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

Kanjana Suriyaprom[1], Talabporn Harnroongroj[2], Pisit Namjuntra[1], Yaovamarn Chantaranipapong[2] and Rungsunn Tungtrongchitr[2]

[1]Faculty of Medical Technology, Rangsit University, Pathum Thani; [2]Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand

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

Correspondence: Rungsunn Tungtrongchitr, Department of Tropical Nutrition and Food Science, Faculty of Tropical Medicine, Mahidol University, 420/6 Ratchawithi Road, Ratchathewi, Bangkok 10400, Thailand.
Tel: 66 (0) 2354-9100 ext 1582, 1584, 2089; Fax: 66 (0) 2644-7934
E-mail: tmrtg@mahidol.ac.th

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 α_1- 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 (m^2).

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 relationship between the BMI and median serum A2M levels in 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 1
Percentage of smokers according to the quantity of cigarettes smoked for the whole period of smoking (Cigarette pack-years).

Quantity of cigarette pack-years	Frequency	Percentage
1-5	66	35.5
6-10	46	24.7
11-15	19	10.2
16-20	18	9.7
>21	37	19.9

Table 2
Median, range and 95% confidence interval (CI) for age, BMI, and biochemical parameter levels in smokers and nonsmokers.

Parameters	Total				p-value
	Smokers (N=186)		Nonsmokers (N=102)		
	Median (range)	95%CI	Median (range)	95%CI	
Age (years)	37.0 (19.0-60.0)	(33.3-40.0)	35.0 (19.0-60.0)	(32.0-36.8)	0.069
BMI (kg/m^2)	22.6 (15.9-35.2)	(21.3-23.1)	23.1 (17.7-35.4)	(22.2-23.8)	0.156
Hemoglobin (g/dl)	14.8 (8.6-17.7)	(14.4-14.9)	14.9 (10.8-17.9)	(14.5-15.1)	0.615
Hematocrit (%)	45.0 (24.5-54.3)	(44.0-45.4)	44.7 (31.4-54.0)	(44.0-45.0)	0.924
RBC (10^{12}/l)	5.2 (2.9-7.2)	(5.0-5.2)	5.2 (4.3-7.1)	(5.1-5.4)	0.153
WBC (10^9/l)	7.0 (2.3-17.7)	(6.5-7.2)	6.0 (3.4-12.9)	(5.7-6.4)	0.000*
Glucose (mg/dl)	84 (50-125)	(77-85)	80 (52-123)	(78-82)	0.313
BUN (mg/dl)	13 (5-22)	(12-13)	12 (5-20)	(11-12)	0.035*
Creatinine (mg/dl)	1.1 (0.5-1.6)	(1.0-1.1)	1.1 (0.5-1.6)	(1.0-1.1)	0.480
Thiocyanate (µmol/l)	60 (5-195)	(37-57)	3 (0-29)	(0-2)	0.000*
Total cholesterol (mg/dl)	200 (103-290)	(187-205)	203 (103-292)	(186-209)	0.750
Triglycerides (mg/dl)	119 (40-292)	(104-120)	125 (40-269)	(95-128)	0.685
AST (U/l)	33 (12-86)	(29-35)	32 (12-80)	(28-33)	0.333
ALT (U/l)	30 (13-93)	(27-32)	30 (13-94)	(27-33)	0.146
ALP (U/l)	73 (33-175)	(67-75)	70 (38-133)	(66-72)	0.268
Total protein (g/dl)	7.8 (6.2-9.9)	(7.6-7.9)	8.1 (6.8-9.9)	(7.9-8.2)	0.000*
Albumin (g/dl)	4.3 (2.4-7.1)	(4.2-4.4)	4.4 (3.1-6.0)	(4.3-4.5)	0.023*
A2M (mg/dl)	250 (159-594)	(240-263)	230 (112-429)	(210-236)	0.004*

*$p<0.05$ 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
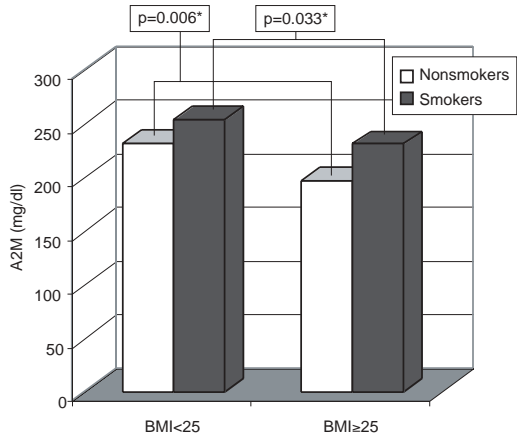Correlation coefficients of A2M with other parameters in smokers.

Variables	Total protein	Albumin	Total cholesterol	Triglycerides	BUN	Creatinine	BMI	Cigarette pack-years
A2M	-0.067	-0.169[a]	-0.284[b]	-0.186[a]	-0.212[b]	-0.097	-0.218[b]	0.206[b]

[a]$p < 0.05$, [b]$p < 0.01$
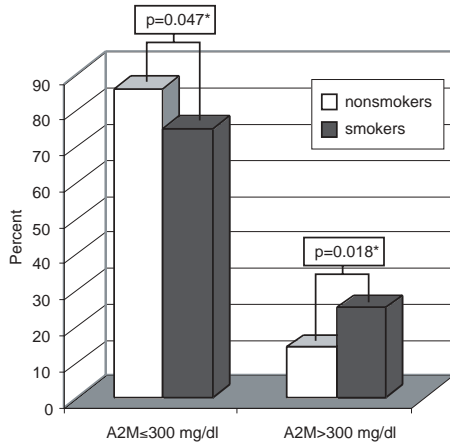BUN = blood urea nitrogen, BMI = body mass index.

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.

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

($r = 0.206$, 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
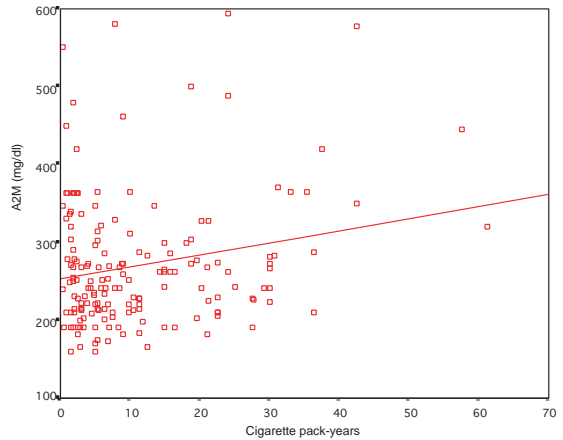

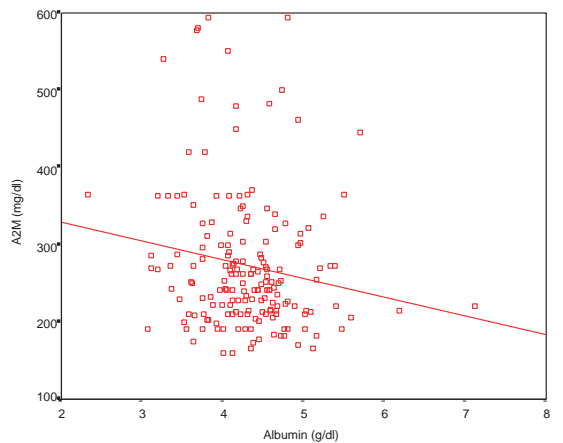Fig 3–Relationship between serum A2M concentration and cigarette pack-years among Thai male smokers.

Fig 4–Relationship between serum A2M concentration and albumin concentration among Thai male smokers.

($Y = 377.89-24.23X$) ($p = 0.029$). 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-

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.

Variables	Unstandardized coefficients		
	B	Standard error	p-value
Constant	344.60	46.34	0.000
Cigarette pack-years	1.46	0.56	0.010
Albumin	-21.15	10.51	0.046

pounds harmful to health, such as nicotine, nitrogen oxides, carbon monoxide, hydrogen cyanide, free radicals, etc (Hoffmann *et al*, 2001). This study used serum thiocyanate concentration to confirm smoking status, because hydrogen cyanide is metabolized by the liver into thiocyanate. Serum thiocyanate concentrations were significantly higher in smokers than nonsmokers, and these concentrations rose substantially with an increase in cigarette consumption. Thiocyanate is present in smokers as well as nonsmokers, but, on average, smokers have double the levels of thiocyanate than nonsmokers (Cohen and Bartsch, 1980). Therefore, serum thiocyanate is potentially more suitable as an index for cigarette smoking. Serum total cholesterol and triglycerides did not differ significantly between smokers and nonsmokers (Marangon *et al*, 1997, Barcin *et al*, 2006), and the present study confirms these findings. Smoking >10 cigarettes/day produced higher triglycerides than in nonsmokers; the levels of total cholesterol were not significantly different between these two groups (Imamura *et al*, 2000). Smokers had higher BUN levels than nonsmokers. BUN is the breakdown product of protein in the blood. Studies have demonstrated that the major hazardous effect of cigarette smoke, which contains some oxidants, is extensive oxidative damage of human plasma proteins and tissue microsomal proteins (Panda *et al*, 1999; Ma *et al*, 2000).

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 α_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 α 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 (H_2O_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 inhibitory 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 α_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 α 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 -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 Sarnet, 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.

ACKNOWLEDGEMENTS

REFERENCES

Barcin C, Tapan S, Kursaklioglu H, *et al.* Effects of non-heavy smoking on high-density lipoprotein cholesterol in healthy Turkish young men. *Acta Cardiol* 2006; 61: 411-5.

Bell DY, Haseman JA, Spock A, McLennan G, Hook GR. Plasma proteins of the bronchoalveolar surface of the lungs of smokers and nonsmokers. *Am Rev Respir Dis* 1981; 124: 72.

Beutler B, Cerami A. Cachectin and tumour necrosis factor as two sides of the same biological coin. *Nature* 1986; 320: 584-8.

Chessman KH, Slater TF. An introduction to free radical biochemistry. *Br Med Bull* 1993; 49: 481-93.

Cohen JD, Bartsch GE. A comparison between carboxyhemoglobin and serum thiocyanate determinations as indicators of cigarette smoking. *Am J Public Health* 1980; 70: 284-6.

Crowell RJ, Sarnet JM. Invited commentary: why does the white blood cell count predict mortality? *Am J Epidemiol* 1995; 142: 499-501.

Dati F, Schumann G, Thomas L, *et al.* Consensus of a group of professional societies and diagnostic companies on guidelines for interim reference ranges for 14 proteins in serum based on the standardization against the IFCC/BCR/CAP Reference Material (CRM 470). International Federation of Clinical Chemistry. Community Bureau of Reference of the Commission of the European Communities. College of American Pathologists. *Eur J Clin Chem Clin Biochem* 1996; 34: 517-20.

Degiampietro P, Peheim E, Drew D, Graf H, Colombo JP. Determination of thiocyanate in plasma and saliva without deproteinisation and its validation as a smoking parameter. *J Clin Chem Clin Biochem* 1987; 25: 711-7.

Diana JN. Tobacco smoking and nutrition. *Ann NY Acad Sci* 1993; 686: 1-11.

Francus T, Romano PM, Manzo G, Fonacier L, Arango N, Szabo P. IL-1, IL-6, and PDGF mRNA expression in alveolar cells following stimulation with a tobacco-derived antigen. *Cell Immunol* 1992; 145: 156-74.

Frohlich M, Sund M, Lowel H, Imhof A, Hoffmeister A, Koenig W. Independent association of various smoking characteristics with markers of systemic inflammation in men. Results from a representative sample of the general population (MONICA Augsburg Survey 1994/95). *Eur Heart J* 2003; 24: 1365-72.

Goldenberg RL, Tamura T, Cliver SP, Cutter GR, Hoffman HJ, Davis RO. Maternal serum alpha 2-macroglobulin and fetal growth retardation. *Obstet Gynecol* 1991; 78: 594-9.

Halliwell B. Albumin – an important extracellular antioxidant? *Biochem Pharmacol* 1988; 37: 569-71.

Hansen LK, Grimm RH Jr, Neaton JD. The relationship of white blood cell count to other cardiovascular risk factors. *Int J Epidemiol* 1990; 19: 881-8.

Hoffmann D, Hoffmann I, El-Bayoumy K. The less harmful cigarette: a controversial issue. A tribute to Ernst L Wynder. *Chem Res Toxicol* 2001; 14: 767-90.

Hu ML, Louie S, Cross CE, Motchnik P, Halliwell B. Antioxidant protection against hypochlorous acid in human plasma. *J Lab Clin Med* 1993; 121: 257-62.

Hunter KA, Garlick PJ, Broom I, Anderson SE, McNurlan MA. Effects of smoking and abstention from smoking on fibrinogen synthesis in

humans. *Clin Sci (Lond)* 2001; 100: 459-65.

Imamura H, Uchida K, Kobata D. Relationship of cigarette smoking with blood pressure, serum lipids and lipoproteins in young Japanese women. *Clin Exp Pharmacol Physiol* 2000; 27: 364-9.

Johnson AM. Amino acids, peptides and proteins. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz textbook of clinical chemistry and molecular diagnostics. 4th ed. Philadelphia: Elsevier, 2006: 553-5.

Kim SH, Kim JS, Shin HS, Keen CL. Influence of smoking on markers of oxidative stress and serum mineral concentrations in teenage girls in Korea. *Nutrition* 2003; 19: 240-3.

Kloczko J, Bielawiec M, Giedrojc J, Radziwon P, Galar M. Human monocytes release plasma serine protease inhibitors *in vitro*. *Haemostasis* 1990; 20: 229-32.

Koj A, Gauldie J, Regoeczi E, Sauder DN, Sweeney GD. The acute-phase response of cultured rat hepatocytes. System characterization and the effect of human cytokines. *Biochem J* 1984; 224: 505-14.

Laurell CB. Electroimmunoassay. *Scand J Clin Lab Invest* 1972; 29: 21-4.

Ma J, Hampl JS, Betts NM. Antioxidant intakes and smoking status: data from the continuing survey of food intakes by individuals 1994-1996. *Am J Clin Nutr* 2000; 71: 774-80.

Marangon K, Herbeth B, Artur Y, Esterbauer H, Siest G. Low and very low-density lipoprotein composition and resistance to copper-induced oxidation are not notably modified in smokers. *Clin Chim Acta* 1997; 265: 1-12.

Margetts BM, Jackson AA. Interactions between people's diet and their smoking habits: the dietary and nutritional survey of British adults. *BMJ* 1993; 307: 1381-4.

Marnett LJ, Riggins JN, West JD. Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. *J Clin Invest* 2003; 111: 583-93.

Molvarec A, Prohaszka Z, Nagy B, *et al*. Association of increased serum heat shock protein 70 and C-reactive protein concentrations and decreased serum alpha(2)-HS glycoprotein concentration with the syndrome of hemolysis, elevated liver enzymes, and low platelet count. *J Reprod Immunol* 2007; 73: 172-9.

Panda K, Chattopadhyay R, Ghosh MK, Chattopadhyay DJ, Chatterjee IB. Vitamin C prevents cigarette smoke induced oxidative damage of proteins and increased proteolysis. *Free Radic Biol Med* 1999; 27: 1064-79.

Plusa T, Tchorzewski H. Analysis of proteolytic enzymes and their natural inhibitors in serum and bronchial lavage fluid in atopic bronchial asthma, chronic bronchitis and pneumonia. *Allergy Immunol* 1985; 31: 169-78.

Pongpaew P, Tungtrongchitr R, Phonrat B, Vudhivai N, Viroonudomphol D, Schelp FP. Tobacco smoking in relation to the phenotype of alpha-1-antitrypsin and serum vitamin C concentration. *J Nutr Environ Med* 2001; 11: 167-73.

Ryan TA, Brian LB, Ryan BF. Minitab student handbook. 2nd ed. Boston: PWS-Kent, 1985. Sherman CB. Health effect of cigarette smoking. *Clin Chest Med* 1991; 12: 643.

Statistic Year Book Thailand. Survey of Thai smokers practice. Bangkok: Ministry of Information and Communication Technology, 2005.

Suriyaprom K, Tungtrongchitr R, Phonrat B, *et al*. Homocysteine and vitamin status in healthy Thai smokers. *J Nutr Environ Med* 2005; 15: 9-21.

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: 1320-7.

Tappia PS, Troughton KL, Langley-Evans SC, Grimble RF. Cigarette smoking influences cytokine production and antioxidant defences. *Clin Sci (Lond)* 1995; 88: 485-9.

Tungtrongchitr R, Pongpaew P, Phonrat B, *et al*. The effect of cigarette smoking on ceruloplasmin and C3 complement: risk of cardiovascular disease (atherosclerosis). *Asian Pac J Allergy Immunol* 2002; 20: 23-8.

Weiss SJ. Tissue destruction by neutrophils. *N Engl J Med* 1989; 320: 365-76.

World Health Organization. Guidelines for controlling and monitoring the tobacco epidemic. Geneva; World Health Organization, 2001: 2-7.