescent formation, or adhesion to Bowman's capsule seen in >30% of all biopsied glomeruli. When sites of sclerosis are totaled and converted to global sclerosis, the sclerosis rate is >50% of all glomeruli. Some glomeruli also show compensatory hypertrophy.

T1: Prominent changes are not seen in the interstitium, renal tubuli, or blood vessels.

T2: Cellular infiltration is slight in the interstitium except around some sclerosed glomeruli. Tubular atrophy is slight, and mild vascular sclerosis is observed.

T3: Interstitial cellular infiltration and tubular atrophy, as well as fibrosis, are seen. Hyperplasia or degeneration is seen in some intrarenal arteriolar walls.

The degree of glomerular matrix accumulation was examined by imaging analysis consisting of the following steps, as described previously [23]: (1) capturing glomeruli on the periodic acid Schiff (PAS) preparation at a magnitude of 200×, (2) tracing the outline of the glomeruli to obtain the whole glomerular area, (3) selecting the PAS-positive area manually with the mouse pointer. Finally, MMI was calculated from the ratio of the PAS-positive area to glomerular area measured as above. The mean of each glomerular MMI in the specimens was regarded as representing the magnitude of matrix accumulation in each case. Control values of MMI were obtained from needle biopsy specimens from patients with minimal-change nephrotic syndrome (MCD) ($n = 39$).

All renal biopsy samples were examined independently by a researcher who was not provided with any clinical information about the patients. The study was approved by the research ethics committee at Fukushima Medical University.

Statistical analysis

All data are presented as mean ± standard deviation (SD). As nonnormal distributions or inequality of variances was present in some variables, nonparametric analysis was performed. Statistical comparisons were performed using Mann–Whitney's U test and chi-square test for independence. Values of $P < 0.05$ were regarded as statistically significant. These calculations were performed with StatView, Ver. 5.0 (Abacus Concepts, Berkeley, CA, USA).

Results

Clinical characteristics of patients with N-IgAN and those with IgAN are shown in Table 1. Urinary protein excretion (0.50 ± 0.66 vs. 0.82 ± 1.26 g/day; $P = 0.01$, respectively) and degree of hematuria (1.33 ± 1.60 vs. 2.50 ± 1.57; $P < 0.001$, respectively) were significantly lower in patients with N-IgAN than in those with IgAN. Creatinine clearance (Ccr) was higher in N-IgAN in IgAN patients (89.4 ± 29.7 vs. 74.4 ± 25.1 ml/min; $P = 0.001$, respectively). Serum concentration of IgA was lower in N-IgAN than IgAN patients (236 ± 107 vs. 404 ± 157 mg/dl; $P < 0.001$, respectively). Age at the time of renal biopsy (40.92 ± 16.00 vs. 36.67 ± 14.94 years old, respectively), gender (male/female 50/34 vs. 59/55, respectively), duration of hematuria and/or proteinuria (7.38 ± 7.86 vs. 7.13 ± 7.58 years, respectively), serum creatinine (0.89 ± 0.32 vs. 1.01 ± 0.58 mg/dl, respectively), and systolic blood pressure (128.02 ± 17.59 vs. 123.42 ± 15.30 mmHg, respectively) did not differ.

The degree of mesangial matrix accumulation measured by MMI was increased by rank of order as in patients with IgAN, N-IgAN, and controls (MCD group) (13.65 ± 5.01, 9.83 ± 3.37, and 3.99 ± 1.57%, respectively $P < 0.001$) (Fig. 1). As shown in Fig. 2a and b, glomerular and tubulointerstitial changes were significantly milder in N-IgAN than IgAN patients (G0, G1, G2, and G3 were 33, 28, 14, and 9; and 18, 20, 44, and 32, respectively; T1, T2, and T3 were 58, 14, and 12; and 39, 46, and 29, respectively), as estimated by chi-square test. There were no obvious differences in vascular changes, although we did no quantitative evaluation.

To examine the time dependency of histological findings, glomerular and tubulointerstitial grades as well as other clinical characteristics in patients with ≥6 years between onset of proteinuria and renal biopsy (longer duration) were compared with those with an interval <6 years (shorter duration) (Tables 2 and 3). IgAN of shorter duration had significantly milder glomerular findings (G0:G1:G2:G3 14:15:25:14 vs. 4:5:19:18, respectively, $P = 0.043$) (Table 2, and Fig. 3a), and creatinine was lower (0.95 ± 0.50 vs. 1.11 ± 0.66 mg/dl, respectively; $P = 0.0188$) and higher (80.53 ± 23.48 vs. 65.31 ± 24.58 ml/min, respectively; $P = 0.0008$) (Table 2). Disease duration did not affect tubulointerstitial findings in

Table 1 Clinical characteristics of patients with mesangioproliferative glomerulopathy without immunoglobulin (Ig)A deposition (N-IgAN) and with IgA nephropathy (IgAN)

	N-IgAN ($n = 84$)	IgAN ($n = 114$)	P value
Age (year)	40.92 ± 16.00	36.67 ± 14.94	0.48
Male:female	50:34	59:55	0.35
Interval from the onset (year)	7.38 ± 7.86	7.13 ± 7.58	0.93
BP (S) mmHg	128.02 ± 17.59	123.42 ± 15.30	0.10
BP (D) mmHg	76.45 ± 11.92	73.55 ± 11.45	0.02
Urinary protein excretion (g/day)	0.50 ± 0.66	0.821 ± 1.26	0.01
Hematuria score	1.33 ± 1.60	2.50 ± 1.57	<0.001
Crea (mg/dl)	0.89 ± 0.32	1.01 ± 0.58	0.07
Ccr (ml/min)	89.35 ± 29.74	74.39 ± 25.08	0.01
IgA (mg/dl)	236 ± 107	404 ± 157	<0.001
G grade (G0:G1:G2:G3)	33:28:14:9	18:20:44:32	<0.001
G grade score	0.99 ± 1.00	1.78 ± 1.03	<0.001
T grade (T1:T2:T3)	58:14:12	39:46:29	<0.001
T grade score	1.45 ± 0.74	1.87 ± 0.64	<0.001
MMI (%)	9.83 ± 3.37	13.65 ± 5.01	<0.001
ACE polymorphism (DD:ID:II)	8:41:35	24:48:42	0.03
PAI-1 polymorphism (4G4G:4G5G:5G5G)	15:36:9	40:54:20	0.38

Serum creatinine in mg/dl may be converted to μmol/l by multiplying by 88.4

BP (S) systolic blood pressure, *BP (D)* diastolic blood pressure, *Crea* creatinine, *Ccr* creatinine clearance, *MMI* mesangial matrix index, *ACE* angiotensin-converting enzyme, *PAI-1* plasminogen activator inhibitor-1

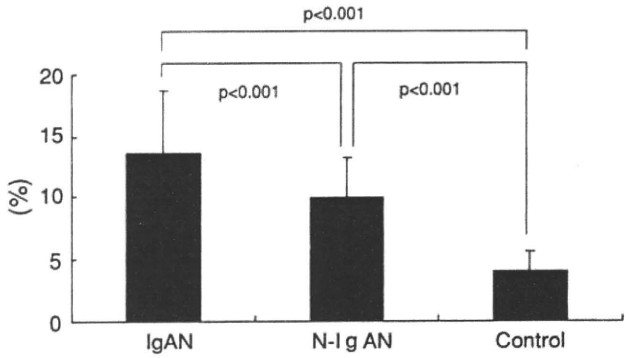

Fig. 1 Degree of mesangial matrix accumulation measured by mesangial matrix index (*MMI*) is significantly increased in patients with immunoglobulin (Ig)A nephropathy (*IgAN*) compared with patients with mesangioproliferative glomerulopathy without IgA deposition (*N-IgAN*). MMI in N-IgAN patients is significantly increased compared with the control group

either group (T1:T2:T3 24:35:9 in shorter duration vs. 8:31:7 in longer duration, respectively, $P = 0.11$) (Table 2). On the other hand, in patients with N-IgAN, there was no difference in the histopathology between those with shorter duration and longer duration (G0:G1:G2:G3 23:11:7:4 vs. 10:17:7:5, respectively, and T1:T2:T3 33:7:5 vs. 25:7:7, respectively) (Table 3; Fig. 3b), suggesting a less progressive nature of N-IgAN compared with IgAN.

Figure 4a demonstrates that the frequency of the DD genotype was significantly lower in patients with N-IgAN (DD:ID+II 8:76) than those with IgAN (DD:ID+II 24:90, $P = 0.0294$) or healthy control volunteers (DD:ID+II 53:217, $P = 0.033$), whereas the PAI-1 gene allele frequency of 4G4G was similar in N-IgAN and IgAN patients and healthy control volunteers (Fig. 4b).

Patients with N-IgAN were followed up for 3.25 ± 1.91 (range 0.8–7) years, having taken essentially no medications. Their renal function did not deteriorate (from 0.89 ± 0.32 to 0.93 ± 0.71 mg/dl, $P = 0.073$), whereas those with IgAN decreased from 1.01 ± 0.58 to 1.17 ± 0.69 mg/dl, $P = 0.134$) with treatment by steroid, ACE-inhibitor, and/or angiotensin receptor blocker (ARB). Data indicate that there were few changes in renal function in patients with N-IgAN without any specific treatment.

Discussion

Mesangioproliferative glomerulonephritis is the most common form of primary glomerulopathies. Among them, IgAN is the most common and well-characterized. In contrast, the clinical course and pathophysiology of N-IgAN has not been well described. This study demonstrated that MesPGN without IgA deposition might take a benign course compared with MesPGN with IgAN (Tables 2, 3; Figs. 2a, b, 3a, b). The clinical and histological characteristics were significantly and clearly different between patients with IgAN and those with N-IgAN (Table 1; Fig. 2a, b), suggesting that the basic pathological processes in N-IgAN are independent of those of IgAN.

In the genotyping assay, the low frequency of the DD genotype in patients wtih N-IgAN may indicate that the pathogenesis of N-IgAN is distinguished from IgAN (Fig. 4a). The genotype of ACE in IgAN patients as well

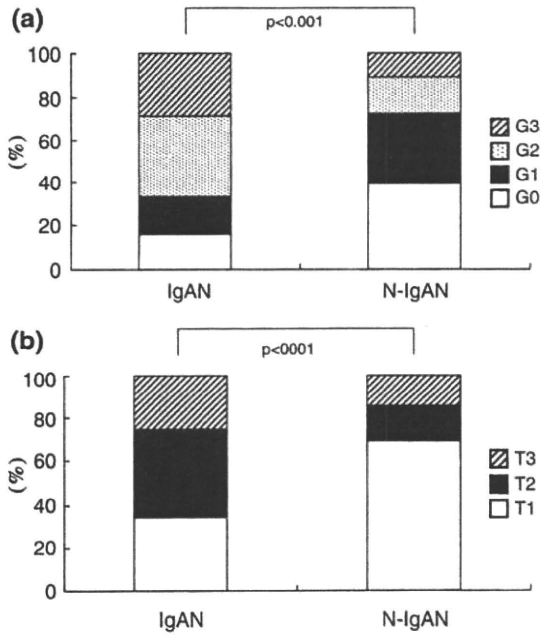

Fig. 2 **a** Degree of pathological glomerular changes are significantly milder in patients with mesangioproliferative glomerulopathy without immunoglobulin (Ig)A deposition (*N-IgAN*) than those with IgA nephropathy (*IgAN*) by chi-square test. As indicated, the ratio of G0+G1 is 72.6% in N-IgAN patients and 33.3% in those with IgAN. **b** Degree of pathological tubulointerstitial changes are significantly milder in patients with mesangioproliferative glomerulopathy without IgA deposition (*N-IgAN*) than those with IgA nephropathy (*IgAN*) by chi-square test. As indicated, the ratio of T1 is 69.0% in N-IgAN patients and 34.2% in IgAN patients

as normal controls, of which samples were examined in parallel with those of patients with N-IgAN, were similar with the average frequency in the Japanese population [9, 24, 25], which indicates that technical bias was unlikely. Moreover, the genotypes of PAI-1, which we previously demonstrated [12] to be associated with the progression of histological changes and a decrease in kidney function, are similar in N-IgAN and IgAN patients, thus suggesting a unique genotypic feature of ACE in N-IgAN patients. The sample size of N-IgAN patients was adequate for statistical analysis of gene polymorphisms of ACE, also considering its distribution in the Japanese population.

Patients with the DD genotype are supposed to have higher local angiotensin II concentrations, and angiotensin II promotes pathological glomerular processes [26]. The small number of DD genotypes in N-IgAN patients may indicate that N-IgAN is a less advanced subgroup of this particular disorder. It could be speculated that N-IgAN is a less pathogenic subtype of IgAN, because angiotensin II has been demonstrated to stimulate mesangial uptake of immune complexes, possibly resulting in IgA accumulation in mesangial cells [27]. However, the frequency of the DD genotype in IgAN patients was not increased compared with the control population in this study, a result reported by others [16, 18]. The influence of the low frequency of the DD genotype in N-IgAN patients should be further investigated.

In conclusion, these findings suggest that N-IgAN is a distinct type of primary glomerular disorder and is clearly distinguished from IgAN by clinical, histological and genetic characteristics, although there should be several limitations because of the relatively small number of patients in this study. The I/D polymorphism of the ACE gene may play some role in the genesis and progression of MesPGN.

Table 2 Comparison of clinical characteristics in patients with ≥6 years between onset of proteinuria and renal biopsy and in those with an interval of <6 years in immunoglobulin (IgA) nephropathy (IgAN)

IgAN	<6 years (n = 68)	≥6 years (n = 46)	P value
Age (year)	35.25 ± 14.99	45.15 ± 13.63	<0.001
Male:female	35:33	24:22	0.9
Interval from onset (year)	2.07 ± 1.55	14.33 ± 6.85	<0.001
BP (S) mmHg	121.68 ± 15.06	126.84 ± 16.33	0.13
BP (D) mmHg	71.52 ± 10.63	76.94 ± 12.19	0.02
Urinary protein excretion (g/day)	0.85 ± 0.66	0.77 ± 1.29	0.68
Hematuria score	2.68 ± 1.43	2.24 ± 1.73	0.15
Crea (mg/dl)	0.95 ± 0.50	1.11 ± 0.66	0.02
Ccr (ml/min)	80.53 ± 23.48	65.31 ± 24.58	<0.001
G grade (G0:G1:G2:G3)	14:15:25:14	4:5:19:18	0.04
G grade score	1.57 ± 1.04	2.11 ± 0.92	0.01
T grade (T1:T2:T3)	24:35:9	8:31:7	0.10
T grade score	1.78 ± 0.67	1.98 ± 0.58	0.09
MMI (%)	13.3 ± 5.1	14.4 ± 4.8	0.21
ACE polymorphism (DD:ID:II)	15:25:28	9:22:15	0.49
PAI-1 polymorphism (4G4G:4G5G:5G5G)	29:30:9	11:24:11	0.08

Serum creatinine in mg/dl may be converted to μmol/l by multiplying by 88.4

BP (S) systolic blood pressure, *BP (D)* diastolic blood pressure, *Crea* creatinine, *Ccr* creatinine clearance, *MMI* mesangial matrix index, *ACE* angiotensin converting enzyme, *PAI-1* plasminogen activator inhibitor-1

Table 3 Comparison of clinical characteristics in patients with ≥6 years between onset of proteinuria and renal biopsy and in those with an interval <6 years in mesangioproliferative glomerulopathy without immunoglobulin (Ig)A deposition (N-IgAN)

N-IgAN	<6 years (n = 45)	≥6 years (n = 39)	P value
Age (year)	37.58 ± 18.56	44.769 ± 10.21	0.05
Male:female	26:19	24:15	0.90
Interval from the onset (year)	1.70 ± 1.62	13.92 ± 7.05	<0.001
BP (S) mmHg	126.44 ± 19.87	129.85 ± 14.58	0.15
BP (D) mmHg	73.38 ± 12.13	80.00 ± 10.77	0.04
Urinary protein excretion (g/day)	0.46 ± 0.69	0.55 ± 0.61	0.18
Hematuria score	1.51 ± 1.83	1.13 ± 1.28	0.60
Crea (mg/dl)	0.86 ± 0.28	0.92 ± 0.35	0.40
Ccr (ml/min)	92.79 ± 32.72	85.38 ± 25.72	0.27
G grade (G0:G1:G2:G3)	23:11:7:4	10:17:7:5	0.10
G grade score	0.82 ± 1.00	1.18 ± 0.97	0.06
T grade (T1:T2:T3)	33:7:5	25:7:7	0.60
T grade score	1.38 ± 0.68	1.54 ± 0.79	0.33
MMI (%)	9.30 ± 3.02	10.45 ± 3.67	0.17
ACE polymorphism (DD:ID:II)	5:19:21	3:22:14	0.43
PAI-1 polymorphism (4G4G:4G5G:5G5G)	19:20:6	20:16:3	0.59

Serum creatinine in mg/dl may be converted to μmol/l by multiplying by 88.4

BP (S) systolic blood pressure, *BP (D)* diastolic blood pressure, *Crea* creatinine, *Ccr* creatinine clearance, *MMI* mesangial matrix index, *ACE* angiotensin converting enzyme, *PAI-1* plasminogen activator inhibitor-1

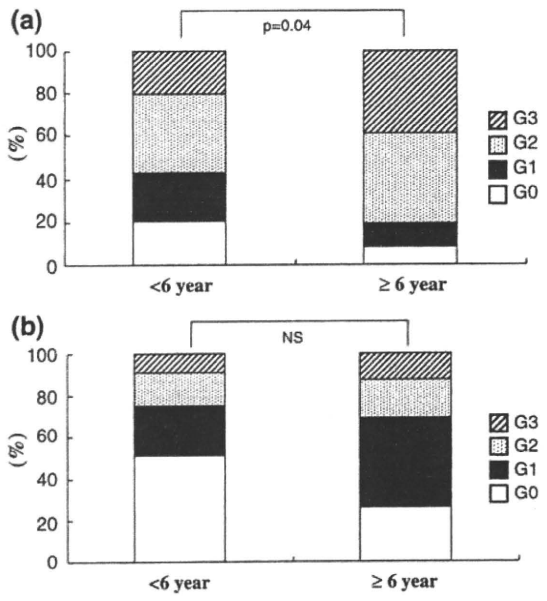

Fig. 3 **a** Degree of pathological glomerular changes are significantly and severely advanced when examined by chi-square test in immunoglobulin (Ig)A nephropathy (*IgAN*) in a time-dependent manner. The ratio of G2+G3 is 80.4% in patients for whom the interval between the onset proteinuria and renal biopsy was ≥6 years. On the other hand, the ratio of G2+G3 was 57.4% in patients with an interval <6 years. **b** Degree of pathological glomerular change is not statistically different according to chi-square test in patients with intervals between the onset of proteinuria and renal biopsy >6 years of mesangioproliferative glomerulopathy without immunoglobulin (Ig)A deposition (N-IgAN) compared with those whose intervals were <6 years. Ratio of G2+G3 was 30.8% in patients with an interval of >6 years. On the other hand, the ratio of G2+G3 was 24.4% in patients with an interval of <6 years

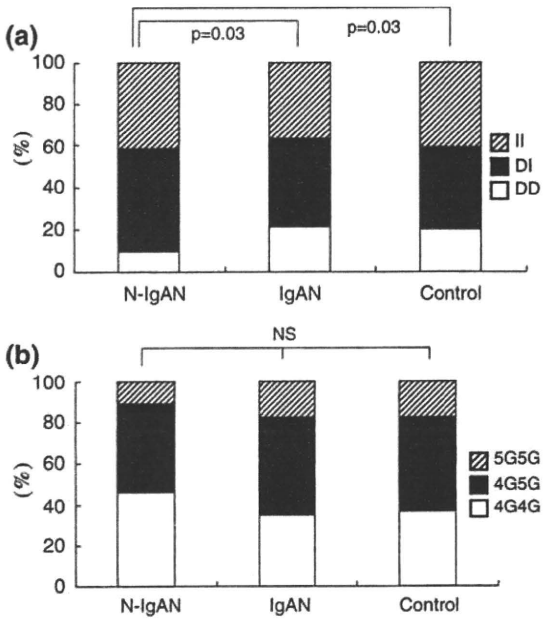

Fig. 4 **a** Frequency of the DD genotype of angiotensin-converting enzyme (*ACE*) was significantly lower in patients with mesangioproliferative glomerulopathy without immunoglobulin (Ig)A deposition (*N-IgAN*) than in those with IgAN or healthy control volunteers. **b** Plasminogen activator inhibitor-1 (PAI-1) gene allele frequency of 4G4G, 4G5G, and 5G5G is similar in patients with mesangioproliferative glomerulopathy without IgA deposition (*N-IgAN*), with IgA nephropathy (*IgAN*), and healthy control volunteers

References

1. Bohle A, Wehrmann M, Bogenschutz O, Batz C, Vogi W, Schmitt H, et al. The long-term prognosis of the primary

glomerulonephritis. A morphological and clinical analysis. Pathol Res Pract. 1992;188:908–24.
2. Kobayashi Y, Tateno S, Hiki Y, Shigematsu H. IgA nephropathy: prognostic significance of proteinuria and histological alterations. Nephron. 1983;34:146–53.
3. Chida Y, Tomura S, Takeuchi J. Renal survival rate of IgA nephropathy. Nephron. 1985;40:189–94.
4. Schena FP. A retrospective analysis of the natural history of primary IgA nephropathy worldwide. Am J Med. 1990;89:209–15.
5. D'Amico G. Influence of clinical and histological features on actuarial renal survival in adult patients with idiopathic IgA nephropathy, membranous nephropathy and membranoproliferative glomerulonephritis: survey of the recent literature. Am J Kidney Dis. 1992;20:315–23.
6. Lee DY, Kim W, Kang SK, Koh GY, Park SK. Angiotensin-converting enzyme gene polymorphism in patients with minimal-change nephrotic syndrome and focal segmental glomerulosclerosis. Nephron. 1997;77:471–3.
7. Gaillard MC, Mahadeva R, Lomas DA. Identification of DNA polymorphisms associated with the V type alpha1-antitrypsin gene. Biochim Biophys Acta. 1999;1444:166–70.
8. Frishberg Y, Toledano H, Becker-Cohen R, Feigin E, Halle D. Genetic polymorphism in paraoxonase is a risk factor for childhood focal segmental glomerulosclerosis. Am J Kidney Dis. 2000;36:1253–61.
9. Yorioka T, Suehiro T, Yasuoka N, Hashimoto K, Kawada M. Polymorphism of the angiotensin converting enzyme gene and clinical aspects of IgA nephropathy. Clin Nephrol. 1995;44:80–5.
10. Yoshida H, Mitarai T, Kawamura T, Kitajima T, Miyazaki Y, Nagasawa R, et al. Role of the deletion of polymorphism of the angiotensin converting enzyme gene in the progression and therapeutic responsiveness of IgA nephropathy. J Clin Invest. 1995;96:2162–9.
11. Harden PN, Geddes C, Rowe PA, McIlroy JH, Boulton-Jones M, Rodger RS, et al. Polymorphisms in angiotensin-converting-enzyme gene and progression of IgA nephropathy. Lancet. 1995;345:1540–2.
12. Suzuki H, Sakuma Y, Kanesaki Y, Eiro M, Asahi K, Sanada H, et al. Close relationship of plasminogen activator inhibitor-1 4G/5G polymorphism and progression of IgA nephropathy. Clin Nephrol. 2004;62:173–9.
13. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature. 1992;359:641–4.
14. Ludwig E, Corneli PS, Anderson JL, Marshall HW, Lalouel JM, Ward RH. Angiotensin-converting enzyme gene polymorphism is associated with myocardial infarction but not with development of coronary stenosis. Circulation. 1995;91:2120–4.
15. Uemura K, Nakura J, Kohara K, Miki T. Association of ACE I/D polymorphism with cardiovascular risk factors. Human Genet. 2000;107:239–42.
16. Schena FP, D'Altri C, Cerullo G, Manno C, Gesualdo L. ACE gene polymorphism and IgA nephropathy: an ethnically homogeneous study and a meta-analysis. Kidney Int. 2001;60:732–40.
17. Suzuki S, Suzuki Y, Kobayashi Y, Harada T, Kawamura T, Yoshida H, et al. Insertion/deletion polymorphism in ACE gene is not associated with renal progression in Japanese patients with IgA nephropathy. Am J Kidney Dis. 2000;35:896–903.
18. Pei Y, Scholey J, Thai K, Suzuki M, Cattran D. Association of angiotensinogen gene T235 variant with progression of immunoglobin A nephropathy in Caucasian patients. J Clin Invest. 1997;100:814–20.
19. Schimidt SE, Sttier R, Hartung G, Stein J, Bahnisch AJ, Woodroffe AR, et al. No association of converting enzyme insertion/deletion polymorphism with immunoglobulin A glomerulonephritis. Am J Kidney Dis. 1995;26:727–31.
20. Sakai H, Abe K, Kobayashi Y, Koyama A, Shigematsu H, Harada T, et al. Joint Committee of Ministry of Health and Welfare of Japan and Japanese Society of Nephrology. Clinical guidelines of IgA nephropathy. Jpn J Nephrol. 1995;37:417–21.
21. Churg J, Sobin LH. Renal disease: classification and atlas of glomerular disease. Tokyo, Japan: Igaku-shoin; 1982.
22. Walburger DK, Afonina IA, Wydro R. An improved real time PCR method for simultaneous detection of C282Y and H63D mutations in the HFE gene associated with hereditary hemochromatosis. Mutat Res. 2001;432:69–78.
23. Kuriki M, Asahi K, Asano K, Sakurai K, Eiro M, Suzuki H, et al. Steroid therapy reduces mesangial matrix accumulation in advanced IgA nephropathy. Nephrol Dial Transplant. 2003;18:1311–5.
24. Ohishi M, Fujii K, Minamino T, Higaki J, Kamitani A, Rakugi H, et al. A potent genetic risk factor for restenosis. Nature Genet. 1993;5:324–5.
25. Higashimori K, Zhao Y, Higaki J, Kamitani A, Katsuya T, Nakura J, et al. Association analysis of a polymorphism of the angiotensin converting enzyme gene with essential hypertension in the Japanese population. Biochem Biophys Res Commun. 1993;191:399–404.
26. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest. 1990;86:1343–6.
27. Singhal PC, Santiago A, Satriano J, Hays RM, Schlondorff D. Effects of vasoactive agents on uptake of immunoglobulin G complexes by mesangial cells. Am J Physiol. 1990;258:F589–96.

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総論―ステージ分類別対処法の概要

ステージ2

KeyWords
○CKDステージ2
○蛋白尿
○微量アルブミン尿
○心血管危険因子

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Headline

1. CKDステージ2の診断に必須の「何らかの腎障害を示唆する所見」として，蛋白尿（アルブミン尿）が特に重要である．
2. CKDステージ2では自覚症状を伴わないことが通常であるが，蛋白尿は末期腎不全（ESRD），心血管系疾患（CVD）発症の危険因子である．
3. CKDステージ2では進行性または寛解・治癒可能な腎疾患を見逃すことのないよう，0.5 g/gクレアチニン以上または2＋以上の蛋白尿が存在する場合や，蛋白尿と血尿がともに陽性（1＋以上）の場合は腎臓専門医に紹介し，腎生検を含めた精査を行う．
4. 紹介基準を満たさないCKDステージ2ではかかりつけ医の役割が重要であり，ESRDやCVD発症のリスクを十分に認識しつつ，生活習慣病に対する十分な介入（禁煙，血糖，血圧，脂質などの厳格な管理）をしながら，蛋白尿（アルブミン尿）の減少，腎機能障害の抑制，CVDのリスク軽減を主眼とした治療を進め，必要に応じて腎臓専門医と連携する．

はじめに

近年，透析・移植を要する末期腎不全（end-stage renal disease；ESRD）患者が先進工業国のみならず全世界的に著しく増加し，国民保健上の問題となるとともに医療経済も圧迫している．さらに，蛋白尿（アルブミン尿）と腎機能低下は心血管疾患（cardiovascular disease；CVD）発症の独立した危険因子であり，生命予後にも重大な影響を及ぼす危険因子であることが改めて認識され，2002年にアメリカNational Kidney foundation，Kidney Disease Outcomes Quality Initiative（K-DOQI）work groupのガイドラインで，アルブミン尿などの腎疾患の徴候または糸球体濾過量（glomerular filtration rat；GFR）が60 mL/min/1.73 m²未満の腎機能低下が3か月以上継続する病態として定義される慢性腎臓病（chronic kidney disease；CKD）の概念と対策の必要性が提唱された．

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CKDはESRD，CVD，死亡（ハードアウトカム）のリスクの高い患者を早期に抽出し，集団的，社会的視点からの予防を目的とする病態論から捉えた症候群であり，原疾患（病因）を問わない腎臓病学の新たな概念といえる．CKDは腎機能別にステージ分類がなされ，各ステージに応じた対策が求められている．本稿ではCKDステージ2への対処にあたっての考え方を中心に総論的に概説する．

CKDステージ2の定義と疫学

CKDのステージ分類における腎機能の評価には，血清クレアチニン（Cr）値に基づく日本人独自の推定糸球体濾過量（eGFR）推算式（$eGFR = 194 \times Cr^{-1.094} \times Age^{-0.287}$．女性の場合，×0.739）を用いる[1]．

CKDステージ2は「血液，尿，画像，病理組織検査で何らかの腎障害を示唆する所見があり，GFRの軽度低下（60～89 mL/min/1.73