Detection of C-reactive protein (CRP), Tumor necrosis factor-alpha (TNF-α) and immunoglobulin A (IgA) serum levels in healthy smokers and nonsmokers

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Received : 03/04/2011 Accepted : 29/05/2011

The smoking habit is one of the main factors that affect the inflammatory system of the body, and it can lead to many inflammatory diseases, such as cardiovascular diseases, cancer, and some respiratory diseases. Smoking can also cause a decrease in the immune system, which affects the body's ability to fight against infectious diseases.

In this study, the serum levels of CRP, TNF-α, and IgA were measured in 40 healthy smokers (25 males and 15 females) and 26 healthy nonsmokers (10 males and 16 females). The study was conducted over two years.

The results showed a significant increase in the serum levels of CRP and TNF-α in smokers compared to nonsmokers. Similarly, the levels of IgA were also increased in smokers compared to nonsmokers.

The correlation coefficient between CRP and TNF-α was 0.4, and between CRP and IgA was 0.37, indicating a positive significant relationship. The correlation between TNF-α and IgA was 0.29, indicating a positive but non-significant relationship.

The study highlights the importance of smoking cessation to improve the health status of smokers and reduce the risk of various health problems.
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ABSTRACT

Smoking is one of the major lifestyle factors influencing the health of human beings. In response to cigarette smoke, activated inflammatory cells produce a great variety of inflammatory mediators, such as CRP and TNF-α, also cigarette smoke affects the IgA levels in serum of smokers. We included in our study 40 healthy smokers (25 males and 15 females), and 16 healthy nonsmokers (10 males and 6 females) as a control group. Smokers group was classified into two subgroups: less than one pack (20 subjects) and more than one pack per day (20 subjects). The serum levels of CRP and TNF-α were assessed by Enzyme-Linked Immuno Sorbent Assay (ELISA). Otherwise IgA levels were assessed by Radial immunodiffusion (RID).

The levels of CRP, TNF-α and IgA in serum were significantly higher in smokers group than non smokers (P< 0.05). CRP and IgA concentrations are significantly higher (P < 0.05) in serum of smokers with more than one pack per day compared with those with less than one pack per day. We also noticed an increased TNF-α concentration in the serum of smokers with more than one pack per day compared with those with less than one pack per day but the result was not significant. We did not found any significant influence of gender in smokers on TNF-α, CRP and IgA levels. On the other hand, the results shown significant increased in the levels of serum CRP with age increased. There was positive correlation between the levels of TNF-α and CRP, the levels of CRP with IgA and weak positive correlation between the level of TNF-α and IgA in the serum of our smoker subjects (r = 0.4), (r = 0.37) and (r = 0.29) respectively. The significant increased CRP and TNF-α serum levels could induce in smokers, suggest the imbalance between proinflammatory and anti-inflammatory factors as a result of tobacco Smoke exposure. Serum levels of TNF-α and CRP might
be useful biomarkers for the selection of heavy smokers with a risk of developing smoke induced diseases.

**INTRODUCTION**

Smoking is one of the major lifestyle factors influencing the health of human beings. Tobacco smoking is associated with increased prevalence of various diseases, both in the respiratory tract and in distal organs. The possibility that tobacco smoke induced changes in immune and inflammatory processes may play a part in the aetiology and pathogenesis of many of these diseases (1).

In response to cigarette smoke, activated inflammatory cells produce a great variety of inflammatory mediators, first of all, acute-phase proteins (APPs) and cytokines (2). The activation of the acute phase response from infection, immune activation or injury is signaled by interleukin-6 (IL-6), which produces proteins such as fibrinogen, C-reactive protein (CRP), and serum amyloid A that lead to inflammatory reactions. CRP is a member of the class of acute-phase reactants, as its levels rise dramatically during inflammatory processes occurring in the body. This increment is due to a rise in the plasma concentration of IL-6, which is produced predominantly by hepatocytes, as well as adipocytes. Localized inflammation can induce CRP expression (3). CRP binds to phosphocholine on microbes. It is thought to assist in complement binding to foreign and damaged cells and enhances phagocytosis by macrophages (opsonin mediated phagocytosis), which express a receptor for CRP. It is also believed to play another important role in innate immunity, as an early defense system against infections (4).

Inhaled cigarette smoke can also induce tumor necrosis factor-alpha (TNF-α) production by alveolar macrophages, which in turn may enhance metalloproteinases (MMPs) production. MMPs have been involved in mediating the inflammation and lung destruction (5). TNF-α was originally described as a factor produced by the endotoxin stimulated macrophages that causes hemorrhagic necrosis of tumors (6). It is a powerful proinflammatory cytokine with pleiotropic properties and a key mediator of inflammation. TNF-α operates by binding to two structurally related cell surface receptors: p55 and p75. However, the p55 receptor seems to be responsible for mediating the majority of TNF-α function (7).
The effects of cigarette smoking on humoral immunity have been studied extensively. Several studies have found that smokers had serum immunoglobulin levels (IgA, IgG, and IgM) 10% to 20% lower than those of nonsmokers. Mili et al (8) found that IgA, IgG, and IgM levels were higher among former smokers than current smokers and increased with duration of smoking cessation. This suggests that the effect was reversible, with a return toward the immunoglobulin levels of nonsmokers (9).

**The present study aimed to** 1. measure the CRP, TNF-alpha, IgA serum levels in healthy smokers compare to healthy nonsmokers, determining the dose-response relationship with cigarette smoke exposure, 2. analyze the possible influence of gender and age on this variables 3. determine a possible association between this variables in healthy smokers.

**MATERIALS AND METHODS**

**Subject:** Forty healthy smokers (25 males and 15 females), their age range between 19-60 years in addition to 16 healthy nonsmokers (10 males and 6 females) age range between 20-59 years as a control group were included in this study. Smokers group was classified into two subgroups: less than one pack (20 subjects) and more than one pack per day (20 subjects).

A clinical and paraclinical evaluation was performed in both groups, without any evidence of infection or chronic obstructive pulmonary disease (COPD).

Details of subject medical histories and smoking status were asked in the questionnaires.

**Specimen collection:** Blood was collected from each individual including in this study, 5 ml of blood was drawn by vein puncture using disposable syringe. The blood was placed in plastic disposable tubes to obtain serum, which were stored at -20 °C till tested.

**Methods:** The serum levels of CRP and TNF-α were assessed by Enzyme-Linked Immuno Sorbent Assay (ELISA) using quantitative CRP in human serum or plasma kit (IBL international, Germany), following the instructions provided by the manufacturer and Assay Max TNF -α ELISA kit (Assaypro, USA) designed for the detection of TNF-α levels in human serum or plasma, also following the instructions provided by the
manufacturer (10). On the other hand, IgA levels were assessed by Radial immunodiffusion according to the guidelines mentioned in the leaflet supplied by the manufacturer (Immuchem, Belgium) (11).

**Statistical analysis:**
Data are expressed as mean ± standard deviation (SD). The statistical analysis system SAS (2004) program used to study the effect of smoking and all of sex and age in TNF-α, CRP and IgA levels between study groups. The least significant difference (LSD) test was used to the significant compare between means, and SAS program was employed for the correlation coefficient calculation to evaluate the associations between variables (12).

**RESULTS AND DISCUSSION**
The levels of CRP and IgA in serum were significantly higher in smokers group than non smokers (p< 0.05). CRP and IgA concentrations in serum of smokers with more than one pack per day compared with those with less than one pack per day also significantly higher (p < 0.05) as it shown in table 1.
The TNF-α serum level was significantly higher in smokers group than in nonsmokers (p<0.05). We also noticed a non significant increased TNF- α concentration in the serum of smokers with more than one pack per day in comparison to those with less than one pack per day (table 1).

**Table -1: Effect of tobacco smoking in the levels of TNF-α, CRP and IgA in the healthy smokers compared with healthy non smokers (control).**

<table>
<thead>
<tr>
<th>Smokers</th>
<th>No.</th>
<th>Mean ± SD</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TNF-alpha (pg/ml)</td>
<td>CRP (µg/ml)</td>
<td>IgA (mg/dl)</td>
<td></td>
</tr>
<tr>
<td>Smokers more than one pack</td>
<td>20</td>
<td>43.0 ± 11.5 a</td>
<td>7.82 ± 0.66 a</td>
<td>240.82 ± 18.01 a</td>
<td></td>
</tr>
<tr>
<td>Smokers less than one pack</td>
<td>20</td>
<td>21.8 ± 7.0 ab</td>
<td>3.93 ± 0.55 b</td>
<td>191.13 ± 14.09 b</td>
<td></td>
</tr>
<tr>
<td>Non smokers</td>
<td>16</td>
<td>9.9 ± 1.6 b</td>
<td>3.69 ± 0.48 b</td>
<td>168.32 ± 17.59 b</td>
<td></td>
</tr>
<tr>
<td>LSD</td>
<td>--</td>
<td>0.025 *</td>
<td>1.702*</td>
<td>49.171*</td>
<td></td>
</tr>
</tbody>
</table>

( P< 0.05) *
Mean values that have different letters have significant difference between them. SD= standard deviation
Detection of C-reactive protein (CRP), Tumor necrosis factor-alpha (TNF-α) and immunoglobulin A (IgA) serum levels in healthy smokers and nonsmokers

Results revealed insignificant influence of gender in smokers on TNF-α, CRP and IgA levels (table 2). We did not able to show any significant differences in smokers TNF-α and IgA levels between age groups. However, table 3 illustrates higher significant (p< 0.05) levels of CRP in serum of third age group (more than 40 years) in expense of the second age group (31-40 years).

Table -2: Effect of gender on smoker TNF-α, CRP and IgA levels.

<table>
<thead>
<tr>
<th>Smokers</th>
<th>No.</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TNF-alpha (pg/ml)</td>
</tr>
<tr>
<td>Male</td>
<td>25</td>
<td>31.9 ± 10.0</td>
</tr>
<tr>
<td>Female</td>
<td>15</td>
<td>23.5 ± 6.5</td>
</tr>
</tbody>
</table>

( P > 0.05). SD= standard deviation

Table -3: Effect of age on smoker TNF-α, CRP and IgA levels.

<table>
<thead>
<tr>
<th>Smokers age group (year)</th>
<th>No.</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>TNF-alpha (pg/ml)</td>
</tr>
<tr>
<td>Less than 30 -30</td>
<td>16</td>
<td>18.3 ± 11.4</td>
</tr>
<tr>
<td>31-40</td>
<td>9</td>
<td>30.7 ± 20.3</td>
</tr>
<tr>
<td>More than 41</td>
<td>15</td>
<td>36.8 ± 9.1</td>
</tr>
<tr>
<td>LSD</td>
<td>--</td>
<td>NS</td>
</tr>
</tbody>
</table>

( P < 0.05) * , NS : not significant. SD: standard deviation
Mean values that have different letters have significant difference between them.

There was positive correlation between the levels of TNF-α and CRP, also between the levels of CRP and IgA in the serum of smoker subjects, r = 0.4 and 0.37, respectively. Whereas there was weak positive correlation between the level of TNF-α and IgA (r = 0.29) as follows in table 4.
In the present study, the levels of CRP in serum were significantly higher in smokers group than non smokers one. This increase in the CRP levels was found to be related to the number of smoked cigarette per day. These results are in agreement with Lao et al (13), who found that smoking is associated with increased CRP and WBC levels in older Chinese men and with Das (14), who stated that male and female smokers showed an acute phase response indicated by significantly raised serum CRP levels than non smokers. On contrary, Helmersson et al (15) observed that the increase in CRP levels in smokers was not statistically significant. Ohsawa et al (16) reported that CRP levels are elevated in smokers while the increased CRP levels unrelated to the number of cigarettes smoked per day. Lowe et al (17) demonstrated that a dose-dependent correlation between CRP levels and number of cigarettes per day. No significant gender – specific differences for smoking and CRP levels were noticed in our findings. Likewise, Fröhlich et al (18) suggested that serum CRP concentrations were significantly higher in male regular smoker than male never smoker, but no significant differences was observed in women. The increased CRP has been found to be associated with increasing age (4).

The acute inflammatory response is induced by cigarette smoking and leads to gross changes in the levels of CRP and other acute phase proteins. The primary regulators of CRP and the acute phase proteins are the cytokines interleukin (IL)-6 and IL-1β and tumor necrosis factor (TNF) - α, which are secreted by neutrophil granulocytes and macrophages at sites of injury. These cytokines bind to cell surface receptors and initiate an intracellular signaling cascade, leading to activation of several transcription factors which is directly responsible for inducing transcription of CRP (19).

Our TNF-α results are in agreement with Petrescu et al (5) who indicated that the serum levels of TNF-α were significantly higher in the smoker group than in the nonsmoker group and the concentration of TNF-α

<table>
<thead>
<tr>
<th>Related Variables</th>
<th>Correlation coefficients (r)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α and CRP</td>
<td>0.40</td>
<td>**</td>
</tr>
<tr>
<td>TNF-α and IgA</td>
<td>0.29</td>
<td>*</td>
</tr>
<tr>
<td>CRP and IgA</td>
<td>0.37</td>
<td>**</td>
</tr>
</tbody>
</table>

*(P<0.05), **(P<0.01)
is elevated in the serum of healthy heavy smokers in cigarette dose-dependent manner also with Wirtz et al. (20) who reported a trend for higher baseline TNF-α levels among healthy smokers. On the other hand, these results are not in agreement with Diez-Pina et al. (21) who mentioned that TNF-α levels in serum did not show either differences between smoker and non-smoker groups. Dissimilarly, Gander et al. (22) findings failed to show significant effects of smoking on TNF-α plasma levels.

The serum levels of TNF-α were also significantly higher in smoker of more one pack per day than less one pack per day are in agreement with the observations of Zoppini et al. (23) who demonstrated a marked increase of TNF-α system activation with an increase in the number of cigarettes smoked per day. Similar results were reported by Fernandez-Real and coworkers (24).

Present work results didn’t found any differences in TNF-α level in serum of two groups according to the age or gender, these results disagreed with the study of Chatila (25) which showed that tobacco smoke effects might be sex dependent. Diez-Pina et al. (21) found higher levels of TNF-α in serum of male smokers, otherwise they have not confirmed any significant influence of age on TNF-α levels. Himmerich et al. (26) showed a significant influence of age on the serum TNF-α levels (increasing with age) and also confirmed a mild influence of gender on the serum TNF-α levels. We were not able to verify the influence of these two factors on the serum levels of TNF-α.

The major role of TNF-α in smoke-induced lung injuries is supported by large number of studies. The components of cigarette smoke that are responsible for local and systemic inflammatory response have not fully elucidated. The bacterial endotoxin contained in tobacco can survive combustion as an active compound of tobacco smoke. Thus, it might be one of the many pathologic substances involved in cigarette smoke-induced inflammatory reaction (27).

The levels of IgA in serum were significantly higher in smokers group than non-smokers. IgA concentrations in serum of smokers with more than one pack per day compared with those with less than one pack per day were also found to be significantly higher. In regard to smoker serum level of IgA, no effect of gender or age was observed. Even as Holt (28) indicated that concentration of the immunoglobulins IgG, IgM and
IgA are reduced by 10 -20% in the serum of smokers. Gonzalez – Quintela et al (29) also found that serum levels of IgA in smokers were not significant different from those of non smokers but agreed with him in the results show that sex- and age-related changes in immunoglobulin concentrations are smoking independent.

IgA levels increment, in response to cigarette smoke, activates inflammatory cells to produce a great variety of inflammatory mediators like circulating cytokines, one of them IL-6 which is a co-factor for immunoglobulin synthesis. Beagley et al (30) confirmed that there was some parallelism between IgA and IgG concentrations and serum concentration of IL-6, a marker of inflammation. Serum IL-6 levels were positively correlated with IgA and IgG concentrations.

The serum levels of TNF-α were positively correlated with CRP levels. Our findings are in agreement with Petrescu et al (5) who observed the same result. Also there is positively correlation between the levels of CRP and IgA in the serum of our smoker subjects; whereas there was a weak positive correlation between the level of TNF-α and IgA.

TNF-α and IL-1β are classical proinflammatory cytokines; together, they initiate a second wave of cytokines, including IL-6, whose activities include stimulating liver production of acute phase protein such as CRP (31)

**Conclusions:** significant increase in CRP and TNF-α serum levels could be induced in smokers, suggest the imbalance between proinflammatory and anti-inflammatory factors as a result of tobacco Smoke exposure. Serum levels of TNF-α and CRP might be useful biomarkers for the selection of heavy smokers with a risk of developing smoke induced diseases.

**REFERENCES**


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