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Original Article

Biochemistry Section

A Study of Serum Apolipoprotein A1, Apolipoprotein B and Lipid Profile in Stroke

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ABSTRACT

Background: Role of Serum Lipids, Lipoproteins and Lipoprotein related variables in the prediction of Stroke is less clear. Abnormalities in plasma Lipoproteins are the most firmly established and best understood risk factors for Atherosclerosis and they are probable risk factors for Ischaemic stroke, largely by their link to Atherosclerosis. Apo B reflects the concentration of potentially atherogenic particles (LDL), and Apo A1 reflects the corresponding concentration of anti-atherogenic particles (HDL), represent additional lipoprotein related variables that may indicate the vascular risk.

Aim: To study serum concentration of Apolipoprotein A1, Apolipoprotein B, Apo B/Apo A1 ratio and Lipid profile in Stroke Cases and to compare with healthy controls.

Design: A total number of 100 subjects within 30 – 70 years were considered for the study. 50 subjects with Stroke (both clinically as well as Computed tomographically proven cases) and 50 age and sex matched healthy individuals were taken for the study.

Material and Methods: Total cholesterol, HDL cholesterol and Triglycerides are estimated by Enzymatic method using Semiautoanalyser. LDL cholesterol is estimated by Friedewald formula. Apo B and Apo A1 are estimated by Immunoturbidimetric method using Semiautoanalyser.

Statistical Analysis: Student 't' test was used to compare the data between cases and controls. Diagnostic validity tests were conducted to assess the Diagnostic efficiency of Apo A1, Apo B and Apo B/Apo A1 ratio.

Results: Total cholesterol, LDL cholesterol and Triglycerides are significantly increased in Cases compared to Controls. HDL – cholesterol is significantly decreased in Cases compared to Controls. Apo B and Apo B/Apo A1 ratio are significantly increased and Apo A1 is significantly decreased in Cases compared to Controls. Diagnostic validity tests showed that, Apo B , Apo A1 and Apo B /Apo A1 ratio have highest Sensitivity, Specificity and Diagnostic efficiency.

Conclusion: Apo B , Apo A1 and Apo B / Apo A1 ratio can be used as predictors of stroke along with traditional lipid profile components.

Key words: Apolipoprotein A1, Apolipoprotein B, Lipid profile, Stroke

INTRODUCTION

Stroke or a Cerebrovascular Accident (CVA) is defined as the "Abrupt onset of a neurological deficit that is attributable to a focal vascular cause". If the neurological signs and symptoms last for < 24 hours, it is called as a Transient Ischaemic Attack (TIA) and if they exceed 24 hours, then it is called as stroke [1]. Stroke is a worldwide health problem. It makes an important contribution to the morbidity, mortality and the disability in the developed as well as the developing countries. A WHO collaborative study which was done in 12 countries, showed that the incidence rates of stroke were 0.2 – 2.5 per 1000 population per year. It is the leading cause of adult disability and the second most common cause of death worldwide. It accounts for 10 – 12% of the total deaths in the developed countries [2].

"Little stroke, big trouble" the theme of the World Health Day, 2008, speaks about the importance of Stroke as a critical warning sign of further more debilitating vascular events or death [3]. Disturbances in the cerebral function in stroke is caused by three morphological abnormalities, which include stenosis, occlusion or rupture of arteries, leading to ischaemia, infarction and cerebral haemorrhage respectively. Abnormal lipid parameters like Total Cholesterol (TC), Low Density Lipoprotein (LDL) cholesterol, High Density Lipoprotein (HDL) Cholesterol and Triglycerides (TG) are the probable risk factors for Ischaemic Stroke, largely due to their link to Atherosclerosis. The apolipoproteins are the protein components of the Lipoproteins. Apolipoprotein B (Apo B), which reflects the concentration of the potentially atherogenic Lipoprotein Particles (LDL) and Apolipoprotein A1(Apo A1), which reflects the concentration of the antiatherogenic particles (HDL), represent the additional Lipoprotein related variables that may indicate a vascular risk [4].

Dyslipidaemia, low Apo A1 and high Apo B are widely accepted as the risk factors for Coronary artery disease. In contrast, the correlation has not been well established for Stroke [5]. So, the present study was undertaken to evaluate the relationship between Apo A1, Apo B and the Lipid profile in Stroke.

MATERIAL AND METHODS

A cross sectional study on the serum Apo A1, Apo B and the Lipid profile in Stroke subjects was carried out for a duration of 1 year. After obtaining the ethical committee's clearance, a total number of 100 subjects within 30 – 70 years were considered for the study. 50 subjects with Stroke (both clinically as well as Computed tomographically proven) were taken as the cases and 50 age and sex matched healthy individuals are taken as the controls [Table/Fig-1]. The patients with Hepatic Disease, Renal diseases, Sepsis and Malignancy and the patients who were on Hypolipidaemic drugs were excluded from the study. All the cases and the controls were selected from the Chigateri General Hospital and the Bapuji Hospital, (both the hospitals are attached to JJM Medical College, Davangere, India) and the study was carried out in the Department of Biochemistry, JJM Medial College, Davangere, India. After

obtaining the informed consents of the cases and the controls, about 6 ml of blood was drawn under aseptic precautions in sterile bulbs after a period of overnight fasting for 12 hours. The serum was separated by centrifugation and it was used for the analysis.

No. of subjects		Controls (n = 50)	Cases (n = 50)
Age (years)	Mean ± SD	58.5 ± 10.8	61.9 ± 7.1
	Range	40-69	40-72
Gender	Male	31	34
	Female	19	16

[Table/Fig-1]: Descriptive information of study subjects

Total cholesterol was estimated by the Enzymatic Cholesterol oxidase Phenol aminoantipyrine method. HDL cholesterol was estimated by the Enzymatic Cholesterol oxidase Phenol amino-antipyrine method after the precipitation of LDL C, Very Low Density Lipoprotein (VLDL) Cholesterol and Chylomicrons with phosphotungastate in the presence of divalent cations such as Magnesium. The triglycerides were estimated by the Enzymatic glycerol phosphate oxidase – Phenol aminoantipyrine method. LDL cholesterol was estimated by using Friedewald's formula. Apo B and Apo A1 were estimated by an immunoturbidimetric method [6]. The estimations are done by using a Semiautoanalyzer (Erba). The statistical analysis was done by using Student's 't' test. A p value of less than 0.05 was considered as statistically significant. Diagnostic validity tests were conducted to discriminate those with Stroke and those without Stroke. The median of the combined groups was used as the cutoff value for discriminating the cases from the Controls.

RESULTS

The serum levels of TC, LDL – C and TG were higher and HDL – C was lower in the Stroke cases than in the Controls and the difference was statistically highly significant with a p value of < 0.001 [Table/Fig-2]. The serum levels of Apo B and the Apo B / Apo A1 ratio were higher and Apo A1 was lower in the Cases as compared to the Controls and the difference was statistically highly significant with a p value of < 0.001 [Table/Fig-3].

Groups	n	TC (mg/dl)	LDL - C (mg/dl)	HDL - C (mg/dl)	TG (mg/dl)
Controls	50	188.0 ± 36.9 (126-280)	113.0 ± 38.3 (54-226)	51.8 ± 15.9 (25-82)	127.7 ± 59.0 (56-453)
Cases	50	274.3 ± 28.8 (173 – 329)	203.7 ± 32.2 (173 – 329)	25.3 ± 6.8 (10 – 42)	220.0 ± 59.6 (116 – 499)
Mean diff.		86.3	90.7	26.5	92.3
t-value		13.08	12.82	10.87	7.78
p-level		< 0.001	< 0.001	< 0.001	< 0.001

[Table/Fig-2]: Comparision of Serum Total cholesterol, LDL cholesterol, HDL cholesterol and Triglycerides in Cases and Controls

For Apo A1, the cutoff value was 114 mg/dl, for Apo B , the cutoff value was 144mg/dl and for the Apo B/Apo A1 ratio, the cutoff value was 1.2. Apo B , Apo A1 and the Apo B/Apo A1 ratio had the highest Sensitivity, Specificity, Positive predictive value and Negative predictive value, with the diagnostic efficiencies for Apo A1, Apo B and the Apo B/Apo A1 ratio being 87%, 95% and 94% respectively [Table/Fig-4].

	ApoB (>144mg/dl)	Apo A-1 (< 114mg/dl)	Apo B/ Apo A1 (> 1.2)
Sensitivity	96%	88%	98%
Specificity	94%	86%	96%
PPV	94%	86%	98%
NPV	95%	86%	96%
Diagnostic efficiency	95%	87%	94%

[Table/Fig-4]: Diagnostic validity of Apolipoprotein A-1, Apolipoprotein B and Apo B/ Apo A-1 ratio for discrimination of Stroke Subjects

DISCUSSION

Stroke is a heterogeneous pathophysiological entity in which many different pathways lead to indistinguishable clinical presentations. The well recognised mechanisms for stroke include cardiac embolism or artery – artery embolism, atherosclerosis of extracranial or intracranial arteries and non atherosclerotic disease of the small diameter penetrating arteries. Atherosclerosis of the extracranial or the intracranial arteries accounts for substantial proportions of stroke via an artery – artery embolisation of the plaque associated thrombi or an in situ atherothrombotic occlusion. Accordingly, the known contributors of atherosclerosis like cholesterol and its subfractions play an important aetiological role which is similar to their role in coronary artery disease [7].

In our study, total cholesterol, LDL cholesterol and the triglycerides were significantly increased in the stroke cases as compared to those in the controls. The results were in accordance with those of the studies which were done by other authors [4,8 – 10]. HDL-cholesterol was significantly decreased in the cases as compared to the controls, which was in accordance with the results of similar studies [4,11-16].

Elevated total cholesterol and LDL cholesterol are usually described in terms of their effects on the pathogenesis of atherosclerosis. Atherosclerosis plays a primary role in the biological aetiology of the most common form of stroke (ischaemic stroke). Myocardial infarction and cerebral infarctions share a common mechanism in which a clot (thrombus) becomes lodged in the coronary/cerebral/carotid artery, which is narrowed by advanced atherosclerosis. The subsequent loss of the blood supply results in a temporary or a permanent loss of the tissue function in the area which is supplied by the affected artery [17]. High resolution ultrasonography has

Groups		Apo B (mg/dl)		Apo A1 (mg/dl)		Apo B/Apo A-1	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Controls	50	106.4 ± 29.8	112-196	158.9 ± 39.6	86 – 266	0.71 ± 0.27	0.31 – 1.58
Cases	50	190.8 ± 39.7	138 – 296	87.1 ± 23.6	41-145	2.39 ± 0.96	1.1 -5.0
Mean diff.		84.4		71.7		1.68	
t-value*		12.49		11.00		11.91	
p-level		< 0.001		< 0.001		< 0.001	

[Table/Fig-3]: Comparision of Serum Apolipoprotein A-1, Apolipoprotein B and the ratio of Apo B/Apo A-1 in Cases and Controls

clearly established that thickening of the carotid artery intima and media is a predictor of stroke. An association between carotid atherosclerosis and LDL – C has been found in many studies [4]. It has also been shown that the elevated LDL – C levels in patients with stroke are more susceptible to lipid peroxidation and that the products of lipid peroxidation are significantly associated with the risk of Stroke [9]. An inverse correlation has been found between the serum HDL – C levels and the risk of Stroke. This could be explained by the antiatherogenic effects of HDL [11]. This can be explained by several mechanisms like – its ability in transporting cholesterol from the peripheral cells to the liver (a reverse cholesterol transport), its ability in preventing lipid peroxidation (antioxidant effects), its ability in limiting the expression of cytokines like TNF – α and interleukin – I (anti-inflammatory effects), its ability in inhibiting platelet activation and aggregation and its ability in improving the endothelial function by prostacyclin release and the release of the endothelium derived relaxing factor [11,12].

In contrast, few other studies have demonstrated a positive association between the serum cholesterol levels and death from non – haemorrhagic Stroke and an inverse correlation between the serum cholesterol levels and the risk of death from Haemorrhagic stroke [18,19]. Higher cholesterol levels are associated with less severe Stroke and a better outcome after the Stroke [20,21].

This can be explained by the fact, that stroke is a heterogeneous syndrome of a different aetiologic origin. Lipid abnormalities may be important for some subtypes of stroke but not for the others. Furthermore, the association may be different for the ischaemic stroke subtypes. Secondly, the lipoprotein subfractions exert varying influences on the stroke risk. It is possible that the protective effect of HDL – C against stroke, weakens the positive association between total cholesterol and stroke. Therefore, it is better to discuss the association between stroke and cholesterol on the basis of the stroke subtypes and the lipoprotein subfractions [22]. The protective effect of cholesterol is also explained by the fact that, cholesterol is an essential component of the cell and organelle membranes and that cholesterol can act as a buffer, in neutralizing a proportion of the free radicals, limiting the extent of the lesions and increasing the cellular recuperation capacity [20].

In the present study, the mean value of Apo B was higher, that of Apo A1 was lower and the Apo B /Apo A1 ratio was higher in the cases as compared to the controls and the difference was statistically significant with a p value of < 0.001. The results were in accordance with those of similar studies [4,23 – 25].

Apo B which is present in VLDL, IDL, large buoyant LDL and small dense LDL, reflects the total number of atherogenic particles. Atheroscerosis leads to the entrapment of these Lipoproteins in the arterial wall. High Apo B levels may indicate an increased number of small dense LDL particles which are easily oxidised and which promote an inflammatory response and the growth of plaques. The larger Apo B containing particles such as VLDL and IDL can enhance the risk of atherothrombosis by inhibiting the fibrinolytic system and by stimulating the cytokine production and the inflammatory response. Apo A1, the major protein in HDL, has a central role in the reverse cholesterol transport and in transferring excess cholesterol from the peripheral cells back to the liver in the HDL particles. The Apo B /Apo A1 ratio reflects the balance of the cholesterol transport in a simple way. The higher the value of the Apo B/Apo A1 ratio, the more cholesterol is likely to be deposited in the arterial wall, thereby provoking the atherogenesis and increasing the vascular risk.

Therefore, the comparison of the apolipoproteins with the lipoproteins shows that they both have similar degrees of predictive abilities. This supports the finding that Apo B , Apo A1 and the Apo B/Apo A1 ratio represent the additional Lipoprotein related variables that estimate the Stroke risk as well as the respective Cholesterol components. A major attribute of employing Apolipoproteins to assess the risk of vascular events is:

- They are measured directly, as opposed to LDL – C, which is usually calculated by using Friedewald's formula.
- The measurements of the Apolipoproteins are also internationally standardised.
- The measurements of the Apolipoproteins are accurate, precise and easily automated.
- They do not require fasting blood samples [4].

Hence, both the lipoproteins and the Apolipoproteins are used as the predictors of Stroke. They have a good sensitivity but they lack specificity. Abnormal levels are reported in various other physiological and pathological conditions like Pregnancy [26], Cardiovascular disease [27,28]. and peripheral arterial diseases [25]. Hence, while implementing the lipoproteins and the Apolipoproteins in predicting the occurrence of Stroke, it is important to consider and to rule out these clinical conditions.

CONCLUSION

The concentrations of the lipid parameters can be changed through meals. But the concentrations of the apolipoproteins are not affected by meals. So, the measurements of the apolipoproteins do not require fasting blood samples. HDL cholesterol sometimes gives misleading results, since the cholesterol composition of HDL can vary in response to various physiological and pathological conditions. Hence, the measurement of Apo A1, the protein part of HDL, is a better predictor of stroke. The LDL particles are heterogenous and they are composed of small and large dense particles. Only the small, dense particles are associated with risk of atherosclerosis. They contain less cholesterol and hence, the measurement of LDL cholesterol does not accurately reflect their plasma concentrations. Apo B (the protein part of LDL) predicts the development of stroke better than LDL cholesterol. This is because each of the atherogenic particles (VLDL, IDL and LDL) contains one molecule of Apo B and therefore, plasma Apo B measures the total number of atherogenic particles. The apolipoproteins have good sensitivity, specificity and a high diagnostic accuracy. So, the current study supports the concept that that Apolipoprotein A1, Apolipoprotein B and the Apo B/Apo A1 ratio can be used as the predictors of Stroke, along with the traditional lipid profile measurements. Further studies are required to to assess the usefulness of the apolipoproteins in indicating the risk of stroke in the patients with transient ischaemic attack and in the patients with abnormal lipid levels.

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