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Relationship between Apo E Phenotypes and Dyslipidemia in the Patients with Non-Insulin Dependent Diabetes Mellitus

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Effects of apo E phenotypes on dyslipidemia in NIDDM patients were evaluated. Apo E phenotypes were determined by isoelectric focusing and immunoblotting techniques in 398 patients with NIDDM, including 255 males and 143 females. Apo E phenotypes were classified into three types: E2, E3 and E4. Blood lipid levels, apolipoproteins and Lp (a) were compared between the three groups. Statistical analyses were conducted using Mann-Whitney's U test and the multiple logistic regression analysis.

The incidence of apo E phenotypes was as follows: 8.8% for E2, 74.4% for E3 and 16.8% for E4. Mean TG levels and apo E concentrations were higher in patients with apo E2 than those with apo E3, but LDL-C levels and LDL-C/HDL-C ratio were significantly lower in the former than the latter. The odds ratio for high LDL-C (LDL-C≥250 mg/dl) and high Lp (a) (Lp (a) ≥30 mg/dl) were lower in patients with apo E2 than in those with apo E3 (0.06, 0.35 respectively). Similar patterns were observed in patients treated with lipid lowering agents. Relative risk of LDL-C and Lp (a) levels was significantly higher in patients with apo E4 than in those with E3 (odds ratio: 1.20 and 1.97, respectively).

These findings suggest that apo E2 has favorable effects on dyslipidemia in NIDDM patients, while apo E4 has unfavorable effects.

Introduction

Many studies have been conducted on the relationship between apo E genes and plasma lipid levels and arteriosclerosis since Utermann et al[1] reported that type III hyperlipidemia is related to apo E2/2 in 1975. However, whether apo E phenotypes are related to arteriosclerosis in diabetic patients remains a controversial issue. In the present study, therefore, we evaluated effects of apo E phenotypes on dyslipidemia in NIDDM patients.

Subjects and Methods

Included in the present study were 398 NIDDM outpatients treated in our center, including 255 males and 143 females. Antidiabetic therapy consisted of diet therapy alone in 136 patients, oral hypoglycemic agents in 143, and insulin in 121. In the assessment of renal function, the diagnosis of normoalbuminuria was made if the amount of albumin in the first urine sample taken early in the morning was less than 12 mg/g・Cr (n=189), microalbuminuria if it was 12～250 mg/

Table 1 Clinical characteristics of NIDDM patients by apo E phenotypes

	E2	E3	E4
Number (%)	35(8.8)	296(74.4)	67(16.8)
Age (yrs)	58.3 ± 2.1	58.3 ± 0.6	60.4 ± 1.2
BMI (kg/m^2)	23.8 ± 0.6	23.6 ± 0.2	23.6 ± 0.4
Systolic Bp (mmHg)	135.9 ± 3.9	137.7 ± 1.2	135.2 ± 2.3
Diastolic Bp (mmHg)	78.0 ± 1.9	80.1 ± 0.7	78.2 ± 1.6
Fasting plasma glucose (mg/dl)	159.8 ± 10.9	156.9 ± 3.1	147.3 ± 5.2
HbA$_{1C}$ (%)	7.1 ± 0.3	7.4 ± 0.1*	6.8 ± 0.2*
Retinopathy [simple/proliferative] (%)	5/7(14.3/20.0)	59/76(20.0/25.8)	17/14(25.4/20.9)
Albuminuria [micro/macro] (%)	11/9(31.4/25.7)	83/64(28.1/21.6)	24/18(35.8/26.9)
Vascular events (%)	11(31.4)	95(32.1)	24(35.8)
Hypertension (%)	12(34.3)	103(34.8)	26(38.8)
Lipid-lowering therapy (%)	9(25.7)	73(24.7)	20(29.9)

Data are means ± SE. * : $p<0.05$ apo E3 vs E4. Number in parenthesis is percent of patients.

g・Cr (n=118), and macroalbuminuria if persistent proteinuria and renal failure were present (n=91). Fasting blood glucose, HbA$_{1c}$, cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), apo A-I, apo B, apo E and Lp(a) levels were also determined using venous blood samples collected from diabetic patients early in the morning.

Apolipoprotein measurement used Turbidlmetric Immunoassay (Daiichi-kagaku, Japan). Lp(a) levels were measured by ELISA using a kit supplied by Biopool (Sweden). Apo E phenotypes were determined by immunoblotting techniques after polyacrylamidegel isoelectric focusing using Phenotyping Apo E (Joko, Japan). Apo E was classified into three groups: E2 (2/2 and 2/3), E3 (3/3) and E4 (3/4 and 4/4). Whether there were significant differences between these three types in blood lipid levels was evaluated. Subjects with apo E2/4 phenotype could not be assigned to any of the groups and were therefore excluded from further analysis. LDL-cholesterol (LDL-C) was calculated by Friedewald's formula.

Multiple inter-group comparison was undertaken using Kruscal–Wallis' rank test. This was followed by Mann–Whitney's U test if significant differences were found at $p<0.05$. Multiple logistic regression analysis was conducted to compare three apo E phenotypes and lipid levels adjusted for various factors (sex, age, duration of diabetes, body mass index (BMI), blood glucose control, renal function, and the use of lipid-lowering drugs). Relative risk (odds ratio) of apo E2 and E4 with respect to apo E3 was calculated. All statistical analysis was carried out using SAS.

Results

The incidence of apo E2, E3 and E4 in NIDDM patients was 8.8% (35/398), 74.4% (296/398) and 16.8% (67/398), respectively. Clinical features of patients with apo E2, E3 and E4 are shown in Table 1. Age, BMI, blood pressure and fasting plasma glucose levels were comparable in the three groups, but HbA$_{1c}$ was higher in patients with apo E3 than in those with apo E4 ($p<0.05$). There were no significant differences between the three groups in the percentage of patients with simple or proliferative retinopathy and those with microalbuminuria or macroalbuminuria. The percentages of patients with hypertension, those treated with lipid lowering agents and those with arteriovascular disease were comparable in all three groups.

Plasma lipid levels, apolipoprotein, LDL-C・HDL-C ratio, apo B・apo A-I ratio and Lp(a) were

Table 2 Comparison of serum lipids, apo-lipoproteins and Lp(a) in NIDDM patients with apo E2, E3 and E4 phenotypes

	E2	E3	E4	p-value	
				E2 vs. E3	E4 vs. E3
Total cholesterol (mg/dl)	211.9 ± 6.1	215.5 ± 2.4	214.0 ± 5.4	ns	ns
Triglyceride (mg/dl)	188.1 ± 23.8	145.5 ± 6.5	189.1 ± 27.0	0.05	ns
LDL-cholesterol (mg/dl)	126.9 ± 6.3	139.8 ± 2.3	138.3 ± 5.0	0.05	ns
HDL-cholesterol (mg/dl)	51.4 ± 3.0	49.3 ± 0.9	45.7 ± 1.8	ns	ns
Apo A-I (mg/dl)	138.4 ± 5.6	129.0 ± 1.6	126.4 ± 3.2	ns	ns
Apo B (mg/dl)	107.7 ± 6.3	113.2 ± 2.1	119.1 ± 5.1	ns	ns
Apo E (mg/dl)	7.2 ± 0.7	5.4 ± 0.1	6.1 ± 0.5	0.002	ns
LDL-C/HDL-C	2.57 ± 0.18	3.07 ± 0.08	3.17 ± 0.14	0.02	ns
Apo B/Apo A-I	0.82 ± 0.06	0.91 ± 0.02	0.97 ± 0.04	ns	ns
La(a) (mg/dl)	23.4 ± 3.1	32.7 ± 1.6	24.7 ± 2.4	0.05	0.05

Data are means ± SE.

Table 3 Adjusted relative risks (and 95% confidence intervals) for apo E phenotypes for selected risk factors among NIDDM patients

	all patients	with lipid-lowering therapy
Numbers	398	102
LDL-cholesterol ≥ 150 mg/dl		
E2	0.06 (0.0076 ~ 0.45)	0.09 (0.011 ~ 0.89)
E3	1.00	1.00
E4	1.30 (0.69 ~ 2.40)	1.20 (0.38 ~ 3.50)
Lipoprotein(a) ≥ 30 mg/dl		
E2	0.35 (0.13 ~ 0.94)	0.54 (0.0055 ~ 0.54)
E3	1.00	1.00
E4	0.55 (0.28 ~ 1.10)	1.97 (0.53 ~ 7.30)

Apo E2/E3 amd E4/E3 odds ratios were calculated after adjustment for sex, age, duration of diabetes, BMI, blood glucose control, renal function and use or non-use of lipid lowering agents. Only statistically significant findings obtained with respect to serum lipids and apoproteins are shown.

compared between the three groups (Table 2). TG and apo E levels were significantly higher but LDL-cholesterol levels and the LDL-C/HDL-C ratio were significantly lower in patients with apo E2 than in those with apo E3. However, there were no significant differences between patients with E3 and those with E4 in any of these parameters. Lp (a) levels were significantly higher in patients with E3 than in those with E2 and E4 ($p<0.05$).

Multivariate analyses were conducted using a logistic regression model in order to determine the degree to which apo E phenotypes are related to arteriosclerosis-related lipids. Only statistically significant findings are presented in Table 3. Overall, risk of LDL-C levels higher than 150 mg/dl was much lower (0.06) in patients with apo E2 than in those with apo E3. Risk of Lp(a) higher than 30 mg/dl was also significantly smaller (0.35) in the former than in the latter. Furthermore, similar findings were obtained in patients with lipid lowering therapy (odds ratio: 0.09 for LDL-C and 0.54 for Lp(a)). On the other hand risk of LDL-C and Lp(a) increasing above 150 mg/dl and 30 mg/dl, respectively, was significantly higher in hypolipidemic agent-treated patients

with apo E4 than in those with apo E3 (odds ratio: 1.20 for LDL-C and 1.97 for Lp(a)).

Discussion

The incidence of apo E genes polymorphism has been reported to be comparable in diabetics and non-diabetics among Japanese[2]. In the present study, the incidence of apo E phenotypes determined in NIDDM was consistent with their reports[2]. Although patients with apo E2 account for only several present of the total, E2 is believed to be liable to impair catabolism of TG-rich lipoprotein due to low affinity with LDL receptors[3]. According to Eto et al[4], the incidence of type III hyperlipidemia (associated with remnant accumulation) and type IV hyperlipidemia (associated with VLDL accumulation) is high among diabetic patients with apo E2, and fasting blood glucose and HbA_{1c} levels increase in apo E2 patients with hyperlipidemia. In our study, the incidence of hyperlipidemia, as well as fasting blood glucose and HbA_{1c} levels, in apo E2 patients were comparable to those in apo E3 and E4 patients. However, mean TG and apo E levels were higher in E2 patients than in E3 patients.

Most investigators believe that apo E4 is related to atherosclerotic diseases. Dallongeville et al[5] conducted meta-analysis of 45 population samples (14,799 persons) from 17 countries and reported that higher cholesterol and TG levels and lower HDL-C levels were found in persons with ε4/ε3 genotype than with ε3/ε3 genotype, suggesting that apo Eε4 allele may increase cardiovascular risk. In the diabetic patients, apo ε4 allele is also reported to increase TC levels[6] and unlikely to respond to diabetic therapy[7]. Our findings also showed that LDL-C levels are liable to increase in NIDDM patients with apo E4 and that the risk of Lp(a) elevation to ≥ 30 mg/dl is about twice as high in NIDDM patients with E4 treated with lipid lowering agents as in those with E3. It is therefore necessary to control both TC and blood glucose levels in NIDDM patients with apo E4[8].

Whether apo E 2 contributes to the progression of arteriosclerosis is a controversial issue. According to Horita et al[9], TG-rich lipoprotein and remnant are likely to accumulate in patients with apo E2, resulting in aggravation of abnormal lipid metabolism in the presence of diabetic renal insufficiency. Our findings obtained in the present study showed, however, that LDL-C and Lp (a) levels are less likely to increase significantly in patients with E2 than those with E3, suggesting that E2 may inhibit risk factors of arteriosclerosis. Recently, Ukkola et al[10] reported that apo E2 may have beneficial effects on micro and macrovascular complications in NIDDM patients. Groop et al[11] also found that decreases in LDL-C and increases in HDL-C occur in the presence of apo E2 allele in type I diabetic patients with nephrosis and suggested that the presence of apo E2 allele may protect dyslipidemia.

In patients with lipid lowering therapy in the present study, the LDL-C and Lp(a) odds ratio was significantly lower in patients with E2 than in those with E3, but was higher in those with E4. Ordovas et al[12] reported that pravastatin shows greater inhibitory effects in patients with apo E2 allele because these patients have greater HMG-CoA reductase activity in the liver. Therefore, the lower LDL-C levels in patients with apo E2 in our study may be partly due to greater sensitivity of these patients to pravastatin effects.

Further studies are necessary in order to determine whether the apo Eε2 allele has beneficial effects on dyslipidemia and acts as an arteriosclerosis inhibitor.

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NIDDMにおける脂質代謝異常とアポE表現型の関連

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NIDDMにおける脂質代謝異常にアポE表現型がいかなる影響を及ぼすかを検討した．398名（男/女：255/143）のNIDDM患者のアポE表現型をisoelectric focusing and immunoblotting techniquesを用いて測定した．アポE表現型はE2, E3, E4の3型に分け，血中脂質，アポリポ蛋白，動脈硬化指数，Lp(a)を3群間で比較した．統計処理はKruscal–Wallis' rank testおよびMann–WhitneyのU検定と，多重ロジスティック回帰分析を行った．

NIDDMにおけるアポE表現型の頻度はE2：8.8%（35/398），E3：74.4%（296/398），E4：16.8%（67/398）であった．E2, E3, E4の3群間で年齢，肥満度，血圧，空腹時血糖値に差はないが，HbA_{1c}がE3で軽度に高値であった．網膜症や腎症の合併率，心・脳血管障害の併発率，および高血圧や抗脂血剤の使用者は3群間で差を認めなかった．アポE2を有する者ではE3を有する者に比しTG，アポE濃度が高値であったが，LDL-CとLDL-C/HDL-C比およびLp(a)がいずれも有意に低値であった．またアポE2をもつものはE3をもつものに比し，高LDL-C（LDL-C≧150 mg/dl）のodds比が0.06，高Lp(a)（Lp(a)≧30 mg/dl）のodds比が0.35と有意に低値であった．そしてこれらはいずれも抗脂血剤服用群において同様に認められた．一方アポE4をもつものでは，高LDL-Cと高Lp(a)の相対危険度がE3をもつものより有意に高かった（odds比は各々1.20と1.97）．

以上よりNIDDMにおける脂質代謝異常にアポE2は有利に働き，E4は不利に働く可能性が示唆された．