

# THE LIPOPROTEIN PROFILE OF YOUNG ADULTS WITH CEREBRAL ATHEROSCLEROSIS

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**Abstract.** In order to elucidate the relationship between the lipoprotein profile and large cerebral artery atherosclerosis in the young adults living in developing Asian countries, the serum lipoprotein profile and the luminal diameter of large cerebral arteries (internal carotid, middle/anterior cerebral and vertebrobasilar arteries) were measured and correlated in 67 young Taiwanese with non-embolic cerebral infarct (CI). Totally 23 (21.9%) patients had atherosclerotic stenosis. A significant elevation of the serum total cholesterol (TC), triglyceride, total lipids,  $\beta$ -lipoprotein ( $\beta$ -LP) and pre- $\beta$ -LP level was found in atherosclerotic CI patients. But multiple regression analysis showed only the serum  $\beta$ -LP ( $p = 0.0041$ ) and TC ( $p = 0.0324$ ) level to be the independent risk factors for atherosclerosis. Secondary dyslipoproteinemia is the main cause for hyperlipoproteinemia in our atherosclerotic patients. Therefore, an abnormal lipoprotein profile is linked to large cerebral artery atherosclerosis in young Asians regardless of ethnic group. A tailored program is recommended to modify the life style and dietary habit, as well as to gain access to secondary control for large cerebral atherosclerosis prevention in developing countries.

## INTRODUCTION

Cerebral infarct (CI) is a worldwide problem with no exception in developing countries. In Taiwan, stroke has been the second most common fatal disease in the past 10 years (Hung, 1993). A rapid and considerable increase of incidence and mortality rate in stroke is also reported by the Public Health Services in Thailand (Viriyavejakul, 1990). In contrast to an earlier view, CI in young adults is actually not uncommon. Experience from the Lausanne Stroke Registry shows that 12.3% of first-ever CI patients are younger than 45 years (Bogousslavsky *et al*, 1988). A collaborative multicenter study in Taipei also revealed about 10% out of 2,289 Taiwanese CI patients to be under 50 years (Hung *et al*, 1988). In our collection of 2,940 patients with cerebral infarct from 1982 to 1993 year, 211 (7.18%) out of them was between 18 to 45 years (personal data).

Large cerebral artery atherosclerosis (CAS) associated with an abnormal lipoprotein (LP) profile is a common risk factor for young CI caucasians (Adams *et al*, 1986; Bevan *et al*, 1990; Lisovski

and Rousseaux, 1991; Smoker *et al*, 1987). However, the role of LP for cerebral atherosclerosis in CI is unclear in young Asians, probably due to a serious shortage of relevant research. In a series of 143 young Indians with stroke, CAS was present in 71% of the group (Jayakumar *et al*, 1991). Therefore, CAS may be an important factor in CI in young Asians which was previously underestimated. As the public health and socioeconomic status are improving in Southeast Asia, a great change of the life style and dietary habit may increase the possibility of CAS in young Asians. It is expected that CAS and CI will become a serious future medical problem in developing Asia. Accordingly, it is necessary to elucidate the inter-relationship between LP and CAS in young Asians in order to establish a schedule for preventing CAS.

## MATERIALS AND METHODS

### Subjects

From 1986 to 1992, individuals aged from 20 to 45 years admitted to Kaohsiung Medical College Hospital (KMCH) on account of cerebral infarct were enrolled. The exclusion criteria were: (1) evidence of a cardioembolic or systemic embolic source, infectious arteritis, a positive lupus test,

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brain tumor or abscess, dysmetabolic, cancerous or mitochondrial encephalopathy; (2) a history of recent head or neck injury; (3) usage of herbal remedy, estrogen, androgen, or corticosteroid; (4) a previous recurrent optic neuritis or unexplained focal neurological deficit with spontaneous recovery; (5) an abnormal renal (serum creatinine > 1.4 mg/dl) or hepatic (SGOT > 40 IU/l, SGPT > 40 IU/l, serum albumin < 3.8 gm/dl) function; (6) an abnormal thyroid or adrenal function; (7) affirmed venous thrombosis, vascular malformations or specific vasculopathy (fibromuscular dysplasia, Moyamoya disease, Takayasa's arteritis, arterial dissection); or (8) an incomplete or absence of cerebral angiography study.

A total of 67 individuals were eligible in this study, consisting of 44 men and 23 women with age ranged from 20 to 45 years. Associated cardiovascular risk factors are listed in Table 1.

For comparison, we selected two age and sex-matched controls for each CI patient. These 134 controls were all in a good physical condition. None of them suffered from focal neurological deficit, unexplained optic deficit, recent head or neck injury, or any familial disease. They had normal hemograms, biochemistry and diurnal cortisol levels, as well as a normal thyroid, renal and hepatic function. All of them were negative on the lupus test. The electrocardiogram, cardiac echogram, and extracranial carotid dopscan were all normal.

Table 1

The associated risk factors in 67 CI patients.

Risk factors	Frequency (%)
Absence of risk factor	16 (28.2)
Presence of risk factor	51 (71.9)
cigarette smoking	27 (40.3)
hypercholesterolemia	26 (38.8)
hypertriglyceridemia	17 (25.4)
alcoholism	15 (22.4)
hypertension	13 (19.4)
hyperviscosity	12 (17.9)
diabetes mellitus	10 (14.9)
hyperfibrinogenemia	10 (14.9)
hyperuricemia	8 (11.9)
hemoconcentration	8 (11.9)

### Blood sample collection and laboratory study

After an overnight fast, the patient remained supine and blood was collected from the antecubital vein. Blood for lipoprotein electrophoresis and total lipids analysis was centrifugated and the serum was stored at -20°C and subjected to batch measurement within 72 hours. Blood samples for total cholesterol (TC), esterified cholesterol (EC), triglyceride (TG), uric acid, total protein and albumin were processed for analysis within 2 hours by the Beckman ASTRA autoanalyser. Total lipid was measured by colorimetric method under 540 nm wavelength.

### Lipoprotein electrophoresis

Five µl serum was applied into each well of a Titan III-Lipo cellulose acetate plate in Tris-barbital-sodium barbital buffer (pH 8.8) and electrophoresis was run. After electrophoresis, lipoproteins were stained with oil red O, then observed and scanned under 525 nm for quantitation. The level of  $\alpha$ ,  $\beta$  and pre- $\beta$ -lipoprotein was determined by multiplying the ratio of each individual with the value of total lipids.

### Cerebral angiography

Biplanar cerebral angiography via the transfemoral approach was done in the study subjects for evaluation the status of the cerebral artery. The internal carotid (ICA), anterior cerebral (ACA), middle cerebral (MCA) and vertebrobasilar arteries (VBAs) were observed and evaluated by a neuro-radiologist (Dr MS Chou) who did not know the clinical and biochemical data. The selection of the portion of each artery was: (1) from the level above the carotid bulb to the MCA and ACA stem in ICA; (2) A1 (horizontal part) in ACA; (3) M1 (sphenoid part) in MCA; (4) vertebral artery segment above atlas (V4) to the bifurcation of the posterior cerebral artery in vertebrobasilar artery (VBA). The luminal diameter was measured by Alter's method (Alter *et al*, 1972). In brief, the luminal width at the narrowest point (A) to the beginning of the stenosis (B) was measured. The degree of stenosis was calculated as  $(B - A)/B$  and expressed as a percentage. In this study, luminal stenosis less than 25% in the ICA, and 35% in the ACA, MCA or VBAs was regarded as insignificant.

### Statistical analysis

Student's *t*-test and multiple variable regression were used in this study. Statistical significance was recorded when the *p* value was < 0.05.

### RESULTS

A total of 51 patients (71.9%) had an associated cardiovascular risk factors in our 67 patients. The most common factor was smoking habit (40.3%), followed by hypercholesterolemia (38.8%), hypertriglyceridemia (25.4%), alcoholism (22.4%), hypertension (19.4%), hyperviscosity (17.9%), diabetes mellitus (14.9%) and hyperfibrinogenemia (14.9%) (Table 1).

A total of 23 (34.3%) patients, 14 men and 9 women, were identified as having atherosclerotic (AST) stenosis of either ICA, ACA, MCA or VBAs by the angiographic criteria.

In CI patients, a significant elevation of serum TC, EC, TG, TL,  $\beta$ -LP, pre- $\beta$ -LP and non- $\alpha$ -LP/TL

level was found (Table 2), suggesting that abnormal lipoprotein profiles are closely related to young CI adults. When these data were further analysed according to the presence of atherosclerosis, all of them were significantly higher in AST than non-atherosclerotic (NAT) subjects or control groups. To minimize the influence of interdependence among these factors, multiple variables regression test was done in 67 CI patients. Our result showed  $\beta$ -LP (*p* = 0.0041) and TC (*p* = 0.0324) to be the two independent lipidic factors for atherosclerosis in our series (Table 3). Otherwise, diabetes mellitus, mean arterial blood pressure, uric acid, total proteins, albumin, whole blood viscosity, hematocrit, hemoglobin, smoking, alcoholism sex and age showed insignificant relationship to atherosclerosis on multiple variable regression.

Totally 60.7% AST, 31.8% NAT and 7.0% controls had a serum TC level > 200 mg/dl. An association of secondary was found in all of hypercholesterolemic AST and 66.7% of normocholesterolemic AST patients (Fig 1). In the hypercholesterolemic AST patients, hemoconcentration (38.3%) was the chief secondary association, fol-

Table 2  
The serum lipidic profile in CI patients and controls.

Lipid profile	CI patients			
	AST (n = 23)	NAT (n = 44)	Total (n = 67)	Control (n = 134)
TC (mg/dl)	216.6 ± 11.5 <sup>***</sup>	186.8 ± 6.63	197.0 ± 5.73 <sup>3</sup>	170.2 ± 2.87 <sup>c</sup>
EC (mg/dl)	165.1 ± 8.21 <sup>***</sup>	138.4 ± 4.64	147.7 ± 4.91 <sup>3</sup>	124.4 ± 2.69 <sup>c</sup>
TC/EC	1.31 ± 0.02 <sup>***</sup>	1.36 ± 0.01	1.34 ± 0.02	1.36 ± 0.008 <sup>c</sup>
TG (mg/dl)	231.1 ± 49.9 <sup>***</sup>	127.7 ± 11.3	202.3 ± 45.0	148.7 ± 8.85 <sup>c</sup>
TL (mg/dl)	867.3 ± 71.0 <sup>***</sup>	606.1 ± 25.9	700.8 ± 33.6 <sup>3</sup>	596.8 ± 24.3 <sup>c</sup>
$\beta$ -LP (mg/dl)	470.7 ± 34.5 <sup>***</sup>	325.5 ± 20.6	378.8 ± 19.6 <sup>3</sup>	294.1 ± 10.4 <sup>c</sup>
pre- $\beta$ -LP (mg/dl)	261.5 ± 15.0 <sup>***</sup>	128.5 ± 17.9	177.2 ± 28.7 <sup>3</sup>	101.6 ± 4.14 <sup>c</sup>
$\alpha$ -LP (mg/dl)	136.0 ± 71.9	152.6 ± 6.63	150.2 ± 4.91 <sup>3</sup>	194.8 ± 4.34 <sup>c</sup>
$\alpha$ -LP/TL (%)	18.8 ± 1.92 <sup>***</sup>	26.9 ± 1.33	23.9 ± 1.64 <sup>3</sup>	33.4 ± 1.03 <sup>c</sup>
$\beta$ -LP/TL (%)	57.2 ± 3.84	53.9 ± 2.65	55.1 ± 1.64 <sup>3</sup>	49.8 ± 1.18 <sup>c</sup>
non- $\alpha$ -LP/TL (%)	81.2 ± 1.92 <sup>***</sup>	73.1 ± 1.33	76.1 ± 1.64 <sup>3</sup>	66.6 ± 1.03 <sup>c</sup>

The data are presented as mean ± SEM.

TC: total cholesterol; EC: esterified cholesterol; TG: triglyceride; TL: total lipids;  $\beta$ -LP:  $\beta$ -lipoprotein;  $\alpha$ -LP:  $\alpha$ -lipoprotein. AST: atherosclerosis; NAT: non-atherosclerosis

AST vs NAT:<sup>\*\*\*</sup>, *p* < 0.01

CI vs control:<sup>3</sup>, *p* < 0.01

AST vs control:<sup>c</sup>, *p* < 0.01

Table 3  
The multiple variables regression test.

Variables	$\beta$ Coefficient	Probability
$\beta$ lipoprotein	0.049417	0.0041
total cholesterol	0.123479	0.0324
whole blood viscosity	7.503011	0.1081
total lipids	0.018100	0.1110
MABP	0.301611	0.1940
age	0.318724	0.3114
alcoholism	6.359306	0.3439
smoking habit	-4.284045	> 0.4
uric acid	1.279677	> 0.4
triglyceride	0.012162	> 0.4
$\alpha$ lipoprotein	-0.044137	> 0.4
albumin	-3.022417	> 0.4
hemoconcentration	-2.402766	> 0.4
pre- $\beta$ lipoprotein	-0.002145	> 0.4
total proteins	-0.713570	> 0.4
sex	-0.238484	> 0.4

lowed by hyperfibrinogenemia (24.5%), hyperviscosity (23.0%), hypertriglyceridemia (17.6%) and smoking (11.9%). On the other hand, smoking (11.1%), alcoholism (11.1%) and hypertension (11.1%) constituted nearly half of all secondary associations in normocholesterolemic AST patients.

In NAT patients, secondary was present in 71.4% of hypercholesterolemic and 53.3% of normocholesterolemic patients. Smoking, hyperviscosity and alcoholism were the common associated ones among them. Therefore, it is clear that secondary is an important cause for hypercholesterolemia and CAS in young Asians living in developing countries.

DISCUSSION

Despite of a mounting evidence for association between hyperlipoproteinemia and large cerebral artery atherosclerosis in industrial countries (Holme *et al*, 1981; Teller *et al*, 1988), a serious shortage of large scale studies, as well as a dim belief of a distinct CAS pattern in Orientals, makes the role of LP in cerebral atherogenesis very obscure in Asians. Although several elegant epidemiologic studies in Asian developing countries support an elevation of serum cholesterol (Hu *et al*, 1988. The Korean Neurological Association, 1993) or triglyceride level (Liu *et al*, 1990) in relation to CI, however, they have been unable to characterize the role of LP in CAS, especially in the young population.

Aging is an important factor for CAS. The serum LP level and peroxidated products increase

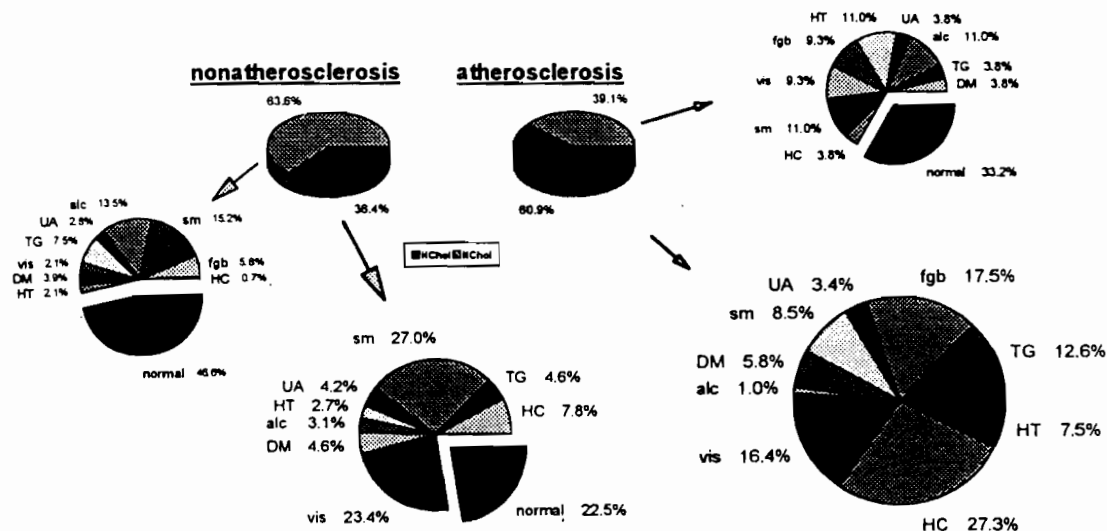


Fig 1- The frequency and etiology of secondary in the 67 cerebral infarct patients. Abbrev. alc: alcoholism; DM: diabetes; fgb: hyperfibrinogenemia; HT: hypertension; SM: smoking; TG: hypertriglyceride mia; UA: hyperuricemia; vis: hyperviscosity; normal: no evitable secondary.

in parallel with age. The LP is thus proposed to exert less relevant effect on CAS in young adults. In our patients, CAS was significantly associated with an increase of serum  $\beta$ -LP and cholesterol levels, suggesting that an excessive cholesterol level is related to CAS in the young population. It is likely that age is not an absolutely crucial factor for the cholesterogenic effect in the process of cerebral atherogenesis.

Increasingly, data suggest an ethnic difference in the pattern of cerebral atherosclerosis. A predominance of intracranial atherosclerosis is observed in Asians. The Honolulu Heart Program is elaborately designed to investigate the cardiovascular risk factors for the Japanese descendants living in Hawaii. These data provide excellent information for understanding life style dietary habit, and ethnicity in association with cerebral atherosclerosis. The relative risk of cholesterol is 1.4 for thromboembolic stroke (Benfante *et al*, 1994). Only Hawaiian Japanese descendants consuming Asian diets showed similar intracranial arterial lesions to Japanese in Japan (Reed *et al*, 1994), implying that environmental factors modify the patterns of CAS, rather than ethnic background. In addition, the serum cholesterol level is remarkably correlated to the severity of atherosclerosis at the large cerebral artery (Reed *et al*, 1988). These results strongly suggest an ambient atherogenicity of cholesterol to large cerebral artery regardless of ethnic group.

Since Japan is a developed and Asian-diet consuming Asian country, the investigation of cholesterol and CAS is a good reference for the interrelationship amongst socio-economic status, cholesterol and CAS in Asians. A national study of atherosclerosis in Japan from autopsied adolescents showed that cholesterol was significantly correlated with the extent and severity of CAS while TG showed no significant correlation (Tanaka *et al*, 1988). These data are similar to those in developed industrial countries, implying that excessive cholesterol may cause CAS with no racial or age difference. Our result also supports a close correlation between cholesterol and CAS in young adults in a developing Asian country. Therefore, it is suggested that in young Asians an increase of serum cholesterol level carries an increased risk for CAS which is independent of age, ethnicity as well as socio-economic development.

Traditional Asian diets that contain low animal

fat and protein, as well as hard labor, is considered to protect individuals from CAS and coronary atherosclerosis. However, poor chronic disease control, smoking and alcoholism, which in turn cause an elevation of serum cholesterol level, are very popular in eastern developing societies. In our series, all atherosclerotic and over three-fourths of non-atherosclerotic patients had secondary associations. These are speculated to be important confounding factors for hypercholesterolemia in young adults. Therefore, a program in public health education to recognize disease control and the risk of smoking and alcoholism is an urgent requirement to prevent CAS and hypercholesterolemia in the young population in the immediate future.

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