—Original—

Relation of Apolipoprotein(a) Phenotypes to Diabetic Retinopathy in Elderly Type 2 Diabetes

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Abstract

The aim of this study was to clarify the relationship between apolipoprotein(a) (apo(a)) phenotypes and diabetic retinopathy in elderly type 2 diabetes. Serum Lp(a) concentrations and apo(a) phenotypes were analyzed in 250 diabetic patients aged 60 to 88 years old. Apo(a) phenotypes were classified into 7 subtypes (F, B, S1, S2, S3, S4, O (Null)) by the method SDS electrophoresis with Western blotting. Patients were divided into two groups according to their apo(a) phenotypes: a low molecular weight (LMW) Lp(a) group, and a high molecular weight (HML) Lp(a) group. Patients were classified as having one of 4 types of diabetic retinopathy: no retinopathy (R0), simple retinopathy (R1), pre-proliferative retinopathy (R2), and proliferative retinopathy (R3). There was a significant association between serum Lp(a) levels and severity of diabetic retinopathy ($p<0.001$). A gradual trend toward increasing serum Lp(a) levels was observed across the groups (from R0 to R3). A significantly greater percentage of LMW Lp(a) was observed in the R1, R2, and R3 groups than in the R0 group (42.9% ($p<0.001$), 27.0% ($p<0.01$), and 27.3% ($p<0.05$) vs. 10.4%). Multiple logistic regression analysis revealed that duration of diabetes and LMW Lp(a) are independent risk factors for diabetic retinopathy. These results provide significant evidence that LMW Lp(a) contributes to an increased risk of diabetic retinopathy in elderly type 2 diabetes.

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Key words: lipoprotein(a), apolipoprotein(a) phenotypes, diabetic retinopathy, type 2 diabetes mellitus, elderly

Introduction

Diabetic retinopathy is the most common chronic complication associated with diabetes and remains a leading cause of vision disability[1]. Most patients with diabetes develop retinopathy, and several factors are now recognized as being associated with the risk of developing diabetic retinopathy, such as length of duration of diabetes, lack of blood glucose control, high lipids levels, and high blood pressure[2–4]. However, these factors do not fully account for the incidence of diabetic retinopathy.

Genetic factors may also be involved, as suggested by the clinical observation that some patients develop severe retinopathy after several years of well-controlled diabetes, while others with similar duration of diabetes but poor glucose control do not

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develop retinopathy[5].

Lipoprotein (a) (Lp (a)) is a plasma complex composed of apolipoprotein (a) (apo(a)) covalently linked to apoB-100[6]. Lp (a) has biological homology with plasminogen and the fibrinolytic system. A high serum Lp (a) concentration has been shown to be an independent and genetically linked risk factor in the development of atherosclerosis and thromboembolic events, especially coronary heart disease[7-13]. Recently, an association between serum Lp(a) concentrations and diabetic retinopathy has been suggested [14-18]. It appears that high serum Lp(a) levels might play a role in capillary occlusion which can lead to severe retinopathy. Moreover, recent work has shown that apo(a) shows a high degree of genetic polymorphism and this polymorphism may have a predictive value greater than the serum Lp(a) concentration, since apo(a) phenotypes are genetic traits not influenced, like serum Lp(a) levels, by several environmental factors[19]. To our knowledge, no studies have examined the relationship between apo(a) phenotypes and diabetic retinopathy in elderly type 2 diabetes. We therefore investigated the relationship between diabetic retinopathy and apo(a) phenotypes in patients with elderly type 2 diabetes.

Materials and Methods

The subjects were 250 elderly type 2 diabetic patients attending to our hospital, 60 to 88 years of age. All type 2 diabetic patients were diagnosed according to World Health Organization (WHO) criteria and the diagnosis of type 2 diabetes was based on clinical characteristics that included no episodes of ketoacidosis and treatment by diet or oral hypoglycemic agents. Patients with thyroid or liver disease, non-diabetic renal disease, acute or chronic inflammatory disease, alcoholism, or evidence of malnutrition were excluded from this study. In addition, patients on any drugs known to influence lipid or lipoprotein metabolism were excluded.

Two blood pressure recordings were obtained with a mercury sphygmomanometer in sitting position after 10 minutes of rest, and the mean of these two values was calculated. Patients with hypertension were defined as those with a systolic BP of 160 mmHg or more, and/or a diastolic pressure of 95 mmHg or more, and/or those who were currently using antihypertensive medication. Patients with hyperlipidemia were defined as those with a total cholesterol level of 220 mg/dl or higher, and/or a triglyceride level of 150 mg/dl or higher, and/or a high density lipoprotein (HDL) cholesterol level of 40 mg/dl or lower, and/or those who were currently using lipid lowering agents. Diabetic nephropathy was defined as a urine albumin-to-creatinine ratio of 30 mg/g. Cr or more. After overnight fasting, blood samples were obtained to determine blood glucose, hemoglobin A_{1C} (Hb A_{1C}), plasma lipids and apo(a) levels. Blood glucose was determined by a glucose oxidase method. Total cholesterol, high density lipoprotein (HDL) cholesterol, and triglyceride, were enzymatically measured with an automatic analyzer. Lp(a) concentrations were determined by the latex agglutination (LA) method. Albuminuria was determined on two occasions by radioimmunoassy of morning spot urine samples and the mean value of the two measurements was calculated. All subjects were confirmed to be free of urinary tract infections. The body mass index was calculated as weight (in kilograms) divided by the square of height (in meters).

Apo(a) phenotyping was performed by sodium dodecyl sulfate-agarose gel electrophoresis (SDS-agarose) with Western blotting. Antigens were localized on nitrocellulose using a double-antibody procedure using gold-labeled anti-rabbit IgG conjugated to rabbit anti-Lp(a) γ-globulin. Apo(a) phenotypes were classified into 7 subtypes (F, B, S1, S2, S3, S4, O (Null))[20]. A conventional cut-off margin was established between 640 and 655 Kda in order to group the low-and high molecular weight apo(a) phenotypes, as previously described by Gazzaruso et al.[12]. Therefore, patients were divided into two groups according to their apo(a) phenotypes:a low molecular weight (S3, S4, O) (LMW) Lp(a) group, and a high molecular weight (F, B, S1, S2) (HML) Lp(a) group. When the patient had a double band, the faster band was used to express the phenotype[21]. All patients underwent a retinal examination by opthalmologists. Patients were classified as having one of four types of diabetic retinopathy based on severity: no retinopathy (R0), simple retinopathy (R1), pre-proliferative

retinopathy (R2), and proliferative retinopathy (R3).

Statistical analysis was carried out using the chi-squared test, Student t test and one-way analysis of variance. The relationship between grade of diabetic retinopathy and Lp(a) concentration was estimated using Spearman's rank correlation. Multiple regression analysis was used to investigate the relationship between diabetic retinopathy and several explanatory variables. The following explanatory variables were examined: sex (female = 0, male = 1), age (in years), duration of diabetes (in years), HbA_{1C} (%), LMW Lp(a) (HML Lp(a) = 0, LMW Lp(a) = 1), diabetic nephropathy (yes = 1, no = 0), hypertension (yes = 1, no = 0) and hyperlipidemia (yes = 1, no = 0). Results were expressed as the mean ± SD and p values less than 0.05 were considered significant.

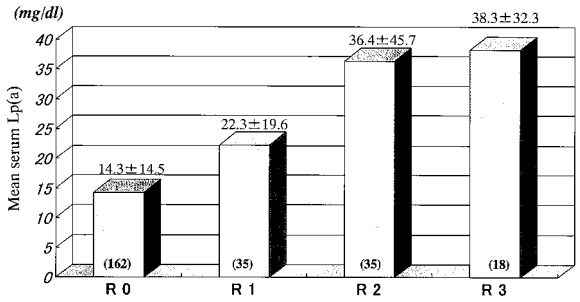

Fig. 1 Mean values of lipoprotein (a) (±SD) in the study population according to grade of diabetic retinopathy. There was a significant association between Lp (a) levels and severity of diabetic retinopathy (Spearman's rank correlation = 0.36; $p<0.001$). A gradual increasing trend from R0 to R3 was observed. ( ); number of subjects

Results

Fig. 1 shows the mean serum Lp(a) concentrations of each of the four groups divided by severity of diabetic retinopathy. There was a significant association between Lp(a) levels and severity of diabetic retinopathy (Spearman's rank correlation = 0.36;

Table 1 Clinical characteristics of Type 2 diabetic patients according to Lp(a) molecular weight.

Variables	*HMW Lp(a) group*	*LMW Lp(a) group*	P
N	205	45	−
Gender (M/F)	110/95	25/20	NS
Age (years)	69 ± 7	69 ± 6	NS
Duration of diabetes (years)	10 ± 9	9 ± 7	NS
Fasting plasma glucose (mg/dl)	170 ± 65	171 ± 65	NS
HbA_{1c} (%)	8.0 ± 1.6	8.1 ± 1.8	NS
Lp(a) (mg/dl)	12 ± 6	53 ± 16	< 0.01
Systolic blood pressure (mmHg)	135 ± 16	138 ± 17	NS
Diastolic blood pressure (mmHg)	79 ± 10	79 ± 10	NS
Total cholesterol (mg/dl)	205 ± 37	207 ± 41	NS
Triglyceride (mg/dl)	126 ± 87	134 ± 90	NS
HDL cholesterol (mg/dl)	58 ± 17	60 ± 19	NS
Apo A1 (mg/dl)	136 ± 28	139 ± 28	NS
Apo B (mg/dl)	109 ± 32	105 ± 29	NS
Cr (mg/dl)	0.9 ± 0.2	1.0 ± 0.5	NS
Uric Acid (mg/dl)	4.9 ± 1.6	5.1 ± 1.5	NS
Body Mass Index (m^2)	23.2 ± 4.2	23.5 ± 4.3	NS
Hypertension (%)	42.9	35.6	NS
Hyperlipidemia (%)	36.3	35.6	NS
Diabetic retinopathy (%)	29.8	66.7	< 0.01
Diabetic nephropathy (%)	31.4	52.9	< 0.05
Treatment of DM (Diet/OHA/Insulin)	39/52/10	13/13/4	NS
Ischemic heart disease (%)	16.0	26.7	< 0.05
Cerebral infarction (%)	22.0	17.8	NS

Abbreviation, HMW Lp(a) : high molecular weight Lp(a), LMW Lp(a) : low molecular weight Lp(a), OHA : Oral Hypoglycemic agent. Mean ± SD.

p<0.001). A gradual increase in Lp(a) levels was observed from R0 to R3.

Table 1 shows the clinical characteristics of the subjects. Serum Lp(a) levels in the LMW Lp(a) group were significantly greater than those in the HMW Lp(a) group. The prevalence of diabetic retinopathy, diabetic nephropathy, and ischemic heart disease was observed to be significantly greater in the LMW Lp(a) group than the HMW Lp(a) group. No significant differences were observed with regard to sex, age, duration of diabetes, fasting plasma glucose levels, HbA_{1C}, systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, HDL cholesterol, Apo A1, Apo B, creatinine, uric acid, BMI, hypertension, hyperlipidemia, or diabetic therapy among the two groups.

Table 2 shows the range and distribution pattern of apo(a) phenotypes and mean Lp(a) concentrations in the HMW Lp(a) and LMW Lp(a) groups.

Fig. 2 shows the percentage of LMW Lp(a) in relation to degree of diabetic retinopathy. The percentage of LMW Lp(a) was significantly greater in the R1, R2, and R3 groups, than in the R0 group (42.9% (p<0.001), 27.0% (p<0.01), and 27.3% (p<0.05) vs. 10.4%).

Table 3 shows the results of a multiple logistic regression analysis of the association between various parameters and the presence of diabetic retinopathy. Duration of diabetes and LMW Lp(a) were significant independent risk factors for diabetic retinopathy (p<0.001 and p<0.01, respectively). Sex, age, HbA_{1C}, diabetic nephropathy, hypertension and hyperlipidemia were not significant predictors in this model.

Discussion

In this cross-sectional study, there was a significant association between serum Lp(a) levels and severity of diabetic retinopathy. Increasing serum Lp(a) levels were observed with increasing severity of retinopathy (an increasing trend in Lp(a) levels from R0 to R3 was observed). Conflicting results have been reported with regard to serum Lp(a) concen-

Table 2 Distribution of apo(a) phenotypes and their Lp(a) range and mean concentration

Apo(a) phenotype	n	Frequency (%)	Mean Lp(a) concentration (mg/dl), (range)
HMW Lp(a) group			
O	6	2.4	3.2 (0.9～11)
S3	51	20.4	17.3 (0.7～33)
S4	55	22.0	11.5 (0.9～36)
S3+S3	6	2.4	31.2 (8～37.6)
S3+S4	57	22.8	22.3 (5～68)
S4+S4	30	12.0	12.4 (4～26.8)
LMW Lp(a) group			
S1	1	0.4	221
S2	5	2.0	30.1 (15.8～71)
B	1	0.4	43
F	1	0.4	85
S1+S3	3	1.2	64.3 (10～94.1)
S1+S4	2	0.8	93 (92～94)
S2+S3	9	3.6	51.3 (8～133.6)
S2+S4	20	8.0	44.9 (19～94.1)
F+B	1	0.4	85.7
B+S4	1	0.4	102
F+S4	1	0.4	54
Sum	250		

Abbreviation, HMW Lp(a) : high molecular weight Lp(a), LMW Lp(a) : low molecular weight Lp(a)

Table 3 Multiple logistic regression analysis of the association between various parameters and the presence of diabetic retinopathy

Variables	β	S.E	Chi-square	P
Sex	0.5197	0.5087	1.044	0.309
Age	0.0287	0.0229	1.568	0.213
Duration of diabetes	0.1191	0.0361	10.846	<0.001
HbA1c	0.2449	0.1478	2.743	0.100
Diabetic nephropathy	0.5277	0.3224	2.678	0.105
LMW Lp(a)	1.3178	0.5085	6.716	<0.01
Hypertension	0.5826	0.5014	1.350	0.248
Hyperlipidemia	0.1672	0.5421	0.095	0.758

Abbreviation, LMW Lp(a) : low molecular weight Lp(a)

trations in patients with diabetic retinopathy. Some studies have revealed that Lp(a) concentrations are elevated in type 2 diabetic patients with diabetic retinopathy. Onuma et al. have shown by multiple regression analysis that Lp(a) might be an independent risk factor for proliferative retinopathy in male patients with type 2 diabetes[15]. Morisaki et al. have reported that Lp(a) concentrations are significantly greater in diabetic patients with retinopathy than in those without. Moreover, multiple logistic regression analysis revealed high levels of Lp(a) as an independent risk factor for pre-proliferative retinopathy in the elderly diabetic patients examined[16]. Similar results have been reported for type 1 diabetic patients with severe retinopathy (pre-proliferative or proliferative retinopathy)[17,18]. Thus, the results of the present study are in agreement with those of previous studies. However, the serum Lp(a) levels of type 1 diabetic patients with retinopathy do not differ significantly from those of type 1 diabetic patients without retinopathy or those of normal subjects[22-24]. Boemi et al. have reported that Lp(a) levels are not associated with retinopathy in either type 1 or type 2 diabetic patients[25]. However, the type 1 and 2 diabetic patients examined in their study were evaluated together and the duration of diabetes within the two groups differed significantly.

These conflicting results seem to be a result of differences in the populations studied, as well as differences in the classification of diabetic retinopathy. Most previous studies that have reported little association between Lp(a) levels and diabetic retinopathy in type 1 diabetic patients have used relatively small

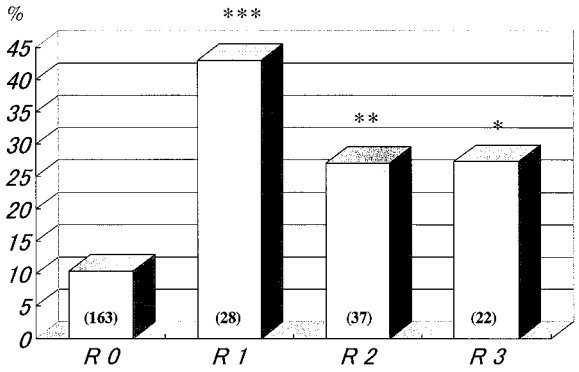

Fig. 2 The percentage distribution of low molecular weight (LMW) Lp (a) according to grade of diabetic retinopathy. The percentage of LMW Lp (a) in the R1, R2, and R3 groups was significantly higher than that of the R0 group. ( ); number of subjects.
*p<0.05, **p<0.01, ***p<0.001 vs. R0 group.

sample sizes. Type 1 and type 2 diabetes mellitus are due to different pathophysiological mechanisms and involve different genetic components. This might explain why Lp(a) seems to play a lesser role in the development of diabetic retinopathy in type 1 diabetic patients[26]. As mentioned, evaluation of the role of Lp(a) in diabetic retinopathy is further complicated by differences in the way diabetic retinopathy is classified in different studies. Some studies[17,18,22,24] have compared patients with or without proliferative retinopathy, while others[16,23,25] have studied patients with or without retinopathy. In a study by Onuma et al.[15], subjects were classified into three groups: patients without retinopathy, those with simple retinopathy, and those with proliferative retinopathy. In our study, patients were classified

as having one of 4 types of diabetic retinopathy: no retinopathy, simple retinopathy, pre-proliferative retinopathy, and proliferative retinopathy.

Furthermore, the conflicting results obtained in previous studies might be partially explained by variations in apo(a) phenotypes, which were not determined. Apo(a) phenotypes show a high degree of genetic polymorphism[27] and Lp(a) levels are inversely related to the molecular weight of their apo (a) phenotypes[28]. In this cross-sectional study, apo (a) phenotypes were classified into 7 subtypes, F, B, S1, S2, S3, S4, and O (Null) and patients were divided into two groups according to their phenotypes: low (S3, S4, O) and high (F, B, S1, S2) molecular weight Lp(a) groups. The percentage of LMW Lp(a) in the R1, R2, and R3 groups was significantly greater than that of the R0 group. In addition, multiple logistic regression analysis revealed that LMW Lp(a) is an independent risk factor for diabetic retinopathy. These results provide significant evidence that low molecular Lp(a) contributes to an increased risk of diabetic retinopathy. Previous studies that have assessed both Lp(a) levels and apo(a) phenotypes in relation to diabetic retinopathy have reported the following: high Lp(a) levels and apo(a) phenotypes of low relative molecular mass might be associated with severe retinopathy in both Type 1 and Type 2 diabetic patients[26,29]. The present results are in agreement with these studies and are the first to show this trend in elderly type 2 diabetic patients. In contrast, Maioli et al. have not found any difference in the Lp(a) levels or apo(a) phenotypes of patients with or without active retinopathy and they have concluded that apo (a) polymorphism is not an important genetic risk factor for severe diabetic retinopathy[30]. However, sample sizes were limited in both our own and in previous studies, so conclusive statements cannot be made with respect to the role of apo(a) polymorphism in diabetic retinopathy. Prospective studies involving large populations are needed in order to clarify the potential role of Lp(a) levels and apo(a) phenotypes as genetic markers for the development of diabetic retinopathy.

Although, there was a trend toward association between HbA_{1c} and diabetic nephropathy with diabetic retinopathy in our study, this trend did not reach statistical significance in multiple regression analysis ($\chi^2 = 2.743$, $p = 0.100$ and $\chi^2 = 2.678$, $p = 0.105$, respectively). There is strong evidence to support the belief that degree of glycemic control influences the development and progression of diabetic retinopathy [31]. Furthermore, a clinical relationship between diabetic retinopathy and diabetic nephropathy has been observed in that both run a parallel natural course and show interdependence with various treatment modalites[32]. Although, most studies appear to show that good long-term blood glucose control is associated with less diabetic retinopathy, no striking relationship has been found in cross-sectional studies, including the present study[31]. Urinary albumin to creatinine ratios obtained from morning spot urine samples were used to assess the degree of diabetic nephropathy in the present study. Several studies have shown a high degree of agreement between albumin excretion measured in this way and direct measurement of albumin excretion over a short timed collection period and with overnight or 24-h urine collection[33,34]. However, there is large intra-individual day-to-day variability in urine albumin excretion with a coefficient of variation of about 45%[35,36]. The relatively weak relationship observed between diabetic retinopathy and diabetic nephropathy in the present study may have been due to variations in levels of albuminuria. This may have caused patients to be misclassified with regard to degree of diabetic nephropathy.

In conclusion, this study suggests that Lp(a) phenotypes of low molecular weight are associated with the presence of diabetic retinopathy in the elderly.

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