Original Article

LDL Subtypes and Its Association With Stroke A Case-Control Study

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Abstract

Cardiovascular disease (CVD) or stroke is one of the most common cause of mortality and long term severe disability. Hyper cholesterolemia facilitate atherosclerosis of cerebral arteries leading to ischaemic stroke. Atherosclerotic vascular disease is directly related to plasma cholesterol level. Traditionally estimation of total serum cholesterol and LDL-c are used as an indicator of atherogenicity. But subject may develop stroke with normal level of LDL cholesterol. So assessment of LDL-cholesterol concentration may not entirely reflect its atherogenic potential. Because LDL-c is not a single entity rather it consist of seven distinct subclasses of different particle size. The size of LDL particle is inversely correlated to their atherogenecity. Smaller LDL particles are consider to have more atherogenic profile than those with larger particles despite of identical total cholesterol levels. Therefore individual having smaller LDL particles are more atherogenic and more at risk to develop stroke even with normal LDL cholesterol concentration. So measurement of small dense LDL particle is more important than any other lipid measure. With this aim 91 stroke patients were selected as case and 40 were healthy control. The mean age of cases and controls were 49.60 ±12.88 years, 40.28 ±11.88 years respectively. Serum apo-B was measured in all study subject. The amount of apo-B is almost similar in every LDL subtype but the amount of cholesterol increases with the increasing particle size. So the ratio of cholesterol to apo-B decreases as the particles size decreases, thus LDL cholesterol /apo-B < 1 indicate the presence of atherogenic small dense LDL. So the prevalence of small dense LDL was evaluated by calculating the ratio of LDL-C/apo-B. The ratio was significantly lower in normolipidaemic stroke cases (0.73+0.23) compared to controls as well as dyslipidaemic stroke cases, indicating the presence of sd LDL in normolipidaemic stroke case. So it can be concluded that sd LDL is isolatedly associated with stroke in normolipidaemic individuals and it is independent of conventional lipid measure.

Key words: Small dense LDL, stroke, apo-B, Lipid profile.

Introduction

Stroke is one of the most common causes of mortality and long-term severe disability. Previous studies indicated that, risk factors for stroke include advanced age, male gender, hypertension, smoking. The current stroke guidelines recommended the assessment of other risk factors in individuals with stroke for further management.[1]

Approximately 80% strokes are ischaemic in nature. Many of these are due to embolization from either the heart (eg. in atrial fibrillation) or from the carotid artery. It is well recognised that carotid artery stenosis is a risk factor for ischemic stroke.[2]

In a prospective study serum total cholesterol was found to be positively associated with ischaemic Stroke rather then haemorrhagic Stroke.[3]

Lipid and Lipoprotein disorders are important metabolic risk factors of atherosclerosis. Another study also demonstrated that not only serum lipid concentration but also the distribution of triglyceride and cholesterol within the major lipoprotein classes are of importance for the development of atherosclerosis.[4]

Elevated LDL-C concentration has been established as a modifiable risk factor for cardiovascular diseases.[5] Dyslipidaemia may play an important role in initiation and progression of atherosclerosis.[6] Clinical practice has traditionally been based on the concentration of cholesterol and triglyceride in plasma as well as on that

of cholesterol contained in low density and high density lipoprotein particles. Among them, LDL-C has been declared to be the most important.[7]

Persons may develop stroke and CHD with normal level of LDL-C and assessment of LDL-C concentration may not entirely reflect its atherogenic potential[8]. Because the total number of LDL particle is more important determinant of risk of vascular disease than any other lipid measures; so, to asses accurately the risk from LDL, one needs to know not only the amount of LDL-C but also the number and size of the LDL particles.[7]

LDL consists of at least seven distinct subclasses of different particle size. One of these subclasses, called sd LDL is associated with carotid atherosclerosis and may be a modifiable risk factor for stroke as well as IHD.[9]

The size of LDL particle is inversely related to its atherogenecity. Patients with predominantly small LDL particles are considered to have more atherogenic profile than those with larger particles, despite of identical total cholesterol levels, Smaller and denser subtypes are lipid depleted particles (less cholesterol containing) but more atherogenic than the larger and more buoyant particles which are relatively lipid rich.[10]

All LDL subtypes and other apo-B containing lipoproteins contain identical and equal apo-B content irrespective of their varied cholesterol content. So, it is the apo-B concentration rather than LDL-C concentration that is more reliable measure of LDL particle number. Therefore LDL cholesterol is an inadequate measure of LDL atherogenecity.[11] Apo-B moiety of LDL is particularly important for its atherogenecity because lipoproteins without apo-B apparently do not produce atherosclerosis.[12]

Apo-B measurement provides a direct estimate of the total number of LDL particles, irrespective of their subtypes. So, only the LDL-C measurement is a poor reflection of its atherogenicity; rather apo-B reflects a complete and comprehensive picture of atherogenic risk.[13]

The amount of apo-B is almost similar in different LDL subtypes but the amount of cholesterol increases with the increasing particle size. So, the ratio of cholesterol to apo-B decreases as the particle size decreases, thus LDL -C / apo-B <1 indicates the presence of atherogenic sd LDL in stroke patients.[14]

Small dense LDL particles are also associated with increased VLDL, TG and apo-B levels and decreased HDL cholesterol[15]. Patient with predominantly smaller LDL particles are considered to have more atherogenic profile than those with larger particles despite of identical total cholesterol level. So, sd LDL is the most common profile in patient with vascular disease and reduction in sd LDL is clearly associated with clinical benefit.[14]

Material and Methods

The case control study was conducted in the department of Bio-Chemistry, BSMMU, Dhaka, Bangladesh during the period from Jan'08 to Jan'09. In this study our aim was to see preponderance of sdLDL in stroke subject.

With this aim 91 diagnosed stroke subject of both sex and 41 age and sex match healthy controls were enrolled. All the study subjects were non diabetic and subject having hepatic failure, renal failure, thyroid disorder were excluded. Patient taking ocp, HRT and lipid lowering drugs were also excluded. On the basis of conventional lipid profile the cases were sub divided into two group as normolipidaemic and dislipidaemic. Dyslipidaemia was defind as total cholesterol > 150 mg/dl, Tg>150mg/dl, LDL cholesterol > 100mg/dl and <40mg/dl in men, 50 mg/dl in women. Apo-B was measured in all study subject. LDL-C/apo-B<1indicate presence of atherogenic sdLDL in stroke patient. Chi-square test was done to compare sdLDL in between and within group.

Result and Observation:

During the period of Jan'08 to Jan'09 a total of 131 subjects of both sexes were selected for the study, of which 91 stoke subjects and 40 healthy controls. The mean age of our cases and control were 49.60 ±12.88 and 40.28 ±11.88 years respectively.

Among case 64 were male and 27 were female. In control group 22 were male and 18 were female.

Table-I: Age & sex distribution of the study subjects

Study subjects	Age (year) Mean ± SD	t-value	p-value	Male	Female
Cases (n=91)	49.60±12.88	1.94	0.243	64	27
Control (n40)	40.28±11.88			22	18

P-value reached by unpaired t-test

Table-II shows small dense LDL status of the cases & controls. Among 91 cases, 60 cases were found to have predominance of Sd LDL & 31 cases found to show no predominance of sd LDL. In control group there was 40 subjects of which 10 subjects was found to have predominance of Sd LDL & 30 subjects found to show no predominance of sd LDL. X2 test of association revealed strong association of Sd LDL with stroke.

Table-II: Small dense LDL status between case & control.

Small dense LDL	Case (n=91)	Control (n40)	P value
Predominant (LDL-C/apoB<1)	60(66%)	10 (25%)	<.001
Not Predominant LDL-C/apoB>1)	31(34%)	30(75%)	

P-value reached by x2 test.

Table-III shows Sd LDL status of the two groups of cases. Among 50 normolipidaemic cases 44 cases had the predominance of sd LDL & the rest 6 cases showed no predominance of Sd LDL & the rest 6 cases showed no predominance of Sd LDL. Among dyslipidaemic cases there was total 41 cases & 16 cases was found to have sd LDL predominance & 25 cases had no predominance of sd LDL. X2 test of association revealed strong association of sd LDL with normolipidaemic stroke compared to dyslipidaemic stroke cases.

Table-III: Small dense LDL status between two groups of cases.

Small dense LDL status	Grouping of the case subjects		P value
	Normolipidaemic n50	Dyslipidaemic n41	
Predominant (LDL-C/apoB<1)	44(88%)	16(39%)	.000
Not Predominant LDL-C/apoB>1)	6(12%)	25(61%)	

P-value reached by x2 test.

Table-IV & Fig-I shows a significant negative correlation of TG with LDL-C/ apoB ratio .Correlation coefficient (r) were - 0.186 and p value <0.005

Table-IV: Correlation of TG with LDL/Apo B ratio in cases

Independent variable / Dependent Variable	r-value	p-value
TG/Ratio	-0.186	<.005

Pearson's correlation coefficient test was done as the test of significance

Discussion

In this case controls study our aim was to see the preponderance of small dense LDL in stroke subjects. With this aim we have measured serum lipid profile and apoB in all study subjects. Preponderance of small dense LDL was evaluated by LDL-C/apoB ratio.

With respect to lipid profile, the mean total TC level in cases were lower than in the controls, but the difference was not significant. The meanplasma TG levels in cases were higher than controls but not significant. No significant differences were found in LDL cholesterol between case and controls. HDL cholesterol was comparable in cases to controls.

With respect to the serum apo protein profile; apo-B was significantly higher in dyslipidaemic stroke cases in comparison to the normolipidaemic stroke cases and controls.[6,16,17] But when we compared between normolipidaemic stroke cases and controls there was no significant difference between these two groups. Though numerically the mean concentration of apo-B was higher in normolipidaemic stroke cases but statistically it was insignificant. By contrast researchers of the atherosclerosis risk communities (ARIC) found that apo-B does not show any association with risk independently, when TAG, LDL & HDL cholesterol are taken into account.[10]

When LDL-C/apo-B ratio was taken into account it was significantly lower in normolipidaemic cases in comparison to the dyslipidaemic cases and in comparison to the controls, indicating the presence of small dense LDL in normolipidaemic stroke cases. Many other studies have demonstrated abnormalities of LDL subfraction profile in patient with stroke .They observed that LDL particles are on average smaller and heterogeneous in stroke patients.[5] But when compared between dyslipidaemic stroke cases and controls there was no significant difference between these two groups.

The possible explanation is that in controls and normolipidaemic cases the mean apo-B is almost equal but ratio is significantly lower in normolipidaemic cases because probably may be the LDL subtype variety may contain less cholesterol than apo-B. Similarly in dyslipidaemic cases apo-B was higher in comparison to controls and normolipidaemic cases but ratio was increased in dyslipidaemic cases because probably the LDL subtype variety may contain more LDL cholesterol than apo-B.

The prevalence of sd LDL was significantly higher in normolipidaemic stroke cases in comparison to the dyslipidaemic cases and controls. But there was no significant difference between controls and dyslipidaemic cases with respect to sd LDL. That means Sd LDL is more predominant in normolipidaemic stroke subjects than dyslipidaemic stroke subjects and it is independent of traditional lipid profile.

Several study also shows association of sd LDL with stroke.[9]

We found isolated association of Sd LDL with stroke even in normolipidaemic case. According to our study it could be that sd LDL isolatedly associated with stroke.

We hope that this study will stimulate & facilitate further broad based large study on this issue and will help to reduce mortality and morbidity due to stroke.

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