

THE EFFECTS OF PASSIVE INHALATION OF CIGARETTE SMOKE ON SERUM LIPID PROFILES IN RAT

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ABSTRACT

Cigarette smoke contains 4720 toxic and mutagenic agents such as CO and aromatic hydrocarbons. Cigarette has two types of smokes; the one is the main smoke which is the smoker inhales and the other one is the lateral smoke which is inhaled by others around a smoker and is more toxic than the main smoke. Lateral cigarette smoke contains five times more CO and 6 times more nicotine compared to the main smoke because cigarette filter has a protective role for smokers. Cigarette smoke contains a broad spectrum of oxidants and free radicals which can increase lipid peroxidation. So in any inhalation of cigarette smoke approximately 10^{14} free radicals enter the lungs. Free radicals can directly or indirectly induce oxidative stress. Adding some aromatic ingredients to cigarette may play an important role in increasing damage and free radicals. Therefore, in this study, the effect of passive cigarette smoke inhalation on serum lipid profiles in rats was evaluated. This study was conducted as comparative and intervention so 16 male rats weighing 200 to 250 g were divided randomly into two groups of eight rats, control and treatment. There was no intervention in the control group, but treatment group was exposed to a cigarette passive smoke daily for a month using a designed vacuum. After a month, blood samples were collected from the rats by decapitating, and then the sera were measured using diagnostic kits and by spectrophotometric method for serum lipid profiles, consisting triglycerides, total cholesterol, LDL and HDL. The results were statistically analyzed using t test. Comparison of results between treatment and control groups showed no significant differences in serum levels of cholesterol, triglycerides, and HDL but an increase in LDL serum levels was confirmed in the treatment group. We can conclude that smoking increases serum LDL, which is considered a risk factor for cardiovascular disease.

Keywords: Cigarette, Passive Smoking, Lipid Profiles, Rat

INTRODUCTION

Despite knowledge of harmful effects of smoking on Health, smoking habit in Human societies continues to expand. According to the World Health Organization, almost one-third of 15 years-old people are smokers (Zenzes *et al.*, 2000). Moreover, 26% and 3%, populations of men and women respectively are smokers in Iran (Ahmadi *et al.*, 2001). Smoking is considered as most important factor for early death in developing countries (Benowitz, 1988). Cigarette smoke contains 4720 toxic and mutagenic agents such as CO, aromatic hydrocarbons, and nicotine (Carvalho *et al.*, 2006). Nicotine as a volatile alkaloid is one of the important components of cigarette smoke and is able to create many hazardous effects. A cigarette has 2 to 24 percent nicotine (Kavitharaj *et al.*, 1999). Nicotine is able to negatively affect different body systems' homeostasis such as cardiovascular, endocrine, and genital systems (Carvalho *et al.*, 2006). Nicotine exerts its cellular effects via nicotinic acetylcholine receptors (nAChRs) (Dasgupta *et al.*, 2009). Smoking has always been considered as one of the oxidative stress factors. Cigarette smoke has several free radicals in a body that damages some vital macromolecules such as proteins and lipids. Also, enzymes are sensitive to free radicals because of their protein structure. So, their structure and activity can be affected by these molecules (Deaton *et al.*, 2003). Free radicals, atoms, single molecules and electrons have a very high reactive power and can damage different macro-molecules such as proteins, lipids, carbohydrates, by continuous circulation in the body and different cell DNA can be damaged as a result of (Ranjbar *et al.*, 2004). Triglyceride-Enriched lipoproteins after a meal are thrombogenic because of

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increased activity of active factor 8 and decreased PAI blood level (Cohn, 1998). Plasma LDL level and concentration the components resulted of nicotine damage the endothelial cells in smokers. Also, it has been confirmed that the coronary arteries are thicker in smokers (Rouzbahani *et al.*, 2009). Cigarette smoke contains a broad spectrum of oxidants and free radicals that can increase lipid peroxidation so in any inhalation of cigarette smoke enter approximately 10^{14} free radicals to the lungs. Free radicals can directly or indirectly induce oxidative stress (Greg, 2003). Therefore, in this study, the effect of passive cigarette smoke inhalation on serum lipid profiles in rats was evaluated.

MATERIALS AND METHODS

This study was conducted as comparative and intervention so 16 male rats weighing 200 to 250 g were divided randomly into two groups of eight rats, control and treatment and were placed into 60×30×30 cm propylene boxes. There was no intervention in the control group, but treatment group was exposed to a cigarette passive smoke daily for a month using a designed vacuum. After a month, all rats were under blood sampling by decapitating followed by centrifuge and serum separation. The sera were measured using diagnostic kits by spectrophotometric method for serum lipid profiles consisting triglycerides, total cholesterol, LDL and HDL and the results were Statistical analyzed using t test. During the study, both groups were exposed to equal environmental condition and light as well as unlimited access to water.

RESULTS AND DISCUSSION

Results

Mean Serum Cholesterol in Control and Smokers Groups: According to Table 1, the mean total cholesterol in the control and smokers groups was 85.12 ± 11.66 mg/dl and 96.25 ± 9.08 mg/dl, respectively. The results were measured according to the equality of variance test $F = 0.033$, confidence level of 95%, and significance level of $p = 0.859$ and it was demonstrated that both groups had an equal variance. Therefore, T test with an equal variance was used so that $T = 2.12$ and $P = 0.052$ at the confidence level of 95% were obtained. Hence, the observed difference in cholesterol mean of both groups was not significant ($P > 0.05$).

Table 1: Mean serum cholesterol per mg/dl of two understudied groups

Group	number	mean	F	P-value	T	df	P-value
Control	8	85.12 ± 11.66	0.033	0.859	2.12	14	0.052
Smokers	8	96.25 ± 9.08					

Mean Serum Triglyceride in Control and Smokers Groups: According to Table 2, the mean total triglyceride in the control and smokers groups was 58 ± 12.906 mg/dl and 73.25 ± 16.96 mg /dl, respectively. The results were measured according to the equality of variance test $F = 0.144$, confidence level of 95%, and significance level of $p = 0.710$ and it was demonstrated that both groups had an equal variance. Therefore, T test with an equal variance was used so that $T = 2.02$ and $P = 0.063$ at the confidence level of 95% were obtained. Hence, the observed difference in triglyceride mean of both groups was not significant ($P > 0.05$).

Table 2: Mean serum triglyceride per mg/dl of two understudied groups

Group	number	mean	F	P-value	T	df	P-value
Control	8	58 ± 12.906	0.144	0.710	2.02	14	0.063
Smokers	8	73.25 ± 16.96					

Mean Serum HDL in Control and Smokers Groups: According to Table 3, the mean total HDL in the control and smokers groups was 54.37 ± 9.21 mg/dl and 51.37 ± 7.38 mg/dl, respectively. The results were measured according to the equality of variances test $F = 0.485$, confidence level of 95%, and

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significance level of $p = 0.497$ and it was demonstrated that both groups had an equal variance. Therefore, T test with an equal variance was used so that $T = 0.719$ and $P = 0.484$ at the confidence level of 95% were obtained. Hence, the observed difference in HDL mean of both groups was not significant ($P > 0.05$).

Table 3: Mean serum HDL per mg/dl of two understudied groups

Group	number	mean	F	P-value	T	df	P-value
Control	8	54.37 ± 9.21					
Smokers	8	51.37 ± 7.38	0.485	0.497	0.719	14	0.484

Mean Serum LDL in Control and Smokers Groups: According to Table 4, the mean total LDL in the control and smokers groups was 17.85 ± 3.93 mg/dl and 27.77 ± 6.68 mg/dl, respectively. The results were measured according to the equality of variances test $F = 0.335$, confidence level of 95%, and significance level of $p = 0.572$ and it was demonstrated that both groups had an equal variance. Therefore, T test with an equal variance was used so that $T = 3.619$ and $P = 0.003$ at the confidence level of 97% were obtained. Hence, the observed difference in LDL mean of both groups was not significant ($P < 0.05$).

Table 4: Mean serum LDL per mg/dl of two understudied groups

Group	number	mean	F	P-value	T	df	P-value
Control	8	17.85 ± 3.93					
Smokers	8	27.77 ± 6.68	0.335	0.572	3.619	14	0.003

Discussion

In a study conducted on university students in Ankara (22 smokers and 22 nonsmokers), there was a significant difference in the levels of MDA, total cholesterol, HDL, LDL, cholesterol, VLDL, between the two groups (Ergunder *et al.*, 2009) that was not consistent with the results of this study on HDL and total cholesterol, but are quite consistent with the results of the present study about LDL. In a study conducted by Antoniades, significant increase was confirmed in total cholesterol, triglyceride and LDL levels and a significant reduction in HDL in smokers compared with controls that isn't consistent with the results of this study on total cholesterol, triglycerides and HDL, but not about LDL (Zamir *et al.*, 2000).

In another study conducted by Ergunder on 11 non-smokers as control group and 14 smokers consuming at least 10 cigarettes per day, a meaningful increase in MDA, total cholesterol and LDL in the smoking group compared with the control group was proved but there was no meaningful difference in HDL of both groups that is not consistent with the results of the present study concerning the level of serum total cholesterol and HDL but the two studies have consistency about LDL (Ergunder *et al.*, 2009).

Tai *et al.*, (2004) demonstrated that there was no difference in smokers and non smokers' total cholesterol and LDL that was consistent with the present study about cholesterol but didn't agree in LDL (Tai *et al.*, 2004).

This difference in the results of the studies can be linked to studies' cases that have genetic and epigenetic diversity (Garrison *et al.*, 1978).

Based on clinical trial conducted by Chattopadhyay on rats, subcutaneous injection of nicotine tartrate single dose (0.9% physiologic serum) at a dose of 3.5mg/kg per body weight daily for 15 days resulted in a significant increase ($P < 0.01$) in total cholesterol levels by 11% and increase significantly plasma triglycerides and LDL, ($p < 0.001$) compared with the control group which is inconsistent with the results of the present study (Chattopadhyay *et al.*, 2008). The study conducted by Chitra *et al.*, (2000) in which rats were exposed to smoke from burning daily for one month, a significant increase ($p < 0.01$) in total cholesterol and triglyceride levels was observed in smokers compared to controls that inconsistent with the results of the present study (Chitra *et al.*, 2000).

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An increase in free radicals and reactive oxygen species (ROS) would occur due to imbalance between the production of these compounds with antioxidant agents in the body that can be resulted of an interfere with the metabolism of fats, proteins, carbohydrates, and nucleotides (Jackson *et al.*, 2009). Pathologic effects of these factors can occur through various mechanisms, including lipid peroxidation, inhibition of protein synthesis and reduce the amount of adenosine triphosphate. Lipid peroxidation causes increased oxidized LDL (Ox-LDL), which may play a role in the synthesis and secretion of interleukin 1 (IL-1) from macrophages and secretion of cytokines. In contrast, HDL-cholesterol may have a protective role to inhibit the damaging effects of cholesterol and oxidative LDL (Oner *et al.*, 2008).

Many previous studies indicate that tobacco use reduces HDL levels, which is consistent with the results of the present study (Tsujii *et al.*, 2004). In conclusion it can be say that passive cigarette smoke may increase serum LDL that is considered one risk factors for cardiovascular disease.

REFERENCES

- Ahmadi G, Khalili H and Jooibar R (2001).** Prevalence of cigarette smoking in Iran. *Psychological Reports* **89**(2) 339-41.
- Akoachere JF, Ndip RN, Chenwi EB, Ndip LM, Njock TE and Anong DN (2002).** Antibacterial effect of *Zingiber officinale* and *Garcinia kola* on respiratory tract pathogens. *East African Medical Journal* **79**(11) 588-592
- Benowitz NL (1988).** Drug therapy. Pharmacologic aspects of cigarette smoking and nicotine addiction. *New England Journal of Medicine* **319** 1318-1330.
- Carvalho CA, Favaro WJ, Padovani CR and Cagnon VH (2006).** Morphometric and ultrastructure features of the ventral prostate of rats (*Rattus norvegicus*) submitted to long term nicotine treatment. *Andrologia* **38** 142-51.
- Chattopadhyay K and Chattopadhyay BD (2008).** Effect of nicotine on lipid profile, peroxidation & antioxidant enzymes in female rats with restricted dietary protein. *Indian Journal of Medical Research* **127** 571-576.
- Chitra R Semmalar and Shyamala Devi CS (2000).** Effect of fish oil on cigarette induced dyslipidemia in rats. *Indian Journal of Pharmacology* **32** 114-119.
- Cohn JS (1998).** Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. *Canadian Journal of Cardiology* **14** 18-27.
- Dasgupta P, Rizwani W, Pillai S, Kinkade R, Kovacs M and Rastogi S *et al.*, (2009).** Nicotine induces cell proliferation, invasion and epithelial mesenchymal transition in a variety of human cancer cell lines. *International Journal of Cancer* **124** 36-45.
- Deaton CM and Marlin DJ (2003).** Exercise-associated oxidative stress. *Clinical Techniques in Equine Practice* **2**(3) 278-91.
- Ergunder, Asli Ucar, Işıl Ariturk, Toker Erguder, Aslihan Avc, Seniha Hasipek and Kadirhan Sunguroglu (2009).** The effects of cigarette smoking on serum oxidant status, and cholesterol, homocysteine, folic acid, copper, and zinc levels in university students. *Turkish Journal of Medical Sciences* **39**(4) 513-517.
- Garrison RJ, Kannel WB, Castelli WP, Feinlieb M, McNamara PM and Padgett SJ (1978).** Cigarette smoking and HDL cholesterol. *Atherosclerosis* **30** 17– 25.
- Greg Kelly (2003).** The Interaction of Cigarette Smoking and Antioxidants. *Ascorbic Acid Alternative Medicine Review* **8**(1) 43- 54.
- Jackson LW, Schisterman EF, Dey-Rao R, Browne R and Armstrong D (2009).** Oxidative stress and endometriosis. *Human Reproduction* **20**(7) 2014–2020.
- Kavitharaj N and Vijayammal P (1999).** Nicotine administration induced changes in the gonadal functions in male rats. *Pharmacology* **58** 2-7.
- Oner Iyidogan Y, Kocak H, Gurdol F, Korkmaz D and Buyru F (2008).** Indices of oxidative stress in eutopic and ectopic endometria of women with endometriosis. *Gynecologic and Obstetric Investigation Journal* **57** 214-217.

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Ranjbar A, Zhand Y, Mirzadeh E, Esmaeili A, Ghasemi Nejad S and Maleki Rad AA (2004). Comparison of oxidative stress in smokers and non smokers. *Journal of Arak Medical School* **7**(3) 7-11.

Rouzbahani R, Asgary S, Naderi GA, Dehghan Nejad M and Rezaei F (2009). Comparison of plasma peroxidants, glycosylated hemoglobin, conjugated dienes and CRP level in smokers and non-smokers men. *Journal of Isfahan Medical School* **27**(93) 115-121.

Tai ES and Tan Genes CE (2001). Diet and serum lipid concentrations Lessons from ethnically diverse populations and their relevance to coronary heart disease in Asia. *Current Opinion in Lipidology* **15** 5–12.

Tsujii S and Kuzuya H (2004). The significance of lifestyle as a factor for the metabolic syndrome. *Nippon Rinsho Journal* **62**(6) 1047-1052.

Zamir Ahmad Akbari, Muhammad Sarwar Bhatti and Muhammad Shakoore (2000). Lipid profile in smoking. *JAMC* **12**(3) 19-21.

Zenzes MT (2000). Smoking and reproduction: gene damage to human gametes and embryos. *Human Reproduction Update* **6** 122-131.