ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Lipid ratios in Heavy smokers.

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Manuscript Info

Manuscript History:

Received: 12 January 2016
Final Accepted: 22 February 2016
Published Online: March 2016

Key words:
Smokers , Lipid ratios.

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Abstract

Smoking is a established cardiovascular disease risk factor .but the mechanism how it cause cardiovascular risk is still not properly understood. 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 heavy smokers under study were 35 and control group consisted of 15 persons. Lipid profile and lipid ratios were studied in smokers and non-smokers. 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.

Introduction:-

Smoking is considered as a major cardiovascular risk factor [1] .There is a dose- response relationship between the number of cigarettes smoked and cardiovascular morbidity and mortality[2]. The mechanism by which cigarette smoking causes atherosclerosis remains obscure, but cigarette smoking have been found to alter the level of lipoproteins[3-7]. Plasma lipoprotein abnormalities are said to be the underlying major risk factors and may even be essential for the common occurrence of atherosclerotic vascular diseases [8].

Nicotine stimulates sympathetic adrenal system leading to increased secretion of catecholamine resulting in increased lipolysis and increased concentration of plasma free fatty acids (FFA) which further result in increased secretion of hepatic FFAs and hepatic triglycerides along with VLDL-C in the blood stream [4]. These changes contribute to the atherosclerotic potential of cigarette smoke. Blood of cigarette smokers routinely displays decreased antioxidant capacity and increased oxidized lipids compared to non-smokers [9]. Oxidative pathway appears to be one important mechanism for modifying LDL, because a wide variety of structurally unrelated antioxidants inhibit atherosclerosis in animal models of hypercholesterolemia [10]. Evidence suggests that oxidatively modified LDL contribute to the pathogenesis of atherosclerosis [11]. Cigarette smoke contains approximately 10^{17} oxidant molecules per puff that can cause damage to lipids, proteins, DNA, carbohydrates, and other bio-molecules [12] . It is becoming increasingly evident that a prooxidant / antioxidant imbalance largely contributes to atherosclerosis processes [13]. It has been postulated that many of the adverse effect of smoking may result from oxidative damage to critical biological substances[14]. Previous reports have demonstrated abnormal endothelial function in chronic smokers [15]. Endothelial dysfunction in turn has been proposed to play a pathogenic role in the initiation of vascular disease [16]. Although smoking induced endothelial dysfunction is very likely multifactorial, more recent clinical and experimental observations strongly point to a potential role of oxygen derived free radicals in mediating this phenomenon [11.]

Dyslipidemia in heavy smokers is very important cause of morbidity and mortality related to cardiovascular risk .It is in facta important independent risk factor for acute coronary syndrome. Hence, ours is an effort to study lipid profiles and lipid ratios in heavy smokers 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 was conducted on 35 healthy male bidis/cigarettes smokers in the age group 25-50 years and it was compared with 15 healthy age, sex and body mass index(BMI) matched non smokers . Persons smoking 15 bidis/cigarettes or more per day continuously for a year were considered as heavy smokers. A detailed physical examination of the subjects of both groups was done.

The following tests were done in each Sample during the Study.
(1) Serum total Cholesterol
(2) Serum HDL
(3) Serum Triglyceride
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.

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.

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.

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

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 O2 . 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 (H2O2). This hydrogen peroxide in presence of peroxidase (POD)couples with 4-aminoantipyrine and phenol to produce red colouredquinoneimine 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 been used in accordance with the outline of Freidewald's Formula. Here VLDL cholesterol can be indirectly ascertained as 1/5th of the triglyceride value.

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) – HDL cholesterol (mg/dl) – Triglyceride/5 (mg/dl)

Results:-

Table 1.Lipid profiles in study and control group

Parameter (mg/dl)	Study group (n=35) (Mean±S.D)	Control group (n=15) (Mean±S.D	p value
Total Cholesterol	171.4 ± 46.5	132.0 ± 20.0	<0.05*
Triglyceride	142.0 ± 21.0	115.0 ± 29.0	<0.05*
HDL-C	29.5 ± 4.5	38.0 ± 7.5	<0.05*
LDL-C	106.00 ± 34.00	75.00 ± 27.50	<0.05*
VLDL-C	28.00 ± 3.00	21.00 ± 5.00	<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=35) (Mean±S.D)	Control group (n=15) (Mean±S.D)	P value
LDL/HDL	3.60 ± 1.24	2.10 ± 0.73	<0.05*
TG/HDL	4.97 ± 1.23	3.09 ± 1.26	<0.05*
TC-HDL/HDL	5.35 ± 2.17	2.79 ± 0.91	<0.05*

***p value <0.05 is considered as statistically significant**

Discussions:-

In the study, the two groups of subjects (smokers and non-smokers) were of comparable sex, age and BMI. They were non-diabetic, non-alcoholic and normotensive subjects.

The biological mechanism linking smoking and atherogenesis, the process leading to cardiovascular diseases, is complex and not fully understood. The current opinion is that atherosclerosis is an immune/ inflammatory response of the intima to endothelial injury [17,18].It is also well known that the injury is mainly initiated by lipid accumulation.[18,19]. Native plasma lipids, in particular native LDLs, can freely enter the intima and are taken up by vascular cells via LDL receptor-mediated endocytosis. Nevertheless, they do not primarily initiate an inflammatory response, they are not phagocytosed by monocytes, and they do not initiate atherosclerotic alterations [19,20]. Oxidation or other modifications of LDL, however, substantially alter its role: oxidized or modified lipids are chemotactic for monocytes, induce migration, initiate inflammatory responses, alter the endothelium, induce differentiation of monocytes into macrophages, and are avidly taken up by macrophages via scavenger receptors [19,21,22.]

Modified LDL is recognized by scavenger receptors distinct from the 'classical' LDL receptors present on all mammalian cells [23]. Scavenger receptors bind and internalize modified low-density lipoprotein (LDL). Because the expression of scavenger receptors is not down-regulated by cholesterol, macrophages expressingscavenger receptors can internalize substantial quantities of cholesteryl ester from oxidized LDL leading to foam cell formation [24].

In this present study, we evaluated the lipid profiles and lipid ratios in heavy smokers and compared it with age, sex and BMI matched healthy controls. We found statistically significant results when lipid ratios of heavy smolers 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 heavy smokers . Dyslipidemia is an independent risk factor for coronary artery disease. Thus ours is an effort to evaluate the lipid ratios in smokers 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 . To conclude, lipid ratios can be used in risk assessment of Dyslipidemia in smokers in resource poor situations.

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