16 wk, even when taking into account of the dose reductions necessary for patients with a dangerous decrease of hemoglobin caused by ribavirin, as often seen in genotype 2 patients with a low hemoglobin level at pretreatment.

In conclusion, the 24-week IFN and ribavirin combination treatment was highly effective and resulted in a remarkably high SVR in Japanese HCV patients with genotype 2 from the retrospective study of ours. The most significant predictor was continuation of the ribavirin treatment for up to 16 wk. These findings are not pertinent to the other different genotypes.

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Association between fast-migrating low-density lipoprotein subfraction as characterized by capillary isotachophoresis and intima-media thickness of carotid artery

Bo Zhang [a], Naoyasu Maeda [b], Kyoko Okada [b], Masafumi Tatsukawa [b], Yasumori Sawayama [b], Akira Matsunaga [a], Koichiro Kumagai [a], Shin-ichiro Miura [a], Takamitsu Nagao [c], Jun Hayashi [b], Keijiro Saku [a,*]

[a] *Department of Cardiology, Fukuoka University School of Medicine, 7-45-1 Nanakuma Jonan-ku, Fukuoka 814-0180, Japan*
[b] *Department of General Medicine, Kyushu University Hospital, Fukuoka, Japan*
[c] *Japan Immunoresearch Laboratories Co., Ltd., Gunma, Japan*

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Abstract

Background: A mildly modified LDL subfraction that is characterized by an increased negative charge exists in plasma. This electronegative LDL separated by ion-exchange chromatography has been shown to be inflammatory and its proportion is increased in patients with hyperlipidemia and diabetes mellitus. The present study examined the association between the level of fast (f)-migrating LDL subfraction characterized by capillary isotachophoresis (cITP) and carotid-artery intima-media thickness (CA-IMT).

Methods and results: This study included 469 subjects who underwent a physical examination. CA-IMT was determined by high-resolution B-model ultrasonoraphy. Levels of charge-based LDL subfractions were measured by cITP on a Beckman P/ACE MDQ system. An increased serum LDL-C level and cITP fLDL level were associated with increased CA-IMT after adjusting for age. The extent of the associations between cITP fLDL and CA-IMT and between LDL-C and CA-IMT were similar as assessed by a receiver-operating characteristic curve analysis. LDL-C, triglyceride, and remnant-like particle cholesterol levels were independently correlated with cITP fLDL, and the LDL-C level had the strongest correlation with cITP fLDL. The association between the cITP fLDL level and CA-IMT was significant in the high LDL-C stratum but not in the low stratum, indicating that it is modified by the LDL-C level. The high-LDL-C-high-fLDL group had the highest relative risk for a high CA-IMT among the groups with each combination of LDL-C and cITP fLDL level.

Conclusion: The cITP fLDL level was associated with CA-IMT and its combination with the LDL-C level is a stronger indicator for a high CA-IMT.

Keywords: Low density lipoprotein (LDL) cholesterol; Intima-media thickness; Carotid atherosclerosis; Capillary isotachophoresis; Fast-migrating LDL subfraction

1. Introduction

Serum level of low-density lipoprotein cholesterol (LDL-C) is an established risk factor of coronary artery disease (CAD), and a reduction in LDL-C levels has been shown to be associated with reduced death rates caused by CAD. LDL is composed of heterogeneous particles that differ in size, composition, and electric charge. Qualitatively modified forms of LDL have been shown to exist in human plasma, including small dense LDL, oxidative modified LDL, glycated LDL, and diasylated LDL [1–5], and they are

* Corresponding author. Tel.: +81 92 801 1011; fax: +81 92 865 2692.
E-mail address: saku-k@cis.fukuoka-u.ac.jp (K. Saku).

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all atherogenic. These forms of modified LDL all have an increased negative charge. The electronegative subfraction of LDL [LDL(−)] has been separated from plasma by anion-exchange chromatography techniques [6,7]. LDL(−) in plasma can also be generated by other processes including enrichment with nonesterified fatty acids or enzymatic modification by phospholipase A2 or cholesteryl esterase/trypsin [7].

Although the origins of LDL(−) are complex and not fully understood, LDL(−) has been shown to have proinflammatory activity on endothelial cells [8] and its proportion is increased in patients with hypertriglyceridemia [9], familial hypercholesterolemia (FH) [9], and diabetes mellitus (DM) [10], patients on hemodialysis [11], and patients with anigographically documented CAD [12].

However, there is still little information available on whether or not the LDL(−) subfraction level is a marker for atherosclerosis and whether or not its association with atherosclerosis is independent of the LDL-C level, partly because of the lack of a routine analytical technique for this modified LDL subfraction.

The current ion-exchange chromatography method for measuring the LDL(−) subfraction gives the proportion of LDL(−) protein content in total LDL separated by ultracentrifugation [6]. Therefore, it is disadvantageous for routine analysis in that it is time-consuming and requires a relatively large amount of samples, and the absolute level of LDL(−) in plasma cannot be determined.

Capillary isotachophoresis (cITP) is another technique that separates and quantifies LDL subfractions according to electric charge. It was originally developed by the research group of Schmitz [13,14]. Fast-migrating LDL (fLDL) carries more negative charge than slow-migrating LDL (sLDL) [13,14]. Since lipoproteins are pre-stained with a fluorescent lipophilic dye, LDL subfractions can be measured directly in plasma and with high sensitivity (only several microliters of sample are necessary). Separation and on-line detection can both be performed within just a few minutes. Therefore, analytical cITP technique may be useful for the routine analysis of lipoprotein profiles. We previously showed that the absolute levels of lipoprotein subfractions can be determined as a peak area relative to an internal marker and the levels of cITP fLDL and sLDL were proportional to the protein content of LDL [15–17].

Measurement of the thickness of the intima and media of carotid arteries by high-resolution B-mode carotid ultrasonography has been used as a non-invasive method for detecting early carotid atherosclerosis [18,19]. Carotid-artery intima-media thickness (CA-IMT) is associated with the prevalence of cardiovascular disease and with cardiovascular risk factors [20].

We investigated the hypothesis that the cITP fLDL subfraction level is associated with CA-IMT. We also hypothesized that the cITP fLDL level contributes to the ability of LDL-C to predict the risk of CA-IMT after controlling for conventional cardiovascular risk factors.

2. Methods

2.1. Subjects

This study included 469 male subjects (aged between 21 and 88 years) who participated in a health examination. This study was approved by the Ethics Committees of Kyushu University Hospital, and samples were collected only after the participants had given their informed consent.

The prevalence of hypertension, diabetes mellitus, and smoker in the study subjects was 39.7% ($n=186$), 13.9% ($n=65$), and 32.6% ($n=153$), respectively. Twelve subjects (2.6%) had a history of stroke, and 12 (2.6%) had a history of coronary heart disease. Hypertension was defined as systolic blood pressure $\geq$140 mmHg, diastolic pressure $\geq$90 mmHg, or treatment with antihypertensive medications. Diabetes mellitus was defined as a self-reported history of diabetes, a fasting plasma glucose concentration $\geq$126 mg/dl, or the use of anti-diabetic drugs. Smokers were defined as those who had smoked past or who were present smokers. Subjects who refused ultrasound examination or who had a fasting blood glucose concentration $\geq$400 mg/dl or triglyceride (TG) level $\geq$400 mg/dl were excluded from the study.

Blood was drawn between 9 and 12 a.m. after an overnight fast and stored at −80 °C until analysis. Storage of samples at −80 °C for up to 5 months does not apparently affect measurements for cITP LDL subfractions [17].

2.2. Ultrasonographic measurement

Common carotid-artery lesions were assessed by high-resolution B-mode ultrasonography with a 7.5 MHz mechanical sector transducer on an Aloka SSD-2000 (Aloka Co. Ltd., Tokyo, Japan), as described previously [21,22]. All assessment of carotid arteries was performed by three specially trained technicians who were unaware of the clinical history or risk factor profile. IMT was measured at points 20, 25, 30 mm proximal to the flow divider on the far wall of the right and left common carotid arteries at the end of the diastolic phase. Using this information, mean CA-IMT was determined for each individual.

2.3. Measurement of serum lipids and lipoproteins

Serum levels of total cholesterol (TC), TG, high-density lipoprotein cholesterol (HDL-C) were measured by enzymatic methods. Serum LDL-C levels were calculated indirectly using the Friedewald formula.

Remnant-like particle cholesterol (RLP-C) levels were measured by an RLP-Cholesterol Immunoseparation Assay using a commercially available kit (JIMRO-II, Japan Immunoresearch Laboratories Co., Ltd., Gunma, Japan) [23,24]. Briefly, the RLP immunoseparation gel was washed before use three times with RLP buffer by low-speed centrifugation and suspended by repeatedly inverting the container. After 150 μl of the suspended gel was aliquoted into Hitachi

microsample cups, 5 μl serum samples were added and the mixture was stirred using a steel bead for 2 h at room temperature on an RLP Mixer J-100 (Otsuka Electrics Co., Ltd, Tokyo, Japan). After the gel had settled for 15 min, the cholesterol level in the supernatant was measured with cholesterol reagents included in the assay kit using an auto-analyzer (Hitachi 7600-020S).

2.4. Quantification of lipoprotein subfractions by cITP

Capillary isotachophoresis of serum lipoproteins was performed on a Beckman P/ACE MDQ system (Beckman-Coulter Inc., Tokyo, Japan) according to the method of Bottcher et al. [13] with some modifications, as previously described [15–17,25,26]. Briefly, 6 μl of serum was diluted with 14 μl of leading buffer consisting of 10 mM HCl and 18 mM ammediol (2-amino-2-methyl-1,3-propanediol) (pH 8.8), prestained with 10 μl 0.1 mg/ml NBD C6-ceramide (Molecular Probe Inc., OR, USA) for 5 min at room temperature, and mixed with 50 μl of the mixture containing leading buffer with 0.35% hydroxypropylmethylcellulose (HPMC), spacers, and 5-carboxy-fluorescein as an internal marker. The spacers were *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES), D-glucuronic acid, 1-octanesulfonic acid sodium salt, 3-(*N*-tris[hydroxymethyl]methylamino)-2-hydroxypropanesulfonic acid (TAPSO), *N*-tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid (TAPS), L-serine, L-glutamine, L-methionine, and glycine. The terminating buffer contained 24 mM β-alanine and 13 mM ammediol, and was adjusted to pH 10.5 with saturated barium hydroxide solution. A dimethylpolysiloxane-modified fused silica capillary (AT^{TM}-1) was purchased from Alltech Japan Inc. (Tokyo, Japan). The sample was injected at 20 psi for 18 s into a 30-cm long capillary (i.d. 180 μm), and separation was performed at a constant 30 μA for 1 min and 10 kV for 7 min. The separated zones were monitored with argon-laser-induced fluorescence detection (excitation, 488 nm; emission, 520 nm). Each peak was identified and the peak area in relative fluorescence units was analyzed using 32 Karat Software version 5.0 (Beckman-Coulter Inc., Tokyo, Japan). Levels of cITP lipoprotein subfractions were expressed as the peak area relative to the internal marker.

2.5. Statistical analysis

All of the statistical analysis was performed using the SAS (Statistical Analysis System) Software Package (Version 9.1, SAS Institute, CA, USA) at the Fukuoka University. The distribution of variables were examined by the Shapiro–Wilk test [27]. The 33.3th and 66.7th percentiles were used to produce tertiles of CA-IMT. Linear trends of risk factors across tertiles of CA-IMT after adjusting for age were examined by an analysis of covariance (ANCOVA) using a general linear model. Correlation between variables was examined by Spearman correlation. Log-transformed values of TG and RLP-C were used in the data analysis. Low and high LDL-C strata were defined as $<$ and $\geq$ the median value of LDL-C (118 mg/dl), respectively, and low and high CA-IMT were defined as $<$ and $\geq$ the median value of CA-IMT (0.77 mm). The strength of the associations between the cITP fLDL and LDL-C levels was compared using a receiver operating characteristic (ROC) curve analysis. An ROC-curve (plot of sensitivity versus 1-specificity) analysis is a powerful tool for assessing the ability of a continuous variable to discriminate between two groups of subjects, and does not depend on the cutoff value selected. The area under the ROC curve represents the probability for a randomly chosen low CA-IMT subject to exhibit a value lower than the level observed among randomly chosen high CA-IMT subjects. A value of 0.5 means that the distributions of the values in the two groups are similar; conversely, a value of 1.0 means that the distributions of the values in the two groups do not overlap. We determined the area under the ROC curve by the trapezoidal rule and evaluated its significance by the Wald chi-square test, as described previously [28]. Stepwise multiple regression analysis was used to examine the independent variables that are related to cITP fLDL. The significance of the association between the combination of LDL-C and cITP fLDL and CA-IMT after controlling for age and other related variables was examined by a multivariate logistic regression analysis using dummy variables. The odds ratio and 95% confidence interval (CI) were given for each combination of LDL-C and cITP fLDL. All p values are two-tailed. The significance level was considered to be 5% unless indicated otherwise.

3. Results

Table 1 shows the mean levels of conventional risk factors of CAD, serum levels of lipids and lipoproteins, and RLP-C levels according to tertiles of CA-IMT. Increased age was associated with increased CA-IMT (tertile III versus tertile II versus tertile I: 64.4 ± 0.9 year versus 59.7 ± 0.9 year versus 48.9 ± 1.0 year, $p < 0.05$, by an analysis of variance). The prevalence of DM and serum levels of TC and LDL-C were positively and significantly associated with CA-IMT after adjusting for age, as assessed by an analysis of covariance (Table 1). Body mass index (BMI), prevalence of HT and smoking, and serum levels of TG, HDL-C, and RLP-C were not significantly associated with CA-IMT after adjusting for age (Table 1).

Fig. 1 shows the typical cITP lipoprotein profiles of subjects with low (0.54 mm) and high CA-IMT (1.17 mm). As shown, capillary isotachophoresis clearly separated lipoproteins into eight fractions within 8 min. Peaks 6 and 7 are the two LDL subfractions with fast and slow electrophoretic mobility. Subject with high CA-IMT had apparently higher levels of both fLDL and sLDL than that with low CA-IMT (Fig. 1).

Table 2 shows that age-adjusted mean levels of intermediate-migrating HDL decreased and cITP fLDL and sLDL increased across tertiles of CA-IMT. This result indi-

Table 1
Age-adjusted mean levels of risk factors according to tertiles of carotid-artery intimal-media thickness (CA-IMT)

	Tertiles of CA-IMT			p^a
	Low (<0.67 mm)	Middle (0.67–0.83 mm)	High (≥0.83 mm)	
No. of subjects	142	159	168	
Age (year)	48.9 ± 1.0	59.7 ± 0.9	64.4 ± 0.9	<0.05
Body mass index (kg/m^2)	22.6 ± 0.3	23.9 ± 0.2	23.5 ± 0.2	n.s.
Hypertension (%)	23	40	53	n.s.
Diabetes mellituss (%)	5	12	22	<0.05
Smoking (%)	37	31	30	n.s.
TC (mg/dl)	191 ± 3	199 ± 2	204 ± 3	<0.05
log(TG)	4.7 ± 0.0	4.8 ± 0.0	4.8 ± 0.0	n.s.
HDL-C (mg/dl)	53 ± 1	54 ± 1	52 ± 1	n.s.
LDL-C (mg/dl)	113 ± 3	118 ± 2	123 ± 2	<0.05
log(RLP-C)	2.7 ± 0.0	2.8 ± 0.0	2.9 ± 0.0	n.s.

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol. The units of TG and RLP-C were mg/dl.

[a] Assessed by an analysis of covariance or logistic regression analysis after adjusting for age. Continuous variables were adjusted for age by means of linear regression, and categorial variables were adjusted for age by means of logistic regression.

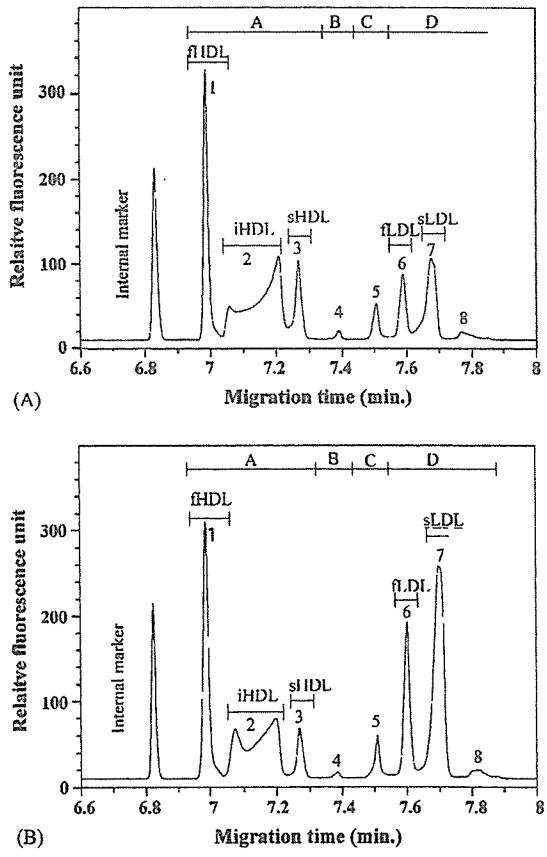

Fig. 1. Lipoprotein profiles as determined by capillary isotachophoresis in serum from subjects with low (A) and high (B) carotid-artery intima-media thickness (CA-IMT: 0.54 and 1.17 mm, respectively). The various lipoprotein subfractions are depicted as follows [13,14]: A, HDL; B, chylomicron/remnants; C, VLDL/IDL; D, LDL. fHDL, fast-migrating HDL; iHDL, intermediate-migrating HDL; sHDL, slow-migrating HDL; fLDL, fast-migrating LDL; sLDL, slow-migrating LDL.

cates that both cITP fLDL and sLDL were positively associated with CA-IMT independent of age. The strength of the associations between cITP fLDL and CA-IMT (two levels) and between LDL-C and CA-IMT were compared by an ROC curve analysis. Fig. 2 shows the plot of sensitivity (true positive) versus 1-specificity (false positive) for the LDL-C level and cITP fLDL level. The area under the ROC curve was similar for cITP fLDL and LDL-C (0.578 and 0.582, respectively).

The cITP fLDL levels were negatively correlated with HDL-C levels ($r=-0.135$, $p<0.01$) and significantly ($p<0.01$) and positively correlated with age, BMI, and serum levels of TC, TG, LDL-C, and RLP-C ($r=0.156$, 0.168, 0.524, 0.208, 0.545, and 0.147, respectively). Stepwise multiple regression analysis selected LDL-C, TG, and RLP-C as independent variables that were related to cITP fLDL (Table 3). The LDL-C level had the strongest correlation with cITP fLDL (Table 3). Fig. 3 shows the correlation between cITP fLDL and LDL-C levels in subjects with low, middle, and high CA-IMT. As shown, cITP fLDL levels were significantly correlated with LDL-C levels in all the three groups of subjects. As also shown in Fig. 3, the regression lines of cITP fLDL versus LDL-C levels in subjects with middle and high CA-IMT (dotted lines) were shifted towards higher cITP fLDL levels as compared with that in subjects with low CA-IMT (solid line). This result indicates that cITP fLDL levels were higher in subjects with middle and high CA-IMT than in subjects with low CA-IMT after controlling for LDL-C levels.

Therefore, LDL-C levels were stratified into low and high strata and the association between cITP LDL and CA-IMT was examined according to LDL-C strata to test its relation to LDL-C levels. As shown in Table 4, the association between cITP fLDL and CA-IMT was significant in the high LDL stratum [odds ratio (95% CI): 2.2 (1.2–3.8)] but not in the low LDL stratum after adjusting for age by a multiple logistic regression analysis. This result indicates that

Table 2
Age-adjusted mean levels of lipoprotein subfractions as measured by capillary isotachophoresis (cITP) according to tertiles of carotid-artery intimal-media thickness (CA-IMT)

	Tertiles of CA-IMT			p^a
	Low (<0.67 mm)	Middle (0.67–0.83 mm)	High (≥0.83 mm)	
cITP fHDL	1.46 ± 0.04	1.47 ± 0.04	1.40 ± 0.04	n.s.
cITP iHDL	2.22 ± 0.04	2.14 ± 0.03	2.10 ± 0.03	<0.05
cITP sHDL	0.41 ± 0.01	0.40 ± 0.01	0.39 ± 0.01	n.s.
cITP VLDL/IDL	0.91 ± 0.04	1.04 ± 0.04	1.01 ± 0.04	n.s.
cITP fLDL	1.09 ± 0.03	1.16 ± 0.02	1.20 ± 0.03	<0.05
cITP sLDL	1.29 ± 0.05	1.36 ± 0.04	1.46 ± 0.04	<0.05

Levels of cITP lipoprotein subfractions are expressed as peak area relative to the internal marker. fHDL, iHDL, and sHDL, fast-intermediate, and slow-migrating high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; fLDL and sLDL, fast- and slow-migrating low-density lipoprotein.
[a] Assessed by an analysis of covariance. Variables were adjusted for age by means of linear regression.

Table 3
Stepwise multivariable regression analysis of the independent variables related to fast-migrating low-density lipoprotein (fLDL) as determined by capillary isotachophoresis (cITP)

Step	Variable entered	Partial correlation coefficient	F	p
1	LDL-C	0.573	190.1	<0.001
5	log(TG)	0.306	67.3	<0.001
4	log(RLP-C)	0.204	25.6	<0.001

Levels of cITP fLDL are expressed as peak area relative to the internal mark.

the association between cITP fLDL and CA-IMT was modified by the LDL-C level. Fig. 4 shows a three-dimensional plot of the age-adjusted relative risk for a high CA-IMT for each combination of cITP fLDL and LDL-C levels. The high-LDL-C-high-fLDL group had the highest risk for a high CA-IMT among the four groups: the low-LDL-C-low-fLDL group, the low-LDL-C-high-fLDL group, the high-LDL-C-low-fLDL group, and the high-LDL-C-high-fLDL group. Similar results were obtained after additionally adjusting for HT, DM, and smoking (data not shown). These results indicate that the combination of cITP fLDL and LDL-C level was a stronger indicator for a high CA-IMT than either cITP fLDL or LDL-C alone.

4. Discussion

With advances in techniques in lipoprotein analysis, a new LDL subfraction in plasma that is characterized by a greater negative charge than native LDL has attracted considerable attention. The electronegative LDL subfraction separated by ion-exchange chromatography has been shown to contain mildly modified LDL that could be produced from multiple origins [7] and is associated with a pathogenic state that is related to atherosclerosis [9–11]. Therefore, this negatively charged LDL subfraction could be a novel marker for atherosclerosis. However, there is still little evidence to support this point because of the lack of routine analytical techniques for this LDL subfraction. Ion-exchange chromatography is excellent for the separation of LDL(−) and for preparative use [6]. However, since it requires the separation of LDL by ultracentrifugation, the absolute level of LDL(−) in plasma cannot be measured with this technique and routine analysis is also difficult.

Analytical capillary isotachophoresis is a new technique for routine analysis of LDL subfractions according to their electric charges, which was established by the research group of Schmit et al. [13,14]. Several microliters of serum or plasma can be directly analyzed and separation and detection of cITP fast- and slow-migrating LDL can be performed within minutes. However, little attention has been paid to this technique [15–17,25,26], and therefore the clinical significance of the cITP fLDL subfraction is still unclear. We have previously shown that cITP can be used to quantify charge-based LDL subfractions [17] and express the absolute levels of cITP lipoprotein subfractions as the peak area relative to an internal marker [15–17,25,26].

We are the first to report that cITP fLDL and sLDL levels are associated with carotid-artery IMT. This finding is not unexpected because serum levels of LDL-C are associated with CA-IMT and levels of cITP LDL subfractions were correlated with LDL-C levels (Table 3, Fig. 3). We also found using an ROC curve analysis that the ability of cITP fLDL to predict for a high CA-IMT was similar to that of LDL-C (Fig. 2).

Table 4
Multiple logistic regression analysis of the association between fast-migrating LDL determined by cITP and carotid-artery intima-media thickness after adjusting for age in low and high LDL-C strata

	Regression coefficient ± S.E.	Odds ratio (95% confidence interval)	Wald chi-square	p
Low LDL-C	0.12 ± 0.37	1.1 (0.54–2.3)	0.10	n.s.
High LDL-C	0.78 ± 0.29	2.2 (1.2–3.8)	7.36	<0.01

The median value of LDL-C (118 mg/dl) was used to produce low and high LDL-C strata.

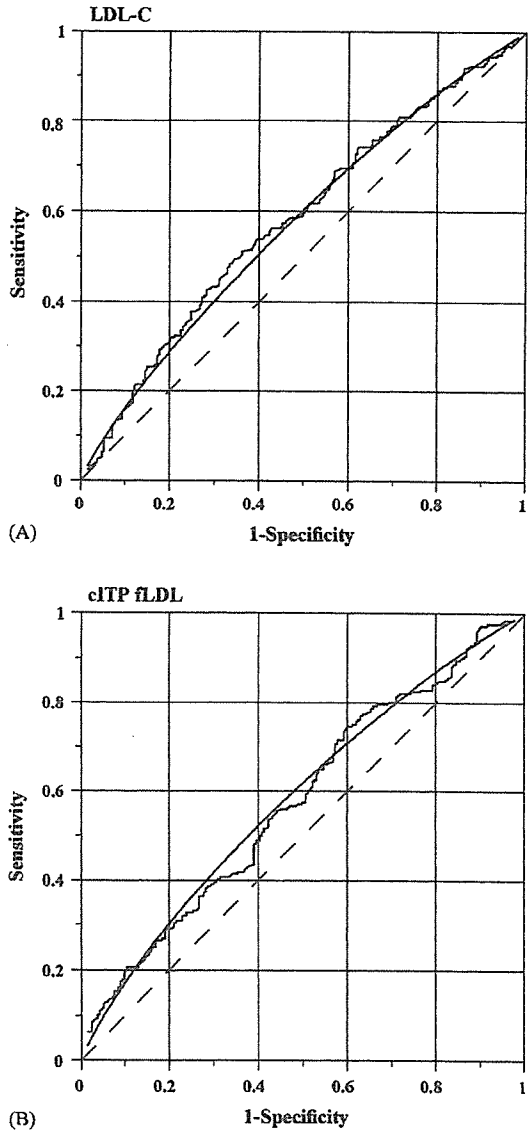

Fig. 2. ROC curves of the true-positive rate (sensitivity) vs. the false-positive rate (1-specificity) for LDL-C (A) and cITP fLDL (B). The smooth curves are model-fitted curves by the method of Swets [31].

Our finding that the cITP fLDL level was significantly related to the serum TG level (Table 3) agrees with that of Sanchez-Quesada et al., who reported that patients with hypertriglyceridemia had an increased proportion of LDL(−) [9]. Therefore, a high TG level could contribute to the increased electronegativity of LDL. We also observed a significant correlation between cITP fLDL and RLP-C levels (Table 3). The RLP-C level has been shown to be associated with CA-IMT independent of LDL-C and TG levels in a group of 50-year-old Caucasian men [29]. In our study subjects who had a wide range of ages, we observed no statistically significant association between the RLP-C level and CA-IMT after adjusting for age. The mechanism by which

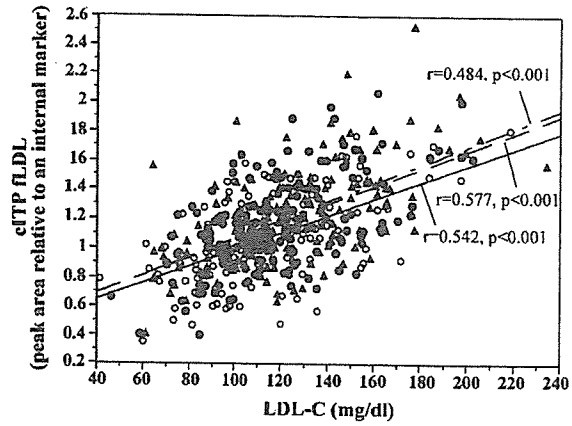

Fig. 3. Correlation between levels of fast-migrating LDL as determined by capillary isotachophoresis (cITP fLDL) and LDL-C levels in subjects with low (○), middle (●) and high (▲) carotid-artery intima-media thickness (CA-IMT).

RLP-C is related to cITP fLDL and whether or not it contributes to the association between cITP fLDL and atherosclerosis need further investigation.

Despite a strong correlation between cITP fLDL and LDL-C levels, we found that the association between the cITP fLDL level and CA-IMT was modified by LDL-C levels (Table 4) and the combination of cITP fLDL and LDL-C levels is a better indicator for a high CA-IMT (Fig. 4). Therefore, increased cITP fLDL could be a potentially useful marker for a high CA-IMT when the LDL-C level is high. Although the result of this cross-sectional study cannot be used to determine whether or not cITP fLDL subfraction is a causal factor for a high CA-IMT, our finding suggests that mildly modified

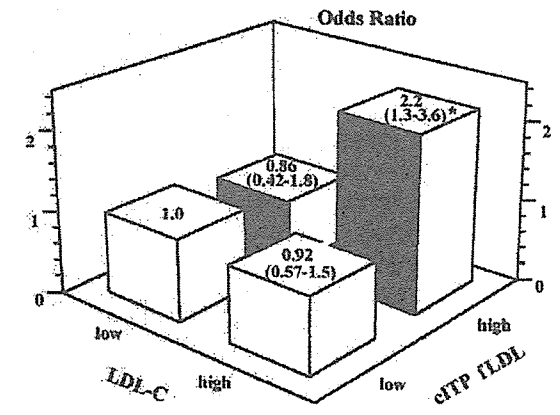

Fig. 4. Age-adjusted odds ratios [95% confidence interval (CI)] for a high carotid-artery intima-media thickness (CA-IMT) in each combination of LDL-C level and cITP fLDL level (low-LDL-C-low-fLDL, low-LDL-C-high fLDL, high-LDL-C-low-fLDL, and high-LDL-C-high-fLDL groups). Two levels of CA-IMT were produced using the median value (given a value of 0 if CA-IMT < 0.77 mm and 1 if CA-IMT ≥ 0.77 mm). The median value of LDL-C (118 mg/dl) and the 66.7th percentile value of cITP fLDL (1.37) were used to make dummy variables for each group. $^{*}p < 0.01$, as assessed by a multiple logistic regression analysis.

LDL in human blood could be important in the pathogenesis of atherosclerosis, especially under a high LDL-C level.

Our finding that cITP fLDL was associated with CA-IMT supports the notion that the electronegative subfraction of LDL is associated with risk factors of CAD, as reported by other authors [8–11], and the prevalence of angiographically documented CAD [12]. However, in the present study, the absolute levels of cITP fLDL were examined in its relation to CA-IMT, while other authors reported an association between the proportion of LDL(−) in total LDL and risk factors for CAD [8–11] or the prevalence of CAD [12]. The absolute plasma level of LDL(−) cannot be determined by anion-exchange chromatography because LDL has to be separated from plasma by ultracentrifugation or other technique before it is used for the separation of LDL(−). Therefore, the proportion of LDL(−) reported in previous studies is not equivalent to the level of cITP fLDL in the present study. However, the more negative-charged LDL subfractions separated by the two different techniques are closely related. We have previously shown that cITP fLDL represents an electronegative fraction of LDL because the cITP sLDL subfraction was converted to the fLDL subfraction when LDL was subjected to in vitro oxidation by $CuSO_4$ [17]. Bittolo-Bon et al., who separated plasma LDL into four subfractions using a different buffer system in capillary isotachophoresis, also reported that the ratio of fast-migrating (LDL1 and LDL2) and slow-migrating (LDL3 and LDL4) LDL subfractions determined by cITP was strongly and positively correlated with the proportion of LDL(−) determined by anion-exchange chromatography [30]. We observed no significant associations between the proportion of cITP fLDL in total cITP calculated from the absolute levels of cITP fLDL and sLDL and the ratio of cITP fLDL to sLDL and CA-IMT (data not shown). Therefore, our findings indicate that the absolute level of cITP fLDL but not the proportion of cITP fLDL in total LDL is important as a marker for a high CA-IMT.

In conclusion, fast LDL as characterized by analytical cITP was associated with carotid-artery intima-media thickness and could be a potentially useful marker for early atherosclerosis in combination with the LDL-C level. Further investigations are needed to clarify whether or not this conclusion can be applied to coronary atheosclerosis.

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Noriko FUKIWAKE[1]
Norihiro FURUSYO[2,3]
Norihiko KUBO[2,3]
Hiroaki TAKEOKA[2,3]
Kazuhiro TOYODA[2,3]
Keisuke MORITA[1]
Satoko SHIBATA[1]
Takeshi NAKAHARA[1]
Makiko KIDO[1]
Sayaka HAYASHIDA[1]
Yoichi MOROI[1]
Kazunori URABE[1]
Jun HAYASHI[2,3]
Masutaka FURUE[1]

[1] Department of Dermatology, Kyushu University Hospital, Maidashi 3-1-1, Higashiku, 812-8582, Fukuoka, Japan
[2] Department of General Medicine, Kyushu University Hospital, Maidashi 3-1-1, Higashiku, 812-8582, Fukuoka, Japan
[3] Department of Environmental Medicine and Infectious Disease, Faculty of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashiku, 812-8582, Fukuoka, Japan

Reprints: N. Fukiwake
Fax: (+81) 92 642 5600
<norikof@dermatol.med.kyushu-u.ac.jp>

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Incidence of atopic dermatitis in nursery school children – A follow-up study from 2001 to 2004, Kyushu University Ishigaki Atopic Dermatitis Study (KIDS)

Atopic dermatitis (AD) is a multifactorial disease that usually decreases the quality of life of affected patients. We monitored the incidence of AD and serum total IgE levels annually among nursery school children in Ishigaki Island, Okinawa, Japan, from 2001 to 2004. A total of 1731 children were enrolled. The prevalence of AD ranged from 3.7 to 11% in each year, with no significant difference between boys and girls. 869 children were examined at least twice. 71.6% (53/74) of AD patients regressed spontaneously, whereas 5.5% (44/795) of non-AD individuals developed AD during the 3-year follow-up. Increases in total IgE levels were greater and more rapid in children with long-term AD than in those who had spontaneously regressed, had newly-developed AD or did not have AD. The regression rate of AD was > 70% while new-onset AD occurred at a rate of 3.67%/person year in nursery school children of Ishigaki Island.

Key words: atopic dermatitis, epidemiology, immunoglobulin E, questionnaires

Atopic dermatitis (AD) is a common and chronic inflammatory skin disease that is characterized by relapsing itch and eczema [1]. It is a major skin disease of children that is increasing in both developed [2-4] and developing countries [5]. A similar trend has been documented in Japan, [6-8] although one study has reported that AD is no longer increasing [8]. There have been many studies of the prevalence of AD [6-15]. However, there are very few population-based epidemiological studies assessing prevalence among children aged 5 years and less. In a previous study, we established the prevalence of AD and serum total and specific IgE levels among children in Ishigaki Island, Okinawa, Japan, in 2001 [16].
In the present study, we monitored children in Ishigaki Island by means of annual physical examinations from 2001 to 2004.

Methods

Study population

We performed physical examinations of children in 15 nursery schools in Ishigaki Island, which has a population of 45,000, in Okinawa Prefecture, Japan. All the children were aged 5 years or less. Approval for the study was obtained from the Ethics Committee of Kyushu University Hospital as well as from the directors and class teachers of the schools. Informed consent to allow participation of the children was obtained from the parents and guardians. The yearly average temperature and humidity were 25.4 °C and 76% on Ishigaki Island.

The physical and laboratory examination was continued annually from 2001 to 2004. The number of children examined was 631 in 2001, 836 in 2002, 844 in 2003, and 764 in 2004 *(table 1)*. Of these, 862 were examined only once; 466 were followed for one year; 297 were followed for 2 years; and 106 were followed for 3 years. 1731 individuals were thus enrolled in total, which represented 42.1% of the 4112 kindergarten pupils in Ishigaki City. The physical and laboratory examination was completed in July and August each year (summer season, average temperature 28 °C).

Physical and laboratory examination

The medical examinations for all children were done by two dermatologists from the Department of Dermatology, Kyushu University Hospital. AD was diagnosed according to the Japanese Dermatological Association criteria *(table 2)* [17]. All children were tested for total and specific IgE antibodies. Total IgE levels were determined by a radioimmunoassay with a detection limit of 20 IU/mL (Shionoria IgE, Shionogi & Co., Ltd. Japan). A total IgE level > 230 IU/mL was considered abnormal for the purpose of statistical analysis. Specific IgE antibodies against aeroallergens such as house dust, Japanese cedar pollen, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Candida*, *Malassezia*, and food allergens, such as chicken egg white, cow's milk, rice, and soy were tested with the Pharmacia Enzyme CAP procedure (Pharmacia CAP System Specific IgE FEIA, Pharmacia Diagnostics AB, Sweden). A level of specific IgE antibodies over 0.7 UA/mL was considered abnormal.

Table 1. Prevalence of atopic dermatitis in nursery school children in Ishigaki Island

	Male		Female		Total		
	Number	AD (%)	Number	AD (%)	Number	AD (%)	Mean age ± SD
2001	342	19 (5.6)	289	20 (6.9)	631	39 (6.2)	3.0 ± 1.3
2002	446	23 (5.2)	390	30 (7.7)	836	53 (6.3)	2.9 ± 1.3
2003	455	44 (9.7)	389	49 (12.6)	844	93 (11.0)	3.2 ± 1.2
2004	412	15 (3.6)	352	13 (3.7)	764	28 (3.7)	3.0 ± 1.3

Statistical analysis

Continuous data were expressed as mean values ± standard deviation (SD) or standard error (SE) of the mean. Unpaired t-tests and Mann-Whitney U-tests were used to compare the means of samples between two groups. The chi-square test or Fisher's exact test was used for categorical variables for comparisons between two groups. The Cochran-Armitage test was used to determine the relationship between the increase or decrease in the prevalence rate of AD and the IgE abnormality rate. $P < 0.05$ was considered statistically significant.

Results

Incidence of AD

Table 1 shows the annual prevalence of AD in the study population, which ranged from 3.7 to 11% (mean, 6.8%) each year, with no significant difference between boys and girls. Of the total of 1731 children examined, 869 were followed up for 1 to 3 years, of whom 74 were diagnosed as having AD, while the remaining 795 were considered to be free of AD at the initial physical examination.

Among the 74 AD cases, 53 were confirmed to have regressed during the 3-year follow-up (71.6%); 31 cases after one year, 16 at 2 years, and the remaining 6 at 3 years *(figure 1)*. In contrast, 44 of the 795 non-AD individuals (5.5%) developed AD newly-developed within this 3-year period *(figure 2)*, indicating that the rate of new onset AD was 3.67%/person year in these nursery school children.

Table 2. Definition and diagnostic criteria for atopic dermatitis by Japanese Dermatological Association

Definition

AD is a pruritic, eczematous dermatosis, the symptoms of which fluctuate chronically with remissions and relapses. Most individuals with AD have atopic diathesis.

Atopic diathesis: (1) personal or family history (asthma, allergic rhinitis and/or conjunctivitis and AD), and/or (2) predisposition to overproduction of immunoglobulin E (IgE) antibodies.

Diagnostic criteria for atopic dermatitis

1. Pruritus

2. Typical morphology and distribution:

(1) Eczematous dermatitis

(a) acute lesions: erythema, exudation, papules, vesiculopapules, scales, crusts

(b) chronic lesions: infiltrated erythema, lichenification, prurigo, scales, crusts

(2) Distribution

(a) symmetrical: predilection sites: forehead, periorbital area, perioral area, lips, periauricular area, neck, joint areas of limbs, trunk

(b) age-related characteristics

- infantile phase: starts on the scalp and face, often spreads to the trunk and extremities
- childhood phase: neck, the flexural surfaces of the arms and legs
- adolescent and adult phase: tendency to be severe on the upper half of body (face, neck, anterior chest and back)

3 .Chronic or chronically relapsing course (usually coexistence of old and new lesions):

(1) More than 2 months in infancy

(2) More than 6 months in childhood, adolescence and adulthood

Definite diagnosis of AD requires the presence of all three features.

Total IgE levels

Total IgE levels were compared in four different groups with or without AD, namely the long-term AD group, the regressed AD group, the newly-developed AD group, and the non-AD group *(figure 3)*. Total IgE levels gradually increased with increasing age in all four groups. However, in the long-term AD group, the increase of total IgE was significantly more rapid and greater than in the other

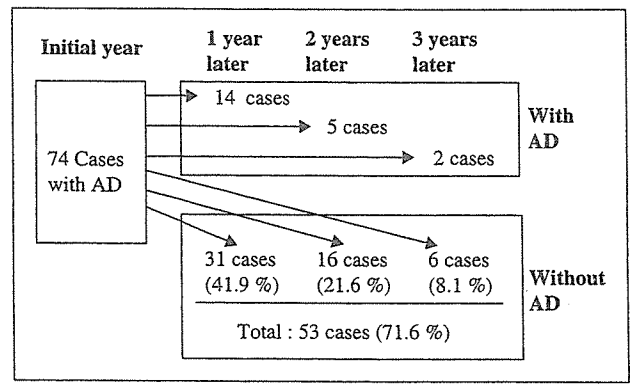

Figure 1. Rate of spontaneous regression in AD of the 74 cases with AD. 53 cases regressed during 3-year follow-up.

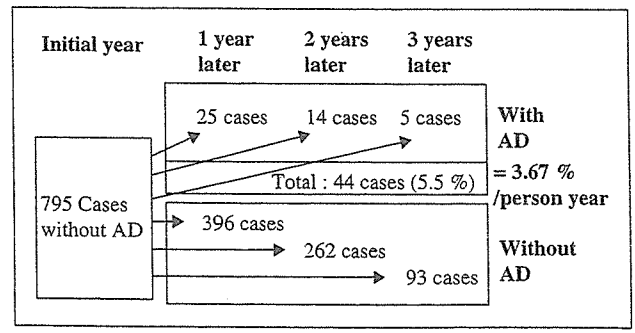

Figure 2. Rate of new-development in AD. The rate was 3.67%/person year.

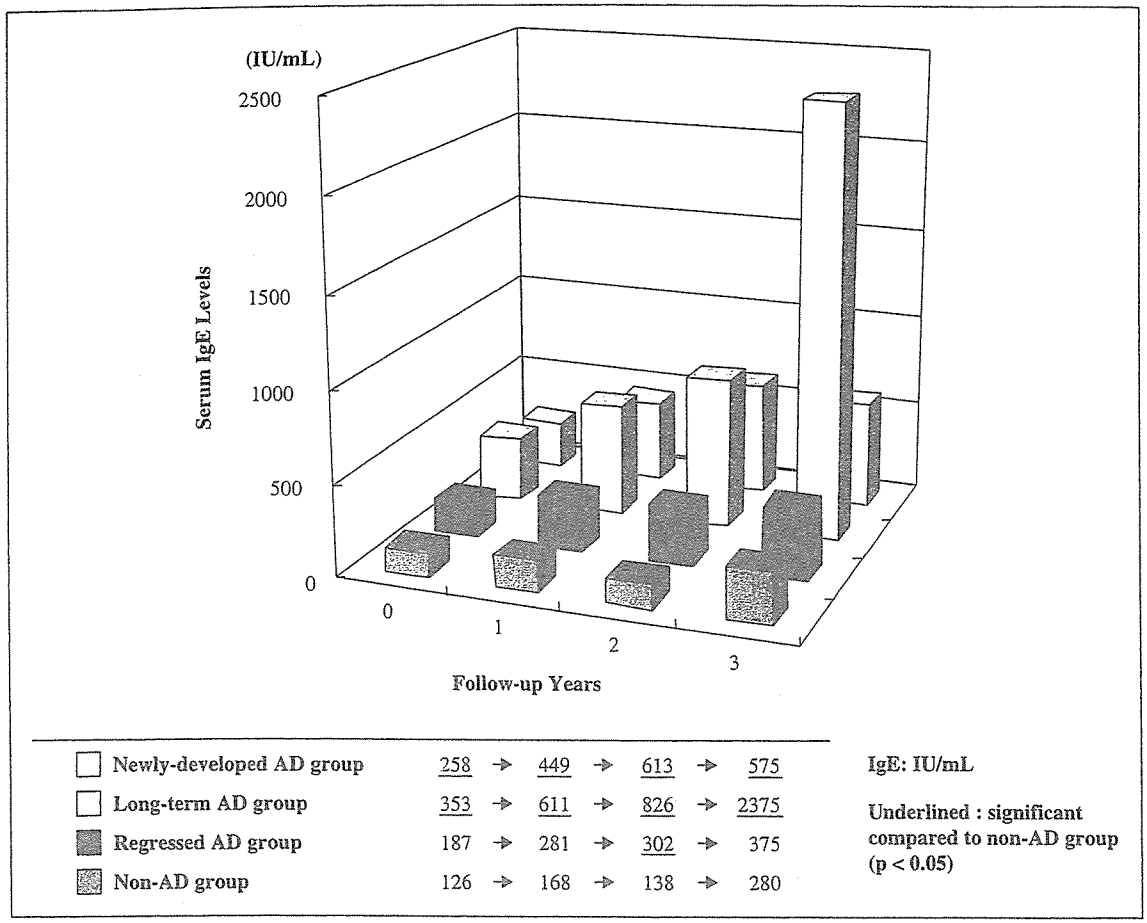

Figure 3. Changes of total IgE levels in the long-term AD group, the regressed AD group, the newly-developed AD group and the non-AD group, respectively, in nursery school children of Ishigaki Island. The increase of total IgE levels in long-term AD sufferers was significantly more marked and more rapid than in the other 3 groups during the 3-year follow-up period.

3 groups over the 3-year follow-up period *(figure 3)*. In contrast, increases in total IgE levels were very slight both in the regressed AD group and in the newly-developed AD group. However, it is interesting that IgE levels were slightly higher in the latter, and also that the IgE levels of the regressed AD group fell almost to the same levels as in children without AD.

Discussion

In the present study, we performed a follow-up study of AD in among children in Ishigaki and found that 71.6% of children with AD experienced remission during the follow-up period. Furthermore, the *de novo* occurrence of AD in these nursery school age children was estimated as 3.67%/person year.

Symptoms become apparent during the first year of life in 65% of children developing AD and in 85% during their first 5 years [18]; it is thus worthwhile to determine the incidence as accurately as possible in nursery school children. The incidence in Japanese elementary school students was around 3% in 1981 to 1983 but increased to around 6 to 7% in the 1990s [7]. In 2000 to 2002, a research team of the Japanese Ministry of Health, Labor and Welfare (chief researcher, Dr. S. Yamamoto) performed physical examinations of 48,072 children living in Asahikawa, Iwate, Tokyo, Gifu, Osaka, Hiroshima, Kochi, and Fukuoka [19, 20]. In that study, it was found that the average national prevalence of AD was 12.8% in 4-month-old children, 9.8% in 18-month-olds, 13.2% in 3-year-olds, 11.8% in 6- to 7-year-olds, 10.0% in 12- to 13-year-olds, and 8.2% in 18-year-old children in Japan. In the present study, the mean prevalence of AD in children under the age of 5 years was 6.8% in Ishigaki Island through 2001 to 2004, which was much lower than that in mainland Japan [19, 20]. Yemaneberhan *et al.* studied the prevalence of AD symptoms and the effects of potential environmental etiologies in rural and urban areas of Jimma in southwestern Ethiopia [21]. Lifetime cumulative prevalence of AD symptoms was generally low with an overall prevalence of 1.2%, but it was higher in the urban (1.5%) than in the rural areas (0.3%; odds ratio = 4.45 [95% CI 2.34-8.47]) indicating a marked urban-rural gradient [21]. In relation to industrialization and urbanization, air pollution is now believed to be undeniably involved in the increase of allergic diseases such as asthma and AD [22-25]. In a recent Spanish epidemiologic study, air pollution was associated with a higher prevalence of AD with a trend toward greater severity as well [25]. In accordance with this notion, air pollution is much lower on Ishigaki Island compared to mainland Japan.

It is generally believed from clinical experience that spontaneous regression occurs in the majority of AD patients in

the early period of life. However, few studies have actually addressed and confirmed this assumption using population-based cohort study methodology. Kohno reported that 80% of 4-month-old AD patients became symptom-free at 18-months [26]. In the present cohort, we determined the regression rate prospectively. Spontaneous regression was observed in 71.6% of AD patients during the 3-year follow-up period in the nursery school children studied here. Such spontaneous regression seemed to occur rather rapidly because 41.9% of patients no longer showed any symptoms as early as 1 year later.

Among the 795 initially symptom-free children, AD developed in 25, 14 and 5 cases, one, 2 and 3 years later, respectively. Thus, 44 of 795 children (5.5%) developed AD over the 3 years (3.67%/person year). Consistent with this finding, it was reported that 60% of 3 year-old AD patients had not shown any symptoms at 4-months [26]. Considering the spontaneous regression and *de novo* development as mentioned above, the clinical course of AD is clearly extremely diverse in nursery school children.

In our previous study, high levels of total IgE were found in only 33.3% of those children diagnosed with AD. However, IgE antibodies specific for one or more allergens were detected in 64.1% of children with AD. The total and specific IgE levels were both significantly higher in children with AD than in those without [16]. In the present cohort, we compared IgE levels in long-term AD patients with those who spontaneously regressed, developed AD *de novo*, or never had AD. The IgE levels tended to increase gradually as the children's ages increased in all of these groups. Nonetheless, a much more marked elevation of IgE was observed in the long-term AD group compared to the others. Other studies have also found that IgE levels were elevated in 80 to 85% of children who developed AD and correlated with disease severity [27, 28]. Recently, Yamamoto *et al.* showed that subcutaneous injection of culture supernatants from keratinocytes potently enhanced IgE secretion by splenocytes and increased *in vivo* IgE levels in mice [29]. Soumelis *et al.* demonstrated that thymic stromal lymphopoietin (TSLP) was highly expressed by keratinocytes from patients with AD, and that TSLP-activated dendritic cells primed naive helper T cells to produce the proallergic cytokines IL-4, IL-5, IL-13 and tumor necrosis factor-alpha, while down-regulating IL-10 and interferon-γ [30]. These results suggest that continuous atopic inflammation of the skin may enhance IgE production by stimulating the secretion of keratinocyte-derived cytokines.

In conclusion, more than 70% of AD children experienced spontaneous regression within the 3-year follow-up period, while new onset was estimated at 3.67%/person year in nursery school age children. ■

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Original Article

The Impact of Peripheral Arterial Disease and Acute Ischemic Stroke

Yasunori SAWAYAMA, Maki HAMADA, Shigeru OTAGURO, Shinji MAEDA, Hachiro OHNISHI, Yuuji TAIRA and Jun HAYASHI

Department of General Medicine, Kyushu University Hospital, and Divisions of Cardiology**, Harasanshin General Hospital*

Abstract Peripheral arterial disease (PAD) is associated with coronary artery disease (CAD) and stroke, but data on the relationship between PAD and acute ischemic stroke are lacking. Therefore, we investigated this relationship. A total of 101 patients were enrolled on admission to Harasanshin General Hospital (Fukuoka, Japan) with their first ischemic stroke. All 101 patients underwent cranial CT and/or brain magnetic resonance imaging, duplex ultrasonography of the extracranial carotid arteries, and transthoracic echocardiography.

The subjects were aged 41 to 92 years. PAD was present in 81/101 patients (80.2%), including 57/73 (78.1%) with small artery occlusion, 11/13 (84.6%) with large artery occlusion, and 13/15 (86.7%) with cardiogenic embolism. In 42 of these 81 patients (51.9%), PAD was asymptomatic. Serum apoprotein A1 levels were significantly higher and the intima-media thickness was significantly greater in the patients with PAD than in those without PAD. The modified Rankin scale score was significantly higher on admission in patients with PAD than in those without PAD. Stepwise logistic regression analysis revealed that the apoprotein A1 level and the modified Rankin scale score on admission were strongly associated with the occurrence of stroke in patients with PAD.

Our results suggest that PAD is frequently associated with acute ischemic stroke. It may be important to perform screening for PAD in patients who have suffered an ischemic stroke.

Key words : peripheral arterial disease, ischemic stroke, stroke subtypes, carotid atherosclerosis

INTRODUCTION

Atherosclerosis is a highly prevalent disease, and is currently the greatest cause of morbidity and mortality in developed societies. Many risk factors are involved in the occurrence of atherosclerosis, which manifests as coronary artery disease (CAD) and myocardial infarction (MI), including hyperlipidemia, hypertension, smoking, and diabetes mellitus[1]. It is also known that peripheral arterial disease (PAD) is associated with CAD and stoke, but data on the relationship between peripheral arterial disease and acute ischemic stroke are lacking. The pulse wave velocity can be used as an indicator of arterial stiffness[2][3], and it is regarded as a marker of vascular damage[4][5]. An instrument was recently developed that can measure the brachial-ankle

Yasunori Sawayama, M.D., Ph.D.
Department of General Medicine, Kyushu University Hospital, Fukuoka, 812-8582, Japan
TEL : +81-92-642-59094
FAX : +81-92-642-5916
E-mail address : sawayama@genmedpr.med.kyushu-u.ac.jp

pulse wave velocity (baPWV) by the volume-rendering method. Yamashina et al. have reported a high validity and reproducibility of baPWV measurements, suggesting that this parameter may be an acceptable indicator of vascular damage and may be suitable for screening large populations to detect vascular disease[6].

Recently, arteriosclerotic disease has been increasing in Japan as the population ages and the lifestyle becomes more westernized. The prevalence of arteriosclerosis is therefore anticipated to increase in Japan and the prevalence of PAD is also expected to increase. However, there have been few epidemiological studies on PAD in Japan and even fewer studies on the prevalence of PAD among stroke patients. This is partly because early PAD is asymptomatic and therefore difficult to diagnose and also because distinguishing PAD from other conditions, such as spinal cord disease, is difficult even after PAD symptoms like nocturnal leg pain become evident. PAD not only interferes with daily activities and reduces the quality of life in stroke patients, but also worsens their survival. PAD is associated with the occurrence of coronary artery disease and cerebrovascular disease[7] Therefore, PAD should be detected and treated as early as possible.

We performed the present prospective study to investigate whether PAD was an independent risk factor for acute ischemic stroke in Japanese patients.

METHODS

Subjects

All of the patients with acute ischemic stroke admitted to the Division of General Medicine at Harasanshin General Hospital (Fukuoka, Japan) during the period from August 1, 2003 to July 31, 2004 were eligible for the present study. As a result, a total of 101 patients who suffered their first ischemic stroke were registered for this study after meeting the following criteria: (a) first ischemic stroke, (b) admission to hospital for treatment, and (c) admission within 72 hours of the onset.

Categorization of Stroke

Stroke was defined according to World Health Organization criteria[8]. Cerebral infarction was diagnosed on the basis of the initial CT and MRI data. All patients underwent ultrasonography of the neck and intracranial arteries. The carotid arteries were assessed by color flow B-mode Doppler ultrasound (SONOS 5500, PHILIP) according to the standard method[9][10]. The vertebrobasilar system was evaluated by radionucleotide angiography to determine the presence/absence of atherosclerotic lesions. Patients without clinical or imaging evidence of atherosclerosis who had atrial fibrillation and/or echocardiographic findings suggestive of possible cardiogenic embolism were classified as having thromboembolic stroke. The other patients were diagnosed as having large artery stroke if there was >50% stenosis of the extracranial carotid artery or an intracranial artery, or as having small artery occlusion if they had a clinical lacunar syndrome associated with appropriate CT changes or a typical clinical syndrome despite normal CT scans. Patients were classified as having undefined stroke if they did not fit any of these categories[11]. Functional outcome was measured using the modified Rankin Scale[12]. During hospitalization, neurological evaluation was always done by a single neurologist who applied the study criteria for classification of the patients. All evaluations were performed at the

Department of Neuroradiology.

Laboratory Tests

All blood samples were stored at -80(C and were analyzed simultaneously by technicians who were unaware of the clinical data of the patients.

Brachial-Ankle Pulse Wave Velocity (baPWV)

The baPWV was measured using a volume plethymograph (PWV/ABI ; Colin, Co., Ltd., Komaki, Japan), which simultaneously recorded the PWV, blood pressure, electrocardiogram, and heart sounds[6]. Each subject was examined in the supine position, with the electrocardiographic leads on both wrists, a microphone for detecting heart sounds taped at the left sternal edge, and cuffs on both arms and ankles. The cuffs were connected to a plethysmograph sensor that determined the pulse volume waveform and to an oscillometric pressure sensor that measured the blood pressure. Pulse volume waveforms were recorded using a semiconductor pressure sensor, with the acquisition frequency set at 1,200 Hz. Waveforms for the arm and ankle were stored in 10-sec batches with automatic gain analysis and quality adjustment. The baPWV data were obtained after the subjects had rested for at least 5 min. The reproducibility of baPWV values obtained in healthy subjects was reported to be reasonable, with an interobserver coefficient of variation of 2.4% (n=15) and an intraobserver coefficient of variation of 5.8% (n=17)[6].

Diagnosis of Peripheral Arterial Disease

PAD was diagnosed as follows: Criterion[1] was severe stenosis or occlusion of a lower extremity artery on MRA and/or no diastolic reverse flow (type II - VI) on lower extremity ultrasonography. Criterion 1 was positive when one of these two factors was detected. Criterion[2] was an ankle-brachial index <0.9, a pulseless artery, and/or symptoms of PAD. Criterion 2 was positive when two of these three factors were detected. PAD was defined as present when both criterion 1 and criterion 2 were positive.

Statistical Analysis

Data were recorded on standard forms and then entered into a database. Results are expressed as percentages or as the mean (standard deviation (SD). A nonparametric test (the Mann-Whitney U test) was used to compare variables between groups. Two-way analysis of variance (ANOVA) was used for comparison of the means of numerical variables between three groups. Multiple comparison with the Kruskal-Wallis test was also employed to compare three groups. The risk of ischemic stroke in patients with PAD was estimated by forward stepwise multiple logistic regression analysis with adjustment for the apoprotein A1 level, carotid intima-media thickness (IMT), and modified Rankin scale score (on admission).

Ethics

The design of this study was approved by the Ethics Committee and the Data Protection Committee of Harasanshin General Hospital (Fukuoka, Japan). Informed consent to participation was obtained from all patients (or their closest relatives).

RESULTS

One hundred and thirty-three patients with stroke were evaluated for enrollment in the study, but 32 patients were excluded because of an unclassified stroke subtype (n=13) or refusal to participate (n=19).

Therefore, 101 patients were investigated.

Characteristics of the Subjects

Table 1 shows the characteristics of the 3 subgroups of stroke patients and their risk factors. The mean age of the small artery occlusion group was significantly higher than that of the patients with cardioembolic stroke. The mean serum triglyceride level of the small artery occlusion group was signifi-

Table 1-A Characteristics of the Stroke Patients

Risk Factors	Small artery occlusion (n=73)	Large artery atherosclerosis (n=13)	Cardioembolic stroke (n=15)	P value	Multiple comparison
Age [years, mean±SD]	68.9±12.1[a)]	75.6±9.1	79.3±10.2[b)]	0.0035	a) vs b)**
Male sex [%]	51(69.9%)	9(69.2%)	7(46.7%)	0.2172	
Blood pressure					
Systolic [mean±SD, mmHg]	159.2±26.4	172.5±45.9	167.5±27.9	0.4158	
Diastolic [mean±SD, mmHg]	85.3±14.4	87.5±21.4	87.6±13.8	0.7922	
BMI [kg/m^2]	22.3±2.7	21.8±2.6	22.9±3.3	0.6084	
Smoking [%]	45(61.6%)	11(84.6%)	12(80.0%)	0.1399	
History:					
Hypertension [%]	67(91.8%)	11(84.6%)	14(93.3%)	0.6679	
Diabetes Mellitus [%]	29(39.7%)	4(330.8%)	2(13.3%)	0.1404	
Hyperlipidemia [%]	60(82.2%)	10(76.9%)	12(80.0%)	0.8972	
PAD [%]	57(78.1%)	11(84.6%)	13(86.7%)	0.5869	

ANOVA ; ** $p<0.01$

Table 1-B Characteristics of the Stroke Patients (contined)

Lipids	Small artery occlusion (n=73)	Large artery atherosclerosis (n=13)	Cardioembolic stroke (n=15)	P value	Multiple comparison
TC [mean±SD, mg/dl]	208.3±46.3	212.6±29.7	196.5±40.3	0.5664	
TG [mean±SD, mg/dl]	113.0±86.3[a)]	100.0±64.5	77.0±60.0[b)]	0.0307	a) vs b)*
HDL-C [mean±SD, mg/dl]	45.5±37.5	43.0±36.0	46.0±36.0	0.8837	
LDL-C [mean±SD, mg/dl]	127.1±106.0	137.0±117.8	138.0±92.2	0.6641	
Lipoprotein(a) [mean±SD, mg/dl]	17.1±10.0	23.3±8.83	12.1±7.7	0.4864	
Apoprotein A1 [mean±SD, md/dl]	124.0±112.0	125.5±103.0	119.0±96.0	0.1736	
Apoptotein B [mean±SD, md/dl]	100.0±84.0	103.5±89.0	103.0±79.0	0.5980	
Apoprotein E [mean±SD, md/dl]	4.364±1.097	4.813±1.092	4.100±0.700	0.3149	
RLP-C [mean±SD, md/dl]	3.3±2.7	4.15±3.3	3.6±2.2	0.2736	

TC; total cholesterol TG ; Kruskal-Wallis test; * $p<0.05$
HDL-C; HDL cholesterol, LDL-C; LDL cholesterol
RLP-C; RLP cholesterol

Table 1-C Others Factors

Lipids	Small artery occlusion (n=73)	Large artery atherosclerosis (n=13)	Cardioembolic stroke (n=15)	P value	Multiple comparison
CRP [mean±SD, mg/dl]	0.0±0.0[a)]	0.0±0.0[b)]	1.0±0.3[c)]	0.0004	a) vs c)** b) vs c)*
D-D [mean±SD, μg/dl]	0.6±0.3[d)]	1.2±0.4	3.1±0.98[e)]	0.0001	d) vs e)**
TAT [mean±SD, ng/dl]	2.50±1.75[f)]	2.60±1.65	8.65±3.58[g)]	0.0051	f) vs g)**
IMT [mean±SD, mm]	1.110±0.928	1.310±1.015	1.14±0.95	0.2481	
ABI [mean±SD]	1.14±1.02	1.04±0.8	1.15±1.08	0.1281	
baPWV [mean±SD, mmHg]	1934±1650	1847±131	2405±1896	0.1816	
modified Rankin Scale [mean±SD]					
on admission	3.0±2.0	4.0±2.0	5.0±5.0	0.0001	h) vs i)**
on discharge	1.0±0.0	2.0±1.0	4.0±2.0	0.0001	j) vs k)** j) vs l)**
Admission period [mean±SD, days]	23.0±18.0	23.5±20.0	2405±1896	0.0022	m) vs n)**

ANOVA; ** $p<0.01$ Kruskal-Wallis test; * $p<0.05$

CRP; C reactive protein
TAT; thrombin-antithrombin III complex
ABI; ankle brachial index
D-D; D-dimer
IMT; intima-media thickness
baPWV; brachial-ankle pulse wave velocity,

cantly higher than that of the cardioembolic stroke group, although there were no significant differences among the D-dimer (D-D) three stroke subtypes with respect to the other serum lipids. Serum C reactive protein (CRP) and thrombin-antithrombin III complex (TAT) levels were significantly higher in the cardioembolic stroke patients than in those with small artery occlusion, while serum CRP was significantly higher in the cardioembolic stroke patients than in those with large artery atherosclerosis. The modified Rankin scale values on admission and discharge were significantly higher and the duration of admission was significantly longer in the cardioembolic stroke patients than in those with small artery occlusion.

Association Between PAD and Risk Factors

The associations between PAD and various risk factors are displayed in Table 2. PAD was present in 81 of the 101 stroke patients (80.2%), including 57 out of 73 patients with small artery occlusion, 11 out of 13 with large artery occlusion, and 13 out of 15 with cardiogenic embolism. In 42 of these 81 patients (51.9%), PAD was asymptomatic. When the associations between PAD and various risk factors were assessed, no significant differences of these risk factors were found between the stroke patients with and without PAD.

The serum apoprotein A1 level was significantly higher in the stroke patients with PAD than in those without PAD. However,

Table 2-A Association Between PAD and Risk Factors

Risk Factors	PAD		P value
	positive (n=81)	negative (n=20)	
Age [years, mean±SD]	72.0±11.9	68.6±12.6	0.2585
Male sex [%]	52(64.2%)	15(75.0%)	0.5148
Blood pressure			
Systolic [mean±SD, mmHg]	162.8±30.1	159.8±29.5	0.6925
Diastolic [mean±SD, mmHg]	86.7±15.6	82.7±13.3	0.2970
BMI [kg/m^2]	22.4±2.8	22.0±2.5	0.5521
Smoking [%]	55(67.9%)	13(65.0%)	1.0000
History:			
Hypertension [%]	75(92.6%)	17(85.0%)	0.5293
Diabetes Mellitus [%]	28(34.6%)	7(35.0%)	1.0000
Hyperlipidemia [%]	68(84.0%)	14(70.0%)	0.2669

Table 2-B Association Between PAD and Risk Factors (continued)

Lipids	PAD		P value
	positive (n=81)	negative (n=20)	
TC [mean±SD, mg/dl]	204.0±176.5	202.0±181.0	0.2585
TG [mean±SD, mg/dl]	107.0±76.3	102.5±79.0	0.6886
HDL-C [mean±SD, mg/dl]	45.0±37.0	55.0±38.0	0.0631
LDL-C [mean±SD, mg/dl]	134.9±106.5	118.8±108.7	0.2833
Lipoprotein(a) [mean±SD, mg/dl]	1.71±10.1	13.1±6.2	0.3644
Apoprotein A1 [mean±SD, md/dl]	121.0±110.0	143.0±116.0	0.0188*
Apoptotein B [mean±SD, md/dl]	100.0±85.5	95.0±81.0	0.4607
Apoprotein E [mean±SD, md/dl]	4.33±1.03	4.52±1.13	0.5266
RLP-C [mean±SD, md/dl]	3.6±2.6	3.7±3.1	0.3438

Mann-Whitney U-test; * $p<0.05$

TC; total cholesterol TG;
HDL-C; HDL cholesterol, LDL-C; LDL cholesterol
RLP-C; RLP cholesterol

Table 2-C Association Between PAD and Risk Factors (continued)

Other Factors	PAD positive (n=81)	PAD negative (n=20)	P value
CRP [mean±SD, mg/dl]	0.2±0.0	0.0±0.0	0.2444
D-D [mean±SD, μg/dl]	0.8±0.4	0.9±0.2	0.4585
TAT [mean±SD, ng/dl]	2.7±1.9	2.4±1.8	0.1809
IMT [mean±SD, mm]	1.15±0.98	0.98±0.83	0.0126*
ABI [mean±SD]	1.13±1.01	1.12±1.08	0.5942
baPWV [mean±SD, mmHg]	1979±1712	1868±1371	0.0608
modified Rankin Scale [mean±SD]			
on admission	3.0±2.0	2.0±1.3	0.0376*
on discharge	1.0±1.0	1.0±0.0	0.1069
Admission period [mean±SD, days]	26.0±19.0	24.0±18.8	0.5650

CRP; C reactive protein D-D; D-dimer Mann-Whitney U-test; * $p<0.05$
TAT; thrombin-antithrombin III complex IMT; intima-media thickness
ABI; ankle brachial index baPWV; brachial-ankle pulse wave velocity,

there were no significant differences of the other lipid parameters between the patients with and without PAD.

The associations between PAD and several other factors are shown in Table 2 The carotid intima-media thickness and the modified Rankin scale score on admission were significantly larger in the stroke patients with PAD than in those without PAD, but there were no significant differences of the other factors between the patients with and without PAD.

Multiple Logistic Regression Analysis

Logistic regression analysis showed that the apoprotein A1 level and the modified Rankin scale score on admission were strongly related to the occurrence of stroke in patients with PAD (Table 3).

DISCUSSION

The present study showed that PAD is frequently associated with acute ischemic stroke due to either large or small artery occlusion, suggesting that it may be important to perform screening for PAD in patients with ischemic stroke. Our study also revealed that the prevalence of PAD is increased in stroke patients, suggesting that detection of PAD may help to improve the prognosis of patients with ischemic stroke. In general, an ABI of less than 0.9 is considered to indicate the presence of PAD Since blood pressure is higher in the lower limbs than in the upper limbs, the normal ABI ranges from 1.0 to 1.5[13]. Detection of PAD by measuring the ABI was previously found to have a 90% sensitivity and 95% specificity, so this method is generally accepted as the gold standard[14]. In many studies conducted in Europe and the USA, PAD was defined as being present when the ABI was less than 0.9[15)~20)]. Alternatively, an ABI greater than 0.90 at rest that decreases by 20% or more after exercise has been proposed to be diagnostic of PAD[21]. This suggests that patients with leg pain on exertion who have ABI values >0.90 should

Table 3 Forward Stepwise Multiple Logistic Regression Analysis Of PAD in Relation to Apoprotein A 1, IMT, and modified Rankin Scale (on admission)

	Coefficient	Odds ratio	95% CI		P value
Apoprotein A1	−0.02138	0.979	0.959	0.999	0.0312
modified Rankin Scale (on admission)	0.5041	1.660	1.01	2.71	0.0318