Serum Lipoprotein(a) Level and Its Distribution in Healthy Japanese Male Subjects

Noboru YOSHIMINE*, Kanichi ASAI*, Fumio KUZUYA* and Hiroshi SHIMOKATA**

Introduction

Since the presence of lipoprotein(a) [Lp(a)] in the blood has been reported more than twenty years before, high serum level of Lp(a) has been suggested to be closely related to the development of arteriosclerotic diseases, especially ischemic coronary artery disease. However, a few literatures are available regarding the distribution of the serum Lp(a) level and relationship with sex, age, and other clinical parameters in the healthy Japanese population. Therefore, by using Lp(a) assay kit, we tried to clarify the normal range of serum Lp(a) level and correlation with various clinical parameters in the healthy Japanese male subjects.

Materials and Methods

Examination of a standard curve

We examined the linearity of a standard curve at 0, 1, 2, 3, 4, 6, 8, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mg/dl by using a Lp(a) assay kit.

Subjects

The subjects were recruited from the male workers of an automobile company located near Nagoya city, Japan. Fasting blood samples were taken from antecubital vein and separated immediately after collecting the blood samples. Subjects with the following findings were excluded from this study: Abnormal ECG finding. Hypertension, systolic pressure 160 mmHg or more and/or diastolic pressure 95 mmHg or more. Hyperglycemia, fasting blood glucose 120 mg/dl or more. Furthermore, those who had been admitted to hospital or under-

went an operation within the last one year were excluded from this study.

Measurement of serum Lp(a) and lipid levels

The Serum Lp(a) value was measured by an enzyme immunoassay (ELISA) method using a Tint-Elize Lp(a) kit of Biopool AB (Umeå, Sweden).

The serum levels of total cholesterol (TC), triglyceride (TG), and phospholipid (PL) were measured enzymatically. High density lipoprotein cholesterol (HDL-C) was measured by precipitation method and low density lipoprotein cholesterol (LDL-C) level was determined using Friedewald equation:

$$\text{LDL-C} = \text{TC} - \text{HDL-C} - \text{TG}/5.$$

Clinical parameters and their correlation with Lp(a) value

The following clinical parameters were assessed: age, sex, height, weight, blood pressure, ECG, serum total protein (TP), albumin, urea nitrogen (BUN), creatinine (CR), uric acid (BUA), sodium, potassium, chloride, calcium (Ca), aspartate- and alanin-transaminase (GOT, GPT), alkaline phosphate, total and direct bilirubin (T- and D-Bil), fibrinogen (Fib), fasting blood glucose, antithrombin III (AT III), white and red blood cell counts (RBC, WBC), hemoglobin (Hb), erythrocyte sedimentation rate. The clinical parameters but age and sex were examined routinely in our laboratory. The correlation of Lp(a) level with other clinical parameters was investigated by Pearson's correlation coefficients and SAS REG procedure¹⁾.

Results

Examination of a standard curve

The standard curve of Lp(a) assay kit showed the linearity from 0 mg/dl to 60 mg/dl. However, the curve did not show the linearity between 0 and 3 mg/dl and over 70 mg/dl of the Lp(a) value. Therefore, serum specimens over 60 mg/dl were measured by diluting two-fold. Five serum samples were assayed in triplicate, with intraassay CVs of 3.3% in 10 mg/dl

* Department of Geriatrics School of Medicine, Nagoya University

** Department of Epidemiology and Social Medicine, Research Institute for Nuclear Medicine and Biology, Hiroshima University

Received: October 21, 1991

Accepted: January 8, 1992

Table 1 Levels of serum lipoprotein(a) and lipids

Age	Lp(a) (mg/dl)	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)
20-29 (164)	15.4±14.0 ^a	173.7±29.3	99.2±72.3	55.1±11.2
30-39 (110)	16.5±16.8	189.2±28.9**	147.1±12.9*	51.6±14.5
40-49 (367)	19.3±16.8	202.6±41.2**	109.6±69.8	53.1±13.7
50-59 (190)	20.5±17.7*	208.3±37.9**	113.3±82.1	52.8±14.7

^a: Mean±SD, *: p<0.05, **: p<0.01

Parentheses show number of subjects.

Fig. 1 Frequency distribution of serum lipoprotein(a).**Table 2** Levels of serum lipoprotein(a) in percentiles (N=831)

Percentile	Lp(a)
0	0 mg/dl
5	2.1
10	3.4
25	7.1
50	14.2
75	24.7
90	39.9
95	51.5
100	97.6

and 2.8% in 40 mg/dl found respectively. It is suggested that the reliability of ELISA method is 3 mg/dl or more in this study.

Subjects

Total number of subjects were 831 Japanese men: 164 men aged 20 to 29 years, 110 aged 30 to 39 years, 367 aged 40 to 49 years, and 190 aged 50 to 59 years. All subjects were healthy without any obvious diseases.

Serum levels of Lp(a) and lipids

The mean levels of Lp(a) and lipids of these groups

are shown in Table 1. The distribution frequency of Lp(a) was skewed towards the lower value and did not show the regular pattern (Fig. 1). Because of the skewed pattern of Lp(a), the percentile range was adopted for determining the normal range of Lp(a) in the healthy subjects (Table 2). As shown in Table 2, median value was 14.2 mg/dl and 95th, 90th, and 10th percentile values were 51.5, 39.9, and 3.4 mg/dl, respectively. The mean value of Lp(a) in all subjects was 18.3 ± 16.6 mg/dl (mean±SD) and increased with age, and showed the significant higher level in the subjects of the fifties than those of the twenties (Table 1). The cumulative frequency curve also shifted toward the higher value in the older subjects (Fig. 2). TC level increased significantly with age, while TG and HDL-C levels did not change.

Clinical parameters and their correlation with Lp(a) value

Pearson correlation coefficients showed a positive correlation between Lp(a) and TC or LDL-C levels, and a negative correlation between Lp(a) and TG levels. Moreover, the Lp(a) level correlated with other items listed in Table 3. From the results of the multivariate analysis in Table 3, however, the Lp(a) level showed a significant positive correlation with TC and LDL-C levels, while no correlation was shown with

Fig. 2 Cumulative frequency curve of lipoprotein(a) in each age: the twenties (—), the thirties (.....), the forties (---), and the fifties (— · — ·).

Table 3 Multivariate correlation between serum lipoprotein(a) and other clinical parameters

AGE	0.228	Cr	1.904
SBP	0.722	BUA	-1.947
DBP	0.396	GOT	0.346
TC	2.524**	GPT	-0.824
LDL-C	2.524**	T-Bil	0.645
HDL-C	0.733	D-Bil	-0.960
TG	-0.249	Fib	1.097
PL	-0.277	RBC	0.083
Ca	-1.909	Hb	-0.952
BUN	-0.628	Tp	-0.715

** : $p < 0.01$

other parameters, including age and blood pressure.

Discussion

Lp(a) is a lipoprotein which closely resembles LDL in lipid composition and its existence in blood was first reported by Berg in 1963²⁾. It is distinguished by the presence of apolipoprotein(a) bound to the apolipoprotein B-100 by disulfide linkage³⁾. It is evident from recent works that a high Lp(a) level is a significant and independent risk factor for ischemic coronary heart disease and cerebrovascular disease⁴⁻⁹⁾. However, little data are available on the normal range and distribution of the Lp(a) level in healthy Japanese adults. Therefore, we assessed the serum Lp(a) level in them with the intention of tentatively setting up the normal range. We also investigated the relationship of the Lp(a) level to other clinical parameters including age. Frequency distribution of Lp(a) showed a

skewed pattern in the American white population, while that of the black population showed a bell pattern¹⁰⁾. Frequency distribution of Lp(a) in this study showed a skewed pattern toward the lower value, which is similar to that of the white population, including Hawaiian of Japanese descent^{7,11)}. Accordingly, we concluded that no difference of the Lp(a) frequency distribution existed between Japanese and the American white adults. The mean value of Lp(a) in this study was 18.3 ± 16.6 mg/dl (mean $\pm$ SD), a similar result to those of other reports^{7,12)}. It is important for us to assess the normal upper range of the serum Lp(a) value because high serum level might be a risk factor for arteriosclerotic vascular diseases. Dahlen reported the high mortality and morbidity of patients with the high value (>48 mg/dl) of the serum Lp(a)¹³⁾. Abe et al. investigated the distribution patterns of serum Lp(a) value of both healthy subjects and patients with ischemic coronary heart diseases and observed the similar patterns skewed to the lower level. However, the higher level of the serum Lp(a) (>40 mg/dl) was observed more in patients with ischemic heart diseases than in healthy subjects¹²⁾. In addition to these reports, we would suggest that it might be suitable to make normal range slightly wide when the patterns between healthy subjects and the diseased are distributed similarly. Namely, by using the wide range of the serum Lp(a) level as a normal one, we should assess the critical range for chronic diseases such as arteriosclerosis, diabetes mellitus, cerebrovascular diseases, hypertension, and others in the future investigation.

From the distribution pattern and percentile value of this study, we would like to consider 40 mg/dl as a normal upper limit in the healthy males tentatively. An increase of the Lp(a) level was observed with age, as was the case with TC and LDL-C levels by Pearson's correlation coefficient. However, no correlation was shown between the Lp(a) level and age or other clinical parameters by multivariate analysis, while a correlation of the Lp(a) level with TC and LDL-C levels remains significant. This may be explained as follows: The influence of age on Lp(a) level is thought to be weaker than that of TC or LDL-C and consequently effect of age on Lp(a) might be masked by multivariate analysis. A significant correlation of Lp(a) level with TC or LDL-C level has not been reported in literatures from other countries. Therefore, further investigation is required to confirm whether there are any differences between the western and Japanese populations. On the basis of these results, we are convinced that the finding of the present study supports the possibility of a significant, though not strong, correlation between the Lp(a) level and TC or LDL-C level. Moreover, since the Lp(a) level correlated with TC and LDL-C levels significantly, it might be a strong risk factor for arteriosclerotic diseases by itself or together with hyperlipidemia. By using the normal range set in this study, longitudinal follow-up study for arteriosclerotic diseases is needed in order to utilize the Lp(a) level as an index to the prevention and management of these diseases.

References

- 1) SAS User's Guide: Statistics, Release 6.03 ed. pp. 723-875, SAS Institute, Carry, NC, U.S.A. (1988).
- 2) Berg, K.: A new serum type system in man—The Lp system. *Acta Path. Microbiol. Scand.*, **59**: 369-382 (1963).
- 3) Scanu, A. M.: Lipoprotein(a)—A potential bridge between the fields of atherosclerosis and thrombosis. *Arch. Pathol. Lab. Med.*, **112**: 1045-1047 (1988).
- 4) Kostner, G. M., Avogaro, P., Cazzolato, G., Marth, E., Bittolo, G. and Quinci, G. B.: Lipoprotein Lp(a) and the risk for myocardial infarction. *Atherosclerosis*, **38**: 51-61 (1981).
- 5) Murai, A., Miyahara, T., Fujimoto, N., Matsuda, M. and Kameyama, M.: Lp(a) lipoprotein as a risk factor for coronary heart disease and cerebral infarction. *Atherosclerosis*, **59**: 199-204 (1986).
- 6) Armstrong, V. W., Cremer, P., Eberle, E., Manke, A., Schulze, F., Wieland, H., Kreuzer, H. and Seidel, D.: The association between serum Lp(a) concentration and angiographically assessed coronary atherosclerosis-dependence on serum LDL levels. *Atherosclerosis*, **62**: 249-257 (1986).
- 7) Rhoads, G. G., Dahlen, G., Berg, K., Morton, N. E. and Dannenberg, A. L.: Lp(a) lipoproteinemia as a risk factor for myocardial infarction. *J.A.M.A.*, **256**: 2540-2544 (1986).
- 8) Rosengren, A., Wilhelmsen, L., Eriksson, E., Risberg, B. and Wedel, H.: Lipoprotein(a) and coronary heart disease: a prospective case-control in a general population sample of middle aged men. *Brit. Med. J.*, **301**: 1248-1251 (1990).
- 9) Hearn, J. A., DeMaio, S. J., Jr., Roubin, G. S., Hammarstrom, M. and Sgoutas, D.: Predictive Value of Lipoprotein(a) and Other Serum Lipoprotein in the Angiographic Diagnosis of Coronary Artery Disease. *Am. J. Cardiol.*, **66**: 1176-1180 (1990).
- 10) Guyton, J. R., Dahlen, G. H., Pasch, W., Kautz, J. A. and Gotto, A. M., Jr.: Relationship of plasma lipoprotein Lp(a) levels to race and to apolipoprotein B. *Arteriosclerosis*, **5**: 265-272 (1985).
- 11) Braendstrom, A., Johnson, O., Dahlen, G. and Ranby, M.: Lp(a) levels in a healthy population measured by a new enzyme linked immunisorbent assay. *Thrombosis and Haemostasis*, **26**: 27-36 (1989).
- 12) Abe, A., Noma, A., Lee, Y. and Yamaguchi, H.: Lp(a) glycoprotein phenotypes in healthy subjects and in patients with angiographically defined coronary artery diseases. *Atherosclerosis* (in press).
- 13) Dahlen, G. H.: Lipoprotein(a) in relation to atherosclerotic diseases. *Recent Aspects of Diagnosis and Treatment of Lipoprotein Disorders*. ed. by Widhalm, K., Naito, H. K., Alan R Liss Inc., New York, p. 27 (1988).

Summary

The serum Lp(a) level was assessed in 831 healthy Japanese males to clarify its distribution and normal range, as well as to analyze the relationship of Lp(a) taking into account age, serum lipids, and other clinical parameters. The mean serum Lp(a) level ($\pm$ SD) was 18.3 ± 16.6 mg/dl, while the frequency distribution was skewed to the lower value. The median, the 90th and the 95th percentile values were 14.2, 39.9 and 51.5 mg/dl, respectively. On the basis of these results, we have set the upper limit of the normal range for Lp(a) at 40 mg/dl tentatively. Multivariate analysis demonstrated a positive cor-

relation of the Lp(a) level with TC and LDL-C levels, while no correlation was found between the serum Lp(a) level and age or the other clinical parameters investigated in this study. Although Lp(a) is considered to be an independent risk factor for arteriosclerotic diseases, we obtained a significant correlation of the Lp(a) level and the TC and LDL-C levels simultaneously. Lp(a) may therefore play an important role by itself or together with hyperlipidemia in the acceleration of arteriosclerotic diseases.

Key words: Lipoprotein(a), total cholesterol, LDL cholesterol.