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LABORATORY STUDY

The proportion and metabolic effects of adiponectin multimeric isoforms in patients with chronic kidney disease on maintenance hemodialysis

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ABSTRACT

Adiponectin circulates at least in three major forms of oligomeric complexes in plasma: a low-molecular-weight (LMW) trimer, a middle-molecular-weight (MMW) hexamer, and high-molecular-weight (HMW) adiponectin. Although it has been reported that adiponectin has the favorable metabolic properties for humans, the roles of these multimers in the patients with the end-stage renal disease (ESRD) were unidentified. We determined the level of total and multimeric adiponectin in 71 patients with nondiabetic ESRD treated with hemodialysis (HD) using a commercially available kit of enzyme-linked immunosorbent assay (ELISA). Correlations between metabolic variables and total and multimeric adiponectin were examined by Spearman's correlations analysis. Forward stepwise multiple linear regression analysis was also performed to determine the factors independently associated with them. Female patients had significantly higher total, HMW, and MMW levels than male patients did. According to homeostasis model of assessment of insulin resistance (HOMA-IR), value was associated not only with HMW but also with MMW and LMW. In multivariate analyses, HMW showed independently and positively associated with high-density lipoprotein cholesterol (HDL-C), body mass index (BMI), and sex as total adiponectin did. Unexpectedly, LMW adiponectin was independently and negatively correlated with TG and high-sensitive C-reactive protein (hs-CRP). Not only HMW adiponectin but also LMW adiponectin track with favorable metabolic effects in the patient with the ESRD.

Keywords: adiponectin; low-molecular-weight trimer; middle-molecular-weight hexamer; high-molecular-weight multimer; hemodialysis

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Adiponectin is an adipose tissue-specific protein that is abundantly present in human plasma.[1] It plays multiple protective roles in insulin sensitivity,[2] anti-inflammation, and antiatherosclerosis.[3] The reduced level of plasma adiponectin has been clinically demonstrated as a risk factor for the development of obesity,[4] type 2 diabetes mellitus,[5] coronary artery disease,[6] and hypertension.[7] Increasing evidence from experimental studies revealed that adiponectin protects against the development of diabetes mellitus, hypertension, and cardiovascular disease.

Abnormal glucose and insulin metabolism are common in patients with chronic renal failure (CRF). In addition, it is well known that increased cardiovascular morbidity and inflammation in these patients.[8] However, it was demonstrated that the level of plasma adiponectin was increased in patients with chronic kidney disease (CKD), transplanted patients,[9] and those with end-stage renal disease (ESRD) both on hemodialysis (HD)[10,11] and peritoneal dialysis.[12] Ohashi et al. reported that higher, rather than lower, plasma adiponectin levels independently predicted total mortality in HD patients.[13] A recent cohort study showed that the level of adiponectin was associated with increased mortality in patients with CKD stages 3 and 4 [mean glomerular filtration rate (GFR) of 33 mL/min/1.73 m^2].[14] It has not yet elucidated the mechanism why high adiponectin levels were associated with total

death. In general, weight loss increases plasma adiponectin levels.[15] An experimental study suggested that adiponectin increased resting energy consumption and induced weight loss through a direct action on the brain.[16] Nutritional states in most of the HD patients become gradually worse and catabolism progresses. As a result, a higher body mass index (BMI), which is expected to be associated with lower adiponectin, paradoxically may confer a survival advantage in HD patients apart from general population.[17]

Adiponectin circulates in three major forms of oligomeric complexes in plasma and these forms are separated by gel filtration chromatography[4,18]; low-molecular-weight (LMW) trimers, middle-molecular-weight (MMW) hexamers, and high-molecular-weight (HMW) multimers.[19] The basic structure of adiponectin is that of a trimer, and the trimers are connected by disulfide bonds at N-terminus in a larger multimer. Adiponectin monomers have an N-terminal collagen-like domain and a C-terminal globular domain.[20] The adiponectin globular head is also detected in the trimeric form in human and mouse plasmas albeit at low concentrations.[21,22] Various mutations of human adiponectin related to diabetes and hypoadiponectinemia were reported[23] and it was revealed that they were associated with impaired multimerization and possibly with impaired secretion and/or impaired action of adiponectin.[19] Recent studies showed that HMW adiponectin, not total adiponectin, was associated with insulin sensitivity,[24,25] and that HMW isoform, specifically, promotes AMP-activated protein kinase (AMPK) in hepatocytes.[19] On the contrary, it was also reported that only LMW form activates AMPK in myocytes, whereas other two forms had no effect.[18] It was confirmed that there are two kinds of receptor for adiponectin, AdipoR1 and AdipoR2.[26] AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver. AdipoR1 is a high-affinity receptor for globular adiponectin, whereas AdipoR2 binds both full-length and globular forms. In this work, the distributions of adiponectin isoforms in patients undergoing HD were evaluated and the correlations between each isoform and metabolic parameters were identified.

PATIENTS AND METHODS

Study population

This study included 71 patients with nondiabetic ESRD (30 males and 41 females, age 40–87, 60 ± 13 years old) on maintenance HD with duration for more than 6 months. The patients received 12–15 hours of HD per week. The renal diseases were chronic glomerulonephritis (n = 48), polycystic kidneys (n = 6), hypertensive nephrosclerosis (n = 9), gout (n = 3), and others (n = 5). Exclusion criteria were diabetes mellitus defined by a self-reported history, a fasting plasma glucose concentration ≥ 126 mg/dL, or the use of antidiabetic agents. Liver dysfunction, thyroid dysfunction, and acute infectious diseases were also excluded. Subjects who had one or more lipid-lowering agents, whose total cholesterol (TC) levels were ≥ 300 mg/dL, and/or whose triglyceride (TG) levels ≥ 350 mg/dL were excluded from this study. Written informed consent was obtained from each patient. This study is approved by our Institutional Review Board (the Ethics Committee of Fukuoka University Hospital).

Determination of lipids and HOMA-IR and hs-CRP

Blood was collected into ethylenediaminetetraacetic acid (EDTA)-containing tubes in the morning after an overnight fasting for more than at least 12 h. Plasma was separated by centrifugation at 2500 × g for 15 min at 4°C, and kept frozen at –80°C until analysis. Serum levels of TC, TG, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by standardized automated enzymatic methods. Plasma-oxidized LDL concentration was determined by sandwich enzyme-linked immunosorbent assay (ELISA) using antioxidized phosphatidyl-choline monoclonal antibody and anti-human apolipoprotein-B antibody as described by Kohno et al.[27] Remnant-like particle cholesterol (RLP-C) was isolated from fresh plasma using RLP-Cholesterol Assay Kits (Jimro-II, Japan Immunoresearch Laboratories, Japan). Insulin resistance as assessed by homeostasis model assessment (HOMA-IR) was calculated using the following formula: HOMA-IR = fasting glucose (mg/dL) × fasting insulin (μU/mL)/405. HOMA-IR values have been shown to closely correlate with those of the euglycemic-hyperinsulinemic glucose clamp technique,[28] and the correlation has been confirmed in patients with renal failure.[29] High-sensitive C-reactive protein (hs-CRP) levels were measured by the latex-enhanced immunonephelometric assay (N Latex CRP, Dade Behring, Liederbach, Germany).[30]

Assays of adiponectin and adiponectin multimeric isoforms

Plasma adiponectin and adiponectin multimers were measured by the originally described by Ebinuma et al. (Human Adiponectin ELISA kit for Total and Multimer, Daiichi Pure Chemicals, Tokyo, Japan).[31] Briefly, the plasma was pretreated by a protease which selectively digested LMW and MMW adiponectin or by a protease which digested only LMW adiponectin. Resulting total, HMW, or HMW, and MMW adiponectin were treated with sodium dodecyl sulfate (SDS)-containing

acid buffer converting the fractionated adiponectin mainly to a dimer form. Each subfraction can be measured by sandwich ELISA system that uses two kinds of anti-human monoclonal antibodies specific to adiponectin. Total and HMW adiponectin concentrations were determined directly. The MMW and LMW adiponectin concentrations were determined indirectly, calculated by subtracting the HMW adiponectin from the combined MMW and HMW adiponectin concentration, and by subtracting the combined MMW and HMW adiponectin concentration from the total adiponectin concentration, respectively. The ELISA had a dynamic range of 0.075–4.8 ng/mL. Intra-assay variations were 5.3% (total), 4.1% (MMW + HMW), and 3.3% (HMW).

Statistical analysis

All of data analyses were performed using SPSS software package (Version 11.0 for windows, Chicago, IL, USA). Continuous variables were expressed as mean ± SD. Comparison two groups were compared by Mann–Whitney *U*-test. Correlations between variables were examined by Spearman's correlations analysis. Forward stepwise multiple linear regression analysis was used to determine factors independently associated with total and adiponectin multimers. Here, distribution was tested for normality using Shapiro–Wilk *W* test; and nonnormally distributed parameters were log-transformed before analysis. The significant level was considered less than 0.05.

RESULTS

Effects of sex on adiponectin and adiponectin multimers

Table 1 summarizes the arthrometric and biochemical characteristics of nondiabetic HD patients. Stratified by sex, no statistical differences in age, BMI, blood pressure, TG, and HOMA-IR were noted. Female patients had significantly higher serum levels of TC, HDL cholesterol, and hs-CRP levels than males did. Females had higher plasma levels of total, HMW, and MMW adiponectin than males, whereas the amounts of LMW adiponectin were similar between men and women. The higher level of total adiponectin in women was mainly caused by the increased amounts of HMW adiponectin. The ratio to total adiponectin was higher in HMW and lower in LMW in women compared to those in men (Table 2).

Correlations between total, multimeric isoforms, and metabolic parameters

Table 3 shows the univariate correlations between each multimers of adiponectin and metabolic parameters including plasma lipids. In univariate correlation analysis, total and all multimers of adiponectin were positively correlated with HDL cholesterol and inversely correlated with TG. Total, HMW, and MMW adiponectin were negatively associated with HOMA-IR. Total, HMW, and LMW adiponectin were negatively associated with hs-CRP. Plasma levels of total and each multimers of adiponectin did not show the correlations with age, duration on HD treatment, blood pressure, LDL cholesterol, and RLP cholesterol. In multiple linear regression analysis, total and HMW adiponectin was independently associated with sex, BMI, and HDL cholesterol. Although LMW adiponectin was independently correlated with TG and hs-CRP (Table 4).

TABLE 1. Antherometric and biochemical characteristics of non-diabetic HD patients.

Number of subjects	71
Gender (M/F)	30/41
Age (years)	64 ± 9
Duration of HD (months)	99 ± 70
BMI (kg/m^2)	20.6 ± 2.7
Systolic blood pressure (mmHg)	148 ± 19
Diastolic blood pressure (mmHg)	76 ± 9
Total cholesterol (mg/dL)	163 ± 29
Triglycerides (mg/dL)	102 ± 29
HDL cholesterol (mg/dL)	49 ± 16
LDL cholesterol (mg/dL)	89 ± 24
Oxidized LDL cholesterol	10.2 ± 9.9
RLP cholesterol (mg/dL)	6.5 ± 4.1
High-sensitive CRP (mg/dL)	1.43 ± 1.98
HOMA-IR	1.70 ± 1.03

Note: Result are expressed as meas ± SD.

TABLE 2. The levels and ratios between adiponectin multimers and total adiponectin.

	Female (n = 41)	Male (n = 30)	p-Value
Total (μg/mL)	15.84 ± 6.70	11.47 ± 5.93	$p < 0.01$
HMW (μg/mL)	9.42 ± 5.36	5.70 ± 3.74	$p < 0.01$
MMW (μg/mL)	2.99 ± 1.39	2.55 ± 1.38	$p < 0.05$
LMW (μg/mL)	3.44 ± 1.01	3.50 ± 1.25	n.s
HMW/Total	0.56 ± 0.10	0.46 ± 0.10	$p < 0.001$
MMW/Total	0.19 ± 0.05	0.19 ± 0.06	n.s
LMW/Total	0.24 ± 0.09	0.35 ± 0.12	$p < 0.001$

Note: Result are expressed as meas ± SD.

TABLE 3. Spearmann correlation between adionectin multimers and other parameter.

Variables	Total	HMW	MMW	LMW
Age (years)	0.071	0.031	0.132	0.119
Duration of HD (months)	−0.030	−0.083	0.018	0.194
BMI (kg/m^2)	−0.570**	−0.546**	−0.524**	−0.276*
Systolic blood pressure (mmHg)	0.082	0.087	0.001	0.227
Diastolic blood pressure (mmHg)	−0.069	−0.036	−0.047	0.016
Total cholesterol (mg/dL)	0.117	0.175	0.091	−0.075
Triglycerides (mg/dL)	−0.515**	−0.471**	−0.408**	−0.430**
HDL cholesterol (mg/dL)	0.558**	0.556**	0.510**	0.327*
LDL cholesterol (mg/dL)	−0.053	0.002	−0.059	−0.162
Oxidized LDL cholesterol	0.006	−0.003	0.018	0.058
RLP cholesterol (mg/dL)	−0.085	−0.071	−0.088	−0.188
High-sensitive CRP (mg/dL)	−0.329**	−0.332**	−0.213	−0.319*
HOMA-IR	−0.407**	−0.385**	−0.385**	−0.228

Notes: * $p < 0.05$, ** $p < 0.01$.

TABLE 4. Forward stepwise multiple regression analysis of dependent variable.

Variables	β	Standard error	t	p	Variables	β	Standard error	t	p
		Total-Ad					HMW-Ad		
HDL-C	0.297	0.002	2.668	0.010	HDL-C	0.278	0.002	2.565	0.013
BMI	−0.326	0.009	−3.073	0.003	BMI	−0.341	0.013	−3.298	0.002
Sex	−0.247	0.048	−2.462	0.016	Sex	−0.296	0.065	−3.021	0.004
		MMW-Ad					LMW-Ad		
BMI	−0.446	0.008	−4.314	0.000	Log TG	−0.364	0.553	−3.407	0.001
Sex	−0.268	0.045	−2.590	0.012	Log hs-CRP	−0.278	0.226	−2.606	0.011

Note: Independent variables tested include age and sex, as well as parameters showing a significant relationship in univariate analysis.

DISCUSSION

The level of plasma adiponectin is increased in patients on maintenance HD. In this study, we evaluated adiponectin oligomeric complex formation in HD patients to clarify whether the increase of plasma adiponectin is accompanied with the alteration of distribution of oligomeric complex compared with that of healthy population. Concerning the comparison between male and female, as many survey supported the consensus that women had higher concentration of total adiponectin,[4,5,10,19,25,31] particularly higher in HMW adiponectin than men,[19,25,31] in this study also the total and HMW adiponectin levels in women were significantly higher than those in men among HD patients. In distribution the ratio of HMW to total adiponectin in female was also higher than that in male (Table 2) as the results of normal populations.[31] These differences between men and women might be resulted from the differences of sex hormones. Testosterone was previously shown to selectively reduce the HMW adiponectin,[32] and plasma estradiol concentration was shown to negatively correlate with total adiponectin level in postmenopausal women.[33]

Regarding distribution difference between healthy persons and HD patients, the proportion of HMW in HD patients was high, and that of MMW was lower (Table 2).[31] Then, we analyzed the correlations between each isoform and metabolic parameters in HD patients. Spearman's correlation analysis showed three isoforms that HMW, MMW, and LMW were associated with BMI, TG, HDL-cholesterol, hs-CRP, and HOMA-IR as total adiponectin showed (Table 2). Clinical data have been accumulated that HMW

adiponectin plays more important roles in insulin sensitivity[24,25,34] and have demonstrated that HMW adiponectin in uremic patients is also closely associated with insulin sensitivity as well as in nonuremic patients. In this work, however, the decrease of total adiponectin levels in high-HOMA-IR subjects were explained by not only HMW but also every isoforms levels decline (Table 3). In fact, significant relationship between HMW and HOMA-IR was not proved in forward stepwise multiple regression analysis. HMW independently has causal relationships with HDL-C, BMI, and sex as total adiponectin has in forward stepwise multiple regression analysis (Table 3). Between MMW and HDL-C, a significant relationship was not recognized.

Unexpectedly, we observed that LMW adiponectin was independently and inversely correlated with TG and hs-CRP. Numerous studies have observed an inverse relationship between plasma TG and adiponectin levels. Using adenovirus-mediated gene transduction technique, mouse model with acute elevations of plasma adiponectin was established. This model mouse showed that an acute increase of circulating adiponectin reduces serum TG. The secretion rates of hepatic VLDL-TG were not altered by elevated plasma adiponectin, but skeletal muscle lipoprotein lipase (LPL) activity and mRNA levels of LPL and VLDL receptor were increased in this mouse.[35] Although the authors demonstrated and described that adenovirus-mediated adiponectin gene transduction proportionally increased all three isoforms of adiponectin, the increase of expression for LMW appeared to be extreme higher than those of others were according to their Western blot analysis. Furthermore, the fact that contrary to in hepatocytes only HMW and MMW activate the AMPK pathway, in myocytes LMW also activates it as the other isoforms does[19] suggested that LMW may play a principal role for decreasing TG. Actually, LMW adiponectin is a very short half-life protein, and more active protein than the HMW and MMW complexes in vitro assay.[36] Moreover, only LMW adiponectin exert anti-inflammatory properties through the inhibition of nuclear factor (NF)-kappa B.[37] Therefore, considering our results of multiple regression analysis, elevated level or prolonged half-life of LMW adiponectin may contribute to have favorable metabolic effects in uremic state. Not only HMW adiponectin but also LMW adiponectin may have favorable biological effects in HD patients.

CONCLUSION

Biological property of total adiponectin for metabolism in HD patients was explained by that of HMW adiponectin. However, LMW adiponectin in HD patients may also have unique advantageous biological effects, which was not confirmed in healthy peoples.

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