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A Study of Serum Fasting Lipid Profile and Serum Total Protein and Fractions in Normotensive Non-Diabetic Glaucoma Patients

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ABSTRACT

Glaucoma, which is the third most common cause of blindness and is responsible for 10% of blindness worldwide, the majority of those affected in developing countries are unaware that they have the disease and visual impairment. This study was done to asses and compare serum fasting lipid profile, serum total protein and fractions concentration in normotensive, non-diabetic glaucoma patients with special reference to normotensive, non-diabetic glaucoma healthy controls.

Mean total cholesterol value in glaucoma patients and controls were (162.52 ± 30.72) mg/dl and (137.07 ± 28.09) mg/dl respectively which is statistically significant ($p < 0.001$). Mean LDL-C value between glaucoma patients and controls were (94.68 ± 24.60) mg/dl and (69.08 24.05) mg/dl respectively and this difference was statistically highly significant (<0.001). The mean total protein value in cases and controls were (7.08 0.54) gm/dl and (7.89 ± 0.33) gm/dl and respectively which is a significant differences ($p < 0.01$). Mean albumin value in cases and controls were (3.23 ± 0.31) gm/dl and (4.09 ± 0.34) gm/dl respectively which is also statistically significant ($p < 0.01$).

There was significant increase in total cholesterol and LDL-C in glaucoma patients compared to controls. However, no significant difference in TGL-C, VLDL-C and HDL-C has been seen between the two groups. It was observed that both the total proteins and serum albumin were decreased in the patients group in comparison to controls group of the study population.

KEYWORDS : Glaucoma, Lipid profile, Cholesterol, Albumin, Intra Ocular Pressure (IOP)

Introduction

Glaucoma is a chronic, progressive optic neuropathy caused by a group of ocular conditions which leads to damage of the optic nerve with loss of visual function. The most common risk factor known is a raised intraocular pressure.[1]

Glaucoma is a leading cause of irreversible blindness throughout the world. World Health Organization statistics, published in 1995, indicate that glaucoma accounts for blindness in 5.1 million persons (13.5%) of global blindness {behind cataract15.8 million (41.8%) and trachoma 5.9 million (15.5%) persons of global blindness).2

Glaucoma, which is the third most common cause of blindness and is responsible for 10% of blindness worldwide, the majority of those affected in developing countries are unaware that they have the disease and visual impairment3.

Many studies have shown association between glaucoma and biochemical parameters like serum lipid profile and serum total protein and fractions in recent years. Similarly, both mean serum total protein and albumin levels were lower in patients compared with controls but only that of albumin was found to be significant4.

The association between lipid profile and glaucoma has been studied by many workers in recent years. An elevated Intra Ocular Pressure (IOP) is an important modifiable risk factor for the developments of glaucoma and by far the most common risk factor for vision loss in glaucoma. However, it is not only factor involved, because people with normal IOP have been shown to experience vision loss from glaucoma. On the other hand, some people with high IOP never develop the optic nerve head damage of glaucoma. The study found significant changes in the total cholesterol in glaucoma patients compared to controls. The study observed increase in cholesterol, a risk factor for atherosclerosis calls for further investigation; more so that homocysteine which is another risk factor was recently reported to be elevated in some types of glaucoma like primary open-angle glaucoma.5

"Negative" acute-phase proteins decrease in inflammation. Examples include Transthyretin (Prealbumin), Albumin and Transferrin. The decrease of such proteins may be used as markers of inflammation. The physiological role of decreased synthesis of such proteins is generally to provide amino acids in order to produce "positive" acute-phase proteins more efficiently. Theoretically, a decrease in transferrin could additionally be decreased by an upregulation of transferrin receptors, but the latter do not appear to change with inflammation. Chronic inflammation can cause elevated IOP by sort of clogging up the drain with debris.6

Gupta MC et al (1985) observed that in the origin of acute glaucoma attack, the neurovascular circulation plays an important role and there is a rise in blood lipid concentration7.

In order to appreciate the above observation and hypothesis, it was taken up this humble piece of work for a period of one year in Assam Medical College and Hospital, Dibrugarh, which is a premiere centre of Upper Assam and the neighbouring states catering patients from different strata. So, we have undertaken this study to evaluate the fasting lipid profile, serum Total Protein and Fractions level in normotensive non-diabetic glaucoma patients with non-glaucoma healthy individuals coming to our hospital in upper Assam. We also want to find out the correlation of serum fasting lipid profile, serum Total Protein and Fractions with the incidence of normotensive, non-diabetic glaucoma patients with non-glaucoma healthy individuals as no previous data is available regarding it in this eastern most part of the Indian subcontinent.

Materials Methods

The study was carried out in the department of Biochemistry, Assam Medical College and Hospital, Dibrugarh over a one year period

amongst newly diagnosed normotensive (normal systolic blood pressure), non diabetic glaucoma patients attending Out-patient Department and also those admitted into the ward in the Department of Ophthalmology, Assam Medical College and Hospital, Dibrugarh.

Inclusion Criteria:
A total of 50 cases with established Glaucoma patients, both male and female, and aged between 30—70 years were taken up for the study. The cases were primarily selected on the basis of detailed history, clinical features, intraocular pressure and gonioscopic findings. Age and Sex matched 50 cases of control was selected from amongst the healthy individuals.

Exclusion Criteria:
The following patients were excluded from the study:

Patients with diabetes mellitus.

All hypertensive patients with blood pressure more than 120/80 mm Hg (according to JNC 7 classification).

Congenital glaucoma
Patients on lipid lowering drugs and anti-glaucoma medication (e.g. topical beta blockers)

Patients with any other systemic disease affecting serum total protein and its fractions.

The venepuncture was done in the cubital fossa. About 3 ml of blood was transferred to sterile empty vials and samples were centrifuged at 5,000 rpm for 10 minutes as soon as after formation of the clot. The supernatant clear serum was then pipetted out using dry piston pipettes with disposable tips. The samples were analysed on the same day. Serum Fasting Lipid profile and serum protein and fractions were estimated in Semi-auto analyzer from the study sample.

Serum Triglyceride estimation (GPO/PAP method)8:
Principle: Triglycerdes are hydrolysed by lipase to glycerol and free fatty acids. Glycerol is phosphorylated by ATP in the presence of glycerol kinase to glycerol 3-phosphate, which is oxidized by the enzyme glycerol 3-phosphate oxidase (GPO) producing hydrogen peroxide. Hydrogen peroxide so formed reacts with phenolic compound and 4-aminoantipyrine to give a red coloured quinoneimine complex which is proportional to the amount of Triglyceride present in the sample.

Serum Cholesterol estimation: (CHOD/PAP method)9:
Principle: Cholesterol esterase (CHE) hydrolyses cholesterol ester to free cholesterol. Free cholesterol is oxidized to hydrogen peroxide. Hydrogen peroxide formed reacts with 4amino antipyrine and phenol in the presence of Peroxidase (POD) to produce red coloured quinoneimine dye complex. Intensity of the colour formed is directly proportional to the amount of cholesterol present in the sample.

HDL cholesterol estimation (PEG/ CHOD/PAP method)10:
Principle: When the serum is reacted with the Polyethylene Glycol contained in the precipitating reagent, all the VLDL and LDL are precipitated. The HDL remains in the supernatant and is then assayed as a sample for cholesterol using the Cholesterol (CHOD/PAP) reagent.

LDL Cholesterol estimation:

LDL Cholesterol is calculated by using Friedwald's formula.

LDL Cholesterol in mg/dl = Total cholesterol/5 - (HDL-Cholesterol)

Estimation of VLDL:
VLDL is the primary triglyceride carrying form in the fasting state; its concentration can be approximated by dividing the amount of plasma triglyceride by 5.

Estimation of Total Protein.11
Principle: Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample.

Estimation of Albumin.12
Principle: Albumin binds with the dye Bromocresol green in a buffered medium to form a green coloured complex. The intensity of the colour formed is directly proportional to the amount of albumin present in the sample.

Results:
The present study is a randomized case control study and results are analysed by using unpaired students t-test. 50 patients of normotensive, non-diabetic glaucoma patients and 50 healthy persons (controls) were included in the present study.

Serum lipid profile (total cholesterol, triglyceride, HDL-C and VLDL-C) were estimated for both cases and controls and the results were compared between the two groups.

In the present study, 50 patients of glaucoma were included. On decade wise grouping, it was found that maximum numbers of patients were between the age group of 51—60 years (34%). Among the 50 glaucoma patients included in the study, 31 patients were male which was higher than the number of female patients (19).

The mean age for total number of patients was: 51.76 ± 11.19

Mean age for male patients was : 51.84± 0.14

Mean age for female patients was :51.63±13.01

In Table–1 it was shown that mean total cholesterol value in glaucoma patients and controls were (162.52 ± 30.72) mg/dl and (137.07 ± 28.09) mg/dl respectively which is statistically significant ($p < 0.001$).

Table–1: **Fasting Lipid profile in Glaucoma patients and Controls**

Parameters	Glaucoma patients (mg/dl)		Controls (mg/dl)		p-value
	Mean	SD	Mean	SD	
Total Cholesterol	162.52	30.72	137.07	28.09	<0.001
Triglyceride	120.03	50.64	122.05	45.10	0.83
HDL-C	41.2	5.05	43.66	8.53	0.08
LDL-C	94.68	24.60	69.08	24.05	<0.001
VLDL-C	24.81	10.73	24.55	8.89	0.89

***SD: Standard Deviation**

Mean triglyceride value in glaucoma patients and control were (120.03 ± 50.6) mg/dl and (122.05 ±45.10) mg/dl respectively. However this difference was statistically not significantly ($p > 0.05$ i.e. $p = 0.83$).

Mean HDL-C values in glaucoma patients were slightly decreased compared to controls, (41.2 ±5.05) mg/dll and (43.66 ±8.53) mg/dl respectively. However this difference is statistically not significant. ($p>0.05$ i.e. $p=0.08$).

Mean LDL value between glaucoma patients and controls were (94.68 ±24.60) mg/dl and (69.08 ±24.05) mg/dl respectively and this difference was statistically highly significant. (<0.001)

There is no significant increase in VLDL-C between the cases and controls, (24.55 ±8.89) mg/dl and (24.81 ±10.73) mg/dl respectively. This is also statistically non-significant ($p > 0.05$ i.e. $p = 0.89$).

In Table–2 it was shown that mean total protein value in cases and controls were (7.08 ± 0.54) gm/dl and (7.89 ± 0.33) gm/dl and respectively which is a significant differences ($p < 0.01$).

Table–2: Total Protein, Albumin and Globulin in Glaucoma patients and Controls

Parameters	Glaucoma patients (mg/dl)		Controls (mg/dl)		p-value
	Mean	SD	Mean	SD	
Total Protein	7.08	0.54	7.89	0.33	<0.01
Albumin	3.23	0.31	4.09	0.34	<0.01
Globulin	3.81	0.65	3.79	0.40	>0.05
*SD: Standard Deviation					

Mean albumin value in cases and controls were (3.23 ± 0.31) gm/dl and (4.09 ± 0.34) gm/dl respectively which is also statistically significant ($p < 0.01$). Mean globulin value in cases and controls were (3.81 ± 0.65) gm/dl and (3.79 ± 0.40) gm/dl and respectively. However this difference is not statistically significant ($p > 0.05$).

Discussion:

The results of the study on the lipid profile in patients with non diabetic, non hypertensive (systemic blood pressure) glaucoma patients showed that there were significant alterations in the lipid profile specially serum total cholesterol (TC) and low density lipoprotein (LDL-C). But there was no alteration of serum triglyceride (TGL-C), very low density lipoprotein (VLDL-C) and high density lipoprotein (HDL-C), the values of which are not significantly higher in cases compared to healthy controls.

In the present study, mean age in glaucoma patients was 51.8 years and the disease was more in males (31 cases) than females (19 cases). It is found that, mean serum Total Cholesterol was (162.52±30.72) and (137± 28.09) in the study group and control group respectively. The mean serum LDL-C was (94.68 ± 24.60) and (69.08±24.05) in the study group and control group respectively. The mean serum Total Cholesterol and serum LDL-C level is significantly higher in glaucoma patients compared to healthy controls ($p<0.001$).

Adekunle B Okesina, Joshua FA et al (2011) have found that mean age of glaucoma patients was 56.9 years and the disease was more in males compared to females (M/F=46/23). The study found that serum total cholesterol was significantly higher ($p=0.01$) in patients with glaucoma than controls. The study also found that serum albumin level was significantly lower in patients with glaucoma than the controls. However, Serum TGL-C, HDL-C, LDL-C had no significant difference in both the groups13.

Shuang W et al (2012) have reported that dyslipidemia was significantly associated with higher IOP ($p<0.001$)14. Gavin S et al has found that IOP was higher in the total population with higher total cholesterol ($p<0.001$) and higher triglyceride ($p<0.002$).The association between these risk factors and higher IOP was also statistically significant in the population of participants without glaucoma15.

In the present study, serum total proteins as well as serum albumins were decreased significantly in glaucoma patients compared to healthy controls. Albumin is the most abundant plasma protein accounting for approximately one half of the plasma protein mass. Albumin is also known as negative acute phase reactant (APR) which has levels decreasing with inflammation. Associated disorders include surgery, trauma, bacterial infection etc. Presumably the APR all play a part in the very complex defensive process of inflammation, particularly complement activation and in control of enzyme activity. However, acute phase reaction is a non-specific reaction to inflammation and is not diagnostic for any given disease. High levels of albumin are partially protective against atherosclerosis, probably because of its promotion of cholesterol efflux from fibroblast and other cells.16

Conclusion:

In this study, serum lipid profile, serum protein and fractions were studied in normotensive non diabetic glaucoma patients and healthy controls to find any association of biochemical alteration with disease process of glaucoma. There was significant increase in total cholesterol and LDL-C in glaucoma patients compared to controls. However, no significant difference in TGL-C, VLDL-C and HDL-C has been seen between the two groups. It was also observed that both the total proteins and the serum albumin were decreased in the patients group in comparison to control group of the study population. The significant increase in blood total cholesterol and LDL-C may lead to atherosclerosis of blood vessels leading to decrease blood flow to optic nerve head which may lead to glaucomatous damage. Decrease level of serum total proteins and the albumin may also contribute towards the pathogenesis of glaucoma. We can conclude that total Cholesterol and LDL-C were significantly higher in cases compared to controls but the TGL-C and HDL-C were not significantly increased.

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