

⟨Research Article⟩

## Comprehensive assessment of oxidative stress degrees and anti-oxidant potential in dialysis patients

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**Summary** When the kidney is compromised, oxidative stress is aggravated by an increased rate of reactive oxygen species generation and a decreased rate of substance elimination. In addition, dialysis treatment itself is reported to be associated with oxidative stress in patients with terminal renal insufficiency. This study was conducted to determine the optimum dialysis treatment. Diacron-reactive oxygen metabolite and biological anti-oxidant potential tests were performed to assess oxidative stress levels. Increased oxidative stress and decreased anti-oxidant potential were found immediately after dialysis. A comparison of dialysis membranes revealed that ABH<sup>®</sup>-PA membranes exhibited a significantly lower rate of decrease in anti-oxidant potential in terms of oxidative stress compared with Maxiflux<sup>®</sup> membranes. Analysis of biochemical parameters identified significant strong positive correlations between diacron-reactive oxygen metabolites test results and serum total protein, serum albumin, total cholesterol, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol for the percentage decrease and between biological anti-oxidant potential test results and inorganic phosphorus for pre-dialysis measurements. ABH<sup>®</sup>-PA should be used as the dialysis membrane to minimize the increase in the degree of oxidative stress caused by dialysis treatment. We recommend measuring serum total protein, serum albumin, total cholesterol, and inorganic phosphorus before and after dialysis sessions to better understand oxidative stress degrees and anti-oxidant potential.

**Key words:** d-ROMs, BAP, Dialysis treatment, Oxidative stress, Anti-oxidant potential

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## 1. Introduction

The kidneys excrete waste products and are involved in various other functions including regulating electrolyte and pH balance, activating vitamin D, and secreting hormones such as erythropoietin and renin. These activities require a considerable amount of oxygen and produce a large quantity of reactive oxygen species (ROS) as a byproduct. When the kidney is compromised, oxidative stress is aggravated by an increased rate of ROS generation and a decreased rate of substance elimination<sup>1-3</sup>. Dialysis treatment itself is reported to increase oxidative stress in patients with terminal renal insufficiency<sup>4-6</sup>. Within minutes of dialysis initiation, the exposure of blood to dialyzer membranes and dialysate triggers the activation of complement factors, platelets, and polymorphonuclear white blood cells, thereby inducing ROS production<sup>7</sup>. The increase in oxidative stress with dialysis treatment is considered to be related to several dialysis treatment-related factors, such as the duration of the dialysis session and the type of dialysis membrane, dialysate, anticoagulant, and medications used<sup>8</sup>.

Currently, there are four methods for assessing oxidative stress: measuring ROS occurring in the body<sup>9-11</sup>, measuring systems that produce ROS<sup>12</sup>, measuring substances produced by ROS<sup>13</sup>, and measuring anti-oxidant substances induced and generated in the body in response to ROS<sup>14,15</sup>. These methods are based on several measurement systems, each with their own advantages and disadvantages.

Two methods used to evaluate oxidative stress are the diacron-reactive oxygen metabolites test (d-ROMs)<sup>16,17</sup>, which is used to assess ROS generation; and the biological anti-oxidant potential test (BAP)<sup>17,18</sup>, which is used to assess the levels of anti-oxidant substances. In this study, we measured oxidative stress before and after dialysis treatment using d-ROMs and BAP and proposed a method for comprehensively improving dialysis treatment.

## 2. Materials and Methods

Prior to conducting this study, we obtained approval from the Ethics Committee of Kinashi Obayashi Hospital (approval number 29-01) and the Ethics Committee of Kagawa Prefectural University of Health Sciences (approval number 252). Written informed consent was also obtained from all participants.

In total, 115 chronic dialysis patients agreed to participate in the study (65 men and 50 women, average age 69.9 [44–93] years). All participants used sodium heparin as an anticoagulant during dialysis sessions. The principal components of the dialysate used included sodium chloride, glucose, anhydrous sodium acetate, and sodium hydrogen carbonate. The dialysis sessions lasted 4 h.

The participants comprised 42 patients with diabetic nephropathy, 36 with chronic glomerulonephritis, 8 with nephrosclerosis, 5 with IgA nephritis, 4 with polycystic kidney disease, 3 with nephrotic syndrome, 8 with known other conditions, and 9 with an uncertain diagnosis. Average dialysis history was 10.8 years (0–47 years). Dialysis methods comprised on-line hemodiafiltration (OHDF; n = 82) and hemodialysis (HD; n = 33). Of the dialysis membranes used by the 82 OHDF patients, 47 were Maxiflux<sup>®</sup> (MFX; Nipro Corp., Osaka, Japan), 17 were Fineflux<sup>®</sup> (FIX; Nipro Corp.), 15 were the ABH<sup>®</sup>-PA hollow-fiber type hemodiafiltration filter (ABH-PA; Asahi Kasei Medical Co., Ltd., Tokyo, Japan), and 3 were Toraysulfone<sup>®</sup> NV TDF-MV (TDF-MV; Toray Medical Co., Ltd., Tokyo, Japan). Of the dialysis membranes used by the 33 HD patients, 19 were VitabranE<sup>®</sup> VPS<sup>®</sup>-HA (VPS-HA; Asahi Kasei Medical Co., Ltd.), 5 were the KF-201 hollow fiber dialyzer (KF-201; Asahi Kasei Medical Co., Ltd.), 5 were the APS<sup>®</sup>-EA hollow-fiber type dialyzer (APS-EA; Asahi Kasei Medical Co., Ltd.), 3 were the Baxter Limited H12 hemodialyzer (Nipro Corp.), and 1 was the FB-eco series triacetate hollow-fiber dialyzer (FB; Nipro Corp.). In addition, 54 patients were taking iron preparations and 6 were taking vitamin C.

Samples were collected in the dialysis room immediately before and immediately after each dialysis session. For sample preparation, blood was allowed to stand at room temperature for 30 min and serum was collected by centrifugation at 3,000 rpm for 5 min. Samples were stored at  $-25^{\circ}\text{C}$  until they were assayed. The clinical features of the control group are shown in Table 1. The control group comprised 30 healthy individuals who were included in our previous study<sup>19</sup>. Their renal function and lipid biochemical test results were within the reference interval and their lipoprotein patterns were the symmetric type on polyacrylamide gel disk electrophoresis<sup>20</sup>.

d-ROMs and BAP were measured before the dialysis session (pre-HD) and after the dialysis session (post-HD) and their relationships with values in the control group, underlying disease, current medical history, age, gender, dialysis history, dialysis methods, dialysis membranes used, and biochemical parameters were examined. The BAP/d-ROMs ratio was calculated as an index of comprehensive anti-oxidant potential, and its relationships with each parameter were assessed. In addition, to determine the influence of an individual dialysis session on various measurements, the relationships

between the percentage decrease and each parameter were also examined. Percentage decrease was calculated as [(pre-HD measurement values – post-HD measurement values) / pre-HD measurement values  $\times$  100] ( $\Delta\%$ ). Dialysis membranes were compared individually for two membranes.

The Free Carriio Duo free radical analyzer (Diacron International, Grosseto, Italy) was used for the d-ROMs and BAP tests (WISHERLL Co., Ltd., Tokyo, Japan), which were performed in accordance with the manufacturer's instructions. The d-ROMs test, a method for comprehensively evaluating oxidative stress in the body<sup>16,17</sup>, does not directly measure active oxygen or free radicals, which cause oxidative stress, but instead measures the resulting active oxygen metabolites (mainly hydroperoxide). BAP evaluates the power of reductive processes to comprehensively inhibit oxidation reactions<sup>17,18</sup>.

Biochemical parameters were measured with a 7180 clinical analyzer (Hitachi High-Tech Corporation, Tokyo, Japan). The items measured were blood urea nitrogen, creatinine, uric acid, calcium, inorganic phosphorus (IP), serum iron, unsaturated iron binding capacity (UIBC), magnesium,  $\beta$ 2-microglobulin, ferritin,  $\alpha$ 1-microglobulin, sodium, potassium, chloride, serum total protein (TP), serum albumin, total bilirubin (T-BIL), total cholesterol (TC), triglycerides, high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), phospholipid, and C-reactive protein (CRP). To measure changes in concentration associated with the dialysis session, pre-HD and post-HD hematocrit values (Ht) were measured as an additional item using an exclusive reagent (only in 18 patients) with an XT-4000i multi-item automatic blood cell analyzer (Sysmex Corporation, Kobe, Japan).

Sample size was confirmed using standard deviation and 95% confidence interval and standard error<sup>21</sup>. The Mann-Whitney U test, Wilcoxon signed-rank test, and Spearman's rank correlation coefficient were used to determine significance<sup>22</sup>, with  $P < 0.05$  considered statistically significant.

Table 1 Clinical characteristics of the control group

Parameter	Value
Numbers of samples(M/F)	30(14/16)
Age(yrs)	45.63 $\pm$ 9.09
Urea nitrogen(mmol/L)	4.77 $\pm$ 1.20
Creatinine( $\mu$ mol/L)	62.8 $\pm$ 9.72
Uric acid( $\mu$ mol/L)	298.6 $\pm$ 55.3
Inorganic phosphorus(mmol/L)	1.09 $\pm$ 0.14
Calcium (mmol/L)	2.31 $\pm$ 0.08
Potassium(mmol/L)	4.18 $\pm$ 0.27
Sodium (mmol/L)	142.6 $\pm$ 1.47
Chloride(mmol/L)	107.5 $\pm$ 1.53
Serum total Protein(g/L)	69.4 $\pm$ 3.7
Serum albumin(g/L)	43.7 $\pm$ 2.4
Total bilirubin( $\mu$ mol/L)	15.05 $\pm$ 4.66
Total cholesterol(mmol/L)	4.58 $\pm$ 0.54
Triglycerides(mmol/L)	0.79 $\pm$ 0.31
HDL cholesterol (mmol/L)	1.73 $\pm$ 0.29
LDL cholesterol(mmol/L)	2.66 $\pm$ 0.49
C-reactive protein(mg/L)	0.50 $\pm$ 2.35
Glucose(mmol/L)	5.03 $\pm$ 0.34

Values are expressed as means  $\pm$  standard deviations.

### 3. Results

Fig. 1 shows the results of d-ROMs, BAP, and the BAP/d-ROMs ratio for pre-HD, post-HD and the control group. The post-HD d-ROMs results were significantly higher. In contrast, post-HD BAP and BAP/d-ROMs ratio were significantly lower (Fig. 1).

There was a significant but weak positive correlation between age and  $\Delta\%$  of d-ROMs ( $r = 0.323, P < 0.001$ ). Age and pre-HD and post-HD BAP had significant but weak negative correlations (pre-HD:  $r = -0.302, P < 0.01$ ; post-HD:  $r = -0.295, P < 0.01$ ). Age and  $\Delta\%$  of the BAP/d-ROMs ratio had a significant but weak negative correlation ( $r = -0.220, P < 0.05$ ).  $\Delta\%$  of BAP and  $\Delta\%$  of the BAP/d-ROMs ratio had a significant but weak positive

correlation (BAP:  $r = 0.283, P < 0.01$ ; BAP/d-ROMs ratio:  $r = 0.231, P < 0.05$ ) in relation to dialysis history. There was no significant relationship of the d-ROMs, BAP, and BAP/d-ROMs ratio results with underlying disease, current medical history, gender, iron preparations, and vitamin C use (data not shown).

The relationships of d-ROMs, BAP, and the BAP/d-ROMs ratio with dialysis methods are shown in Fig. 2 and Table 2. Post-HD BAP results were significantly lower for OHDF than for HD (Table 2).  $\Delta\%$  of BAP and  $\Delta\%$  of BAP/d-ROMs ratio were significantly higher for OHDF than for HD (Fig. 2). In contrast, in terms of the relationship with the dialysis membrane,  $\Delta\%$  of the BAP/d-ROMs ratio of ABH-PA was significantly lower than that of MFX (Fig. 3). However, there was no significant

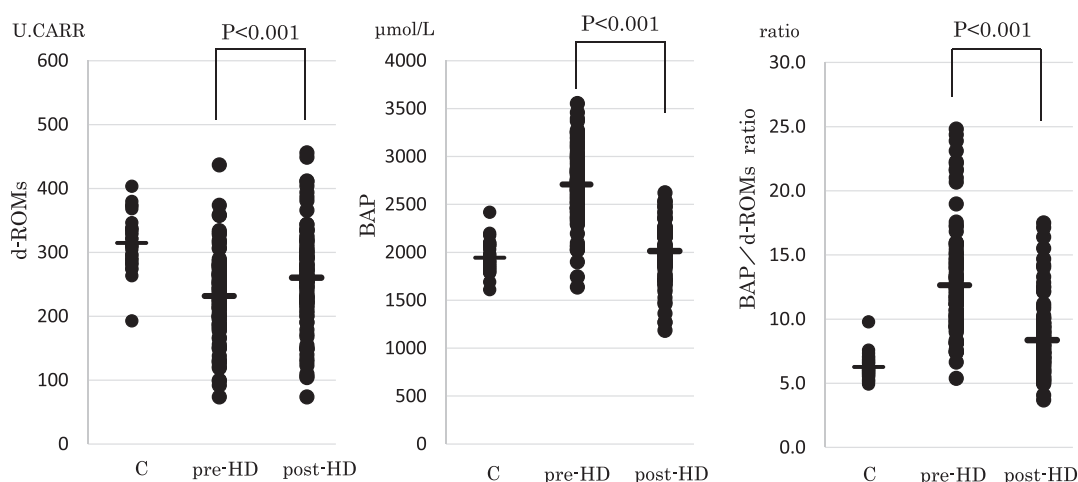


Fig. 1 Relationship between pre- and post-HD results for d-ROMs, BAP, and BAP/d-ROMs ratio. Scatter plot of d-ROMs, BAP, and BAP/d-ROMs ratio between the control, pre-HD, and post-HD groups. Statistical analysis: Wilcoxon signed-rank sum test. d-ROMs, diacron-reactive oxygen metabolites test; BAP, biological anti-oxidant potential test; pre-HD, before dialysis session; post-HD, after dialysis session; C, control group. Numbers of samples: pre-HD and post-HD (n=115) and C (n=30).

Table 2 Relationship of d-ROMs, BAP, and BAP/d-ROM sratio results with dialy sismethod

	d-ROMs(U.CARR)			BAP(µmol/L)			BAP/d-ROMs ratio		
	HD	OHDF	P value	HD	OHDF	P value	HD	OHDF	P value
pre-HD	229.2	232.7	P=0.97	2719	2703	P=0.92	13.25	12.40	P=0.74
post-HD	260.8	260.2	P=0.60	2107	1974	P<0.05	9.04	8.1	P=0.38
$\Delta\%$	-13.72%	-11.83%	P=0.48	22.09%	26.74%	P<0.05	31.22%	34.16%	P<0.05

Average values of the measurement results of HD and OHDF with P values.

$\Delta\%$ , percentage decrease;  $\Delta\%$ , calculated as (pre-HD measurement values – post-HD measurement values)/ pre-HD measurement values  $\times 100$ .

Numbers of samples: All (n=115)

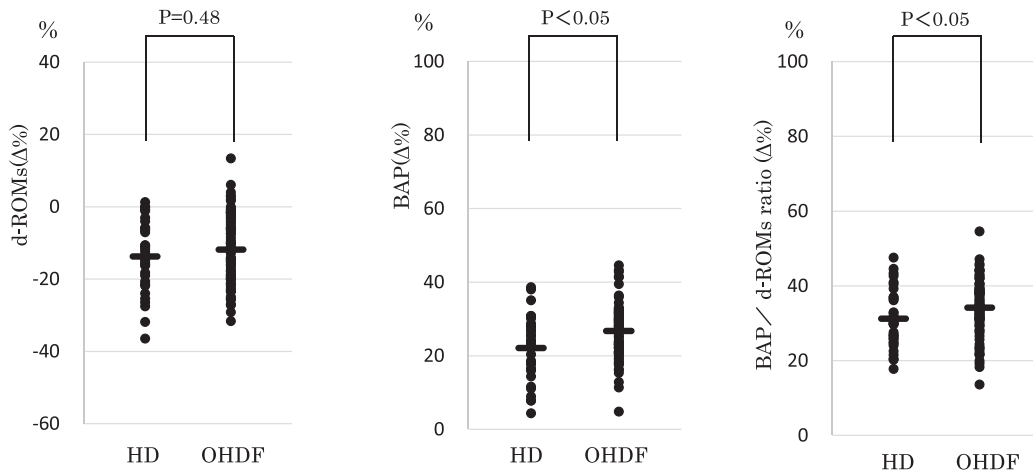


Fig. 2 Relationship of d-ROMs ( $\Delta\%$ ), BAP ( $\Delta\%$ ), and BAP/d-ROMs ratio ( $\Delta\%$ ) results with dialysis method. Scatter plot of d-ROMs ( $\Delta\%$ ), BAP ( $\Delta\%$ ), and BAP/d-ROMs ratio ( $\Delta\%$ ) between HD and OHDF. HD, hemodialysis; OHDF, on-line hemodiafiltration. Numbers of samples: HD (n=33) and OHDF (n=82).  $\Delta\%$ , percentage decrease;  $\Delta\%$ , calculated as (pre-HD measurement values – post-HD measurement values)/ pre-HD measurement values  $\times$  100. Statistical analysis: Mann-Whitney test.  $\Delta\%$  of BAP and  $\Delta\%$  of BAP/d-ROMs ratio were significantly higher for OHDF than for HD.

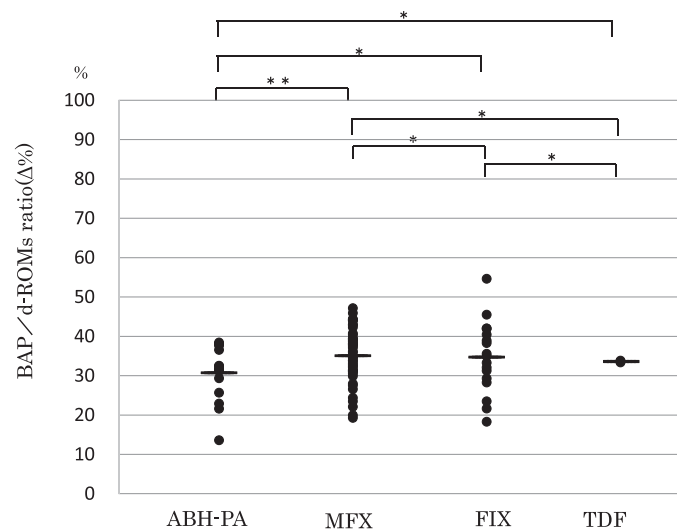


Fig. 3 Comparison among OHDF dialysis membranes for the BAP/d-ROMs ratio  $\Delta\%$ . Numbers of samples: ABH-PA (n=15), MFX (n=47), FIX (n=17), and TDF (n=3). \*No significant difference between the BAP/d-ROMs ratio  $\Delta\%$ . \*\*Significant difference ( $P < 0.05$ ) between the BAP/d-ROMs ratio  $\Delta\%$ . Statistical analysis: Mann-Whitney test. The smaller the  $\Delta\%$ , the smaller the influence of oxidative stress.

difference in age or dialysis history between ABH-PA users and MFX, FIX, and TDF-MV users (data not shown). There was no significant difference between the HD dialysis membranes (data not shown).

Table 3 shows the pre- and post-HD biochemical

parameters and  $\Delta\%$ . In relation to d-ROMs,  $\Delta\%$  showed significant strong positive correlations with TP, serum albumin, TC, HDL-C, and LDL-C (Fig. 4). IP showed a significant strong positive correlation with pre-HD BAP and a significant positive correlation with post-HD BAP (Fig. 5). On the other

Table 3 Biochemical results for the pre-and post-HD group sand Δ%

Parameter		pre-HD	post-HD	Δ%
Urea nitrogen	(mmol/L)	22.61±6.35	6.08±2.87	73.36±8.13
Creatinine	(μmol/L)	837.2±282.9	284.7±137.0	66.87±8.23
Uric acid	(μmol/L)	383.7±88.03	94.0±37.47	75.69±7.28
Inorganic phosphorus	(mmol/L)	1.67±0.53	0.66±0.23	59.66±10.56
Magnesium	(mmol/L)	1.00±0.14	0.82±0.06	17.07±8.36
β <sub>2</sub> -Microglobulin	(mg/L)	27.33±8.13	10.10±11.00	65.14±16.31
α <sub>1</sub> -Microglobulin	(mg/L)	114.6±18.35	107.1±24.26	6.30±15.76
Potassium	(mmol/L)	4.61±0.78	3.28±0.49	27.94±10.01
Calcium	(mmol/L)	2.13±0.12	2.33±0.13	-9.75±6.25
Serum iron	(μmol/L)	9.34±5.25	11.75±5.77	-36.14±42.61
Sodium	(mmol/L)	139.9±3.34	138.1±1.86	1.23±2.10
Chloride	(mmol/L)	103.3±3.63	99.3±2.18	3.76±3.08
Serum total protein	(g/L)	62.9±4.9	70.7±8.2	-12.49±10.57
Serum albumin	(g/L)	32.8±4.6	36.5±6.6	-11.10±11.10
Total bilirubin	(mmol/L)	5.64±2.91	8.38±3.76	-52.61±41.72
Total cholesterol	(mmol/L)	4.06±1.0	4.64±1.25	-14.00±10.78
Triglycerides	(mmol/L)	1.28±0.69	0.98±0.59	18.68±30.30
HDL cholesterol	(mmol/L)	1.24±0.42	1.37±0.49	-10.59±14.68
LDL cholesterol	(mmol/L)	2.24±0.79	2.86±1.07	-28.16±18.00
Phospholipid	(mmol/L)	2.44±0.49	2.86±0.65	-16.72±8.00
C-reactive protein	(mg/L)	6.3±12.6	6.4±13.3	-10.59±23.11
Unsaturated iron binding capacity	(μmol/L)	34.34±12.73	—	—
Ferritin	(μg/L)	109.9±161.9	—	—

Values are expressed as means ± standard deviations.  
 Δ%, minus value means an increase.  
 Numbers of samples: Phospholipid (n=18), other parameters (n=115)

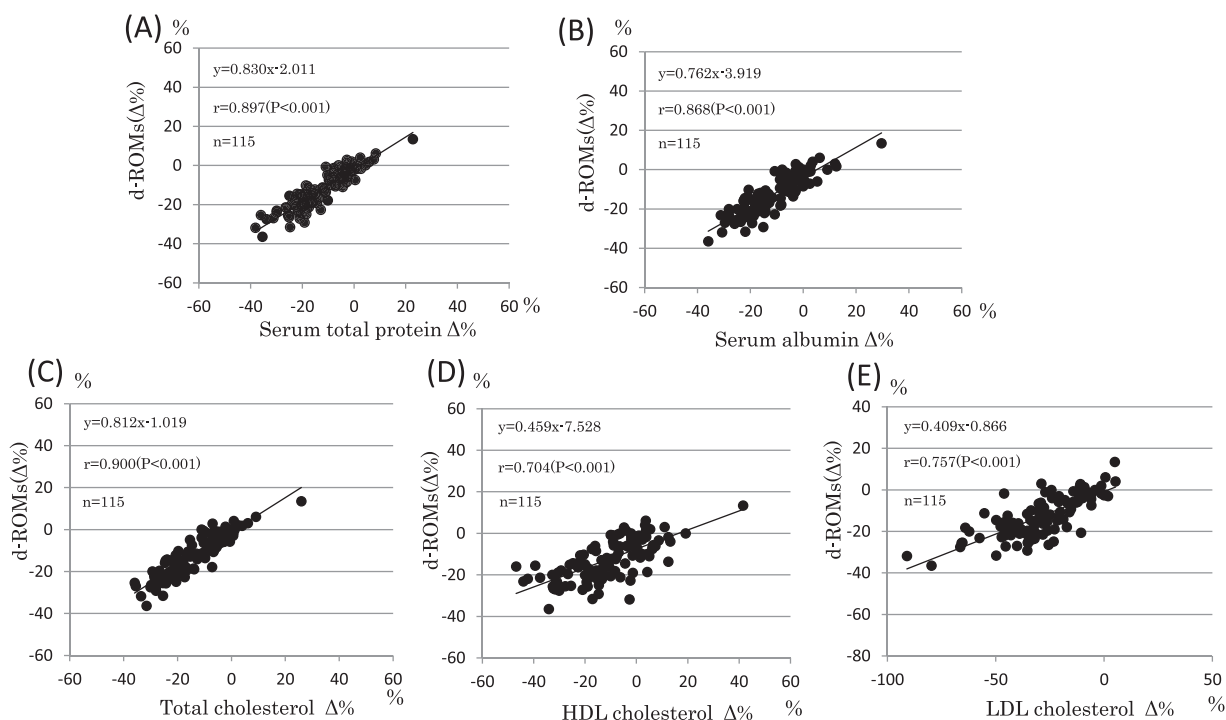


Fig. 4 Correlations in Δ% between d-ROMs and (A) serum total protein, (B) serum albumin, (C) total cholesterol, (D) HDL-cholesterol, and (E) LDL-cholesterol. Axis shows Δ% of d-ROMs. Statistical analysis: Spearman’s rank correlation coefficient test. Δ%, minus value means an increase.

hand, the average change in Ht concentration of 18 patients calculated between pre- and post-HD was 108.36%. A significant strong positive correlation was seen between the change in Ht concentration and

change in d-ROMs, which was calculated as (post-HD measurement value / pre-HD measurement value) × 100 (r = 0.82; P < 0.001; Fig. 6). Furthermore, Δ% of five parameters-TP, serum albumin, TC, HDL-C,

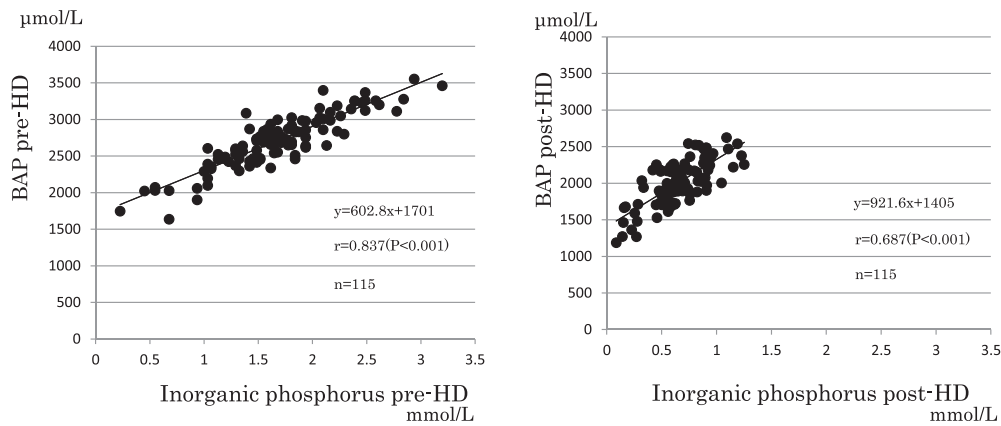


Fig. 5 Relationship between inorganic phosphorus pre-HD and BAP pre-HD and between inorganic phosphorus post-HD and BAP post-HD. Statistical analysis: Spearman’s rank correlation coefficient test. There were significant strong positive correlations between inorganic phosphorus pre-HD and BAP pre-HD and between inorganic phosphorus post-HD and BAP post-HD.

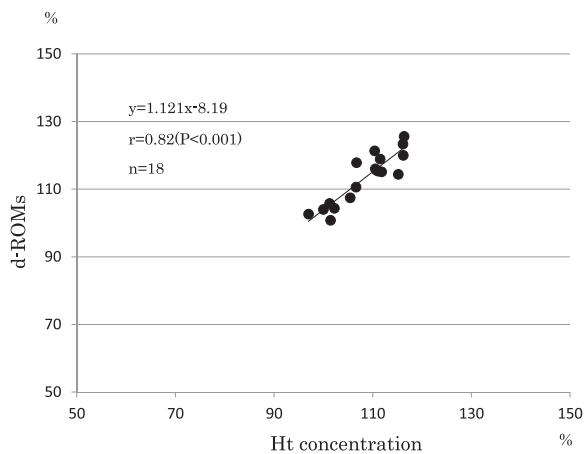


Fig. 6 Relationship between changes in Ht concentration by dialysis session and changes in d-ROMs. Statistical analysis: Spearman’s rank correlation coefficient test. Ht: hematocrit values. There was a significant strong positive correlation between changes in Ht concentration by dialysis session and changes in d-ROMs.

and LDL-C-were significantly positively correlated with d-ROMs. Although relationships with the corrected results were identified based on the change in Ht concentration, significant correlations were not seen with any of the parameters (data not shown).

#### 4. Discussion

One of the most notable results of this study is

that the dialysis session increases oxidative stress and decreases anti-oxidant potential (Fig. 1). In contrast, it has previously been reported<sup>23</sup> that oxidative stress is reduced post-HD by the purification effect. This is due to decreased levels of oxidized albumin and increased levels of mercaptalbumin (reduced form). This difference may be attributable to the comprehensive evaluation method of oxidative stress and antioxidant activity used in this study. The increase in oxidative stress is possibly due to draining-related changes in the concentration of the circulating blood because there was a significant strong positive correlation between the change in Ht concentration by dialysis session and the change in d-ROMs (Fig. 6). The fall in anti-oxidant potential is possibly due to the simultaneous removal of various anti-oxidants along with waste products during dialysis. A comparison of the results between the OHDF and HD methods revealed that OHDF, which can remove more waste products, was associated with a significantly higher rate of decrease in anti-oxidant potential post-HD (Fig. 2). These results suggest that HD is better for mitigating the decrease in anti-oxidant potential caused by the dialysis session. However, OHDF<sup>24,25</sup> has the advantage of removing low-molecular-weight proteins and water in addition to small- to medium-molecular-weight materials compared with HD, which removes only small-

molecular-weight materials and water. Recently, the use of OHDF has been increasing<sup>26-28</sup>. Although there was no significant difference, the reduction rate of oxidative stress tended to be greater in OHDF than in HD (Fig. 2). Taken together, the results suggest the superiority of OHDF. Because the dialysate used contains acetic acid but not citric acid, the measurements were not affected; there are currently no reports on the influence of acetic acid on dialysis. The sample size used confirmed that the error calculated using each standard deviation and 95% confidence interval was 6% or less of each average value.

The second most noteworthy finding is that ABH-PA was suggested to be an excellent dialysis membrane. This is because a comparison of dialysis membranes showed that ABH-PA users undergoing OHDF dialysis had a significantly lower rate of decrease in anti-oxidant potential in terms of oxidative stress compared with MFX users (Fig. 3). Both sample sizes were calculated from the standard deviation, 95% confidence interval, and 5% error and were confirmed to be statistically appropriate. There was no significant relationship between underlying disease, current medical history, gender, or the use of iron preparations. Based on the findings of the relationship between age and dialysis history, the influence of oxidative stress due to dialysis decreases with age, whereas the oxidative stress influence due to dialysis increases with a longer dialysis history. However, there was no significant difference in age or dialysis history between ABH-PA dialysis membrane users and MFX dialysis membrane users (data not shown). The material of the ABH-PA hollow-fiber membrane is polysulfone and polyvinylpyrrolidone; that of the MFX hollow-fiber membrane is polyethersulfone; and that of the FIX hollow-fiber membrane is cellulose triacetate. Our result is thus consistent with several studies reporting that treatment with polysulfone membranes is associated with reduced lipid peroxides, reduced ROS production, and significantly higher serum levels of anti-oxidants such as vitamins and catalase<sup>8</sup>. In those reports, there was no mention of vitamin E fixation for OHDF membranes.

The third most noteworthy result is that IP measurements could be used to understand comprehensive anti-oxidant potential indicators. This is because IP and BAP showed a significant strong positive correlation pre-HD ( $r = 0.837$ ) and a significant positive correlation post-HD ( $r = 0.687$ ; Fig. 5). The excretion of IP by the kidney is affected by renal failure; however, the excretion of other substances affected by renal failure, such as creatinine and blood urea nitrogen, did not have as strong a positive correlation as that of IP. Because IP is found in phospholipids, which are constituents of cell membranes, this relationship was investigated further. However, no association with BAP was found (data not shown). It is thus currently unclear why BAP and IP have a significant strong positive correlation.

The final noteworthy finding is that the rate of change in TP, serum albumin, TC, HDL-C, and LDL-C with dialysis session could be used to understand the overall rate of increase in oxidative stress due to the dialysis session. This is because the  $\Delta\%$  values of TP, serum albumin, TC, HDL-C, and LDL-C were significantly positively correlated with the  $\Delta\%$  value of d-ROMs (Fig. 4; a minus  $\Delta\%$  value indicates an increased rate). However, it was suggested that changes in d-ROMs, TP, serum albumin, TC, HDL-C, and LDL-C due to the dialysis session were related to the change in concentration of circulating blood induced by water removal because there was a significant strong positive correlation between the change in Ht concentration by dialysis session and change in d-ROMs (Fig. 6). In addition, TC had a considerably stronger correlation ( $r = 0.90$ ;  $P < 0.001$ ) compared with the relationship between HDL-C and LDL-C, and was similar to the measured value (rate of increase) of d-ROMs (Fig. 4). The increase in TC may be due to only draining-related changes in the concentration of the circulating blood. The increases in HDL-C and LDL-C, on the other hand, are possibly due to such draining-related changes as well as the oxidative stress and anti-oxidant potential associated with the dialysis session, which may differ depending on the lipoprotein patterns, and also possibly due to the



measurement principles for HDL-C and LDL-C [Homogeneous method: MetaboLead® HDL-C (Hitachi Chemical Diagnostics Systems Co. Ltd.), MetaboLead® LDL-C (Hitachi Chemical Diagnostics Systems Co. Ltd.)]<sup>29</sup>. Taken together, these results suggest that the rate of change in TP, serum albumin, and TC with dialysis session could be used to better understand the overall rate of increase in oxidative stress due to the dialysis session.

These results indicate that a single dialysis session increases oxidative stress and decreases anti-oxidant potential. However, the results do not show significant differences due to underlying disease, current medical history, oxidative stress, anti-oxidant potential, or degree of oxidative stress. About 30,000 dialysis patients die every year in Japan<sup>26-28</sup>. The leading cause of death is heart failure, followed by infection and malignant tumors<sup>26-28</sup>. Several studies have reported that heart failure and malignant tumors are related to oxidative stress<sup>30-33</sup>. The above results suggest that efficient dialysis treatment as well as minimization of the level of oxidative stress caused by the dialysis session are essential. In addition, it is necessary to understand oxidative stress degrees and anti-oxidant potential.

The results of this study show that ABH-PA is the most appropriate OHDF dialysis membrane because it minimizes the increase in oxidative stress caused by dialysis treatment. In addition, we recommend measuring pre- and post-HD TP, serum albumin, TC, and IP to better understand oxidative stress degrees and anti-oxidant potential.

### Conflict of interest

The authors declare that there are no conflicts of interest.

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