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Lowering of Oxidative Stress in Hemodialysis Patients by Dialysate Cleaning: in Relation to Arteriosclerosis

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Abstract: The objective of this study was to investigate changes in oxidative stress associated with the cleaning of the dialysate. Thirty-six dialysis patients were studied. Changes in soluble CD-14 (sCD-14), malondialdehyde-low-density lipoprotein (MDA-LDL), and oxidized-LDL (Ox-LDL) were monitored for 1 year before and 1 year after dialysate cleaning. The mean endotoxin (ET) level in the dialysate had previously been confirmed to decrease from 39.0 EU/L to an undetectable level after the cleaning. The mean levels of sCD-14, MDA-LDL, and Ox-LDL decreased significantly after the cleaning (sCD-14, $P < 0.0001$; MDA-LDL, $P < 0.001$; Ox-LDL, $P < 0.001$). One year after the cleaning, six cases still showed high levels of MDA-LDL and Ox-LDL. Cardiovascular events occurred in four of those six cases within 2.8 years after the cleaning. These four patients suffered from strong oxidative stress during dialysis, even after the cleaning. We therefore concluded that high levels of MDA-LDL and Ox-LDL are improved in dialysis patients by cleaning of the dialysate. These results indicate that even a dialysate containing 50 EU/L or less ET may stimulate monocytes and cause oxidative stress. They also suggest that even low levels of ET may aggravate arteriosclerosis in dialysis patients. Thus, in order to prevent cardiovascular events in dialysis patients, it is necessary to purify the dialysate. **Key Words:** Arteriosclerosis, Endotoxin, Hemodialysis, Malondialdehyde-low-density lipoprotein, Oxidative stress, Oxidized-low-density lipoprotein.

Dialysis patients are exposed to a great deal of oxidative stress (1), and it has been suggested that endotoxin (ET) in the dialysate is an important source of oxidative stress (2). As an index of this oxidative stress, malondialdehyde-low-density lipoprotein (MDA-LDL) and oxidized-LDL (Ox-LDL) are attracting attention, particularly in relation to arteriosclerosis.

Vascular events associated with arteriosclerosis, such as cerebral stroke and myocardial infarction, are known as two of the main causes of death in dialysis patients (3). It has been postulated that oxidative stress plays a central role in the induction of arteriosclerosis. The importance of dialysate cleaning and its clinical effects have been reported frequently (4–6), but the relationship between cleaning of the dialysate and the incidence of arteriosclerosis has not yet been suggested clinically.

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In the present study, the concentration of endotoxin in the dialysate was reduced to an undetectable level by dialysate cleaning, and the levels of MDA-LDL and Ox-LDL in the blood of dialysis patients was investigated. In addition, the clinical laboratory data obtained simultaneously were examined in relation to cardiovascular events.

SUBJECTS AND METHODS

The subjects included in this study were 37 patients with stable disease conditions, who had been treated with ordinary chronic maintenance dialysis for 1 year or longer using high performance dialyzers at our hospital. To avoid the influence of residual renal function, the patients who had just started the introduction of dialysis were excluded from the study. The subjects studied were 18 men and 19 women, and informed consent to participate in this study was obtained from every patient. Their age ranged from 44.0 to 76.5 years (mean 59.9 years), and the period of dialysis from 1.2 to 26.6 years (mean 11.0 years).

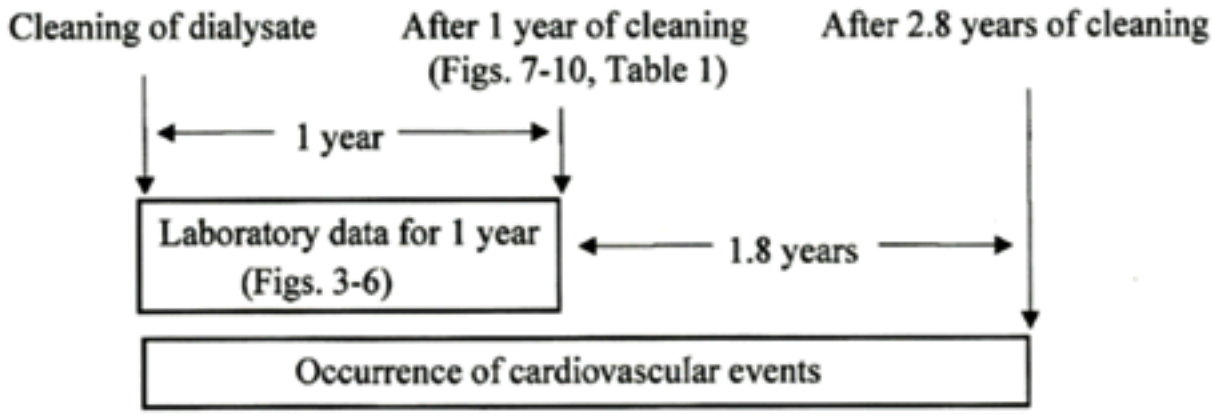

FIG. 1. Time schedule for the study.

The primary disease was chronic nephritis in 29 patients and diabetic nephropathy in eight patients. All of the patients were dialyzed three times a week for 3.5–5.0 h each time. Dialysis conditions, dialyzers and medications were the same throughout the study period.

Because one patient died soon after the start of the study, 36 patients were monitored for 2.8 years after cleaning of the dialysate (Fig. 1).

At our hospital, the dialysate supply system was improved in December 1999 as follows: (i) the pre-filter (PUF) was placed before the reverse osmosis (RO) unit; (ii) piping was totally improved, shortened and simplified; and (iii) an endotoxin-cut filter (MOLSEP FF03-FL-FUS 1041; Daicen Maintenance and Engineering Co., Ltd., Tokyo, Japan) was placed behind the central unit for many patients, or the endotoxin-cut filter (EF-01; NIKKISO Co., Ltd., Tokyo, Japan) was placed before the dialyzer for individual use.

Various tests including the measurement of MDA-LDL and Ox-LDL were conducted before and after the above cleaning of the dialysate, and changes of data with time were observed for 1 year. Blood samples were collected before and after the first dialysis of the week, using the arterial side of the circuit. As a quantitative assay of endotoxin, the Endotoxin-Specific Test (Endospacy ES-50M; Seikagakukogyou Co., Ltd., Tokyo, Japan) was used. Soluble CD-14 (sCD-14) and interleukin (IL)-10 were assayed using an enzyme immunoassay (EIA), IL-6 was assayed using enzyme-linked immunosorbent assay (ELISA), and MDA-LDL and Ox-LDL were assayed using the thiobarbituric acid (TBA) method (Yagi method) and ELISA, respectively. The standard ranges of MDA-LDL and Ox-LDL in dialysis patients were ≤5.0 nmol/mg LDL protein for MDA-LDL and ≤2.00 ng/μg LDL protein for Ox-LDL.

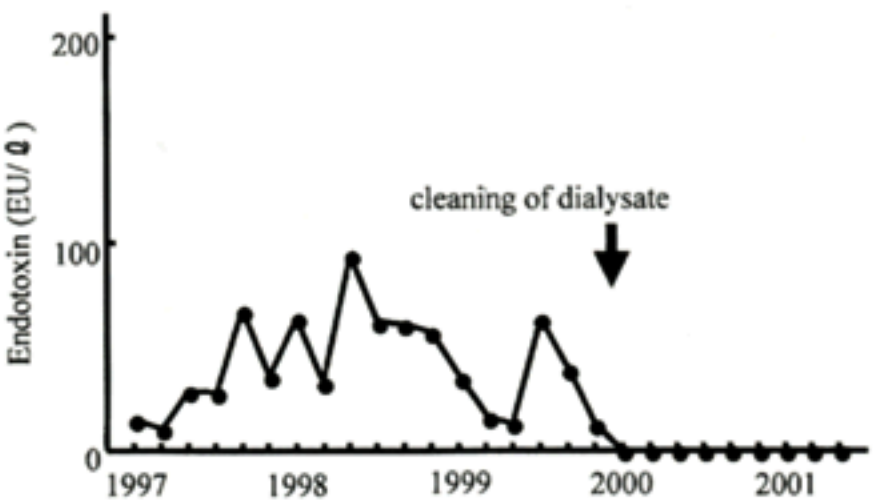

FIG. 2. Ultrapurification of the dialysate and level of endotoxin in the dialysate. In December 1999, the dialysate supply system was completely improved. The level of endotoxin in the dialysate in our hospital before and after the improvement of the system was greatly reduced.

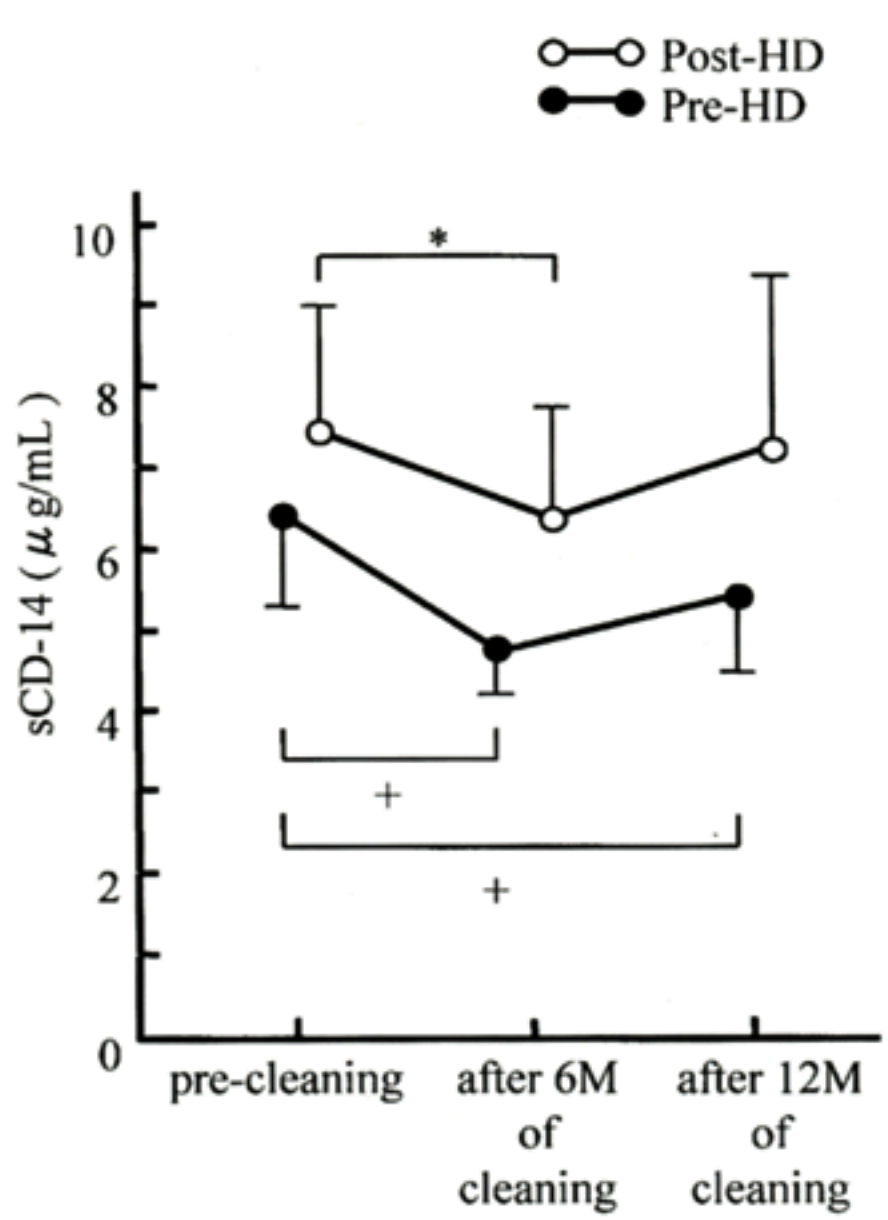

FIG. 3. Changes in soluble CD-14 (sCD-14) 1 year after dialysate cleaning. Mean ± SD, $N = 29$. $*P < 0.01$; $+P < 0.0001$; (●) pre-hemodialysis; (○) post-hemodialysis.

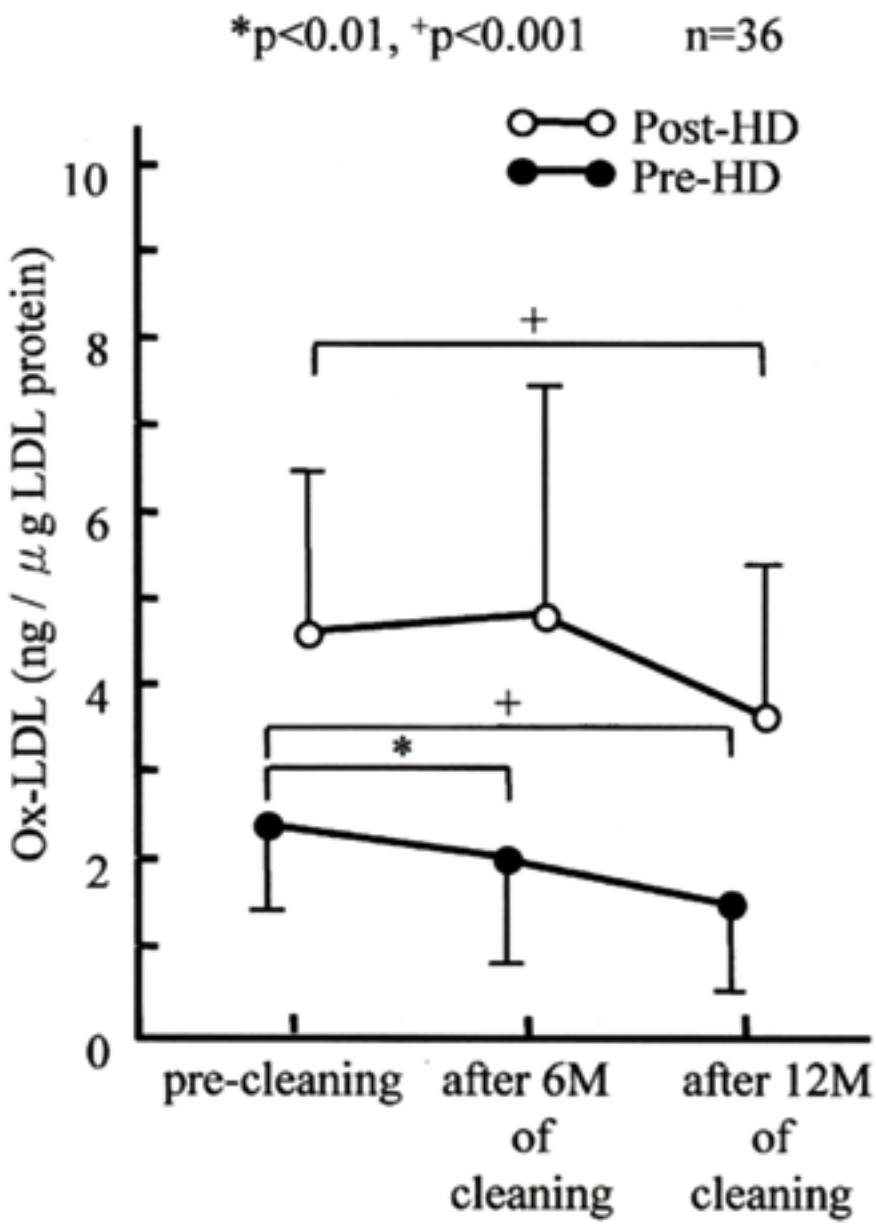

FIG. 4. Changes in oxidized-low-density lipoprotein (Ox-LDL) 1 year after dialysate cleaning. Mean ± SD, $N = 36$. *P <0.01; +P < 0.0001; (●) pre-hemodialysis; (○) post-hemodialysis.

All data observed were expressed as the mean value ± SD. The paired t-test was used to compare data before and after dialysis cleaning, and the unpaired t-test was used to compare data from patients with and without cardiovascular events, using StatView-J 4.5 (Abacus Concepts Inc., Berkeley, CA, USA). Differences were regarded as statistically significant at $P < 0.05$.

RESULTS

The mean endotoxin concentration in the dialysate decreased from 39.0 to an undetectable level (Fig. 2). CD-14, a receptor for endotoxin, expresses on the surface of monocytes. Soluble CD-14 is shed from monocytes after their activation (7). The mean sCD-14 level at predialysis was decreased significantly after the cleaning (from 6.35 ± 1.10 μg/mL to 5.40 ± 0.86 μg/mL, $P < 0.0001$) (Fig. 3).

The mean Ox-LDL level was significantly decreased 1 year after dialysate cleaning (from 2.39 ± 1.00 ng/μg LDL protein to 1.54 ± 1.03 ng/μg LDL protein, $P < 0.001$) (Fig. 4). The mean MDA-LDL level was also significantly decreased after dialysate cleaning (from 5.7 ± 1.5 nmol/mg LDL protein to 4.7 ± 1.3 nmol/mg LDL protein, $P < 0.001$) (Fig. 5). Interleukin-10 is a suppressor of antigen-induced inflammation. It reduces lymphokine production by Th2 cells. IL-10 increased significantly after the dialysate cleaning (from 2.1 ± 2.3 pg/mL to 3.6 ± 3.7 pg/mL, $P < 0.01$) (Fig. 6).

The patients experienced various cardiovascular events during the observation period of 2.8 years, such as cerebral apoplexy, acute myocardial infarction and gangrene of the limbs. We defined these events as cardiovascular events, and those due to microangiopathy were excluded.

New cardiovascular events occurred in four patients during this period, and consisted of intracerebral hemorrhage in two, acute myocardial infarction in one and gangrene of the limbs in one patient. We investigated cardiovascular events and levels of MDA-LDL and Ox-LDL at 1 year after dialysate cleaning (Fig. 7). Only six patients had MDA-LDL and Ox-LDL levels remaining higher than the standard values. New cardiovascular events occurred in four of the six patients. Thirty patients, not including

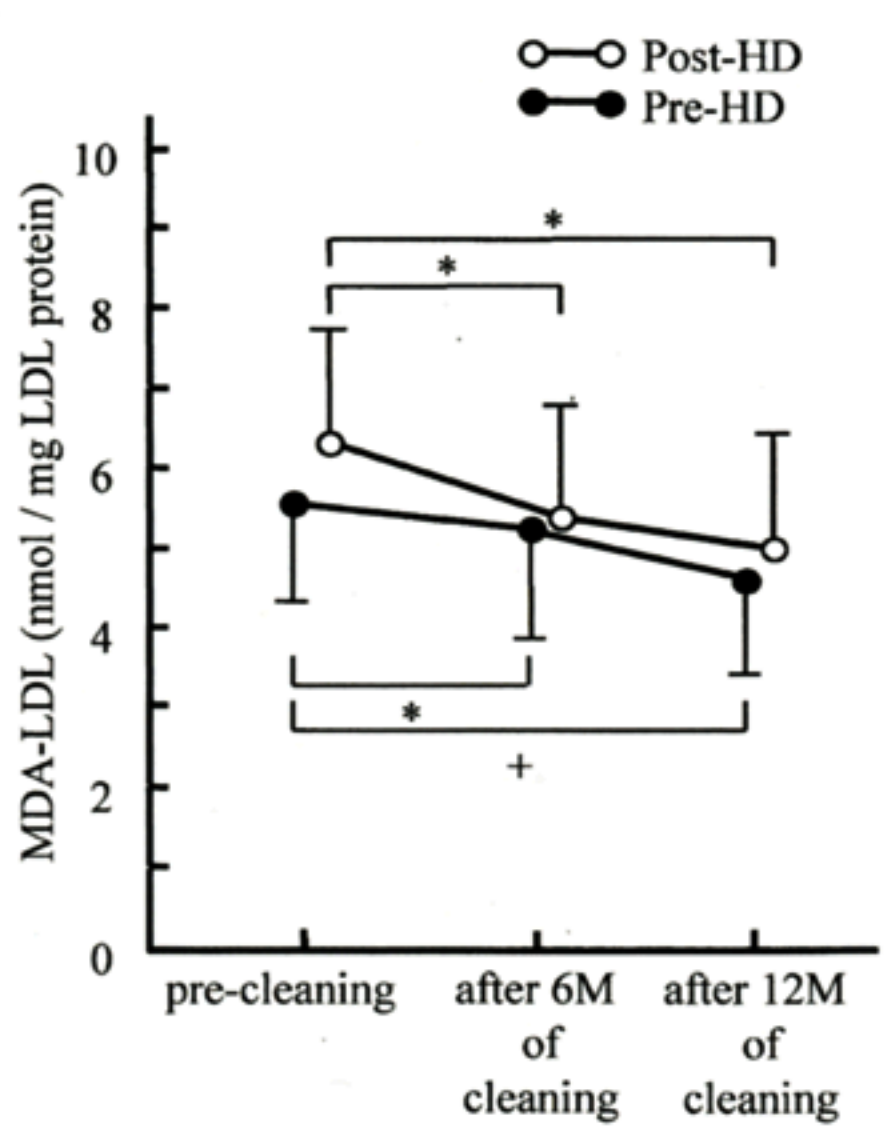

FIG. 5. Changes in malondialdehyde-low-density lipoprotein (MDA-LDL) 1 year after dialysate cleaning. Mean ± SD, $N = 36$. *P <0.01; +P < 0.0001; (●) pre-hemodialysis; (○) post-hemodialysis.

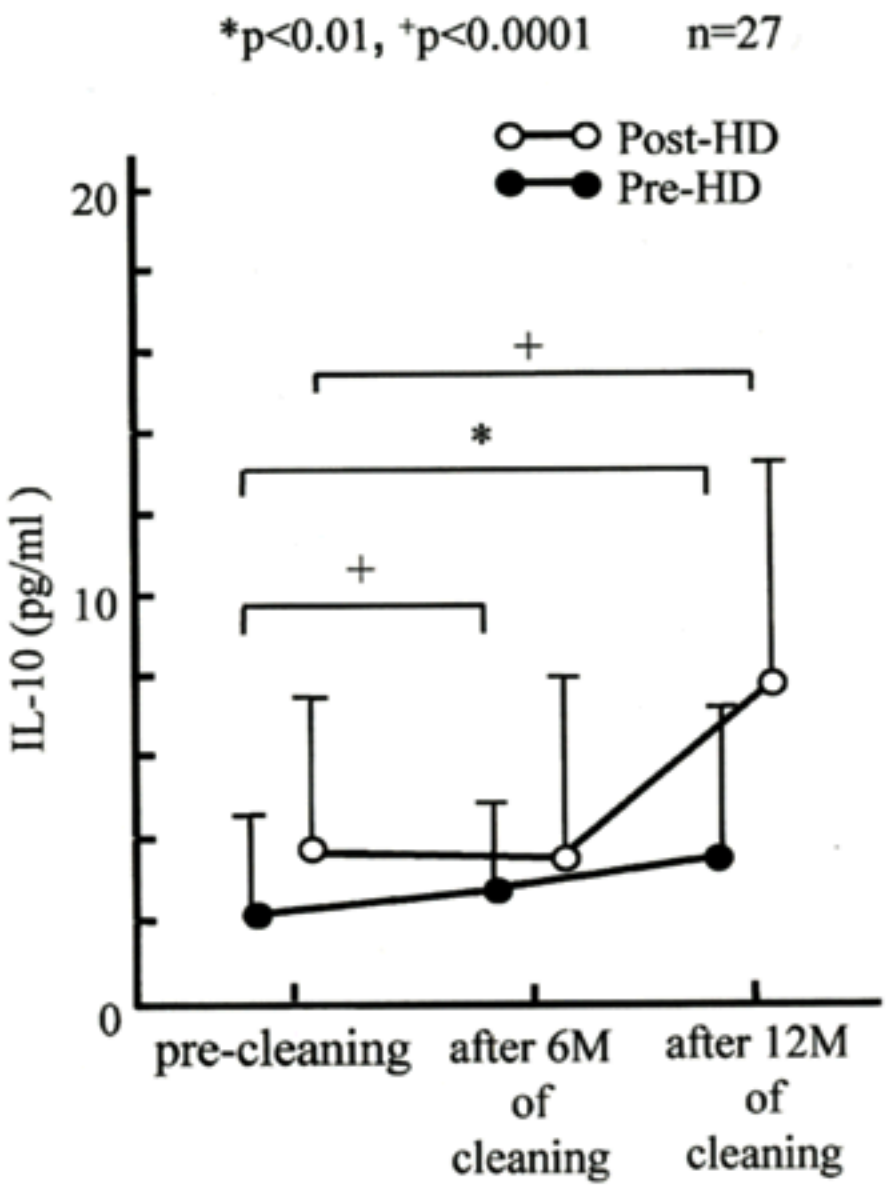

FIG. 6. Changes in interleukin-10 1 year after dialysate cleaning. Mean ± SD, $N = 27$. *$P < 0.01$; +$P < 0.0001$; (●) pre-hemodialysis; (○) post-hemodialysis.

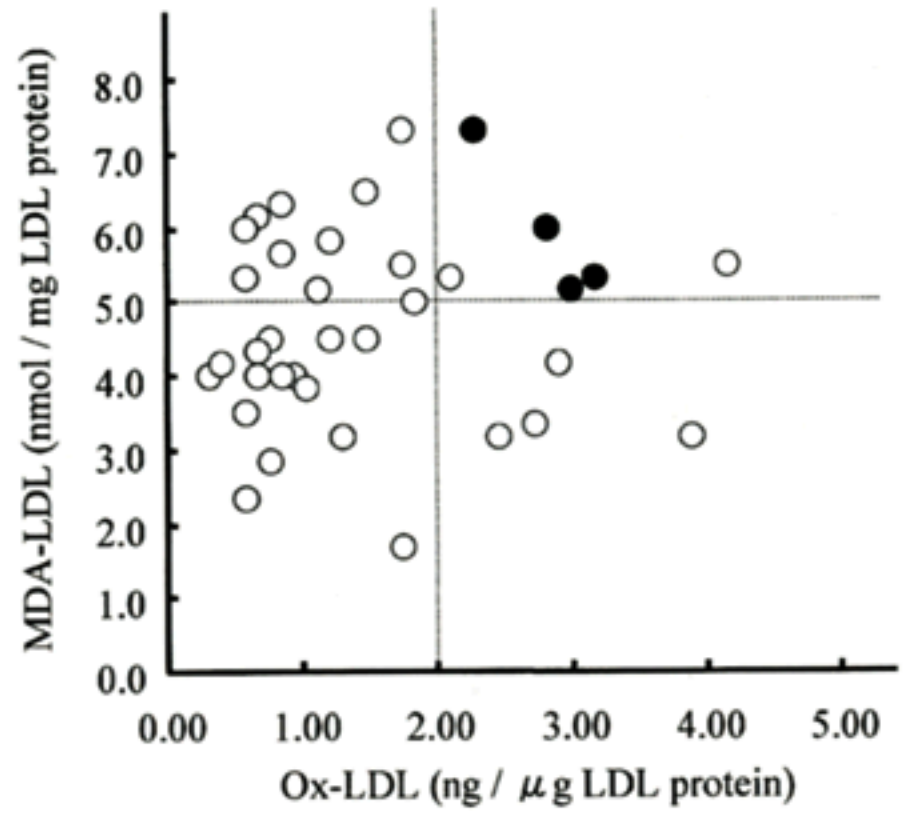

FIG. 7. Malondialdehyde-low-density lipoprotein (MDA-LDL) and oxidized-LDL (Ox-LDL) values at 1 year after dialysate cleaning and the occurrence of cardiovascular events. Standard ranges of Ox-LDL in dialysis patients: ≤5.0 nmol/mg LDL protein for MDA-LDL, ≤2.00 ng/ig LDL protein for Ox-LDL ($N = 36$). (●) Patients who showed cardiovascular events ($N = 4$); (○) patients who did not show cardiovascular events ($N = 32$).

the six patients with sustained high levels of oxidative stress, did not experience cardiovascular events during the observation period of 2.8 years.

The levels of sCD-14 in the four patients with cardiovascular events were significantly higher than those in patients who did not develop cardiovascular events, especially after hemodialysis (predialysis, $P < 0.01$; postdialysis, $P < 0.001$) (Fig. 8). Activation of monocytes was higher in cases with events.

The levels of Ox-LDL in the four patients with cardiovascular events were significantly higher than those in patients without cardiovascular events (predialysis, $P < 0.01$; postdialysis, $P < 0.01$). In these four patients, the levels of Ox-LDL were significantly increased after hemodialysis (Fig. 9). Oxidative

TABLE 1. *Baseline clinical characteristics of the six patients who showed MDA-LDL and Ox-LDL levels higher than standard values after 1 year of dialysate cleaning*

No.	1	2	3	4	5	6
Event	+	+	+	+	–	–
DM	–	–	–	+	–	–
Age (years)	64	65	53	71	60	64
Duration of HD (years)	26	24	11	7	7	2
Systolic BP (mm Hg)	132	164	166	148	160	152
Diastolic BP (mm Hg)	80	80	84	80	80	70
IL-6 (pg/mL)	4.63	5.48	8.25	11.33	2.52	5.40
IL-10 (pg/mL)	2.9	1.2	1.7	1.3	0.5	2.3
sCD-14 (μg/mL)	7.8	7.4	7.9	7.7	6.5	4.7
Pre-albumin (mg/dL)	36.2	30.2	32.7	32.9	41.3	37.9
Albumin (g/dL)	3.9	4	4.1	4.2	4.2	4.4
Total cholesterol (mg/dL)	185	180	189	160	242	151
Triglyceride (mg/dL)	95	136	292	126	110	51

These data were as of December 2000, i.e. after 1 year of dialysate cleaning. Compared to the two patients without cardiovascular events, the duration of HD was longer; sCD-14, which indicates the strength of activation of monocytes, was higher; IL-6, which indicates the strength of inflammation, was slightly higher; and, Pre-albumin and albumin, which indicate the state of nutrition, were slightly lower in the four patients with events. BP, blood pressure; DM, diabetes mellitus; HD, hemodialysis; IL, interleukin; MDA-LDL, malondialdehyde-low-density lipoprotein; Ox-LDL, oxidized-low-density lipoprotein; sCD-14, soluble CD-14.

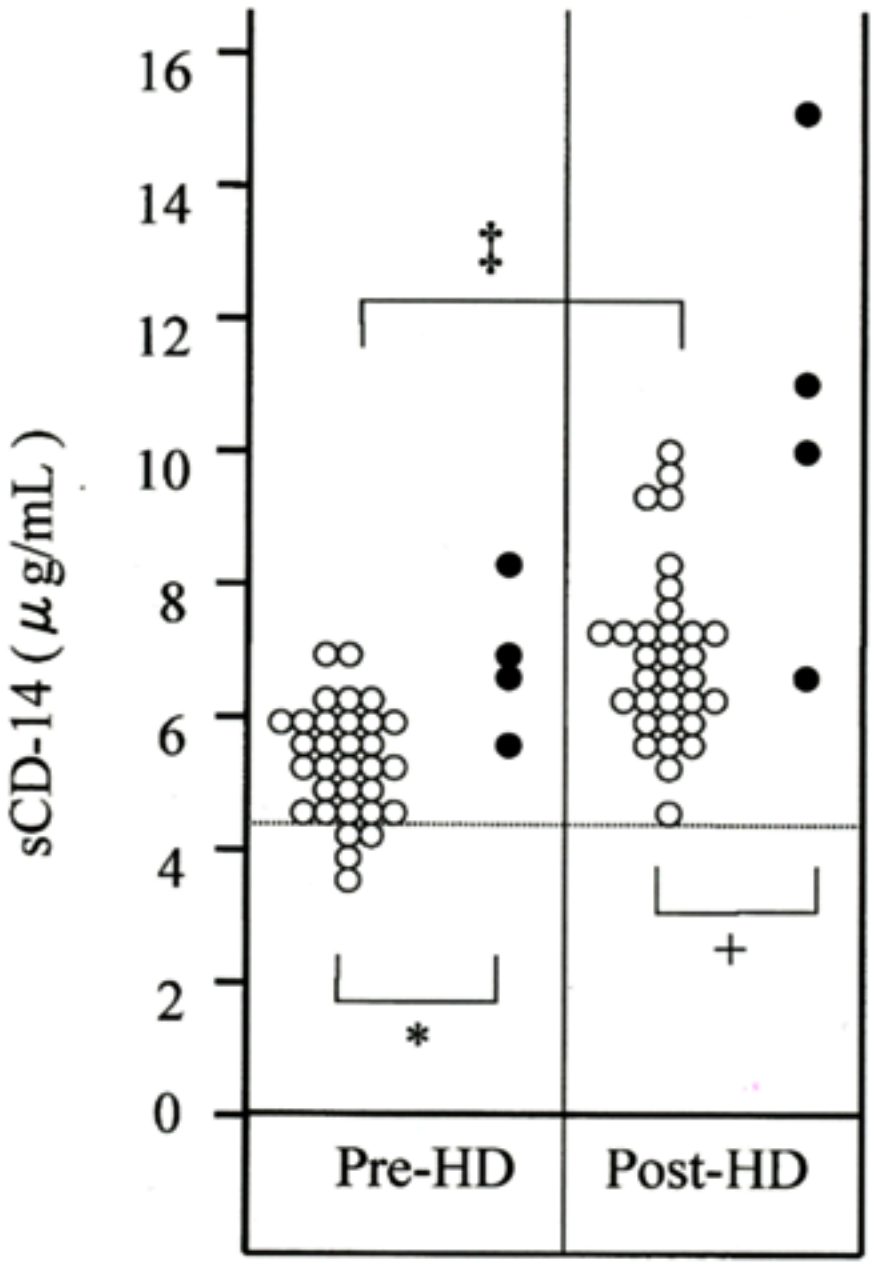

FIG. 8. Soluble CD-14 (sCD-14) values (1 year after dialysate cleaning) and the occurrence of cardiovascular events Standard range of sCD-14: 2.1 – 4.3 μg/mL, $N = 29$. Pre-hemodialysis (HD): ● vs. ○, $P < 0.01$; post-HD: ● vs. ○, $P < 0.001$; (●) pre-HD vs. post-HD, NS; (○) pre-HD vs. post-HD, $P < 0.0001$.

stress due to one session of dialysis was stronger in patients with cardiovascular events. MDA-LDL prior to dialysis was significantly higher in patients with events compared to those without events. ($P < 0.05$) (Fig. 10). In patients with events, oxidative stress was persistently high.

Many factors, other than the levels of MDA-LDL and Ox-LDL, such as age, duration of hemodialysis, inflammation, malnutrition, hypotension and diabetes mellitus, are well known to be associated with cardiovascular events. The baseline clinical characteristics of these six patients who presented with persistently higher values of MDA-LDL and Ox-LDL are described in Table 1.

DISCUSSION

Soluble CD-14 is a parameter that indicates the activity of monocytes, and monocytes are activated by immunological stimuli, such as endotoxin and contact with the dialyzer (8). The comparison of pre-dialysis data from before cleaning of the dialysate and 1 year after cleaning showed a significant decrease in sCD-14 ($P < 0.0001$), indicating that even a dialysate containing 50 EU/L or less of ET may stimulate monocytes and cause oxidative stress. The

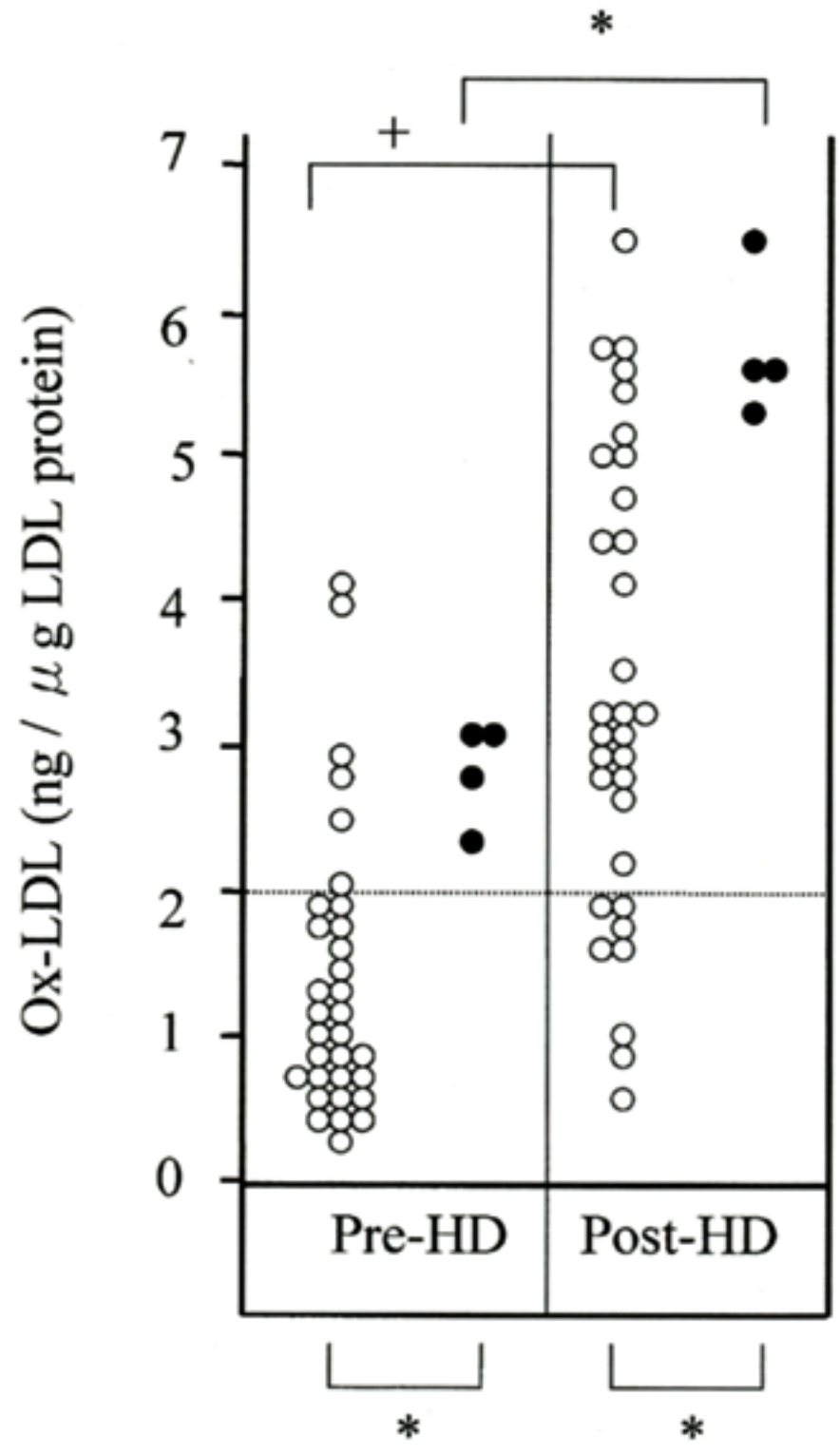

FIG. 9. Oxidized-LDL (Ox-LDL) values (1 year after dialysate cleaning) and the occurrence of cardiovascular events ($N = 36$). Pre-hemodialysis (HD): ● vs. ○, $P < 0.01$; post-HD: ● vs. ○, $P < 0.01$; (●) pre-HD vs. post-HD, $P < 0.01$;(○) pre-HD vs. post-HD, $P < 0.0001$.

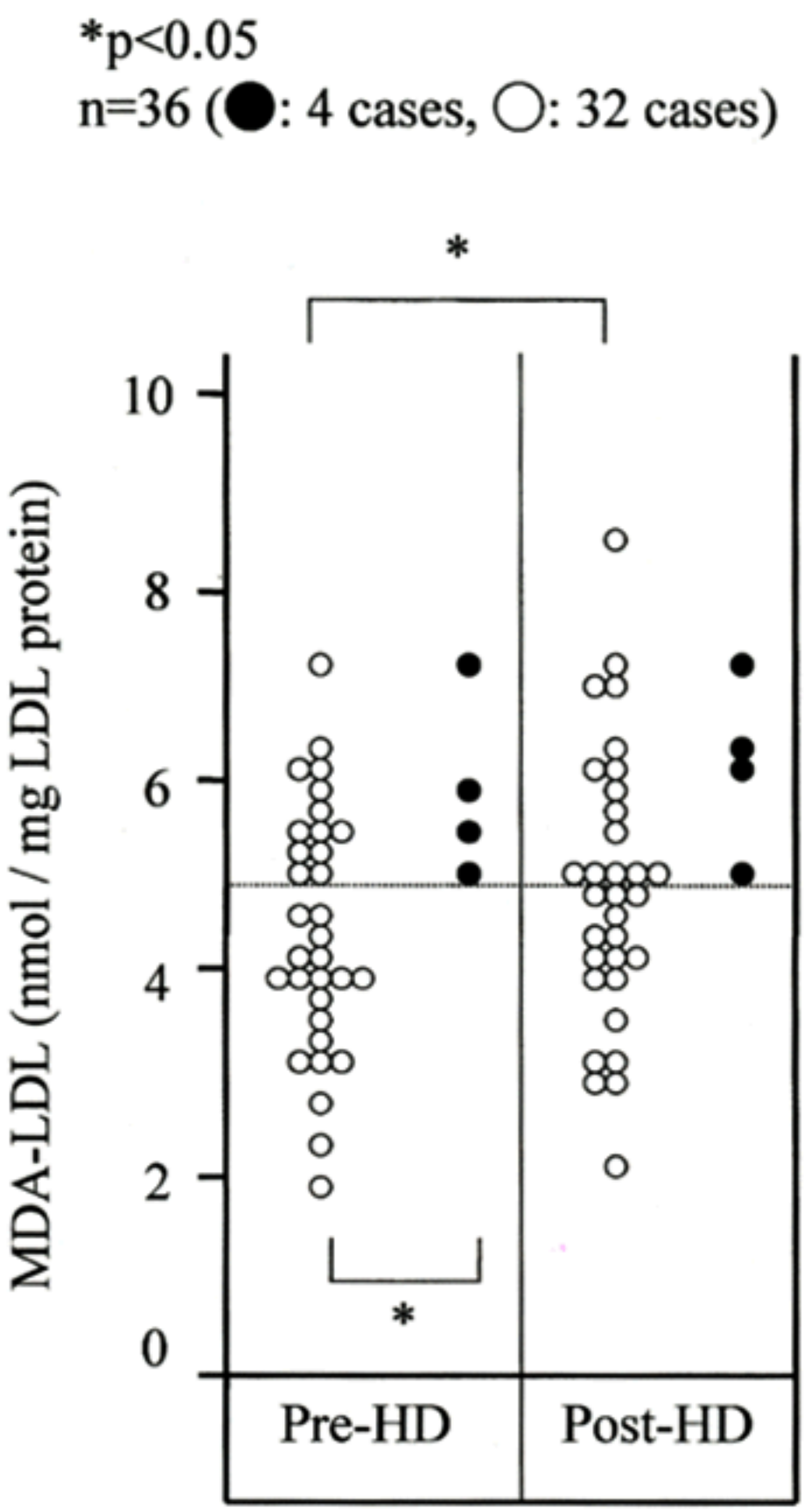

FIG. 10. Malondialdehyde-low-density lipoprotein (MDA-LDL) values (1 year after dialysate cleaning) and the occurrence of cardiovascular events ($N = 36$). Pre-hemodialysis (HD): ● vs. ○, $P < 0.05$; post-HD: ● vs. ○, NS; (●) pre-HD vs. post-HD, NS; (○) pre-HD vs. post-HD, $P < 0.05$.

endotoxin in the dialysate may cause oxidative stress in hemodialysis patients.

At present, MDA-LDL and Ox-LDL are assayed as oxidatively modified LDL. Although LDL-cholesterol is widely known to contribute to the pathogenesis of arteriosclerosis, oxidatively modified LDL has been noted to play a more important role in the progression of arterioclerosis (9). Monitoring of serum MDA-LDL and Ox-LDL is also expected to become a clinically useful index for coronary vascular disorders (9,10) and cerebrovascular disorders.

For 1 year after the dialysate cleaning, both MDA-LDL and Ox-LDL decreased significantly, showing that oxidative stress in dialysis patients could be decreased by cleaning of the dialysate. IL-10 is one of the anti-inflammatory cytokines, and its genotype protects dialysis patients from cardiovascular events (11). The level of IL-10 increased after dialysate cleaning in this study.

Whether the reduction of oxidative stress by dialysate cleaning alone is sufficiently effective to improve the prognosis of dialysis patients is a question that requires further investigation. However, dialysate cleaning is considered to be indispensable at least to prevent complications in patients under dialysis therapy over a long period of time and thereby improve their prognosis.

We investigated data at 1 year after dialysate cleaning and cardiovascular events during 2.8 years (i.e. 1 year after the dialysate cleaning and 1.8 years thereafter until the completion of this study) (Fig. 1).

Only six patients showed both MDA-LDL and Ox-LDL levels higher than the standard values; four of these six patients developed cardiovascular events (e.g. intracerebral hemorrhage) after cleaning of the dialysate. The levels of sCD-14, MDA-LDL and Ox-LDL were significantly higher in the patients who developed cardiovascular events, suggesting that monocytes were activated in such patients and that they were subjected to strong oxidative stress. However, the levels of Ox-LDL in those four patients increased significantly after hemodialysis, suggesting that they were subjected to strong oxidative stress after each session of hemodialysis.

For cases in whom persistently high levels of MDA-LDL and Ox-LDL were observed even after dialysate cleaning, some counter measures must be taken to reduce oxidative stresses. Various drugs with antioxidant activity and improved dialyzers are available for this purpose, but extensive investigations should be conducted using MDA-LDL and Ox-LDL as indexes.

Although the assay for MDA-LDL and Ox-LDL is not commonly used at present, it may be said that they will serve to monitor patients under chronic maintenance hemodialysis.

CONCLUSION

Even low levels of ET in the dialysate may stimulate monocytes and cause oxidative stress, potentially aggravating arteriosclerosis in patients under long-term hemodialysis. In such patients oxidative stress can be reduced by dialysate cleaning. However, four of the patients in this study with cardiovascular events were under strong oxidative stress even after dialysate cleaning.

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