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Comparative study of lipid profile and lipid indices in stroke patients and healthy subjects

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Abstract: *Background:* Stroke is a serious, life-threatening medical condition that occurs when the blood supply to part of the brain is cut off. Strokes are a medical emergency and urgent treatment is essential because sooner the person receives treatment for stroke, thereby reducing the serious consequence associated with it. The relationship between atherosclerosis and elevated serum lipids are not well established. *Objectives:* The study was taken up to decipher whether there is any correlation between lipid profile parameters and pathogenesis of stroke in young and elderly cases of stroke. *Methods:* The study population consisted of 91 clinically diagnosed stroke cases, of either gender between 25-80 years of age. The relevant clinical history was taken. Around 5 ml of venous blood sample was collected in yellow vacutainer, after a period of 12 hours overnight fasting, then serum was separated. Total cholesterol, triglyceride, high density lipoprotein (HDL-Cholesterol), low density lipoprotein (LDL- cholesterol) and serum creatinine were estimated by on cobas® 6000 analyzer - Roche Diagnostics. Estimation of GFR - eGFR was calculated by using MDRD formula. The atherogenic index and lipid ratios were calculated using the following established formulas. *Results*: In the study there was increase in LDL- cholesterol (LDL-C) values in old age stroke cases as compared to young age stroke cases. An increase in serum creatinine value was observed in old age cases in comparison to young age cases. In old male stroke cases there was increase in serum cholesterol, triglyceride, LDL- cholesterol , Tc: HDL-cholesterol ratio, CR II, atherogenic coefficient, TG/ HDL-Cholesterol ratio and serum creatinine levels. The study shows higher predisposition to atherogenesis in older age cases as compared to young age stroke cases. There was decrease in HDL-Cholesterol (HDL-C) and eGFR values in older male and female stroke cases in comparison to younger male and female stroke cases. *Conclusion:* Stroke patients with dyslipidemia need a comprehensive health care approach involving dietician, physician and good biochemistry back up.
Keywords: Atherogenesis, atherogenic index of plasma, dyslipidemia, eGFR, stroke.

Introduction

Stroke is defined as abrupt onset of neurological deficit, which is attributable to a focal vascular cause and presents as brain infarction or hemorrhage. It being a medical emergency, is one of the leading cause of mortality and morbidity throughout the world [1]. Due to difficulty in its treatment, the ability to forecast stroke shall be critical and has become challenging making the detailed study of predisposing factors essential.

The relationship between atherosclerosis and elevated serum lipids are not well established. Elevated plasma concentration of low-density lipoproteins (LDL- cholesterol) and low level of high-density lipoprotein (HDL-cholesterol) concentration are associated with increased risk of atherosclerosis. While there is an overwhelming amount of evidence relating high levels of serum total and LDL-cholesterol and low levels of HDL-cholesterol with coronary atherosclerosis, the relation between serum lipids, lipoproteins and cerebrovascular atherosclerosis is not very clear. Studies of cholesterol levels in stroke patients have revealed results varying from insignificant changes to a moderate elevation. Various studies have shown dyslipidemia to be associated with stroke. It has been reported aggressive treatment of dyslipidemia decreases the risk of stroke [2].

Several other risk factors of stroke include diabetes mellitus, hypertension, smoking, atherosclerosis, age and others. Cerebral

ischemia is due to reduced blood flow for several seconds, if it persists for little longer duration, infarct or brain tissue death occurs [3].

Intracranial hemorrhage due to bleeding into or around the brain may produces stroke. Stroke is caused by two major mechanisms, ischemia and hemorrhage. Ischemic stroke constitutes 80% of total stroke. Ischemic stroke is due to diminished or absent blood supply to the neurons resulting in deprivation of necessary substrate to neurons [4].

The study was taken up to decipher whether there is any correlation between lipid profile parameters and pathogenesis of stroke in young and elderly cases of stroke.

Material and Methods

The study population consisted of 91 clinically diagnosed stroke cases, of either gender between 25-80 years of age with clinical findings, brain CT-scan or MRI indicative of cerebral infraction or intra-cerebral hemorrhage were enrolled for this study. The patients with underlying condition of liver disease, familial hypercholesterolemia and hypothyroidism, on drugs for dyslipidemia, cerebral hemorrhage secondary to cerebral tumor, trauma or previous coagulation disorders were excluded from the study.

The ethical clearance was obtained from the ethical review board of the institution. An informed consent was taken from patients before collection of sample. The relevant clinical history was taken. Clinical examination of the patients was performed and vitals were recorded.

Around 5 ml of venous blood sample was collected in yellow vacutainer, after a period of 12 hours overnight fasting. The sample was allowed to stand for 20 minutes. Serum was separated from the sample after centrifugation at 3500 rpm for 15 minutes. Lipid profile and serum creatinine level were estimated in the sample.

Total cholesterol, triglyceride, high density lipoprotein (HDL-cholesterol), and low density lipoprotein (LDL- cholesterol) were estimated on cobas® 6000 analyzer - Roche Diagnostics. Estimation of GFR - eGFR (estimated Glomerular Filtration Rate) was calculated by using MDRD (Modification of diet in renal disease) formula. eGFR (mL/min/1.73 m^2) = 186 x (serum creatinine mg/dL) $^{-1.154}$ x (age) $^{-0.203}$ x (1.210 if black) x (0.742 if female) [5]. eGFR greater than 90ml/min/1.73 m^2 was considered to be normal.

The atherogenic index and lipid ratios were calculated using the following established formulas, Atherogenic index was calculated by using the following formula: $\log_{10}$ (TG/HDL-C). It can be classified according to the values obtained: −0.3 to 0.1 for low risk, 0.1 to 0.24 for medium, and more than 0.24 for high risk of CVD. Castelli's Risk Index I (CRI-I) was calculated by the formula: CRI-I = TC/HDL-C, Non - HDL-C = Total Cholesterol - HDL-C, Castelli's Risk Index II (CRI-II) was calculated by the formula: CRI-II = LDL-C/HDL-C. Atherogenic coefficient was calculated using the formula AC= TC-HDL-C/HDL-C [6].

Statistical Methods The results were expressed as mean ± SD. Significance was assessed at 5% level of significance. Categorical data was represented in the form of number (n). Student "t" test (two tailed, independent) was used to find the significance of study parameters. Fischer Exact chart was used for lipid profiles between young and old age stroke cases. Statistical analysis was performed using SPSS 12.0 software.

Results

The study was carried out to study lipid profile, atherogenic indices and serum creatinine level in stroke cases. 91 stroke cases who were admitted to hospital were recruited for the study as per inclusion and exclusion criteria. Further they were subdivided according to their age, between 20-45 years as young age stroke cases and between 46-80 years as older stroke cases.

In Table 1 age, cholesterol, triglycerides, HDL-cholesterol, LDL- cholesterol, non HDL-cholesterol, Atherogenic index of plasma, atherogenic coefficient, Castelli's risk index I and II, serum Creatinine and eGFR are represented.

Table-1: Comparision of age, serum creatinine and lipid indices between young and old age stroke cases (Mean±SD)					
	Young (ALL) Mean	**SD(±)**	**Old (ALL) Mean**	**SD(±)**	**p value**
Age yrs	37.34	5.99	63.52	11.34	0.000
Total Cholesterol mg/dl	164.72	48.14	159.69	50.07	0.626
Triglycerides mg/dl	168.17	103.67	136.53	61.03	0.082
HDL-C mg/dl	34.70	10.80	35.49	11.95	0.936
VLDL mg/dl	33.63	20.73	27.31	12.21	0.082
LDL-C mg/dl	70.89	44.05	91.68	46.57	0.041
TC:HDL-C	4.95	1.40	4.98	2.52	0.775
CRI I= TC/HDL-C	4.95	1.40	4.98	2.52	0.775
Non HDL-C= TC-HDL-C mg/dl	130.01	43.14	124.19	46.22	0.572
CRI II= LDL-C/HDL-C	2.15	1.27	2.90	2.11	0.074
Ac (TC-HDL-C)/ HDL-C	3.95	1.40	3.98	2.52	0.775
AIP = log TG/HDL-C	0.65	0.25	0.57	0.28	0.260
TG/HDL –C	5.20	2.73	4.68	4.10	0.940
S. Creatinine mg/dl	0.89	0.16	1.11	0.66	0.028
GFR (MDRD) ml/min	97.11	18.36	73.55	23.49	0.000
n=		47		44	

Fig -1: Diagram showing age (years) in young and old age stroke cases

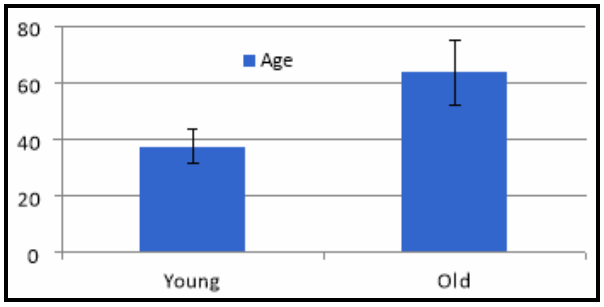


Fig-2: Diagram showing serum LDL-Cholesterol levels (mg/dl) in young and old age stroke cases

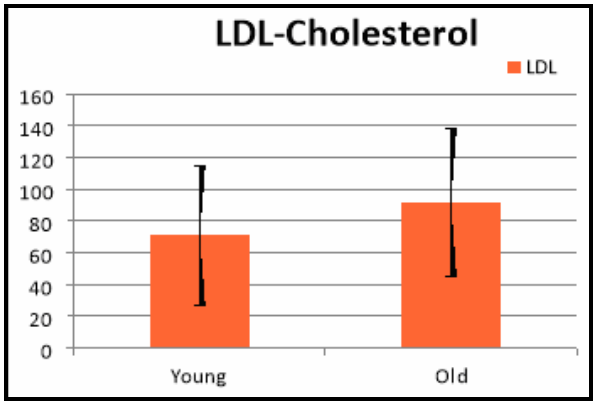
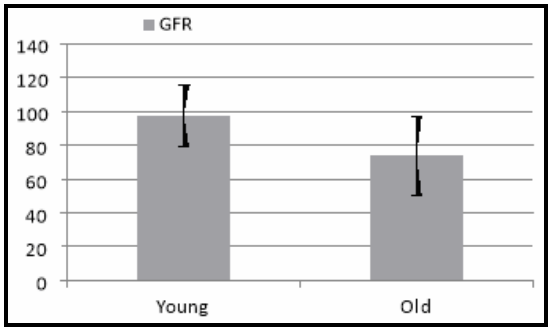


Fig-3: Diagram showing GFR (ml/min) in young and old age stroke cases



The average age of young stroke cases was 37±5.99 years and old age stroke cases were 63.52±11.34 years (Fig1). In the study there was increase in LDL- cholesterol values in old age stroke cases (91.68±46.57) mg/dl as compared to young age stroke cases (70.89±44.05) mg/dl (Fig 2). An increase in serum creatinine value is observed in old age cases in comparison to young age cases with p value of 0.028. GFR is decreased in old age stroke cases as compared to young cases with p value of <0.01 (Fig 3).

Table-2: Comparision of age, serum creatinine and lipid indices between young males and old age males stroke cases(Mean±SD)					
	Young Males Mean	**SD(±)**	**Old males Mean**	**SD(±)**	**p value**
Age yrs	37.81	5.50	64.23	11.20	0.000
Total Cholesterol mg/dl	162.55	53.17	156.63	55.33	0.669
Triglycerides mg/dl	176.89	115.61	140.00	63.83	0.129
HDL –C mg/dl	32.79	9.85	30.87	10.65	0.462
VLDL mg/dl	69.98	55.68	40.51	34.78	0.129
LDL-C mg/dl	71.43	45.36	91.07	49.28	0.110
TC:HDL-C	5.12	1.43	5.57	2.76	0.418
CRI I= TC/HDL-C	5.12	1.43	5.57	2.76	0.418
Non HDL-C= TC-HDL-C mg/dl	129.76	47.68	125.77	49.99	0.749
CRI II= LDL-C/HDL-C	2.24	1.28	3.10	2.44	0.087
Ac (TC-HDL-C)/ HDL-C	4.12	1.43	4.57	2.76	0.418
AIP = log TG/HDL-C	0.70	0.22	0.65	0.30	0.504
TG/HDL –C	5.64	2.85	6.00	6.01	0.764
S. Creatinine mg/dl	0.93	0.12	1.24	0.76	0.028
GFR (MDRD) ml/min	100.09	17.38	73.60	25.58	0.028
N=		32		30	

Table-3: Comparision of age, serum creatinine and lipid indices between young males and young females stroke cases(Mean±SD)			
	Young males Mean ± SD	**Young females Mean ± SD**	**p value**
Age yrs	37.81 ± 5.50	36.33 ± 7.04	0.436
Total Cholesterol mg/dl	162.55 ± 53.17	169.34 ± 36.37	0.657
Triglycerides mg/dl	176.89 ± 115.61	149.57 ± 71.95	0.406
HDL-C mg/dl	32.79 ± 9.85	38.78 ± 11.92	0.076
VLDL	35.38 ± 23.12	29.91 ± 14.39	0.406
LDL-C mg/dl	71.43 ± 45.36	69.71 ± 42.64	0.902
TC:HDL-C	5.12 ± 1.43	4.60 ± 1.32	0.234
CRI I= TC/HDL-C	5.12 ± 1.43	4.60 ± 1.32	0.234
Non HDL-C= TC-HDL-C mg/dl	129.76 ± 47.68	130.56 ± 32.87	0.953
CRI II= LDL-C/HDL-C	2.24 ± 1.28	1.94 ± 1.26	0.455
Ac (TC-HDL-C)/ HDL-C	4.12 ± 1.43	3.60 ± 1.32	0.234
AIP = log TG/HDL-C	0.70 ± 0.22	0.56 ± 0.27	0.065
TG/HDL –C	5.64 ± 2.85	4.24 ± 2.27	0.102
S. Creatinine mg/dl	0.93 ± 0.12	0.80 ± 0.21	0.006
GFR (MDRD) ml/min	100.09 ±17.38	90.73 ± 19.36	0.104
N=	32	15	

Table 2 shows comparative study between young and old male stroke cases. In old male stroke cases there is increase in serum cholesterol, triglyceride, LDL- cholesterol, Tc: HDL-cholesterol, CR II, atherogenic coefficient, TG/ HDL-cholesterol ratio and serum creatinine levels. However, there is decrease in HDL-cholesterol, eGFR in older cases. There is no significant change in values of various parameters except for slight increase in serum creatinine in young males as compared to young female stroke cases (Table 3). This rise in serum creatinine can be due to increase in muscle mass in young males as compared to their female counter parts (Fig 4).

Fig-4: Diagram showing serum creatinine (mg/dl) in young males and old age males stroke cases

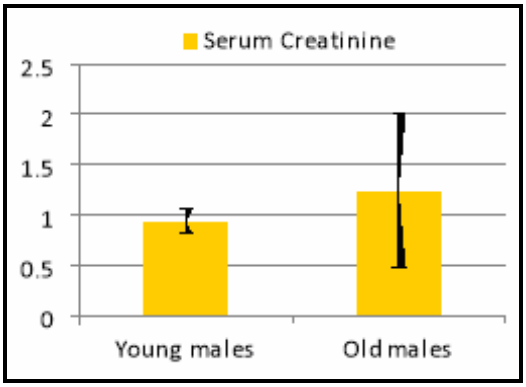


Table-4: Comparision of age, serum creatinine and lipid indices between young and old age females stroke cases(Mean±SD)

	Young females Mean	SD(±)	Old females Mean	SD(±)	p value
Age yrs	36.33	7.04	62.00	11.91	0.000
Total Cholesterol mg/dl	169.34	36.37	166.23	37.36	0.822
Triglycerides mg/dl	149.57	71.95	129.08	56.08	0.402
HDL-C mg/dl	38.78	11.92	43.55	12.57	0.304
VLDL mg/dl	73.33	56.45	38.50	33.03	0.402
LDL-C mg/dl	69.71	42.64	90.12	42.66	0.209
TC:HDL-C	4.60	1.32	4.00	1.11	0.203
CRI I= TC/HDL-C	4.60	1.32	4.00	1.11	0.203
Non HDL-C= TC-HDL-C mg/dl	130.56	32.87	118.42	29.90	0.319
CRI II= LDL-C/HDL-C	1.94	1.26	2.19	1.16	0.583
Ac (TC-HDL-C)/ HDL-C	3.60	1.32	2.63	1.18	0.047
AIP = log TG/HDL-C	0.56	0.27	0.45	0.26	0.283
TG/HDL –C	4.24	2.27	3.27	1.72	0.206
S. Creatinine mg/dl	0.80	0.21	0.84	0.19	0.562
GFR (MDRD) ml/min	90.73	19.36	73.43	19.12	0.023
N=		15		14	

Fig-5: Diagram showing Ac ratio in young females and old age female stroke cases

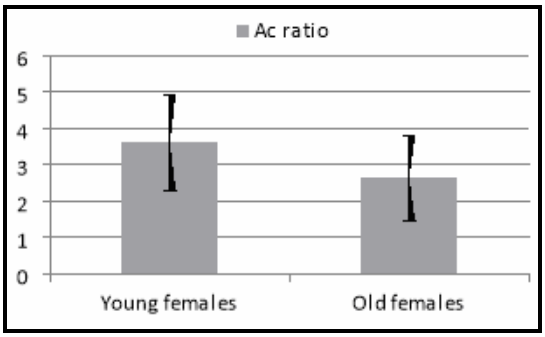


Fig-6: Diagram showing serum HDL- Cholesterol (mg/dl) in old age males and females stroke cases

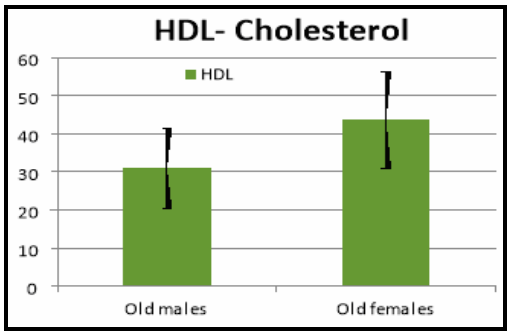
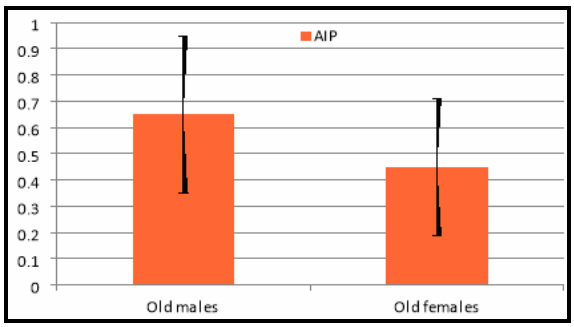


Table-5: Comparision of age, serum creatinine and lipid indices between old age males and old age females stroke cases(Mean±SD)					
	Old males Mean	**SD(±)**	**Old females Mean**	**SD(±)**	**p value**
Age yrs	64.23	11.20	62.00	11.91	0.549
Total Cholesterol mg/dl	156.63	55.33	166.23	37.36	0.560
Triglycerides mg/dl	140.00	63.83	129.08	56.08	0.586
HDL-C mg/dl	30.87	10.65	43.55	12.57	0.001
VLDL mg/dl	28.00	12.77	25.82	11.22	0.586
LDL-C mg/dl	91.07	49.28	90.12	42.66	0.951
TC:HDL-C	5.57	2.76	4.00	1.11	0.047
CRI I= TC/HDL-C	5.57	2.76	4.00	1.11	0.047
Non HDL-C= TC-HDL-C mg/dl	125.77	49.99	118.42	29.90	0.626
CRI II= LDL-C/HDL-C	3.10	2.44	2.19	1.16	0.196
Ac (TC-HDL-C)/ HDL-C	4.57	2.76	2.63	1.18	0.016
AIP = log TG/HDL-C	0.65	0.30	0.45	0.26	0.035
TG/HDL-C	6.00	6.01	3.27	1.72	0.104
S. Creatinine mg/dl	1.24	0.76	0.84	0.19	0.062
GFR (MDRD) ml/min	73.60	25.58	73.43	19.12	0.982
n =		30		14	

Table-6: Fischer Exact chart of lipid profiles between young and old age stroke cases					
	Groups	**Values**	**Young (N=47)**	**Old (N=44)**	**P value**
Triglycerides mg/dl	Normal	<150	25	29	0.4945
	Borderline high	150 – 199	13	8	
	High	200 – 499	8	7	
	Very high	≥ 500	1	0	
Total Cholesterol mg/dl	Desirable	< 200	36	35	1.0
	Borderline high	201 – 239	8	7	
	High	≥240	3	2	
LDL cholesterol mg/dl	Optimal	<100	33	25	0.1096
	Near optimal	100 – 129	9	8	
	Borderline high	130 – 159	3	9	
	High	160 – 189	2	0	
	Very high	≥190	0	2	
HDL cholesterol mg/dl	Low	< 40	30	31	0.7358
		40 - 59	16	12	
	High	≥ 60	1	1	
Non HDL cholesterol mg/dl	Goal	<130	23	26	0.3948
		≥130	24	18	
TC:HDL cholesterol ratio	No risk	<5	25	26	0.5215
	risk	≥5	22	18	

Fig-7: Diagram showing atherogenic index of plasma (AIP) in old age males and females stroke cases



Between the female stroke cases, there is slight increase in atherogenic coefficient in young females as compared to older females (Fig 5), as older females had better HDL-cholesterol values as compared to young female stroke cases (Table 4). However, young female cases had eGFR within physiological limits as compared to older female cases due to better perfusion of kidney.

Table 5, shows older male stroke cases had higher atherogenic coefficient and atherogenic index of plasma as compared to older female stroke cases (Fig 7), due to higher TGL and lower HDL-cholesterol (Fig 6) value as compared to their female counter parts. In the study an average of 68.1% of stroke cases were male and 31.9% of stroke cases were female. As per Fisher Extract no significant correlation could be established between various components of lipid profile and the incidence of stoke in the present study (Table 6).

Discussion

The study was taken up to find if there is any relevant relation between change in lipid profile values and the incidence of stroke. The further it was tried to establish if there is any significant relation between lipid profile and alteration in renal profiles between young and elderly stroke cases. Lipid profile changes are thought to be a risk factor in the occurrence of stroke. On the other hand, stroke itself is also associated with changes in the lipid levels probably because of the accompanying stress and catecholamine overproduction that occurs during an acute stroke.

The relationship between serum cholesterol levels and the risk of stroke is not clear. There are reports of varied correlation between the level of serum total cholesterol and the risk of stroke. Diabetes mellitus because of its common association with dyslipidemia is a common cause of stroke. However, little is known regarding the clinical pattern, outcome and predictors of early mortality after stroke in patients without diabetes. Dyslipidemia is also one of the major risk factors noted in patients of stroke without diabetes mellitus.

During the study period 91 stroke cases, between 20-80 years of either gender who were admitted to hospital were recruited for the study. 47 stroke cases in the study were between 20-45 years and 44 stroke cases were between 46- 80 years of age. In our study, though male cases (59) with stroke were more as compared to their female counter parts (32). This could be due to selection bias at a tertiary care center and hence further epidemiological studies will be needed to prove this hypothesis.

This finding was similar to study by Roquer et.al [7], where they found that the mean age of stroke affected patients was higher in women. The serum total cholesterol levels in 5 patients having stroke were high (Total cholesterol > 240mg% according to the Adult Treatment Panel (ATP) – III guidelines of National Cholesterol Education Program (NCEP)). Rastenyte et.al [8] found that serum cholesterol levels are not related to risk of death from stroke.

The levels of serum LDL cholesterol was significant in our study conducted on elderly stroke cases. Sreedharan et.al [9] showed raised levels of serum LDL cholesterol had significant risk of ischemic stroke in non-diabetics. Huxley et.al [10] have showed positive correlation between LDL cholesterol levels and risk of stroke.

The rise in serum creatinine level in old age stroke cases is seen as compared to young age stroke cases with p value of 0.028. GFR is decreased in old age stroke case due to decrease in renal perfusion as compared to young age stroke cases in the study. There is increase in total cholesterol, triglyceride, LDL- cholesterol and atherogenic coefficient in older male stroke cases as young male stroke cases. There is increase in atherogenic

coefficient in older female stroke cases as compared to younger female stoke cases, and other profiles were not significantly changed. The study shows higher predisposition to atherogenesis in older age cases in comparison to young age stroke cases. Similarly, decrease in HDL-cholesterol and GFR is seen in older male and female stroke cases in comparison to younger male and female stroke cases.

Findings of the present study indicating no role for TG in the ischemic strokes are on the contrary to that of certain previous studies. Pandey et.al [11] found a positive correlation of serum triglyceride levels with patients suffering from atherothrombotic strokes and Transient Ischemic attacks (TIA) as compared to control subjects. However, Sridharan [12] has not found any correlation between triglycerides and stroke.

Cholesterol can be differently involved in stroke, depending on the etiologic subtype. In this view, a large part of the inconsistency of observational data from this study on the relation between cholesterol and stroke can be due to gathering data by having both types of stroke together in a same group. This can explain the absence of any detectable association between cholesterol and stroke. Older females had increase in HDL-cholesterol as compared to older male stroke cases. Older males also had higher atherogenic coefficient due to increased triglycerides and decreased HDL-cholesterol. This may the reason for increased number of elderly male stroke cases as compared to their female counterparts. Kurth et.al [13] showed remarkable increase in serum LDL- cholesterol levels in ischemic stroke patients.

Due to the possible differences in the effects of cholesterol at different vascular sites, could be the reason of complex association between serum cholesterol levels and stroke [14]. The origin of the internal carotid artery is probably the most common site of atherosclerosis that leads to Transient Ischemic Attack (TIA) or stroke.

The atherogenic index of plasma (AIP) is a critical index that can be used as a stand-alone index for cardiac risk estimation. AIP which is defined as logarithm [log] of the ratio of plasma concentration of triglyceride to HDL-cholesterol and is strongly correlated with cardiovascular risks. It can act as an adjunct over the individual lipid profile. AIP is the best determinant for fractionated esterification rate of HDL-cholesterol and more useful than routine lipid parameters. It can be used as a diagnostic indicator when the other atherogenic risk parameters appear normal. The AIP calculation estimates the values of "zone of atherogenic risk". AIP value were found higher in old male stroke cases as compared to older female cases, however no significant changes were observed between young male and female stroke cases. Existing guidelines, however, do take advantage of non–HDL cholesterol as an index of risk associated with this combined dyslipidemia [15].

The recognition of this index is not new; this "beta" lipoprotein cholesterol fraction has been associated with increased CHD mortality in certain studies. Non–HDL cholesterol is simply defined as the difference between total and HDL cholesterol and, thus, represents cholesterol carried on all of the potentially proatherogenic apo B-containing particles [i.e. VLDL, IDL, and LDL- cholesterol as well as chylomicron remnants and lipoprotein].

The Atherosclerosis Risk in Communities (ARIC) study has concluded that the relation of circulating cholesterol to ischemic stroke does not resemble its well-known relation to coronary heart disease [16]. Our study found a positive correlation between serum HDL-cholesterol, AC, AIP levels and risk of stroke in certain groups only. Our study could not establish correlation between serum triglycerides, cholesterol, LDL- cholesterol, and VLDL levels with risk of stroke. Additional studies focused on HDL-cholesterol and VLDL levels might show some correlation.

Stroke patients with dyslipidemia need a comprehensive health care approach involving dietician, physician and good biochemistry back up. In Indian scenario, where majority of the patients belong to the low socio-economic status, life style modification plays a more important role in prevention and management of stroke and dyslipidemia in contrast to high cost of lipid lowering agents. Dyslipidemia is

a tip in iceberg. Dyslipidemia, being a modifiable risk factor for stroke if properly treated can decrease the incidence of stroke. This can decrease morbidity and mortality due to stroke leading to a healthier society. Dyslipidemia are found to be associated with 1.8 - 2.6 times, relative risk of stroke. Stroke prevention by aggressive reduction in cholesterol levels (SPARCL)-9 trial showed reduction in secondary stroke levels in patients with recent stroke on TIA.

Conclusion

The study suggests annual health checkups from younger age for lipid profile and associated profiles. This shall help to identify and to reduce modifiable risk factors with life style changes thereby diminish incidence of stroke in younger age group population.

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