ORIGINAL ARTICLE

Assessment of α_1-antitrypsin and α_2-macroglobulin levels in obese patients

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KEY WORDS

α_1-antitrypsin, α_2-macroglobulin, metabolic syndrome, obesity

ABSTRACT

INTRODUCTION Epidemiologic data show a higher frequency of thromboembolic incidents in obese individuals compared with normal weight subjects. Pro-inflammatory factors seem to play an important role in their development. It has not been fully explained so far how α_1-antitrypsin (α_1ATp) and α_2-macroglobulin (α_2MG) act in obese subjects. Both proteins participate directly and indirectly in regulation of inflammation, coagulation and fibrinolysis. Thus alterations in serum levels of these protease inhibitors may play an important role in the development of vascular incidents in obesity.

OBJECTIVES To assess serum α_1ATp and α_2MG levels in obese patients.

PATIENTS AND METHODS The study involved 16 subjects with obesity and metabolic syndrome and 14 obese subjects with no disturbances of glucose and lipid profile or arterial hypertension. 20 healthy volunteers served as the control group. Levels of α_1ATp and α_2MG were determined in all subjects using immunonephelometry.

RESULTS No significant differences in α_1ATp and α_2MG levels between the patients and the control group were observed. Comparison of the tested parameters in the obese with metabolic syndrome and those without metabolic disturbances showed higher values of α_1ATp levels in the former group. In this group positive correlations between α_1ATp levels and fasting insulin levels were found.

CONCLUSIONS Metabolic disturbances in obesity are associated with an elevated level of α_1ATp, which might confirm its important role in the development of vascular incidents in obese patients. An increased risk of vascular pathological lesions in obesity is probably not associated with α_2MG levels.

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INTRODUCTION A markedly higher incidence of vascular thromboembolic episodes in the obese patients as compared to the normal weight subjects has been observed in a number of epidemiological studies. These incidents affect both venous and arterial systems and are the main causes of death in obese patients. Metabolic disturbances occurring in obesity seem to play a major role in the development of thromboembolic episodes. It is well known that hyperglycemia, insulin resistance with secondary hyperinsulinemia or arterial hypertension adversely affect hemostasis at several levels.[1-5] Numerous reports have confirmed an increase in coagulation factors (VIII, IX, XI, von Willebrand) and an increased plasminogen activator inhibitor type 1 activity in obese patients.[6-15] Growing attention is being paid to the inflammatory etiology of the development of vascular lesions. Recent reports have confirmed the contribution of pro-inflammatory factors to the development of atherosclerosis.[16-18] Available data provide numerous reports evaluating levels of acute-phase proteins, i.e. C-reactive protein (CRP), pro-inflammatory cytokines or fibrinogen in obese subjects.[19,20] There are several ambiguities concerning the action of endogenous inhibitors of proteases, which belong to acute-phase proteins, including α_1-antitrypsin (α_1ATp) or α_2-macroglobulin (α_2MG), in the development of vascular complications. A particular

TABLE 1 Characteristics of analyzed parameters in obese patients

Parameter	Obese patients with metabolic syndrome		Obese patients without metabolic syndrome	
	mean	SD	mean	SD
Age (years)	44.47	14.28	33.21	13.95
BMI (kg/m^2)	36.19	5.29	34.1	3.72
α_1-antitrypsin (g/l)	1.48	0.17	1.08	0.23
α_2-macroglobulin (g/l)	1.84	0.63	1.65	0.41
Total cholesterol (mmol/l)	5.95	1.33	5.2	0.9
Triglycerides (mmol/l)	1.68	0.68	1.39	0.38
LDL cholesterol (mmol/l)	3.89	1.25	3.4	0.9
HDL cholesterol (mmol/l)	1.28	0.37	1.46	0.55
Glucose (mmol/l)	6.25	1.72	5.3	0.9
Insulin (pmol/l)	147.49	64.05	132.52	55.5

Abbreviations: BMI – body mass index, HDL – high density lipoproteins, LDL – low density lipoproteins, NS – not significant, SD – standard deviation

TABLE 2 Characteristics of the analyzed parameters in obese patients and in the control group

Parameter	Obese patients		Control group		
	mean	SD	mean	SD	p
α_1-antitrypsin (g/l)	1.29	0.27	1.37	0.19	NS
α_2-macroglobulin (g/l)	1.78	0.52	1.71	0.32	NS

Abbreviations: see **TABLE 1**

TABLE 3 Characteristics of the analyzed parameters in obese patients with and without metabolic syndrome

Parameter	Obese patients with metabolic syndrome		Obese patients without metabolic syndrome		
	mean	SD	mean	SD	p
α_1-antitrypsin (g/l)	1.48	0.17	1.08	0.23	<0.01
α_2-macroglobulin (g/l)	1.84	0.63	1.65	0.41	NS

Abbreviations: see **TABLE 1**

role in these events plays α_1ATp, as, apart from its contribution to the inflammatory process, it also seems to affect hemostasis. It is well known that it indirectly increases the activity of coagulation factors, i.e. II, V, VIII, XII and XIII and inhibits activated protein C, which represents the endogenous anticoagulant system.[21,22] The impact of α_2MG on hemostasis activity is also significant. Similarly to α_1ATp, it inhibits activated protein C and affects the inactivation of prothrombin and plasminogen activators.[23]

Therefore, changes in the activity and levels of both serine protease inhibitors may indirectly influence the development of vascular complications obesity. Thus it seems that the assessment of the particular parameters in this group of patients is fully justified.

The aim of the present study was to determine serum α_1ATp and α_2MG levels in obese patients (body mass index [BMI] ≥30 kg/m^2).

PATIENTS AND METHODS 30 patients (20 women and 10 men, mean age 38.2 ±14.3 years) with obesity (BMI ≥30 kg/m^2) admitted to the Department of Endocrinology and Internal Medicine, Medical Academy, Gdańsk, were enrolled in the study, in order to perform hormonal evaluation. Patients with acute or chronic infection, cancer, systemic connective tissue disease, liver damage, women on contraceptives or on hormone replacement therapy and patients with a history of a thromboembolic episode over a period of 6 months were excluded from the study based on the thorough subjective and objective examination, and on the results of laboratory tests. There were 4 patients with hypercortisolemia, 3 patients with symptoms of hyperandrogenism and 1 patient with the post-steroidal adrenal failure in the analyzed group. 16 patients met the criteria for metabolic syndrome, whereas 14 patients did not show either disturbances in carbohydrate and lipid metabolism or arterial hypertension. Metabolic syndrome was diagnosed on the basis on the criteria issued by the International Diabetes Federation (IDF 2005), which include co-existence of central obesity (waist circumference in men ≥94 cm, in women ≥80 cm) with at least 2 metabolic abnormalities, i.e. high triglycerides levels (≥1.71 mmol/l [150 mg/dl]), decreased high-density lipoprotein levels in women (<1.3 mmol/l [50 mg/dl]) and in men (<1 mmol/l [40 mg/dl]), increased arterial blood pressure (≥130/85 mmHg) and the fasting glucose level (≥5.55 mmol/l [100 mg/dl]).

The control group consisted of 20 healthy volunteers (health care workers, members of their family) with a normal weight matched for their gender and age. A subjective and objective examination, including basic anthropometric evaluation, was performed in all participants of the study.

Blood for the laboratory tests was drawn from the basilic vein, following at least the 30-minute rest at supine position, in a fasting state, in the morning. The serum obtained by spinning down the blood samples was divided into 2 portions and stored in sterile plastic tubes at –70°C, and then transferred into room temperature just prior to the determination of the individual parameters.

Levels of α_1ATp and α_2MG were determined in all individuals. The tests were performed in the Central Clinical Laboratory of Academic Clinical Centre, Medical Academy, Gdańsk. The levels of α_1ATp and α_2MG were tested using immunonefelometry and the following reagents, N Anti-sera α_1 Antitrypsin and α_2 Macroglobulin (Dade-Behring, Germany), according to the manufacturer's instructions. Intra- and interassay variability were as follows: for α_1ATp 2.0, 3.2% and for α_2MG 1.6, 3.0%, respectively. Parameters of the coagulation system (activated partial thromboplastin time, international normalized ratio, fibrinogen level, anti-thrombin and protein C activity), lipid profile and carbohydrate metabolism parameters, i.e. glycemia and fasting

TABLE 4 Characteristics of the analyzed parameters in men and women

Parameter	Women					Men				
	Obese patients		Controls			Obese patients		Controls		
	mean	SD	mean	SD	p	mean	SD	mean	SD	p
α_1-antitrypsin (g/l)	1.25	0.29	1.40	0.20	NS	1.37	0.22	1.35	0.15	NS
α_2-macroglobulin (g/l)	1.85	0.56	1.71	0.30	NS	1.63	0.39	1.70	0.43	NS

Abbreviations: see TABLE 1

insulin levels, occasional glycemia, glucose tolerance test (in patients whose glucose levels were in the range of 100 to 125 mg/dl) were measured in obese patients using standard methods.

The Ethics Committee approved the study. Participants gave their written consent.

In the analysis of the variables the methods of statistical conclusion with the use of the STATISTICA computer program were applied. The mean values and standard deviations were calculated. The Saphiro-Wilk test was used to evaluate the distribution of variables. To compare the differences between the groups, the variables were further analyzed using the non-parametric Mann-Whitney U test and Spearman's test evaluating the correlations between the variables. A $p < 0.05$ was considered significant.

RESULTS The characteristics of the study group have been shown in TABLE 1.

The comparison of α_1ATp and α_2MG levels in obese and normal individuals did not show any statistically significant differences between both groups (TABLE 2). However, analysis of the measured parameters in subgroups, with regard to the metabolic disturbances present, showed significantly higher α_1ATp levels in patients fulfilling the criteria for metabolic syndrome as compared to the obese patients without concomitant metabolic disturbances. There was no such correlation for α_2MG. (TABLE 3).

The analysis of parameters showed no statistically significant differences in the groups of the same gender (TABLE 4). In the subgroups formed with regard to the metabolic disturbances within a group of the same gender statistical analysis was not performed as the number of patients was too small.

Next, the relationship between the parameters determined and the BMI and waist to hip ratio index was analyzed, and no significant correlation among the studied parameters was found.

The results obtained were further analyzed in relation to the co-existing metabolic disturbances. In patients with metabolic syndrome, a statistically significant correlation between the level of α_1ATp and the fasting level of insulin was observed. No statistically significant relationship was found between the levels of α_1ATp and α_2MG and lipid parameters.

DISCUSSION Available data provide numerous reports on the factors increasing the risk of vascular thromboembolic episodes in obese individuals. A body of evidence points to an increase in the activity of clotting factors, increased plasminogen activator inhibitors and an enhanced blood platelet aggregation in this group of patients.[6-15] In the recent years, more and more attention has been paid to the influence of pro-inflammatory cytokines on the hemostasis system and the development of vascular complications in obese patients.[19,20] There are few papers on obese individuals evaluating levels of α_1ATp and α_2MG, which are the endogenous serine protease inhibitors. In the present study no differences in tested parameters between the group of obese patients and controls were observed. However, the study demonstrated a significantly higher level of α_1ATp in patients with metabolic syndrome than in obese normotensive individuals without disturbances in metabolism of carbohydrates and lipids. Patients from the latter group, described in available data as "metabolically healthy but obese", present a normal metabolic profile despite the obesity.[24] The comparison of the results obtained during the current study with those provided by available data is difficult as most authors have evaluated in their studies a group of 5 proteins associated with the inflammation (inflammation-sensitive plasma proteins – ISPs), i.e. ceruloplasmin, α_1ATp, haptoglobin, fibrinogen and plasma acidic glycoprotein – orosomucoid, without taking into account the roles of particular proteins in their analyses. It has been consistently reported that increased levels of ISPs are linked to an increased risk of vascular complications.[25-29] More data on the action of particular protease inhibitors was provided by the study by Karelis et al., who found significantly higher levels of inflammation-sensitive plasma proteins, like, for instance, α_1ATp, in obese postmenopausal women with insulin resistance and dyslipidemia as compared to women without metabolic disturbances.[30] At the same time, Engstrom reported a negative correlation between the level of α_1ATp and BMI in men with classic cardiovascular risk factors.[25] In the current study there was no significant correlation between the α_1ATp level and BMI. However, the study showed a significant positive correlation between the α_1ATp level and the fasting level of insulin. Therefore, increased α_1ATp levels do not seem to depend directly on the degree of obesity, but on the co-existing metabolic disorders. These results are confirmed by Faraj et al.,

who showed a significant correlation between α_1ATp levels and the HOMA index in obese postmenopausal patients.[31] In turn, Cymerys et al. did not find significantly higher levels of this parameter in patients with arterial hypertension.[32] Over recent years, growing emphasis has been placed on the role of pro-inflammatory factors in atherogenesis in metabolic disorders. Several lines of evidence have demonstrated an increase in CRP, fibrinogen or pro-inflammatory cytokine levels in patients with hyperglycemia, insulin resistance or dyslipidemia.[33,34] It is important to stress that α_1ATp also belongs to a family of acute-phase proteins. Its increased levels seem to confirm a significant role of an inflammatory process in the development of vascular lesions in patients with metabolic syndrome. The effect of α_1ATp on hemostasis is also significant. It abolishes elastase action on particular parameters of coagulation and the fibrinolysis due to its inactivation, including a decrease in coagulation factors II, V, VIII, XII, XIII activity and pro-fibrinolytic effect.[21] α_1ATp directly inhibits CRP, an endogenous anticoagulant, and the levels of complexes of active protein C and α_1ATp may serve as a marker of high risk of thrombosis.[21,35] Therefore α_1ATp, either directly, by elastase inactivation, or indirectly, by stimulation of thrombin generation and inhibition of fibrinolysis, leads to enhanced blood clotting.

A definitely fewer number of papers relates to the profile of α_2MG levels in a group of obese patients. Relevant data are controversial. The authors of recent reports have obtained conflicting results. Bogdański et al. showed significantly higher α_2MG levels in patients with metabolic syndrome than in controls. They did not find any significant correlation between levels of the protein tested and BMI or the waist circumference.[36] On the other hand, Rugsarash et al. showed significantly lower α_2MG levels in obese Thais, without evaluation of concomitant metabolic disturbances.[37] The current study did not find significant differences in levels of the protein tested between the group of the obese patients and the controls, regardless of whether they were accompanied with metabolic disorders or not. However, it should be noted that obese patients are not a uniform group; they present with various co-existing disturbances of not only a metabolic nature but also of endocrine origin, which may affect the changes in α_2MG levels to a different degree and with varying dynamics. Elucidation of these issues obviously requires further studies on larger groups of patients.

The results of the current study do not answer all the questions, but they refer to a very important problem regarding the action of selected inhibitors of serine proteases in obese patients.

The findings of the studies performed and available data suggest that increased levels of α_1ATp are associated with the incidence of metabolic disturbances in obese subjects, which may indirectly indicate its contribution to the development of vascular complications in these patients. However, α_2MG is probably not responsible for the high risk of vascular lesions in obesity.

REFERENCES

1 Świątkowska-Stodulska R, Kazimierska E, Sworczak K, Częstochowska E. [Hemostatic disturbances in obesity]. Wiad Lek. 2007; 60: 185-188. Polish.

2 Hori Y, Gabazza EC, Yano Y, et al. Insulin resistance is associated with increased circulating level of thrombin-activatable fibrinolysis inhibitor in type 2 diabetic patients. J Clin Endocrinol Metab. 2002; 87: 660-665.

3 Kohler HP. Insulin resistance syndrome: interaction with coagulation and fibrinolysis. Swiss Med Wkly. 2002; 132: 241-252.

4 Anand SS, Yi Q, Gerstein H, et al. Relationship of metabolic syndrome and fibrinolytic dysfunction to cardiovascular disease. Circulation. 2003; 108: 420-425.

5 Alessi MC, Bastelica D, Morange P, et al. Plasminogen activator inhibitor 1, transforming growth factor-β_1 and BMI are closely associated in human adipose tissue during morbid obesity. Diabetes. 2000; 49: 1374-1380.

6 Avellone G, Di Garbo V, Cordova R, et al. Coagulation, fibrinolysis and haemorheology in premenopausal obese women with different body fat distribution. Thromb Res. 1994; 75: 223-231.

7 Balleisen L, Bailey J, Epping PH, et al. Epidemiological study on factor VII, factor VIII, and fibrinogen in an industrial population: I. Baseline data on the relation to age, gender, body-weight, smoking, alcohol, pill-using, and menopause. Thromb Haemost. 1985; 54: 475-479.

8 Bastard JP, Pieroni L. Plasma plasminogen activator inhibitor 1, insulin resistance and android obesity. Biomed Pharmacother. 1999; 53: 455-461.

9 Cigolini M, Targher G, Bergamo IA, et al. Visceral fat accumulation and its relation to plasma hemostatic factors in healthy men. Arterioscler Thromb Vasc Biol. 1996; 16: 368-374.

10 Conlan MG, Folsom AR, Finch A, et al. Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. Thromb Haemost. 1993; 70: 380-385.

11 De Pergola G, Pannacciulli N. Coagulation and fibrinolysis abnormalities in obesity. J Endocrinol Invest. 2002; 25: 899-904.

12 Folsom AR, Qamhieh HT, Wing RR, et al. Impact of weight loss on plasminogen activator inhibitor (PAI-1), factor VII, and other hemostatic factors in moderately overweight adults. Arterioscler Thromb. 1993;13:162-169.

13 Mutch NJ, Wilson HM, Booth NA. Plasminogen activator inhibitor-1 and haemostasis in obesity. Proc Nutr Soc. 2001; 60: 341-347.

14 Rosito GA, D'Agostino RB, Massaro J, et al. Association between obesity and a prothrombotic state: The Framingham Offspring Study. Thromb Haemost. 2004; 91: 683-689.

15 Bowles LK, Cooper JA, Howarth DJ, et al. Associations of haemostatic variables with body mass index: a community-based study. Blood Coagul Fibrinolysis. 2003;14: 569-573.

16 Mahmoudi M, Curzen N, Gallagher PJ. Atherogenesis: the role of inflammation and infection. Histopathology. 2007; 50: 535-546.

17 Lubas W, Gutkowski K. C-reactive protein in cardiovascular diseases. Przegl Lek. 2006; 63: 562-566.

18 Miłosz D, Czupryniak L, Saryusz-Wolska M, et al. Adiponectinemia, inflammatory process activity, and endothelial dysfunction in patients with type 2 diabetes and acute coronary syndrome with ST elevation in relation to the severity of lesions in the coronary arteries. Pol Arch Med Wewn. 2007; 117:343-349.

19 Bastard JP, Jardel C, Bruckert E, et al. Elevated levels of interleukin 6 are reduced in serum and adipose tissue of obese women after weight loss. J Clin Endocrinol Metab. 2000; 85: 3338-3342.

20 Dandona P, Weinstock R, Thusu K, et al. Tumor necrosis factor-α in sera of obese patients: fall with weight loss. J Clin Endocrionol Metab. 1998; 83: 2907-2910.

21 Brower MS, Harpel PC. α-1-antitrypsin-human leukocyte elastase complexes in blood: quantification by an enzyme-linked differential antibody immunosorbent assay and comparison with α-2-plasmin inhibitor-plasmin complexes. Blood. 1983; 61: 842-849.

22 Heeb MJ, Griffin JH. Physiologic inhibition of human activated protein C by α_1-antitrypsin. J Biol Chem. 1988; 263: 11613-11616.

23 Beheiri A, Langer C, During C, et al. Role of elevated α_2 –macroglobulin revisited: results of a case-control study in children with symptomatic thromboembolism. J Thromb Haemost. 2007; 5: 1179-1184.

24 Karelis AD, Brochu M, Rabasa-Lhoret R. Can we identify metabolically healthy but obese individuals (MHO)? Diabetes Metab. 2004; 30: 569-572.

25 Engström G, Stavenow L, Hedblad B, et al. Inflammation-sensitive plasma proteins and incidence of myocardial infarction in men with low cardiovascular risk. Arterioscler Thromb Vasc Biol. 2003; 23: 2247-2251.

26 Engström G, Hedblad B, Stavenow L, et al. Inflammation-sensitive plasma proteins are associated with future weight gain. Diabetes. 2003; 52: 2097-2101.

27 Engström G, Stavenow L, Hedblad B, et al. Inflammation-sensitive plasma proteins, diabetes, and mortality and incidence of myocardial infarction and stroke: a population-based study. Diabetes. 2003; 52: 442-447.

28 Engström G, Hedblad B, Stavenow L, et al. Fatality of future coronary events is related to inflammation-sensitive plasma proteins: a population-based prospective cohort study. Circulation. 2004; 110: 27-31.

29 Engström G, Hedblad B, Stavenow L, et al. Incidence of obesity-associated cardiovascular disease is related to inflammation-sensitive plasma proteins: a population-based cohort study. Arterioscler Thromb Vasc Biol. 2004; 24: 1498-1502.

30 Karelis AD, Faraj M, Bastard JP, et al. The metabolically healthy but obese individual presents a favorable inflammation profile. J Clin Endocrinol Metab. 2005; 90: 4145-4450.

31 Faraj M, Messier L, Bastard JP, et al. Apolipoprotein B: a predictor of inflammatory status in postmenopausal overweight and obese women. Diabetologia. 2006; 49: 1637-1646.

32 Cymerys M, Chyrek R, Bogdański P, et al. [Evaluation of acute-phase protein levels in obese patients with hypertension]. Pol Merk Lek. 2003; 88: 352-355. Polish.

33 Festa A, D'Agostino R Jr, Tracy RP, Haffner SM. Insulin Resistance Atherosclerosis Study. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes. 2002; 51: 1131-1137.

34 Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X Diabetologia. 1997; 40: 1286-1292.

35 España F, Gilabert J, Vicente V, et al. Activated protein C: α_1-antitrypsin (APC: α_1 AT) complex as a marker for in vitro diagnosis of prethrombotic states. Thromb Res. 1992; 66: 499-508.

36 Bogdański P, Chyrek R, Pupek-Musialik D, Jabłecka A. [Evaluation of selected acute phase proteins in patients with metabolic syndrome]. Pol Merk Lek. 2006; 21: 12-14. Polish.

37 Rugsarash W, Tungtrongchitr R, Petmitr S, et al. The genetic association between alpha-2-macroglobulin (A2M) gene deletion polymorphism and low serum A2M concentration in overweight/obese Thais Nutr Neurosci. 2006; 9: 93-98.