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Original Article

A Study of Lipid Profile in Non-diabetics with Stroke

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Abstract

Aim: The aim is to study the serum lipid profile in non-diabetics with stroke and to determine the significant correlation between them.

Materials and Methods: A cross-sectional study was conducted on patients period of 12 months from May 2018–June 2019, Mahatma Gandhi Memorial Hospital for 6 months. Patients and controls were tested for fasting lipid profile 12 h after overnight fast. Participants were 60 patients of non-diabetic stroke and 60 controls. Among the 60 patients, 37 were male and 23 were female. In controls, there were 37 males and 23 females. Age- and sex-matched controls were selected. Stroke patients with infarct or hemorrhage in computed tomography brain were included in the study.

Results: In this study, total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, and triglycerides were significantly associated with the risk of stroke. In this study, 56.7% of patients had high-density lipoprotein <40 mg/dl, 41.7% had TC >200 mg/dl, 65% of them had LDL cholesterol >100 mg/dl, and 43.3% of patients had very LDL >30 mg/dl.

Key words: Dyslipidemia, Lipid profile, Non-diabetic stroke

INTRODUCTION

Stroke or a cerebrovascular accident is an acute neurological injury which occurs due to vascular pathology and presents as a brain infarction or hemorrhage. Stroke is a medical emergency. The risk factors of stroke have been identified. The modification of risk factors in stroke has brought down both mortality and morbidity of stroke remarkably in the past 30 years.

Dyslipidemia, as a major risk factor for stroke, is studied for many years. Various studies in different population have shown dyslipidemia that is associated with stroke. Dyslipidemia is a correctable risk factor. It has been shown that the reduction of total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, triglycerides, very LDL (VLDL) cholesterol, and increasing high-density lipoprotein (HDL) cholesterol by drugs has decreased the incidence of stroke.

In our study, lipid profile was studied in non-diabetic patients with stroke. Diabetes itself is associated with hyperlipidemia and increased atherosclerosis which makes it an undisputed risk factor for stroke. The atherogenicity of diabetics and non-diabetics is different. Hence, non-diabetic patients were included in the study.

The study is titled as "A STUDY OF LIPID PROFILE IN NON-DIABETICS WITH STROKE."

Aim

This study aims to study the serum lipid profile in non-diabetics with stroke and to determine the significant correlation between them.

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MATERIALS AND METHODS

The study was conducted on 60 non-diabetic stroke patients and 60 age- and sex-matched controls who did not have a stroke after obtaining informed consent.

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This is a cross-sectional study conducted over a period of 6 months in Mahatma Gandhi Memorial Hospital. Detailed history, clinical examination, radiological examination, serum TC, LDL, VLDL, HDL, and triglycerides were estimated by enzymatic method.

Inclusion Criteria

All patients with stroke with hemorrhage or infarct in computed tomography brain were included in the study.

Exclusion Criteria

The following criteria were excluded from the study:

- Patients with diabetes mellitus
- Patients on drugs for dyslipidemia
- Patients on dietary modification for dyslipidemia cerebral infarct associated with trauma or tumor.

Collection of Blood Sample

Blood samples were collected from all patients after an overnight fast of minimum 12 h. The previous day patient was advised to have light fat-free diet. Sample collected from cubital fossa. Tourniquet was released just before sample collection to avoid increased serum lipids artifactually. Ten milliliters of blood were drawn in sterile syringes and blood was transferred to dry glass tubes.

Preparation of Serum

Serum for HDL was separated within 2 h of collection. The sample was centrifuged at 5000 rpm for 10 min in a centrifuge tube. The clear serum was pipetted out and stored at 4°C. Samples were analyzed within 24 h.

Serum TC Estimation

Serum TC is measured by cholesterol peroxidase method. This method has extended stability. Reconstituted reagent is stored at 2–8°C and is stable for 90 days. This method is linear up to 500 mg/dl.

Principle

Enzymatic calorimetric method of the determination of TC is by the following reactions:

$$\text{Cholesterol} + \text{water} \xrightarrow{\text{Cholesterolesterase}} \text{fattyacid} + \text{cholesterol}$$

$$\text{Cholesterol} + \text{oxygen} \xrightarrow{\text{Cholesterolesterase}} 4\text{-cholesten-3-one} + H_2O_2$$

$$2H_2O_2 + \text{phenol} + 4\text{-aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{redquninone} + 4H_{20}$$

The reagent is stable when stored at 2–8°C up to expiry date. The reagent is linear up to the value of 500 mg/dl, if the concentration is >500 mg/dl, the sample has to be diluted with normal saline and the assay has to be repeated and the result has to be repeated with dilution fraction.

Reagents

- CHOLESTEROL R1: 2 ml × 50 ml/4 ml × 50 ml/4 ml ×100 ml/2 ml × 405 ml Phenol – 24 mmol/L
  - Sodium cholate – 0.2 mmol/L Sipes buffer
  - pH (6.9) – 50 mmol/L.
- CHOLESTEROL R2: 2 ml × 50 ml/4 ml × 50 ml/4 ml × 100 ml/8 ml × 100 ml
  - Cholesterol esterase >200 U/L
  - Peroxidase >1000 U/L
  - Cholesterol oxidase <250 U/L
  - 4-aminoantipirine – 0.5 mmol/L.
- CHOLESTEROL STANDARD: 1 ml × 5 ml/1 ml × 5 ml/1 ml × 5 ml/2 ml × 5 ml.
  - Cholesterol standard concentration 200 mg/dl.

Preparation of Working Reagent

Dissolve reagent R1 and R2 of cholesterol is shown on the label sample – serum.

Mix the contents and incubate at 37°C for 5 min. Absorbance of the standard and sample to be measured against reagent blank.

Table 1: Preparation of working reagent

Parameter	Blank	Standard	Sample
Working reagent	1000 µL	1000µL	1000µL
Standard	-	10 µL	-
Sample	-	-	10 µL

Table 2: Procedure

Sample	300 µL
High-density lipoprotein reagent	300 µL

Table 3: Working Reagent

Parameter	Blank	Standard	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Standard (HDL)	-	50 µL	-
Sample (HDL supernatant)	-	-	50 µL

HDL: High-density lipoprotein

Table 4: Preparation of working reagent

Parameter	Blank	Standard	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Standard	-	10 µL	-
Sample	-	-	10 µL

Table 5: Sex X diagnosis

Gender	Non-diabetics with stroke	Control
Male	61.7	61.7
Female	38.3	38.3

Calculation

Cholesterol conc. (mg/dl) = Absorbance of sample/absorbance of standard × 200.

Estimation of Serum HDL Cholesterol

The reagent measures HDL cholesterol in serum/plasma by precipitation method, linear up to 125 mg/dl.

Reagent Composition

- HDL CHOLESTEROL REAGENT: 4 ml × 25 ml
  - Magnesium chloride – 1 mmol/L
  - Phosphotungstate – 14 mmol/L.
- HDL CHOLESTEROL CONCENTRATION STANDARD – 1 ml × 5 ml
- HDL CHOLESTEROL CONCENTRATION: 50 mg/L.

Principle

VLDL, LDL, and chylomicrons are precipitated by magnesium and phosphotungstate. HDLs are concentrated in the supernatant; the following centrifugation is measured by enzymatic methods.

Reagent

Reagent is stored at 2–8°C and is stable up to expiry date. The reagent is linear up to the value of 125 mg/dl, if the concentration is >125 mg/dl, the sample has to be diluted with normal saline and the assay has to be repeated and the result has to be repeated with dilution fraction.

The reagent can be used readily.

Sample: Serum/plasma.

Procedure

Mix both reagent and sample allow it to stand for 10 min at room temperature. Remix and centrifuge for 10 min at 4000 rpm. Separate the clear precipitant within 1 h and HDL cholesterol concentration has to be determined.

Mix the contents and incubate at 37°C for 5 min. Absorbance of the standard and sample to be measured against reagent blank.

Calculation

HDL cholesterol conc. (mg/dl) = Absorbance of sample/absorbance of standard × N ×2.

- Where, 2 is dilution factor of sample
- N is the standard concentration.

Serum LDL

The immunological principle together with enzymatic assay of cholesterol is used for estimation of LDL directly.

LDL = TC–(HDL cholesterol+triglyceride/5).

Serum Triglyceride

Glycerol phosphate oxidase-p-aminophenazone methodology is used for measuring triglycerides in serum or plasma.

Reagent is stored at 2–8°C and is stable up to expiry date. The reagent is linear up to the value of 1000 mg/dl, if the concentration is >1000 mg/dl, the sample has to be diluted with normal saline and the assay has to be repeated and the result has to be repeated with dilution fraction.

Principles

Enzymatic determination of triglycerides is by the following reaction:

$$\text{Triglyceride} + \text{water} \xrightarrow{\text{Lipoproteinlipase}} \text{glycerol} + \text{fattyacid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerolkinase}} \text{glycerol} - 3 - \text{phosphate} + \text{ADP}$$

$$\text{Glycerol3phosphate} + \text{oxygen} \xrightarrow{\text{Glycerol3phosphateoxidase}} \text{dihydroxylacetonephosphate} + H_2O_2$$

$$H_2O_2 + 4 - \text{aminoantipirine} + \text{P} - \text{chlorophenol} \xrightarrow{\text{POD}} \text{redquinoneimine}$$

TRIGLYCERIDESTANDARDCONCENTRATION – 200 mg / dl

Sample – serum/plasma.

Mix the contents and incubate at 37°C for 5 min. Absorbance of the standard and sample to be measured against reagent blank.

Triglyceride concentration (mg/dl) = Absorbance of sample/absorbance of standard ×200

VLDL concentration (mg/dl) = Triglyceride/5.

Age X Diagnosis

In both control groups and people with non-diabetic stroke, <40 years are 8.3%. In 41–60 years, it is 48.3% and >60 years is 43.3%. Maximum number of patients in 41–60 years group is 48.3%.

Sex X Diagnosis

In both groups, 61.7% of the patients were male and 38.3% were female. Male-to-female ratio is 1.61:1.

Body Mass Index X Diagnosis

Body mass index	Non-diabetics with stroke	Control
Underweight	1.7	0
Normal	46.7	66.7
Overweight	50	33.3
Obese	1.7	0

In non-diabetics, 1.7% was undernourished, 46.7% was normal, 50% overweight, and 1.7% obese. Maximum number of patients – 50% was overweight. In control, 66.7% was normal and 33.3% was overweight.

Smoking X Diagnosis

Smoking	Non-diabetics with stroke	Control
Yes	23.3	0
No	76.7	100

In non-diabetics with stroke, 23.3% were smokers and 76.7% were non-smokers. In control group, 100% were smokers.

Hypertension X Diagnosis

Table 6 shows that 23.3% of non-diabetics with stroke had hypertension and 76.7% of the same group were normotensives. All controls 100% were normotensives.

TC X Diagnosis

About 43.3% of non-diabetics with stroke have TC < 200 About 16.7% of the same group had cholesterol 200–240. About 40% of the same group has cholesterol more than 240%. Maximum patients, 43.3% has normal levels of TC. In controls, 90% have normal cholesterol values <200. About 5% has cholesterol 200–240. About 5% has cholesterol more than 240.

Triglycerides X Diagnosis

Triglycerides	Non-diabetics with stroke	Control
<150	58.3	86.7
150–199	26.7	6.7
>200	15	6.7

Table 6: Hypertension X diagnosis

Hypertension	Non-diabetics with stroke	Control
Yes	23.3	0
No	76.7	100

Table 7: Total cholesterol X diagnosis

Total cholesterol	Non-diabetics with stroke	Control
<200	43.3	90
200–240	16.7	5
>240	40	5

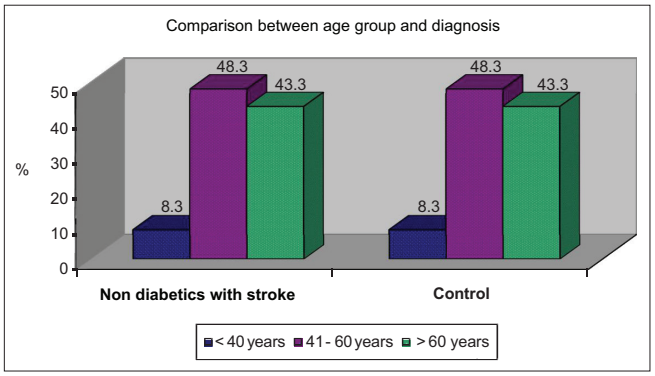

Figure 1: Age X diagnosis

In non-diabetics with stroke, <150 triglyceride value was 58.3%; 150–199 was 26.7%; and >200 was 15%. Maximum patients, 58.3% has normal triglycerides. In controls, 86% has triglyceride <150, 6% has 150–199%, and in 6% more than 200. Maximum controls, 86% has normal triglyceride values.

In non-diabetics with stroke, 53.3% had HDL cholesterol <40 and 46.7% had HDL cholesterol >40%. Maximum number of patients 53.3% had low HDL cholesterol. In control group, 36.7% had HDL <40 and 63.3% had HDL >40%. Maximum number of controls 63.3% had normal HDL values.

In non-diabetics with stroke, 71.7% had HDL/LDL ratio <0.39. Only 28.3% of the same group had HDL/LDL ratio >0.4%. In control group, 83.3% had HDL/LDL ratio >0.4 and 16.7% had HDL/LDL ratio >0.4.

In non-diabetic stroke males, 56.8% has TC/HDL >4.5 and 43.2% has <4.4. Maximum number of patients had TC/HDL ratio. In the control group, 86.5% has TC/HDL ratio in males <4.4 and 13.5% had >4.5.

In non-diabetic stroke with females, maximum number of patients had 78.3% TC/HDL ratio >4 and 21.7% had ratio <3.9%. In the control group, maximum number of controls, 65.2% has TC/HDL <3.9. 35.8% has TC/HDL more than 4.

In non-diabetics with stroke, 56.7% had VLDL <30 and 43.3% had VLDL >30%. Maximum number of patients has VLDL normal values. About 86.7% of controls had normal VLDL <30%. About 13.3% of controls have high VLDL values >30 [Figure 1 and Tables 1-7].

DISCUSSION

Association of TC to Non-diabetics with Stroke

In our study conducted on 60 patients showed TC was elevated in non-diabetics with stroke compared to the control group was highly significant $P < 0.001$.

A study on lipid profile in non-diabetics with stroke done by Sridharan,[1] in 2010, showed a definite increase in serum TC in non-diabetic stroke patients when compared to control groups. In his study, he showed that both ischemic and hemorrhagic strokes are associated with increased cholesterol levels.

Benfante *et al.* (stroke 1994) showed that elevated serum cholesterol is a risk factor for both coronary heart disease and thromboembolic stroke in Hawaiian Japanese men.

Mascio *et al.* showed a positive association between risk of stroke and serum cholesterol.

Iso *et al.* emphasized an inverse association between serum cholesterol level and hemorrhagic stroke, but in his study, there was a positive association with non-hemorrhagic stroke.

Tanizaki *et al.* had showed that TC was an addition risk factor for cardioembolic stroke in females.

Strorn *et al.*, in 1994, showed that low TC levels decrease stroke in coronary artery disease patient.

Mohankar *et al.*, 1993, observed that increase TC leads to increase the incidence of atherosclerosis of large vessels. Atherosclerosis is a definite risk factor for stroke.

Triglycerides Association with Non-diabetic Stroke

The serum triglycerides were high in our patients compared to the control group of our study showing statistical significance ($P < 0.05$).

Sridharan[1] in his study showed that 80% of non-diabetic stroke patients with serum triglyceride >200 mg/dl had ischemic stroke and the remaining 20% had hemorrhagic stroke.

Tilvis *et al.*,[2] in his study, had showed that serum triglyceride is higher in ischemic stroke. Farid *et al.* also had similar results in his study in 1972.

Albucher *et al.*,[3] 2000, have showed serum triglycerides in normal range in his study on stroke.

Hachinski *et al.* showed a positive association of triglycerides in patients of atherothrombotic stroke and transient ischemic attacks.

Association of Serum HDL Cholesterol

The levels of serum HDL cholesterol are not significant in this study conducted on 60 non-diabetic stroke patients.

Simons *et al.* study revealed that HDL cholesterol had protective effect on ischemic stroke.

The northern Manhattan study on stroke in 2001 concluded that higher values of HDL cholesterol were associated with reduced risk of stroke.

Mohankar *et al.*,[4] in 1993, showed that increased LDL levels and low HDL levels were associated with atherosclerosis.

Mithee *et al.* had shown that high HDL levels were associated with decreased non-fatal stroke risk.

A study by Rubens *et al.*, in 2001, showed Gemfibrozil which raises HDL cholesterol level decrease ischemic stroke by 31% in men.

Albucher *et al.*[3] study clearly indicated HDL cholesterol as the only lipid associated with stroke risk. He emphasized the need for the management of low HDL cholesterol in young patients regardless of atherosclerosis.

Association of Serum LDL Cholesterol

The levels of serum LDL cholesterol were highly significant in our study conducted on 60 non-diabetics with stroke ($P < 0.001$).

Sridharan[1] showed that raised levels of serum LDL cholesterol had significant risk of ischemic stroke in non-diabetics.

Bolet *et al.* and Hachinski *et al.*[5] have showed positive correlation between LDL cholesterol levels and risk of stroke.

Ansell,[6] in 2000, showed that patients with established atherosclerosis showed are treated with statins to lower LDL cholesterol levels <100 mg to decrease the incidence of stroke.

Kurth *et al.*,[7] 2007, showed remarkable increase in serum LDL levels in ischemic stroke patients.

VLDL

VLDL levels were significantly elevated in our study conducted on 60 non-diabetics with stroke and control group.

Bidyadhar *et al.*,[8] 1984, showed that VLDL was raised in their study on stroke.

Sridharan,[1] in his study, showed that high VLDL was not associated with risk of stroke in non-diabetic patients.

CONCLUSION

Our study was conducted on 60 non-diabetic stroke patients and 60 controls. Exclusion was done because diabetes is associated with hyperlipidemia and atherosclerosis.

This study showed a significant association of TC, triglycerides, and LDL cholesterol in non-diabetics with stroke. High levels of TC, triglycerides, and LDL cholesterol are associated with higher risk of stroke.

Lowered HDL cholesterol levels were not significantly associated with stroke. The ratio of HDL/LDL cholesterol and TG/HDL cholesterol was calculated.

Dyslipidemia is a tip in iceberg. Dyslipidemia if properly treated being a modifiable risk factor for stroke it decreasing the incidence of stroke due to dyslipidemia. This leads to decrease morbidity and mortality, leading to a healthier society.

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