

Effect of Smoking on Serum Amyloid A-Low Density Lipoprotein in Sprague Dawley Rats

¹Aneela Jamil, ¹Amir Rashid, ²Abdul Khaliq Naveed and ¹Asifa Majeed

¹Department of Biochemistry and Molecular Biology, Army Medical College, National University of Sciences and Technology Islamabad, Pakistan

²Department of Biochemistry, Islamic International Medical College, Riphah International University Rawalpindi, Pakistan

Abstract: *Objective:* To investigate the effect of cigarette smoking on SAA-LDL in Sprague Dawley rats. *Study Design:* A randomized control trial. *Place and Duration of Study:* Army Medical College, Rawalpindi and National Institute of Health, Islamabad, from July 2014 to January 2015. *Methodology:* Seventy healthy Sprague Dawley rats were randomly divided into two groups. Group-I rats were not exposed while group-II rats were exposed to cigarette smoke for 3months. SAA-LDL levels were determined in both these groups using commercially available enzyme linked immunosorbent assay (ELISA) kit. Results revealed that higher levels of SAA-LDL were found in smoke exposed as compared to that of control rats. Mean values of SAA-LDL in control and smoke exposed groups were 1.51 ± 0.29 and 2.99 ± 0.59 respectively. The difference was statistically significant ($p < 0.05$). *Conclusion:* Positive association was found between cigarette smoking and SAA-LDL. This shows that SAA-LDL, an oxidative stress marker, may be used as an early indicator of oxidative damage in smoke related disorders.

Key words: Cigarette Smoking • Oxidized LDL • SAA-LDL • Oxidative Stress

INTRODUCTION

Tobacco use is one of the greatest public health hazards the world has faced [1]. Tobacco is commonly smoked in different ways including cigarette, cigar and waterpipe. Tobacco smoke remains the main preventable cause of mortality and morbidity worldwide. WHO estimates that the number of smokers worldwide will increase from 1.1 to 1.6 billion by 2025 [2]. Globally more than 5 million people per year die from tobacco use. According to a report, the prevalence of smoking in Pakistan was 23% [3]. Cigarette smoking is an important risk factor for developing heart disease, chronic obstructive pulmonary disease, stroke and acute respiratory diseases [4, 5]; therefore it is important to understand the mechanisms through which smoking increases vulnerability to these disorders as it may establish new avenues for prevention or treatment of complex smoke related disorders [6]. The mechanisms by which tobacco smoking might cause these negative health

effects include increased tissue inflammation and the production of high amount of free radicals in smokers [7, 8].

Reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2) are continually produced by endogenous oxygen metabolism in all living species [9, 10]. Cigarette smoking is an important exogenous factor leading to the production of ROS [11]. Cigarette smoke itself is a source of free radicals; moreover, inhalation of particles causes recruitment of inflammatory cells, neutrophils and macrophages, to the lung tissue. These cells undergo activation resulting in synthesis and release of ROS and in up regulation of factors involved in inflammation and fibrosis [12]. One puff of cigarette smoke contains 10^{18} free radicals [13]. Production of ROS in excess of the endogenous antioxidant capacity of a cell is referred to as oxidative stress [14]. Increase ROS cause oxidation of DNA, protein and lipids and are implicated in the pathogenesis of several diseases including atherosclerosis.

ROS are highly reactive and short lived [15] and therefore the best way to estimate oxidative stress is to quantify the products of their reaction with lipids, proteins and nucleic acids [16]. Elevated levels of oxidative stress biomarkers in smokers are due to both increased ROS production from smoke exposure as well as an impaired antioxidant defence system.

Serum amyloid A low density lipoprotein (SAA-LDL) is formed by oxidative modification of LDL [17, 18]. Polyunsaturated fatty acid residues of lipids and apolipoprotein B100 in LDL are the main targets of oxidation [19]. Oxidized LDL is an accepted oxidative stress marker [20]. Serum amyloid-A (SAA), an acute phase protein, is produced in the liver in response to inflammation, injury, infection, or stress. SAA-LDL complex is considered a new and unique marker of oxidatively-modified LDL particles, which is associated with atherosclerotic conditions [21]. Kotani K *et. al* showed that SAA-LDL measurements may be useful for the assessment of the risk of cardiovascular disease as a biochemical marker [22]. The aim of this study was to investigate effect of smoking on SAA-LDL.

MATERIALS AND METHODS

The study was designed as a randomized control trial conducted at Department of biochemistry and Molecular Biology, Army Medical College Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad and Centre for Research in Experimental and Applied Medicine (CREAM), Army Medical College, Rawalpindi. The protocol of the study was approved by Ethical Committee of CREAM, Army Medical College, Rawalpindi. Total number of 70 healthy Sprague Dawley rats was acquired from NIH, Islamabad. Rats were kept under standard conditions with a daily photoperiod of 12 hours light and 12 hours dark at animal house. All rats were fed normal pellet diet (NPD) and water ad libitum, they were randomly distributed among the groups with 35 rats in each group: group I (control) and group II (exposed). Rats in group-II were placed in smoke chamber and were exposed to cigarette smoke (10 cigarettes/day) for 12 weeks. Lit cigarette was introduced into the smoke chamber. Each cigarette burnt for 10-12mins. A break of 15 minutes was given before the start of the next cigarette, to allow the entry of a little amount of fresh air. For comparison control rats were exposed to only fresh air in an identical chamber, where cigarette smoke was not introduced.

Blood sample measuring 3 ml was collected from each rat of both the groups at the end of 12 weeks by intracardiac puncture. Blood was taken in vacuum tubes containing clot activator. Serum obtained by centrifugation was stored at -80°C. SAA-LDL levels were determined using a commercially available ELISA kit at CREAM, Army Medical College. All data were analysed by SPSS version 17 and represented as mean \pm standard deviation. The statistical differences between the smoke exposed and control groups were calculated by using independent sample t-test. Significant *p* value was < 0.05 .

RESULTS

The comparison of mean of Serum Amyloid A-Low Density Lipoprotein (SAA-LDL) levels between two groups at the end of 12 weeks is shown in table 1. The mean SAA-LDL level was 1.51 ± 0.29 for control group rats while it was 2.99 ± 0.59 was for group II rats. The difference between control group and group II was statistically significant ($p < 0.05$). The mean SAA-LDL level was significantly increased in exposed as compared to that of control group rats.

Table 1: Mean of SAA-LDL levels in group I and II

Variable	Mean \pm SD values of both groups		
	Group I (n = 35)	Group II (n = 35)	<i>p</i> Value
SAA-LDL	1.51 ± 0.29	2.99 ± 0.59	< 0.05

DISCUSSION

The present study was conducted to ascertain whether cigarette smoke induces oxidative stress using a relatively new oxidative stress marker, SAA-LDL. Biomarkers of oxidative stress can be grouped as molecules that are modified by ROS and antioxidant molecules. Molecules that can be modified by interaction with excessive ROS include DNA, lipids and proteins. Factors which influence the clinical applicability of a oxidative stress biomarker include the ease of acquiring a suitable biological specimen; the stability of the biomarker throughout specimen preparation steps; the specificity, sensitivity and reproducibility of the assay used to measure the modification.

Consistent with previous studies, the present study, using the new oxidized LDL related marker, SAA-LDL, increases understanding of smoking-lipoprotein associations. In this study, we found a strong correlation between SAA-LDL and cigarette smoking ($p < 0.05$).

The mean SAA-LDL of smoker group rats is 1.98-fold higher than that of control group rats. The findings reflect that SAA-LDL levels are high in smoker group rats than in control rats which indicate that cigarette smoking leads to oxidative LDL modification thus linking oxidized LDL (ox-LDL) with smoke related diseases. It has been proposed that circulating ox-LDL plays a critical role in cardiovascular diseases such as atherosclerosis [23]. Some biological explanations of linking ox-LDL with smoke related diseases can be the following. First, an increase in ox-LDL levels activates chemotaxis of neutrophils and monocytes [24]. Second, ox-LDL has been shown to increase the expression or secretion of pro-inflammatory cytokines [25]. Third, oxidized lipids are thought to promote a shift from acute to chronic inflammation. Forth, ox-LDL increases intracellular ROS production thereby increasing oxidative stress [26].

Most studies observed positive association between cigarette smoking and oxidative stress using different oxidative stress markers. Elevated levels of oxidative stress biomarkers in smokers are due to both increased ROS production from smoke exposure as well as an impaired antioxidant defence system. In our study SAA-LDL was used as an oxidative stress marker. Our results are consistent with findings of most other studies that smoking increases oxidative stress. A study published in 2011 showed that significantly higher SAA-LDL levels were observed in current smokers versus non-smokers [17]. Wada H *et. al* also showed that serum levels of SAA-LDL were significantly increased in current compared to non-current smokers [18]. Nawrot TS *et. al* showed in his study that plasma oxidized-LDL level was higher in smokers than in non-smokers [27]. Another study showed that smoking oxidizes low-density lipoprotein (LDL) particles [28]. Malondialdehyde (MDA) and oxLDL were both found to be high in smokers compared to non-smokers in a study by Bloomer RJ (14). Campos KK *et. al* found that the oxidative damage was significantly higher in cigarette smoke exposed mice compared to the control group [29]. According to a study published in 2012, reactive oxygen species and lipid peroxidation levels in lung tissue were significantly increased in mice exposed to cigarette smoke [30]. Increased concentrations of both reactive oxygen species and lipid peroxidation products were found in sperm cells of mice exposed to cigarette smoke in a study by La Maestra *et al.* [31]. Another study carried out by Ramesh T *et al* concluded that rats exposed to cigarette smoke

showed significant increase in oxidative stress markers like hydroperoxides (HP) and MDA levels with associated decrease in reduced glutathione (GSH), vitamin C, vitamin E and antioxidant enzymes [32].

A study carried out in 2014 showed high levels of urinary MDA and down-regulation of expressions of antioxidant related genes in smokers as compared to non-smokers ($p < 0.05$) [33]. Another study showed significant difference ($p < 0.001$) in the MDA levels in control and smoker groups. This study also demonstrated significant reduction in antioxidant enzymes in smoker group as compared to healthy controls [34]. A case control study carried out in 2013 revealed decreased superoxide dismutase levels in cases compared to control group [35]. Another study showed that smoking habits independently induces the formation of F2-isoprostanes and increases the oxidative stress level [36]. Nagamma T *et al* observed a significant rise in plasma MDA and low levels of antioxidants in breast cancer patients with smoking habit as compared to non-smoking breast cancer patients [37]. Wozniak A *et. al* found that superoxide dismutase activity was 40% higher ($p < 0.05$) while glutathione peroxidase was 41% lower ($p < 0.001$) in smokers than in controls.

CONCLUSION

The present study concluded that cigarette smoking is associated with increased levels of oxidative stress as measured by SAA-LDL which can therefore be used as an oxidative stress marker predictive of smoke related disorders. However, more detailed studies are needed to clarify the clinical relevance and the mechanisms associated with these findings.

REFERENCES

1. Uma, A., R. Pajanivel and S. Raj Lokeshmaran, 2011. Smoking-induced satellite associations in a rural population of south India: An in vitro study. *International journal of applied and basic medical research*, 1(2): 75-9.
2. Pattemore, P.K., 2013. Tobacco or Healthy Children: The Two Cannot Co-Exist. *Frontiers in pediatrics*. 1: 20.
3. Moosazadeh, M., H. Ziaaddini, A. Mirzazadeh, A. Ashrafi-Asgarabad and A.A. Haghdooost, 2014. Meta-analysis of Smoking Prevalence in Iran. *Addiction and health*, 2013; 5(3-4): 140-53.

4. Chen, R.J., L.W. Chang, P. Lin and Y.J. Wang, 2011. Epigenetic effects and molecular mechanisms of tumorigenesis induced by cigarette smoke: an overview. *Journal of oncology*, 2011; 2011: 654931.
5. Chang, S.A., 2013. Smoking and type 2 diabetes mellitus. *Diabetes and metabolism journal*. 2012; 36(6): 399-403.
6. Dogan, M.V., B. Shields, C. Cutrona, L. Gao, F.X. Gibbons, R. Simons, M. Monick, G.H. Brody, K. Tan, S.R. Beach and R.A. Philibert, 2014. The effect of smoking on DNA methylation of peripheral blood mononuclear cells from African American women. *BMC genomics*, 15: 151.
7. Khabour, O.F., K.H. Alzoubi, M. Bani-Ahmad, A. Dodin, T. Eissenberg and A. Shihadeh, 2012. Acute exposure to waterpipe tobacco smoke induces changes in the oxidative and inflammatory markers in mouse lung. *Inhalation toxicology*. 2012; 24(10): 667-75.
8. Tanni, S.E., C.R. Correa, A.Y. Angeleli, S.A. Vale, L.S. Coelho and I. Godoy, 2012. Increased production of hydrogen peroxide by peripheral blood monocytes associated with smoking exposure intensity in smokers. *J. Inflamm. (Lond)*, 2012; 9(1): 45.
9. Chia, T., C.Y. Hsu and H.L. Chen, 2008. Oxidative damage of workers in secondary metal recovery plants affected by smoking status and joining the smelting work. *Industrial health*. 2008; 46(2): 174-82.
10. Margaret, A.L., E. Syahrudin and S.I. Wanandi, 2011. Low activity of manganese superoxide dismutase (MnSOD) in blood of lung cancer patients with smoking history: relationship to oxidative stress. *Asian Pacific journal of cancer prevention: APJCP*. 2011; 12(11): 3049-53.
11. Rajendrasozhan, S., S.R. Yang, I. Edirisinghe, H. Yao, D. Adenuga and I. Rahman, 2008. Deacetylases and NF-kappaB in redox regulation of cigarette smoke-induced lung inflammation: epigenetics in pathogenesis of COPD. *Antioxidants and redox signaling*, 2008; 10(4): 799-811.
12. Ignatowicz, E., A. Wozniak, M. Kulza, M. Senczuk-Przybylowska, F. Cimino, W. Piekoszewski, M. Chuchracki and E. Florek, 2013. Exposure to alcohol and tobacco smoke causes oxidative stress in rats. *Pharmacological reports: PR*. 2013; 65(4): 906-13.
13. Padmavathi, P., V.D. Reddy, P. Maturu and N. Varadacharyulu, 2010. Smoking-induced alterations in platelet membrane fluidity and Na(+)/K(+)-ATPase activity in chronic cigarette smokers. *Journal of atherosclerosis and thrombosis*. 2010, 17(6): 619-27.
14. Bloomer, R.J., 2007. Decreased blood antioxidant capacity and increased lipid peroxidation in young cigarette smokers compared to nonsmokers: Impact of dietary intake. *Nutrition journal*, 2007, 6: 39.
15. Asano, H., T. Horinouchi, Y. Mai, O. Sawada, S. Fujii, T. Nishiya, M. Minami, T. Katayama, T. Iwanaga, K. Terada and S. Miwa, 2012. Nicotine-and tar-free cigarette smoke induces cell damage through reactive oxygen species newly generated by PKC-dependent activation of NADPH oxidase. *Journal of pharmacological sciences*, 2012, 118(2): 275-87.
16. Arja, C., K.M. Surapaneni, P. Raya, C. Adimoolam, B. Balisetty and K.R. Kanala, 2013. Oxidative stress and antioxidant enzyme activity in South Indian male smokers with chronic obstructive pulmonary disease. *Respirology*, 2013, 18(7): 1069-75.
17. Kotani, K., N. Satoh-Asahara, Y. Kato, R. Araki, A. Himeno, H. Yamakage, K. Koyama, M. Tanabe, M. Oishi, T. Okajima and A. Shimatsu, 2011. Serum amyloid A low-density lipoprotein levels and smoking status in obese Japanese patients. *The Journal of international medical research*, 2011, 39(5): 1917-22.
18. Wada, H., S. Ura, N. Satoh-Asahara, S. Kitaoka, S. Mashiba, M. Akao, M. Abe, K. Ono, T. Morimoto, M. Fujita, A. Shimatsu, Y. Takahashi and K. Hasegawa, 2012. Alpha1-Antitrypsin low-density-lipoprotein serves as a marker of smoking-specific oxidative stress. *Journal of atherosclerosis and thrombosis*, 2012, 19(1): 47-58.
19. Arai, H., 2014. Oxidative modification of lipoproteins. *Sub-cellular biochemistry*, 2014, 77: 103-14.
20. Barbosa, K.B., A.C. Volp, H.H. Hermsdorff, I. Navarro-Blasco, M.A. Zulet, J.A. Martinez and J. Bressan, 2011. Relationship of oxidized low density lipoprotein with lipid profile and oxidative stress markers in healthy young adults: a translational study. *Lipids in health and disease*, 2011, 10: 61.
21. Kotani, K., T. Yamada, M. Miyamoto, S. Ishibashi, N. Taniguchi and A. Gugliucci, 2012. Influence of atorvastatin on serum amyloid A-low density lipoprotein complex in hypercholesterolemic patients. *Pharmacological reports: PR*. 2012; 64(1): 212-6.

22. Kotani, K., H. Koibuchi, T. Yamada and N. Taniguchi, 2009. The effects of lifestyle modification on a new oxidized low-density lipoprotein marker, serum amyloid A-LDL, in subjects with primary lipid disorder. *Clinica chimica acta; international journal of clinical chemistry*, 2009, 409(1-2): 67-9.
23. Shen, Y., T. Yang, S. Guo, X. Li, L. Chen, T. Wang, F. Wen, 2013. Increased serum ox-LDL levels correlated with lung function, inflammation and oxidative stress in COPD. *Mediators of inflammation*, 2013, 2013: 972347.
24. Behnes, M., M. Brueckmann, V. Liebe, C. Liebetrau, S. Lang, C. Putensen, M. Borggrefe and U. Hoffmann, 2008. Levels of oxidized low-density lipoproteins are increased in patients with severe sepsis. *Journal of critical care*, 2008; 23(4): 537-41.
25. Greig, F.H., S. Kennedy and C.M. Spickett, 2012. Physiological effects of oxidized phospholipids and their cellular signaling mechanisms in inflammation. *Free radical biology and medicine*, 2012; 52(2): 266-80.
26. Min, K.J., K.H. Cho and T.K. Kwon, 2012. The effect of oxidized low density lipoprotein (oxLDL)-induced heme oxygenase-1 on LPS-induced inflammation in RAW 264.7 macrophage cells. *Cellular signalling*, 2012; 24(6): 1215-21.
27. Nawrot, T.S., J.A. Staessen, P. Holvoet, H.A. Struijker-Boudier, P. Schiffers, L.M. Van Bortel, R.H. Faqard, J.P. Gardner, M. Kimura and A. Aviv, 2010. Telomere length and its associations with oxidized-LDL, carotid artery distensibility and smoking. *Front Biosci (Elite Ed)*, 2010; 2: 1164-8.
28. Urahama, N., G. Iguchi, M. Shimizu, K. Fujihira, S. Kobayashi, H. Baba, 2008. Smoking and small, dense low-density lipoprotein particles: cross-sectional study. *Nicotine and tobacco research: official journal of the Society for Research on Nicotine and Tobacco*, 2008; 10(8): 1391-5.
29. Campos, K.K., R.G. Manso, E.G. Goncalves, M.E. Silva, W.G. de Lima, C.A. Menezes, F.S. Bezerra, 2013. Temporal analysis of oxidative effects on the pulmonary inflammatory response in mice exposed to cigarette smoke. *Cellular immunology*, 2013; 284(1-2): 29-36.
30. Nemmar, A., H. Raza, D. Subramaniyan, A. John, M. Elwasila, B.H. Ali, E. Adeghate, 2012. Evaluation of the pulmonary effects of short-term nose-only cigarette smoke exposure in mice. *Exp Biol. Med. (Maywood)*, 2012; 237(12): 1449-56.
31. La Maestra, S., S. De Flora, R.T. Micale, 2015. Effect of cigarette smoke on DNA damage, oxidative stress and morphological alterations in mouse testis and spermatozoa. *International journal of hygiene and environmental health*, 2015; 218(1): 117-22.
32. Ramesh, T., C. Sureka, S. Bhuvana, V.H. Begum, 2014. Oxidative stress in the brain of cigarette smoke-induced noxiousness: neuroprotective role of *Sesbania grandiflora*. *Metabolic brain disease*, 2014.
33. Na, H.K., M. Kim, S.S. Chang, S.Y. Kim, J.Y. Park, M.W. Chung and M. Yang, 2014. Tobacco smoking-response genes in blood and buccal cells. *Toxicology letters*, 2014.
34. Waseem, S.M., M.H. Mobarak, N. Islam and Z. Ahmad, 2012. Comparative study of pulmonary functions and oxidative stress in smokers and non-smokers. *Indian journal of physiology and pharmacology*, 2012; 56(4): 345-52.
35. Naga Sirisha, C.V. and R.M. Manohar, 2013. Study of antioxidant enzymes superoxide dismutase and glutathione peroxidase levels in tobacco chewers and smokers: a pilot study. *Journal of cancer research and therapeutics*, 2013; 9(2): 210-4.
36. Romanazzi, V., V. Pirro, V. Bellisario, G. Mengozzi, M. Peluso, M. Pazzi, M. Buqiani, G. Verlato and R. Bono, 2013. 15-F(2)t isoprostane as biomarker of oxidative stress induced by tobacco smoke and occupational exposure to formaldehyde in workers of plastic laminates. *The Science of the total environment*, 2013; 442: 20-5.
37. Nagamma, T., J. Baxi and P. Singh, 2014. Status of oxidative stress and antioxidant levels in smokers with breast cancer from Western Nepal. *Asian Pacific journal of cancer prevention: APJCP.*, 2014; 15(21): 9467-70.