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Alteration of Serum Homocysteine and Lipid Profile Levels in Stroke Patients

Mrs. Mandakini Kshirsagar[1], Dr.Ajit Sontakke[2]

[1]M.Sc. Medical Biochemistry,
[2]MD Biochemistry

[1]Department of Biochemistry, Krishna Institute of Medical Sciences Deemed University, Karad-415539, Dist. Satara, (Maharashtra), India

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ABSTRACT:

Background: The dyslipidemia and hyperhomocysteinemia are the major risk factors for cardiovascular disease (CVD) and stroke patients. Hyperhomocysteinemia induces thrombogenicity and promotes the proliferation of smooth muscle cells, which could lead to vascular constriction and stiffness are well documented in literature. *Aim and Objectives:* The main aim of our study to see the serum homocysteine and lipid profile levels in stroke patients of our hospital. *Material and Methods:* Thirty stroke patients admitted to medicine ward of Krishna hospital, Karad (Maharashtra), were included and compared with same age and sex matched healthy controls. **Results**: Serum homocysteine level (191.8%), ratios of total cholesterol/High density lipoprotein (TC/HDL) (27.08%), and low density lipoprotein /High density lipoprotein (LDL/HDL) (32.66%) were significantly increased in stroke patients as compared to controls. HDL (-17.1%) was significantly decreased in stroke patients as compared to controls. Serum Triglycerides (TG) level (7.18%), Very low density lipoprotein VLDL (8.46%), TC (2.02%) and LDL (10.28%), were not significantly increased in stroke patients as compared to controls. *Conclusions:* It concludes that the increased serum homocysteine level in this study might be possible cause for the stroke. Along with lipid profile parameters the estimation of homocysteine is also now days beneficial to find out the risk of stroke.

Keywords: homocysteine, lipid profile, stroke.

1. Introduction

Strokes occur mainly due to the poor blood supply to the brain, because of blockage in blood supply or a ruptures of blood vessel within the brain or brain tissue damage. The brain tissue does not receive enough oxygen or nutrients in a stroke. Mainly there are three types of stroke,
i.e ischemic strokes, hemorrhagic strokes and transient ischemic attacks [1].
Ischemic stroke is the most common form of stroke, accounting for around 85% of strokes and caused by blockages or narrowing of the arteries that provide blood to the brain. These blockages are may be due to blood clots or deposition of fat in the arteries [1, 2].
Hemorrhagic stroke are mainly due to leaking blood or bursting arteries in the brain. The leaked blood puts pressure on brain cells cause the brain tissue damage. Rupturing of arteries may be due to hypertension, trauma, blood-thinning medications and aneurysms (weaknesses in blood vessel walls). In transient ischemic attack the flow of blood to the brain is only briefly interrupted and similar to ischemic strokes [1, 2]. According to the Centers for Disease Control and Prevention (CDC), over a third of people who got transient ischemic attack go on to have a major stroke within a year if they have not received any treatment [3].
Stroke is one of the major causes of death and disability in India and approximately prevalence rate of stroke range, 84-262/100,000 in rural and 334-424/100,000 in urban areas. The incidence rate is 119-145/100,000 based on the recent population based studies [4]. The incidence of stroke is increase because of smoking, hypertension, dyslipidemia, diabetes mellitus and aging. Early identification of these risk factors may be useful to prevent the stroke and reduced the mortality rates [5, 6].
Previous studies have shown that serum triglycerides (TGs) and total cholesterol (TC) and lipoproteins are contributed to the development of atherosclerosis, which is the precursor to stroke. Hypercholesterolemia is a moderate risk factor for stroke. Elevated plasma concentration of low-density lipoproteins (LDL) and decreased high-density lipoprotein concentration (HDL) is associated with an increased risk of atherosclerosis [7].
Earlier studies shown that plasma homocysteine is an independent risk factor for coronary heart disease and stroke [8-12] and increased homocysteine levels have been found to cause oxidative
Stress, endothelial dysfunction, and atherothrombosis [13] and associated with higher mortality rates from stroke and coronary heart disease [14-15].

Therefore, this present study was designed to evaluate the serum lipid profile and homocysteine levels of patients who had experienced an acute stroke during the first 24-hour and compared these levels with healthy individuals.

2. Material and Methods

Study Design:

The study was carried out in the department of Biochemistry and Medicine, Krishna hospital, Karad (Maharashtra), India. In this study 30 stroke patients admitted to medicine ward were taken and compared with 30 same age and sex matched healthy controls. All the stroke patients were diagnosed by doing detailed medical and physical examinations by physician.
Several diseases such as renal and thyroid dysfunction, cancer, psoriasis, and diabetes as well as various drugs, alcohol, tobacco, coffee, and menopause, are believed to be associated with moderately elevated homocysteine concentrations are not included in our study.

Biochemical Parameters

Overnight fasting blood samples were collected for estimation of homocysteine and lipid profile.

Homocysteine: Serum homocysteine was measured by Autopure homocysteine enzymatic kit method by Accurex biomedical Pvt. Ltd, using the principle of conversion of oxidized Homocysteine to reduced form and then converting into S-adenosyl-L-Homocysteine [16].

Cholesterol: Serum cholesterol was estimated by using Cholesterol Oxidase Peroxidase (CHOD-PAP) method. Cholesterol esters are enzymatically hydrolysed by cholesterol esterase to cholesterol and free fatty acids. Free cholesterol then oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide.The hydrogen peroxide combines with 4-aminoantipyrine to form a chromophore (quinoneimine dye) which was measured at 505 nm. [17]

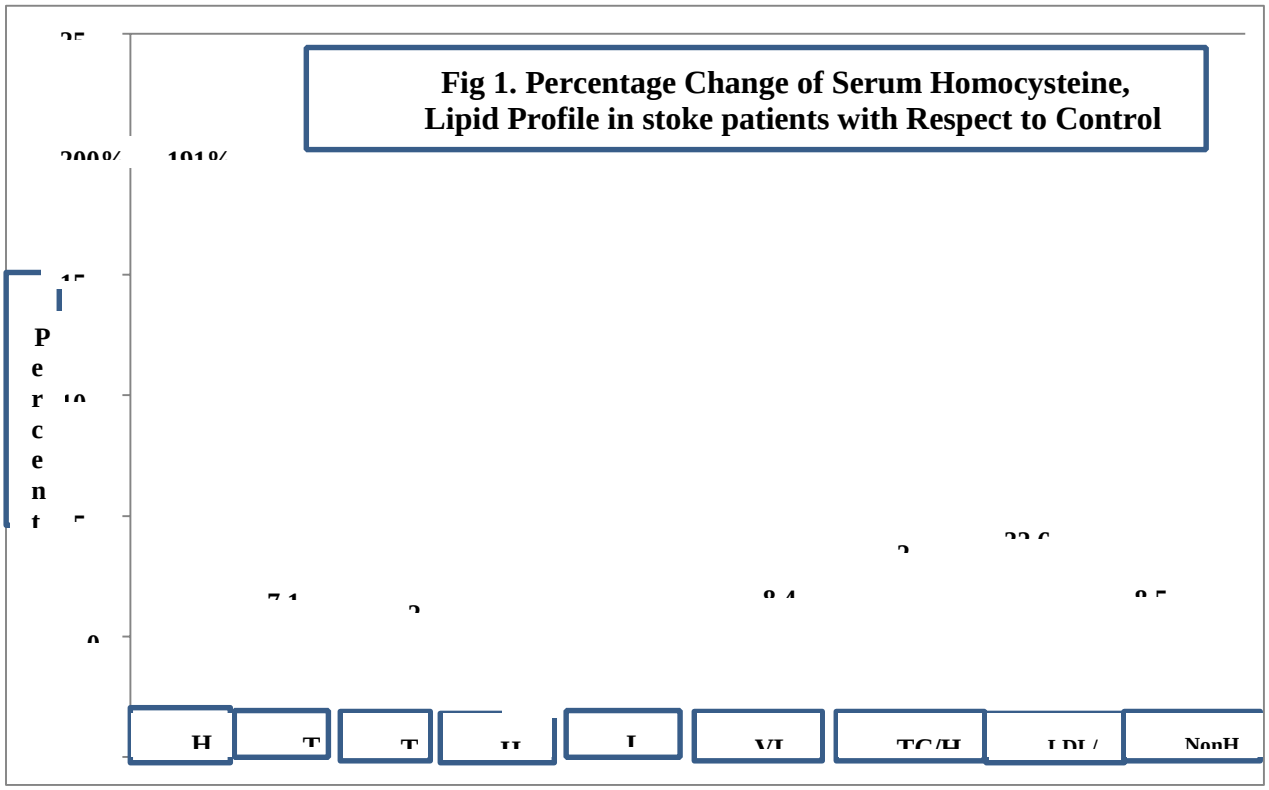
Triglycerides: Serum triglycerides estimated by (GPO / PAP Method) using the principal Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol is converted to glycerol 3 phosphate in the presence of glycerol kinase and ATP, which is oxidised by the enzyme glycerol phosphate oxidase to form hydrogen peroxide. The glycerol-3-phosphate is oxidized by molecular oxygen in the presence of GPO (Glycerol Phosphate Oxidase) to produce hydrogen peroxide (H2O2) and dihydroxyacetone phosphate. The formed H2O2 reacts with 4- aminophenazone and N,N-bis(4-sulfobutyl)-3,5-dimethylaniline, disodium salt (MADB) in the
Presence of peroxidase (POD) to produce a chromophore, which is read at 660/800nm. The increase in absorbance at 660/800 nm is proportional to the triglyceride content of the sample [18].
HDL: Serum HDL was estimated by using modified Polyvinyl Sulfonic acid (PVS) and polyethylene-glycol-methyl ether (PEGME) coupled classic precipitation method [19] LDL, VLDL and chylomicron (CM) react with PVS and PEGME and the reaction results in inaccessibility of LDL, VLDL and CM by cholesterol oxidase (CHOD) and cholesterol esterase (CHER). The enzymes selectively react with HDL to produce H_2O_2 which is detected through a Trinder reaction.
LDL, VLDL and Non- HDL: Serum LDL and VLDL were calculated by using Friedwald's Equation.
[LDL- Cholesterol] = [Total Cholesterol] − [HDL-Cholesterol] − [Triacylglycerol] / 5.
VLDL = Triglycerides / 5
Serum Non-HDL Cholesterol concentrations were calculated by subtracting HDL-C concentrations from total cholesterol concentrations. [20].
Statistical analysis: Statistical significance of this study were calculated by student't' test and percentage change by using instant graph pad software.

3. Results

Table 1: Mean and SD of Serum Homocysteine and Lipid Profile of Healthy Control Subjects and Stroke

Sr. No	Parameters	Control Group (N=30)	Stroke (N=30)
1	Homocysteine (µmol/lit)	15.63 ± 4.1	*** 16.42 ± 5.4
9	Non HDL	112 ± 22.7	122 ± 32.4
2	Total Cholesterol (mg/dl)	157.8 ± 22.3	161 ± 35.3
3	Triglyceride (mg/dl)	104 ± 31.5	112 ± 44.7
4	HDL (mg/dl)	46.6 ± 9.2	*** 38.6 ± 8.5
5	LDL (mg/dl)	90 ± 20.7	100 ± 32.3
6	VLDL (mg/dl)	20 ± 6.5	22 ± 9
7	TC / HDL	3.36 ± 0.63	*** 4.27 ± 1.10
8	LDL / HDL	1.99 ± 0.64	** 2.64 ± 0.96

Figures in table indicate mean and SD.
$^{**}p<0.01$, $^{***}p< 0.001$, ·Non Significant as compared to control group.



4. Discussion

Serum homocysteine level was significantly increased in stroke patients ($p< 0.001$, 191% %) as compared to control subjects. The exact cause of hyperhomocysteinemia in these stroke patients is not known, since serum homocysteine level is increased by various reasons, it may be due to inherited genetic defects of Cystathionine β-Synthase [CBS] and deficiency of coenzyme i.e vitamin B6, this enzyme and coenzyme are required for conversion of homocysteine to cystathioinine. Secondly due the deficiency of enzymes and coenzymes required for the
Synthesis of methionine from homocysteine such as N^5 N^{10} Methylenetetrahydrofolate reductase, methionine synthase, vitamins folic acid and B_{12}. The most common one that is detected worldwide and has a high incidence in different populations, is single nucleotide polymorphisms of $N^{5,}$ N^{10}-methylenetetrahydrofolate reductase which has been associated with mild (13–24 μM) and moderate (25–60 μM) hyperhomocysteinemia [21]. We are unable to rule out the exact cause of hyperhomocysteinemia in stroke patients, since we have not measured these serum enzymes and coenzymes levels due to fund constraint.
Hyperhomocysteinemia may be a strong indicator of stroke risk among people with heart disease reported in several studies [22-25]. The heart patients with the four time increased homocysteine levels are likely to suffer the most common type of stroke compared with those with the lowest homocysteine levels reported in literature [26]. The homocysteine interferes with the formation of intermolecular cross-links that help stabilize the collagen macromolecular network. It induced the free radical generation and also causes the thrombosis, mental retardation, osteoporosis, atherosclerosis, myocardial infarction, stroke, pulmonary embolism are well documented in literature. The oxidative damage to the vascular endothelium and the proliferation of the vascular smooth muscle create a prothrombotic condition, which contributes to the development of premature atherosclerosis reported in earlier studies [27-29].
Therefore, the increased serum homocysteine level in our study may be induced more generation of free radicals, which might be the cause of development of atherosclerosis and results the stroke.

Population studies show that too much homocysteine in the blood is related to a higher risk of cardiovascular disease and stroke, so high homocysteine may be a marker of increased risk [26].
Previous studies have shown that homocysteine levels can be effectively lowered by consuming more folic acid and vitamin B_{12}. Natural sources of these nutrients include citrus fruits, tomatoes, vegetables, and grain products [26].
Hence, from the earlier reports and present results, it indicates that the increased serum homocysteine level might be cause of stroke in our study.
The lipid profile status in stroke patients were estimated in our study and observed the significantly increased ratios of TC/HDL ($p<0.001$, 27%) and LDL/HDL ($p<0.01$, 32.60%) as compared to control subjects. Serum HDL level ($p<0.001$, -17%), was significantly decreased
And serum total cholesterol, triglyceride, VLDL, LDL and Non- HDL levels were not altered in stroke patients as compared to control group.
Some of the earlier studies have found total cholesterol and triglycerides levels to be elevated in patients with stroke [30]. However, in our study we could not find any significant differences in serum total cholesterol and triglycerides levels as compared to control and these results clearly indicates that the stroke is not due to the cholesterol and triglycerides.
We have calculated the ratios of Total Cholesterol (TC) / High-Density Lipoprotein Cholesterol (HDL-C) and Low-Density Lipoprotein Cholesterol (LDL-C) / HDL-C, since these ratios of TC/HDL and LDL/HDL are used to predict ischemic heart disease risk. But there is, no consensus on which of these 2 indices is superior [31].
Elevated LDL-C concentration in plasma is atherogenic, whereas a high HDL-C level is cardio protective reported in earlier study [32, 33], the measurement and interpretation of LDL-C and HDL-C levels is emphasized in the US National Cholesterol Education Program guidelines [34]. According to these guidelines, LDL-C concentration should be considered the primary therapeutic target, whereas HDL-C levels may also be critical in the assessment of CHD risk [34].
Results of prospective studies have suggested that a high LDL-C/HDL-C ratio combined with hypertriglyceridemia is associated with highest CHD risk. This dyslipidemic state has been described as atherogenic dyslipidemia [35 - 37].
Serum HDL level ($p<0.001$, -17%), was significantly decreased in this study might be one reason to increased the ratios of TC/HDL and LDL/HDL. Low levels of HDL cholesterol raise stroke risk because HDL cholesterol carries cholesterol away from the arteries and back to the liver where it is removed from the body.
Low levels of LDL-C and high levels of HDL-C did not protect the patients against the homocysteine induced coronary artery disease reported by several studies [38]. Therefore, from the earlier reports and present results, it concludes that the increased serum homocysteine level in this study might be possible cause for the stroke. Along with lipid profile parameters the estimation of homocysteine is also now days beneficial to find out the risk of stroke

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