Original Article

Hemodialysis raises oxidative stress through carbon-centered radicals despite improved biocompatibility

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Leukocyte activation and the resulting oxidative stress induced by bioincompatible materials during hemodialysis impact the prognosis of patients. Despite multiple advances in hemodialysis dialyzers, the prognosis of hemodialysis patients with complications deeply related to oxidative stress, such as diabetes mellitus, remains poor. Thus, we re-evaluated the effects of hemodialysis on multiple reactive oxygen species using electron spin resonance-based methods for further improvement of biocompatibility in hemodialysis. We enrolled 31 patients in a stable condition undergoing hemodialysis using high-flux polysulfone dialyzers. The effects of hemodialysis on reactive oxygen species were evaluated by two methods: MULTIS, which evaluates serum scavenging activities against multiple hydrophilic reactive oxygen species, and i-STrap, which detects lipophilic carbon-center radicals. Similar to previous studies, we found that serum hydroxyl radical scavenging activity significantly improved after hemodialysis. Unlike previous studies, we discovered that scavenging activity against alkoxyl radical was significantly reduced after hemodialysis. Moreover, patients with diabetes mellitus showed a decrease in serum scavenging activity against alkyl peroxyl radicals and an increase in lipophilic carbon-center radicals after hemodialysis. These results suggest that despite extensive improvements in dialyzer membranes, the forms of reactive oxygen species that can be eliminated during dialysis are limited, and multiple reactive oxygen species still remain at increased levels during hemodialysis.

***Key Words*: hemodialysis, biocompatibility, alkyl peroxyl radical, electron spin resonance, MULTIS, i-STrap**

Chronic kidney disease (CKD) is known to be associated with high oxidative stress.[1–4] Accumulation of uremic toxins with pro-oxidative properties leading to microinflammation caused by the elevation of pro-inflammatory cytokines is the key feature of CKD pathophysiology.[5–7] Hemodialysis (HD), a widely conducted renal replacement therapy, acts as a double-edged sword to reduce oxidative stress in patients with CKD. Activation of polymorphonuclear leukocytes, due to contact with the membrane or other artificial surfaces in HD circuits, is the main cause of oxidative stress induced by HD.[8–11] Conversely, the removal of pro-oxidative uremic toxins, especially low-molecular weight hydrophilic compounds such as trimethylamine-*N*-oxide or guanidino compounds, may improve the antioxidative nature of HD, leading to long-term improvement of survival rate and quality of life in patients with CKD.[12,13] In addition, correction of acidosis leading to re-activation of antioxidative enzymes and reduction of volume overload leading to prevention of cardiovascular complications are antioxidative mechanisms.[14] Thus, it is necessary for HD materials to minimize leukocyte activation and maximize antioxidative effects. In order to overcome this problem, antioxidative and biocompatible HD materials exemplified by the vitamin E-coated dialyzer have been developed.[15–17] However, despite the numerous advances in HD dialyzers, the prognosis of HD patients remains unsatisfactory, especially those with diabetes mellitus (DM), a disease that promotes a highly oxidative state.[18]

These concerns are partly due to the fact that the details of widely varied and complexed *in vivo* oxidative stress-related reactions have not been sufficiently analyzed. Thus, this study aimed to clarify the effect of HD on reactive oxygen species (ROS) dynamics, as the upstream events of oxidative stress reactions, based on the concept that each target site of oxidative and antioxidant reactions in the body needs to be biochemically described in a specific time and space. One explanation for this is a lack of detailed analysis of the upstream side of oxidative stress-related reactions that stimulates these cellular reactions. The identification of ROS that act as stimulators of oxidative stress reactions or interactions among ROS to generate oxidative stimulators is difficult due to the high reaction rates and complex reaction chains. Since ROS are not uniform and individual ROS have specific characteristics during *in vivo* reactions, an analysis of multiple ROS is required.[19–21] Thus, to improve biocompatibility based on the use of antioxidative and biocompatible HD materials, one must understand the changes in ROS dynamics caused by HD.

To investigate ROS dynamics during HD, we employed two newly developed electron spin resonance (ESR)-based methods: the multiple hydrophilic free-radical scavenging assay (MULTIS) and the lipophilic radical detection assay on whole blood (i-STrap). Although research on ROS scavenging activity by ESR is not extensive, it remains the only method available for identifying the type and examining the dynamics of ROS. The MULTIS method combines a high-performance liquid chromatography-type flow system to an ESR system and employs 5-(2,2-dimethyl-

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Table 1. Photolytic ROS production methods used in MULTIS measurements

Free radical	Spin trap	Precursor/Sensitizer	UV/VL	Irradiation period	Antioxidant equivalent
$^{\bullet}OH$	CYPMPO	H_2O_2 10 mM	UV	5 s	GSH
$O_2^{\bullet-}$	CYPMPO	Riboflavin 20 μM	VL	60 s	SOD
$RO^{\bullet}$	CYPMPO	AAPH 10 mM	UV	5 s	Trolox
$ROO^{\bullet}$	CYPMPO	*t*BHP 10 mM	UV	5 s	α-lipoic acid
$^1O_2$	TEMP	Rose bengal 200 μM	VL	30 s	GSH

ROS, reactive oxygen species; MULTIS, multiple hydrophilic free-radical scavenging assay; UV, ultraviolet (300–400 nm); VL, visual light (500–600 nm); AAPH, 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride; *t*BHP, *tert*-butyl hydroperoxide; GSH, glutathione; SOD, superoxide dismutase; CYPMPO, 5-(2,2-dimethyl-1,3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline *N*-oxide; TEMP, 4-hydroxy-2,2,6,6-tetramethylpiperidine.

1,3-propoxy cyclophosphoryl)-5-methyl-1-pyrroline *N*-oxide (CYPMPO) as the spin trap. MULTIS provides higher sensitivity and stability for the ROS scavenging measurement than conventional ESR assays.[22–24] One of our co-authors has previously reported MULTIS-measured ROS scavenging activities in patients with stage 5 CKD. However, they did not evaluate the effect of HD.[22] Additionally, the i-STrap method is an ESR-based method that detects ROS in whole blood using 2-diphenylphosphinoyl-2-methyl-3,4-dihydro-2H-pyrrole *N*-oxide (DPhPMPO) as its spin trap and measures ESR after an organic extraction, thus reflecting lipophilic ROS.[25,26] In this study, we found that the levels of several ROS were increased and not eliminated by HD, indicating that radical chain reactions are still not adequately controlled following HD. This may affect the prognosis of patients with CKD.

Materials and Methods

Subjects and informed consent. All procedures conducted on human subjects were performed after obtaining individual written consent. The protocol that was used was approved by the Tsukuba University of Technology Committee (Authorization No. 201809). This is an observational study and, therefore, no interventions were performed on the patients.

A total of 31 stable HD patients were included in the study. We obtained and recorded clinical and demographic information for each patient. All patients were treated with high-flux polysulfone membrane dialyzers (Toray Medical Co., Ltd., Tokyo, Japan) with various membrane surface areas, adapted to the body constitution of each individual. The patients were divided into two groups depending on the cause of their end-stage renal disease: the non-diabetic group (non-DM group, $n = 17$) and the diabetic group (DM group, $n = 14$).

Sample collection. Blood samples were obtained at the onset and end of the dialysis treatment from the sampling port located on the arterial side of the HD circuit. For MULTIS measurements, sera were separated and stored in a freezer at –80°C until the measurement was performed; for i-STrap measurements, whole-blood samples were collected in heparin-coated tubes that were allowed to stand for one hour before measurement.

Measurements of multiple free radical scavenging activity in serum. Hydrophilic ROS scavenging activities were measured with the MULTIS method using previously described protocols with minor modifications.[22,27] Scavenging activities against five ROS, namely hydroxyl radical ($^{\bullet}OH$), superoxide ($O_2^{\bullet-}$), alkoxyl radical ($RO^{\bullet}$), alkyl peroxyl radical ($ROO^{\bullet}$), and singlet oxygen ($^1O_2$), were measured. An X-band ESR spectrometer (RR-X1 ESR; Radical Research Inc., Tokyo, Japan) employing 100 kHz field modulation and WIN-RAD operation software (Radical Research Inc.) was used. The ESR spin trapping reagents used were CYPMPO for $^{\bullet}OH$, $O_2^{\bullet}$, $RO^{\bullet}$, and $ROO^{\bullet}$ and 4-hydroxy-2,2,6,6-tetramethylpiperidine (TEMP) for $^1O_2$. The typical spectrometer settings were as follows: field modulation width, 0.1 mT; microwave power, 10 mW; field scan width and rate, ±7.5 mT/2 min; and time constant, 0.1 s. Each ROS was generated via *in situ* illumination with UV/visible light from an illuminator (RUVF-203SR UV illuminator; Radical Research Inc.) equipped with a 200 W medium-pressure mercury/xenon arc lamp and a quartz light-guide, connected to the resonator cavity. The light sources, illumination times, precursors, and photosensitizers used to produce ROS are summarized in Table 1. The ROS scavenging activities were calculated according to a previously described method[22] and converted into the unit equivalent to known pure scavengers: glutathione (GSH) for $^{\bullet}OH$ and $^1O_2$, superoxide dismutase (SOD) for $O_2^{\bullet-}$, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) for $RO^{\bullet}$, and α-lipoic acid for $ROO^{\bullet}$. MULTIS measurements for each ROS were performed in triplicate.

Measurement of lipophilic ROS scavenging activity in whole blood using i-Strap. Lipophilic ROS scavenging activity was measured using the i-STrap ESR measurement kit (Dojin-Glocal/Dojindo, Kumamoto, Japan) based on the manufacture's protocol and a previous report.[25] This method is based on the competitive reaction between antioxidants in whole blood and DPhPMPO with the tert-butyl hydroperoxide (BuOOH) radical. Whole blood samples were incubated with DPhPMPO (10 mM) and tert-BuOOH (10 mM) for 30 min at room temperature. After incubation, the spin adducts in the organic phase were extracted using chloroform and methanol solutions; ESR measurements were conducted using these organic samples. The ESR measuring conditions were the same as for the MULTIS measurement, except for the field scan width and rate, set at ±5.0 mT/2 min. There were no specific antioxidants for i-STrap measurement; therefore, scavenging activity was expressed as an I0/I−1 value, where "I0" denotes the signal intensity without a sample and "I" denotes the signal intensity with a sample.[28]

Reagents. CYPMPO was obtained from RR INC. (Tokyo, Japan); riboflavin, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH), *tert*-BuOOH, dimethyl sulfoxide (DMSO), and rose bengal were purchased from Sigma-Aldrich Japan (Tokyo, Japan); and TEMP was purchased from Tokyo Chemical Industry (Tokyo, Japan). Hydrogen peroxide and buffers were obtained from Wako Chemical Co. (Osaka, Japan).

Statistical analysis. Statistical analysis was performed using Prism 6 for Mac OS X computer software (GraphPad Software Inc., La Jolla, CA). Data were tested using Student's paired *t* test. Data were expressed as mean values with 95% confidence interval (95% CI).

Results

Profile of the patients group. Clinical demographic information on the patients are summarized in Table 2. There were no significant differences in age, male/female ratio, and duration of renal replacement therapy between the two groups.

Estimation of ESR spectra in MULTIS and i-STrap. The ESR spectra of the $^{\bullet}OH$, $O_2^{\bullet-}$, $RO^{\bullet}$, $ROO^{\bullet}$, and $^1O_2$ adducts of the spin-trapping agent observed in the process of the MULTIS

Table 2. Clinical profile of the patients

	Total	Non-DM	DM
Number	31	17	14
Age (95% CI, years)	68.1 (63.7–72.5)	67.7 (60.8–72.5)	68.6 (62.6–74.6)
Male/Female	17/14	9/8	8/6
HD Duration (mo.)	53.0 (33.8–72.2)	52.6 (22.7–82.6)	53.5 (26.0–81.0)

Cause of CKD in non-DM patients: chronic glomerulonephritis 8, nephrosclerosis 5, lupus nephritis 1, myeloma kidney 1, and unknown 2. All patients were treated with polysulfone dialyzers with various surface areas. DM, diabetes mellitus; HD, hemodialysis; 95% CI, 95% confidence interval.

Table 3. Serum scavenging activities against multiple ROS, before and after HD

ROS		Pre-HD Mean (95% CI)	Post-HD Mean (95% CI)	*p*
$^{\bullet}OH$	*mM-GSHeq*	6.37 (4.16–8.59)	13.3 (9.41–17.2)	<0.001
$RO^{\bullet}$	*μM-TROLOXeq*	1,180 (948–1,412)	752 (586–919)	<0.001
$ROO^{\bullet}$	*μM-αLA eq*	1,417 (1,057–1,777)	934 (644–1,223)	0.002
$O_2^{\bullet-}$	*U/ml-SODeq*	5.97 (4.94–7.01)	6.67 (5.79–7.55)	0.124
$^1O_2$	*μM-GSHeq*	47.4 (33.1–61.7)	43.4 (25.4–61.4)	0.675
LCCR	*I0/I−1*	1.96 (1.75–2.16)	1.89 (1.67–2.10)	0.301

The scavenging activities are converted to the equivalent unit of the specific scavenger against each ROS. The scavenging activity against the lipophilic carbon-centered radical is expressed as I0/I−1 value (see Materials and Methods). HD, hemodialysis; ROS, reactive oxygen species; 95% CI, 95% confidence interval; GSH, glutathione; αLA, α-lipoic acid; TROLOX, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; SOD, superoxide dismutase; LCCR, lipophilic carbon-centered radical.

and the i-STrap measurements were in concordance with the previously reported spectra of the corresponding radical adducts, confirmed by hyperfine coupling constants (Supplemental Fig. 1*).[23,27] The ESR spectra obtained from the i-STrap measurements were also in agreement with the data from a previous report.[25] In both methods, the signal intensity increased with an increase in the concentration of the relevant spin trapping reagent and decreased with the addition of sera, suggesting that these methods are based on competitive reactions of antioxidants in sera or whole blood.

Effect of HD on multiple ROS scavenging activities measured by MULTIS and i-STrap. First, we analyzed the changes of multiple ROS scavenging activities before and after HD in the entire patient cohort using the MULTIS method. The individual scavenging activity values are shown in Table 3, and the graphic depictions of changes in the pre- to post-HD values are shown in Fig. 1A–F. The $^{\bullet}OH$ scavenging activity of the HD patients before dialysis was 6.37 mM-GSHeq (95% CI: 4.16 to 8.59), a number consistent with the previously reported $^{\bullet}OH$ scavenging activity of patients with stage 5 CKD.[22] Scavenging activity was significantly improved after one HD session, and the difference between pre- and post-HD values was significant (mean difference: 6.94, 95% CI 3.96 to 9.91, $p<0.0001$; Fig. 1A).

In contrast, scavenging activities against $RO^{\bullet}$ and $ROO^{\bullet}$ were significantly altered after one HD treatment (for $RO^{\bullet}$, mean difference –441, 95% CI of difference –665 to –216, $p<0.001$; Fig. 1B; for $ROO^{\bullet}$, mean difference –526, 95% CI of difference –845 to –208, $p = 0.002$; Fig. 1C). There were no significant differences between pre- and post- HD serum scavenging activities against $O_2^{\bullet-}$ (mean difference 0.698, 95% CI of difference –0.202 to 1.60, $p = 0.124$; Fig. 1D) and $^1O_2$ (mean difference –3.98, 95% CI of difference –23.2 to 25.2, $p = 0.675$; Fig. 1E). The lipophilic scavenging activity showed no significant difference during the HD session (mean difference 0.0106, 95% CI of difference –0.00988 to 0.0310, $p = 0.300$; Fig. 1F).

Differences in the effect of hemodialysis on ROS scavenging activities depending on the presence or absence of diabetes. Since diabetes considerably contributes to the pathophysiological status of HD patients, we examined the impact of diabetes on the ROS scavenging activities. An increase in $^{\bullet}OH$ scavenging activity was observed in both the non-DM and DM groups (mean difference 5.48, 95% CI 1.72 to 9.23, $p = 0.007$ for the non-DM group; mean difference 9.08, 95% CI 3.79 to 14.40, $p = 0.003$ for the DM group; Fig. 2A). Concurrently, a decrease in $RO^{\bullet}$ scavenging activity was observed in both the non-DM and DM groups (mean difference –579, 95% CI –927 to –231, $p = 0.003$ for the non-DM group; mean difference –245, 95% CI –488 to –1.85, $p = 0.043$ for the DM group; Fig. 2B).

Conversely, a significant decrease in $ROO^{\bullet}$ was only observed in the DM group (mean difference –642, 95% CI –1113 to –170, $p = 0.012$ for the DM group; mean difference –433, 95% CI –911 to 45.1, $p = 0.073$ for the non-DM group; Fig. 2C). This tendency was the same for the lipophilic carbon-centered radical (displayed as the I0/I−1 value), and the scavenging activity was significantly reduced in only the DM group (mean difference 0.034, 95% CI 0.002 to 0.065, $p = 0.039$ for the DM group; mean difference –0.005, 95% CI –0.031 to 0.021, $p = 0.671$ for the non-DM group; Fig. 2F). There were no significant differences between pre- and post- HD serum scavenging activities against $O_2^{\bullet-}$ (mean difference 0.621, 95% CI of difference –0.506 to 1.750, $p = 0.260$ for the non-DM group; mean difference 0.792, 95% CI of difference –0.838 to 2.421, $p = 0.313$ for the DM group; Fig. 2D) and $^1O_2$ (mean difference 11.2, 95% CI of difference –32.9 to 10.5, $p = 0.291$ for the non-DM group; mean difference 8.98, 95% CI of difference –32.2 to 50.2, $p = 0.641$ for the DM group; Fig. 2E). The individual scavenging activity values are shown in Table 4. To clarify the redox effect of hemodialysis, a radar chart summarizing the changes in ROS scavenging activities caused by one HD session is shown in Fig. 3.

Discussion

Previous reports investigating the effects of HD treatment on the antioxidative status have yielded controversial results. Among these reports, few studies have investigated ROS involvement in the upstream oxidative stress-related reactions that occur during HD. Most reports investigating ROS studied non-specific radicals, and only few reports identified the type of ROS in detail. Moreover, most of these studies were limited to $^{\bullet}OH$ and $O_2^{\bullet-}$.[10,11,29,30] Previous reports evaluated $^{\bullet}OH$ and $O_2^{\bullet-}$ scavenging activities

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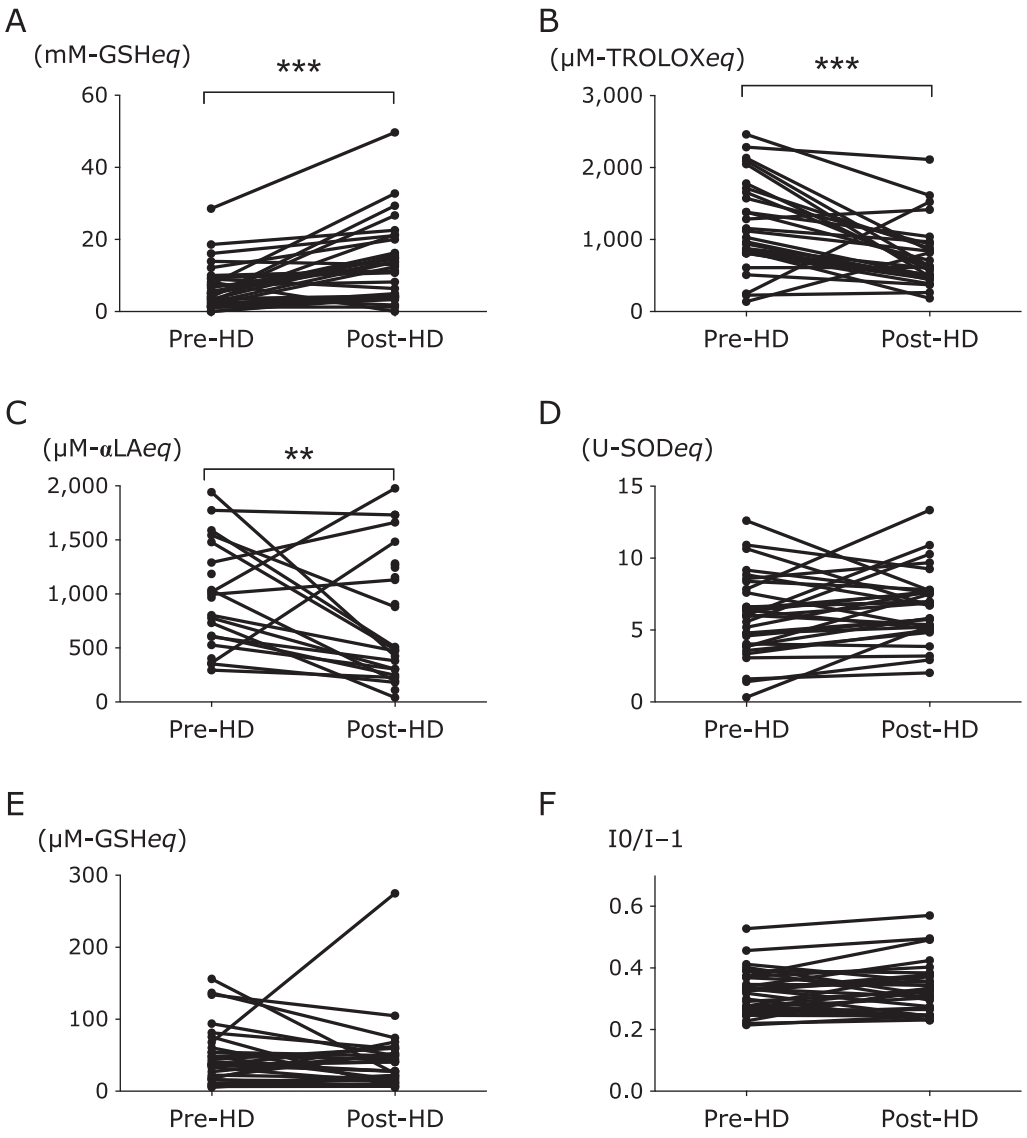

Fig. 1. Effect of HD on radical scavenging activities in serum. Effect of HD on serum scavenging activities against: (A) $^{\bullet}OH$, (B) $RO^{\bullet}$, (C) $ROO^{\bullet}$, (D) $O_2^{\bullet-}$, (E) $^1O_2$, and (F) lipophilic carbon-centered radical. Scavenging activities before and after HD of all included patients are shown and converted into the equivalent unit of the specific scavenger against each ROS, except those against the lipophilic carbon-centered radical, as I0/I−1 value (see Materials and Methods). *p<0.05, **p<0.01, ***p<0.001. The detailed values are shown in Table 3. HD, hemodialysis; ROS, reactive oxygen species; GSH, glutathione; TROLOX, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; αLA, α-lipoic acid; SOD, superoxide dismutase.

using the ESR spin-trapping technique.[22,31]

Consistent with our current result, we have previously reported that serum $^{\bullet}OH$ scavenging activity is reduced in HD patients and that a single HD session can restore this activity to the level equivalent to that seen in a healthy human.[31] Although contradictory results have been reported on the effect of HD on $^{\bullet}OH$ scavenging activity, previous studies using ESR have shown an increase in the scavenging activity, regardless of the generation method of the hydroxyl radicals.[32–36] Thus, we strongly suggest that the $^{\bullet}OH$ scavenging activity may be restored by HD.

The effect of HD on $O_2^{\bullet-}$ dynamics remains controversial. Several previous studies found an increased production of $O_2^{\bullet-}$ in patients after HD.[37,38] A previous report from our co-author revealed that $O_2^{\bullet-}$ scavenging activity was enhanced in the sera of HD patients.[22] Conversely, the $O_2^{\bullet-}$ scavenging activity of HD patients, analyzed by direct scavenging measurements or the $O_2^{\bullet-}$ dismutase (SOD) enzyme assay, is reportedly higher in patients with CKD than in healthy individuals.[22,38,39] Consistent with these reports, our results did not show remarkable changes in $O_2^{\bullet-}$ scavenging activity after a single session of HD. Moreover, long-term usage of the vitamin E-coated dialyzer, which directly scavenges intradialyzer $O_2^{\bullet-}$, has enhanced $O_2^{\bullet-}$ scavenging activity.[15] Since the uremic condition itself is a highly oxidative state,[15] we suggest that the constant improvement against uremic oxidative stress is more influential than the $O_2^{\bullet-}$ production brought about by a single HD process.[1–3,4,40]

The influences of $RO^{\bullet}$ and $ROO^{\bullet}$ in kidney diseases have rarely been investigated,[41–43] and no study has analyzed their association with HD. Our results showed that both $ROO^{\bullet}$ and $RO^{\bullet}$ scavenging activities deteriorated after a single HD session, suggesting an uncontrolled production of both radicals during HD. Although limited studies have investigated the pathophysiological role of $ROO^{\bullet}$ in diseases, established reports have indicated their strong cytotoxicity, leading to carcinogenesis and cardiovascular damage.[41,44,45] $ROO^{\bullet}$ are generated in a reaction between heme iron and lipid peroxide produced by spontaneous oxidation of

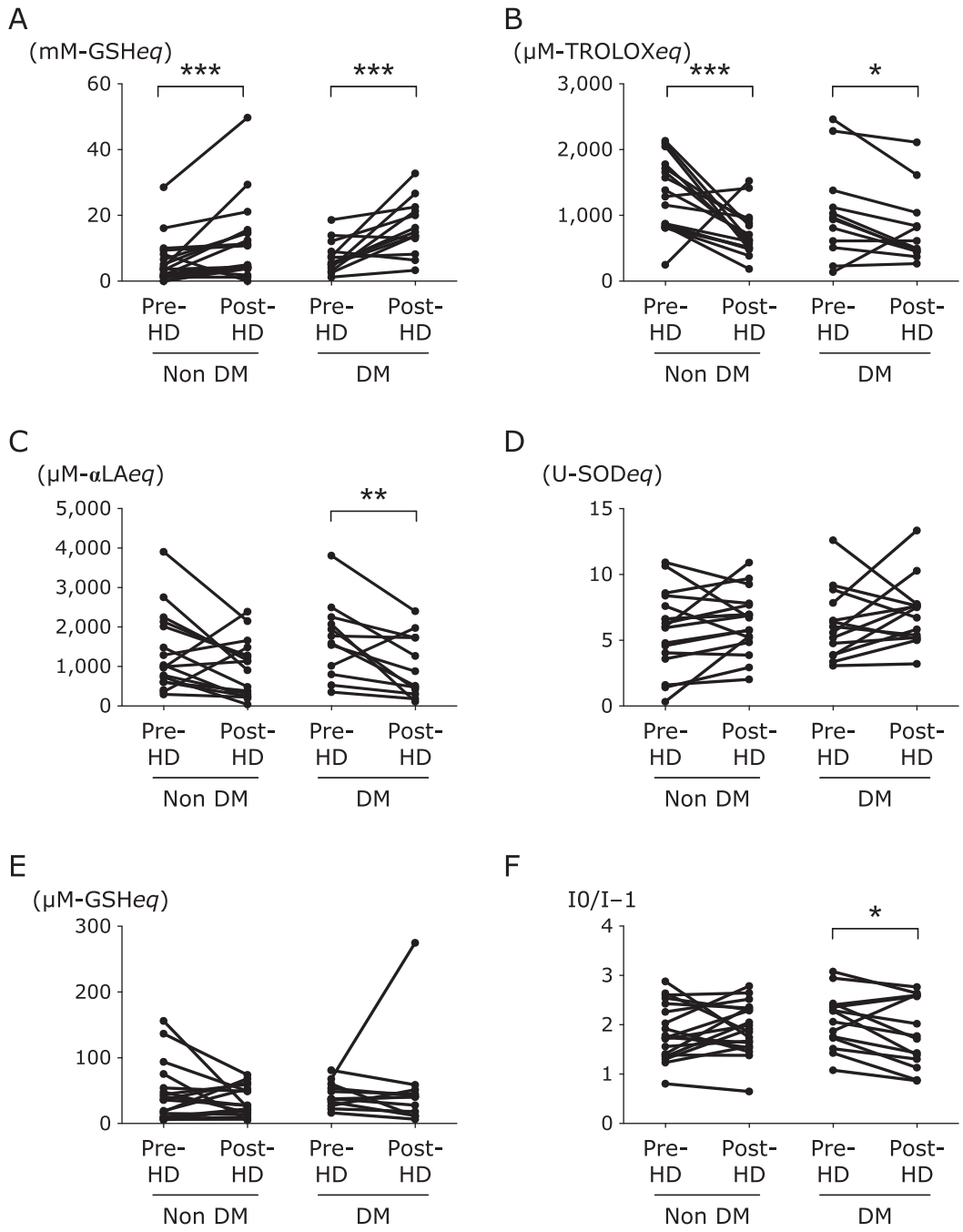

Fig. 2. Effect of HD on serum radical scavenging activities in non-DM and DM groups. Panels show the scavenging activities against: (A) $^{\bullet}OH$, (B) $RO^{\bullet}$, (C) $ROO^{\bullet}$, (D) $O_2^{\bullet-}$, (E) $^1O_2$, and (F) lipophilic carbon-centered radical. Scavenging activities before and after HD of all included patients are presented. The scavenging activities are converted to the equivalent unit of the specific scavenger. *p<0.05, **p<0.01, ***p<0.001. The detailed values are shown in Table 4. HD, hemodialysis; DM, diabetes mellitus; GSH, glutathione; TROLOX, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; αLA, α-lipoic acid; SOD, superoxide dismutase.

unsaturated fatty acid.[46] In a ferric nitrilotriacetate (Fe-NTA)-induced renal carcinoma rat model, $ROO^{\bullet}$ was detected in renal tissue.[41] Moreover, $ROO^{\bullet}$ has a longer half-life *in vivo* than other radicals in biological circumstances,[46] leading to further production of carbon-centered radicals.[47] Therefore, the control of $ROO^{\bullet}$ generation may be an important strategy for improving the survival rate of HD patients. Moreover, lipophilic carbon-centered radicals are cytotoxic and have relatively long half-lives. These radicals have been reported in acute lung injury and in rat models of chronic alcohol-induced pancreatitis.[48,49] Scavenging activities against these two ROS may serve as crucial markers for the evaluation of biocompatibility.

There are several limitations in this study. First, our method evaluated ROS scavenging activity but did not directly detect the ROS themselves. Direct detection of ROS in biological samples is difficult, and there is a certain discrepancy between the dynamics of ROS and ROS scavenging activity. However, measurement of scavenging activity against a specific ROS is a well-established method and is able to serve as an evaluating tool for ROS dynamics.[22,50–52] Thus, the evaluation of ROS scavenging activity

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Table 4. Differences in the effects of hemodialysis on the various serum ROS scavenging activities, depending on the presence or absence of diabetes

ROS		Non-DM			DM		
		Pre HD Mean (95% CI)	Post HD Mean (95% CI)	*p*	Pre HD Mean (95% CI)	Post HD Mean (95% CI)	*p*
•OH	*mM-GSHeq*	5.73 (2.41–9.04)	11.2 (5.4–17)	0.007	7.32 (4.3–10.3)	16.4 (11.4–21.4)	0.003
RO•	*μM-TROLOXeq*	1,274 (1,000–1,547)	722 (543–900)	0.003	1,040 (583–1,497)	795 (442–1,148)	0.049
ROO•	*μM-αLA eq*	1,331 (823–1,838)	956 (568–1,344)	0.073	1,548 (922–2,174)	906 (406–1,407)	0.012
$O_2^{\bullet-}$	*U/ml-SODeq*	5.77 (4.21–7.32)	6.39 (5.19–7.59)	0.26	6.22 (4.69–7.76)	7.02 (5.56–8.47)	0.313
$^1O_2$	*μM-GSHeq*	45.1 (23–67.2)	33.9 (22.7–45.1)	0.291	43.5 (31.2–55.7)	52.5 (6.65–98.3)	0.641
LCCR	*I0/I−1*	1.86 (1.59–2.13)	1.93 (1.68–2.18)	0.671	2.09 (1.76–2.41)	1.83 (1.43–2.24)	0.039

The scavenging activities are converted to the equivalent unit of the specific scavenger against each ROS. The scavenging activity against the lipophilic carbon-centered radical is expressed as I0/I−1 value (see Materials and Methods). HD, hemodialysis; DM, diabetes mellitus; ROS, reactive oxygen species; 95% CI, 95% confidence interval; GSH, glutathione; TROLOX, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; αLA, α-lipoic acid; SOD, superoxide dismutase; LCCR, lipophilic carbon-centered radical.

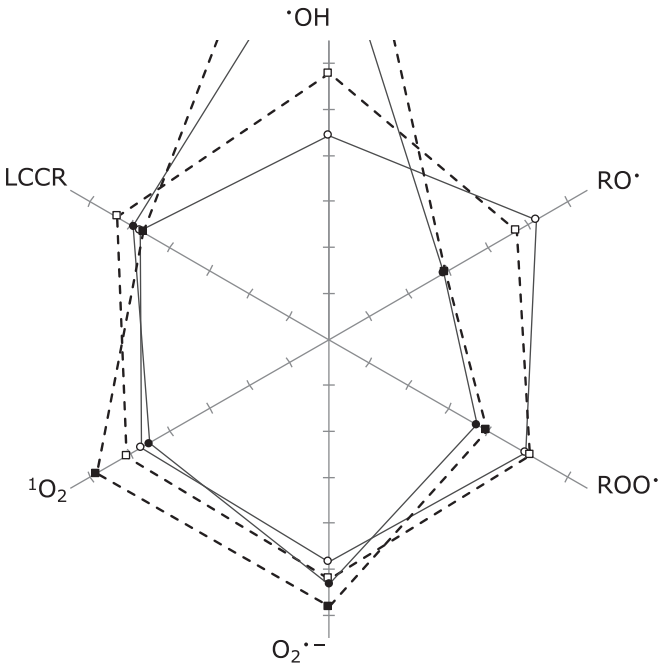

Fig. 3. A radar chart of serum scavenging activities comparing the non-DM and DM groups. Solid lines with circle markers (○●) indicate non-DM patients and broken lines with square markers (□■) indicate DM patients. Open markers (○□) indicate pre-HD activities and filled markers (●■) indicate post-HD activities of the corresponding group. Percentage differences in the scavenging activities are shown with respect to those of all patients before HD. HD, hemodialysis; DM, diabetes mellitus; LCCR, lipophilic carbon-centered radical.

in this paper reflects the ROS dynamics in the upstream region prior to cellular reactions against oxidative stress reactions. Second, our sample size is limited, and we only employed dialyzers containing polysulfone membranes. However, the characteristics of our study population are similar to that of the population of Japanese HD patients in terms of age, dialysis history, and distribution of primary diseases.[53] Polysulfone dialyzers are the most biocompatible dialyzer and are widely used.[36,53,54] Thus, we believe that our study cohort reflects the general population of dialysis patients. Evaluations with other membrane materials are required in further studies.

In our results, scavenging activities against alkyl and lipophilic carbon-centered radicals measured by i-STrap were reduced only in the patients with DM, indicating that the deterioration of ROS scavenging activity was more remarkable in the DM group than in the non-DM group. Patients with DM have more unfavorable prognoses than non-DM patients due to higher cardiovascular complication rates. The pathophysiology of these complications is strongly associated with oxidative stress.[55–57] Thus, our results suggest that to improve the prognosis in HD patients with DM, the control of ROO• radical and carbon-center radical is a promising strategy.

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Conflict of Interest

The authors report no biomedical financial interests or potential conflicts of interest relevant to this study.

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