

**EFFECT OF SMOKING ON C-REACTIVE PROTEIN LEVELS:  
COMPARISON BETWEEN MALE SMOKERS AND NON-SMOKERS  
IN THE NATIONAL RIBAT UNIVERSITY, FACULTY OF MEDICAL  
LABORATORY SCIENCES, KHARTOUM, SUDAN.**

**Mohamed Mahmoud Khidr<sup>1\*</sup>, Rasha Elbushra Abdulhameed<sup>2</sup>, Fatima Ahmed<sup>3</sup>,  
Salma Hassan<sup>3</sup> and Tahra Murtada<sup>3</sup>**

<sup>1,2\*</sup>Faculty of Medical Laboratory Sciences, Department of Immunology, The National Ribat  
University, Khartoum, Sudan.

<sup>3</sup>Faculty of Medical Laboratory Sciences, Department of Haematology and Immunology, The  
National Ribat University, Khartoum, Sudan.

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**\*Correspondence for  
Author**

**Mohamed Mahmoud  
Khidr**

Faculty of Medical  
Laboratory Sciences,  
Department of  
Immunology, The  
National Ribat University,  
Khartoum, Sudan.

**ABSTRACT**

**Objective:** The effects of both cigarettes and hookah (shisha) smoke on human health are serious and in many cases, deadly. The aim of the present study was to assess the extent of adverse effects of smoking on C-reactive protein levels in male students who attend The National Ribat University, Faculty of Medical Laboratory Sciences, Khartoum, Sudan. **Subjects and Methods:** Sixty eight male students participated in this study, 48 were smoker males students and the remaining 20 were non smokers (controls). CRP levels were measured by latex card and positives samples which showed visible agglutination were further investigated by latex semi quantitative method. **Results:** Out of the 48 smoker males, 11(22.9%) showed visible agglutination by latex card (positive results) while only one (5%) non smoker student showed positive result. There was no significant correlation between the CRP titer levels and the duration of smoking and/ or number of cigarettes

smoked/day. The mean WBC count among smokers and controls were  $5.7 \times 10^3 \pm 1.67$  and  $6.43 \times 10^3 \pm 1.77$ , respectively and this results indicate normal levels of both. **Conclusion:** In conclusion, the findings of the study showed that continuous tobacco smoking could have severe adverse effects on serum CRP level and these alterations might be associated with a greater risk for developing inflammatory disease and/or cardiovascular diseases. The small

number of samples collected in the study was due to lack of the financial support therefore further study and larger samples would be of value.

**KEYWORDS:** Cigarettes smoking, hookah (shisha), C-reactive protein (CRP), latex card, inflammation.

## INTRODUCTION

Tobacco smoking is associated with increased prevalence of various diseases, both in the respiratory tract and in distal organs. It is well recognized that tobacco smoke induced changes in immune and inflammatory processes and may play a part in the etiology and pathogenesis of many diseases. It is also widely known that smokers have higher risk for cardiovascular diseases, hypertension, stroke, clotting disorder, respiratory disease and inflammation.<sup>[1]</sup>

Inflammation, acute and systemic, is part of our immune reaction and leads to the release of C-reactive protein (CRP) into the blood stream. However, chronically increased serum CRP is a risk factor for the development of cardiovascular diseases.<sup>[2]</sup> Understanding the mechanisms through which cigarettes smoking may affect CRP levels in young smokers is central to the prevention of early inflammatory diseases. Serum CRP, the gold standard measurement of low grade inflammation, can predict future cardiac events, even in healthy individuals. Much attention has been given to CRP as a potential biomarker for pathologies involving inflammation. As one of the first reactants released into plasma, its measurement has been considered a classic indicator for the acute phase response in inflammation.<sup>[3]</sup>

Many of the healthcare consequences of nicotine in cigarettes could be related to its ability to compromise the immune system and a constant low level of infection that may be responsible for the pathogenesis of inflammatory disease induced by smoking. It was demonstrated that cigarettes tobacco smoke causes direct vascular injury, subsequently leading to an immunological response. There are also experimental evidence that points towards smoke as an immunosuppressant for the function of immune cells including respiratory epithelial cells, macrophage, neutrophils and lymphocytes.<sup>[4]</sup> Smoking also causes activation of resident cells and the recruitment of inflammatory cells into the lungs, which leads to release of pro-inflammatory cytokines, chemotactic factors, oxygen radicals and proteases which alter the function of immune cells.<sup>[5]</sup>

The cigarettes smoke contains more than 45,000 chemicals, which have various toxic, mutagenic and carcinogenic effects. The content and concentration of chemical ingredients can vary widely in the different cigarettes brands.<sup>[6]</sup> The major components of smoke that lead to many of the deleterious and toxic effects include nicotine, tar, ammonia, carbon monoxide, carbon dioxide, formaldehyde, acrolein, acetone, benzopyrenes, hydroxyquinone, nitrogen oxides and cadmium.<sup>[7]</sup> Cigarettes smoke that contains high levels of tar and nicotine induce greater immunologic changes than cigarettes smoke that contains lower levels of these compounds.

Although it is thought that shisha smoking has fewer health risks than smoking cigarettes, that it is a major misconception in due to various toxic ingredients which have shown that shisha smoking is far more dangerous and even after passing through water it still contains high levels of toxic compounds, including carbon monoxide, heavy metals and cancer-causing chemicals (carcinogens).<sup>[8]</sup> Researches revealed that a single session of shisha smoking which takes up approximately 45 minutes to 1 hour, yields nicotine intake as much smoke as a cigarettes smoker would inhale consuming 100 or more cigarettes.<sup>[9]</sup>

CRP is synthesized in the liver in response to interleukin (IL-6, IL-1) and tumor necrosis factor (TNF) released from the inflamed tissue. It plays a key role in activating the complement system, inducing adhesion molecule expression, enhancing phagocytosis and macrophage and leukocyte activation and opsonization. CRP levels may directly reflect the expression of an IL-6 polymorphic genotype.<sup>[3]</sup>

The relation between smoking and complete blood count has been well established. In a number of studies, it has been found that tobacco smoke may change blood profile by altering the complete blood count causing an elevated peripheral white blood cell count, erythrocytes, platelet and hemoglobin levels.<sup>[10]</sup>

## **MATERIALS AND METHODS**

### **Data collection**

Sixty eight consented males (age between 16-30 years) were enrolled in this study and 48 blood samples were collected from those who smoke cigarettes and/or hookah. Serum was obtained from blood samples by centrifugation at 1500 rpm for 5 minutes. Another 20 age-matched non smoker males also were included for control purposes. All eligible study

participants completed a structured, in-person interview and questionnaire, which was designed to maintain all valuable information's concerning smoking habits.

### **Total white blood cell count**

Total white blood count was performed using manual blood count by microscope. 0.2 ml of blood was added to 0.38ml of diluted 2% glacial acetic acid (2% GAA) and mixed well. Under 10x magnification leukocytes were counted in the four outside large squares of counting chamber. Cells were counted starting in the upper the left top large corner square then, the upper right corner square after that, the bottom right corner square and end in the bottom left corner square. Final cell count was reported as the number of white blood cells per micro-liter (WBC/ $\mu$ L) using the following formula.

$$\text{WBC}/\mu\text{L} = \frac{\text{Average of cells} \times \text{Correction for dilution}}{\text{No. of squares counted} \times \text{Volume of one square}}$$

### **C-reactive protein measurement**

#### **Qualitative method**

Fifty microliter (approximately one drop) of each smoker serum was transferred into the test circle on the latex card and one drop of the C-reactive protein (CRP) latex reagent was added. The drops were mixed using disposable wooden stick and the test circle was covered by the mixture. Gently and evenly, the test card was rotated for 5 minutes. Positives samples which showed visible agglutination were further investigated by latex semi quantitative method.

#### **Semi quantitative method**

Using isotonic saline solution serial dilution of the smokers samples was prepared (1/2, 1/4, 1/8, 1/16, 1/32, 1/64). 50 $\mu$ L of each sample was transferred and one drop of the CRP latex reagent was added. The drops were mixed and gently rotated for 5 minutes. After that, agglutination was examined under a strong light source after 5 minutes.

The serum CRP level was calculated by multiplying the dilution factor by the detection limit, to give the number mg/dl concentration as follow

$$6 \times \text{CRP titer} = \text{mg/L}.$$

### **RESULTS**

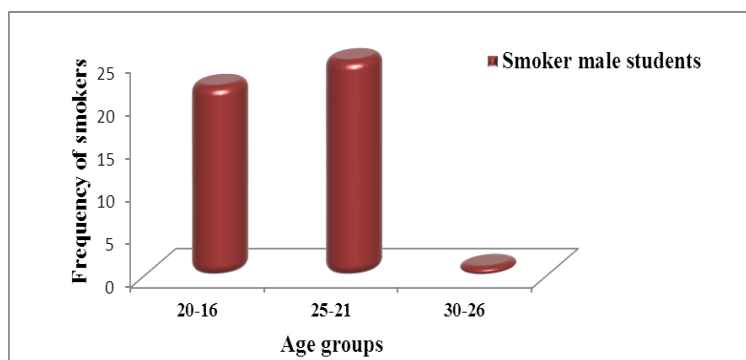
A total number of 68 male students (age between 16-30 years) were included in the study, 48 samples were collected from those who smoke cigarettes and/or shisha while the remaining

20 samples were non smokers. The study showed that most of smoker students were between the ages of 21-25 years while the least number of smokers were among 26-30 years old, figure 1. Out of the 48 smokers, 11(22.9%) showed visible agglutination by latex card (positive result) while only one non-smoker student showed positive result, figure 2. There was no significant correlation between the CRP titer levels and number of cigarettes smoker/day or/and type of cigarettes brand.

The demographic data of the smoker students revealed that 32 students smoke about 1-10 cigarettes/day, of them 28% were detected to be having positive CRP results. While among 13 students who smoke 11-20 cigarettes/day, 23% showed positive CRP levels. The most number of cigarettes smoked/day was among 3 students who smoke 21-30 cigarettes/day, otherwise they all showed negative CRP results, figure 3. Bringe brand was shown to be the most popular cigarettes brand used among smokers, representing (43%) of smoker students and (33%) were using both hookah (Shisha) and Bringe brand. While (4%) of students used Penson brand. In the other hand Lord has shown to be the least type of brand used among smokers in which only (2%) of students declared to be using it, in the other hand (6%) of the smokers were using hookah as an alternative to cigarettes smoke. The rest of the students were using different combination between cigarettes brands, figure 4.

Interviewing the smoker students who participated in this study revealed that 31 of them have been smoking for 1-5 years, while 13 smokers admitted that they've been smoking for 6 to 10 years and the remaining 4 smokers were smoking for more than 11 years. There was no significant correlation between the CRP titre levels and the duration of smoking, figure 5.

Manual white blood cells count (WBCs) using microscope showed normal levels among smokers and non smokers, in which the mean WBCs count were  $5.7 \times 10^3 \pm 1.67$  and  $6.43 \times 10^3 \pm 1.77$ , respectively.



**Figure 1: Distribution of smokers among the studied males according to their ages.**

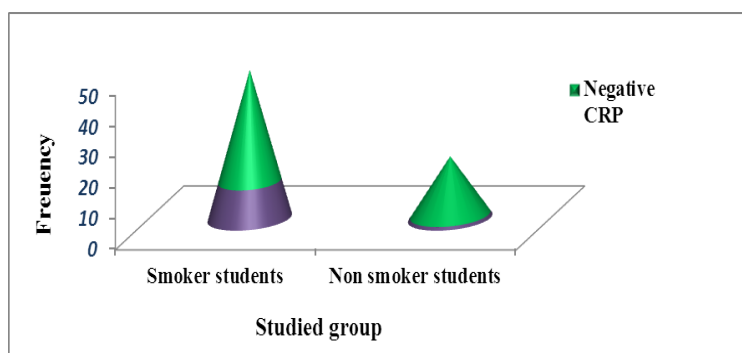


Figure 2: Comparison between CRP results among smokers and controls.

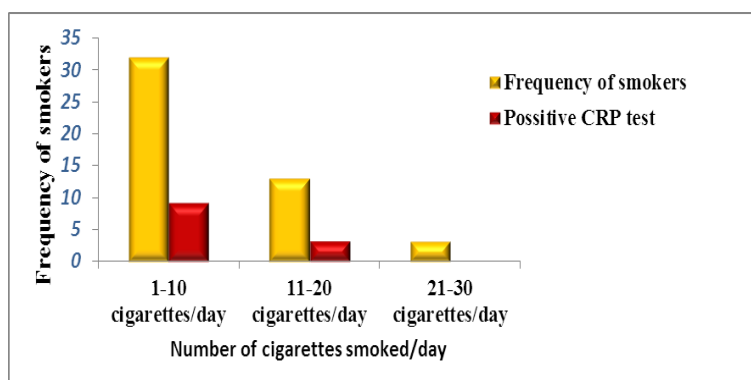


Figure 3: Frequency of smokers in relation to number of cigarettes smoked/day.

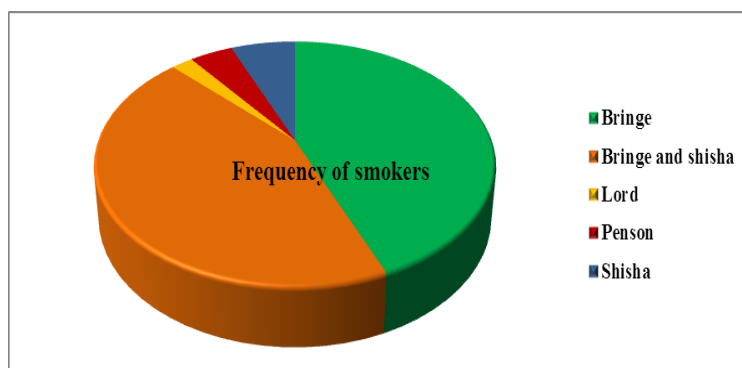


Figure 4: The frequency of cigarettes brand used among smoker students.

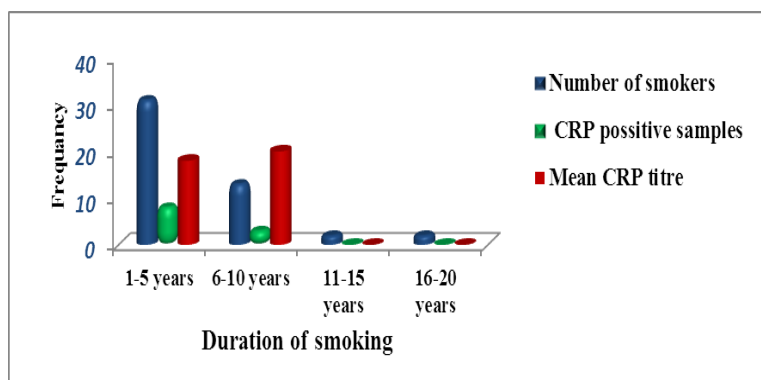


Figure 5: The relationship between the duration of smoking and CRP positive results.

## DISCUSSION

Tobacco smoking has been correlated to cause several major morphological and biochemical problems in individuals. In this study C-reactive protein had been used for comparative analysis between smokers and non-smokers among students of The National Ribat University, Faculty of Medical Laboratory Sciences, Khartoum, Sudan. Among the random selected smokers in this study it was found that age play a major role in smoking habit; for students younger than 25 years, 98% were tobacco smokers whereas for students older than 26 years only 2% were smokers. This finding agrees with previous studies which have found that nearly all first use of tobacco takes place before high school graduation and teens are easy targets for the tobacco industry, in which they're often influenced by TV, movies, the Internet, advertising and by what their friends do and say.<sup>[11]</sup> Smokers at younger age do not realize what a struggle it can be to quit and exposing themselves to smoking-related health problems like cancer, emphysema, blindness, or impotence may not seem like real concerns and at that age, smokers are either not well informed or they do not consciously process information on the health hazards of smoking.

C-reactive protein test by latex card is perhaps the most useful, inexpensive and simple biomarker for inflammation. We found that regular smokers had slight increased CRP levels compared to non smokers in which 11(22.9%) smoker students showed visible agglutination by latex card indicating a positive result while only one non smoker had positive results. This is explained by previous investigations which have demonstrated that increased CRP levels are a secondary effect of cigarettes smoking and reflect tissue injury in which cigarettes smoking has been associated with systemic inflammation, it is also has been hypothesized that components of cigarettes smoke may trigger a complex pro-inflammatory response through the recruitment of leukocytes to the site of inflammation via cytokine signaling (such as IL-1 $\beta$  and TNF- $\alpha$ ), thus leading to increased level of CRP.<sup>[12]</sup>

The present study demonstrated that Bringe brand was the most used by smoker students when compared to other cigarettes brands, representing (43%) of total participant, this might be explained by a recent study done in 2015, that the reason which makes it so special is the strength of them. They are quite strong, much stronger than Marlborough reds. So once a person is used to such heavy cigarettes such as Bringi, nothing else will do.<sup>[13]</sup>

The experimental results did not show an obvious relationship between the duration of smoking and the number of cigarettes smoker per day in relation to CRP positive results and



this is contrary to other research findings which revealed an associations between cigarettes smoked per day and CRP levels, which have been described previously, this might be explained by the small number of samples collected in the study due to lack of the financial support therefore further study and larger samples would be of value.<sup>[14]</sup>

Result of mean WBCs count of smokers and non-smokers were both of normal levels. These findings are contrary to previous researches which demonstrated and proved an increase in the WBCs count among tobacco consumers which causes an activation of resident cells and the recruitment of inflammatory cells.<sup>[15]</sup> Furthermore genetic diversity may influence variation in the complete blood count as well as common environmental factors such as dietary iron intake and exercise are important in influencing and regulating complete blood count.<sup>[4]</sup>

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