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EFFECT OF SMOKING ON INTERLEUKIN-6 IN SPRAGUE DAWLEY RATS

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ABSTRACT

Objective: To investigate the effect of cigarette smoking on interleukin-6 in Sprague Dawley rats.
Study Design: Randomized controlled study.
Place and Duration of Study: Study was conducted at Army Medical College Rawalpindi, from Jan to Apr 2015.
Material and Methods: Seventy healthy male Sprague Dawley rats were randomly divided into two groups at National Institute of Health (NIH). Group-I rats were not exposed while group-II rats were exposed to cigarette smoke for 3 months. Interleukin-6 (IL-6) was determined using commercially available enzyme linked immunosorbent assay (ELISA) kit. Data was analyzed using t-test.
Results: Higher levels of IL-6 were observed in exposed than in non-exposed. The mean of IL-6 levels of control and smoker groups was 40.84 ± 9.09 and 49.48 ± 19.69 respectively. The difference was statistically significant (p<0.05).
Conclusion: Positive association was found between cigarette smoking and IL-6. This shows that IL-6 may be used as a biochemical marker to assess inflammatory damage in smoke related diseases.

Keywords: Cigarette, IL-6, Inflammation, Interleukin, Smoking.

INTRODUCTION

Millions of people are involved in the habit of smoking worldwide. Cigarette, cigar, and water pipe are different modes of tobacco use. Major form of tobacco use is cigarette smoking[1]. Cigarette smoking is the process of combustion of tobacco in cigarettes resulting in release of nicotine, along with massive chemicals that are harmful and addictive to the human body. Thus cigarette smoke is the concentrated cocktail of toxins and chemicals. Chemicals are present in the cigarette smoke in particulate phase and the gas phase. Volatile chemical components such as hydrocarbons and gases are present mainly in the gas phase. Polycyclic aromatic hydrocarbons and nitrosamines are present in the particulate phase[2]. More than 5 million people die from tobacco use annually worldwide. One billion smokers consume six billion cigarettes worldwide annually[3]. A large proportion of smokers belong to the developing countries where smoking related mortality and morbidity is greatest[4]. It is estimated in a study that the prevalence of tobacco smoking is 23% in Pakistan; 36% for males and 9% for females[5]. Efforts are being made to minimize the tobacco use over the past 50 years; but more new tobacco products, such as electronic cigarettes, are being introduced into the market[6].

Majority of deaths due to chronic obstructive pulmonary disease (COPD), cancer and coronary diseases are attributable to smoking. According to WHO data, smoking is responsible for 85% of deaths due to COPD, 30% due to cancer and 25% of deaths from coronary artery disease[7]. Cigarette smoking is a known risk factor for many inflammatory diseases, particularly chronic obstructive pulmonary disease of the airways. Cigarette smoke damages antibacterial defense as it causes harm to the ciliated airway epithelium leading to loss of physical integrity and impairs uptake of bacteria by phagocytic cells like macrophages thus increasing the susceptibility to bacterial infection of the airways. Cigarrete smoke also activates inflammatory and immune cells such as macrophages and T cells that

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provoke airway inflammation[8]. Alveolar macrophages are particularly important in this regard as they initiate the process of inflammation in response to cigarette smoke by releasing inflammatory cytokines and chemokines. Inflammatory cytokines then mobilize additional inflammatory cells, including monocytes and neutrophils, to the lungs, thus intensifying the inflammatory response[9].

Tobacco smoke particularly affects the lungs and airways, but it also induces low-grade systemic inflammation, and several inflammatory biomarkers have been associated with clinical outcomes in this regard. Thus smoking is the strong generator of the inflammatory mediators[10]. Chemotactic cytokines play a central role in the inflammatory response induced by cigarette smoke. Cigarette smoke induced inflammation is related to the increase in the activation of intracellular signaling molecules, including mitogen-activated protein kinase and nuclear factor-κB leading to increase in the expression of many pro-inflammatory chemokines and cytokines[11].

Interest is rising in finding biomarkers of smoke related disorders in asymptomatic persons as it may help in the prevention or treatment of those disorders. Although full mechanism is not clear, serum IL-6 may provide a partial understanding of inflammatory changes with respect to smoking[12]. Cytokines are small extracellular signaling proteins produced by various cells types in the body. Cytokines are the chief mediators of the inflammatory response of the body. Numerous cytokines are known now a days. They are classified into different classes based on their structural analysis. Cytokines can also be classified according to the type of receptor they bind[13].

IL-6 is a multi-functional glycoprotein. Both leukocytes and non-leukocytes produce IL-6 in response to cell damage. IL-6 is a cytokine originally identified as a B-cell differentiation factor in 1985, a factor that induced the maturation of B cells into antibody producing cells. IL-6 binds to cell surface receptor followed by receptor dimerization and activation of signaling cascade[14]. Serum IL-6 levels are raised in many inflammatory conditions. Therefore IL-6 is believed to be an inflammatory marker. IL-6 and CRP are among the most commonly used indicators of inflammation. IL-6 plays a central role in host defense by stimulating various cell populations. However, impaired IL-6 production contributes to the pathophysiology of numerous immune and inflammatory disorders and it is evident in many animal models of diseases[15].

MATERIAL AND METHODS

The study was a randomized controlled trial conducted at Army Medical College (AMC) Rawalpindi, from Jan 2015 to Apr 2015 in collaboration with National Institute of Health (NIH), Islamabad. The Research Ethics Committee of the AMC approved the study. A total number of 70 healthy Sprague Dawley rats weighing 220 ± 30g were kept under standard conditions at animal house of NIH, Islamabad. All procedures of animal handling were performed according to the Guidelines for the Care and Use of Laboratory Animals of the National Institute of Health. Rats were randomly distributed among the following groups.

Group-I (Control, number = 35), animals in this group were provided with normal pellet diet and water ad libitum for a period of 12 weeks. This group was not exposed to smoke. Group-II (Smokers, number = 35). In addition to diet and water this group was exposed to smoke (10cig/day for 12 weeks). They were placed in smoke chambers. Five rats were placed in one smoke chamber. Transparent plastic chambers of size 14" x 20" x 14" were built with two holes, each 3cm in diameter in two opposite walls to provide unforced exchange of fresh air and through these holes the head of burning cigarette was introduced into the chamber, by keeping it on the iron rod stand specially built for the purpose. Cigarette was attached to the stand by means of scotch tape. Cigarettes were burnt and introduced into the smoke chambers, and animals

were exposed to the cigarette smoke for 5 days a week for 12 weeks. Each cigarette burnt for 10-12 min. 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.

At the end of study period the rat cages were taken to the laboratory of animal house which was well equipped with all necessary equipment to anaesthetize and draw samples. Blood sample measuring 3ml was collected from each rat of both the groups at the end of 12 weeks by intracardiac puncture. Serum obtained by centrifugation was stored at -80°C. Serum IL-6 levels were determined using a commercially available enzyme linked immunosorbent assay (ELISA) kit at CREAM.

Data Analysis

Data was analyzed by SPSS version 17 and represented as mean ± standard deviation. The statistical differences between the smoker and control groups were calculated by using student t-test. Significant *p*-value was <0.05.

RESULTS

The comparison of mean of Interleukin-6 levels between two groups at the end of 12 weeks is shown in table. The mean IL-6 level was 40.84 ± 9.09 for control group rats while it was 49.48 ± 19.69 was for group-II rats. The difference between control group and group-II was statistically significant ($p<0.05$). The mean IL-6 level was significantly increased in exposed as compared to non-exposed rats.

Table: Mean of IL-6 levels in group-I and II.

Mean ± SD values of Interleukin-6			
Variable	**Group-I (n=35)**	**Group-II (n=35)**	***p*-value**
IL-6	40.84 ± 9.09	49.48 ± 19.69	<0.05

DISCUSSION

Smoking prevalence in developing countries like Pakistan is alarming because of lack of educational programs. If production and consumption of tobacco products continues then this trend is projected to cause approximately one billion deaths by the end of the 21st century[16]. Smokers are 2-4 times more prone to develop cardiovascular disorders and stroke and 25 times more prone to suffer from lung cancer as compared to that of non-smokers. In addition multiple other abnormalities are related to smoking as diabetes mellitus, asthma, atherosclerosis, impaired fertility and reduced immune response of the body leading to increased risk of infections[17].

Smoking-induced inflammation and immune modification are appearing as potentially important mechanisms in the development of cancer and other systemic chronic diseases. Oxidative stress and systemic inflammation are increased in smokers and are responsible for the severity and progression of smoke related diseases like COPD[18]. However, whether specific inflammatory blood markers are associated with systemic response to smoke or not, is currently unknown. The effects of tobacco smoke on systemic inflammatory and immune markers may help in the understanding of the mechanisms by which cigarette smoking causes disease. Interleukin-6 behaves as both a pro-inflammatory and an anti-inflammatory cytokine that participates in infection and inflammation. In chronic inflammation IL-6 has got a pro-inflammatory role as it activates lymphocytes[19].

Many studies have illustrated that cigarette smoking stimulates various inflammatory signaling pathways and it is manifested by increased concentration of plasma leukocytes, neutrophils and raised levels of acute phase reactants like C-reactive protein and fibrinogen. Nicotine content of cigarettes may be responsible for cigarette smoke induced inflammation[20]. Studies have documented different mechanisms that how nicotine induces inflammation in smokers. Nicotine causes chemotaxis of leukocytes and promotes their adhesion to

vascular endothelium. It increases the genetic expression of many proinflammatory cytokines. Nicotine also stimulates T cell activity which further augments cytokine production. NF-κB is considered to be an important signaling pathway by which smoking produces an inflammatory response as activation of this pathway leads to increased transcription of genes involved in the synthesis of inflammatory mediators and the synthesis of adhesion molecules[20].

A study by Metcalfe et al showed that IL-6 production was inhibited by exposure to cigarette smoke extract[20]. A study by Szlagatys-Sidorkiewicz et al also had similar results showing no significant differences in IL-6 levels between nonsmoking and smoking women[21]. In a study by Piskin et al breast milk was obtained from 25 smoker and 27 non-smoker women during their postpartum visit. Different cytokines including IL-6 were determined in the samples. Results showed that IL-6 levels were significantly low in the breast milk of smokers[22].

Utiyama et al conducted his study in which IL-6 concentration was determined in nasal lavage fluid of the study subjects. Study participants included both smokers and smoke quitters. Results demonstrated no change in the concentration of IL-6between the two groups[23].

Except few studies, most of the studies, however, showed positive association between smoking and IL-6. Results of our study also showed significant increase in IL-6 among smokers than in non-smokers (p<0.05).

Meng et al carried out their study on male Sprague Dawley rats and demonstrated that rats exposed to cigarette smoke showed increased levels of mRNA of IL-6 versus that of control group[24]. Khanna et al also conducted their study on rats. Lewis rats were randomly divided into two groups. Experimental group was exposed to cigarette smoke for six weeks. Control rats were not exposed to cigarette smoke. Results showed that IL-6 gene expression was upregulated in brain sections of exposed group as compared to that in controls[25]. Opposite results were shown by McEvoy et al who found no significant association between smoking and IL-6 when compared between smokers and never smokers[26].

Kianoush et al assessed association between smoking and inflammation by measuring the levels of C reactive protein (CRP). About 1844 current smokers, 4121 former smokers, and 8138 never smokers were enrolled in the study. Current smokers were those who smoked cigarettes within the last 30 days, former smokers had not smoked cigarettes for last 30 days while never smokers had history of consumption of <100 cigarettes throughout their life. The inflammatory biomarker CRP was measured via immuno chemistry. It was found that mean CRP levels were significantly higher in current smokers and former smokers compared with never smokers[27]. Consistent with our results a study was carried out by Silkoff et al. Thirty healthy nonsmokers and 30 healthy asymptomatic smokers participated in the study. Non smokers were those who had not smoked in the previous year and had a ≤10 pack-year history of smoking. On the other hand smokers were actively smoking with a history of ≥20pack-years. Results showed increased quantity of leukocytes and neutrophils in blood and sputum of smokers than in nonsmokers. Serum C-reactive protein was also significantly increased in smokers compared to nonsmokers (p<0.05). According to this study active smoking was associated with changes in inflammatory markers including elevated blood leukocyte and neutrophil count and sputum neutrophil quantity[28].

Meng et al carried out a study on male Wistar rats and showed that IL-6 levels in serum and bronchoalveolar lavage fluid were significantly increased in the smoke-exposed group compared with the 24 week. Control group[29]. IL-6 levels were significantly raised in cigarette smoke exposure group Balb/c mice as compared with that of controls shown by Duan et al[30]. Cigarette smoke was shown to augment the production of numerous pro-inflammatory cytokines such as TNF-alpha, IL-1, IL-6 and IL-8 by Arnson et al[31]. The present study, however,

has several limitations, such as the sample size, animal subjects and the limited duration of smoke exposure. Additional studies are needed to determine whether IL-6 may be utilized as a novel biomarker in the future for earlier diagnosis of smoke related diseases.

CONCLUSION

Positive association was found between cigarette smoking and IL-6. This shows that IL-6 may be used as a biochemical marker to assess inflammatory damage in smoke related diseases.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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