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Effects of Cigarette Smoking on Some Immunological and Hematological Parameters in Male Smokers in Erbil City

Farhang A. Aula and Fikry A. Qadir*

Biology Department, College of Science, Salahaddin University, Iraq

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Abstract

The present study was done to investigate the effect of cigarette smoking (CS) on some immunological and hematological parameters in Erbil city. The study is carried out on fifty male smokers, who smoked at least 10 cigarettes per day for at least10 years. Depending on the age of the smokers, they were divided into two groups. The first group includes smokers with age range between 25-35 years, and the second group includes smokers with age range between 36-45 years. Two control (non-smokers) groups were collected with the same range of age for statistical comparison. The results of the study revealed a significant increase of interferon-gamma (IFN-γ) level in both age groups when compared with their controls. A significant decrease of the total immunoglobulin A (IgA) level was recorded in both age groups when compared with their control. Furthermore the level of malondialdehyde (MDA) which is an indicator of lipid peroxidation (LPO) and oxidative stress significantly increased in cigarette smokers in both age groups when compared with control groups. While the level of Carbohydrate Antigen 19-9 (CA 19-9 Ag) increased in the age group 25-35 years, but this increase is not significant when compared with its control group; the level of CA 19-9 Ag significantly increased in age group 36-45 years when compared with its control group. Moreover the results revealed a significant increase in the total white blood cells (WBC), Neutrophil, Eosinophil, Basophil, Monocyte, and Lymphocyte count in both age groups when compared with their control groups. However, the basophil in the first group (25-35 years), the increase was not significant. While the number of platelets count did not statistically change in both age groups.

Keywords: Cigarette Smoking, IFN-γ, IgA, Hematology

1. Introduction

Smoking is one of the most common addictions of modern times. It has been implicated as an etiological agent for various chronic diseases, including a variety of infections, cancers, heart diseases, and respiratory illnesses such as chronic obstructive pulmonary disease (COPD), that have impairment in the balance between cell growth and cell death, which, put together, are the leading causes of morbidity and mortality in today's society (Zhong *et al.,* 2008; Mehta *et al.,* 2008). Unless current smoking patterns are reversed, the World Health Organization (WHO) estimates that, by the decade 2020-2030, tobacco will be responsible for 10 million deaths per year, with 70% of them occurring in developing countries (WHO, 2001; Suriyaprom *et al.,* 2007).

Cigarette tobacco smoke contains over 4000 compounds, including at least 200 toxicants, 80 known or suspected carcinogens, large quantities of oxidants and free radicals that induce oxidative stress, oxidative lung injury and apoptosis (Zhong *et al*., 2008; Soldin *et al.,* 2011).

Smoking generates many toxic and carcinogenic compounds harmful to the health, such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide, and free radicals (Hoffmann *et al.,* 2001). Smoking is associated with increased oxidative stress and exerts an inflammatory stimulus on lung macrophages which may, like bacterial and viral infection, result in the production of free radicals and the inflammatory cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), IFN-□ and tumor necrosis factor α (TNF-α); these may be the precursors to the diseases associated with smoking (Francus *et al.,* 1992; Takajo *et al.,* 2001).

Chronic inhalation of CS alters a wide range of immunological functions, including innate and adaptive immune responses. It has been speculated that many of the health consequences of chronic inhalation of CS might be due to its adverse effects on the immune system. The possibility that the increased prevalence of diseases that are associated with CS might, in part, be due to tobacco-smoke-induced changes in the immune and inflammatory processes, was recognized first in the 1960s (Sopori, 2002).

Cigarette smoke (CS) affects a wide range of immunological functions in humans and experimental

* Corresponding author. e-mail: fikrybio@gmail.com.

animals, including both the humoral and cell-mediated immune responses. It is postulated that this increased susceptibility reflects cigarette smoke-induced impairment of the immune system (Sopori and Kozak, 1998; Kalra *et al.*, 2000).

The acute effects of CS on markers of oxidative stress have been analyzed in exhaled air, broncho-alveolar lavage fluid (BALF) and blood, even though that, in chronic smoking, the numbers of neutrophils are increased in the blood and BALF. In addition, CS causes an acute inflammatory reaction characterized by the accumulation of neutrophils and macrophages in the membranous bronchioles and alveoli of the lungs, leading to destruction of the peribronchiolar alveolar attachments and eventually pulmonary dysfunction (Sopori, 2002).

Interferon-gamma (IFN-γ) dimerized soluble cytokine that is the only member of the type II class of IFNs (Abbas and Andrew, 2005). This IFN was later called macrophage-activating factor, a term now used to describe a larger family of proteins to which IFN-γ belongs. IFN-γ is a cytokine that is critical for innate and adaptive immunity against viral and intracellular bacterial infections and for tumor control. Aberrant IFN-γ expression is associated with a number of autoinflammatory and autoimmune diseases. The importance of IFN-γ in the immune system stems, in part, from its ability to inhibit viral replication directly, and most importantly from its immunostimulatory and immunomodulatory effects. IFN-γ is produced predominantly by natural killer (NK) as part of the innate immune response, and by CD4 Th1 and CD8 cytotoxic T lymphocyte (CTL) effector T cells once Ag-specific immunity develops (Schoenborn and Wilson, 2007).

Secretory IgA (sIgA) is the main Ig isotype mediating humoral immunity in human secretions at luminal sites including oral, gastrointestinal, respiratory passages as well as in the eye. sIgA production is triggered after translocation of Ag from the lumen to mucosa-associated lymphoid tissue. The presence of IgG Abs at the mucosal surface may be protective and it has been show that IgG2 subtype predominates. IgG in secretions is derived from serum or mucosa. Studies show tobacco smoke impacts both the systemic and mucosal immunity with changes demonstrated in mucosal Ab production and systemic Ab production. This includes passive smoking, which increases the risk of respiratory disease (Bouvet *et al.*, 2002).

CA 19-9 is biochemically identified as 36 kD sialyl derivative of lacto- N-fucopentaose II, hapten of human Lewis blood group carbohydrate antigenic determinant occurring as monosialoganglioside/glycolipid in tissue or as circulating mucin with a molecular weight >106 D in serum (Lamerz, 1999; Rottenberg *et al.*, 2009). This carbohydrate Ag is expressed in bronchiolar epithelial cells and found in bronchoalveolar lavage in patients with pulmonary fibrosis. Purified CA19-9 stimulated neutrophil chemotaxis to C5a and IL-8. Normally, CA19-9 may be found in healthy individuals and it increases in benign hepatobiliary disease, with the highest levels in excretory ductal pancreatic adenocarcinoma, biliary, hepatocellular and cholangiocarcinoma cancer. CA19-9 is associated as a tumor marker with metastatic disease in pancreatic cancer, colorectal cancer, biliary cancer, urothelial cancer and melanoma. Neoplasm transformation is induced by high expression of CA19-9. Extravasations of tumor cells from the bloodstream and formation of metastatic disease were associated with CA19-9. The mechanism is probably due to its interaction with E-selectin expressed on endothelial cells (Miki *et al.*, 1995; Rottenberg *et al*., 2009).

Oxidative stress is implicated in CS-induced airway diseases, such as COPD (Rahman and MacNee, 1999). It is suggested that oxidative stress is an important trigger in the up regulation of pro-inflammatory genes (Van den Berg *et al.*, 2001)

Cigarette smoke (CS) contains numerous oxidants that have the capability of interacting with various biomolecules to cause adverse biological effects. Some of the more prominent targets of CS include DNA, RNA, lipids, amino acids, proteins, dietary antioxidants and various endogenously synthesized biomolecules such as glutathione and α1-antiprotease (Traber *et al.*, 2000; Bruno, 2004).

Exposure to CS causes cellular oxidative stress, a key feature in smoking-induced lung inflammation (Rahman, 2003). Oxidative stress can enhance nuclear factor (NF-κB) DNA binding activity (Schreck *et al*., 1991). NF-κB is a critical transcription factor regulating many cytokines, including IL-8, IL-6, TNF-α, granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein (MIP)-1, and macrophage chemotactic protein-1 (MCP-1). Enhanced NF-κB activation has been shown in bronchial biopsies from smokers and in guinea pigs exposed to CS, with a subsequent increase in IL-8 release. Activator protein (AP)-1, like NF-κB, regulates many of the inflammatory genes that are overexpressed in response to CS (Li *et al*., 2009).

The aim of the present study is to evaluate the effects of CS on some immunological parameters, like IFN-□, total IgA, oxidative stress (represented by MDA), and CA19-9 Ag, which is a tumor marker in different organs in the human body, and also to evaluate its effects on some hematological parameters in male smokers.

2. Materials and Methods

2.1. Design of the Study

Fifty male smokers were used in this study; they smoked at least 10 cigarettes per day for at least10 years. The smokers were collected in Erbil city during the period (May 2012 - July 2012).

Depending on the age of the smokers, they were divided into two groups. The first group includes smokers with age range between 25-35 years, and the second group includes smokers with age range between 36-45 years. Two control (non-smokers) groups were collected with the same range of age for statistical comparison.

2.2. Blood Sampling

Blood samples were taken by 5 cc syringe and put into chilled tubes with and without ethylene diamine tetra acetic acid (EDTA) (4.5mM) as anticoagulant and

centrifuged at 3000rpm at 4°C for 15 minute; then the sera were stored at -40C°.

2.3. Estimation of Interferon-Gamma (IFN-γ)

Estimation of interferon-gamma (IFN-γ) was done by enzyme-linked immuno sorbent assay (ELISA) technique, which obtained from Cusabio Company (China).

2.4. Detection of Serum Total IgA by Immune Precipitation Technique

The plate from its envelope was removed and left to stand at room temperature for few minutes so that any condensed water in the wells could evaporate. The wells were filled with 5 µl of sample and/or controls and a period of time of waiting was required till it was completely absorbed before handing the plate. The plate was closed and placed in a moist chamber. 72 h period of waiting was required for incubation. The concentration value corresponding to the precipitating ring diameter was read on the enclosed reference table.

2.5. Determination of Serum Malondialdehyde (MDA)

The assessment of the lipid peroxidation process is achieved via determining the end product MDA. The level of serum MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution. In brief, to 150 µl serum sample the following were added: 1ml (17.5%) trichloroacetic acid (TCA) and 1ml of 0.66% TBA, mixed well by vortex, incubated in boiling water for 15 minutes, and then allowed to cool. One ml of 70% TCA was added and the mixture allowed to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, the supernatant was taken out for scanning spectrophotometrically at (532nm) (Muslih *et al.*, 2002).

The concentration of MDA calculated as follow:

$$\text{MDA (µmol/L)} = \frac{\text{Absorbance at532nm}}{\text{L×E0}} \times D \times 10^6$$

L: light path (1cm)

E_0: Extinction coefficient 1.56×10^5 M^{-1} .Cm^{-1}

D: Dilution factor = 1 ml Vol. Used in ref./0.15 =6.7

2.6. Estimation of Carbohydrate Antigen 19-9 (CA 19-9 Ag)

Estimation CA 19-9 Ag was done by enzyme-linked immunesorbent assay (ELISA) technique, obtained from Human Gesellschaft Company (Germany).

2.7. Hematological Analysis

Blood parameters were immediately determined by using automated hematology analyzer (Coulter counter, Sysmex xt-2000i Japan) for determining total WBC count, deferential leukocyte, and platelet numbers.

2.8. Statistical Analysis

All data are expressed as mean± standard error (M±S.E), and statistical analysis was carried out using statistically available software (SPSS). Comparisons between groups were made using independent samples T test at (P<0.05).

3. Results

3.1. Serum Level of Interferon-γ (IFN-γ)

A significant increase of IFN-γ level was recorded in smokers 25-35 years group with mean value (48.73± 0.854) when compared with its control (37.81± 1.796) (Table 1). Furthermore, the level of IFN-γ significantly increased in smokers 36-45 years group with mean value (55.63± 0.997) when compared with its control (41.20± 0.734) (Table 2).

3.2. Serum Level of Total IgA

A significant decrease of total IgA level was recorded in smokers 25-35 years group with mean value (222.2± 13.79) when compared with its control (346.6± 21.53) (Table 1). Furthermore, the level of total IgA significantly decreased in smokers 36-45 years group with mean value (183.2± 18.71) when compared with its control (319.5± 14.34) (Table 2).

Table 1. Mean ± SE of effect of CS on IFN-γ and total IgA in male smokers (25-35 years)

Parameters	Control 25-35 years	Smoker 25-35 years	Statistical evaluation (*P*-value)
IFN-γ (pg/ml)	37.81± 1.796	48.73± 0.854	0.000
IgA (mg/dl)	346.6± 21.53	222.2± 13.79	0.000

P<0.05

Table 2. Mean ± SE of effect of CS on IFN-γ and total IgA in male smokers (36-45 years)

Parameters	Control 36-45 years	Smoker 36-45 years	Statistical evaluation (*P*-value)
IFN-γ (pg/ml)	41.20± 0.734	55.63± 0.997	0.000
IgA (mg/dl)	319.5± 14.34	183.2± 18.71	0.000

P<0.05

3.3. Serum Malondialdehyde (MDA) Level

The level of MDA in smokers 25-35 years group was significantly increased with mean value (3.982± 0.131) when compared with its control group (2.939± 0.153) (Table 3). Besides the level of MDA in smokers 36-45 years group was significantly increased with mean value (4.289± 0.318) when compared with its control group (3.196± 0.126) (Table 4).

3.4. Serum Level of Carbohydrate Antigen 19-9 (CA 19-9 Ag)

The level of CA 19-9 Ag in smokers 25-35 years group was increased with mean value (19.18± 3.434) when compared with its control group (11.87± 1.861) but this increasing in CA 19-9Ag level is not significant (Table 3), while the level of CA19-9 Ag in smokers 36-45 years group was increased significantly with mean value (23.97± 4.178) when compared with its control group (14.13± 1.276) (Table 4).

Table 3. Mean ± SE of effect of CS on MDA and CA 19-9 Ag in male smokers (25-35 years)

Parameters	Control 25 -35 years	Smoker 25-35 years	Statistical evaluation (*P*-value)
MDA (µmol/L)	2.939± 0.153	3.982± 0.131	0.000
Ca19-9 (U/ml)	11.87± 1.861	19.18± 3.434	0.068

P<0.05

Table 4 . Mean ± SE of effect of CS on MDA and CA 19-9 Ag in male smokers (36-45 years)

Parameters	Control 36-45 years	Smoker 36-45 years	Statistical evaluation (*P*-value)
MDA (µmol/L)	3.196± 0.126	4.289± 0.318	0.004
Ca19-9 (U/ml)	14.13± 1.276	23.97± 4.178	0.029

P<0.05

3.5. Haematological Analysis

A significant increase was recorded in most of haemtological parameters, in smokers 25-35 years group, the total WBC count increased significantly with mean value (9.177± 0.619) when compared with its control group (7.063± 0.468); the neutrophils increased significantly with mean value (5.372± 0.547), when compared with its control group (3.840± 0.284). Likewise, a significant increase was recorded in eosinophil numbers with mean value (0.216± 0.032) when compared with its control group (0.094± 0.013), while basophil numbers statistically did not change with mean value (0.056± 0.006) when compared with its control group (0.046± 0.006). On the other hand, a significant increase was observed in monocyte count with mean value (0.765± 0.036) when compared with its control group (0.551± 0.027). Moreover lymphocyte numbers significantly increased with mean value (2.761± 0.133) when compared with those in their control group (1.967± 0.096) (Table 5).

In smokers 36-45 years group, the total WBC count increased significantly with mean value (8.697± 0.302) when compared with its control group (6.550± 0.281), and neutrophils increased significantly with mean value (4.741± 0.291) when compared with its control group (3.948± 0.253). Likewise, a significant increase was recorded in eosinophil numbers with mean value (0.265± 0.020) when compared with its control group (0.132± 0.017). The basophil numbers significantly increased with mean value (0.072± 0.008) when compared with their control group (0.041± 0.006). On the other hand, a significant increase was observed in monocyte count with mean value (0.751± 0.025) when compared with those in their control group (0.534± 0.034). Moreover lymphocyte numbers significantly increased with mean value (2.698± 0.173) when compared with those in their control group (1.829± 0.078) (Table 6).

In smokers 25-35 years group, the numbers of platelets count statistically did not change with mean value (271.6 ±12.89) when compared with its control groups (245.8± 8.911) (Table 5).

Likewise, in smokers 36-45 years group, the numbers of platelets count statistically did not change with mean value (221.5±10.09) when compared with its control groups (241.0±10.34) (Table 6).

Table 5 . Mean ± SE of effect of CS on hematological parameters in male smokers (25-35 years)

Parameters	Control 25-35 years $x10^3/\mu l$	Smokers 25-35 years $x10^3/\mu l$	Statistical evaluation (*P*-value)
WBC	7.063± 0.468	9.177± 0.619	0.009
Neutrophil	3.840± 0.284	5.372± 0.547	0.016
Eosinophil	0.094± 0.013	0.216± 0.032	0.001
Basophil	0.046± 0.006	0.056± 0.006	0.239
Monocyte	0.551± 0.027	0.765± 0.036	0.000
Lymphocyte	1.967± 0.096	2.761± 0.133	0.000
Platelets	245.8± 8.911	271.6 ± 12.89	0.105

P<0.05

Table 6. Mean ± SE of effect of CS on hematological parameters in male smokers (36-45 years)

Parameters	Control 36-45 years $x10^3/\mu l$	Smokers 36-45 years $x10^3/\mu l$	Statistical evaluation (*P*-value)
WBC	6.550± 0.281	8.697± 0.302	0.000
Neutrophil	3.948± 0.253	4.741± 0.291	0.045
Eosinophil	0.132± 0.017	0.265± 0.020	0.000
Basophil	0.041± 0.006	0.072± 0.008	0.002
Monocyte	0.534± 0.034	0.751± 0.025	0.000
Lymphocyte	1.829± 0.078	2.698± 0.173	0.000
Platelets	241.0± 10.34	221.5± 10.09	0.183

P<0.05

4. Discussion

4.1. Serum Level of Interferon-γ (IFN-γ)

The results of the present study showed a significant increase in the level of IFN-γ in both age groups. The results of previous study showed dissimilar results about the level of IFN-γ when compared with other studies.

With regard to CS and immune function, *in vitro* and *in vivo* studies on the effects of CS toxins and nicotine on both IFN-γ are inconclusive. While some *in vitro* rodent studies suggest that nicotine may decrease IFN-γ (Hallquist *et al.*, 2000; Nouri-Shirazi and Guinet, 2003), other studies suggest just the opposite; exposure to tobacco smoke also appears to increase IFN-γ (Petro *et al.*, 1992) and IL-10 secretion (Zhang and Petro, 1996).

Although IFN-γ secreting cells were decreased in smokers' airways compared to nonsmokers' (Hagiwara *et al.*, 2001), no statistically significant difference in IFN-γ levels were found in smoking vs. nonsmoking (Cozen *et al.*, 2004; Zavitz *et al.*, 2008). Further, Zeidel *et al.* (2002) reported an increase in IFN-γ without an increase in the balancing effect of IL-10 in the peripheral blood of cigarette smokers.

Whetzel *et al.* (2007) reported that IFN-γ levels were higher among female smoker. Ouyang *et al.* (2000) reported a significant down regulation of IFN-γ and TNF-α in cultured peripheral blood mononuclear cells treated with CS extract, while Nordskog *et al.* (2005) revealed that the secretion of IL-6, IL-8, IL-4, IL-2, IFN-γ and GM-CSF was stimulated in response to CS condensate.

Increasing evidence suggests that macrophages and lymphocytes are important immunocytes in the smoke induced chronic inflammatory process. Activated T cells could release IFN-γ, which acts as macrophage- activating factor. Activated macrophages secrete many inflammatory proteins that may orchestrate the inflammatory process. Macrophages have the capacity to release the chemokines IFN-γ inducible protein (IP-10), IFN-inducible T-cell α-chemoattractant (I-TAC), and monokine induced by IFN-γ (Mig), which may be chemotactic for CD8+ T cells via interaction with the CCR5(markers of T helper 1 cells) receptor- CCL5 in smokers (Costa *et al.*, 2008).

4.2. Serum Level of Total IgA

The present study indicates a significant decrease in total IgA in both age groups. This result is analogous with other studies on the effects of smoking on the immune system (Schwartz and Weiss, 1994; Sopori and Kozak, 1998; Arnson *et al.*, 2010).

Griesel *et al.* (1999) determined sIgA levels in people who stopped smoking for at least 2 weeks. Transient decrease in sIgA occurred followed by a return to normal values within 2 weeks of stopping smoking.

Several studies have found that smokers had serum Ig levels (IgA, IgG, and IgM) up to 10e20% lower than those of non-smokers (Ussher *et al.*, 2004).

There is increasing evidence that chronic nicotine treatment leads to inhibition of the Ab response indicating that nicotine is a major immunosuppressive component in CS (Geng *et al.*, 1996; Sopori and Kozak, 1998).

4.3. Serum MDA Level

The results of the present study show a significant increase in the level of MDA in both studied age groups. This result is similar to the previous studies like Schmid *et al.* (1996) and Durak *et al.* (2002).

Cigarette smoke (CS) is known to contain a large number of oxidants; it has been hypothesized that many of the adverse effects of smoking may result from oxidative damage to critical biologic substances (Skurnik and Shoenfeld, 1998). Two major phases were identified in CS: a tar phase and a gas phase; both phases are rich in oxygen-centered, carbon-centered and nitrogen-centered free radicals as well as non-radical oxidants. From the analysis of each phase, it was estimated that a single cigarette puff contains approximately 10^{14} free radicals in the tar phase, and 10^{15} radicals in the gas phase. These include various compounds, which are capable of causing an increase in the generation of various ROS like superoxide ($O_2^{\bullet}-$) hydrogen peroxide (H_2O_2), hydroxyl ($OH^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals. These ROS, in turn, are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (Pasupathi *et al.*, 2009). Moreover, copper and iron elements in CS have been found to promote hydroxyl radical ($OH^{\bullet}$) formation by Fenton-type reactions in activated cells (Durak *et al.*, 2002).

Durak *et al.* (2002) suggested that smoking creates a significant oxidant load in the erythrocytes. As a result, toxic free radicals and other oxidant substances in CS damages unsaturated fatty acids and some other oxidation- sensitive structures in the erythrocytes leading to MDA level increase.

Oxidative stress plays a critical role in the inflammatory response to CS through up regulation of redox-sensitive transcription factors and subsequently pro-inflammatory gene expression (Gilks *et al.*, 1998; Keating *et al.*, 1999). Inflammation itself also induces oxidative stress in the lungs and polymorphisms of genes for inflammatory mediators or antioxidant genes may have a role in individual susceptibility to the effects of CS (Smith and Harrison, 1997).

The redox-sensitive transcription factor NF-κB, which can be activated by oxidants and inhibited by antioxidants (Sen and Packer, 1996), plays an important role in the coordinated expression of inflammatory genes induced by CS (van den Berg, 2001).

Exposure to CS causes a cellular oxidative stress, a key feature in smoking-induced lung inflammation (Rahman and MacNee, 1999; Rahman, 2003). Oxidative stress (particularly hydrogen peroxide) can enhance NF-κB DNA binding activity (Schreck *et al.*, 1991). NF-κB is a critical transcription factor regulating many cytokines, including IL-8, IL-6, TNF-α, GM-CSF, macrophage inflammatory protein (MIP)-1, and MCP-1(Di Stefano *et al.*, 2002).

4.4. Serum Level of Carbohydrate Antigen 19-9 (CA 19-9 Ag)

The results of this study show that the level of CA19-9 in age group 25-35 years smokers increased but this increase is not significant, while the level of CA 19-9 increased significantly in age group 36-45 years smokers. This result is similar to what is obtained by Kawai *et al.* (2008), Rottenberg *et al.* (2009), and Wang *et al.* (2012). Lee *et al.* (1998) reported a significant association between CA19-9 and average number of cigarette consumed per day.

CA 19-9 is a carbohydrate antigen expressed in bronchiolar epithelial cells and found in bronchoalveolar lavage in patients with pulmonary fibrosis. Purified CA19-9 stimulated neutrophil chemotaxis to C5a, and IL-8. Normally, CA19-9 may be found in healthy individuals and it increases in benign hepatobiliary disease, with the highest levels in excretory ductal pancreatic adenocarcinoma, biliary, hepatocellular and cholangiocarcinoma cancer (Rottenberg *et al.*, 2009).

The presence of elevated CA19-9 levels in patients with lung cancer has been described in Japan. Most of these patients were diagnosed with adenocarcinoma. In

addition, cell lines from poorly differentiated adenocarcinoma of the lung may produce this tumor marker (Nagami *et al.*, 1988).

Correlation between CA19-9 and the size of the pancreatic adenocarcinoma was reported by Ferrone *et al*. (2006). CA19-9 is associated as a tumor marker with metastatic disease in pancreatic cancer, colorectal cancer, urothelial cancer and melanoma. Neoplasm transformation is induced by high expression of CA19-9. Extravasations of tumor cells from the bloodstream and formation of metastatic disease were associated with CA19-9. The mechanism is probably due to its interaction with E-selection expressed on endothelial cells.

Rottenberg *et al*. (2009) concluded that CA19-9 may be elevated not only in the case of pancreatic cancer, but also in patients with non-small cell lung cancer. And due to the similar demographic and environmental risk factors for both conditions, it is important to interpret the CA19-9 results in light of the clinical presentation as well as chest and abdomen imaging.

4.5. Haematological Parameters:

The results show a significant increase in the total WBC, neutrophil, eosinophil, basophil, monocyte, and the lymphocyte count in both groups, except basophil which statistically did not show any change in group 25-36 years; this result is similar to what is obtained by Schwartz and Weiss (1994), Freedman *et al.* (1996).

Cigarette smoking (CS) has been shown to be associated with an elevated peripheral blood leucocyte count (Schwartz and Weiss, 1994). One of the possible mechanisms of increasing of total WBC may be due to the glycoprotein from the tobacco leaf which can stimulate lymphocyte proliferation and differentiation by interacting with a specific membrane component, as occurs in antigenic response (Freedman *et al.*, 1996).

Cigarette smoke (CS) can induce cyclooxygenase-2 (COX-2) expression and lead to prostaglandin-E3 (PGE2) release from many cell types, such as fibroblasts, blood monocytes, AMs, lung dendritic cells and neutrophils, most of which are immune cells. In addition, the ability of CS and its carcinogens, nicotine in particular, to promote the production of COX-2-derived PGE2 has also been demonstrated in various types of human cancer cells (Badawi *et al.*, 2002).

Arachidonic acid (AA) is liberated from cell membrane phospholipids and converted by COXs to unstable PGG2 and PGH2, which are further metabolized by cell specific prostanoid synthases to biologically active prostanoids, mainly including PGE2, TxA2 and PGI2. TxA2 can bind to its receptor to activate COX-2 gene regulators NF-κB which induce pro- inflammatory cytokines that cause increasing in WBC count (Huang and Chen, 2011)

The eosinophil is likely to be primarily important in terms of IgE-mediated airway injury. Eosinophils are attracted to the lung by elevations in serum IgE, which increases in cigarette smokers. Cytokine activation, particularly IL-3 and-5, may be important in attracting eosinophils to injured airways. CS was shown to activate IL- 2 and may activate other cytokines. In addition, CS is known to be associated with protean immunologic effects, such as increased CD4 cells in light smokers, increased CD8 cells in heavy smokers, and decreased IgG, IgM, and IgA. Thus, other cells and immunologic mechanisms may also be important in airway inflammation due to CS (Schwartz and Weiss, 1994).

It has often been hypothesized that AMs, a key innate immune cell in the lungs, are the orchestrators of CS-induced inflammation. Work by D'Hulst (2005), and Botelho *et al*. (2009) demonstrate that mechanisms that drive CS-induced inflammation are associated with innate immunity. It is acceptable that innate immune mechanisms initially drive CS induced inflammatory processes, and that the engagement of adaptive immunity occurs in a more chronic setting (Nikota and Stämpfli 2012).

The net effect of CS on neutrophils is an elevation of the neutrophils count and a reduction of their functionality. The systemic inflammatory response triggered by exposure to CS is characterized by the stimulation of the hematopoietic system, specifically the bone marrow, which results in the release of leukocytes and platelets into the circulation (Arnson *et al.*, 2010).

Nicotine has been shown to inhibit formation of free oxygen radicals in PMN. Thus, it is likely that nicotine also has the capacity to suppress several neutrophil-mediated inflammatory actions. PMN from the peripheral blood of smokers also exhibits depressed migration and chemotaxis compared with PMN from non-smokers (Nguyen *et al.*, 2001).

The results of the present study show that there is no significant change in the platelets count PDW% and PLCR % between the control and smokers in both groups. This result is in agreement with previous results by Butkiewicz *et al.* (2006), who reported that there is no statistically significant difference in platelet count between male smokers and non-smokers. Suwansaksri *et al.* (2004) observes no alterations in platelets in male smokers and non-smokers. According to Blann *et al.* (1998), smoking two cigarettes a day by chronic smokers of both sexes do not affect the platelet count; Hawkins (1972) also appears to have substantiated the findings of the present study. She observed no significant difference between the platelet counts of nonsmokers, light smokers, and heavy smokers. Various reports have focused on the influence of smoking on platelets because of a possible association between smoking and alteration of blood platelets. Some of these results showed an increase of platelets turnover and a decrease of platelet survival in smokers; increased destruction of platelets, however, was not sufficient to reduce the number of circulating platelets (Fuster *et al.*, 1981).

5. Conclusion

From the present study, we can concluded that cigarette smoking increases the risk of cancer in different organs by the evidence of increasing of level of CA 19-9 and free radicals which have an important role in cancer. Also cigarette smoking increases inflammation responses which are represented by increasing the level of IFN-γ and WBC count.

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