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Skin and Plasma Autofluorescence During Hemodialysis: A Pilot Study

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Abstract: Skin autofluorescence (AF) is related to the accumulation of advanced glycation end products (AGEs) and is one of the strongest prognostic markers of mortality in hemodialysis (HD) patients. The aim of this pilot study was to investigate whether changes in skin AF appear after a single HD session and if they might be related to changes in plasma AF. Skin and plasma AF were measured before and after HD in 35 patients on maintenance HD therapy (nine women and 26 men, median age 68 years, range 33–83). Median dialysis time was 4 h (range 3–5.5). Skin AF was measured noninvasively with an AGE Reader, and plasma AF was measured before and after HD at 460 nm after excitation at 370 nm. The HD patients had on average a 65% higher skin AF value than age-matched healthy persons ($P < 0.001$). Plasma AF was reduced by 14% ($P < 0.001$), whereas skin AF was not changed after a single HD treatment. No significant influence of the reduced plasma AF on skin AF levels was found. This suggests that the measurement of skin AF can be performed during the whole dialysis period and is not directly influenced by the changes in plasma AF during HD. **Key Words:** Hemodialysis—Skin autofluorescence—Plasma autofluorescence—Advanced glycation end products.

Well-functioning kidneys contribute to the elimination of advanced glycation end products (AGE). In contrast, retention of AGE is found in plasma and tissue of uremic subjects even in the absence of hyperglycemia. This is related to the lack of both breakdown and removal of AGEs by the impaired kidneys (1–3). The consequence will be a progressive damage of, for example, vascular cells such as endothelial cells, and subsequently a progression of atherosclerosis and cardiovascular disease (4,5).

Tissue autofluorescence (AF) is related to the accumulation of AGEs in the skin (6). Nowadays, a new optical tool, the AGE Reader, is available to measure skin AF, which makes it possible to recognize noninvasively the state of increased skin AGE (7,8).

Skin AF, measured using a prototype of the AGE Reader, was shown to be a strong independent predictor of overall and cardiovascular mortality after 3 years of follow-up. Skin AF was a stronger predictor of cardiovascular mortality than of overall mortality (1). The prototype of the AGE Reader, the AF Reader, was validated by comparing skin AF values with AGE content measured in extracts from skin biopsies. Thereby, the fluorescent AGE pentosidine and the nonfluorescent AGEs carboxy(m)ethyllysine were significantly related to Skin AF (1,8,9).

Plasma AGEs show AF in the same range (excitation wavelength: 370 nm, emission wavelength: 440 nm) and has increased values in patients on hemodialysis (HD) as compared with controls (10,11). These strongly increased values of the circulating AGE pentosidine and other AGEs in HD patients are

a consequence of the lack of removal (10,12–15). Henle et al. (2) showed that plasma AGE levels could be decreased after HD.

Therefore, the aim of the present pilot study was to investigate whether changes in skin AF appear after a single HD session and if they might be related to changes in plasma AF.

PATIENTS AND METHODS

This study included 35 consecutive patients on chronic HD from which data of skin AF and plasma AF were measured both before and after a dialysis session. Men ($n = 26$) did not differ from women ($n = 9$) in relation to age (mean 67.6 ± 11.5 vs. 59.6 ± 14.8 years) or HD vintage (30.3 ± 18.4 vs. 40.2 ± 34.2 months). The baseline skin AF data were compared with the available age-adjusted AGE Reader measurements on healthy individuals as a function of age, AF = 0.83 + 0.024 age (16).

The reason for dialysis was diabetic nephropathy ($n = 7$: three with diabetes mellitus type 1 and four with type 2), hypertension and/or renovascular disease ($n = 8$), primary glomerular disorders ($n = 8$), and other reasons ($n = 12$). Three of 34 patients were current tobacco users (9%; data missing for one patient), and 20 of 33 were previous tobacco users (57%; data missing for two patients). None of the patients had hepatitis B or C, eight had had a previous myocardial infarction (23%), five had had a previous stroke (14%), 18 had a history of cardiovascular disease (51%), 14 had diabetes mellitus (40%), 29 had hypertension (91%, data missing in three), and three had had a prior kidney transplant (9%). Median duration of HD was 22 months (range 1–106). Patients were informed and consented to participate in the study that had been approved by the local ethical committee.

Three measurements of skin AF at slightly different skin sites on the same forearm were performed at room temperature with the AGE Reader both before and after HD in a room with windows covered by curtains to avoid direct sun illumination (semidark environment). Areas containing scars were avoided, that is, arteriovenous fistula or graft operation scars/arm. The AGE Reader (DiagnOptics Technologies BV, Groningen, The Netherlands) illuminates a skin surface of ~4 cm^2, guarded against surrounding light, with a light source that mainly provides excitation light around a peak wavelength of 370 nm (range 350–420 nm). Reflected light from the skin and AF were measured simultaneously, using a built-in AvaSpec 2048 spectrometer (Avantes, Apeldoorn, The Netherlands) within the instrument. Skin AF was based on the ratio of the average light intensity per nanometer in the range between 420 and 600 nm and the average light intensity per nanometer in the range between 300 and 420 nm (AF in arbitrary units [AUs]). Version 2.3 of the AGE Reader software was used to work out the measurements. In this updated latest version, the effect of skin color was compensated for in the calculation of skin AF (17).

AGEs were determined from freshly frozen plasma samples. Plasma samples were taken before and after dialysis according to the protocol of Schwedler et al. (14). Plasma samples were diluted 50 times in phosphate buffered saline. Plasma fluorescence, plasma AF, was measured at 460 nm after excitation at 370 nm using a FLUOstar Optima plate-reader (BMG Lab Technologies, Durham, NC, USA).

At initiation of the study, there was no knowledge if HD influences skin AF, either in a positive or negative way. We therefore decided to use a heterogeneous material to eventually get some hints for future studies regarding flux and dialysis procedures such as dialysis time (median 4, range 3–5.5 h/session) and dialyzers (FX80 [$n = 15$] and FX10 [$n = 4$], Fresenius, Bad Homburg, Germany; Polyflux 210H [$n = 8$], 140H [$n = 7$], and 17 L [$n = 1$], Gambro, Hechingen, Germany). All patients were dialyzed using 5 mmol glucose/L of the dialysate (Biosol A201.25 glucose 5 and Biosol A301.25 glucose 5) that was provided by Meda AB (Solna, Sweden). The dialysate had been heat sterilized. All patients were offered a light meal during dialysis.

For skin AF, median values for each triple measurement were used. Based on the Kolmogorov–Smirnov test results, a comparison between groups was performed with Wilcoxon nonparametric test for paired data and Student's *t*-test for normal distributions. Spearman's correlation coefficient was used. Paired statistical analyses were made by the paired Student's *t*-test for normally distributed data. Serum albumin was measured in samples before and after HD to correct for the eventual effect of ultrafiltration (Plasma AF after HD × albumin at the start/albumin after). SPSS statistical software (version 19.0; SPSS, Inc., Chicago, IL, USA) was used for the analysis, and a two-tailed P value of less than 0.05 was considered significant.

RESULTS

Before dialysis, patients had skin AF values that were 1.64 times higher than that for age-matched healthy persons ($P < 0.001$), as shown in Fig. 1, which is 65% higher on average.

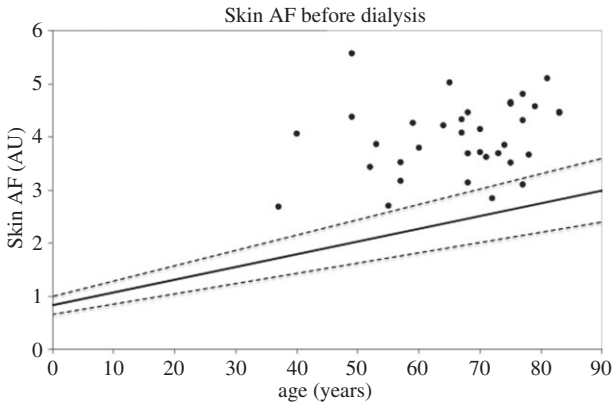

FIG. 1. Skin AF of each patient before hemodialysis as a function of age; the central line shows the mean value and the hatched lines show the standard deviation for the healthy age-matched persons.

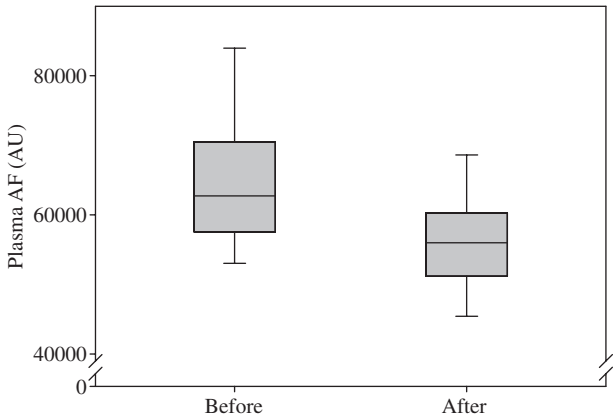

FIG. 3. Boxplot for plasma AF before and after hemodialysis. The paired test showed a significant difference ($P < 0.001$).

Analyses of the whole group revealed that HD resulted in a nonsignificant 3% reduction in skin AF from 3.99 ± 0.7 to 3.88 ± 0.7 after dialysis (Fig. 2).

Plasma AF was reduced from $65\,262 \pm 12\,398$ AU before the start of dialysis to $56\,438 \pm 9049$ after HD, which was a reduction of 14% (Wilcoxon test, $P < 0.001$) (Fig. 3). The urea reduction during HD in these patients was at a median 70%. There was a correlation (Spearman's test) between the dialysis time (hours/session) and the removal of plasma fluorescence (uncorrected values for ultrafiltration: $r = 0.40$, $P = 0.033$ and the corrected data adjusting for change of albumin ratio: $r = 0.60$, $P = 0.001$).

No correlation was found between the decrease in skin AF and the decrease in plasma AF.

No effects of gender were observed in the reduction of skin AF and plasma AF. Women had significantly lower values of skin AF than men, 3.5 ± 0.6 versus 4.2 ± 0.7 ($P = 0.006$) before HD, also after correction for age ($P < 0.05$), for which the results in healthy controls were used as given by the slope for control subjects in Fig. 1. This lower level for women was also found after HD ($P < 0.05$).

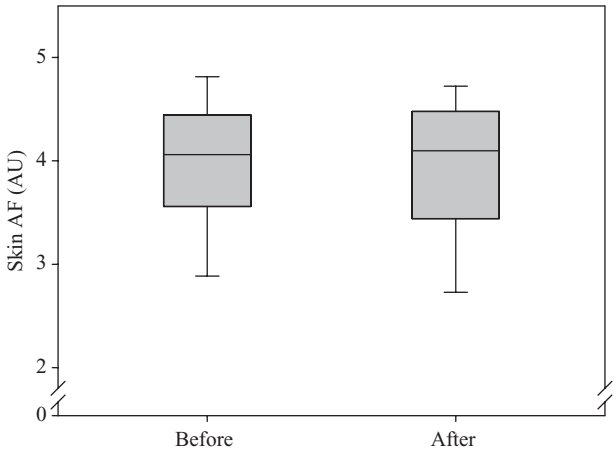

FIG. 2. Boxplot for skin AF before and after hemodialysis. The paired test showed no significant difference ($P = 0.19$).

DISCUSSION

To our knowledge, this is the first intervention study that investigates the effect of HD on changes in skin AF and plasma AF in comparable wavelength ranges. Notable was that skin AF was not significantly reduced by a single HD, although plasma AF decreased by 14%. In another study using high flux dialyzers, the reduction of plasma AF was 30% (2). In both studies, the reduction of plasma AF by HD is limited in regard to the reduction of 70% that is achieved for small water-soluble compounds, such as urea. This difference supports a substantial protein binding in HD that was also shown by others (18). Although plasma AF levels poorly or even negatively relate to mortality (14,15), it seems important to find methods for substantial removal of AGEs from the plasma of HD patients and when possible from their tissue. First, because there is a seven- to ninefold higher rate of increase of skin AF with aging in HD patients as compared with healthy subjects (19) and second because of a strong correlation between skin AF and mortality (1). The present study showed that prolonged dialysis time correlates with removal of plasma AF. Assuming that these products are linked to tissue AGEs, a more effective dialysis may decrease and even normalize the rate of accumulation of tissue AGEs, as measured by skin AF. This has to be clarified in future research.

CONCLUSION

The results indicate that there is no influence of the reduced plasma autofluorescence of one single hemodialysis session on the skin AF level. This suggests that the measurement of skin AF is not directly influenced by the changes in plasma AF during HD.

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Conflict of Interest: R. Graaff is cofounder and stockholder of DiagnOptics Technologies B.V., the manufacturer of the AGE Reader. No economical support was provided by any company.

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