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Lipid ratios in Type 2 Diabetes Mellitus

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ABSTRACT

Diabetes mellitus is a group of metabolic disorder characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative insulin deficiency. The present study was carried out to study the lipid profile and lipid ratios as an assessment tool for coronary artery disease risk profile in resource poor situations. The total numbers of patients under study were 36 and control group consisted of 15 persons. Lipid profile and lipid ratios were studied in cases and controls. Results of different parameters were tabulated and compared and p value <0.05 were considered as statistically significant. Lipid ratios can be used as risk assessment tool for coronary artery disease risk profile in resource poor situations.

Key words: - Diabetes Mellitus, Lipid ratios.

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INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by chronic hyperglycemia and disturbances of carbohydrate, fat and protein metabolism associated with absolute or relative insulin deficiency. Diabetes mellitus is of two types –Type 1 and Type 2 diabetes mellitus. Type 2 diabetes characterized by target tissue resistance to Insulin is epidemic in industrialized societies and is strongly associated with obesity, however the mechanism by which increased adiposity causes insulin resistance is unclear [1-3].

Studies showed that Adipocytes secrete a unique signaling molecule which they have named Resistin for resistance to Insulin. Circulating Resistin levels are decreased by the anti-diabetic drug Rosiglitazone and increased in diet induced and genetic forms of obesity. They also observed that Insulin stimulated glucose uptake by adipocytes is enhanced by neutralization of Resistin and is reduced by Resistin treatment [4-9].

Metabolic syndrome includes glucose intolerance or type 2 diabetes, dyslipidemia and arterial hypertension which are classical cardiovascular risk factors. Insulin resistance is the cornerstone of Metabolic Syndrome X, with secondary hyperinsulinism. Upper body obesity is associated with metabolic syndrome X.

According to [2], Diet is the first step in treating these patients and reducing caloric intake is necessary in most of them. He suggested Polyunsaturated and monounsaturated fats in place of Saturated fat, also long chain carbohydrates with low glycemic index and regular physical activity. If these life style modifications fail, Drugs like Biguanide and glitazone are good [10-19].

Dyslipidemia in diabetes mellitus is very important cause of morbidity and mortality related to cardiovascular risk .It is infact a important independent risk factor for acute coronary syndrome.

Hence, ours is an effort to study lipid profiles and lipid ratios of patients of type 2 diabetes mellitus so that it can be used as an assessment tool for risk profile for coronary artery disease in resource poor situations.

MATERIALS AND METHODS

The present work has been conducted in the Department of Biochemistry, Patna Medical College, Patna. The Cases studied were selected at random from Patna Medical College and Hospital, Patna, Outdoor, Indoor wards of Medicine Department and also from Diabetic clinic of PMCH. The total number of Cases in study was 36 and Normal persons were 15.

The following tests were done in each Sample during the Study.

(1) Serum total Cholesterol
(2) Serum HDL
(3) Serum Triglyceride
(4) Plasma glucose – Fasting - 2½ hrs after 75 gm glucose

Selection of Cases:-

Only those persons were included in the present study, who had no other existing diseases, which were known to influence lipid metabolism and thereby affecting the lipid profile. Disease like jaundice, renal disease, Hypertension, thyroid were discarded. A list of questions was asked as given in the case sheet proforma.

Collection of Blood Sample:-

A morning sample of venous blood after overnight fast was collected in a dry and sterile syringe. First arm was extended and a rubber tourniquet applied a few inches above the elbow. Skin over the antecubital vein was cleaned by rubbing with spirit. A well sharp sterile hypodermic needle fixed on to a syringe of 5ml capacity was inserted into vein and Plunger was withdrawn slightly. As soon as blood appears Tourniquet was released and 5ml of blood was withdrawn into the Syringe. A small cotton swab soaked in spirit was placed at the Needle insertion site and needle withdrawn. Cotton swab held firmly for few minute until bleeding stopped. Needle removed from the Syringe. 3 ml of blood transferred to Plain sterile vial and 2 ml of blood transferred to Sodium Fluoride Containing vial.

Blood in Plain sterile vial was kept inclined to allow to clot. When Blood clotted, Vial was kept vertical for some time. Clot shrinks and serum separated. This serum was centrifuged at 3000 r.p.m for 5 minutes to separate RBC from Serum. Clear Supernatant Serum was pipette out and was used for various Serum lipid profile estimation.

Sodium fluoride containing Blood was centrifuged at 3000 rpm for 5 min to separate red cells from clear supernatant Plasma. Supernatant Plasma was pipette out and used for sugar estimation in Plasma. Patient was then given 75 gm glucose orally in 200 ml water and another blood sample of 2 ml was taken 2½ hrs after meal. Plasma was separated by centrifugation in a similar way and postprandial sugar in plasma was estimated.

Method of Serum Triglyceride estimation

Merck's Kit used for estimation of Serum Triglyceride level. It is based on GPO-POD method, an enzymatic method.

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Principle:-

Triglyceride is hydrolyzed to Glycerol and Free fatty acid by Lipoprotein lipase. In the presence of ATP and Glycerokinase (GK) the glycerol is converted to Glycerol -3. Phosphate and ADP. Glycerol-3- Phosphate is then oxidized by Glycerol 3 Phosphate oxidase (GPO) to yield Hydrogen peroxide and Dihydroxyacetone phosphate in presence of O_2 .

Hydrogen peroxide in presence of peroxidase (POD) enzyme reacts with chromogen (4 Chlorphenol +4 aminoantipyrine) to form coloured complex (chinonimine). The intensity of colour developed is proportional to the triglyceride concentration which is measured in a Photocolorimeter with green filter 520 nm.

Method for Total Cholesterol estimation.

For total cholesterol estimation, Accurex kit was used. It is based on enzymatic method.

Principle :-

Cholesterol esterase (CHE) hydrolyses cholesterol esters into free cholesterol and fatty acids. Free cholesterol is oxidized by the cholesterol oxidase (CHO) to Cholest-4-en-3-one and hydrogen peroxide (H_2O_2). This hydrogen peroxide in presence of peroxidase (POD)couples with 4-aminoantipyrine and phenol to produce red coloured quinoneimine dye. The intensity of colour produced is proportional to the cholesterol concentration.

Method for HDL Cholesterol estimation

CREST Biosystems Kit used for estimation of Serum High density lipoprotein cholesterol. It is based on PEG Precipitation Method.

Principle :-

Very low density lipoprotein (VLDL) and Low density lipoprotein (LDL) of Serum precipitates out when Serum reacts with polyethylene glycol contained in the Precipitating reagent. High density lipoprotein (HDL) remains in the Supernatant fluid which is then determined by using working cholesterol reagent.

Determination of Serum LDL cholesterol and VLDL cholesterol

In absence of a separate estimation of LDL and VLDL cholesterol indirect method has 0 been used in accordance with the outline of Freidewald's Formula.

Here VLDL cholesterol can be indirectly ascertained as $1/5^{th}$ of the triglyceride value

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VLDL cholesterol = Triglycerides/5

The LDL cholesterol has been calculated from the estimated values of triglyceride, Total cholesterol, HDL cholesterol which were directly measured in serum by enzymatic method as described above.

The value of LDL cholesterol is calculated as LDL cholesterol (mg/dl) = Total cholesterol (mg/dl)

$$\text{– HDL cholesterol (mg/dl)} - \frac{\text{Triglyceride}}{5} \text{ (mg/dl)}$$

Estimation of Blood Sugar by O – Toluidine method

Principle :-

Glucose condenses with O – toluidine in glacial acetic acid when heated at 100˚c. The product formed is a blue green N – glucosylamine, the colour so produced is proportional to the amount of glucose concentration which is measured at 630 nm (red filter).

RESULTS

Table 1.Lipid profiles in study and control group

Parameter (mg/dl)	Study group (n=36) (Mean±S.D)	Control group (n=15) (Mean±S.D)	p value
Total Cholesterol	151.5 ± 36.5	137.0 ± 25.0	<0.05*
Triglyceride	125.0 ± 21.0	113.0 ± 32.0	<0.05*
HDL-C	22.5 ± 3.5	35.0 ± 7.5	<0.05*
LDL-C	102.00 ± 36.00	77.00 ± 25.50	<0.05*
VLDL-C	24.00 ± 4.00	22.00 ± 6.00	<0.05*

*p value <0.05 is considered as statistically significant.

Table 2.Lipid ratios in study and control group

Lipid ratios	Study group (n=36) (Mean±S.D)	Control group (n=15) (Mean±S.D)	P value
LDL/HDL	4.10 ± 1.25	2.17 ± 0.73	<0.05*
TG/HDL	5.17 ± 1.23	3.15 ± 1.27	<0.05*
TC-HDL/HDL	5.55 ± 2.15	2.99 ± 0.97	<0.05*

*p value <0.05 is considered as statistically significant.

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DISCUSSIONS

In this present study, we evaluated the lipid profiles and lipid ratios in type 2 diabetes mellitus patients and compared it with age and sex matched healthy controls. We found statistically significant results when lipid ratios of cases were compared with controls as evident by the p value which is found to be less than 0.05.

Dyslipidemia is a major cause of morbidity and mortality in diabetes mellitus patients both in developed and developing countries. Diabetic Dyslipidemia is an independent risk factor for coronary artery disease. Thus ours is an effort to evaluate the lipid ratios in type 2 diabetes mellitus and evaluate the risk of predisposing risk towards coronary artery disease.

Recently these lipid ratios gaining importance over simple lipid profile in assessment of risk profile of diabetes mellitus patients.

To conclude, lipid ratios can be used in risk assessment of Dyslipidemia in diabetes mellitus patients in resource poor situations.

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