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, 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-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 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-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.

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

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-kB) 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>
<thead>
<tr>
<th>No</th>
<th>Kits</th>
<th>Company</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TNF-α Kit</td>
<td>Euro immune</td>
<td>Germany</td>
</tr>
<tr>
<td>2</td>
<td>IFN-γ Kit</td>
<td>Euro immune</td>
<td>Germany</td>
</tr>
<tr>
<td>3</td>
<td>IL-17-A</td>
<td>Euro immune</td>
<td>Germany</td>
</tr>
<tr>
<td>4</td>
<td>Cholesterol</td>
<td>Fortress diagnostics</td>
<td>U.K</td>
</tr>
<tr>
<td>5</td>
<td>Triglyceride</td>
<td>Stanbio</td>
<td>U.K</td>
</tr>
<tr>
<td>6</td>
<td>HDL-chol.</td>
<td>Biomeriux</td>
<td>France</td>
</tr>
</tbody>
</table>

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 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), anticoagulated 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-α, IFN-γ, IL-17A) during smoking are given in Table 1 respectively. Significantly, both of 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>
<thead>
<tr>
<th>Inflammatory y markers</th>
<th>Non-smokers (M ± SE)</th>
<th>Smoking during five years SE</th>
<th>Smoking during ten years (M ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>8.7144±</td>
<td>10.1844±</td>
<td>13.564±</td>
</tr>
<tr>
<td></td>
<td>(pg/dl)</td>
<td>(pg/dl)</td>
<td>(pg/dl)</td>
</tr>
<tr>
<td></td>
<td>0.58357</td>
<td>0.65128</td>
<td>0.77283</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>29.1978</td>
<td>37.7244</td>
<td>44.661±</td>
</tr>
<tr>
<td></td>
<td>(pg/dl)</td>
<td>(pg/dl)</td>
<td>(pg/dl)</td>
</tr>
<tr>
<td></td>
<td>3.3813</td>
<td>4.58929</td>
<td>5.53714</td>
</tr>
<tr>
<td>IL-17A</td>
<td>35.178</td>
<td>38.61</td>
<td>46.2944</td>
</tr>
<tr>
<td></td>
<td>(pg/dl)</td>
<td>(pg/dl)</td>
<td>(pg/dl)</td>
</tr>
<tr>
<td></td>
<td>4.48139</td>
<td>4.71623</td>
<td>5.15783</td>
</tr>
<tr>
<td>ESR</td>
<td>12.0000*</td>
<td>18.8767*</td>
<td>24.9933*</td>
</tr>
</tbody>
</table>

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

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

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

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-α, IFN-γ, IL-17A) may contribute to decrease the anti-inflammatory cytokines in favor of the later. In the current study, the mean concentration of TNF-α 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-γ 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 which was 29.0867pg/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 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 which was 29.0867pg/dl. Statistically, there were significant differences (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].

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 the inducible expression of inflammatory cytokines [23].

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


15. Dobrovolskaia E, Gam A, Slater JE. Competition enzyme-linked immunosorbant 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.


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