

## **The changes in some inflammatory markers and biochemical aspects during smoking in males.**

Ahmed Farhan Shallal\*

Department of Medical Laboratory Science, University of Raparin, Rania, KRG, Iraq

### **Abstract**

**Background:** Smoking is one of a risk factor which can alter normal processes inside human and form disease. Thus, the aim of this study was to investigate the changes in some immunological markers and biochemical aspects during the smoking process. The immunological parameters; include (Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interferon-gamma (IFN- $\gamma$ ), interleukin-17A (IL-17A)) as well as erythrocyte sedimentation rate (ESR). The physiological parameter; included lipid profile (Cholesterol, triglycerides, low density lipoprotein (LDL), very low density lipoprotein (VLDL), high density lipoprotein (HDL)).

**Methods:** The sera were collected from 30 samples. The samples were separated into three groups. The first group includes smokers during 5 years and the second group includes smokers during 10 years as well as, one control (non-smokers) group. These tests were done on sample of thirty healthy males and their ages were 20-30 years. Enzyme-linked immune sorbent assay (ELISA) technique was used to measure cytokines levels while Westergren method was used to measure erythrocyte sedimentation rate. The lipid profile was estimated by using enzymatic colorimetric method.

**Results:** There was an increase in levels of inflammatory cytokines and erythrocyte sedimentation rate. The differences were significant ( $P < 0.05$ ) in the case of TNF- $\alpha$ , IFN- $\gamma$ , however, it was not significant in the case of IL-17A. The current study showed that the ESR level increased significantly ( $P < 0.05$ ) in comparing with non-smokers during the smoking process. The concentration of the cholesterol, triglycerides, (LDL) and very low density lipoprotein (VLDL) were increased while the level of (HDL) was decreased.

**Conclusion:** Smoking also attracts the inflammatory cells which lead to the release of inflammatory cytokines. Smoking affects the levels of inflammatory markers and lipid profile where the concentrations of them were changed.

**Keywords:** Smoking, TNF- $\alpha$ , IFN- $\gamma$ , IL-17A, ESR, Lipid profile.

*Accepted December 30, 2019*

### **Introduction**

The smoking process is the most common addictions and has negative health effects. It is also responsible for many other cancers and health problems. These include infections, lung disease, heart and blood vessel disease, stroke and cataracts, are the leading to cause of the sickness and death in today's communities [1-4].

Cigarette smoke has many compounds, including at least toxicants, carcinogens, and large quantities of oxidants and free radicals that stimulate oxidative stress, lung injury and programmed death of cells (apoptosis) [5,6]. Smoking causes exerts an inflammatory stimulus on macrophages which may, like viral and bacterial infection, lead to production of inflammatory mediators these may be the precursors to the diseases associated with smoking [7]. Smoking has a clear effect on healthy cases in the world. Health issues that connected with smoking may be related to its ability to penetrate and weak the immune system, as well as, low level of infection that cause the inflammation and forming disease

stimulated by smoking [8]. Exposure to the acute smoking leads to increase the cellular oxidative stress, in which lead to induce the inflammation [9]. Smoking process leads to weak the natural killer cell (NK) in diagnosis and surveillance tumor cell, in which the immune response to immunogen is altered in these cells. Thus, the cigarette smoking may change the number and type of lymphocyte, activation and expression of cytokines (inflammatory and anti-inflammatory) in the body. This, in turn, leads to functional impairs in adaptive immune response to the infections [10].

Cells of both the innate and adaptive immune system activate several signaling pathways in response to bacterial infection. The smoking stimulates cytokine production in cultured cell lines and primary cells. Cells of smokers are more sensitive, and have a faster kinetic activation of nuclear factor-kappa B (NF- $\kappa$ B) in comparison to cells of non-smokers [11]. Smoking process that possesses high levels of nicotine and tar induce greater immunologic changes than cigarette that contains lower levels of these materials [12]. LDL-C plays important role in forming of the thermogenesis and associated with increased

mortality that caused by vascular diseases while little concentration of HDL-C is predictor of coronary artery diseases [13]. This study aimed to evaluate the effect of smoking on some immunological and biochemical aspects.

## Material and Methods

### Samples

Thirty healthy males at 20-30 y of age entered in this the study. Women were excluded from this study. Also, subjects were healthy without any acute or chronic diseases, during the study period. Male smokers were used in this study; they smoked at least 10-15 cigarettes daily. The smokers were collected in Baghdad city during the period (March 2019). The groups were divided into three collections. The first group was smokers during 5 y and, the second group also was smokers but during 10 y as well as one non-smokers group as control was collected.

### Material

The kits that used in this study are:

**Table 1.** Kits that used in this study.

No	Kits	Company	Country
1	TNF- $\alpha$ Kit	Euro immune	Germany
2	IFN- $\gamma$ Kit	Euro immune	Germany
3	IL-17-A	Euro immune	Germany
4	Cholesterol	Fortress diagnostics	U.K
5	Triglyceride	Stanbio	U.K
6	HDL-chol.	Biomeriux	France

Solution for erythrocyte sedimentation rate estimation (Trisodium citrate).

### Methods

The samples (8 ml from each participant, 3 ml was for ESR test) were collected in plain tubes. Sera were separated by using centrifugation at 1000 g for 20 min at room temperature. Samples were immediately separated into aliquots and stored at (-20°C) until analysed. A sandwich type (ELISA) was used to measure serum (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) concentrations. In addition to the level of ESR was determined by using Westergren method. Enzymatic colorimetric method was used to estimate concentration of the lipid profile.

**A-Measurement of serum (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A,) levels by enzyme-linked immune sorbent assay (ELISA) technique:** The immune assay is a sandwich type assay with immunological steps. The first step leads to the capture of (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) by the monoclonal anti-(IL-TNF- $\alpha$ , IFN- $\gamma$ , 17A) antibody bound to the wells of the microtiter plate. In the second step anti (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) with conjugate is added which will bind to solid phase complex.

After incubation the wells were washed and a chromogenic substrate added, the intensity of the coloration was proportional to the (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) concentration of the sample and standard. The principle and procedure were similar with simple differences regarding standard concentrations and stop solution, and monoclonal Ab specificity [14-16].

**B-Detection of erythrocyte sedimentation rate levels by using Westergren method:** Westergren method for determining erythrocyte sedimentation rate (ESR), anti-coagulated blood is diluted with 0.85% saline and aspirated into a calibrated tube. The cells are allowed to settle for a period of one hour [17-19].

**C-Estimation of lipid profile levels by enzymatic colorimetric method:** Lipid profile measured enzymatically in serum in a series of coupled reactions [20,21].

### Statistical analysis

The analysis of data was done by using a one way of analysis ANOVA table. The value of (P<0.05) was considered significant for all analyses tests. A statistical analysis was performed by statistical Package for Social Science (SPSS) V22.

## Results

In this study, we found that there are changes in inflammatory cytokines (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) during smoking are given in Table 1 respectively. Significantly, both of TNF- $\alpha$  and IFN- $\gamma$  increased (P<0.05) during smoking compared with non-smokers while IL-7A was not significantly differed. Changes in the level of ESR during the smoking process are shown in Table 1. The increasing was significant (P<0.05) compared to control group. Table 2 shows the significant changing (P<0.05) in the levels of lipid profile except the (LDL) was non-significant during ten years of smoking. Also the result of lipid profile was not significant at fifth years of smoking.

**Table 2.** The means of TNF- $\alpha$ , IFN- $\gamma$ , IL-7A and ESR in both of non-smokers and smokers subjects.

Inflammatory y markers	Non-smokers (M $\pm$ SE)	Smoking during five years (M $\pm$ SE)	Smoking during ten years (M $\pm$ SE)
TNF- $\alpha$	8.7144*	10.1844*	13.5644*
	(pg/dl)	(pg/dl)	(pg/dl)
	0.58357	0.65128	0.77283
IFN- $\gamma$	29.1978	37.7244	44.6611*
	(pg/dl)	(pg/dl)	(pg/dl)
	3.3818	4.58929	5.53714
IL-17A	35.178	38.61	46.2944
	(pg/dl)	(pg/dl)	(pg/dl)
	4.48139	4.71623	5.15783
ESR	12.0000*	18.8767*	24.9933*

(mm/h)	(mm/h)	(mm/h)
0.54457	0.8444	1.27888

\*It means there is significant difference in means at the 0.05 level.

**Table 3.** The mean level (mmoL) of lipid profile in both of non-smokers and smokers subjects.

Biochemical parameters	Non-smokers (M ± SE)	Smoking during five years (M ± SE)	Smoking during ten years (M ± SE)
Cholesterol	3.20 ± 0.16	3.3 ± 0.17	3.9 ± 0.17 a
Triglycerides	1.7 ± 0.20	1.75 ± 0.3	2.5 ± 0.29 a
LDL	2.7 ± 0.2	2.75 ± 0.3	2.8 ± 0.19
VLDL	0.9 ± 0.5	0.93 ± 0.6	1.6 ± 0.18 a
HDL	2.3 ± 0.25	2.35 ± 0.3	1.25 ± 0.7 a

## Discussion

This study was aimed to assess impact of smoking on some immunological and biochemical indicators. The inflammatory markers were estimated via blood analysis of various inflammatory cytokines. In addition to, erythrocyte sedimentation rate test. Findings show that the elevated serum concentration of inflammatory mediators (TNF- $\alpha$ , IFN- $\gamma$ , IL-17A) may contribute to decrease the anti-inflammatory cytokines in favor of the later. In the current study, the mean concentration of TNF- $\alpha$  for control group was 8.7144 pg/dl while it increased for five and ten years of smoking to be 10.1844 pg/dl, 13.5644 pg/dl respectively Table 2. Statistical analysis demonstrated that the differences between values were significant. Our study has been shown that smoking causes increasing in IFN- $\gamma$  level. The results of five years of were 37.7244pg/dl while after ten years of smoking were 44.6611pg/dl in comparison to its value at the non-smoker group which was 29.0867pg/dl. Statistically, there were significant differences (P<0.05). Table 2 displays the effect of smoking on IFN- $\gamma$  concentration.

The results obtained from this study revealed an increase in the mean values of IL-17A in smokers but not reach to the levels of significance where the mean for control group, five years and ten years groups were 35.178 pg/dl, 38.61pg/dl and 46.2944 pg/dl respectively. The mean ratio of ESR for control group was 12.0000 mm/h while mean values of the five and ten years of smoking were 18.8767 mm/h, 28.99 mm/h, respectively.

According to lipid profile, the current study reported that smoking causes changing in levels of lipid profile, the mean value of five years of smoking for cholesterol became 3.3 mmol/L and that of ten years became 3.9 mmol/L compared to its baseline level at the non-smoker which was 3.20 mmol/L (Table 3). There was a tendency of increasing in the concentration of Triglycerides during smoking period. The result of five years of smoking was 1.75 mmol/L while their value after ten years of smoking was 2.5 mmol/L. In comparison to its value at the non-smoking period which was

1.7 mmol/L (Table 3). In comparison to the normal level of LDL at the control group which was 2.7 mmol/L, it increased under effect of smoking. The mean values of fifth and tenth years of smoking were 2.75 mmol/L, 2.8 mmol/L, respectively (Table 3). The results revealed obvious elevation in the level of VLDL during the smoking. The mean value of the fifth year was 0.9 mmol/L while that of tenth year of smoking was 0.93 mmol/L compared to its level at the non-smoker group which was 1.6 mmol/L (Table 3). In the present study, the smoking caused tendency of decreasing in concentration of HDL. The means of HDL for the non-smokers, smoking during five and ten years were 2.3 mmol/L, 2.35 mmol/L 1.25 mmol/L, respectively (Table 3).

Other studies have also reported similar findings who reported that the serum IFN- $\gamma$  concentration was increased significantly (P<0.05) in smokers when compared with its control [22]. On the other hand, in an animal study, who showed that the exposure to nicotine leads to decreases the inducible expression of inflammatory cytokines [23].

The previous study [24] showed disagreement with our result, it has been showed that the nicotine inhibition of the release of tumor necrosis factor from Macrophage. According to my opinion, the explanation for this finding is that during period of smoking and because of high compounds of tobacco that include over 4000 components that induce oxidative stress, oxidative injury and apoptosis that means the smoking has a broad impact on immune (including both types of adaptive immune responses) and metabolism functions in metazoan, Which leads to weak immune system and the incidence of diseases is assumed [25-29].

In conclusion, this study explained that smoking has effect on the cells functions, where attracts the inflammatory cells which lead to produce of inflammatory cytokines. The effect of smoking on the concentration of lipid profile may be closely related to the biochemical response to smoking, where the concentration was increased in the case of cholesterol, triglycerides, (LDL), and very low density lipoprotein (VLDL) while the (HDL) was decreased.

## References

1. Aryanpur M, Yousefifard M, Hosseini M, Oraii A, Heydari G, Kazempour-Dizaji M, Sharifi H, Hessami Z, Hassanzad M. Effect of Active and Passive Exposure to Cigarette Smoke on Lipid Profile of Children and Adolescents; A Systematic Review and Meta-Analysis. *Int J Pediatr* 2018 ; 6: 7575-88.
2. Arcavi L, Benowitz NL. Cigarette smoking and infection. *Arch Intern Med* 2004; 164: 2206-2216.
3. Birrell MA, Wong S, Catley MC, Belvisi MG. Impact of tobacco-smoke on key signaling pathways in the innate immune response in lung macrophages. *J Cell Physiol* 2008; 214: 27-37.
4. Takajo Y, Ikeda H, Haramaki N, Murohara T, Imaizumi T. Augmented oxidative stress of platelets in chronic smokers. Mechanisms of impaired platelet-derived nitric oxide

- bioactivity and augmented platelet agreeability. *J Am Coll Cardiol* 2001; 38: 1320-1327.
5. World Health Organization (WHO). Guidelines for controlling and monitoring the tobacco epidemic. Geneva WHO 2001; 2-7.
  6. Mehta H, Nazzal K, Sadikot RT. Cigarette smoking and innate immunity. *Inflamm Res* 2008; 57: 497-503.
  7. Noakes PS, Hale J, Thomas R, Lane C, Devadason SG, Prescott SL. Maternal smoking is associated with impaired neonatal tolllike-receptor-mediated immune responses. *Eur Respir J* 2006; 28: 721-729.
  8. Lu LM, Zavitz CC, Chen B, Kianpour S, Wan Y, Stämpfli MR. Cigarette smoke impairs NK cell-dependent tumor immune surveillance. *J Immunol* 2007; 178: 936-943.
  9. Mochida-Nishimura K, Surewicz K, Cross JV, Hejal R, Templeton D, Rich EA, Toossi Z. Differential activation of MAP kinase signaling pathways and nuclear factor-kappaB in bronchoalveolar cells of smokers and nonsmokers. *Mol Med* 2001; 7: 177-185.
  10. Droemann D, Goldmann T, Tiedje T, Zabel P, Dalhoff K, Schaaf B. Toll-like receptor 2 expression is decreased on alveolar macrophages in cigarette smokers and COPD patients. *Respir Res* 2005; 6: 68.
  11. Zhong CY, Zhou YM, Pinkerton KE. NF-kappaB inhibition is involved in tobacco smoke-induced apoptosis in the lungs of rats. *Toxicol Appl Pharmacol* 2008; 230: 150-158.
  12. Haapakoski R, Karisola P, Fyhrquist N. Toll-like receptor activation during cutaneous allergen sensitization blocks development of asthma through IFN-gamma-dependent mechanisms. *J Invest Dermatol* 2013; 133: 964-972.
  13. Elhashimi EH, Haala MG, Zakya AA, Abdalla EA. Effect of cigarette smoking on lipid profile in male at Collage of Police and Low Khartoum, Sudan. *Asian J Biomed Pharm Sci* 2013; 3: 28-31.
  14. Mariani M, Luzzi E, Proietti D. A competitive enzyme-linked immunosorbent assay for measuring the levels of serum antibody to Haemophilus influenzae type b. *Clin Diagn Lab Immunol* 1988; 5: 667-674.
  15. Dobrovolskaia E, Gam A, Slater JE. Competition enzyme-linked immunosorbent assay (ELISA) can be a sensitive method for the specific detection of small quantities of allergen in a complex mixture. *Clin Exp Allergy* 2006; 36: 525-530.
  16. Lequin RM. Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clin Chem* 2005; 51: 2415-2418.
  17. CLSI. Procedures for the erythrocyte sedimentation rate test. Standard (5th Edn.). CLSI Document H02-A5 Wayne, PA Laboratory Standards Institute 2011.
  18. Sader HS, Flamm RK, Jones RN. Antimicrobial activity of daptomycin tested against Gram-positive pathogens collected in Europe, Latin America, and selected countries in the Asia-Pacific Region (2011). *Diagn Microbiol Infect Dis* 2013; 75: 417-422.
  19. Curvers J, Kooren J, Laan M, van Lierop E, van de Kerkhof DSV. Evaluation of the Ves-Matic Cube 200 erythrocyte sedimentation method: comparison with Westergren based methods. *Am J Clin Pathol* 2010; 134: 653-660.
  20. Dissanayake DM. A rapid method for testing the erythrocyte sedimentation rate. *Journal of Diagnostic Pathol* 2006; 47-51.
  21. Hallit S, Zoghbi M, Hallit R, Youssef L, Costantine R, Kheir N, Salameh P. Effect of exclusive cigarette smoking and in combination with waterpipe smoking on lipoproteins. *J Epidemiol Global Health* 2017; 269-275.
  22. Pascale SalamehBachorik PS, Albers JJ. Precipitation methods for quantification of lipoproteins. Academic Press, Orlando 1986; 129: 78-100.
  23. Bachorik PS. Measurement of total cholesterol, HDL-cholesterol and LDL-cholesterol in Clinics in Laboratory Medicine. New York 1989; 9: 61-72.
  24. Farhang AA, Fikry AQ. Effects of cigarette smoking on some immunological and hematological parameters in male smokers in Erbil city. *Jordan J Biol Sci* 2013; 6: 159-166.
  25. Roma K, Shashi PS, Juan CPP, Raymond JL, Seddigheh RB, Mohan LS. Immunosuppressive and Anti-inflammatory effects of nicotine administered by patch in an animal model. *Clin Diagn Lab Immunol* 2004; 11: 563-568.
  26. Rahman I. Oxidative stress, chromatin remodeling and gene transcription in inflammation and chronic lung diseases. *J Biochem Mol Biol* 2003; 36: 95-109.
  27. Sopori ML, Kozak W. Immunomodulatory effects of cigarette smoke. *J Neuroimmunol* 1998; 83: 148-156.
  28. Nikota JK, Stampfli MR. Cigarette smoke-induced inflammation and respiratory host defense: Insights from animal models. *Pulm Pharmacol Ther* 2012; 25: 257-262.
  29. Soldin OP, Makambi KH, Soldin SJ, OMara DM. Steroid hormone levels associated with passive and active smoking. *Steroids* 2011; 76: 653-659.
  30. Sopori M. Effects of cigarette smoke on the immune system. *Nat Rev Immunol* 2002; 2: 372-377.
  31. Zhong CY, Zhou YM, Pinkerton KE. NF-kappaB inhibition is involved in tobacco smoke-induced apoptosis in the lungs of rats. *Toxicol Appl Pharmacol* 2008; 230: 150-158.

#### \*Correspondence to

Ahmed Farhan Shallal  
 Biology Department  
 College of Science  
 University of Raparin  
 Iraq