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A Study on the Role of Antioxidant Enzymes and Lipid Profile of Cigarette Smokers in Different Age Groups

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Abstract

The aim of the dispense study was to evaluate the involvement in the smokers in different age people's alteration of lipid profile and antioxidant enzyme level was measured in the blood sample. The serum biomolecules were estimation of total cholesterol, triglycerides, HDL, LDL, VLDL, assay of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, was assayed by IFCC/kinetic method, assay of glutathione peroxidase (glutathione: hydrogen peroxide oxidoreductase, glutathione peroxidase, assay of superoxide dismutase the enzyme was assayed in serum based on the oxidation of epinephrine adenochrome transition by the enzyme. In this study, these finding provide innovative information on total cholesterol levels were found to be elevated only about 3% in age groups of 20 to 40 yrs while smokers in age groups of 41 to 55 years showed increase of about 5 to 10 %. HDL cholesterol levels are lowered by 6 to 8% in smokers in age group of 20 to 30 yrs and by 9 to 14% in age groups between 31 to 55 yrs. These are the indications of increased risks of CHD and are found to be parallel to antioxidant changes, lipid peroxidation in plasma and RBC. The progressive decrease in HDL production in different age groups indicates the increasing risk of (CHD) Coronary Heart Disease.

Keywords: Nicotine, Total cholesterol, peroxidase, lipid profile and triglycerides.

Introduction

Nicotine is a tiny molecule that dissolves in both fatty & water based substances. This rapidly absorbed through the skin and carried through the bloodstream to target receptors of the nervous system & other organs. Cigarette smokers generally get about 1 to 2mg nicotine from each cigarette, with the rest either being left in tobacco butt or going up in smoke [1]. Other tobacco products vary widely in their content and delivery of nicotine. The amount of nicotine in tobacco may not seem like much, but nicotine is very potent. Potency refers to the amount of a drug it takes to produce a given effect – the smaller the amount of drug needed to produce the effect, the more potent is the drug. Nicotine is about 5 to 10 times potent than Cocaine or Morphine in producing psychoactive effects in humans or modifying behavior in animals [2].

Nicotine increases heart rate and blood pressure. It may produce transient contraction of coronary artery which may trigger a thromboembolic attack in coronary vessels. Cigarette smoke contains 5% carbon monoxide which depletes oxygen in RBCs and restricts oxygen availability to vital areas including heart [3]. The small size of nicotine molecules along with its many synthetic activities, liver is responsible for the continuous production of VLDL which in fasting state represents the body's primary source of circulating triglyceride energy. LDL is ultimately removed from the circulation by the high affinity receptor pathway or by scavenger mechanisms thought to lead to the incorporation of LDL cholesterol into atheromatous plaques. Hypercholesterolemia has clearly been identified as a major risk factor for premature heart disease. The probability of cardiovascular disease increases as LDL cholesterol decreases. HDL deficiency is an independent risk factor for premature cardiovascular disease [4]. Two separate mechanisms like as proposed that HDL played a role in cholesterol metabolism by facilitating removal of cholesterol from peripheral cells and transporting to liver for degradation. This has been termed as "reverse cholesterol transport". VLDL is metabolized to LDL which enters the peripheral cells for degradation. An additional mechanism has recently been proposed to explain the inter-relationship between LDL and HDL. In these studies, HDL was demonstrated to influence the binding and uptake of LDL by the peripheral cells. In in-vitro experiments, in fibroblast, endothelial cells, lymphocytes and arterial smooth muscle cells in tissue culture [5-7].

Cholesterol has been long held the molecular root of Heart diseases. Recently, it has been found that C-Reactive Protein (CRP), is a marker of inflammation that has emerged alongside cholesterol deposition and clogged arteries as a significant factor in understanding heart disease. CRP is present in arteriosclerotic lesions and that it functions as a chemo attractant to lure monocytes to the site [8]. It has also been implicated directly in increasing the expression of adhesion molecules. It also can apparently activate immune system components known as "Complement Proteins" which are mediators of inflammation. These radicals are scavenged by antioxidant enzymes present in the cells, which causes depletion of these enzymes. Superoxide radicals [O_2^-, HO_2^-] and hydroxyl radicals (OH^-) are converted to H_2O_2 by 'Superoxide Dismutase'. Thus, accumulated H_2O_2 is removed by catalase and glutathione peroxidase [9].

Cigarette smoking causes an immediate increase in lipid peroxidation, which leads to cell damage and cell death. Elevated levels of lipid peroxides and decreased levels of antioxidant enzymes have been reported. These levels were directly related to severity of the disease of heart and

lung. Increased erythrocyte lipid peroxide levels were observed in chronic smokers when compared to non-smokers, the components present in cigarette smoke, on exposure to human respiratory tract increases the burden of "reactive Oxygen Species" (ROS). It is presumed that the free radicals generated in smokers would attack the phospholipids of membrane, resulting in increased lipid peroxide in smokers. Moreover, the association of increased free radical production is associated with lung cancer and IHD [8,10].

The activities of erythrocyte antioxidant enzymes were also decreased in chronic smokers when compared to healthy individuals and this may be due to elevated lipid peroxide contents and decreased levels of phospholipids in smokers. SOD was inactivated by OH^- radicals acting on active site of the enzyme [11,12]. Catalase plays an important role in destruction of hydroperoxy radicals and inactivation of catalase activity has been shown to be caused by H_2O_2 and superoxide radicals. Selenium present in glutathione peroxidase provides second line of defense against hydro peroxide before they can damage membranes and other cellular components [13-15].

Materials and Methods

Subjects and Sample Collection: 20 male volunteers, aged between 20-55 years who had a smoking history of 5-20 years were taken for this study. The smokers were classified into groups after assigning a unit value 10 each smoker based on the reported number of cigarettes smoked/day and the number of years of smoking habit. Those smoking less than 10 cig/day were assigned a unit score of 8, 11-15 cig/day a unit score of 12, 16-20 cig/day a unit score of 15 and 21-25 cig/day a unit score of 20.

Group		No. of Samples	
A	I a 5-10 cig/day	Control	21
		I a	20
B	II a 11-15 cig/day II b 16-20 cig/day	Control	18
		II a	15
		II b	19
C	III a 10 cig/day III b 11-15 cig/day III c 16-20 cig/day III d 21-25 cig/day	Control	29
		III a	39
		III b	25
		III c	26
		III d	15

Estimation of Lipid Profile

Estimation of Cholesterol – Cholesterol Oxidase Method: The major constituents of plasma lipids are cholesterol and triglycerides. Cholesterol is an important compound of cell membrane and precursor for the synthesis of bile salts and steroid hormones.

Estimation of HDL – Cholesterol: LDL, VLDL and Chylomicrons are precipitated by poly anions in the presence of metal ions to leave HDL in solution. The Cholesterol content of the supernatant fluid is then determined. As the HDL-cholesterol is a minor part of the total cholesterol, it is determined by enzymatic method.

Estimation: Triglycerides were estimated enzymatically by Bucolo and David in 1973. The method was modified to a calorimetric test by Magraw et.al., in 1979. Assay of aspartate transaminase/ serum glutamate oxaloacetate transaminase serum glutamate pyruvate transaminase/ alanine transferase,glutamate pyruvate transaminase was assayed by ifcc / kinetic method, assay of glutathione peroxidase (glutathione: hydrogen peroxide oxidoreductase, e.c.1.11.1.9),glutathione peroxidase enzyme was assayed according the method of rotruck et. al., (1973),assay of superoxide dismutase (super oxide: reductase, e.c.1.15.1.1) the enzyme was assayed in serum according to the method of misra and fridovih (1972), based on the oxidation of epinephrine adenochrome transition by the enzyme.

Test Methods for Lipid Profile

The following formula is used to find out the ratio of cholesterol to HDL. Ratio = Total Cholesterol/ HDL Cholesterol. The triglycerides value is divided by 5, HDL value is added to that and the whole thing is subtracting from total cholesterol value to get LDL value. **LDL = (**Triglycerides/5) +HDL – Total Cholesterol

Results

Antioxidant status and transaminase activity in serum of smokers and age related non-smokers – a comparative study

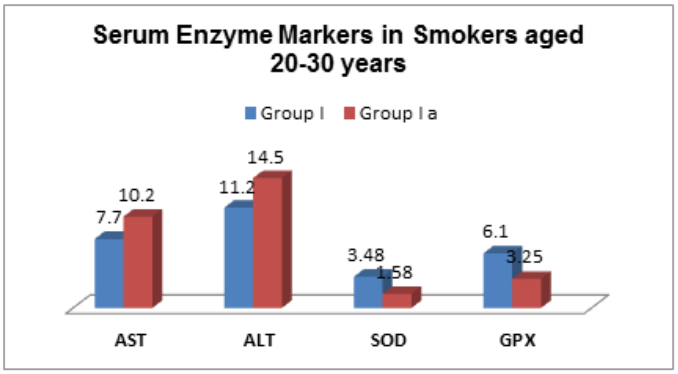

Figure 1

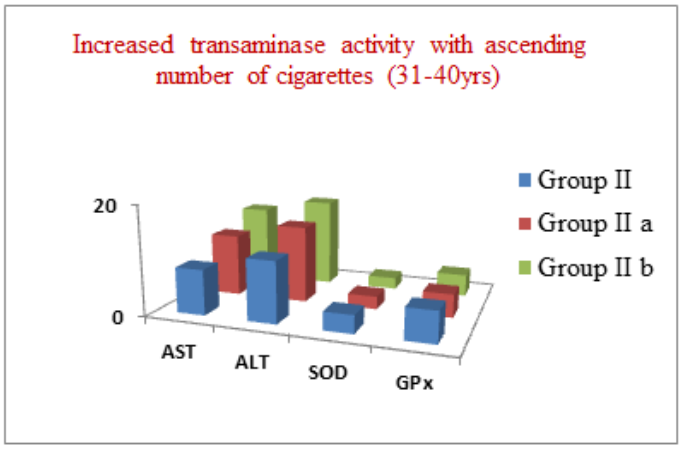

Figure 2

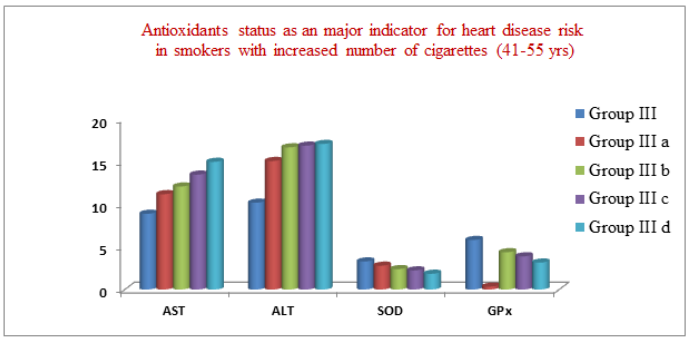

Figure 3

Lipids and Lipoprotein Distribution in Serum of Smokers and Age Related Non-Smokers

Table 1: (20-30 Years)

	Cholesterol	TG	HDL	LDL	VLDL
Group I	216±12	108±12	53 ± 5.3	110 ± 6	53.6 ± 5.20
Group I a	250 ± 9	130 ± 6	39±3.90	132±8	64±5.50

Table 2: (31-40 Years)

	Cholesterol	TG	HDL	LDL	VLDL
Group II	205±14	105±11	54± 5	98±12	24.1± 1.40
Group II a	240 ±8	135±13	41± 3.1	128±12	62.9±4.50
Group II b	252±9	142±11.5	38± 3.3	135±11	63± 5.10

Table 3: (41-55 Years)

	Cholesterol	TG	HDL	LDL	VLDL
Group III	188±15	110±14	60±4	90±9	49.5±5.20
Group III a	239±12	162±15	50±2.9	118 ±7	66.5±5.50
Group III b	238±10	169±17	49.9±2.2	120±8	68.2±6.5
Group III c	235±15	172±16.58	44.2 ± 3.1	122 ± 6	72 ± 5.86
Group III d	245 ± 13	178 ± 15.2	41.3 ± 3.8	117 ± 8	73.4 ± 5.50

Units - Cholesterol, TG, HDL, LDL, VLDL – mg/dl

Discussion

Cholesterol is an amphipathic lipid and such an essential structural component of membranes and outer layer of plasma lipoproteins. Additionally lipoproteins transport free cholesterol in the circulation where it readily equilibrates with cholesterol in other lipoproteins and in membranes. Cholesterol ester is a storage form of cholesterol found in most tissues. It is transported in the core of the lipoproteins [16]. LDL is the mediator of cholesterol and cholesterol ester uptake into many tissues.

Free cholesterol is removed from the tissues by HDL and transported to the liver for conversion to bile acids. The chief role in pathologic process is a factor in the genesis of atherosclerosis of vital arteries, causing Cerebrovascular, coronary and peripheral vascular disease. Coronary atherosclerosis correlates with high plasma LDL: HDL cholesterol ratio [17]. A comparison of smokers and non-smokers of different age groups in the present study revealed that smokers and non-smokers are comparable in age, the total number of cigarettes smoked per day and the total number of years they are addicted to smoking [18]. The extent of the effect is related to the number of cigarettes smoked and to the amount of smoke inhaled. Plasma lipids appear to be significantly affected in smokers with triglycerides and free fatty acids levels increasing in all the age groups studied. The gradual changes provide support for the grading of smokers used in the present study. Elevation of plasma free fatty acids will lead to increased VLDL production by the liver, involving extra triacylglycerol and cholesterol output into circulation. Nicotine from cigarette smoking could also be a factor leading to higher fluctuating levels of free fatty acids [19,20].

The serum cholesterol is correlated with incidence of atherosclerosis and coronary heart disease. Serum triacylglycerol also shows similar correlations. The present investigation shows high levels of LDL, VLDL fraction of lipoprotein, cholesterol and decrease in the levels of HDL. The elevations of these fractions (Hyperlipidemia) can be accompanied by sever arterial disease. Cholesterol and phospholipids form an important constituent of the cell membrane and are essential to maintain normal permeability and structural integrity of the membrane. Studies on erythrocytes have shown that changes in permeability are controlled by Cholesterol: Phospholipids ratio [21-23]. The amount of carboxy hemoglobin may exceed 10% of the total Hb in heavy smokers and increased number of cells compensates for impaired ability if the red cells to transport oxygen. The free radical increase in these subjects could be assessed in terms of lipid peroxidation products, loss of antioxidant activities, abnormalities in plasma lipids and lipoproteins in cell membrane composition [24].

SOD and peroxide motivating enzymes like GPx were found to be affected by smoking, SOD activity was progressively reduced in smokers when compared to control subjects (non-smokers). The decline was also observed in GPx. The decline is antioxidant enzyme profile of smokers with respect to the intensity and duration, there is a sharp decline in age group 20-30 years a plateau in middle years and a sharp progressive decline in age group 41-55 years. It may be postulated that the body tries to cope with the free radical challenge during the middle year of life in the age group of 31-40 years and loses control in the later years of life. The decline in activity could be due to inactivation of the enzymes and due to reduced synthesis where age of the individuals may be a contributing factor [25]. Cigarette smoking is one that exposes the body cells to an enormous free radical challenge and this being a repetitive behavior appears to have a marked effect. The lungs of a smoker are being repeatedly exposed to a variety of toxins and heavy doses of free radicals in the smoke. In addition , in the process of engulfing and digesting the residual components in the smoke, the alveolar macrophages are active, leading to ‘Respiratory Burst’, which results in the release of peroxide and superoxide anions and also hydroxy radicals [26]. SOD and the peroxide inactivating enzymes are needed to combat the inflammatory process arising out of the release of these anions. A decline in the activity of SOD in the present study can be attributes to accumulation of superoxide and H_2O_2 which in turn can lead to the

formation of hydroxyl radicals. The hydroxyl radicals can bring about a number of reactions which can be harmful to the tissues [27-28].

LDL modification in the present study is directly proportional to the rate of free radical production. The small steady increase in LDL cholesterol observed in the smoking group implies an increase in the concentration of substrate and this further enhances the probability of LDL oxidation as suggested by Rosenfield (1991).Oxidative modification of LDL can occur in vivo following interaction of the lipoproteins with cells of the arterial wall, blood cells and immune complexes of plasma cells and immune complexes of plasma constituents of the arterial wall matrix [29]. Along with it heterogenous products that include oxidised fatty acids and their breakdown products, oxidised sterols, oxidised phospholipids are also seen. Oxidised LDL however may also contribute importantly to cardiovascular disease states via mechanisms above and beyond that of contributing cholesterol deposition in the plaque narrowing of the vessel lumen. Analysis of plasma lipid and cholesterol distributions in the lipoprotein fractions of the smokers of this study bring into focus the increased plasma triglycerides and decreased HDL cholesterol in smokers. Similar finding in smokers have also been reported earlier. The changes have also been identified as increasing risk of "Coronary heart disease" [30].

In this study, total cholesterol levels were found to be elevated only about 3% in age groups of 20 to 40 yrs while smokers in age groups of 41 to 55 years showed increase of about 5 to 10 %. HDL cholesterol levels are lowered by 6 to 8% in smokers in age group of 20 to 30 yrs and by 9 to 14% in age groups between 31 to 55 yrs. Increases in LDL cholesterol ranged between 4 to 32% in smokers. VLDL cholesterol showed greater increase than LDL. In smokers, between age groups of 20 and 40 the increase was lesser than older smokers in the age group of 41-55 yrs. The elevations were progressively marked. The progressive decrease in HDL level in smokers indicates that they are at an increased risk of developing cardiovascular diseases as reported [6]. In smokers, circulating Triglycerides and free fatty acids were significantly raised. Free fatty acid levels in blood are known to be increased with β-adrenergic stimulation. As nicotine stimulates the β-adrenergic response, the raised levels of FFA in plasma may have a raise from the nicotine that is taken up during smoking acting in adipose tissue, through β-adrenergic stimulation. Repeated β-adrenergic stimulation is also known to increase plasma triglycerides [31].

Smoking is an irritant and increases the number and size of bronchial mucous gland and evokes an inflammatory response. An important component of this is elastin, a structural protein that can undergo elasticity. This elasticity is a pivotal factor in heating compliance of lungs. Smoking may cause an imbalance in function of protease-antiprotease enzyme system. Antiprotease function is comprised because toxic oxygen radical found in cigarette smoke damages a pivotal methione residue in antitrypsis, increasing its ability to bind and inhibit elastase. Enzyme like ALT which is a marker of liver function, also show a marginal elevation along with increase in severity and duration of smoking. The rise in serum AST is significant at the earliest in the age group 20-30 yrs and a marked increase in age group 41-55yrs. This increase may be the cause of abnormal formation of lipid and enhanced functioning of the liver [8, 32].

Conclusion

Smoking through the action of nicotine affect several biochemical constituents of the body. The extent of the effect is related to the number of cigarettes smoked and to the amount of smoke inhaled. The plasma lipoproteins

(HDL, LDL,and VLDL) and triglyceride concentrations are higher and HDL is lower in smokers than non-smokers of different age groups. Superoxide Dismutase (SOD) and peroxide inactivating enzymes GPx were found to be decreased in smokers of different age groups. The decrease in antioxidant enzymes may lead to an increase in free radical production which could also be a contribution factor for coronary heart disease. The modification in LDL production in this study is directly proportional to the rate of free radical production. The progressive decrease in HDL production in different age groups indicates the increasing risk of (CHD) Coronary Heart Disease.

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