

Skin autofluorescence, a marker for advanced glycation end product accumulation, is associated with arterial stiffness in patients with end-stage renal disease

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Received 22 January 2008; accepted 20 May 2008

Abstract

Elevated cardiovascular mortality has been shown to be associated with increased arterial stiffness. However, the contribution of tissue accumulation of advanced glycation end products (AGEs) to increased arterial stiffness is unclear. We examined whether skin autofluorescence, a recently developed marker of tissue accumulation of AGEs, is associated with arterial stiffness in 120 Japanese patients with end-stage renal disease (ESRD) and 110 age- and sex-matched control subjects. The ESRD patients had significantly higher pulse wave velocity (PWV), a noninvasive measure of arterial stiffness, and skin autofluorescence than the control subjects. Skin autofluorescence was significantly associated with age in the group of all subjects ($R_s = 0.255$, Spearman rank correlation test) and that of control subjects ($R_s = 0.493$), but not in the group of ESRD subjects ($R_s = 0.046$). The PWV was significantly and positively associated with skin autofluorescence in the group of all subjects ($R_s = 0.335$), controls ($R_s = 0.246$), and ESRD subjects ($R_s = 0.205$). Multiple regression analyses showed that, in the group of all subjects, association of skin autofluorescence with PWV was significant even after adjustment for other covariates including the presence of ESRD and age. Moreover, for ESRD subjects, a significant association between skin autofluorescence and PWV was found, independent of age. Our findings demonstrate the potential usefulness of skin autofluorescence in people of color and demonstrate clinically for the first time the potential involvement of tissue accumulation of AGEs in the pathophysiology of arterial stiffness.

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1. Introduction

Aortic stiffness, an important pathophysiologic aspect of large-artery damage, is a predictor of all-cause and cardiovascular mortality [1–3]. Arterial stiffening occurs with aging, diabetes mellitus, renal diseases including end-stage renal disease (ESRD), and other high-risk conditions for cardiovascular diseases, although the pathophysiologic mechanisms underlying it are not fully understood.

Endogenous nonenzymatic glycoxidation of proteins and lipids leads to the formation of heterogeneous products

collectively termed *advanced glycation end products* (AGEs) [4–6]. The initial product of this reaction is a Schiff base, which spontaneously rearranges itself into an Amadori product, as is the case of the well-known glycated hemoglobin A_{1c} (HbA_{1c}). These initial reactions are reversible, depending on the concentration of the reactants. A series of subsequent reactions, including successive dehydrations, oxidation-reduction reactions, and other arrangements, leads to the formation of AGEs. Several compounds, for example, *N*-carboxymethyl-lysine (CML), pentosidine, and methylglyoxal derivatives, are examples of well-characterized and widely studied AGEs. The AGEs also accumulate in the course of many degenerative diseases [4], including ESRD [7]. Increased *de novo* generation of AGEs due to enhanced oxidative stress and accumulation of

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Table 1
Characteristics of the subjects

	Control	ESRD	P
No. of subjects	110	120	–
Age (y)	57.1 ± 10.5	58.1 ± 9.3	.47
Sex (% male)	64.5	73.3	.15
Smoker (%)	54.5	27.5	<.0001
Hemodialysis vintage (y)	–	13.0 (0.1–33.0)	–
Systolic blood pressure (mm Hg)	129 ± 21	149 ± 15	<.0001
Diastolic blood pressure (mm Hg)	73 ± 10	79 ± 7	<.0001
Serum creatinine (μmol/L)	62 ± 12	1142 ± 214	<.0001
Non-HDL cholesterol (mmol/L)	3.84 ± 0.94	2.96 ± 0.86	<.0001
HDL cholesterol (mmol/L)	1.52 ± 0.43	1.25 ± 0.33	<.0001
Vascular complications (%)			
Coronary heart diseases	4 (3.6)	12 (10.0)	.052
Cerebrovascular diseases	0 (0)	17 (14.2)	<.0001
Peripheral artery diseases	0 (0)	5 (4.2)	.034
Use of ACEI or ARB (%)	NA	38 (31.7)	–
PWV (cm/s)	1421 ± 226	1792 ± 449	<.0001
Skin autofluorescence	0.013 ± 0.005	0.018 ± 0.007	<.0001

Continuous variables are summarized as mean ± SD, whereas median values (limits of observed values) are shown for variables with skewed distribution. Prevalences are reported as percentages. The Student *t* test or χ^2 test was used for comparison between groups. NA indicates data not available; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

reactive precursors as well as decreased renal clearance of AGE precursors may further increase the accumulation of end products in ESRD [7,8].

The accumulation of AGEs on tissue proteins has been implicated in the pathogenesis of complications of diabetes [9–11]. The AGEs may also be involved in arterial stiffness, which results from nonenzymatic protein glycation to form irreversible cross-links between long-lived proteins such as collagen and elastin. The AGE-linked extracellular matrix is stiffer and less susceptible to hydrolytic turnover, resulting in the accumulation of structurally inadequate matrix molecules. The AGE cross-link breaker is successfully shown to improve arterial compliance in humans [12]. However, little is known concerning the relationships between tissue glycation and direct measures of arterial stiffness in humans.

A noninvasive device, the autofluorescence reader (AFR), for measuring skin autofluorescence was recently developed to estimate accumulation of AGEs in humans [13]. Because routine skin biopsy is difficult in medical practice and blood samples do not necessarily reflect tissue AGE levels, noninvasive measurement of skin AGEs has been an issue of particular interest. Skin autofluorescence has been found to be increased in patients with diabetes or ESRD [14,15]. Skin autofluorescence has been shown to correlate with tissue levels of pentosidine and CML [13] and with the presence of long-term complications in diabetic patients [15,16]. Moreover, skin autofluorescence is a predictor of cardiovascular mortality in diabetes and ESRD [17,18]. However, it is unclear whether skin autofluorescence is a predictor of quantitatively measured atherosclerosis.

In the present study, we examined for the first time the association between arterial stiffness and skin autofluorescence in 120 nondiabetic ESRD patients and 110 age-

and sex-matched control subjects with neither renal disease nor diabetes. We show here that, in ESRD patients, skin autofluorescence is an independent determinant of arterial stiffness, as determined by arterial pulse wave velocity (PWV).

2. Subjects and methods

2.1. Subjects

This study was approved by the Ethics Committee at Osaka City University Graduate School of Medicine (approval no. 808). The present study included 120 nondiabetic ESRD patients and 110 age- and sex -matched control subjects with neither renal disease nor diabetes. The ESRD patients had been treated by regular hemodialysis thrice (*n* = 117) or twice (*n* = 3) a week at Inoue Hospital, Suita, Japan. Healthy subjects were the participants of a medical check program performed at the Osaka Health Promotion Center, Osaka, Japan. Diabetic subjects were excluded from the study based on the following criteria: fasting plasma glucose greater than 126 mg/dL (7 mmol/L), casual plasma glucose greater than 200 mg/dL (11.1 mmol/L), or history of treatment of diabetes. Presence of vascular complications was diagnosed as described in detail [2]. Table 1 summarizes baseline characteristics of the subjects.

2.2. Brachial-ankle PWV

Brachial-ankle PWV was measured by the BP203RPE automatic waveform analyzer (Colin, Komaki, Japan) as previously described [19]. In brief, measurements were performed in the supine position after 5-minute bed rest. Cuffs for occlusion and sensing were adapted to both arms and both ankles. The electrocardiogram was monitored with electrodes placed on both wrists. Heart sounds S₁ and S₂ were detected using a microphone placed on the left edge of the sternum at the third intercostal space. Pulse pressure waveforms of the brachial and tibial arteries were simultaneously recorded to measure the time interval between the initial rises of these waveforms (T_{ba}, in seconds). The waveform analyzer calculated PWV using the following formula: PWV (in centimeters per second) = (D_{ha} – D_{hb})/T_{ba}, where D_{ha} and D_{hb} are the distances from aortic orifice to the ankle sensor and the brachial sensor, respectively. The D_{ha} and D_{hb} were calculated as follows: D_{ha} (in centimeters) = [0.5643 × height (in centimeters) – 18.381] + (0.2486 × height + 30.709); D_{hb} = 0.2195 × height – 2.0734.

2.3. Skin autofluorescence

Skin autofluorescence was assessed by the AFR (AGE Reader; Diagnostics, Groningen, the Netherlands) as previously described in detail [13]. The measure of autofluorescence used was the average light intensity per nanometer in the range of 420 to 600 nm divided by the

Table 2
Analyses of factors associated with skin autofluorescence

	All	Control	ESRD
Age	0.255 [†]	0.493 [†]	0.046
Sex (M = 0, F = 1)	0.273 [†]	0.578 [†]	0.104
Smoker (non = 0, smoker = 1)	−0.309 [†]	−0.325 [†]	−0.157
ESRD (no = 0, yes = 1)	0.341 [†]	—	—
Hemodialysis vintage	—	—	0.176
Systolic blood pressure	0.244 [†]	0.228*	−0.023
Diastolic blood pressure	0.098	0.104	−0.154
Non-HDL cholesterol	−0.176 [†]	−0.015	0.056
HDL cholesterol	−0.064	0.408 [†]	0.017
Presence of CAD (no = 0, yes = 1)	—	—	0.048
Presence of CVD (no = 0, yes = 1)	—	—	−0.062
Presence of PAD (no = 0, yes = 1)	—	—	−0.090
Use of ACEI or ARB (no = 0, yes = 1)	—	—	0.085

Spearman rank correlation test was performed to examine the associations between factors. R_s values are shown. CAD indicates coronary artery disease; CVD, cerebrovascular disease; PAD, peripheral artery disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker.

* $P < .05$.

[†] $P < .01$.

average light intensity per nanometer in the range of 300 to 420 nm. The intraassay coefficient of variation based on repeated AFR measurements on the same day was 2.8% ($n = 5$).

2.4. Biochemical analyses

Serum levels of total cholesterol and high-density lipoprotein (HDL) cholesterol were measured by enzymatic methods adapted to an autoanalyzer (Hitachi 7470; Hitachi, Tokyo, Japan). The non-HDL cholesterol was calculated by subtracting HDL cholesterol from total cholesterol.

2.5. Statistical analyses

Statistical analyses were performed with the use of StatView V software (SAS, Cary, NC). The Student t test or χ^2 test was performed for comparisons among groups. To evaluate relationship between factors, the Spearman rank correlation test or multiple regression analysis was per-

Table 3
Multiple regression analyses of factors associated with skin autofluorescence

	All	Control	ESRD
Age (y)	0.165 [†]	0.279 [†]	0.096
Sex (M = 0, F = 1)	0.184 [†]	0.424 [†]	0.097
Smoker (non = 0, smoker = 1)	−0.102	0.051	−0.120
Systolic blood pressure (mm Hg)	0.012	0.007	0.057
Non-HDL cholesterol (mmol/L)	0.019	0.155*	−0.067
HDL cholesterol (mmol/L)	0.056	0.252 [†]	−0.066
ESRD (no = 0, yes = 1)	0.360 [†]	—	—
Hemodialysis vintage (short = 0, long = 1)	—	—	0.166
R^2	0.245 [†]	0.466 [†]	0.078

The β values are shown. Because of the skewed distribution, hemodialysis vintage was categorized into short (13 years or shorter) or long duration (>13 years) according to the median. ESRD indicates end-stage renal disease.

* $P < .05$.

[†] $P < .01$.

formed. For multiple regression analyses, potential risk predictors for AGE accumulation and arteriosclerosis (age, sex, smoking, blood pressure, dyslipidemia, presence of ESRD [analyses for all subjects], and hemodialysis vintage [analyses for ESRD subjects]) were used as covariates. Findings of P less than .05 were considered significant.

3. Results

Table 1 shows the characteristics of the subjects enrolled in the study. The ESRD patients had significantly higher systolic and diastolic blood pressure than control subjects. The percentage of smokers and non-HDL and HDL cholesterol levels were significantly lower in ESRD patients than in control subjects. Both PWV and skin autofluorescence were significantly higher in the ESRD than in the control group.

Tables 2 and 3 and Fig. 1A show analyses of the factors associated with skin autofluorescence in the groups of all, control, and ESRD subjects. In the group of all subjects, presence of ESRD was significantly associated with skin autofluorescence (Table 2), which was independent of the other covariates in a multiple regression model (Table 3). In the group of all and control subjects, age and systolic blood pressure were significantly and positively associated with skin autofluorescence, whereas age, but not systolic blood pressure, remained an independent factor associated with it in a multiple regression analysis. In the same group, women

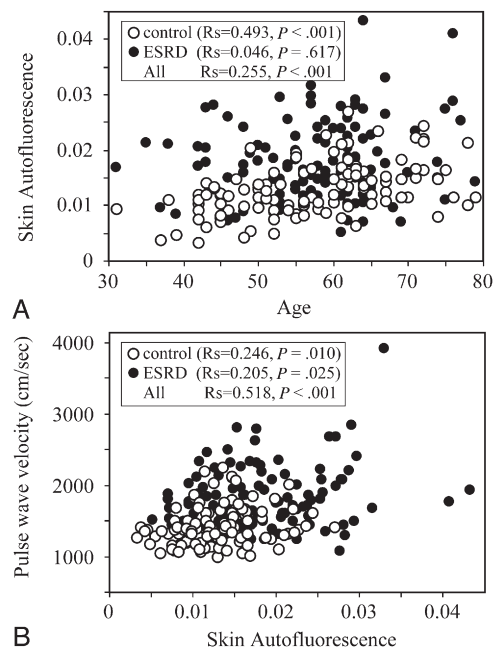


Fig. 1. A, Skin autofluorescence was strongly and positively associated with age in the control but not the ESRD subjects. B, Skin autofluorescence was significantly associated with PWV in both the control and ESRD groups. Spearman rank correlation test was performed for both analyses. Open circles, control subjects; closed circles, ESRD subjects.

Table 4
Analyses of factors associated with PWV

	All	Control	ESRD
Age	0.518 [†]	0.584 [†]	0.520 [†]
Sex (M = 0, F = 1)	0.065	0.133	0.127
Smoker (non = 0, smoker = 1)	−0.186 [†]	−0.122	−0.012
ESRD (no = 0, yes = 1)	0.467 [†]	—	—
Hemodialysis vintage	—	—	−0.078
Systolic blood pressure	0.549 [†]	0.596 [†]	0.165
Diastolic blood pressure	0.255 [†]	0.191*	0.034
Non-HDL cholesterol	−0.275 [†]	0.043	−0.027
HDL cholesterol	−0.099	0.111	−0.019
Skin autofluorescence	0.335 [†]	0.246*	0.205*

Spearman rank correlation test was performed to examine the associations between factors. R_s values are shown. ESRD indicates end-stage renal disease.

* $P < .05$.

[†] $P < .01$.

had higher skin autofluorescence than men, as reported in a white population [15]. This association was still significant even after adjustment for other covariates. In the group of all subjects, non-HDL cholesterol was inversely correlated with skin autofluorescence; but it was not an independent predictor of skin autofluorescence when it was adjusted for other covariates. In the control group alone, HDL cholesterol showed a positive correlation with skin autofluorescence. The HDL cholesterol was also an independent determinant of skin autofluorescence in a multiple regression model in control subjects. In contrast, in the group of ESRD subjects, neither age nor blood pressure was significantly correlated with skin autofluorescence. Sex-related differences in autofluorescence were also not observed in the ESRD group. Hemodialysis vintage exhibited a tendency toward positive correlation with skin autofluorescence ($P = .055$). The uses of different hemodialysis membranes did not significantly affect skin autofluorescence in the group of ESRD subjects ($P = .13$, analysis of variance). Although angiotensin-converting enzyme inhibitor (ACEI) or angiotensin II receptor blocker (ARB) was shown to reduce accumulation of AGEs [20,21], skin autofluorescence was not significantly different between the patients treated with and without ACEI or ARB. None of the covariates were significant independent determinants of skin autofluorescence in ESRD subjects in a multiple regression model. Smokers had less skin autofluorescence than nonsmokers in the group of all and that of control subjects, although this difference disappeared upon adjustment for other covariates, particularly sex (Table 3). Independent effects of age, sex, HDL cholesterol (analyses for control subjects), and presence of ESRD (analyses for all subjects) in multiple regression analyses were still significant even after adjustment with other covariates (data not shown).

Tables 4 and 5 show analyses of the factors associated with PWV. In the group of all subjects, age, presence of ESRD, and systolic and diastolic blood pressures were significantly and positively associated with PWV, whereas smoking and non-HDL cholesterol were inversely corre-

lated with it (Table 4). Multiple regression analysis revealed that age, systolic blood pressure, and presence of ESRD were each independently and significantly associated with PWV (Table 5). Smoking and non-HDL cholesterol were not independently associated with PWV in this model. In the group of control subjects, age and systolic blood pressure each exhibited a strong positive correlation with PWV (Table 4). In multiple regression analysis, both age and systolic blood pressure remained independently and positively associated with PWV. In the group of ESRD subjects, blood pressures exhibited tendencies toward correlation with PWV, although they were not significant ($P = .073$). Age was also strongly and positively correlated with PWV in the group of ESRD subjects. As shown in Table 4 and Fig. 1B, skin autofluorescence was positively correlated with PWV in the groups of all, control, and ESRD subjects. In the groups of all and ESRD subjects, skin autofluorescence remained significantly and independently associated with PWV when classic risk predictors (age, sex, smoking, systolic blood pressure, and non-HDL and HDL cholesterol) were included as covariates in a multivariate regression model. This association remains significant even after adjustment with other covariates including serum creatinine, presence of vascular complications, or use of ACEI or ARB (data not shown). However, in the group of control subjects, the association between skin autofluorescence and PWV was not significant in this multiple regression analysis.

4. Discussion

This study is the first to demonstrate the association between skin autofluorescence, a noninvasive measure to estimate tissue AGE accumulation, and arterial stiffening particularly in subjects with ESRD.

It has been proposed that AGE accumulation plays roles in the mechanisms that increase arterial stiffness. Vishwanath et al [9] first examined the level of Amadori

Table 5
Multiple regression analyses of factors associated with PWV

	All	control	ESRD
Age (y)	0.350 [†]	0.438 [†]	0.422 [†]
Sex (M = 0, F = 1)	−0.007	−0.168	0.157
Smoker (non = 0, smoker = 1)	0.032	0.015	0.013
Systolic blood pressure (mm Hg)	0.237 [†]	0.437 [†]	0.156
Non-HDL cholesterol (mmol/L)	0.009	0.072	−0.085
HDL cholesterol (mmol/L)	−0.062	0.096	−0.146
Skin autofluorescence	0.136*	−0.005	0.185*
ESRD (no = 0, yes = 1)	0.262 [†]	—	—
Hemodialysis vintage (years)	—	—	−0.013
R^2	0.440 [†]	0.485 [†]	0.322 [†]

The β values are shown. Because of the skewed distribution, hemodialysis vintage was categorized as short (13 years or shorter) or long duration (>13 years) according to the median. ESRD indicates end-stage renal disease.

* $P < .05$.

[†] $P < .01$.

products and pentosidine in skin biopsy samples in 41 subjects with type 1 diabetes mellitus and in 25 age-matched controls and found that skin pentosidine level was associated with arterial stiffness as determined by pulse pressure. Direct analyses of the human aorta revealed that increased arterial stiffness could be attributed to glycation-induced intermolecular cross-linking [22]. Increased serum levels of AGEs have also been shown to be associated with pulse pressure in patients with type 1 diabetes mellitus [23]. However, few studies have examined the association between AGEs and PWV, which is now considered a criterion standard for measurement of arterial stiffness. Yoshida et al [24] recently demonstrated a significant positive association between serum pentosidine level and PWV in patients with type 2 diabetes mellitus. We have shown here for the first time that arterial stiffness measured by PWV is positively and independently associated with skin autofluorescence, using a method recently developed to noninvasively estimate AGE accumulation in humans. In healthy subjects, the association between arterial stiffness and skin autofluorescence disappeared upon adjustment for age, suggesting mutual interaction between age and accumulation of AGEs in regulation of arterial stiffness. In ESRD subjects, skin autofluorescence was associated with PWV even after adjustment with age. This implies the existence of additional mechanisms for accumulation of AGEs in ESRD, for example, enhanced generation and decreased renal clearance of AGE precursors [7,8].

Skin autofluorescence has been shown to be related to metabolic stress (HbA_{1c} and hyperlipidemia) and the accumulation of AGEs, including pentosidine, CML, and carboxyethyl-lysine [13]. The AGEs accumulate as a result of increased de novo generation of AGEs due to enhanced oxidative stress and accumulation of reactive precursors as well as decreased renal clearance of AGEs precursors [7,8], which may contribute to the significantly higher skin autofluorescence in subjects with ESRD. Hyperlipidemia may contribute to the tissue accumulation of advanced lipoxidation end products [25], which may in turn contribute to tissue and skin autofluorescence. In our study, however, neither non-HDL nor HDL cholesterol was significantly correlated with skin autofluorescence in ESRD subjects. The serum triglyceride level might be the predominant determinant for AGE accumulation [25], which was not examined in our current study. It has been shown in diabetic subjects that HbA_{1c} makes a small but independent contribution to skin autofluorescence [15]. In our ESRD cohort study recently published [26], neither plasma pentosidine nor CML level was positively correlated with HbA_{1c}. The restricted relationship between glycemic control and accumulation of AGEs has been explained by the shorter turnover time of HbA_{1c}.

In this study, age was a major determinant of skin autofluorescence in nondiabetic healthy subjects. However,

this relationship was completely absent in the group of nondiabetic ESRD subjects, a finding not consistent with that for 109 white subjects with ESRD in whom age was found to be an independent determinant of skin autofluorescence [17]. This discrepancy in findings may be due to the difference in ethnicity. In healthy subjects, sex was found to be an independent determinant of skin autofluorescence, as previously described for subjects with type 2 diabetes mellitus [15]. Another large study of type 2 diabetes mellitus found a similar independent positive association between female sex and plasma levels of low-molecular weight AGEs [27]. This has been suggested to be an estrogen-related effect. Among ESRD subjects, no significant difference in skin autofluorescence was found between men and women. The lack of sex effect in this population may be due to ovarian dysfunction, which is frequently noted in ESRD subjects.

There remain several limitations to the study. The chemical nature of skin autofluorescence, especially its relationship to specific AGE moieties, is not well understood. A variety of skin fluorophores other than AGE moieties may affect skin autofluorescence [17], and some AGEs (eg, *N*-carboxymethyl-lysine) are not fluorescent. It is also unclear whether the present skin autofluorescence represents AGE modification in injured cardiovascular, renal, or osteoarticular tissues, although skin AGEs could be associated with those in several other tissues including kidney and vessel walls [28]. It might also be suggested that the difference we observed between ESRD and control subjects in the relationship between age and skin autofluorescence could also be explained by a qualitative difference in the AGEs that are accumulated or by a difference in the tissues within which AGEs are accumulated in ESRD. The complexities in these interrelationships are hinted at by our observations but should be directly addressed in further researches.

Acknowledgment

This work was supported by a Grant-in-Aid for Scientific Research (17590946 to HK) from the Japan Society for the Promotion of Science and by a grant from the Osaka Kidney Foundation (OKF06-0007 to HK).

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