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

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Abstract

Background: Smoking is one of a risk factor which can alter normal processes inside human and form disease. Thus, this study aimed to investigate the changes in some immunological markers and biochemical aspects during the smoking process. The immunological parameters; include (Tumor necrosis factor-α (TNF-α), interferon-gamma (IFN-γ), 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 y and the second group includes smokers during 10 y as well as, one control (non-smokers) group. These tests were done on sample of thirty healthy males and their ages were 20-30 y. 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 the 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-α, IFN-γ, 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.

Conclusions: 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-α, IFN-γ, IL-17A, ESR, Lipid profile.

1. 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 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 exert an inflammatory stimulus on macrophages which may, like viral and bacterial infection, lead to the 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 causes 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]. The 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, 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 impair in an 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-κB) in comparison to cells of non-smokers [11]. A smoking process that possesses high levels of nicotine and tar induce greater immunologic changes than a cigarette that contains lower levels of these materials [12]. LDL-C played essential role in forming of the atherogenesis and associated with increased mortality that caused by vascular diseases while little concentration of HDL-C is a predictor of coronary artery

diseases [13]. This study aimed to evaluate the effect of smoking on some immunological and biochemical aspects.

2. 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 2018. 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-α Kit	Euro immune	Germany
2	IFN-γ 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-α, IFN-γ, IL-17A) concentrations. In addition to the level of ESR was determined by using Westergren method. Enzymatic colorimetric method was used to estimate the concentration of the lipid profile.

A-Measurement of serum (TNF-α, IFN-γ, 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-α, IFN-γ, IL-17A) by the monoclonal anti-(IL-TNF-α, IFN-γ, 17A) antibody bound to the wells of the microtiter plate. In the second step anti (TNF-α, IFN-γ, 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-α, IFN-γ, 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 one way of analysis ANOVA table. The value of (P<0.05) was considered significant for all analyses tests. Statistical analysis was performed by Statistical Package for Social Science (SPSS) V22.

3. Results

In this study, we found that there are changes in inflammatory cytokines (TNF-α, IFN-γ, IL-17A) during smoking are given in Table 1 respectively. Significantly, both TNF-α and IFN-γ 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-α, IFN-γ, IL-7A and ESR in both of non-smokers and smokers subjects.*

Inflammatory markers	Non-smokers (M ± SE)	Smoking during five years (M ± SE)	Smoking during ten years (M ± SE)
TNF-α	7.6933*	9.9733*	12.4533*
	0.47246	0.54917	0.66172
IFN-γ	28.0867	36.6133	43.5500*
	2.2797	3.47818	4.42693
IL-7A	34.3067	37.5	45.1833
	3.37029	3.60512	4.05672
ESR	12.0000*	18.8767*	24.9933*
	0.54457	0.8444	1.27888

*It means there is significant difference in means at the 0.05 level. The unit of cytokine is (pg/dl) and (mm/h) is for ESR.

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

Biochemical Parameters	Non-smokers (M ± SE)	Smokingduring five years (M ± SE)	Smokingduring 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

4. Discussion

This study was aimed to assess the 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-α, IFN-γ, IL-17A) may contribute to decreasing the anti-inflammatory cytokines in favor of the later. In the current study, the mean concentration of TNF-α for control group was 7.6933 pg/dl while it increased for five and ten years of smoking to be 9.9733 pg/dl, 12.4533 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-γ level. The results of five years of were 36.6133 pg/dl while after ten years of smoking were 43.5500 pg/dl in comparison to its value at the non-smoker group which was 28.0867 pg/dl. Statistically, there were significant differences ($P<0.05$). Table 2 displays the effect of smoking on IFN-γ 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 significancy where the mean for the control group, five years and ten years groups were 34.3067 pg/dl, 37.5000 pg/dl and 45.1833 pg/dl respectively. The mean ratio of ESR for the 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 changes 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 the 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 average level of LDL at the control group which was 2.7 mmol\L, it increased under the 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 the 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 a tendency of decreasing in the 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-γ 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 in 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 a weak immune system and the incidence of diseases is assumed [25-29].

In conclusion, this study explained that smoking has effect on the functions of the cell, where attracts the inflammatory cells which lead to production 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.

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