EFFECTS OF TOBACCO SMOKING ON ALPHA-2-MACROGLOBULIN AND SOME BIOCHEMICAL PARAMETERS IN THAI MALES

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Abstract. This cross-sectional study was carried out among smokers and nonsmokers from suburban and urban residential areas in Bangkok, Thailand. One hundred eighty-six smokers and 102 nonsmokers, who voluntarily participated in the study, were investigated. The levels of alpha-2-macroglobulin (A2M), albumin, total protein, and other biochemical and hematological parameters as well as body mass index (BMI) measurements were taken. The levels of A2M, BUN and WBC counts were significantly higher in smokers than nonsmokers. Total protein and albumin concentrations were significantly lower in smokers than nonsmokers, but the levels of other biochemical parameters did not differ between the two groups. The relationship between BMI and median A2M levels in the smoker and nonsmoker groups showed the higher the BMI, the lower the serum A2M levels. Smokers had a higher percentage of hyperalpha-2-macroglobulinemia than nonsmokers. A2M concentrations correlated inversely with BMI, BUN, albumin, total cholesterol, triglycerides, and the quantity of cigarettes smoked for the total period of smoking (cigarette pack-years). Multiple regression analysis revealed that albumin and cigarette pack-years were the most closely related variables to A2M concentrations among smokers. These findings suggest cigarette smoking affects inflammation markers, increasing A2M and WBC and decreasing albumin. This effect may be the mechanism responsible for the development of chronic disease states associated with smoking since cigarette smoke contains many toxic compounds harmful to health.

INTRODUCTION

Unless current smoking patterns are reversed, the 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). In Thailand, males smoke much more than females, and the prevalence of current smokers has been reported to be greater than 9 million, or 19.5% of 49.4 million, in 2005. Cigarette smoking has been implicated as the cause of many chronic diseases, including cardiovascular diseases, emphysema, bronchitis, lung cancer, and various types of malignancies (Sherman, 1991; Diana, 1993). It generates many toxic and carcinogenic compounds harmful to 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 response.
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), and tumor necrosis factor α (TNFα); these may be the precursors to the diseases associated with smoking (Francus et al, 1992; Takajo et al, 2001).

IL-6 and TNFα can stimulate acute-phase protein synthesis (Beutler and Cerami, 1986). Increases in acute-phase protein production can be used as a measure of the degree of inflammation or tissue damage (Tappia et al, 1995). Alpha-2-macroglobulin (A2M) is a positive acute phase protein, the synthesis of which is induced by inflammatory stimuli (tissue injury, infection) in hepatocytes (Molvarec et al, 2007). Other cells, including monocytes and macrophages, also synthesize and secrete A2M (Kloczko et al, 1990).

A2M is a plasma glycoprotein with a molecular weight of 720 kDa that can stimulate diverse immune functions, interact with various vasoactive substances, and serve as a carrier protein for zinc and other metals (Goldenberg et al, 1991). It is also a key member of the proteinase inhibitors, which can be distinguished from other proteinase inhibitors in both mode of inhibition and range of specificity.

Albumin is a well-known “negative” acute-phase protein that acts as a marker of inflammation and has antioxidant properties. Changes in concentrations of acute-phase proteins, C-reactive protein, ceruloplasmin and α1-antitrypsin have already been studied in Thai smokers, (Pongpaew et al, 2001; Tungtrongchitr et al, 2002) but the underlying biochemical mechanisms in many pathological conditions associated with smoking are not fully understood. Therefore, this study was conducted to investigate the effect of smoking on the inflammatory markers A2M, albumin, and WBC count and other biochemical parameters, specifically total protein, glucose, blood urea nitrogen (BUN), creatinine, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), thiocyanate and hematological parameters among Thai smokers, compared with nonsmokers, and to determine the relationships between A2M and other parameters.

MATERIALS AND METHODS

Subjects

The sample population consisted of 288 men aged 19-60 years from urban and suburban residential areas of Bangkok, Thailand; 186 were smokers and 102 were nonsmokers. All volunteers were interviewed by questionnaire about lifestyle pattern and health history. The subjects were excluded if they suffered from major ailments such as severe hypertension, lung, diabetes mellitus, liver, kidney and cardiovascular diseases, which were diagnosed by a physician. Smoking characteristics, such as age at onset of smoking, number of cigarettes smoked and duration of smoking (years), were obtained by questionnaire. The quantity of cigarettes smoked for the whole period of smoking or cigarette pack-years, was calculated as the duration of smoking (years) multiplied by the number of smoked cigarettes, divided by 20 cigarettes per pack. The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok. Serum was used to determine biochemical variables, namely A2M, albumin, total protein, glucose, blood urea nitrogen (BUN), creatinine, total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and thiocyanate. EDTA blood was used to determine hematological variables, namely hemoglobin, hematocrit, red blood cell count (RBC) and white blood cell count (WBC). Anthropometric measurements, ie weight and height, were
recorded. The body mass index (BMI) was expressed as weight (kg)/height (m²).

Laboratory techniques

About 10 ml of venous blood from each subject were drawn in the morning after an overnight fast. The red blood cell count, white blood cell count, hemoglobin and hematocrit were measured (Coulter Counter). A2M was determined by rocket immunoelectrophoresis (Laurell, 1972). Total protein and albumin were measured by Biuret and Bromocresol Green methods, respectively. Glucose, BUN, creatinine, total cholesterol, triglycerides, AST, ALT and ALP were measured using enzymatic methods by DADE Dimension® AR. Serum thiocyanate concentrations were assessed by colorimetric method (Degiampietro et al, 1987).

To minimize analytical variation, the same technician performed all assays and single lots of reagents were used. The between-run coefficients of variation for each parameter were <5% (n= 30/runs), corresponding to a between-run variance = 0.002.

Statistical analysis

All data were analyzed using the Minitab statistical computer program (Ryan et al, 1985). The data were not normally distributed, so an appropriate non-parametric test was chosen. The median, range and 95% confidence interval (CI) were calculated. Comparisons were made using the Mann-Whitney U-Wilcoxon rank sum W test and a value of p < 0.05 was considered to be statistically significant. The Spearman rank was used to calculate correlation among the variables.

To determine whether cigarette pack-years and albumin were directly related to A2M levels, multiple regression analysis was done, where cigarette pack-years and albumin were taken as independent variables and A2M as a dependent variable.

RESULTS

For this study, 288 subjects (186 male smokers and 102 male nonsmokers) were investigated. The age distribution was 19-60 years. Most smokers started smoking before 20 years of age. The average duration of cigarette smoking was nearly 20 years. Table 1 shows the distribution of the percentage of smokers according to the quantity of cigarettes smoked for the whole period of smoking (cigarette pack-years). The median, range and 95% confidence interval (%CI) for age, BMI, and biochemical measurements in smokers and in nonsmokers, are shown in Table 2. There was no significant age difference between the smoker and nonsmoker groups. Serum thiocyanate concentration was significantly higher in smokers than nonsmokers. The BMI of the smokers were not statistically significantly different from the nonsmokers. Serum total protein and albumin concentrations were significantly lower in smokers than nonsmokers, whereas significantly higher levels of A2M, BUN, and WBC counts were observed in smokers than nonsmokers. The BMI of the smokers and nonsmokers was determined, and revealed that the higher the BMI, the lower the serum A2M levels in these two groups (Fig 1). When an A2M concentration of 300 mg/dl was used as the cutoff value, approximately 25% of smokers and 13% of nonsmokers were classified as having

<table>
<thead>
<tr>
<th>Quantity of cigarette pack-years</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-5</td>
<td>66</td>
<td>35.5</td>
</tr>
<tr>
<td>6-10</td>
<td>46</td>
<td>24.7</td>
</tr>
<tr>
<td>11-15</td>
<td>19</td>
<td>10.2</td>
</tr>
<tr>
<td>16-20</td>
<td>18</td>
<td>9.7</td>
</tr>
<tr>
<td>&gt;21</td>
<td>37</td>
<td>19.9</td>
</tr>
</tbody>
</table>
Table 2
Median, range and 95% confidence interval (CI) for age, BMI, and biochemical parameter levels in smokers and nonsmokers.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Smokers (N=186)</th>
<th>Total</th>
<th>Nonsmokers (N=102)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (range)</td>
<td>95%CI</td>
<td>Median (range)</td>
<td>95%CI</td>
</tr>
<tr>
<td>Age (years)</td>
<td>37.0 (19.0-60.0)</td>
<td>(33.3-40.0)</td>
<td>35.0 (19.0-60.0)</td>
<td>(32.0-36.8)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.6 (15.9-35.2)</td>
<td>(21.3-23.1)</td>
<td>23.1 (17.7-35.4)</td>
<td>(22.2-23.8)</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>14.8 (8.6-17.7)</td>
<td>(14.4-14.9)</td>
<td>14.9 (10.8-17.9)</td>
<td>(14.5-15.1)</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>45.0 (24.5-54.3)</td>
<td>(44.0-45.4)</td>
<td>44.7 (31.4-54.0)</td>
<td>(44.0-45.0)</td>
</tr>
<tr>
<td>RBC (10¹²/l)</td>
<td>5.2 (2.9-7.2)</td>
<td>(5.0-5.2)</td>
<td>5.2 (4.3-7.1)</td>
<td>(5.1-5.4)</td>
</tr>
<tr>
<td>WBC (10⁹/l)</td>
<td>7.0 (2.3-17.7)</td>
<td>(6.5-7.2)</td>
<td>6.0 (3.4-12.9)</td>
<td>(5.7-6.4)</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>84 (50-125)</td>
<td>(77-85)</td>
<td>80 (52-123)</td>
<td>(78-82)</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>13 (5-22)</td>
<td>(12-13)</td>
<td>12 (5-20)</td>
<td>(11-12)</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.1 (0.5-1.6)</td>
<td>(1.0-1.1)</td>
<td>1.1 (0.5-1.6)</td>
<td>(1.0-1.1)</td>
</tr>
<tr>
<td>Thioctyanate (µmol/l)</td>
<td>60 (5-195)</td>
<td>(37-57)</td>
<td>3 (0-29)</td>
<td>(0-2)</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>200 (103-290)</td>
<td>(187-205)</td>
<td>203 (103-292)</td>
<td>(186-209)</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>119 (40-292)</td>
<td>(104-120)</td>
<td>125 (40-269)</td>
<td>(95-128)</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>33 (12-86)</td>
<td>(29-35)</td>
<td>32 (12-80)</td>
<td>(28-33)</td>
</tr>
<tr>
<td>ALT (U/l)</td>
<td>30 (13-93)</td>
<td>(27-32)</td>
<td>30 (13-94)</td>
<td>(27-33)</td>
</tr>
<tr>
<td>ALP (U/l)</td>
<td>73 (33-175)</td>
<td>(67-75)</td>
<td>70 (38-133)</td>
<td>(66-72)</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>7.8 (6.2-9.9)</td>
<td>(7.6-7.9)</td>
<td>8.1 (6.8-9.9)</td>
<td>(7.9-8.2)</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.3 (2.4-7.1)</td>
<td>(4.2-4.4)</td>
<td>4.4 (3.1-6.0)</td>
<td>(4.3-4.5)</td>
</tr>
<tr>
<td>A2M (mg/dl)</td>
<td>250 (159-594)</td>
<td>(240-263)</td>
<td>230 (112-429)</td>
<td>(210-236)</td>
</tr>
</tbody>
</table>

*p<0.05 by using Mann-Whitney U-Wilcoxon Rank Sum W test (Two-tailed).

BUN = blood urea nitrogen, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, RBC = red blood cell count, WBC = white blood cell count, BMI = body mass index.

Table 3
Correlation coefficients of A2M with other parameters in smokers.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Total protein</th>
<th>Albumin</th>
<th>Total cholesterol</th>
<th>Triglycerides</th>
<th>BUN</th>
<th>Creatinine</th>
<th>BMI</th>
<th>Cigarette pack-years</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2M</td>
<td>-0.067</td>
<td>-0.169a</td>
<td>-0.284b</td>
<td>-0.186a</td>
<td>-0.212b</td>
<td>-0.097</td>
<td>-0.218b</td>
<td>0.206b</td>
</tr>
</tbody>
</table>

*a p < 0.05, b p < 0.01

BUN = blood urea nitrogen, BMI = body mass index.

Hyperalpha-2-macroglobulinemia (p=0.018) (Fig 2). The levels of other biochemical parameters–glucose, creatinine, total cholesterol, triglycerides, AST, ALT, and ALP–were not different between the two groups. Various parameters were correlated with each other in smokers, as shown in Table 3. A2M concentrations were inversely correlated with albumin (r = -0.169, p < 0.05), total cholesterol (r = -0.284, p < 0.01), triglycerides (r = -0.186, p <0.05), BUN (r = -0.212, p < 0.01), BMI (r = -0.218, p < 0.01) and cigarette pack-years.
Fig 1–Median serum A2M levels in smokers and nonsmokers, by BMI.

\*p < 0.05 by using Mann-Whitney U-Wilcoxon rank sum W test (Two-tailed).

Fig 1–Median serum A2M levels in smokers and nonsmokers, by BMI.

Fig 2–Percentage of hyperalpha-2-macroglobulinemia among smokers and nonsmokers.

\*p < 0.05 by using Mann-Whitney U-Wilcoxon rank sum W test (Two-tailed).

\(r = 0.206, p < 0.01\). Fig 3 shows that the relationship between A2M concentration (Y) and cigarette pack-years (X) appears to be linear (Y = 253.50 + 1.508X) (p = 0.007). The relationship between A2M concentration (Y) and albumin concentration (X) is shown in Fig 4 (Y = 377.89 - 24.23X) (p = 0.029). Multiple regression analysis revealed that the best related variables for A2M concentrations in smokers were albumin and cigarette pack-years (Table 4).

**DISCUSSION**

The majority of Thai smokers began smoking at age < 20 years. Cigarette smoke contains many toxic and carcinogenic com-
Alpha-2-Macroglobulin in Smokers

Table 4

Multivariate regression analysis in smokers with A2M as a dependent variable and the quantity of cigarettes smoked for the whole period of smoking (cigarette pack-years) as well as albumin as independent variables.

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>Standard error</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>344.60</td>
<td>46.34</td>
<td>0.000</td>
</tr>
<tr>
<td>Cigarette pack-years</td>
<td>1.46</td>
<td>0.56</td>
<td>0.010</td>
</tr>
<tr>
<td>Albumin</td>
<td>-21.15</td>
<td>10.51</td>
<td>0.046</td>
</tr>
</tbody>
</table>

Therefore, the increased BUN levels in smokers compared with nonsmokers may be due to the increased proteolytic degradation of oxidized proteins.

A2M, albumin, and WBC concentrations were studied. They are considered to be markers of inflammation. Smoking in its own right increases inflammation and oxidative stress (Takajo et al, 2001). In this study, cigarette smoking was associated with increased WBC count levels as well as A2M, but decreased levels of serum albumin. The rate of hyperalpha-2-macroglobulinemia in smokers was more than 1.5 times that of nonsmokers. A2M is a positive acute-phase protein, the synthesis of which is induced by inflammatory stimuli (tissue injury, infection) (Molvarec et al, 2007). It is also a proteinase inhibitor and has been known to have the capacity to inhibit proteolytic enzymes. Increased concentrations of A2M in tissue fluids or serum have been demonstrated in a number of inflammatory diseases, such as pulmonary emphysema, pneumonia, and chronic airflow obstruction (Bell et al, 1981; Plusa and Tchorzewski, 1985). The recommended reference range for A2M for adults is 130-300 mg/dl (Dati et al, 1996). Goldenberg et al (1991) found that high A2M levels were associated with a statistically significant decrease in birthweight. Tappia et al (1995) also found increased concentrations of acute-phase proteins in the plasma of smokers compared with nonsmokers.
Pongpaew et al (2001) reported that \( \alpha_1 \)-antitrypsin levels were also higher in smokers than nonsmokers, and serum ceruloplasmin concentrations were significantly higher in smokers than nonsmokers (Tungtrongchitr et al, 2002). Smokers had higher levels of inflammatory cytokines IL-6 and TNF \( \alpha \) than nonsmokers (Tappia et al, 1995), which may stimulate acute-phase protein synthesis in hepatic cells (Beutler and Cerami, 1986). Increases in acute-phase protein production can thus be used to measure the degree of inflammation or tissue damage (Tappia et al, 1995). Therefore, the increased A2M concentrations in smokers may be a bodily response mechanism to protect proteins and tissues from damage, and limit the inflammatory stimulus of cigarette smoke.

Many toxic substances, especially reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) and hypochlorous acid (HOCl), can damage cellular constituents leading to cell inflammation and injury (Marnett et al, 2003). The concentration of antioxidants in the smokers was lower than the nonsmokers (Tappia et al, 1995; Kim et al, 2003); these results were similar to our previous study of vitamin C concentrations in Thai smokers (Pongpaew et al, 2001; Suriyaprom et al, 2005). The low antioxidant status of cigarette smokers may predispose them to oxidant-inflicted tissue damage and disease. Oxidation can dissociate A2M tetramer into dysfunctional dimers and inhibit activity is reduced or eradicated by increased levels of oxidants (such as those from cigarette smoke or neutrophils) or decreased levels of antioxidants (Johnson, 2006). Many studies have shown that ROS-induced damage to antiproteinase inhibitors, such as \( \alpha_1 \)-proteinase inhibitor, secretory leukocyte proteinase inhibitor and A2M, destroyed the inhibitory activity of these antiproteinases, while enhancing the proteolytic activity of latent collagenase (Weiss, 1989), and this effect may contribute to tissue destruction in the pathogenesis of several diseases (Chessman and Slater, 1993). Therefore, smokers may tend to lose proteinase-inhibitor activity, although the levels of A2M will be higher in smokers than nonsmokers. However, further studies should be conducted to determine the relationship between the levels of A2M and its activity in smokers.

In contrast, albumin is a well-known “negative” acute-phase protein and acts as a marker of inflammation. The amount of albumin is decreased by cytokines IL-1, IL-6, and TNF \( \alpha \) during the acute-phase response (Koj et al, 1984). Serum albumin concentration was found to be significantly lower in smokers than nonsmokers; low albumin levels are correlated with smoking (Margetts and Jackson, 1993; Hunter et al, 2001). The results of the present study in Thai subjects confirm these findings and indicate that albumin concentrations were also inversely correlated with A2M concentrations. Cigarette smoking is associated with increased oxidative stress. Albumin also has antioxidant properties, by binding copper ions and scavenging HOCl. The scavenging of HOCl by albumin may well be due to the rapid reaction with \(-\text{SH}\) groups on this protein, and oxidized albumin may be cleared rapidly from the circulation and degraded (Halliwell, 1988; Hu et al, 1993). Cigarette smoking has a powerful influence on WBC count (Hansen et al, 1990; Frohlich et al, 2003), which may be a marker of exposure to oxidants, the inflammatory response, and/or host susceptibility to inflammatory stimuli (Crowell and Sarret, 1995). This study confirms higher WBC counts in smokers than nonsmokers. Our study indicates that cigarette smoking is associated with changes in inflammatory marker levels, such as A2M, albumin, and WBC count, and these may be due to cigarette smoke containing many toxic compounds that can induce inflammatory processes. These observations may provide some insight into the biological
mechanisms underlying the pathology associated with smoking, and promote antismoking education.

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