Original article

Study of serum non-HDL cholesterol in cerebrovascular disease

Parvin S[1], Hoque MM[2], Sultana N[3], Naoshin Z[4]

Abstract

Background: Non-HDL cholesterol is a potential newer risk factor for cerebrovascular diseases (CVD). ***Objective:*** To explore the association of non-HDL cholesterol with cerebrovascular disease. ***Methods:*** This case control study was carried out in the Department of Biochemistry, BSMMU, Dhaka during the period of January to December 2007 to evaluate the association of non-HDL cholesterol with CVD in Bangladeshi population. A total number of 135 subjects of both sexes were grouped as Group-I (CVD cases) and Group-II (Healthy controls). Group-I include 85 cases of which 59 were ischaemic cerebrovascular diseases (ICVD) and 26 were haemorrhagic cerebrovascular diseases (HCVD). By taking the history and doing clinical examination and laboratory investigations, diabetes mellitus, malignant disease, renal disease, liver disease and diuretic medication were excluded from study subjects. Serum non-HDL cholesterol was measured in all study subjects. Statistical analysis was performed by using SPSS for windows version 12.0. Mean values of the findings were compared between groups. One way ANOVA test and multiple comparison (Bonferroni't') test were used to see the level of significance. ***Results:*** Serum non-HDL cholesterol found significantly increased in CVD, ICVD and HCVD cases in comparison to control subjects. But ICVD and HCVD cases did not differ with respect to serum non-HDLcholesterol. ***Conclusion:*** The result shows that elevated non-HDL cholesterol is associated with CVD. Prospective study with large sample size is required to evaluate the elevated Non-HDL cholesterol as a risk factor of CVD.

Key words: Non-HDL cholesterol, Cerebrovascular disease, Ischaemic cerebrovascular disease, Haemorrhagic cerebrovascular disease.

Introduction

Cerebrovascular diseases (CVD) or strokes are one of the most common causes of mortality and long-term severe disability. It is the third leading cause of death after coronary heart disease (CHD) and malignancy and important cause of hospital admission in global perspective[1]. In Japan and other Asian countries including Bangladesh, CVD remains the most common disease. Despite the recent remarkable decrease in mortality, the proportion of patients treated for stroke as well as the prevalence of stroke has remained unchanged or even tended to increase[2]. Stroke accounts for 10-12% of all deaths in industrial countries. According to the consensus' statements on stroke, every 5 minutes someone in UK is having a stroke causing one in eight deaths and constitutes a formidable burden of disability and misery for patients, their caregivers and the wider community[3]. In USA, there are approximately 500,000 cases of stroke each year and of these 150,000 cases are left with mental and physical impairment requiring assistance

1. *Shamima Parvin, Lecturer, Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh.
2. Md. Mozammel Hoque, Professor, Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.
3. Nasima Sultana, Associate Professor, Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh.
4. Zinnia Naoshin, Lecturer, Department of Biochemistry and Molecular Biology, Primeasia University, Dhaka, Bangladesh.

Corresponds to:** Dr. Shamima Parvin., Lecturer, Department of Biochemistry, Dhaka Medical College, Dhaka, Bangladesh. ***Cell phone: +8801746398238. ***E-mail:*** drskabir@yahoo.com.

for activities of daily living. The cost of acute and long term care for stroke patients is about 30 billion dollar per year in USA[4]. There are two classical type of CVD from pathophysiological point of view: Ischemic cerebrovascular diseases (ICVD) and Hemorrhagic cerebrovascular diseases (HCVD). In ICVD that is caused by occlusion of cerebral vessels thromboembolic phenomenon is primarily responsible, where hyperlipidemia and atherosclerosis plays a central role[5]. In HCVD that happens following the rupture of intracerebral or subarachnoid vessels chronic hypertension plays a central role[6]. Hypertension is the most consistently powerful predictor of stroke and it is a factor in nearly 70% stroke of both types[7, 8].

It is now apparently clear that hyperlipidemia leading to atherosclerosis predispose ICVD, although chronic hypertension may enhance the atherosclerotic plaque formation in hyperlipidemic subjects. In contrast, hypertension seems to be the sole factor to be incriminating in HCVD. Atherosclerosis is claimed to be involved with coronary artery disease (CAD), cerebrovascular disease (CVD) and peripheral vascular disease (PVD). Lipids and lipoprotein disorders are important metabolic risk factors of atherosclerosis. There is overwhelming convincing evidences relating hypercholesterolemia, increased LDL cholesterol, decreased HDL cholesterol and increased total cholesterol and HDL cholesterol ratio (TC/HDL) with CAD but their relation to CVD is controversial. Some studies showed positive correlation of ICVD with total cholesterol, LDL cholesterol, TAG and (TC/HDL) but negative correlation with HDL cholesterol[9, 10, 11]. In contrast, several other studies reported no association between stroke and total cholesterol, LDL cholesterol, HDL cholesterol, TAG[12, 13, 14]. When this paradox is jet to be resolved numerous researchers by several lines of biological evidence suggested the positive role of non-HDL cholesterol in pathophysiology of CVD and other atherosclerotic vascular diseases. The main stay of atherosclerotic vascular diseases (e.g. CVD) is the atherogenic hyperlipidemia where LDL cholesterol is the main culprit. Apart from LDL cholesterol, there are several other atherogenic lipoproteins like very low density lipoprotein (VLDL), chylomicron remnant (CMR), intermediate density lipoprotein (IDL) , lipoprotein a [$LP_{(a)}$] etc. The only antiatherogenic lipoprotein is the HDL cholesterol. Plasma total cholesterol represent sum of the cholesterol content of all circulating lipoproteins irrespective of their atherogenic potential. So non-HDL cholesterol (total cholesterol minus HDL cholesterol) is more comprehensive measure of atherogenic lipoprotein than LDL cholesterol alone since it (non-HDL cholesterol) includes LDL cholesterol, VLDL, CMR, IDL and $LP_{(a)}$. Therefore, measuring non-HDL cholesterol reflects atherogenic risk not captured by LDL cholesterol alone particularly in the context of hypertriglyceridemia, where there is increased concentration of CM, VLDL, CMR and IDL. Recently non-HDL cholesterol has shown to be a better predictor of cardiovascular death than LDL cholesterol even in patient with TAG concentrations <200mg/dl. So, although plasma LDL cholesterol is well established as a predictor of CAD, it may not be the best circulating marker, rather non-HDL cholesterol could be the right choice. To calculate non-HDL cholesterol, it is enough to measure total cholesterol and HDL cholesterol for which there is no need to put the patient in fasting state so as in LDL cholesterol measurement, which need fasting sample. So, non-HDL cholesterol is more universal and technically more compliant measure to assess atherogenic potential of an individual[15]. Relation of non-HDL cholesterol, LDL cholesterol and atherogenic risk was addressed by the NCEP-ATP III. When serum TAG is

>150mg/dl, LDL cholesterol alone is not sufficient to define the risk associated with atherogenic lipoproteins, where non-HDL cholesterol is the best choice[16].

In Bangladesh, CVD stands for quite a sizeable number of mortality and morbidity posing a major socioeconomic challenge in the rehabilitation of stroke survivours but the best option for stroke still remains in its prevention. So, we should take appropriate measures for prevention. Traditional modifiable risk factors are now being treated but there is a pressing need to identify additional treatable newer risk factors that are easily measured and highly prevalent in general population. Non-HDL cholesterol is such type of potentially modifiable newer risk factor. It is possible to reduce the probability of stroke by lowering down the raised non-HDL cholesterol, as it is related to stroke. Some limited studies of serum lipid and lipoprotein in CVD have been done but the studies involving non-HDL cholesterol are scanty in our population. So, the aim of this study is to evaluate the association between elevated non-HDL cholesterol with stroke in our population.

Materials and Methods

This case-control study was carried out in the Department of Biochemistry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka during the period of January to December 2007. 135 subjects of both sexes were grouped as Group-I (CVD cases) and Group-II (Healthy control). Group-I included 85 cases, of which 59 had ICVD and 26 had HCVD. Among ICVD cases, 42 were male and 17 were female and among HCVD cases, 19 were male and 7 were female. Group-II included 50 healthy controls of which 37 were male and 13 were female. By taking the history and doing clinical examination and laboratory investigations, diabetes mellitus, malignant disease, renal disease, thyroid disorder, liver disease and diuretic medication were excluded from study subjects. Ethical clearance for the study was taken from the Departmental and Central Ethical Committee, BSMMU, Dhaka. Permission for the study was taken from the concerned departments from where we collected our study subjects. Informed written consent was taken from patient / attendants of all study subjects. 5 ml fasting venous blood was collected from all study subjects with full aseptic precaution blood was allowed to clot and then centrifuged. Separated serum was then collected and preserved at –35°c and later on used for the measurement of lipid profile, creatinine and sugar concentration.

Table I: Serum non-HDL cholesterol concentration among the groups of study subject*

Parameter (mg/dl)	CVD Cases (n=85)	ICVD Cases (n=59)	HCVD Cases (n=26)	Control (n=50)
	Mean(SD)	Mean(SD)	Mean(SD)	Mean(SD)
Non-HDL cholesterol	198.48(34.82) (129-358)	200.14(37.09) (152-358)	194.73(29.34) (129-258)	129.42(27.46) (51-161)

**Lower parentheses show the range.*

Table-II: Comparison of serum non-HDL cholesterol level between cases and controls**

Parameter (mg/dl)	Cases (n=85) Mean(SD)	Control (n=50) Mean(SD)	t value	P value
Non-HDL cholesterol	198.48(34.82)	129.42(27.46)	12.00	<0.001

***Unpaired student's 't'-test was done as the test of significance.*

Table III: Comparison of serum non-HDL cholesterol level among the three groups of study subjects (ICVD, HCVD and controls) ***

Parameter (mg/dl)	ICVD (n=59) mean(SD)	HCVD (n=26) mean(SD)	Control (n=50) mean(SD)	F value	P value
Non-HDL cholesterol	200.14(37.08)	194.73(29.34)	129.42(27.46)	71.93	<0.001

****One way ANOVA test was done as the test of significance.*

Table IV: Comparison of serum non-HDL cholesterol between different groups of study subjects****

Grouping of study subjects		Level of significance
ICVD	HCVD	P≥0.05
	Control	p≥0.001
HCVD	ICVD	p≥0.05
	Control	p≥0.001
Control	ICVD	p≥0.05
	HCVD	p≥0.001

*****Multiple Comparison (Bonferroni't') test was done as the test of significance.*

All data were recorded systematically in a preformed data collection form and were expressed as mean (SD). Statistical analyses were performed by using SPSS for windows version 12.0. Mean values of the findings were compared between groups. One way ANOVA test and multiple comparison (Bonferroni't') test were used to see the level of significance. 95% confidence limit ($p<0.05$) was taken as level of significance.

Results

Total 135 subjects of both sexes were grouped as Group-I (CVD cases) and Group-II (Healthy control). The serum non-HDL cholesterol concentration of all the study subjects was estimated and the results were expressed as mean(SD). The units of measurements were mg/dl. Group-I included 85 cases, 59 were ICVD and 26 were HCVD. Among ICVD cases 42 were male and 17 were female with mean age of 58.34(7.01) years and age range of 40-68 years and among HCVD cases 19 were male and 7 were female with mean age of 59.31(6.73) years and age range of 45-68 years. Group-II included 50 healthy controls, 37 were male and 13 were female with mean age of 50.48(5.50) years and age range of 40-62 years. The mean(SD) of non-HDL-c concentration of CVD, ICVD and HCVD cases and control subjects were 198.48(34.82) mg/dl with the range 129-358 mg/dl, 200.14(37.09) mg/dl with the range 152-358 mg/dl, 194.73(29.34) mg/dl with the range 129-258 mg/dl and 129.42(27.46) mg/dl with the range 51-161 mg/dl, respectively.

Discussion

In this present case control study, the serum non-HDL cholesterol concentration was measured in 85 diagnosed CVD patients and 50 healthy control subjects to evaluate the association of non-HDL cholesterol with CVD. The mean non-HDL cholesterol level found to be significantly high (p□0.001) in CVD cases compared to their control value. This finding is consistent with other similar studies done abroad[17,18]. They concluded that elevated levels of serum triglycerides and non-HDL are associated with large arteries' atherosclerotic stroke. Those with the highest triglycerides were 2.7 times and those with highest non-HDL were 2.4 times more likely to have a large artery stroke. Recently, the use of non-HDL cholesterol level has been suggested as a

better tool for risk assessment and treatment than LDL cholesterol level because non-HDL cholesterol includes all cholesterol present in lipoprotein particles considered to be atherogenic, including LDL, IDL, VLDL, CMR, $LP_{(a)}$ etc. and estimation of LDL cholesterol level using the formula can be inaccurate, when TAG is high. Despite the potential usefulness of non-HDL cholesterol level, only a few studies were done that have demonstrated that elevated non-HDL cholesterol level is associated with an increased risk for development of CVD[19]. In the present study, we also found high non-HDL cholesterol level in both ICVD and HCVD cases compare to control. But no statistically significant difference was found in between ICVD and HCVD cases with respect to non-HDL cholesterol. So, elevated non-HDL cholesterol level found to be associated with an increased risk for CVD irrespective of their clinical types. Serum TC, TAG & LDL cholesterol concentration were also found to be elevated significantly in CVD cases in comparison to control, which is consistent with that of other studies done abroad[20,21,22]. We have found serum HDL-C, significantly low in CVD cases in comparison to control which is supported by Sacco et al.[23]. Iso et al.[24] surprisingly found that the lowering of serum total cholesterol does not reduces the stroke mortality and morbidity, rather predispose HCVD, which is a consequence of weakening of intracerebral arterial endothelium. Sridharan[20] found low HDL cholesterol level and a high total cholesterol and HDL cholesterol ratio (TC/HDL) among the stroke patients. Sacco et al.[23] compared cholesterol levels in stroke patients with control subjects and found an inverse association between HDL cholesterol and the risk of ischemic stroke. Ebrahim et al.[25] studied blood cholesterol in a large cohort of young and middle aged Korean civil servants and found that low concentrations of cholesterol were associated with hemorrhagic stroke while high concentrations were associated with ischemic stroke. Higher total cholesterol and lower HDL cholesterol levels were associated with increased risk of ischemic stroke. The lowest levels of total cholesterol were associated with an increased risk of all hemorrhagic strokes[21.] There is mounting epidemiologic evidence to support the relationship of lipids as a risk factor for ischemic stroke[26]. A 10 years follow up study of Japanese men and women demonstrated that lower HDL cholesterol levels were related significantly and independently to increased risk of all stroke incidences including the ischemic stroke incidence[27]. A new analysis of 61 prospective observational studies has failed to find any association of total cholesterol with stroke mortality[28]. LDL cholesterol was the common risk factor for ischemic stroke in men and women, whereas, non-HDL cholesterol, total cholesterol and their ratio were related to ischaemic stroke as risk factors only in women[22]. Another study done by Kurth et al.[39] revealed that total cholesterol, LDL cholesterol, TC/HDL, and non-HDL cholesterol are risk factors for ischaemic stroke only in women. These studies provide convincing evidence that atherogenic lipoproteins are strongly associated with atherogenicity, hypertension and CVD. Total cholesterol as well as LDL cholesterol is found to provide myopic view of the total atherogenic potential. Since non-HDL-c reflects the total plasma level of all atherogenic lipoproteins, it is especially promising as an acceptable, easy way to measure surrogate biomarker of atherogenic risk and thus, can be integrated into clinical practice as a comprehensive risk factor for CVD.

References

1. Bonita R. Epidemiology of stroke. *Lancet.* 1992; 339: 342-4.

2. Shinohara Y. Life style is not the only cause of stroke – risk factors recently attracting attention. *JAMA*. 2001; 44: 177-81.

3. Shaper AG, Philips AN, Pocock SJ, Walker M, Macfarlane PW. Risk factors for stroke in middle aged British Men. *BMJ.* 1991; 302: 1111-5.

4. Bronner LL, Kanter DS and Manson JE. Primary Prevention of Stroke. *N Eng J Med.* 1995; 333: 1392-1400.

5. Allen CMC, Lueck CJ. Disease of the nervous system. In: Haslet R, Chilver ER, Hunter JAA, Boon NA. eds. *Davidson's principles & practice of medicine.* 18th ed. London: Churchill Livingstone; 1999. pp. 923-1023.

6. Smith WS, Hauser SL, Easton JD. Cerebrovascular diseases, In: Braunwald E, Fauci AS, Kasper DL, Hauser SL, Longo DL, Jameson JL. eds. *Harrison's principles of medicine.* 15th ed. USA: McGraw-Hill Company; 2001. pp. 2369.

7. Dunbabin DW, Sandercock PAG. Preventing stroke by modification of risk factors. *Stroke.* 1990; 21(IV): 36-9.

8. Brown MM. Stroke: epidemiology and clinical features. *Medicine International 2000 (Bangladesh edition).* 4: 45-51.

9. Pedro-Botet J, Senti M, Nogues X, Rubies-Prat J, Roquer J, D'Olhaberriague L, et al. Lipoprotein and apolipoprotein profile in men with ischemic stroke: role of lipoprotein$_{(a)}$, triglyceride-rich lipoproteins and apolipoprotein E polymorphism. *Stroke.* 1992; 23: 1556-62.

10. Lindenstrom E, Boysen G, Nyboe J. Influence of total cholesterol, high density lipoprotein cholesterol, and triglycerides on risk of cerebrovascular disease: the Copenhagen city heart study. *BMJ.* 1994; 309: 11-5.

11. Hachinski V, Graffagnino C, Beaudry M, Bernier AAG, Buck C, Donner A, et al. Lipids and stroke: a paradox resolved. *Arch Neurol.* 1996; 53: 303-8.

12. Zenker G, Koltringer P, Bone G, Niederkorn K, Pfeiffer K, Jurgens G. Lipoprotein $_{(a)}$ as a strong indicator for cerebrovascular disease. *Stroke.* 1986; 17: 942-5.

13. Woo J, Lau E, Lam CW, Kay R, Teoh R, Wong HY, Prall WY, Kreel L, Nicholls MG. Hypertension, lipoprotein$_{(a)}$, and apolipoprotein A_1 as risk factors for stroke in the Chinese. *Stroke.* 1991; 22: 203-8.

14. Christopher R, Kailasanatha KM, Nagaraja D, Tripathi M. Case control study of LP $_{(a)}$, apo-A_1 and apo-B in stroke in the young. *Acta Neurol Scand.* 1996; 94: 127-30.

15. Hirsch GA, Vaid N, Blumenthal RS. The significance of measuring non-HDL cholesterol. *Preventive Cardiology.* 2002; 5(3): 156-9.

16. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) (Adult Treatment Panel III). *JAMA.* 2001; 285: 2486-97.

17. Ovbiagele B. 26th December, 2007. *Neurology.* (on line). Retrieved 3rd March, 2008. from http://www.healthcentral.com/heart-disease/news-197949-66.html.

18. Bang OY, Saver JL, Liebeskind DS, Pineda S, Ovbiagele B. Association of serum lipid indices with large artery atherosclerotic stroke. *Neurology.* 2008; 70: 841-7.

19. Cui Y, Blumenthal RS, Flaws JA, Whiteman MK, Langenberg P, Bachorik PS, et al. Non-HDL cholesterol level as a predictor of cardiovascular disease mortality. *Arch Intern Med.* 2001; 161:1413.

20. Sridharan R. Risk factors for ischaemic stroke: a case control analysis. *Neuroepidemiology.* 1992; 11: 24-30.

21. Tirschwell DL, Smith NL, Heckbert SR, Lemaitre RN, Longstreth Jr. WT, Psaty BM. Association of cholesterol with stroke risk varies in stroke subtypes and patient subgroups. *Neurology.* 2004; 63: 1868-75.

22. Xingang Z, Zhaoqing S, Xinzhong Z, Liqiang Z, Shuangshuang L, Changlun X, et al. Gender differences in blood lipids and the risk of ischaemic stroke among the hypertensive adults in rural China. *Neurology India.* 2007; 55: 338-42.

23. Sacco RL, Benson RT, Kargman DE, Boden-Albala B, Tuck C, Lin IF, et al. High-density lipoprotein cholesterol and ischaemic stroke in the elderly: the Northern Manhattan Stroke Study. *JAMA.* 2001; 285: 2729-35.

24. Iso H, Jacobs DR, Wentworth D, Neaton JD, Cohen JD. Serum cholesterol levels and six-year mortality from stroke in 350,977men screened for the multiple risk factor intervention trial. *N Eng J Med.* 1989; 320: 904-10.

25. Ebrahim S, Sung J, Song YM, Ferrer RL, Lwalor DA, Davey SG. Serum cholesterol, haemorrhagic stroke, ischaemic stroke, and myocardial infarction: Korean national health system prospective cohort study. *BMJ.* 2006; 333: 22-7.

26. Gorelick PB, Schneck M, Berglund LF, Feinberg W, Goldstone J. Status of Lipids as a Risk Factor for Stroke. *Neuroepidemiology.* 1997; 16: 107-15.

27. Soyama Y, Miura K, Morikawa Y, Nishijo M, Nakanishi Y, Naruse Y, et al. High-density lipoprotein cholesterol and risk of stroke in Japanese men and women: the Oyabe Study. *Stroke.* 2003; 34: 863-8.

28. Amarenco P, Steg PG. The paradox of cholesterol and stroke. *Lancet.* 2007; 370: 1803-4.

29. Kurth T, Everett BM, Buring JE, Kase CS, Ridker PM, Gaziano JM. Lipid levels and the risk of ischaemic stroke in women. *Neurology.* 2007; 68: 556-62.