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ORIGINAL ARTICLE

Hemodialysis biomarkers: total advanced glycation end products (AGEs) against oxidized human serum albumin (HSAox)

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Abstract

Aims Nephropathic patients show higher levels of advanced glycation end products (AGEs) and oxidized human serum albumin (HSAox) compared to healthy subjects. These two classes of compounds are formed as the result of oxidative insults; for this reason, they can be useful oxidative stress biomarkers. The present study examines the variation of AGEs and HSAox in hemodialysis (HD) patients before and after dialysis session, evaluating the impact of different dialytic techniques and filters on their removal.

Methods A total of 50 healthy subjects (control group) and 130 HD patients were enrolled in the study. Hemodialysis patients were subdivided based on dialytic techniques: 109 in diffusive technique and 22 in convective technique. We monitored HSAox, AGEs and other laboratory parameters at early morning in healthy subjects and in HD patients before and after the dialysis procedures.

Results The level of HSAox decreases after a single dialytic session (from $58.5 \pm 8.8\%$ to $41.5 \pm 11.1\%$), but the concentration of total AGEs increases regardless of adopted dialytic techniques (from 6.8 ± 5.2 µg/ml to 9.2 ± 4.4 µg/ml). In our study, levels of HSAox and total AGEs are similar in diabetic and non-diabetic HD patients. The increase in total AGEs after dialysis was only observed using polysulfone filters but was absent with polymethacrylate filters.

Conclusions HSAox is a simple and immediate method to verify the beneficial effect of a single dialysis session on the redox imbalance, always present in HD patients. Total AGEs assayed by ELISA procedure seem to be a less reliable biomarker in this population.

Keywords Advanced glycation end products · Oxidized human serum albumin · Hemodialysis · Oxidative stress · Biomarker

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Abbreviations

AGEs	Advanced glycation end products
BSA	Bovine serum albumin
CKD	Chronic kidney disease
DTNB	5,5′-Dithiobis(2-nitrobenzoic) acid
ESRD	End-stage renal disease
e-GFR	Estimated glomerular filtration rate
GFR	Glomerular filtration rate
Glo 1	Glyoxalase 1
HD	Hemodialysis
HF	Hemoperfusion
HSAox	Oxidized human serum albumin
IGF-1	Insulin growth factor-1
IL-1β	Interleukin-1β
MG	Methylglyoxal
NADPH	Nicotinamide adenine dinucleotide phosphate
Pdiast	Diastolic blood pressure
Psyst	Systolic blood pressure
PTV	Policlinico Tor Vergata
RAGE	AGE cell receptor
RCTs	Randomized clinical trials
ROS	Reactive oxygen species
SD	Standard deviation
sRAGE	AGE soluble receptor
TNF-α	Tumor necrosis factor-α

Introduction

Advanced glycation end products (AGEs) are a heterogeneous group of compounds characterized by different protein and amino acid adducts that span from < 1 to ~ 70 kDa [1–3]. The genesis of AGEs is due to non-enzymatic reaction(s) of reducing sugars and related metabolites with proteins, amino acids, lipids and DNA [1]. It is possible to distinguish between two separate classes of AGEs: the protein-bound AGEs (higher molecular weight) and AGE free adducts (lowest molecular weight) [1]. The latter are formed by cellular proteolysis of AGE-modified proteins and are identified as glycated amino acids. AGEs are formed not only in the presence of hyperglycemia, but also in the course of diseases associated with an increased oxidative stress, like chronic kidney disease (CKD) [4]. The protein glycation process is characterized by a series of complex reactions that are generally referred to "Maillard reaction" that occurs in all body tissues and fluids. The majority of AGEs is represented by: hydroimidazolones derived from arginine residues modified by glyoxal, monolysyl adducts, bis(lysyl)imidazolium crosslinks, fluorophores (e.g., pentosidine crosslink) and others [5, 6].

Moreover, AGEs and their cell receptor (RAGE) are implicated in the pathogenesis of several chronic diseases such as atherosclerosis, CKD, cardiovascular disease and diabetes mellitus. The adverse effects of AGEs are induced by non-receptor- and receptor-mediated mechanisms [7]. In particular, the interaction between AGEs and RAGE induces an increased production of reactive oxygen species (ROS) through the activation of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase that in turn activates NF-κβ [8]. This interaction accelerates monocyte migration into the sub-endothelial space resulting in endothelial dysfunction, causing an increased generation of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), as well as enhancing the expression of platelet-derived growth factor (PDGF) and insulin-like growth factor-1 (IGF-1) [2, 7]. The interaction between AGE and RAGE can be counteracted by the soluble receptor for AGE (sRAGE). In fact, sRAGE is a competitive inhibitor for AGE–RAGE interaction; if necessary, it can become a scavenger receptor for circulating AGEs and has a cytoprotective action against the adverse effects of AGE–RAGE interaction [7, 9].

The human body possesses other endogenous biochemical defense mechanisms such as the enzymatic degradation of AGEs (e.g., glyoxalase system), AGE-receptor-mediated degradation (e.g., binding of AGEs to other cell surface AGE receptors such as AGER1, AGER2, AGER3) or renal excretion depending of kidney function [10]. In fact, AGE free adducts accumulate in the plasma of CKD patients proportionally to the reduction in glomerular filtration rate (GFR) [1], and in uremic patients, there is an increase in AGEs compared to healthy subjects. Furthermore, abnormal amounts of methylglyoxal (MG) and related carbonyl compounds have been observed in CKD patients, probably due to a down-regulation of glyoxalase 1 (Glo1) [1, 6]. Dicarbonyl stress and the RAGE interaction with AGEs may be considered a driver of CKD development [1]. Notably, it is also possible to exogenously counteract the effects of AGEs by acting directly on lifestyle factors (e.g., reducing the consumption of foods with a high content of AGEs, avoiding smoking, changing cooking methods, etc.) [7].

Nowadays, few studies are focused on the roles of protein-bound AGEs in CKD, and probably it is due to the complex mechanism derived from the interaction among AGEs and receptors that induce endothelial dysfunction [1]. Interactions between AGEs and their receptors, including RAGE, trigger various intracellular events, such as oxidative stress and inflammation, leading to cardiovascular complications [4]. Recently, the scientific community was interested in AGEs and their relation with the development of renal diseases and complications in CKD patients [4]. In fact, in advanced CKD and in dialyzed patients, a 2–20-fold increase in AGEs was observed [4]. Thus, AGEs are thought to represent a useful biomarker to monitor the oxidative stress of CKD patients and/or the efficiency of dialysis treatments.

The determination of AGEs remains experimentally expensive and time-consuming if assays able to discriminate the two classes of AGEs (protein-bound AGEs and free AGEs) are used. Mass spectrometry, HPLC and specific immunoassays are the most adopted procedures for this purpose. Furthermore, ELISA is also available, but it cannot distinguish between the two classes [11].

In the present clinical study on CKD patients, we compared the variation of total serum AGEs (measured through ELISA) with a typical biomarker of oxidative stress like oxidized human serum albumin (HSAox), before and after dialysis treatment.

HSA is a monomeric single polypeptide chain protein with 17 disulfide bonds and one free cysteine (i.e., Cys34), which has a variety of functions (e.g., enzymatic, ligand binding, etc.) [12–15]. In healthy subjects, about 70% of Cys34 is present in reduced form, while 25–30% is found in reversible oxidized form (i.e., mixed disulfide mainly with cysteine) [16, 17]. Under pathologic conditions, like kidney dysfunction or liver diseases, the level of HSAox may increase up to 70% [18–22]. Recently, HSAox level was evaluated in kidney transplanted patients, and it was used as a short-term biomarker of oxidative stress in this population [16]. Our method for oxidized albumin determination is simple and cheap and requires only a few minutes for analysis and allows to quantify the entire pool of oxidized albumin forms, essential for the diagnosis of oxidative stress [23]. The aim of this study is to analyze the concentration of total AGEs and HSAox before and after dialytic procedures in 131 hemodialysis (HD) patients, evaluating the impact of different dialytic techniques on these biomarkers. We are aware that in hemodialysis many factors may affect the redox equilibrium, such as non-dialysis-related (lifestyle) factors (like smoking, diet, fluid status) and dialysis-related factors (like HD solution, type and dosage of heparin, anemia, iron status, erythropoiesis-stimulating agents (ESA), etc.) [7, 24, 25]; however, we limited our observations to the changes of levels of AGEs and HSAox during the single dialytic treatment, regardless of the cause determining the different levels of these biomarkers in patients before each dialytic session.

Materials and methods

Design of the study and patients

The control group was made up of 50 volunteers from the Transfusion Medicine Section of the Policlinico Tor Vergata (PTV). One hundred and thirty hemodialysis patients were enrolled from the Department of Internal Medicine-Center of Hypertension, Nephrology Unit, PTV. Two different dialysis techniques were performed: 109 patients with diffusive technique and 22 patients with convective technique. Furthermore, two filter types were used: polysulfone ($N = 121$ patients) and polymethacrylate ($N = 10$ patients).

Inclusion criteria were age > 18 years, end-stage renal disease (ESRD) in hemodialysis treatment for at least 6 months and the same hemodialysis technique in the last 3 months.

Exclusion criteria in both healthy controls and HD patients were a clinical history of virus hepatitis B, C and HIV, active rheumatologic disorders (such as systemic lupus erythematosus), active cancer and pregnancy.

Laboratory parameters

Early morning blood samples were taken from each patient for biochemical screening test after 12 h overnight fasting. Blood samples were collected into tubes (Vacutainer, BD, Plymouth, UK) containing ethylenediaminetetraacetate. Blood for the control group was obtained via venipuncture from the antecubital vein. Blood samples for HD patients were collected from the arterial site of the vascular access before and after the dialysis session and at the end of the long interdialytic interval, for monitoring HSAox, AGEs, albuminemia and azotemia.

All samples were placed on ice and plasma and were separated by centrifugation at 1600×*g* for 10 min at 4 °C. Samples were stored with the same modality for HD patients and the control group.

AGEs and HSAox were analyzed on the same blood sample within 1 h from delivery; serum samples selected for intra-assay and inter-assay analysis were stored at − 20 °C (for HSAox and total AGEs) and − 80 °C (for HSAox).

An automated hematology analyzer XE-2100 (Sysmex, Kobe, Japan) was used for hemoglobin (Hb) level quantification. All other routine parameters were determined using Dimension VISTA 1500 (Siemens Healthcare Diagnostics, Milano, Italy). The lipid profile (total cholesterol, triglyceride, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol) was determined by standard enzymatic colorimetric techniques (Roche modular P800, Roche diagnostics, Indianapolis, IN, USA). All other parameters were analyzed according to standard procedures in the Clinical Chemical Laboratories of the PTV.

Clinical parameters

Systolic (Psyst) and diastolic (Pdiast) blood pressures were registered before and after dialytic sessions.

Chemicals and reagents

We used cystamine and Ellman's reagent-5,5′-dithiobis(2-nitrobenzoic) acid (DTNB) (Sigma-Aldrich, St. Louis, MO,

USA), as well as sterile phosphate saline buffer (EuroClone, Pero, Milan, Italy).

Oxidized human serum albumin

HSAox was determined by subtracting the value of reduced HSA from the total HSA estimated from routine clinical assay. Ellman's reagent reacts slowly with Cys34; this amino acid represents the real chemical determinant for the titration of reduced HSA. The level of reduced HSA cannot be evaluated directly. Therefore, we used a modified procedure [15, 16] based on the fast reaction of cystamine with Cys34. The released cysteamine is stoichiometric with Cys34 and fast determined with DTNB ($\varepsilon_{412\,nm}$ of $TNBS^- = 14{,}100\ M^{-1}\ cm^{-1}$). The assay was performed with a Kontron Uvikon 941 Plus spectrophotometer (Kontron Instruments) at 412 nm (25 °C). 50 µl of human serum was diluted in 890 µl of potassium phosphate buffer 0.1 M pH 8.0, recording an autozero sample. After that, 50 µl of DTNB (50 µM final concentration) and 10 µl of cystamine (1 mM final concentration) were added to the solution. After an incubation of around 15 min at room temperature, the absorbance was recorded. Control group healthy subjects were selected for intra-assay ($N = 20$) and inter-assay ($N = 20$). The intra-assay coefficient of variation was 3.2% (five replicates for each sample on the same day within 2 h). The inter-assay coefficient of variation was 2.3% (3 days at the same time) and 2.4% (3 days at the same time) for samples stored at − 20 °C and − 80 °C, respectively.

AGE determination

Cell Biolabs' OxiSelect™ AGE Competitive ELISA Kit enzyme immunoassay (STA-817, Cell Biolabs, Inc., San Diego, CA) was used for the rapid detection and quantification of AGE protein adducts. The quantity of AGE adduct (µg/ml) in serum samples was determined by comparing its absorbance with that of a known AGE–bovine serum albumin (BSA) standard curve. An AGE conjugate was coated on an ELISA plate. The unknown AGE samples or AGE-BSA standards were added to the AGE conjugate preabsorbed ELISA plate. After a brief incubation, an anti-AGE polyclonal antibody was added, followed by horseradish peroxidase-conjugated secondary antibody. The content of AGE protein adducts in unknown samples was determined by comparison with a pre-determined AGE-BSA standard curve [10]. Control group healthy subjects were selected for intra-assay ($N = 20$) and inter-assay ($N = 20$). The intra-assay coefficient of variation was 6.9% (three replicates for each sample on the same day). The inter-assay coefficient of variation was 9.2% (3 days at the same time).

Statistical and graphical analysis

All data were initially entered into an Excel spreadsheet (Microsoft, Redmond, WA, USA), and the statistical analysis was performed using the Statistical Package for the Social Sciences Windows, version 15.0 (SPSS, Chicago, Illinois, USA). The descriptive statistics consisted of the average ± standard deviation (SD) for parameters with normal distribution (after confirmation with histogram and/or Kolmogorov–Smirnov test), the median and the interval (minimum, maximum) for variables with non-normal distributions. The comparison of normal coupled parameters was performed with ANOVA for repeated measurements, while for the comparison between independent data, ANOVA one-way analysis was performed (if the groups compared were greater than two, the Bonferroni post hoc test was also performed). Comparisons between coupled non-normal distribution variables were performed using Wilcoxon's test, while for the comparisons between independent datasets, the Kruskal–Wallis H test was applied. Finally, comparisons between occurrence variables were executed using χ^2 test or Fisher's exact test if cell number was < 5. A value of $p < 0.05$ was considered statistically significant. Correlation analysis was performed by the Pearson correlation R-coefficient. The results and graphic visualization were obtained by GraphPad Prism (La Jolla, CA, USA).

Results

The epidemiological and clinical features of hemodialysis patients are summarized in Table 1.

The routine pre-dialysis laboratory parameters of HD patients are reported in Table 2. The lipid profile parameters have been divided into the following categories: total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides. All the laboratory parameters were similar for the two different dialytic techniques by one-way analysis.

We also compared all clinical parameters in the pre- and post-dialysis sessions. Their statistical significance is reported in Table 3. The clinical parameters examined displayed significant differences between pre- and post-dialysis values (except for the diastolic pressure and heart rate) with both diffusive and convective techniques.

In Table 4, we show HSAox and AGE values, monitored in the pre- and post-dialysis sessions in all HD enrolled patients. Both before and after dialysis, HSAox and AGE levels are significantly enhanced in comparison with the values measured in healthy subjects (control group) except for HSAox.

This parameter is similar between the control group and the post-dialysis convective group. Surprisingly, the AGE

Table 1 Epidemiological and clinical features of hemodialysis patients

Number of patients, *N*	131
Male/female, *N*	96/35
Age, years*	69 ± 14
Dialytic age, months**	36 (6–345)
Dialysis session, weekly frequency*	3.0 ± 0.3
Diabetes mellitus, *N* (%)	33 (25)
Arterial hypertension, *N* (%)	47 (37)
Dialytic techniques	
(a) Convective, *N* (%)	22 (17)
(b) Diffusive, *N* (%)	109 (83)
Hemodialysis filter type	
(a) Polysulfone, *N* (%)	121 (92)
(b) Polymethacrylate, *N* (%)	10 (8)
Primitive cause of ESRD	
(a) Chronic glomerulonephritis, *N* (%)	16 (12)
(b) Nephroangiosclerosis, *N* (%)	43 (33)
(c) ADPKD, *N* (%)	5 (4)
(d) Chronic pyelonephritis, *N* (%)	4 (3)
(e) Diabetic nephropathy, *N* (%)	24 (19)
(f) Kidney cancer, *N* (%)	3 (2)
(g) Unknown cause of ESRD, *N* (%)	36 (27)

ESRD end-stage renal disease, *ADPKD* autosomal dominant polycystic kidney disease

*Data expressed as average ± standard deviation

**Data expressed as median (range minimum–maximum)

Table 2 Pre-dialysis laboratory parameters of 131 hemodialysis patients

Laboratory parameters	Average ± SD
Total cholesterol (mg/dl)	147.2 ± 42.7
LDL cholesterol (mg/dl)	74.0 ± 35.7
HDL cholesterol (mg/dl)	39.4 ± 11.8
Triglycerides (mg/dl)	161.4 ± 89.7
CRP (μg/dl)	2.0 ± 2.2
Hemoglobin (g/dl)	10.7 ± 1.2
Leukocytes ($N \times 10^6$)	6456.0 ± 1884.6
Hematocrit (%)	32.7 ± 4.3
Azotemia (mg/dl)	158.0 ± 33.8

Data expressed as mean ± SD

LDL low-density lipoprotein, *HDL* high-density lipoprotein, *CRP* C-reactive protein

levels after dialysis were higher than those found before dialysis, while an opposite trend was observed for HSAox. Even these differences are statistically significant as confirmed by ANOVA.

Subdividing the sample into two groups based on dialytic techniques (convective and diffusive), we observed

Table 3 Clinical parameters pre- and post-dialysis of patients, subdivided according to two different dialysis techniques

Clinical parameters	Pre-dialysis	Post-dialysis	*p*
Body weight (kg)			
(1) Total HD patients	71.3 ± 15.8	68.9 ± 15.6	0.001
(2) Convective	72.1 ± 11.4	68.9 ± 11.5	0.002
(3) Diffusive	71.1 ± 16.6	68.8 ± 16.3	0.002
Albumin (g/dl)			
(1) Total HD patients	3.8 ± 0.5	3.9 ± 0.5	0.022
(2) Convective	3.8 ± 0.5	4.0 ± 0.4	0.022
(3) Diffusive	3.8 ± 0.5	3.9 ± 0.5	0.022
Psyst (mmHg)			
(1) Total HD patients	138.5 ± 23.6	133.5 ± 21.5	0.001
(2) Convective	136.9 ± 29.4	121.8 ± 23.6	0.034
(3) Diffusive	138.8 ± 22.7	135.5 ± 20.7	0.034
Pdiast (mmHg)			
(1) Total HD patients	63.9 ± 13.3	63.6 ± 13.4	ns
(2) Convective	63.9 ± 15.8	60.8 ± 15.3	ns
(3) Diffusive	63.6 ± 12.9	64.1 ± 13.1	ns
Heart rate (bpm)			
(1) Total HD patients	69.1 ± 10.1	71.2 ± 10.4	ns
(2) Convective	69.5 ± 9.5	70.1 ± 9.6	ns
(3) Diffusive	69.0 ± 10.4	71.4 ± 10.6	ns
Azotemia (mg/dl)			
(1) Total HD patients	158.0 ± 33.8	53.8 ± 17.7	0.001
(2) Convective	174.0 ± 38.0	53.6 ± 22.1	0.001
(3) Diffusive	155.4 ± 32.8	52.6 ± 17.2	0.001

Data expressed as average ± standard deviation

HD hemodialysis, *Psyst* systolic pressure, *Pdiast* diastolic pressure

ns not significant; *p* values < 0.05 are considered as statistically significant

Table 4 HSAox and total AGE values in patients subdivided according to two different dialysis techniques

Laboratory parameters	Pre-dialysis	Post-dialysis	*p*
HSAox (%)			
Control value 38 ± 5			
(1) Total HD patients	58.5 ± 8.8	41.5 ± 11.1	< 0.001
(2) Convective	56.0 ± 9.1	37.8 ± 13.6	< 0.001
(3) Diffusive	59.0 ± 8.7	42.3 ± 10.4	< 0.001
AGEs (μg/mL)			
Control value 0.6 ± 0.2			
(1) Total HD patients	6.8 ± 5.2	9.2 ± 4.4	0.008
(2) Convective	8.6 ± 4.8	10.5 ± 2.9	0.008
(3) Diffusive	6.23 ± 5.3	8.9 ± 4.7	0.008

Data expressed as average ± standard deviation

HSAox oxidized human serum albumin, *HD* Hemodialysis, *AGEs* Advanced glycation end products

ns not significant; *p* values < 0.05 are considered as statistically significant

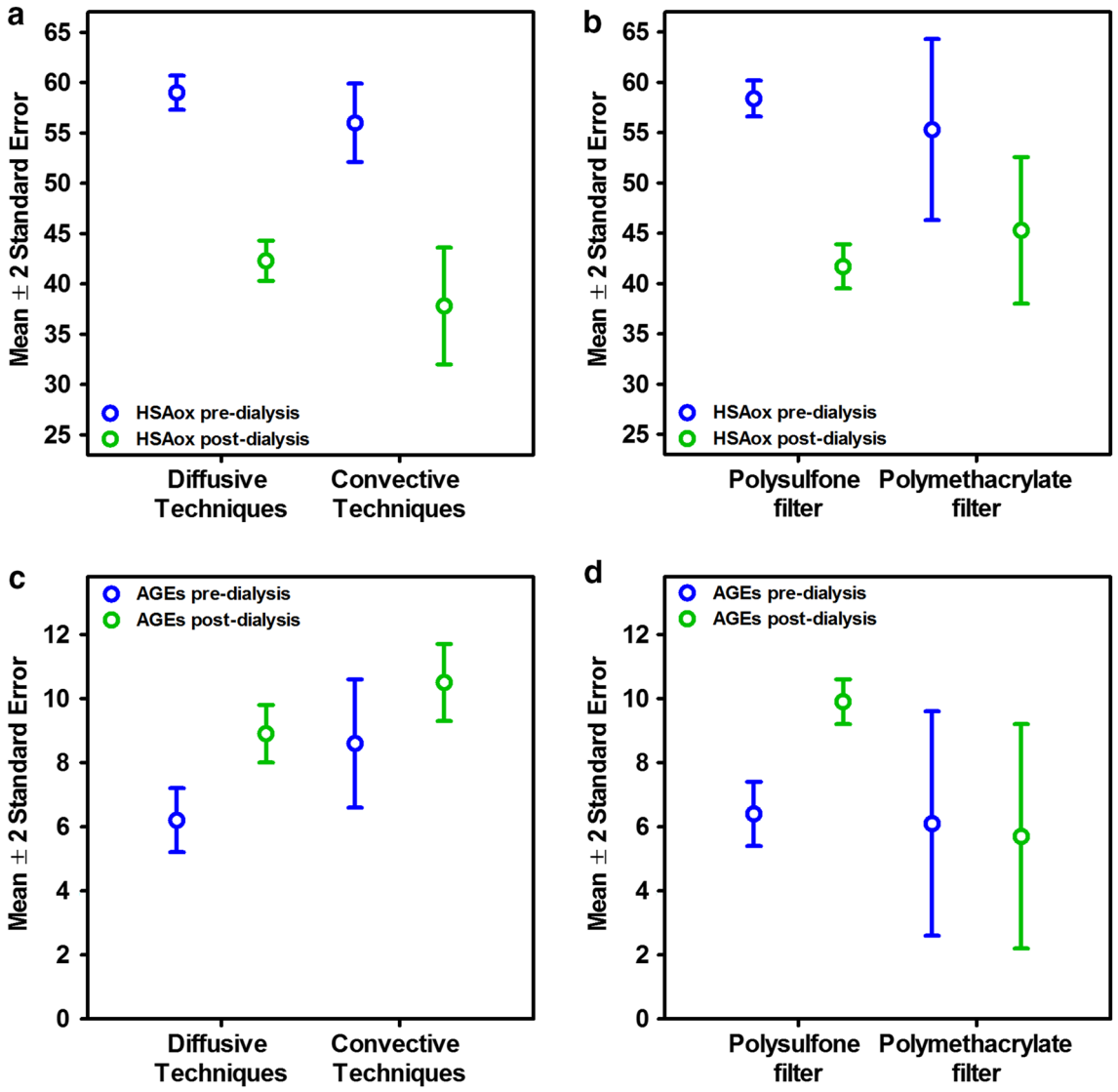

Fig. 1 Oxidized human serum albumin percentage values and AGE values. **a** HSAox percentages pre- (blue) and post-dialysis (green) based on dialytic techniques and **b** on dialysis filters. In panels a and b, data of HSAox (%) (see in "Materials and methods" section) are reported as mean ± 2 standard error (95% confidence interval). **c** AGE values pre- (blue) and post-dialysis (green) based on dialytic procedures and **d** on dialysis filters. In panels c and d, data of AGE (μg/mL) (see in "Materials and methods" section) are reported as mean ± 2 standard error (95% confidence interval) (color figure online)

a significant reduction in HSAox percentage post-dialysis compared to pre-dialysis in both groups (Fig. 1a, b). Conversely, AGE post-dialysis levels increased significantly in all HD patients compared to pre-dialysis values.

Clinical parameters as well as HSAox and AGE pre- and post-dialysis were analyzed by ANOVA test to establish the influence of diabetes mellitus. Data reported in Table 5 likely indicate that diabetes does not appear as a factor influencing the changes in HSAox and total AGE levels before and after dialysis session.

We also performed a Pearson correlation between AGE values pre- and post-dialytic procedures and HSAox percentage pre- and post-dialysis with some clinical parameters (such as dialytic age, leukocyte, hematocrit, C-reactive protein, total proteins and total cholesterol), but we did not find any significant correlation.

To assess whether the dialysis filters or dialytic procedures have some effect on hematic AGE concentration, we performed an ANOVA test for repeated measurements with the Bonferroni test (Fig. 1c, d); the dialytic procedures did not influence the values of AGEs, while the filters seemed to influence their concentration. Specifically, there is no difference between AGE concentration pre- and post-dialysis if patients are treated with a polymethacrylate filter, while we observed

Table 5 Clinical parameters pre- and post-dialysis of HD patients divided by the presence or absence of diabetes mellitus

Clinical parameters	Pre-dialysis	Post-dialysis	*p*
Body weight (kg)			
(1) Total HD patients	71.0 ± 15.6	68.6 ± 15.4	0.001
(2) Diabetic	76.1 ± 14.9	73.4 ± 14.7	0.001
(3) Non-diabetic	69.2 ± 15.5	66.9 ± 15.4	0.001
Albumin (g/dl)			
(1) Total HD patients	3.8 ± 0.5	3.9 ± 0.5	0.01
(2) Diabetic	3.8 ± 0.4	3.9 ± 0.4	0.01
(3) Non-diabetic	3.8 ± 0.5	3.9 ± 0.5	0.01
Psyst (mmHg)			
(1) Total HD patients	137.9 ± 23.4	133.4 ± 21.5	ns
(2) Diabetic	144.8 ± 22.6	140.2 ± 19.7	ns
(3) Non-diabetic	134.8 ± 23.2	130.4 ± 21.7	ns
Pdiast (mmHg)			
(1) Total HD patients	63.9 ± 13.2	63.5 ± 13.3	ns
(2) Diabetic	61.6 ± 11.4	62.1 ± 12.7	ns
(3) Non-diabetic	65.0 ± 13.8	64.1 ± 13.6	ns
Heart rate (bpm)			
(1) Total HD patients	69.1 ± 10.6	71.2 ± 10.7	ns
(2) Diabetic	69.0 ± 11.1	70.9 ± 10.4	ns
(3) Non-diabetic	69.2 ± 10.5	71.2 ± 10.9	ns
Azotemia (mg/dl)			
(1) Total HD patients	158.0 ± 33.8	53.6 ± 21.0	0.001
(2) Diabetic	166.9 ± 30.6	56.7 ± 17.1	0.001
(3) Non-diabetic	156.4 ± 38.5	53.1 ± 21.8	0.001
HSAox (%)			
(1) Total HD patients	58.1 ± 9.2	41.8 ± 11.4	<0.001
(2) Diabetic	58.8 ± 8.2	43.9 ± 11.5	<0.001
(3) Non-diabetic	57.8 ± 9.7	41.0 ± 11.3	<0.001
AGEs (μg/ml)			
(1) Total HD patients	6.9 ± 5.2	9.2 ± 4.5	0.0005
(2) Diabetic	6.0 ± 5.0	9.9 ± 3.3	ns
(3) Non-diabetic	7.1 ± 5.2	9.1 ± 4.8	0.0155

Data expressed as average ± standard deviation

Psyst systolic pressure, *Pdiast* diastolic pressure

ns not significant; *p* values < 0.05 are considered as statistically significant

a significant increase in AGE concentration in patients treated with a polysulfone filter ($p = 0.017$).

Discussion

This study examines the possible impact of different dialytic techniques and filters on several clinical parameters including total AGEs and HSAox. Apart from the well-known changes in clinical parameters before and after dialysis (like weight loss, decreased albumin and azotemia, etc.), we observed that after dialysis procedure, HSAox decreases in both dialytic techniques (convective and diffusive) likewise in the different filters examined. Hemodialysis removes many low molecular weight toxins and metabolic byproducts which may generate oxidative stress. As recently described, the cystine/cysteine ratio is the main determinant for the redox state of Cys34 in albumin [15]. An efficient cystine removal from the blood through the dialysis procedure is the reliable cause of the observed decrease in HSAox after dialytic treatment. Kinetic constants for the reaction of Cys34 with cysteine/cystine are fully compatible with the time of single dialytic session (about 4 h) [15].

Conversely, a statistically significant increase in total AGEs occurs after dialysis. An unlikely explanation of this finding may be due to extracorporeal circulation that generates oxidative stress and a consequent AGE increase. In fact, this possibility can be discarded observing the reduction in HSAox after dialysis. It is useful to remember that our ELISA does not distinguish between protein-bound and free AGEs.

Moreover, HD is efficient to remove small uremic and water-soluble toxins (such as creatinine and azotemia). Its removal capacity is limited for compounds bound to macromolecules like protein-bound AGEs [26].

The AGE increase can be partly explained by the proinflammatory environment that results from the extracorporeal circulation, as well as by the poor removal capacity of AGEs through dialysis membranes. The ability to remove AGEs with diffusive or convective techniques (like hemofiltration or hemodiafiltration) seems to be limited only to about 30% [27].

Moreover, it should be considered that the plasma water ultrafiltration causes relative enhancement in compounds bound to proteins.

An increase in protein-bound AGEs has been previously reported [7, 16, 21]. In fact, a previous study demonstrated that only the combination of HD and hemoperfusion (HF) is able to significantly decrease AGE levels after dialytic procedures [28]. Therefore, this dialytic combination could be the best approach to remove protein-bound uremic toxins.

In conclusion, it appears that HSAox is a simple and reliable biomarker to verify the beneficial effect of a single dialysis session on the redox imbalance, a common condition in ESRD patients.

Conversely, measurement of total AGEs as performed using ELISA gives confounding results. In this context, convective dialysis appears to be a better technique to ameliorate the hematic redox imbalance compared to diffusive dialysis. In fact, the patients undergoing convective techniques showed lower mean values of HSAox compared to patients in diffusive therapy (Fig. 1a, b). Moreover, post-dialytic percentage of HSAox in HD convective patients seems to be similar to the values observed in the control group (healthy

subjects). The convective dialysis seems to be the best depurative method for removing oxidizing toxins.

A further comment must be made about the dialytic efficiency of different filters. High-flux filters (like polymethacrylate) seem to be able to remove a greater amount of AGEs. Our data confirmed the results obtained by De Smet et al. [29]. These authors observed that super-flux cellulose triacetate membranes were more efficient with respect to low flux cellulose triacetate membranes in removing most protein-bound compounds, especially indoxyl sulphate [29]. Conversely, low flux membranes were related to increased levels of AGEs in post-dialytic session with respect to pre-dialytic values (Fig. 1c, d).

Randomized clinical trials (RCTs) will be needed to confirm the usefulness and diagnostic power of HSAox as a biomarker of oxidative stress. These RCTs should be conducted on a larger number of patients and should compare HSAox with other oxidative stress biomarkers such as serum activity extracellular superoxide dismutase [30] and the expression/activation of sirtuin-1 [31].

Conclusion

HSAox represents a more reliable biomarker in the evaluation of oxidative stress in ESRD patients with respect to AGEs. HSAox is also useful to check the beneficial effects of hemodialysis using different techniques and filters against the oxidative stress due to impaired kidney function.

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Data availability The data used to support the findings of this study are available from the corresponding author upon request.

Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Standard Statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee "Comitato Etico Indipendente"—Azienda Ospedaliera Universitaria Policlinico Tor Vergata (experimentation Register Number 60/16) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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