ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (2002) 20: 23-28

The Effect of Cigarette Smoking on Ceruloplasmin and C3 Complement: Risk of Cardiovascular Disease (Atherosclerosis)

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SUMMARY **Serum ceruloplasmin, C3 complement and albumin in 119 male smokers and 65 male non-smokers from a military unit in Bangkok were investigated in this study. The serum ceruloplasmin concentration was found to be significantly higher in smokers than in non-smokers. However, the serum albumin concentration in smokers was statistically significantly lower than in non-smokers. Significant associations were also found between ages, albumin levels and the quantity of cigarettes smoked. There was a significant positive correlation between serum ceruloplasmin and C3 complement concentrations. An association between the quantity of cigarettes smoked and albumin was also found, as well as a significant relationship between smoking and the quantities of cigarettes smoked to serum ceruloplasmin levels when smoking and the quantity of cigarettes smoked were taken as independent variables, and the serum ceruloplasmin levels as a dependent variable. This might suggest that high concentrations of the acute-phase protein, *i.e.* ceruloplasmin, might constitute a risk of developing atherosclerosis or cardiovascular disease in smokers.**

Tobacco smoking is known to cause lipid peroxidation and protein oxidation in the lung tissue.[1] Besides being a risk factor for developing lung cancer,[2] leukemia[3] and breast cancer,[4] cigarette smoking is also instrumental in the development of atherosclerosis. However, the link between cigarette smoke and atherosclerosis is not yet well known. One of the pathways by which cigarette smoking leads to atherosclerosis might involve ceruloplasmin. Concentrations of this acute-phase protein, ceruloplasmin, were found to be 12% higher in the plasma of smokers than in non-smokers ($p < 0.01$).[5] Ceruloplasmin is also known to be linked to the risk of developing cardiovascular disease.[6-7]

The serum complement protein C3 is vital to all three complement activation pathways[8] and plays a crucial role in the defense of the human body against invading microorganisms. However, recent evidence suggests that the activation of the complement system may, in the long run, also have detrimental effects, such as increased risk of cardiovascular disease.[9] Albumin is the main protein synthesized by the liver. Acute changes in serum albumin may be produced by large reductions in protein intake or by trauma or infectious diseases.[10-11] The functions of serum albumin are 1) to maintain osmotic pressure, 2) to act as a transport vehicle for amino acids and other substances to peripheral tissues, and 3) to serve as a temporary amino acid storage site. Injury and inflammation can cause acute decline in serum albumin concentrations.[12]

This study was performed in order to investigate the relation-

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ship between acute phase protein, ceruloplasmin, C3 concentration, and albumin in smokers and non-smokers. The results suggest that ceruloplasmin may play a role as one of the risk factors for atherosclerosis or cardiovascular disease.

MATERIALS AND METHODS

Subjects

One hundred and nineteen male smokers from a military unit in Bangkok participated voluntarily in the study. Sixty-five male non-smokers from the same unit were selected as controls. Socio-demographics and family smoking habits were assessed by means of a questionnaire as well as by a history of illness. The number of cigarettes smoked per day and the duration of cigarette smoking were multiplied together and expressed as "cigarette-years".

The study protocol was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University, and informed consent was obtained from each participant.

Analytical methods

About twenty milliliters of venous blood was drawn from each of the subjects under study in the morning after an overnight fast. For the quantitative determination of ceruloplasmin, the rocket immunoelectrophoresis method was used;[13] C3 concentration was determined by using immunodiffusion technique.[14] Serum albumin was assessed using the colorimetric method.[15]

Statistical analysis

The results were expressed as median and range. For data processing, the Minitab Computer Programme was utilized.[16] The Mann-Whitney U-Wilcoxon Rank Sum W test was used to compare the differences between smokers and non-smokers for continuous variables. The Chi's square test was used to compare proportions.

RESULTS

Table 1 shows the distribution of the percentage of smokers according to the quantity of cigarettes smoked (units in numbers of cigarette-years). Age and serum concentration of ceruloplasmin, C3 complement and albumin are presented in Table 2. The serum ceruloplasmin concentration was found to be significantly higher in smokers than in non-smokers. However, the serum albumin concentration in smokers was statistically significantly lower than in non-smokers.

Table 3 shows the correlation between age, ceruloplasmin, C3 complement, albumin and the quantity of cigarettes smoked by smokers. Significant associations were found between ages, albumin levels and quantity of cigarettes smoked. There were significant positive correlations between serum ceruloplasmin and C3 complement concentrations.

Table 1 Percentage of smokers according to the quantity of cigarettes smoked for the whole period of smoking (unit in number of cigarette-year)*

Quantity of cigarette-year*	N	Percent
1-5	36	29.3
6-10	34	27.6
11-15	30	24.4
16-20	18	14.6
>21	5	4.1

*number of cigarettes per day multiplied by duration of smoking (years)

Table 2 Median and range of age, ceruloplasmin, C3 complement, albumin in smokers and non-smokers

Parameters	Smokers (N = 119)		Non-smokers (N = 65)		*p*-value
	Median (range)	95% CI	Median (range)	95% CI	
Age (years)	41 (19-68)	38-42	37.5 (19-59)	32-42	0.326
Ceruloplasmin	40.5 (22.2-58.8)	38.7-41.5	35.4 (23.7-49.5)	33.0-36.6	0.000*
C3 complement	130 (81-200)	121.0-135.0	135 (70-202)	125.0-141.5	0.686
Albumin (g/dl)	4.5 (4.0-4.9)	4.5-4.6	4.6 (4.3-4.9)	4.6-4.7	0.001*

*Mann Whitney U-Wilcoxon Rank Sum W test (Two-tailed)

Table 3 Correlation between age, ceruloplasmin, C3 complement, albumin, quantity of cigarettes smoked in smokers

Parameters	Age	Ceruloplasmin	C3 complement	Albumin	Quantity of cigarettes smoked
Age	1.000	0.115	0.085	-0.444**	0.686**
Ceruloplasmin	0.115	1.000	0.359**	0.024	0.174
C3 complement	0.085	0.359**	1.000	0.081	0.187**
Albumin	-0.444**	0.024	0.081	1.000	-0.374**
Quantity of cigarettes smoked	0.686*	0.174	0.187**	-0.374**	1.000

*p-value < 0.05
**p- value < 0.01

Table 4 Covariance analysis of smoking as independent variables and serum ceruloplasmin as dependent variable, quantity of cigarette smoked as an additional independent variable to the model

Model	Unstandardized coefficients		Standardized coefficients	t	Significance
	B	Standard error	Beta		
1 (Constant)	45.060	1.522		29.608	0.000
Smoking	-4.894	1.060	-0.326	-4.616	0.000
2 (Constant)	41.327	2.372		17.423	0.000
Smoking	-3.071	1.380	-0.205	-2.226	0.027
Quantity of cigarette smoked	0.198	0.097	0.188	2.039	0.043

To determine whether smoking and the quantity of cigarettes smoked are related to an increased ceruloplasmin concentration, a covariance analysis was conducted. Smoking and the quantity of cigarettes smoked were taken as independent variables, and serum ceruloplasmin as the dependent variable (Table 4). A significant relationship between smoking and the quantities of cigarette smoked, to serum ceruloplasmin, was found.

DISCUSSION

Systemic defences against the damaging effects of free radicals in biological systems result from the complex interaction between antioxidants derived from the diet and endogenously synthesized antioxidants and antioxidant enzymes. The interaction of free radicals derived from cigarette smoke with this system, is rather complex. Cigarette smoke contains more than 1,015 organic free radicals in the gas phase of each inhalation;[4] these radicals cause widespread biological damage in the lung, which includes inactivation of human alpha-1-proteinase inhibitor, leading to emphysematous lesions and loss of lung elasticity.[17] Smoking also exerts an inflammatory stimulus on lung macrophages which may, like bacterial and viral infection, bring about the production of free radicals and inflammatory cytokines.[18] This might be an early event in the development of the disease states associated with smoking.[19-20] In our study, a higher serum concentration of ceruloplasmin was observed in smokers. Ceruloplasmin is an abundant, blue plasma copper protein with a mean, unevoked concentration in adults of about 300 μg/ml.[2] It is an acute phase reactant protein, exhibiting a moderate response (2- to 3-fold increase), but its physiological functions are not known with certainty.[3] Several activities of ceruloplasmin have been described, including copper transport, oxidation

of organic amines, oxidation of Fe^{2+} to Fe^{3+} for subsequent uptake by transferrin, and antioxidant activity against lipid peroxidation.[2,21] The antioxidant activity has been reported by many laboratories and has been suggested as a critical function of ceruloplasmin during inflammatory and acute phase responses.[22-27] However, Samokyszyn *et al.* [26] have shown the first report of ceruloplasmin pro-oxidant activity; the ferroxidase activity of ceruloplasmin that elicits antioxidant activity may, under well-defined experimental conditions, cause lipid oxidation.

Ceruloplasmin may influence the course of cardiovascular disease via lipid and lipoprotein oxidation. The first report of a positive correlation between ceruloplasmin and cardiovascular disease was shown in 1956.[28] Elevated serum ceruloplasmin levels were found in patients with multiple cardiovascular disorders, including arteriosclerosis,[29] abdominal aortic aneurysms,[30] unstable angina,[31] vasculitis and peripheral arterial disease.[32] While these correlations may be partly explained by the acute phase response accompanying the pathologies, several prospective studies have indicated that serum ceruloplasmin may be an independent risk factor for cardiovascular disease. A case-control analysis of a Dutch cohort showed that the risk of death from cardiovascular disease was four times higher in subjects with the highest serum copper quintile.[33] A prospective Finnish study verified that serum copper concentration (which is usually proportional to serum ceruloplasmin[3]) was an independent risk factor for ischemic heart disease.[34]

The following sequence of events may occur in smokers. The chronic production of cytokines in response to smoking is a strong possibility. The inflammatory stimulus of cigarette smoke activates alveolar macrophages to produce tumor necrosis factor (TNF) and interleukin-6.[5] Interleukin-6 might increase hepatic acute-phase protein production, including ceruloplasmin, and TNF induces free radical release from polymorphonuclear cells. This may lead to a greater oxidative burden and depletion of antioxidant defences.

The possibility that ceruloplasmin has a role in the oxidation of low density lipoprotein (LDL) within the vessel wall depends on the presence of a sufficient amount of the pro-oxidant, intact form of ceruloplasmin, and also on appropriate conditions in which ceruloplasmin can express this activity. The presence of a measurable amount of ceruloplasmin, and other acute phase proteins, in human atherosclerotic lesions has been reported.[35]

Ceruloplasmin-induced oxidation of LDL *in vitro* has been inhibited by some proteins such as albumin.[2] Injury and inflammation cause acute declines in serum albumin concentrations.[11-12] Alcohol intake causes an acute reduction in albumin production and serum albumin concentrations are often low in patients with alcoholic cirrhosis.[36] This might cause the low level of serum albumin concentration in smokers. Roubenoff *et al.*[12] observed that serum albumin, as well as body cell mass, is reduced in patients with rheumatoid arthritis. They hypothesized that other chronic inflammatory conditions also alter the protein metabolism, and could underlie the decreases seen in both serum albumin and muscle mass with aging. Inflammatory illnesses and illnesses that cause the acute-phase response to reduce the albumin gene expression, alter the intra- and extravascular distribution of albumin, and increase the rate of degradation.[36] Other acute phase proteins, such as cytokine and complement, could serve as markers and mediators for inflammation-based alterations in the protein metabolism.[12] However, in our study, only positive correlations between serum ceruloplasmin and C3 complement concentration were found, except for albumin.

Although no statistically significant difference in C3 complement concentration between smokers and non-smokers was found, serum ceruloplasmin concentrations in smokers were found to be higher than in non-smokers, while positive correlations between serum ceruloplasmin and C3 complement were also found. This suggests that the C3 complement might be affected by smoking. It has been reported that individuals with angiographically documented coronary artery disease are characterized by insulin resistance[37] and elevated serum C3, C4 and IgA levels.[38] In addition, components of the complement, such as C3 and members of the membrane attack complex, are found in atherosclerotic plaques, and parts of the atheromatous debris, such as cholesterol particles, are potent activators of the complement.[39]

ACKNOWLEDGEMENTS

The authors wish to express their sincere thanks to volunteers from Military Unit 13, Samsen Road, Bangkok, who participated in this project, and to all staff of the Department of Tropical Nutrition and Food Science, Faculty of Tropi-

cal Medicine, Mahidol University for their cooperation in this research. This work was partly supported by funds from the Institution of Tobacco Consumption Control, Ministry of Public Health, Thailand and the Free University, Berlin, Germany.

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