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ORIGINAL ARTICLE

Evaluation of some immunological and inflammatory indices in cigarette smokers of Basra province

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ABSTRACT

Objectives: The aim of the present study was to find out the effect of cigarettes smoking on several haematological, immunological parameters and inflammatory proteins C-reactive proteins (CRP) and complement (C3 and C4).

Methods: Whole blood and serum samples were taken from 32 healthy smokers and 31 non-smokers male as control (19-40 years) in order to detect the total WBCs and differential count, phagocytosis activity and serum inflammatory proteins both of CRPs and complements (C3 and C4).

Results: The total number of WBCs and neutrophils were significantly higher in smokers, but their phagocytic activity was significantly decreased. In relation to inflammatory proteins, both of CRPs and complement proteins (C3 & C4) were increased significantly higher in smokers. The number of smokers has a significant increase in urban rather than rural area. In addition, no significant differences were observed in the numbers of lymphocytes (p= 0.118) and mass body index (MBI p= 0.367) between smokers and non-smokers.

Conclusion: Cigarette smoking has severe adverse effects on different immunologic parameters as it leads to increase in all of the total WBCs count, neutrophils and lymphocytes as well as both of the inflammatory proteins (CRPs and complement (C3 and C4)). While on the other hand, phagocytic activity was decreased.

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INTRODUCTION

It is well clear now day, cigarette smoking is one of the most prevalence problems among young and adult individuals and it was considered as causative agent for several chronic diseases like heart diseases, cancers and respiratory illnesses like chronic obstructive pulmonary disease (COPD)[1]. The World Health Organization (WHO) estimated that tobacco using is one of the causes of death and by 2030 it may be leading to kill more than 8 million approximately 80% of them would occur in low- and middle-income countries[2]. Tobacco smoking contains over 4000 different complex chemical compounds, and over 50 of which are known carcinogenic, co-carcinogenic and/or mutagenic[3]. Also tobacco considers as a source for large quantities of free radicals and oxidants which responsible for oxidative stress, oxidative lung injury and apoptosis[4]. In 2001, Hoffmann and his team found smoking will be generating many carcinogenic and toxic compounds

which are healthy harmful like carbon monoxide, hydrogen cyanide, nicotine, nitrogen oxides, some alkenes and some aromatic hydrocarbons component[5].

Cigarette smoking (CS) has harmful effects on different hematologic parameters and a wide range of immunologic functions include (both humoral and cell mediated immunity)[6].

Neutrophil has an important role in defense functions against microorganisms and it will act as a core element in innate immunity[7]. Many studies revealed that cigarette smoking had an influence on phagocytic functional activity in whole blood, in which often seems to be altered[8,9,10].

The primary function of neutrophil response is carried out by a series of rapid and coordinated responses in phagocytic cells[11]. In the presence of O2, PMNs can be stimulated which leads to free radicals (oxygen reactive intermediates) production; which are form superoxide radical[12] and that can measure by using of Nitro-Blue Tetrazolium test (NBT). NBT is one of the most common tests used to evaluate phagocytic activity of neutrophils depend on optical density changes due to NBT reduction[13].

Numerous studies have been showed increase levels in the white blood cells (WBCs) count and in both of neutrophils and lymphocytes in the healthy smokers as compared with nonsmokers[8,10,14,15,16].

In addition to that cigarette smoking causes elevation in level of both inflammatory markers: C-reactive proteins (CRP) and complement protein (C3&C4)[17,18,19].

C-reactive protein is one of the acute phase proteins produced by liver cells[20]. It structure is distincting from the immunoglobulin; but it shares with it by many biological activities like: activation of complement (acts as an opsonin) and participation in cytokines generating which enhanced the inflammation[21]. CRP level will increase rapidly during infections, inflammation and trauma, then it will decrease rapidly with the resolution of these conditions[21]. As well as, the presence of CRP high level in smoking individuals helps in identification of unusual airway abrasions that may be leading to lung cancer[22].

On the other hand, complement proteins both of (C3 and C4) which are collection of circulating and cell proteins synthesis membrane proteins, have an important function in host defense against microbes[23]. Complement will increase during inflammation and infections and that will help in diagnosis of certain rheumatic and other immunologic disease; but it synthesis and circulating level may be reduce because of defect in genetic polymorphism, and that makes the individual ready to get to infection[24]. Cigarette smoking inhalation causes activation of complement and that leads to chemo-attraction of leukocyte in the lung fluid[25].

The aim of this study is to evaluate the effects of cigarette smoking on some haematological, immunological parameters and inflammatory proteins (CRP, C3 and C4).

MATERIALS AND METHODS

Samples collection

Studied groups

Studied groups involved 63 healthy adult volunteers male with no clinical signs. (32 are smokers while 31 are non-smokers considered as the control group); there ages ranged from 18-40 years in Basra province. Present work was done during the period from January to December 2016. Participants' information was taken according to special designed questionnaire prepared for the study.

Blood samples

Five ml of blood was taken from each man participated in the study by using sterile a disposable syringe. The blood sample was divided into three parts: the first part is one ml of blood was put in a tube with heparin as anticoagulant for direct measurement of phagocytic activity .The second part (1.5 ml) was put in an EDTA tube as anticoagulant which was used for enumerating of total and differential WBCs count. Finally, the remained 2.5 ml of blood was put in a plane tube without anticoagulant, then serum was isolated by centrifugation at 3000 rpm for five minutes and it was stored at -20 C° until it was used for measurement of CRP and complement (C3 and C4).

Determination of total and differential WBCs count

It was done by using automated haematology analyzer (Coulter counter, Sysmex xt-2000i Japan) in a Basra child hospital for cancer.

Determination of phagocytic activity by 0.1 % Nitroblue tetrazolium (NBT) in Tris-HCl solution

Nitroblue tetrazaolium (NBT) (Sigma) is an electron acceptor was used for indirect detection of the super oxide radical (O–) production by PMNs during stimulation and this will provide a quantitative means identify cells O- production by spectrophotometer[13,26].

i-**Solution preparation:** The stain consists of 0.1 % NBT in Tris-HCl solution (which was prepared by dissolving of 24.4 gm of Tris –HCl in 1000 ml DW). 50 ml of that solution added to 41.4 ml of 0.2N HCl, complete the volume to 200 ml with DW and adjustment PH of Tis-HCl to 7.4. After that 0.001 gm of NBT stain was dissolved in 1ml of Tis-HCl[13,26].

ii-**NPT protocol:** The test sample contains 0.5ml of whole blood with heparin and 0.5ml of prepared NBT stain mixed up together gently and incubated for one hour at 37C^{o}[13].

After incubation spin the tubes for 3 minutes at 3000 rpm. The supernatant was removed carefully then placed it in the spectrophotometer cuvette. The optical density (% absorption) was measured at 515 nm using Philips spectrophotometer (type PU8620)[13].

C-reactive protein measurement

Serum C- reactive protein was determined according to a standard method using NycoCard CRP single test system form (Axis-Shield poC AS UK). The well device of the test consists of a membrane coated with immobilised CRP- specific monoclonal antibodies. The test is performed by adding 50 µl of the serum sample to the well. The serum flows through the membrane and

CRP binds with the specific antibody. The result appears with colour changing of membrane from red to brown proportional to CRP concentration in the sample. The colour intensity was measured with NycoCard Reader II.

Serum complement proteins (C3 & C4) measurement by using single radial immune diffusion (SRID)

Immunodiffusion plate was used for detection of C3 and C4 concentration and the process was done as described by the manufacturer (LTA Srl Milano 15-Italy). Serum samples were added to the wells of the plate and incubated from 48 to 72 h. The diameters of the precipitin circle were measured by suitable device (eye piece) after that the concentration was evaluated by using of reference tables. The normal value of C3 ranged between 91 to 156 mg/dl and for C4 it was between 20 to 50 mg/dl.

Body mass index (BMI)

It was done by dividing the weight of an individual in (kg) on the square of the height measured in meters (all measurements were done with light clothes and without shoes) . The results were classified as following: (1) underweight - BMI < 18.5 kg/m^2; (2) normal – BMI 18.5 to 24.9 kg/m^2; (3) overweight – BMI 25 to 29.9 kg/m^2; (4) obese – BMI >= 30 kg/m^2 [(27)].

Smoking Index

SI was calculated by multiplying the number of cigarettes/day by the years of smoking. It will be defined as mild <200 SI, moderate 200–600 SI and heavy >600 SI [28].

Statistical analysis

All results are expressed as mean ± SD and independent sample T-test was used for comparison between the two groups. Analysis was done using SPSS software, version 8 for windows.

RESULTS

The studied populations consist of 63 healthy adult male, which they were divided in to 32 smokers and 31 are non-smokers. The demographic characteristic features of them were illustrated in Table 1 which showed there are no significant differences between two groups in related to their age, education and BMI , but significant differences are present in urban residence between them.

Table 1: **Demographic characteristics of studied groups.**

Parameters	Smokers =32	Non-smokers=31	P-value
Age	29.5 ± 6.9	29 ± 5.4	0.753 *
Education	3.2 ± 0.97	3.1 ± 0.97	0.731 *
BMI	25.66 ± 4.9	24.7 ± 2.8	0.367 *
Urban residence	1.13 ± 0.34	1.4 ± 0.5	0.026 **

* P> 0.05 is not significant ** P<0.05 is significant

Analysis of blood parameters showed a highly significant increase in total WBCs count and neutrophils in smokers, while phagocytic activity was significantly decreased in them when compare with the non-smokers men. Also the lymphocytes count was increased in smokers; but it was not significant (Table 2).

Table 2: **Hematological parameters and phagocytic activity in both studied groups.**

Parameter	Smokers =32	Non-smokers=31	P-value
Total WBCs	7352 ± 1815.57	5860 ± 1357.35	0.001**
Neutrophils	4430 ± 1264.25	3323 ± 1140.4	0.003**
Lymphocytes	2240 ± 504.7	2068 ± 332.43	0.118***
Phagocytosis	0.636 ± 0.16	0.734 ± 0.18	0.028 *

* P< 0.05 is significant
** P<0.01is a highly significant
*** P> 0.05 is not significant

Both of the acute phase proteins: (CRPs) and complement (C3 and C4) were increased significantly in the smokers when compared with the non-smokers (Table 3).

Table 3: **CRP and complement protein (C3 & C4) concentrations (mg/dl) in smokers and non-smokers.**

parameters	Smokers = 32	Non-smokers = 31	P-value
CRP	6.88 ± 0.852	3.16 ± 0.562	0.000 *
C3	223.45 ± 33.41	127.63 ± 14.85	0.000 *
C4	41.27 ± 4.65	24.21 ± 5.31	0.000 *

* P<0.01 is a highly significant

According to the smoking index the recent data indicated that about 18 individuals are mild (<200), 11 are moderate (200-600), while only 3 are considered as sever case (>600).No significant differences were recorded in relations of smoking index with different hematological and immunological parameters.

Discussion

Cigarette smoking responsible for causing several health problems in population people and the documented results here highlighted to some of them.

In the current study, the CS has no significant differences in age and education in smokers as compared with the nonsmokers and that agreed with Jasim and Al-Asadi study in 2010 on students' university of Basra[29] and also with Majeed (2014) [(15)]and disagreed with Shipa *et al* ,in 2017[30].

Although CS has an adverse effect on MBI as it associated with reduce of the body weight in smokers[27,30]; but in this study it was increased insignificantly in smokers and that probably due to that the majority of smokers individuals are young. The smoking behavior has a significant association with urban residence and that similar to Jasim and Al-Asadi (2010) who found that the people who live in the urban area are more ready to get smoking habit[29].

Related to hematological and immunological parameters the data here recorded a significant increasing in the total WBC , neutrophils as well as the lymphocyte count and that agreed with other authors[10,14,16,30] but disagreement with Abbas and Ali, 2015 who found a decreasing in lymphocyte counts in smokers and impairment of lymphocyte functions[8]. The mechanism is not clear for that increasing; Deutsch and his team in 2007 suggested in smokers; nicotine-induced releasing of catecholamines and steroid hormones from the core of the adrenal as well as the increasing level of certain endogenic hormone like a cortisol result in increasing in

the number of leukocytes[31]. While other researchers found the irritating effect of the CS causing inflammation in the respiratory tree resulting in chronic inflammation contributes to increase in the number of leukocytes[32].

NBT test is an easy method for screening oxidative metabolism in PMNs[12]. In spite of a significant increasing in the number of neutrophil; but their phagocytic activity was reduced significantly and these results are agreed with Mehta *et al* . in 2008[33] and with Al-Hamadany *et al.* in 2016[9] who study the effect of smoking on clinical laboratory workers and disagreed with Abbas and Ali in 2015[8] who found there was an increasing in phagocytic activity in smoking individuals. The failure of the NBT reduction inside PMNs may attribute to the reduction of NADPH capacity inside the cell which causes rapid depletion and a very little O_2 production when stimulated[12]. The cause of that may be due to the effect of nicotine on PMNs[34].

Furthermore, the mean complement (C3 and C4) levels was observed to be higher in serum for the men with cigarette smoking than control. Many other studies have also show the same findings like Gemeel (2008) in Iraq[17]; Sanai and Hussain (2011) in Pakistan[18] and Al-Mehdar (2013) in Jadaa[19] and this may be due to activation of alternative complement pathway[25].

The next inflammatory mark which was measured in recent work is C-reactive protein and the data analysis indicated that smoking men who are using cigarette possess the higher level than non-smoking men. Similar findings were recorded by Sanai and Hussain , 2011[18], Al-Mehdar 2013[19], Laleh *et al.*, 2004[35] & Kumar *et al.*, 2015[36]. Mahrukh & Nageen in 2011[37] attributed that to monocyte activation and complement induction which leads to secretion of more inflammatory cytokines and these may lead to release more CRP from liver in the blood.

The relation of the smoking index was depended on the number of cigarette / day and smoking duration has recorded no significant differences with different hematological and immunological parameters and that may be due to the small size of smoking individuals.

Conclusions

Cigarette smoking has adverse effects on immunity in different forms. Some parameters were increased like total white blood cell count, neutrophils, lymphocyte ratios and decrease in phagocytic activity which play an important role in defense mechanisms. Also CS affecting inflammatory proteins, both of c-reactive proteins as well as complement proteins (C3 and C4), both of them are increased with higher significant in smokers.

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