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Improvement of Dyslipoproteinemia in Uremic Patients by Hemofiltration Therapy

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Abstract Uremic patients on prolonged maintenance hemodialysis (HD) therapy are at high risk for atherosclerotic cardiovascular complications. Dyslipoproteinemia which was composed of an accumulation of low density lipoprotein (LDL) subfractions, most probably due to deficiency of very low density lipoprotein (VLDL) catabolism, and an extreme decrease of high density lipoprotein (HDL) has been detected in most uremic patients mainteined HD. Three uremic patients with dyslipoproteinemia were transferred from maintenance HD to hemofiltration (HF). The influences of HF on their serum lipoprotein (Lp) profiles were investigated using polyacrylamide-gel (PAG) disc Lp electrophoresis, ultracentrifugal analysis and chemical methods. Improvement of dyslipoproteinemia which consisted of a disappearance or reduction of the accumulated LDL subfractions (Sf 10-19 LDL) and a gradual increase of HDL was observed in two of the three patients within three or four weeks after introduction of HF. The results of the present study indicate that Lp profiles in some HD patients may be improved by HF therapy.

Key Words: Dyslipoproteinemia; uremia, hemodialysis. Uremia; lipoprotein, hemofiltration. Lipoprotein; hemofiltration, ultrafiltrate

Introduction

High incidence of hypertriglyceridemia[1] and atherosclerotic cardiovascular complications[2,3] had been reported in uremic patients on prolonged hemodialysis (HD) therapy. The early study[4] from the authors' clinic demonstrated that more than 58% of HD patients showed an unique abnormality in serum lipoprotein (Lp), broad mid band Lp pattern (BMP)[4] on polyacylamide gel(PAG) electrophoresis. Recent studies[5-7] from the authors' clinic revealed that BMP is more frequent in HD patiens than previously reported. BMP was ascribed to the presence of a discrete series of abnormal Lp subfractions whose chemical and physical properties are intermediate ones between those of very low density Lp (VLDL) and low density Lp (LDL)[6]. Immunoreactive high density Lp (HDL) as well as HDL-cholesterol (HDL-Ch) decreased greatly in HD patients who

showed BMP as compared with non-uremic healthy persons[7]. From the current concept of Lp metabolism, dyslipoproteinemia in uremic patients might be due to an accumulation of the remnant of VLDL catabolism and reduction of HDL[8-10].

Recently hemofiltration (HF) has been introduced to clinical practice and the clinical experience of HF has been reported[11)-19]. Recent studies[14-16] have shwn a significant decrease in serum triglyceride (TG) level in uremic patients transferred from HD to HF therapy. The objective of the present study was to evaluate the effects of HF on the dyslipoproteinemia in uremic patients.

Materials and Methods

Three uremic patients who were transferred from HD to HF therapy were studied. Lp profile on PAG disc electrophoresis of these patients was BMP-a according to author's definition reported previously[7]. Serum Lp profiles of HD patients had been classified previously into four patterns including BMP-a, PMP-b, BMP-c and normal pattern by visual observation of PAG disc electrophoretogram[7]. Examples of each of three patterns and their variants are shown in Fig. 1. These patients were selected because VLDL catabolism might be most defective and HDL concentration should be most severely reduced in uremic patients with BMP-a. These patients had neither nephrotic syndrome nor diabetes mellitus. Informations pertinent to the patients are summarized in Table 1. The clinical status of these patients had been stable during their respective schedules of HD and HF. The patients had been maintained by HD for 6 yrs prior to transferring from HD to HF. In two patients (Case-1 and Case-2), HF was performed for 22 wk and in the other (Case-3) for 5 wk.

HD run in the authors' clinic was conducted using Kolff or Hollow Fiber type dialyzer and commercially available dialysate (Kindaly Solution-3, Fuso Pharmaceuticals, Osaka). The dialysate was composed of Na^+, 132 mEq/1; K^+, 2 mEq/1; Cl^-, 104 mEq/1; Ca^{2+}, 3.5 mEq/1; Mg^{2+}, 1.5 mEq/1; acetate, 35 mEq/1 and glucose, 200 mg/100 ml. The flow rate of dialysate, average weekly hours of HD and infusion rate of heparin were 500 ml/min, 18 h three time per wk, and 1,000-1,200 units per h, respectively.

HF was performed by means of an RP-6 hemofilter (Rhone-Poulenc, Paris) with a cut-off for substances with a molecular weight of 20,000 daltons using post-dilution mode. Average duration of HF, infusion rate of heparin, and ultrafiltrate were 18 h three times per wk, 1,000-1,200 unit per h, and 20 1, respectively. The substitution fluid administered into venous line was composed of Na^+, 140 mEq/1; K^+, 2 mEq/1; Cl^-, 107 mEq/1; Ca^{2+}, 3.5 mEq/1; Mg^{2+}, 1.5 mEq/1; and lactate, 40 mEq/1.

As estimated on the basis of diet charts recorded by the patients, average daily nutrient intake consisted of protein 1.3-1.6 g, lipid 1.6-1.8 g and carbohydrate 6.0-7.0 g/k body weight, corresponding to daily caloric intake of 45-50 kcal per kg.

Blood samples were obtained after an overnight fast of 14-16 h. The sample period was 48-72 h after the previous HD or HF. For determination of lipid and electrophoresis of Lp, sera were kept in a cold room (2-4°C) and the analyses were completed within 72 h. For ultracentrifugal studies, disodium EDTA (1 mM) and Thiemerosal (0.05%) were added to serum to prevent denaturation of Lps during preparative and analytical procedures. For analytical ultracentrifugal studies, Lp fractions (VLDL, dl .006< ; LDL, dl .006-1.063 ; and HDL, dl .063-1.21) were isolated in an RP 80 T angle rotor mounted on a 80P ultracentrifuge. The fractions were dialyzed against the salt solutions with various densities which were prepared by adding a crystalline KBr to a 0.154 M NaCl solution. The density of the various solutions was determined by a pycnometer. To one sector of the double-sectored alumium cell for the analytical ultracentrifugation, 0.4 ml of a Lp solution (LDL or HDL) was placed and to another sector, 0.45 ml of the salt solution with respective density. A Hitachi RA60H rotor mounted on a UCA-1A machine was operated at 43,700 rpm (138,777 g) at 20.0±0.5°C The Schlieren patterns were recorded on X-ray films and analyzed by using a Nikon profile projector equipped with micrometers (Nippon Opticals, Co., Tokyo) and by planimeter. Sf rate of LDL or LDL subfractions and F rate of HDL were determined by the procedures described previously[20]. Equation derived by Pickels[21] for the Schlieren optical system of the analytical ultracentrifugation was used to convert peak area of LDL subfrac-

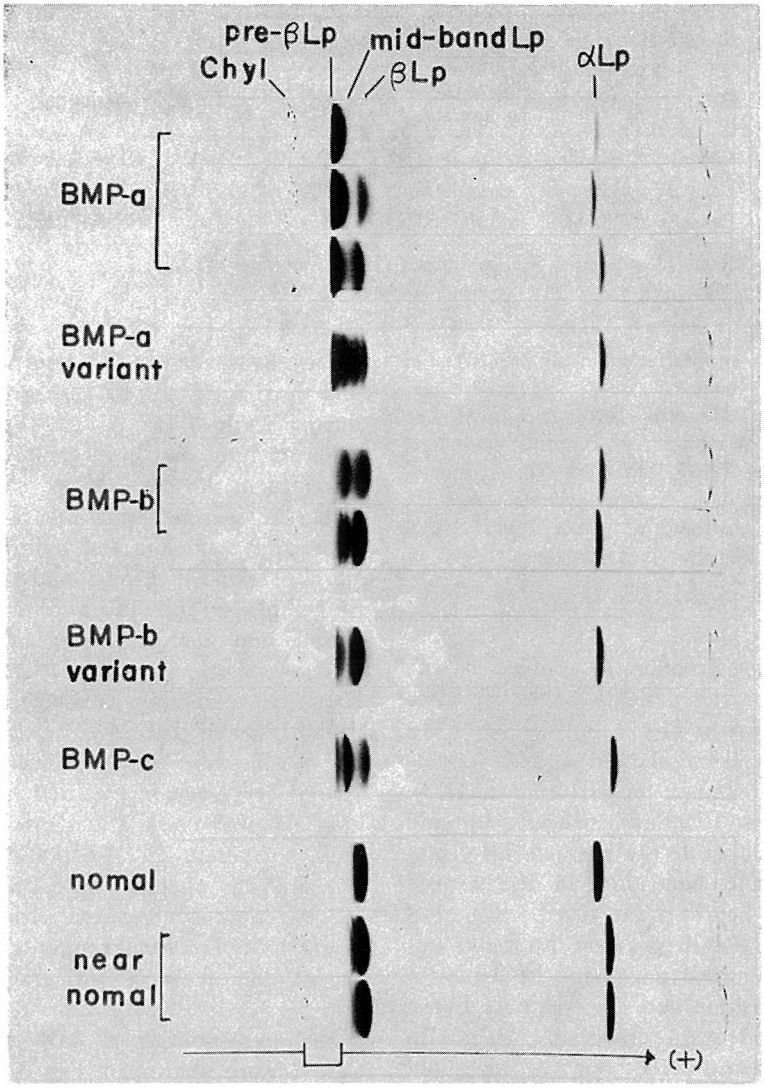

Fig. 1 Four representative PAG disc electrophoretic patterns (BMP-a, BMP-b, BMP-c and normal pattern) and their variants of serum lipoproteins in hemodialysis patients (Sudan black-B prestained). Chyl: Chylomicrons, Pre-β Lp: pre-beta lipoprotein, mid-band Lp: mid-band lipoproteins, β Lp: beta lipoprotein, α Lp: alpha lipoprotein. sample gel, spacer gel and separating ge l: PAG layers in disc electrophoresis. Anode (+) is to the right.

Table 1 Identification, Age, Sex, Cause of Uremia, Duration of Hemodialysis and Hemofiltration, and used Hemofilter

Patients	Age-Sex	Cause of uremia	Duration of HD**	Duration of HF***	Hemofilter
Case-1	56male	chronic GN*	6 yr	22wk	RP-6(1.2m²)
Case-2	55female	chronic GN*	6 yr	22wk	RP-6(1.2m²)
Case-3	45male	chronic GN*	6 yr	5 wk	RP-6(1.2m²)

*Glomerulonephritis, **hemodialysis (18h 3times per wk), ***Hemofiltration (18h 3times per wk).

tions or HDL to concentration in mg per 100 ml of original serum sample.

Methods for PAG disc electrophoresis of Lps were reported previously[22]. TG and cholesterol (Ch) contents of VLDL, LDL and HDL were determined by the methods described elsewhere[7,23]. Methods for measurement of serum lipid[23,24] and protein[25] were also described elsewhere.

Results

PAG disc electrophoretic profiles of the serum Lp from the patients before or after HF were shown in Fig. 2.

In Case-1, serum showed a BMP-a pattern during treatment of HD (before HF). Three wk after HF, dense pre-beta Lp and mid band Lp close to the pre-beta Lp disappeared and mid band Lps in the central position and beta Lp increased. Six or 8 wk after HF, a thin pre-beta Lp band appeared and a broad mid band Lp band in the central position and a moderately dense beta Lp band were observed. Alpha Lp decreased during HD, but it increased gradually during HF.

In Case-2, a BMP-a pattern observed before HF did not change in three wk after introduction of HF. Six or 8 wk after HF, pre-beta Lp and mid band Lps detected in the position close to pre-beta Lp were reduced or disappeared and moderately dense beta Lp appeared as in Case-1. Alpha Lp did not change after 3 wk of HF. A slight increase in alpha Lp was observed 6 wk after HF.

Case-3 showed a BMP-a pattern before and during HF. Alpha Lp increased slightly during 5 wk of HF.

Serum lipid and lipid compositions of Lp fractions in the patients are summarized in Table 2. Serum lipid before HF showed hypertriglyceridemia and normal total cholesterol (TC) level in all the patients. Normal ranges of TG and TC were 70.3±17.4mg/100 ml, and 154.3±29.3mg/100ml, respectively, as reported previously by the author[7]. Hypertriglyceridemia is ascribed to TG level in VLDL and LDL. LDL of the patients before HF were rich in TG. During HF, serum TG level (i.e. VLDL-and LDL-TG level) showed marked declines to a normal level, but serum TC, VLDL-Ch, and HDL-TG did not change. HDL-Ch level before HF were low as compared with our previous values for its normal range, 45.8±11.2mg/100 ml, but it increased gradually during HF.

Schlieren profiles of LDL and HDL in Case-1 were shown in Fig. 3. Before HF, LDL Schlieren profile showed three peaks of LDL subfractions (upper panel of Fig. 3). Sf rate of these LDL subfractions was Sf 19, Sf 13 and Sf 4. Three wk after beginning HF, these LDL subfractions disappeared and were replaced by Sf 7 and Sf 6 LDL. Eight wk after HF, a small amount of the Sf 13 LDL subfraction had reappeared, but after 14 wk this LDL subfraction disappeared and Sf 4 and Sf 7 LDL subfractions were observed. Schlieren profiles of LDL and HDL

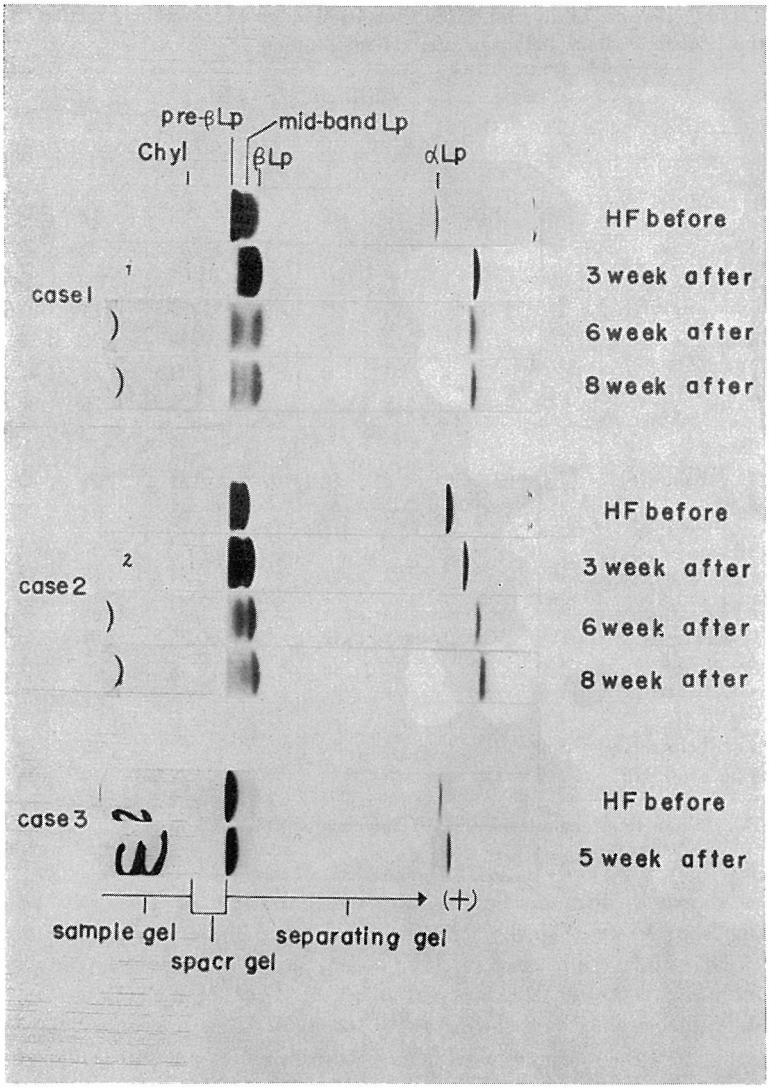

Fig. 2 Representative PAG disc electrophoretograms of serum lipoproteins in the uremic patients before or after hemofiltration. Case numbers are identical to those in Table 1. Lp profiles of all the patients represented BMP-a pattern before hemofiltration. Abbreviations are same as in Fig. 1.

Table 2 Serum Lipid and Lipid Composition of Lipoprotein Fraction in the Uremic Patients before or after Hemofiltration

	Plasma		VLDL		LDL		HDL	
	TC (mg/100ml)	TG	Ch (mg/100ml)	TG	Ch (mg/100ml)	TG	Ch (mg/100ml)	TG
Case-1								
before HF	196	197	10	72	118	92	19	33
3 wk	169	82	10	5	133	47	26	30
4 wk	168	55	—	—	—	—	—	—
6 wk after HF	178	80	14	22	140	26	24	32
8 wk	180	111	10	52	142	29	28	30
14 wk	129	72	3	0	103	35	23	37
22 wk	133	91	15	32	130	23	26	36
Case-2								
before FH	158	246	6	85	85	121	16	40
3 wk	188	134	6	64	101	35	34	35
4 wk	152	81	—	—	—	—	—	—
6 wk after HF	152	78	2	21	132	25	18	32
8 wk	148	86	11	27	117	24	20	35
14 wk	196	84	13	13	163	26	20	35
22 wk	183	84	5	48	134	9	44	37
Case-3								
before HF	162	210	74	97	73	92	15	21
5 wk after HF	169	150	80	75	74	27	15	28

TC. Serum total cholesterol, TG: Triglyceride, Ch: Cholesterol.

in Case-2 were shown in Fig. 4. Schlieren profile of LDL showed two peaks of Sf 16 and Sf 10 LDL subfractions before HF (upper panel of Fig. 4). After HF was introduced, LDL subfractions with large Sf rates (Sf 16 and Sf 14) were reduced or disappeared gradually. Schlieren profiles of LDL and HDL in Case-3 were shown in Fig. 5. LDL subfractions were not altered during HF (upper panel of Fig. 5). In Schlieren profiles of HDLs, peak area, which implies concentration of HDL, was increased gradually in all the patients during HF as shown in lower panels of Fig. 3-5. Total LDL (LDL subfractions and normal LDL) concentration of the two patients (Case-1 and Case-2) determined by analytical ultracentrifugal analysis was reduced after three wk or 4 wk of HF (Table 3). On the contrary, in these patients HDL concentration determined by analytical ultracentrifugal analysis increased gradually during HF (Tabel 3). A shift from large Sf rate of LDL subfractions to lower ones was demonstrated in two of the three patients.

In summary accumulated LDL subfractions composed of remnant Lps disappeared or were reduced, and HDL concentration was increased gradually in two of the three patients during HF therapy.

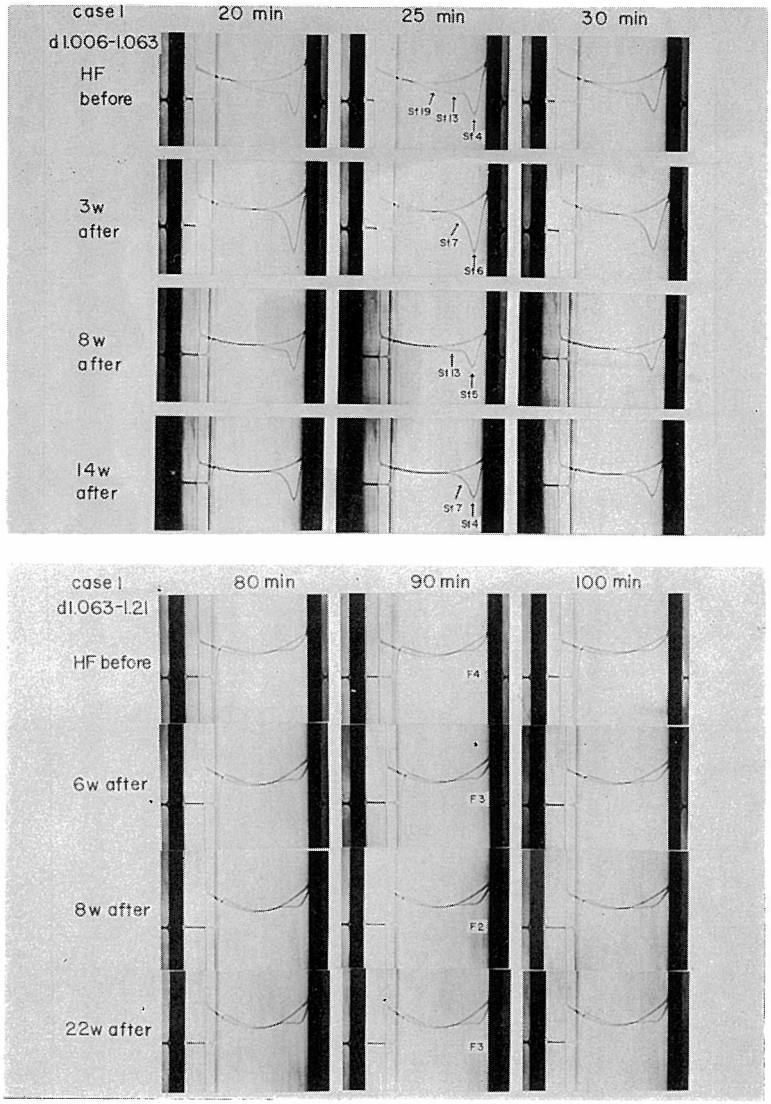

Fig. 3 Ultracetrifugal Schlieren profiles of LDL (d 1.006–1.063) at density 1.063 (upper panel) and HDL (d 1.063–1.21) at density 1.21 (lower panel) isolated from Case–1 before or after hemofiltration. Numbers in the frames indicate Sf rate or F rate of the Schlieren peaks. Photograms were obtained at 20, 25, 30, 80, 90, and 100 min during ultracentrifugal run at 43,700 rpm and at 20.0±0.5°C.

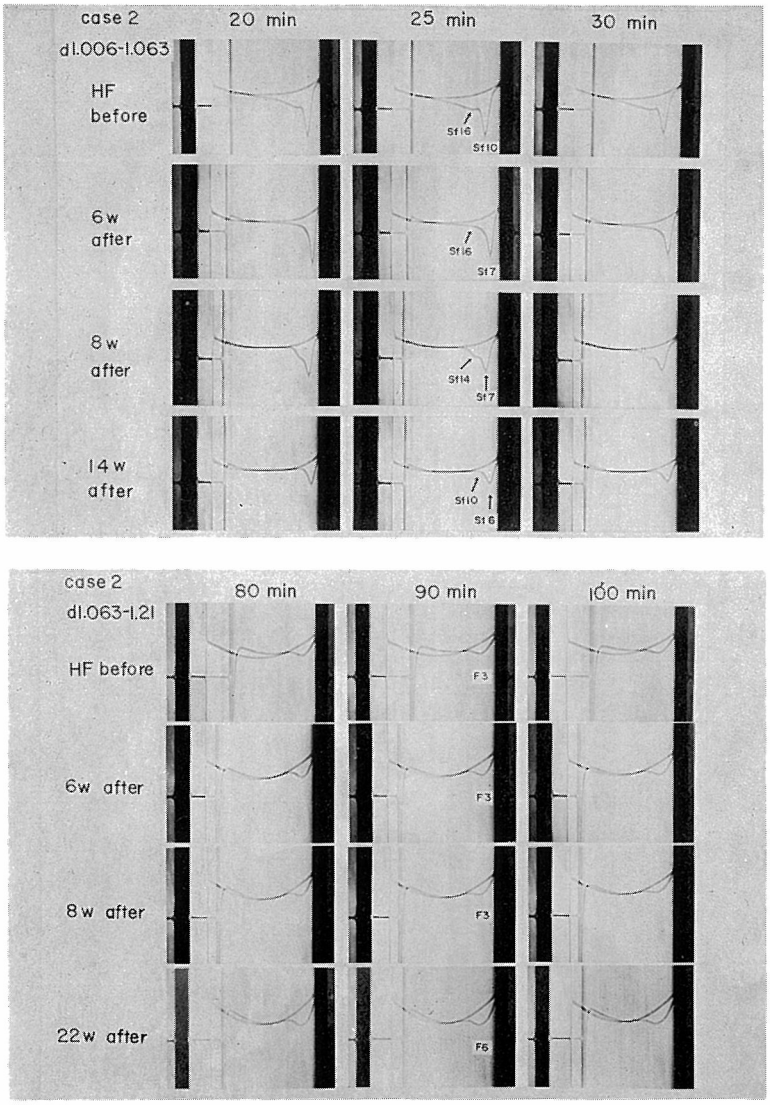

Fig. 4 Ultracentrifugal Schlieren profiles of LDL (d 1.006–1.063) at density 1.063 (upper panel) and HDL (d 1.063–1.21)at density 1.21 (lower panel) isolated from Case–2 before or after hemofiltration. Abbreviations are same as in Fig. 3.

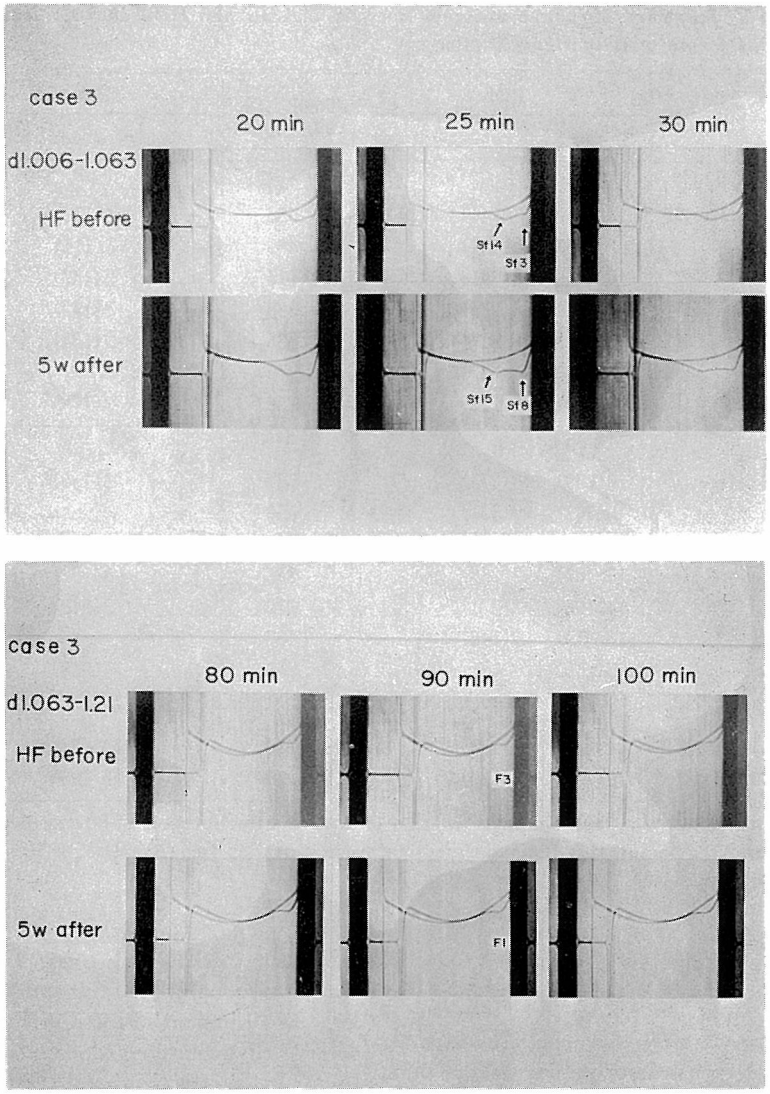

Fig. 5 Ultracentrifugal Schlieren profiles of LDL (d 1.006-1.063) at density 1.063 (upper panel) and HDL (d 1.063-1.21) at density 1.21 (lower panel) isolated from Case-3 before or after hemofiltration. Abbreviations are same as in Fig. 3.

Table 3 Summary of Ultracentrifugal Analysis of LDL and HDL in the Uremic Patients before or after Hemofiltration

	LDL			Total LDL (mg/100ml)	HDL
	Sf rate (Lp concentration)				F rate (Lp concentration)
Case-1					
before HF	19 (74)	13(144)	4(310)	526	4(113)
3 wk	7(105)	6(304)		409	3(115)
4 wk	9(115)	6(201)		316	3(147)
6 wk after HF	20(143)	4(246)		389	3(192)
8 wk	13 (78)	5(257)		335	2(164)
14 wk	7 (65)	4(280)		375	—
22 wk	10 (70)	3(180)		250	3(179)
Case-2					
before HF	16(295)	10(273)		567	3(159)
3 wk	18(247)	6(331)		580	—
4 wk	9 (84)	7(153)		237	3(158)
6 wk after HF	16 (97)	7(288)		385	3(182)
8 wk	14 (98)	7(221)		319	3(146)
14 wk	10 (19)	6(148)		167	—
22 wk	5(250)			250	6(268)
Case-3					
before HF	14 (85)	3(123)		208	3 (74)
5 wk after HF	15(117)	8(136)		253	1(125)

Numerals in the table indicate Sf rate of LDL subfractions or F rate of HDL. Numerals in parentheses indicate lipoprotein concentration (mg/100ml) of LDL subfractions or HDL.

Discussion

Employing paper and PAG disc electrophoresis and preparative ultracentrifugation, Wada et al[4]. demonstrated an occurrence of gross abnormality in serum Lps of HD patients. The abnormality which occurred in 58% of the patients appeard as a broad mid band Lp pattern (BMP)[4]. Decrease of alpha Lp and its momentary increase during HD therapy were also observed. By the latest studies[5,6] from the authors' clinic, it was revealed that the BMP is ascribed to the presence of a discrete series of the mid band Lps of Sf 13-15 and Sf 10. From the current concepts[8,26] of human serum Lps and their metabolism, these LDL subfractions are probably the intermediate density Lp[27] or remnant Lp (s) of VLDL (may include chylomicrons) catabolism. A recent study[7] from the authors' clinic indicated that a significant decrease of immuno-reactive moiety as well as lipid moiety of HDL was observed in HD patients with BMP. This occurred in 87% of the patients.

As a possible mechanism for the genesis of BMP and hence for the accumulation of remnant Lps, Wada et al[4]. proposed that enhanced intravascular lipolysis provoked by systemic heparinization in the circumstance of high glucose availability, inherent to HD therapy, may result in endogenous resynthe-

sis of the mid band Lps. Samar et al[28]. suggested that the square-meter hour (m^2 of dialysis membrane) of dialysis may be an important factor in the genesis of lipid and Lp abnormalities in HD patients. Futhermore, Gonzaleg et al[29]. suggested that a cause of hypertriglyceridemia in HD patients could be the synthesis of TG from acetate, which is transferred to the patients from the dialysate. However, Savdie et al[30]. reported that dyslipoproteinemia in uremic patients could not be improved markedly by bicarbonate substituted for acetate in the dialysate. Influences of administration of anti-lipidemic drugs and of diet therapy on lipid and Lp metabolism had been suggested[31-33] but had not been proved. Recently, Murase et al[34] reported an inhibitor of Lp lipase (LPL) in Lp free plasma. Futhermore, Mordasini et al [35] reported a selective deficiency of hepatic TG lipase in uremic patients. These may be involved in the pathogenesis of dyslipoproteinemia in uremic patients.

Since HF was designed by Henderson et al[36]. in 1967 in a manner analogous to the kidney glomeruli and tubules. HF had been introduced to clinical practice in uremic patients to remove the accumulation of toxic quantities of solutes termed middle molecules[37] in the molecular range of 300 to 3,000 dalton, because HD is inefficient in the extraction of middle molecular substances.

Clinical experiences of HF had been reported on its effect on symptomes[11], hypertension[12,13], triglyceride[14,16], electroencephalogram[17,18], and hormones[19] in uremic patients. Qullhorst[14], Schneider et al.[15], and Maekawa et al.[16] showed a significant decrease in plasma TG level in uremic patients transferring from maintenance HD to HF. However, no clinical investigation concerning Lp profiles in uremic patients transferring from HD to HF has been reported.

The present study demonstrated that accumulation of remnant Lps consisting with Sf 10-19 LDL subfractions (appeared as BMP in PAG disc electrophoretogram in the HD patients) disappeared or was reduced, and changed to lower Sf rate LDL subfractions and that HDL increased gradully during HF therapy. It was therefore, suggested that Lp metabolism in some of HD patients may be improved by HF therapy.

There are three major different factors between HD and HF; (1) the substances removed from plasma which are below 500 dalton in molecular weight by dialyzer of HD and below 20,000 dalton by hemofilter of HF, (2) lactate contained in substitution fluid used in HF was substituted for acetate contained in dialysate used in HD, (3) high glucose concentration (200 mg/100 ml), the dialysate used in HD contained but the substitution fluid used in HF did not. The amounts of heparin infused into patients on HD or HF is not different. Hence it appears that systemic heparinization could not be causative for dyslipoproteinemia in HD patients. If a LPL inhibitor in plasma of uremic patients proposed by Murase et al.[34] is in a molecular weight range from 500 to 20,000 dalton, the inhibitor could be removed from patients' plasma to ultrafiltrate by hemofilter with cut-off for substances with a molecular weight of 20,000 dalton, and Lp metabolism may be improved during HF therapy.

Search for the substances in the ultrafiltrate having a molecular weight range from 500 to 20,000 dalton is obviously required.

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