International Journal of Biomedical and Advance Research
ISSN: 2229-3809 (Online); 2455-0558 (Print)
Journal DOI: 10.7439/ijbar
CODEN: IJBABN

Original Research Article

Evaluation of lipid parameters, Liver Function Test, CRP and MDA (as a marker of lipid peroxidation) in chronic cigarette smokers

Sudeep Kumar[*1], Roshan Kumar Mahat[1] and Jyoti Batra[2]

[1]*Department of Biochemistry, Muzaffarnagar Medical College, Muzaffarnagar (UP), India*
[2]*Department of Biochemistry, Santosh Medical College, Ghaziabad (NCR), India*

***Correspondence Info:**
Dr. Sudeep Kumar
Department of Biochemistry,
Muzaffarnagar Medical College, Muzaffarnagar (UP), India
E-mail: sudeepty@gmail.com

Abstract

Background: The addictive liability and pharmacological effects of smoking are primarily mediated by the major tobacco alkaloid nicotine. Cigarette smoke may promote atherogenesis by producing oxygen-derived free radicals that damage lipids. Cigarette smoking is associated with impaired endothelium-dependent vasodilatation and cardiovascular disease (CVD).

Aim: The Aim of our study was to determine the Level of lipid profile, liver function, CRP and MDA in cigarette smokers.

Method: A Total No. of 100 subjects were selected, out of which 50 were healthy individual and 50 were chronic smokers. The lipid parameter and Liver function were estimated by enzymatic method, CRP were estimated by Turbidometric kit method and MDA level were estimated by thiobarbituric Acid (TBA) method.

Result: Smokers have a significant higher level of liver enzyme such as *Aspartate Transaminase* (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP), lipid parameter such as cholesterol (CHO), triglycerides (TG) and Low Density Lipoprotein (LDL), Malondialdehyde (MDA) and C - reactive protein (CRP); where as decreased significant level of High Density Lipoprotein (HDL) and serum protein.

Keywords: Cigarette Smokers, MDA, CRP, lipid parameter

1.Introduction

Smoking may be explained as habit of inhalation of smoke arising from the burning of tobacco in a pipe or in the form of a cigar or cigarette. Nowadays the cigarette smoking is fashion but it causes many illnesses. A smoker is one who smokes more than 2 packets or packs or twenty cigarettes each day regularly ones a long period[1]. There are more than one billion smokers in the world with an increased smoking habbit[2]. Smokers are at greater risk for cardiovascular diseases, respiratory disorders, cancer, peptic ulcers, bone matrix loss and hepatotoxicity.[3][4] It has been recognised that CVD contains a component of inflammation and has even been referred to as an inflammatory disease[5]. In addition, a link has been established between several other chronic inflammatory diseases and smoking, including chronic obstructive pulmonary disease (COPD)[6], rheumatoid arthritis, systemic lupus erythematosus[7] and Crohn's disease[8]. Although the mechanisms linking smoking to these diseases are not well understood, interest in the relationship between inflammatory markers and smoking has been gathering pace in an attempt to provide explanations for smoking-mediated morbidity and mortality.

Cigarette smoke contains numerous compounds, many of which are oxidants and prooxidants, capable of producing free radicals and enhancing the oxidative stress[9]. Cigarette smoke is a complex mixture of over 7000 chemical compounds[10][11]. Each puff of cigarette smoke contains 10 reactive oxygen species (ROS)[12][13] like superoxide (O_2•), hydrogen peroxide (H_2O_2), hydroxyl (OH•) and peroxyl (ROO•) radicals. Free

radicals are highly reactive molecules generated by biochemical redox reactions that occur as a part of normal cell metabolism. Cigarette smoke contains hundreds of potentially toxic components and, as an impure mixture, has multiple actions in the human body. Both phases of cigarette smoke, particulate and gaseous, contain extremely high concentrations of free radicals. These toxins and free radicals can interact with DNA and could cause genetic mutations and gene activation responsible for the development of an autoimmune disease [14][15].

Free radicals may be produced by the human body, for instance by neutrophils, which produce reactive oxygen species and reactive nitrogen species, with a high oxidizing power[16]. Once they have been produced, these oxidizing species must be eliminated by the body's antioxidants[17]. When free radicals are overproduced or when the antioxidant defences are disrupted, a disturbance of the balance between pro-oxidants and antioxidants called oxidative stress is the result. The accumulation of free radicals leads to an altered oxidative status that may end in the oxidation and damage of biomolecules such as lipids, proteins or DNA. This damage can be assessed by determination of lipid hydroperoxides (LOOH), malonyldialdehyde (MDA) and 4-hydroxy-2nonenal for lipids while the analysis of carbonyl proteins (CPs) and 8-OH guanine is used to measure protein and DNA damage, respectively[18].

Nicotine stimulates sympathetic adrenal system leading to increased secretion of catecholamines 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 blood stream. Fall in oestrogen levels occur due to smoking which further leads to decreased HDL cholesterol. Presence of hyperinsulinaemia in smokers leads to increased cholesterol, LDL-C, VLDL-C and TG due to decreased activity of lipoprotein lipases.

The objective of the present study was to determine the levels of lipid peroxidation(MDA), CRP, liver function and lipid parameter in smokers and nonsmokers

2. Materials and Methods

The present study was carried out jointly in the Department of Biochemistry and Department of Medicine, Muzaffarnagar Medical College Muzaffarnagar in the period from January 2013 to December-2013. This study was approved by the Local Ethical Committee and informed consent was taken from the subjects, prior to study. Control group consists of 50 healthy individual and case group consists of 50 cigarette smokers. Total no. of subject we taken 100.

2.1 Inclusion criteria

The individual of control group were non-smokers. It contains 50 healthy individuals all were males with no clinical history. The individuals of case group were smokers. Case group contains 50 individuals; all were males with no clinical history.

2.2 Exclusion criteria

Patients with Diabetes, asthma, chronic obstructive pulmonary disease (COPD), malignancies, patients with STDs, patients with cardiac disease, patients with renal diseases, patients with hepatic diseases, patients with myocardial infarction, patients with gout and arthritis were excluded from the study.

2.3 Collection of sample

Blood from overnight fasting subjects was collected into tubes without anticoagulant in order to obtain serum. Blood was obtained by venous arm puncture and serum was separated by centrifugation at 3000 rpm for 15 min.[1] Separated serum was used for biochemical analysis.

2.4 Biochemical Analysis

Serum C - reactive protein (CRP) was estimated by using the Turbidometric kit method (SPINREACT, Spain). MDA level was measured as per thiobarbituric Acid (TBA) method described by Nourooz-Zadeh *et al*(1995)[19]. The liver function test, total cholesterol, HDL and triglycerides were estimated by enzymatic method. (Siemens, Gujarat, India). Low Density lipoprotein (LDL) and (Very Low Density lipoprotein) VLDL were calculated by Friedwald's Equation.

2.5 Statistical analysis

The statistical package Graph pad Prism 6 for windows was used for data analysis. Variables distributed normally are presented as mean and standard deviation and Pearson's correlation study were done.

3. Result

The clinical data for control and smoker patients are presented in table-1. Liver function, lipid parameter, MDA and CRP data are showing statistically significant values, however the γGT is non-significant. Analysing the table1, for MDA, those subject with smokers are having higher MDA level as compared to control ($p<.0001$) The smokers are having significantly ($p<.0001$) elevated level of CRP as compared to control subject.

Table-1 shows that the cases are having higher levels of liver enzymes function than smokers and which is highly significant (p<.0001). The lipid parameter (CHO, TG, LDL and VLDL) is higher in smokers then compared to nonsmokers (p<.0001) while HDL level is lower in smokers as compared to nonsmokers (p<.0001).

Table-1: Enzymes and biochemical changes in smokers

Variables	Control	Smoker	p-Value
Cholesterol (mg/dl)	172.28±17.36	284.48±16.25	<.0001*
Triglyceride (mg/dl)	136.92±10.41	183.32±17.88	<.0001*
HDL(mg/dl)	44.78±5.43	25.16±3.09	<.0001*
LDL(mg/dl)	98.12±25.12	223.12±18.09	<.0001*
VLDL(mg/dl)	27.38±2.08	36.67±3.58	<.0001*
Total Bilirubin (mg/dl)	0.82±0.22	0.49±0.13	<.0001*
AST (IU/L)	35.26±3.87	56.18±3.43	<.0001*
ALT (IU/L)	28.34±4.92	44.18±5.37	<.0001*
ALP(IU/L)	92.18±13.86	143.22±10.89	<.0001*
γGT(U/L)	16.04±2.84	16.5±3.15	=0.44
Total Protein (g/dl)	7.62±0.41	5.32±0.45	<.0001*
Albumin (g/dl)	4.22±0.33	2.95±0.32	<.0001*
MDA (nmol/ml)	1.6±0.54	2.82±0.72	<.0001*
CRP (mg/dl)	1.91±0.31	3.99±0.21	<.0001*

*P-Value <.0001 considered as significant

4. Discussion

In the present study we examined the enzymes and biochemical changes in smokers and non-smokers (Table 1). Cigarette smoking is a classical and a major risk factor in the development of several diseases with an inflammatory component, including cardiovascular disease and chronic obstructive pulmonary disease. Improvements in assays for protein markers of inflammation have led to many studies on these factors and their roles in disease.

Cigarette smoke contains high concentration of the gaseous compounds, like carbon monoxide (CO) Nitric oxide (NO) and Nitrogen dioxide (NO_2) and other substances in cigarettes such as aldehydes, hydrogen cyanide, lead, cadmium etc., Cigarette smoke has been reported to stimulate the production of hydrogen peroxides by the pulmonary alveolar macrophage cells. The formed peroxides can react with oxides of nitrogen (NO and NO_2) in cigarette smoke to form hydroxyl radicals (OH•)[1].

Cigarette smokers have high lipid peroxidation, which leads to damage the biomembranes and increased the plasma enzymes that reflect the protein concentration in plasma[20]. It was demonstrated that tobacco smoking is a major predictor of plasma MDA and F2-isoprostanes, two commonly used biomarkers of lipid peroxidation[21].

The Result of the present study showed that the serum malondialdehyde level was significantly higher in smoker when compared with non smoker. Our result was in agreement of Razaq et al who also found that MDA level was significantly higher in smokers as compared to nonsmokers. There are several reasons why smokers would be expected to have a higher level of lipid peroxidation compared with non-smokers. First, smokers are prone to oxidation from the inhalation of large numbers of gas-phase and other radicals giving rise to increased oxidative damage. Second, depletion of plasma antioxidants otherwise protecting against oxidative damage such as lipid peroxidation has consistently been observed among smokers.[22]

We found that the level of cholesterol, triglyceride, LDL and VLDL is significantly increases in smoker as compared to nonsmoker while HDL decreases significantly in smoker as compared to nonsmokers. Our results support study done by Alsalhen et al who observed that the significance increase in cholesterol, triglyceride. Various mechanism leading to lipid alteration by smoking are (a) Nicotine increased sympathetic adrenal system leading to increased secretion of catecholamines resulting in increased lipolysis and increased concentration of plasma free fatty acid which further result in increased secretion of hepatic free fatty acids and hepatic triglycerides along with VLDL-cholesterol in the blood stream (b) Presence of hyperinsulinaemia in smokers leads to increased activity of lipoprotein which leads to increased levels of lipid in smokers[23].

In our study we found that the level of liver enzymes (AST, ALT, ALP) were significantly increased in smokers as compared to nonsmokers while level of serum bilirubin, total protein and albumin decreases in smokers as compared to nonsmokers. Our result also in consistent with Alsalhen et al[23], they found that the liver enzymes (AST, ALT, ALP) significantly increases and total bilirubin, total protein, albumin and globulin decreases significantly in smoker as compared to nonsmoker. The protein depression in the blood may be due to loss of protein by reduce protein synthesis or increased proteolytic activity due to cigarette smoke exposure or via release of high levels of cellular oxidative free radicals could result increased proteolytic activity[24]. Also, the observed decrease in plasma protein of smokers could be attributed in

part to the damaging effect of harmful compounds from cigarette smoking on liver cells as confirmed by the increase in the activities of plasma ALT, AST and ALP in this present study (Table 1).

Although our results showed elevated ALP levels in the current smoker compared to never having smoked (Table 1). ALP was strongly influenced by smoking, consistent with other studies[25][26] concerning osteoporosis have documented increased serum ALP levels in current smokers, as a mainly marker of the liver and bones turnover, but is also present in the kidneys and leukocytes count.

We have also found increased CRP levels in study group as compared to control group. Many other studies have also shown similar findings. Joshi et al found increased CRP levels in higher percentage of subjects in study group as compared to control group[27]. Mahrukh et al also found raised CRP as well as complements like C3, C4 in smokers. They have attributed it to activation of monocytes and complement recruitment, resulting in the secretion of inflammatory cytokines which could further lead to release of CRP from liver in the blood[28]. Loughlin et al studied the association between cigarette smoking and CRP in adolescent girls and boys and found a positive relationship. They also showed a linear association of CRP with the number of cigarettes smoked per day[29]. A study by Pinto-Plata et al also supported our study[30].

5. Conclusion

In the present study, we found that smokers have higher plasma concentrations of MDA compared with non-smokers. Heavy smoking was associated with low total protein, globulin and albumin levels and raised AST, ALT and ALP levels. The levels of cholesterol and triglycerides were significantly increased in all smokers as compared to non- smokers. The association of smoking habits and liver functions test should be carefully analyzed and interpreted. Cigarette smoking decreases serum bilirubin. Further studies are needed to determine if measuring bilirubin is effective in detecting free radicals produced by smoking, which contribute to serious diseases. Thus our study concludes that smokers have higher risk than that of non-smokers.

References

[1] Ramamurthy V, Raveendran S, Thirumeni S, Krishnaveni S. Biochemical changes of cigarette smokers and non-cigarette smokers. *Int J Adv Lif Sci* 2012; 1:68-72.

[2] Aurelio, L. Biochemical markers of cardiovascular damage from tobacco smoke. *Curr. Pharm. Des.* 2005; 11:2190-2208.

[3] Witschi, H. A short history of lung cancer. *Toxicol. Sci.* 2001; 64: 4-6.

[4] Spiro S. G. and Silvestri, G. A. One hundred years of lung cancer. *Am. J.Respir. Crit. Care Med*. 2005; 172: 523-529.

[5] Tonstad S, Cowan J.L. C-reactive protein as a predictor of disease in smokers and former smokers: A review. *Int J Clin Pract* 2009; 63: 1550–3.

[6] Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. *Lancet* 2007; 370: 765–73.

[7] Majka DS, Holers VM. Cigarette smoking and the risk of systemic lupus erythematosus and rheumatoid arthritis. *Ann Rheum Dis* 2006; 65: 561–3.

[8] Lakatos PL, Szamosi T, Lakatos L. Smoking in inflammatory bowel diseases: good, bad or ugly? *World J Gastroenterol* 2007; 13: 6134–9.

[9] Arinola O.G., Akinosun O.M. and Olaniyi J.A., Passive- and active- cigarette smoking: Effects on the levels of antioxidant vitamins, immunoglobulin classes and acute phase reactants, *African J Biotech* 2011; 10: 6130-6132.

[10] Nagamma T., Anjaneyulu K., Baxi J., Dayaram P. and Singh P.P., Effects of Cigarette Smoking on Lipid Peroxidation and Antioxidant Status in Cancer Patients from Western Nepal, *Asian Pasific J Cancer Prev* 2011; 12: 313-316.

[11] Rodgman A. and Perfetti T.A., The chemical components of tobacco and tobacco smoke. Boca Raton, FL: CRC Press, Taylor and Francis group, Boca Raton. 2009.

[12] Risal S., Adhikari D., Alurkar V.M. and Singh P.P., Oxidative stress and antioxidant status in cardiovascular diseases in population of western Nepal. *Kath Univ Med J* 2006; 4: 271-74.

[13] Block G., Dietrich M., Norkus E.P., Morrow J.D., Hudes M., Caan B. and Packer L., Factors associated with oxidative stress in human population, *Am J of Epidem* 2002; 156: 274-285.

[14] Costenbader KH, Karlson EW. Cigarette smoking and autoimmune disease: what can we learn from epidemiology? *Lupus* 2006; 15:737-745.

[15] Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive

rheumatoid arthritis. *Arthritis Rheum* 2004; 50: 3085-92.

[16] Cedergren J, Forslund T, Sundqvist T, Skogh T. Intracellular oxidative activation in synovial fluid neutrophils from patients with rheumatoid arthritis but not from other arthritis patients. *J Rheumatol* 2007; 34:2162-2170.

[17] Valko M, Leibfritz D, Moncola J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol* 2007; 39:44-84.

[18] Seven A, Gu¨ zel S, Aslan M, Hamuryudan V. Lipid, protein, DNA oxidation and antioxidant status in rheumatoid arthritis. *Clin Biochem* 2008; 41:538-43.

[19] Nourooz-zadeh J, Tajaddini Sarmadi J, MC Carthy. S. et al. Elevated levels of authentic plasma hydroperoxides in NIDDM, *Diabetes* 1995; 44: 1054-1058.

[20] Sinus, B., Lzquierdo, M. and Vigaera, P. Chromosome aberrations aberrations and urinary thioethers in smokers. *Mut. Res.* 1990; 240:289 - 293.

[21] Lykkesfeldt J,Viscovich, M and Poulsen H.E, Plasma malondialdehyde is induced by smoking: a study with balanced antioxidant profiles. *British Journal of Nutrition* 2004; 92: 203–206.

[22] Razaq S.N.A, Ahmed BM Effect of cigarette smoking on liver function test and some other related parameters. *Zanco J Med Sci* 2013; 17 (3): 556-562.

[23] Alsalhen K.S, Abdalsalam R.D. Effect of cigarette smoking on liver functions: a comparative study conducted among smokers and non-smokers male in El-beida City, Libya. *International Current Pharmaceutical Journal*, June 2014, 3(7): 291-295.

[24] Tetley, T. D. (2006). Proteinase inhibitors/secretory leukoprotease inhibitor and elafin. In: Laurent GJ, Shapiro SD, editors. Encyclopedia of respira-tory medicine. London: Academic Press.

[25] Cheung, B. M., Ong, K. L.,Wong, L. Y. Elevated serum alkaline phosphatase and peripheral arterial disease in the United States National Health and Nutrition Examination Survey 1999–2004. *Int. J. Cardiol*. 2009; 135(2): 156-161.

[26] Wannamethee SG, Lowe GDO, Shaper AG, Rumley A, Lennon L, Whincup PH: Associations between cigarette smoking, pipe/cigar smoking, and smoking cessation, and haemostatic and inflammatory markers for cardiovascular disease. *Eur Heart J* 2005, 26:1765-1773.

[27] Joshi A.R, Salvi S, Phadke A.V Platelet Aggregability and C - reactive protein In Male Smokers. *International Journal of Basic and Applied Physiology*. 2013; 2(1): 109-113.

[28] Mahrukh S, Nageen H. Levels of inflammatory markers complement C3, complement C4 and C-reactive protein insmokers. *African Journal of Biotechnology*. 2011; 10(82): 19211-19217.

[29] Loughlin J O, Lambert M, Karper I, MacGrath J: Association between cigarette smoking and C-reactive protein in a representative, population-based sample of adolescents. *Nic Tob Res* 2008; 10(3):525-532.

[30] Pinto Plata VM, Mullerova H, Toso JF, Feudjo-Tepie M, Soriano JB, Vessey RS. C-reactive protein in patients with COPD in control smokers and non-smokers. *Thorax*. 2006; 61(1):23-28.