ECRONICON OPEN ACCESS

DENTAL SCIENCE
Research Article

Impact of Passive Smoke on Tongue Blood Vessels, Blood Cell Count and Blood Glutathione Level (Experimental Study)

Rasha H Al-Serwi[1], Samah H Elmeadawy[2] and Lobna RS Radwan[1]*

[1]*Department of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt*

[2]*Departments of Oral Medicine and Periodontology, Faculty of Dentistry, Mansoura University, Egypt*

***Corresponding Author:** Lobna RS Radwan, Department of Oral Biology, Faculty of Dentistry, Mansoura University, Egypt.

Received: September 19, 2015; **Published:** November 05, 2015

Abstract

Introduction: Passive smoke has found to produce local and systemic effects and impair the function of many organs. Passive smoke or second hand smoke is an involuntary exposure to the burn off end of the cigarette smoke which is directly released to the environment. Passive smokers can face the same health problems as smokers.

Aim of the work: To evaluate the effect of passive cigarette smoke on the tongue blood vessels in rats and investigate its effect on blood cell count and glutathione level in blood.

Materials and methods: 30 male Wister albino rats were used and divided into three groups (ten in each): Group I, control group. Group II & III exposed to passive cigarette smoke in closed smoking device (4 cigarettes twice daily) for 30 days and 60 days respectively. Complete blood cell count and glutathione level were measured. The animals were euthanized, tongue specimens were taken, prepared for histological (H & E and Trichrome stain) and immunohistochemical study for detection of α-smooth muscle actin.

Results: Tongue blood vessels exposed to passive smoke showed abnormal dilated blood vessels congested with blood, degenerating endothelial cells and decrease in the pericytes all around the blood vessel. Also, destruction and loss of the subintimal collagen layer were detected with Gomori's trichrome stain. Immunohistochemical study revealed mild to minimum immunoreaction for α SMA on the blood vessels wall in comparison with the intense reaction of the control group. There were significant increases of WBCs count, RBCs count, Hb concentration and hematochrit values in passive smoke. Also, there was significant decrease in glutathione levels.

Conclusion: Passive smoke may lead to blood vessel damage, significant changes in blood cells and glutathione levels. These changes may lead to greater risk for developing atherosclerosis and cardiovascular diseases.

Keywords: *Glutathione; Passive smokers; Cigarette smoke; Hematochrit value; Immunohistochemical study*

Introduction

Smoking is the leading cause of morbidity and mortality in the developed world, causing over 4.8 million deaths per year globally [1]. Morbidity and death rate are likely even higher than current knowledge, due to the largely unknown drawbacks of passive smoke [2]. Active smoking is a hazard factor for conditions including cardiovascular disease, respiratory tract infection, lung, tracheal and bronchial cancers, mucosal alternations and periodontitis [3-8].

Passive smoking has found to produce local and systemic effects and to impair the function of many organs. Passive smoke produces the impairment of the growth and development of children, and increases the incidence of cancer [9], respiratory tract infections in children [10,11], premature vascular aging, and sudden infant death [12]. As regards to the effect of passive smoking on oral tissues, it was found to be associated with periodontal diseases [13,14], caries, alterations in salivary flow rate and protein levels and decrease in salivary PH in children [15].

Cigarette smoke contains over 4000 chemicals, many of which have toxic, carcinogenic effects on different biological systems [16]. Nicotine, the most pharmacologically active compound, is mostly absorbed through the lung alveoli. It is also absorbed in sufficient amount even in a slower rate through the oral mucosa to have a pharmacological effect [17].

Smoking increases the heart rate, cardiac output, and blood pressure and produce peripheral vasoconstriction by autonomic stimulation [18]. Moreover, nicotine also acts directly on blood vessels and capillaries to produce vasoconstriction [19]. Evidence suggests that smoking cause compromised vasodilatation, decreased blood flow to gingiva due to the direct vasoconstricting actions of nicotine [20,21]. Another study used a laser Doppler technique to measure gingival blood flow, and its results showed that smoking doesn't compromise blood flow in the periodontal tissues [22]. Thus, there might be some controversy regarding the effect of tobacco consumption on the gingival blood vessels.

Cigarette smoke contains a large number of free radicals [23]. Free radical may be defined as "any species capable of independent existence that contains one or more unpaired electrons." Reactive oxygen species (ROS) have the ability to produce direct damage to proteins, DNA, carbohydrates and lipids [24]. The main targets of reactive oxygen species (ROS) are polyunsaturated fatty acids present in membrane lipids causing lipid peroxidation and malondialdehyde (MDA) is formed [25].

The antioxidants as vitamin-E, vitamin-C, glutathione peroxidase and superoxide dismutase prevent tissue damage induced by free radicals [26]. Glutathione peroxidase reduces hydrogen peroxide and/or lipid hydrogen peroxides by the oxidation of reduced glutathione or s-nitrosoglutathione [27]. In inflammatory diseases and periodontitis, reduced levels of GSH are detected [28], indicating the involvement of oxidative stress. GSH depletion is also associated with the augment of a pro-inflammatory signal by up-regulating ROS [29].

Therefore, the present study was aimed to evaluate the effect of passive cigarette smoke on the tongue blood vessels in rats and investigate its effects on blood cell count and glutathione level in blood.

Materials and Methods

Animals

Thirty male Wister albino rats (200-250g) were used in this study. The rats were housed in an animal house under standard laboratory conditions (temperature of the room: 22 + 2, humidity: 55 + 5L and 12 hours light\dark cycle). They were allowed commercial standard diet and water ad-libitum.

Experimental protocol

Rats were divided into three groups 10 rats in each: Group I, control group. Group II, exposed to cigarette smoke (Cleopatra) in closed smoking device (4 cigarettes twice daily) for 30 days. Group III, exposed to cigarette smoke (Cleopatra) in closed smoking device (4 cigarettes twice daily) for 60 days.

Smoking device

Closed glass container with ventilation hole at the top and attached metal tube to one side by which main stream of cigarette enters the device and another opening at the top for handling of rats before and after exposure.

Laboratory assessment

Blood samples were taken for complete blood cell count and glutathione level estimation [30].

Histological study

The animals were euthanized; tongue specimens were processed for histological evaluation by routine H&E and Gomori's trichrome stains for detection of collagen fibers. Immunolabeling of α-SMA was performed using the Dako kit. After deparaffinization of the sections in xylene, the sections were rehydrated in ethanol and water. The sections were immersed in 0.1% H_2O_2 for 30 minutes to avoid the activity of the endogenous peroxidase enzyme. Then the sections were washed with phosphate-buffered solution (PBS) and were immersed in ethylene diaminetetraacetic acid (EDTA) buffer or sodium citrate for 10 minutes at 95ºC. Then the sections were cooled to room temperature and were incubated for 20 minutes in 10% normal goat serum to avoid the background activity. After the addition of primary antibodies for (α-SMA), the sections were incubated with mouse or rabbit secondary antibody for 30 minutes at room temperature, and washed in PBS. Finally the sections were stained with diaminobenzidine tetrahydrochloride solution (DAB), and counterstained with hematoxylin [31].

Statistical analysis

Data expressed as mean ± SD In the statistical comparison between the different groups, the significance of difference was tested using student's t-test to compare between mean of two groups of numerical (parametric) data. Pearson correlation coefficient® test was used to correlate different parameters. A P value < 0.05 was considered statistically significant. Analysis was done using the computer program SPSS (Statistical package for social science) version 17.

Results

Histological results

Hematoxylin and eosin stain findings

Group I, showed normal architecture of the blood vessels with endothelial cells lining and normal pericyte arrangement Figure 1A. Group II, showed dilated blood vessels, congested with blood, degenerating endothelial cells with loss of their nuclei and decrease in the pericytes all around the blood vessel Figure 1B. Group III, showed abnormal wavy surface, enlarged and engorged blood vessel with increase the loss of nuclei in the endothelium and the absence of the pericytes in most parts of the blood vessels Figure 1C.

Gomori's trichrome stain findings

Group I showed normal arrangement of the collagen fibers stained light green in the subintimal region and the muscles stained red (Figure 2A). Group II, showed destruction of the subintimal collagen layer Figure (2B) Group III, showed significant loss of the subintimal collagen Figure 2C.

Immunohistochemical Findings

Group I, showed intense immunoreactivity for α SMA in the blood vessel walls Figure 3A. Group II, showed mild immunoreaction for α-SMA in the blood vessel walls, Group III, showed minimum immunoreaction for α-SMA in the blood vessel walls.

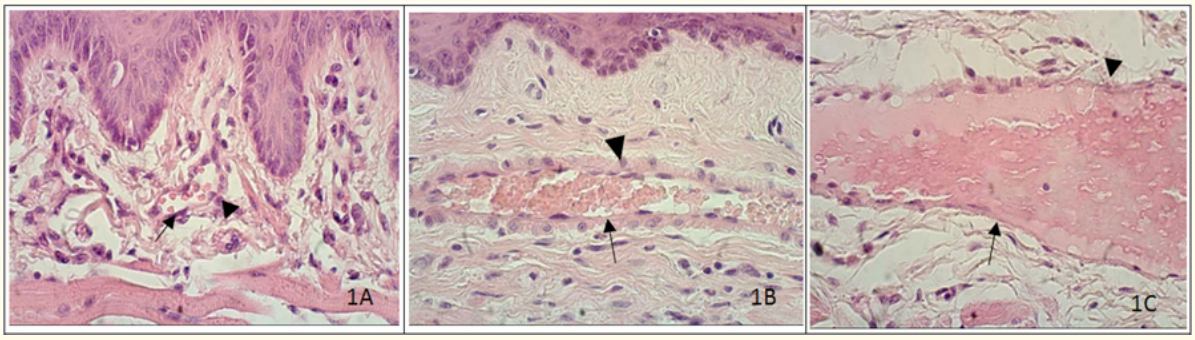

Figure 1: *(1A) Photomicrogragh of group I, showing normal architecture of the blood vessels with endothelial cells lining (arrow) and normal pericyte arrangement (arrow head).*

(1B) Photomicrogragh of group II, showingdilated blood vessel ,congested with blood, degenerating endothelial cells with loss of their nuclei (arrow) and decrease in the pericytes all around the blood vessel (arrow head) .

(1C) Photomicrogragh of group III, showing abnormal wavy surface, enlarged and engorgement blood vessel with increase the loss of nuclei in the endothelium (arrow) and absence of the pericytes in most parts of the blood vessels (arrow head). (H&E X 400)

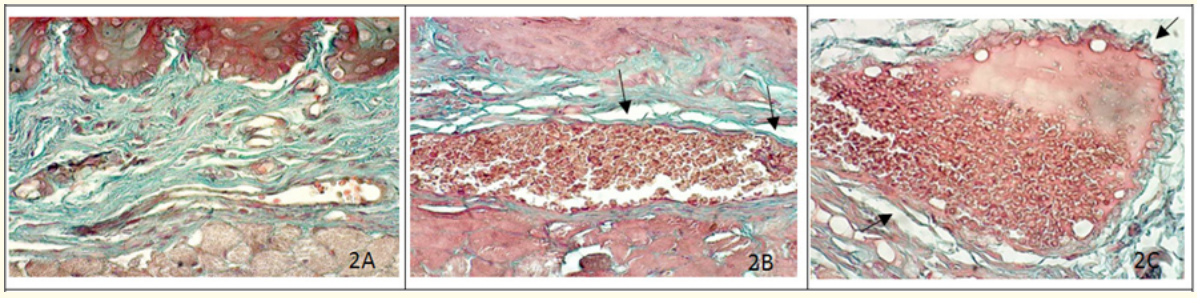

Figure 2: *(2A) Photomicrogragh of group I showing normal arrangement of the collagen fibers stained light green in the subintimal region and the muscles stained red .*

(2B)Photomicrograph of group II, showing destruction of the subintimal collagen layer (arrows).

(2C) Photomicrograph of group III, showing significant loss of the subintimal collagen (arrows)

(Gömöritrichrome X 400).

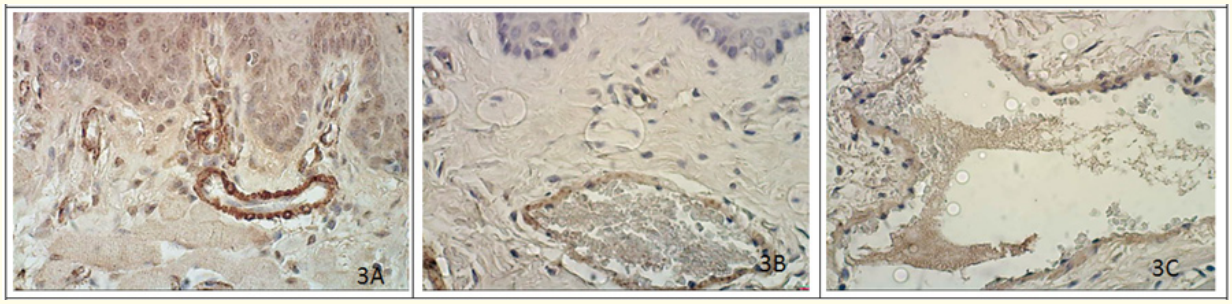

Figure 3: *(3A) Photomicrograph of group I, showing intense immunoreactivity for α SMA in the blood vessel wall.*

(3B) Photomicrograph of group II, showing mild immunoreaction for α SMA in the blood vessel wall.

(3C) Photomicrograph of group III, showing minimum immunoreaction for α SMA in the blood vessel wall. (IHC X 400)

Laboratory Results

There were significant differences in WBCs, HB concentration, RBCs, and hematochrit value of smokers after 30 days and non smokers with p values = 0.00001, 0.006625, 0.001 and 0.008563. There was non significant difference between platelet count of smokers after 30 days and non smoker (P = 0.6883). While after 60 days of passive smoking, there were significant differences between WBCs, HB, RBCs, hematochrit, and platelet counts of smokers and non smokers (P < 0.00001, 0.000353, 0.000142, 0.021663, and 0.000114). There were significant differences between glutathione levels of smokers after 30 days and 60 days and non smokers (P < 0.00001) and there was a non significant difference between glutathione level of smokers at different times (P = 0.6) (Table 1)

	Time	Smoking	Mean	Std. Deviation	P value
30 day	WBCS	Non smokers	6290.2865	1001.19870	< 0.00001
		Smokers	15611.2109	2396.69998	
	Hb	Non smokers	9.0103	1.91767	0.006625
		Smokers	11.9368	2.32831	
	RBCs	Non smokers	4440336.3881	819250.53016	< 0.001
		Smokers	6939426.4710	1198469.30958	
	HCT	Non smokers	32.8804	8.81385	0.008563
		Smokers	44.3919	8.63537	
	Platelet	Non smokers	502877.4302	99425.02909	0.6883
		Smokers	522304.0966	113234.63917	
	Glutathione	Non smokers	0.1557	0.0178	< 0.00001
		Smokers	0.0881	0.0158	

60 day	WBCS	Non smokers	6290.2865	1001.19870	< 0.00001
		Smokers	15585.2545	1924.34632	
	Hb	Non smokers	9.0103	1.91767	0.000353
		Smokers	15.2196	4.04010	
	RBCs	Non smokers	4440336.3881	819250.53016	0.000142
		Smokers	7269497.2334	1671941.39824	
	HCT	Non smokers	32.8804	8.81385	0.021663
		Smokers	42.4489	8.19539	
	Platelet	Non smokers	502877.4302	99425.02909	0.000114
		Smokers	745381.4885	120658.87905	
	Glutathione	Non smokers	0.1557	0.0178	< 0.00001
		Smokers	0.0990	0.0197	

Table1: *Effects of passive smoking on blood cell counts, HB concentration, haematocrit value and glutathione level.*

Discussion

Cigarette smoke contains more than 4000 substances, many of which have toxic, carcinogenic effects on different biological systems [17]. In this study, smoking was found to be associated with dilated blood vessels which were congested with blood. This result was coincidental with the results of another study that assessed gingival blood flow using laser Doppler and they found that smoking does not compromise blood flow in the periodontium [23]. Muller et al found that smoking produces vasoconstriction of blood vessels that is preceded by vasodilatation. Moreover, they suggested that the produced effect may be due to the degree of inhalation of the tobacco smoke and the rate of nicotine absorption [32].

This vasodilatation effect of smoking shown in this study was in contrast with another study that found that nicotine produce vasoconstriction by activating the sympathetic ganglia to secrete catecholamine [33] which affect the alpha-receptors on blood vessels or by acting directly on blood vessels and capillaries [20]. Another study showed the vasoconstrictive direct effect of the tar component of the smoke on the capillaries [34]. This vasoconstrictive effect of nicotine may be responsible for the decreased clinical signs of gingival inflammation such as redness, exudation and bleeding in smokers. Another study found that gingival bleeding was decreased in smokers than in non smokers due to vasoconstriction of gingival vessels [35,36]. Passive smoke has dose- related impairment of endothelium-dependent dilatation in healthy young adults suggesting early arterial damage [37]. Essentially, some of the reported effect discrepancies of smoking can be explained by the use of different preparations and concentration of cigarette smoke chemicals and different cell types by different studies.

The destruction of the subintimal collagen layer of smoker shown by Gomori's trichrome stain may be due to the effect of nicotine that stimulates collagenase enzyme and decrease type I collagen [38]. Another study mentioned that nicotine exposed fibroblast showed decreased growth rate, abnormal shapes, alternations of microtubules, vimentin and other cytoskeletal elements. Additionally, Nicotine decreased cell-signaling molecules and decreased cell migration rates by 50% in cultured gingival fibroblast cells compared with controls [39].

The degenerative changes and death of vascular endothelium may be due to the deposition of smoke chemicals. These degenerative changes occur in the form of reversible contraction of endothelial cells, which is mediated by oxidation and the collapse of the tubulin system [40]. Cigarette smoke' chemicals were found to induce different forms of cell death as necrosis, programmed necrosis, autophagy and apoptosis [41]. Recent data indicate that cadmium present in cigarette smoke may contribute to endothelial cell death [42,43]. The changes of endothelial cell structure and the trigger of cell death decrease endothelial functions and may play a role in thrombogenic events [41].

Passive cigarette smoke induced decrease immunoreactivity for α SMA on the blood vessel wall . This was in contrast to previous study on pulmonary vascular remodelling in smokers and patients with mild chronic obstructive pulmonary disease (COPD), cigarette smoke induced thickened intima and positive immunoreactivity to α-SMA antibody [44]. This is might be explained by Magnus study that demonstrated increased expression of α-SMA positive cells in the large airways in COPD, which was not related to smoking, but to the presence of airway obstruction [45].

In the present study, the glutathione level in passive smokers after 30 and 60 days was significantly lower than that of non smokers. This result was coincidental with Singh et al who found that smoking significantly decreases the levels of reduced glutathione and total antioxidant activity in coronary artery disease patients [46]. Furthermore, Abdelghany., *et al*. Showed that exposure of Bovine aortic endothelial cells to cigarette smoke extract at different concentrations for two hours decreased NO production with a concomitant increase in O_2– generation and depletion of cellular reduced glutathione [47]. 28-day second hand tobacco smoke exposure was found to be associated with increased oxidative stress [48].

The reduction of the glutathione antioxidant in smokers may be the result of their consumption in response to increased oxidative stress produced by cigarette smoke. Moreover, Serebreni., *et al*. 2014 concluded that cigarette smoke increased oxidative stress inducing apoptosis in endothelial cells [49] and other studies detected that the accumulation of nicotine and heavy metals in the cells, inducing genetic alterations, amongst others [50,51].

This may explain that smoking increases the oxidative stress, which may be responsible for tissues destructions.

In this study, it was observed that passive smokers have higher blood WBCs counts than do non-smokers. This result was in accordance with the study of Song., *et al*. who found that smokers have high peripheral blood PMNs levels [52]. Another study reported that smokers had increased levels of neutrophils, lymphocytes, and monocytes when compared with non smokers [53].

Smokers had significantly elevated white blood cell counts, which was correlated to the atherosclerotic plaque formation in the carotid artery [54]. Therefore, smoking was found to affect and stimulate the immune system, both systemically and locally. Systemic immunologic alterations induced by smoking were found to correlate with localized inflammation and increased expression of matrix metalloproteinase, which lead to atherosclerotic plaque formation in the vascular wall [55]. Moreover, not only active smokers can produce proinflammatory systemic effects, but also passive smokers showed increased inflammatory markers concentrations [56].

Passive smoke was found to be associated with increased number of platelets after 60 days of passive smoke. This result was in accordance to the results of numerous studies that reported that cigarette smoke stimulates platelets, activates the coagulation cascade,

and decreases fibrinolysis [57,58]. Another study found that smoking increases the number, activates platelets, increases adhesion molecule expression on the surface of endothelial cells and induces the proatherogenic cytokine release, such as interleukin-6 and interleukin-8 [59]. Therefore, smokers showed a significant shift toward a prothrombotic state in the vascular wall [60].

Finally, passive smoke resulted in significant increase in RBCs, Hb concentration and haematocrit Hct (erythrocyte volume fraction which depends on the number and size of RBCs). These results were in agreement with other studies which investigated the smokers had significantly higher levels of white blood cells, red blood cells, haemoglobin and haematocrit [61-63]. These results can be explained as the smoke causes a persistent state of hypoxia due to smoke contents which cause an increase in erythrocyte count and Hb [62]. These changes might lead to greater danger for developing polycythemia vera, atherosclerosis, chronic obstructive pulmonary disease and\or cardiovascular diseases [61].

Conclusion

In conclusion, passive smoke might lead to blood vessel damage and significant changes in blood cells. It was noticed that the glutathione antioxidant level was significantly lower in passive smokers than non-smokers. Thus, smoking might play a role in enhancing oxidative stress. Also, passive smokers showed higher blood WBCs and platelet counts than do non-smokers which may increase the incidence of atherosclerotic plaque formation and thrombotic state in the vascular wall. So far active and passive smoke has dangerous effects and should be avoided and prevented.

Acknowledgment

We gratefully acknowledge Prof. Dr. Youssry M El-Hawary Prof. of Oral Biology, faculty of dentistry, Mansoura University for his help, effort and revision of this study and thanks to Mona Hassaan senior teaching assistant for her help in this research.

Bibliography

1. Ezzati M and Lopez AD. "Regional disease specific patterns of smoking-attributable mortality in 2000". *Tobacco Control* 13.4 (2004): 388-395.
2. Zavitz CC., *et al*. "Impact of cigarette smoke on T and B cell responsiveness". *Cellular Immunology* 253.1-2 (2008): 38-44.
3. Banoczy J., *et al*. "Tobacco use and oral leukoplakia". *Journal of Dental Education* 65.4 (2001): 322-327.
4. Bokor-Bratic M. "Prevalence of oral leukoplakia". *Med Pregl* 56.11 (2003): 552-555.
5. Ezzati SJ., *et al*. "Role of smoking in global and regional cancer epidemiology: current patterns and data needs". *International Journal of Cancer* 116.6 (2005): 963-971.
6. Tonetti MS. "Cigarette smoking and periodontal diseases: etiology and management of disease". *Annals Periodontology* 3 (1998): 88-101.
7. Birrenbach T and Bocker U. "Inflammatory bowel disease and smoking: a review of epidemiology, pathophysiology, and therapeutic implications". *Inflammatory bowel disease* 10 (2004): 848-859.
8. Costenbader KH., *et al*. "Smoking intensity, duration, and cessation, and the risk of rheumatoid arthritis in women". *American Journal of Medicine* 119 (2006): 503-509.

9. Bartal M. "Health effects of tobacco use and exposure". *Monaldi archives for chest disease* 56.6 (2001): 545-554.
10. Chatzimichael A., *et al*. "The role of breast feeding and passive smoking on the development of severe bronchiolitis in infants". *Minerva Pediatrica* 59.3 (2007): 199-206.
11. Keskinoglu P., *et al*. "The impact of passive smoking on the development of lower respiratory tract infections in children". *Journal of Tropical Pediatrics* 53.5 (2007): 319-24.
12. Loddenkemper R and Kreuter M. "The Tobacco Epidemic". *Karger* 42 (2015): 97-109.
13. Arbes SJJ., *et al*. "Environmental tobacco smoke and periodontal disease in the United States". *American Journal of Public Health* 91.2 (2001): 253-257.
14. Erdemir EO., *et al*. "Periodontal health in children exposed to passive smoking". *Journal of Clinical Periodontology* 37 (2010): 160-164.
15. Avsąr A., *et al*. "Association of passive smoking with caries and related salivary biomarkers in young children". *Archives of Oral Biology* 53.10 (2008): 969-974.
16. Smith CJ and Hansch C. "The relative toxicity of compounds in mainstream cigarette smoke condensate". F*ood and Chemical Toxicology* 38.7 (2000): 637-646.
17. Wallstrom M., *et al*. "The long-term effect of nicotine on the oral mucosa". *Journal of Periodontology* 94.3 (1999): 417-423.
18. Primatesta P., *et al*. "Association between smoking and blood pressure". *Hypertension* 37.2 (2001): 187-193.
19. Napier K. "Cigarettes: What the warning label doesn't tell you". *American Council on Science and Health* 25 (1996): 34-41.
20. Clarke NG., *et al*. "The effects of intra-arterial epinephrine and nicotine on gingival circulation". *Oral Surgery, Oral Medicine, Oral Pathology* 52.6 (1981): 577–582.
21. Pindborg JJ. "Tobacco and gingivitis. Statististical examination of the significance of tobacco in the development of ulceromembranous gingivitis and the formation of calculus". *Journal of Dental Research* 26. 3 (1947): 261-265.
22. Palmer RM., *et al*. "Potential mechanisms of susceptibility to periodontitis in tobacco smokers". *Journal of Periodontal Research* 34.7 (1999): 363-369.
23. Anil S. "Study of the patterns of periodontal destruction in smokers with chronic periodontitis". *Indian Journal of Dental Research* 9.2 (2008): 124-128.
24. Babior BM. "Phagocytes and oxidative stress". *American Journal of Medicine* 109.1 (2000): 33-44.
25. Tsai CC., *et al*. "Lipid peroxidation: A possible role in the induction and progression of chronic periodontitis". *Journal of Periodontal Research* 40.5 (2005):378-384.
26. Chappel ILC and Mathews JB. "The role of reactive oxygen and antioxidant species in periodontal tissue destruction". *Periodontol* 2000 43 (2007): 160-232.
27. Agnihotri R., *et al*. "Association of cigarette smoking with superoxide dismutase enzyme levels in subjects with chronic periodontitis". *Journal of Periodontology* 80.4 (2009): 657-662.
28. Macdonald J., *et al*. "Oxidative stress and gene expression in sepsis". *Oxford Journals | Medicine & Health* 90.2 (2003): 221-232.
29. Haddad JJ., *et al*. "Evidence for the involvement of an inhibitory-kappa B/ nuclear factor-kappaB-sensitive pathway in alveolar epithelial cells". *Journal of Pharmacology and Experimental Therapeutics* 300.2 (2002): 567-576.

30. Raffa M., *et al*. "Decreased glutathione levels and impaired antioxidant enzyme activities in drug-naïve first-episode schizophrenic patients". *BMC Psychiatry* 11 (2011): 124.
31. Liu S., *et al*. "Rac1 expression by fibroblasts is required for tissue repair *in vivo*". *American Journal of Pathology* 174.5 (2009): 1847-1856.
32. Muller HP., *et al*. "Longitudinal association between plaque and gingival bleeding in smokers and non-smokers". *Journal of Clinical Periodontology* 29.4 (2002): 287-294.
33. Trauth JA., *et al*. "Adolescent nicotine exposure produces immediate and long-term changes in CNS noradrenergic and dopaminergic function". *Brain Research* 892.2 (2001): 269-280.
34. Lindeboom JA., *et al*. "Effect of smoking on the gingival capillary density: assessment of gingival capillary density with orthogonal polarization spectral imaging". *Journal of Clinical Periodontology* 32.12 (2005): 1208-1212.
35. Clark NG and Hirsch RS. "Personalized risk factors for generalized periodontitis". *Journal of Clinical Periodontology* 22.2 (1995): 136-145.
36. Bergstrom J and Floderus-Myrhed B. "Co-twin study of the relationship between smoking and some periodontal disease factors". *Community Dentistry and Oral Epidemiology* 11.2 (1983): 113-116.
37. Celermajor DS., *et al*. "Passive Smoking and Impaired Endothelium-Dependent Arterial Dilatation in Healthy Young Adults". *The New England Journal of Medicine* 334.3 (1996): 150-155.
38. Tipton DA and Dabbous MK. "Effects of nicotine on proliferation and extracellular matrix production of human gingival fibroblasts *in vitro*". *Journal of Periodontology* 66.12 (1995): 1056–10 64.
39. Fang Y and Svoboda KK. "Nicotine inhibits human gingival fibroblast migration via modulation of Rac signalling pathways". *Journal of Clinical Periodontology* 32.12(2005): 1200–1207.
40. Bernhard D., *et al*. "Cigarette smoke metal-catalyzed protein oxidation leads to vascular endothelial cell contraction by depolymerization of microtubules". *The FASEB Journal* 19.9 (2005): 1096–1107.
41. Messner B and Bernhard D. "Smoking and Cardiovascular Disease -Mechanisms of Endothelial Dysfunction and Early Atherogenesis". *Arteriosclerosis, Thrombosis, and Vascular Biology*.
42. Messner B and Bernhard D. "Cadmium and cardiovascular diseases: cell biology, pathophysiology, and epidemiological relevance". *Biometals* 23.5 (2010): 811–822.
43. Messner B., *et al*. "Cadmium activates a programmed, lysosomal membrane permeabilization-dependent necrosis pathway". *Toxicology Letters* 212.3 (2012): 268–275.
44. Santos S., *et al*. "Characterization of pulmonary vascular remodelling in smokers and patients with mild COPD". *European Respiratory Journal* 19.4 (2002): 632–638.
45. Löfdahl M., *et al*. "Tenascin-C and alpha-smooth muscle actin positive cells are increased in the large airways in patients with COPD". *Respiratory Research* 12.1 (2011): 48.
46. Palazhy SS., *et al*. "Elevated oxidative stress among coronary artery disease patients on statin therapy: A cross sectional study". *Indian Heart Journal* 67.3 (2015): 227 -232.

47. Abdelghany TM., et al. "Cigarette smoke extract causes endothelial nitric oxide synthase dysfunction through S-glutathionylation". *The FASEB Journal* 27 (2013): 890.11.
48. Meister A and Larsson A. "Glutathione synthetase deficiency and other disorders of the g-glutamyl cycle". *The metabolic basis of inherited disease* 6th Edition (1989): 855-868.
49. Serebreni LBS., *et al*. "Cdk5 Regulates oxidative stress in cigarette smoke induced apoptosis in endothelial cells". *American Journal of respiratory Critcal Care Medicine* 189 (2014): A4298.
50. Avunduk AM., *et al*. "Determinations of some trace and heavy metals in rat lenses after tobacco smoke exposure and their relationships to lens injury". *Experimental Eye Research* 65.3 (1997): 417-423.
51. Barley RD., *et al*. "Tobacco-related-compound-induced nitrosative stress injury in the hamster cheek pouch". *Journal of Dental Research* 83.12 (2004): 903-908.
52. Song DJ., *et al*. "Toll-like receptor-9 agonist inhibits airway inflammation, remodeling and hyperreactivity in mice exposed to chronic environmental tobacco smoke and allergen". *International Archives Allergy Immunology* 151.4 (2010): 285-296.
53. Lavi S., *et al*. "Smoking is associated with epicardial coronary endothelial dysfunction and elevated white blood cell count in patients with chest pain and early coronary artery disease". *Circulation* 115.20 (2007): 2621-2627.
54. Ishizaka N., *et al*. "Association between white blood cell count and carotid arteriosclerosis in Japanese smokers". *Atherosclerosis* 175.1 (2004): 95-100.
55. Kangavari S., *et al*. "Smoking increases inflammation and metalloproteinase expression in human carotid atherosclerotic plaques". *Journal Cardiovascular Pharmacology Therapy* 9.4 (2004): 291-298.
56. Jefferis BJ., *et al*. "Wannamethee SG, Cook DG, and Whincup PH. Secondhand smoke (SHS) exposure is associated with circulating markers of inflammation and endothelial function in adult men and women". *Atherosclerosis* 208.2 (2010): 550-556.
57. Csordas A and Bernhard D. "The biology behind the atherothrombotic effects of cigarette smoke". *Nature Reviews Cardiology* 10.4 (2013): 219-230.
58. Barua RS and Ambrose JA. "Mechanisms of coronary thrombosis in cigarette smoke exposure". *Arteriosclerosis Thrombosis Vascular Biology* 33.7 (2013): 1460-1467.
59. Bernhard D., *et al*. "Cigarette smoke metal-catalyzed protein oxidation leads to vascular endothelial cell contraction by depolymerization of microtubules". *FASEB Journal* 19.9 (2005): 1096-1107.
60. Csordas A and Bernhard D. "The biology behind the atherothrombotic effects of cigarette smoke". *Nature Review Cardiology* 10.4 (2013): 219-230.
61. Asif M., *et al*. "Effect of cigarette smoking based on hematological parameters: comparison between male smokers and non-smokers". *Turk J Biokemiya Dergisi* 38.1 (2013): 75-80.
62. Muhammad I., *et al*. "Effect of smoking on red blood cells count, Hemoglobin concentration and red cell indices.
63. Anandha Lakshmi S., *et al*. "Effect of Intensity of Cigarette Smoking on Hematological and Lipid Parameters". *Journal of Clinical Diagnostics Research* 8.7 (2014): BC11-BC13.

Volume 2 Issue 6 October 2015