

Received: 2011.09.15
Accepted: 2011.12.12
Published: 2012.05.01

Influence of hypertension, obesity and nicotine abuse on quantitative and qualitative changes in acute-phase proteins in patients with essential hypertension

Authors' Contribution:

- A** Study Design
- B** Data Collection
- C** Statistical Analysis
- D** Data Interpretation
- E** Manuscript Preparation
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Source of support: Departmental sources

Summary

Background:

Hypertension is a powerful risk factor for cardiovascular disease and frequently occurs in conjunction with obesity. Accumulative evidence suggests a link between inflammation and hypertension. The aim of study was to evaluate whether blood pressure, obesity and smoking may influence acute-phase response.

Material/Methods:

Ninety-two patients with essential hypertension and 75 healthy volunteers as a control group were studied. In all subjects assessment of hsCRP, α 1-acid glycoprotein (AGP), α 1-antichymotrypsin, transferrin, α 1-antitrypsin, and C3 and C4 complement were performed. Evaluation of glycosylation profile and reactivity coefficient (RC) for AGP was done by means of affinity immunoelectrophoresis with concanavalin A as a ligand.

Results:

When compared to the controls, hypertensive subjects presented significantly higher hsCRP concentrations and lower transferrin level. Hypertensive patients had elevated AGP-AC. The intensification of the inflammatory reaction was greater in the subgroup of hypertensive patients smoking cigarettes. In obese hypertensives, elevated serum C3 complement level was found.

Conclusions:

We conclude that arterial hypertension may evoke the acute-phase response in humans. Markers of acute-phase response are particularly strongly expressed in smokers. Serum C3 complement, but not other APPs, is elevated in hypertension coexisting with obesity.

key words:

arterial hypertension • obesity • nicotine • acute-phase proteins

Full-text PDF:

<http://www.medscimonit.com/fulltxt.php?ICID=882740>

Word count:

2445

Tables:

5

Figures:

—

References:

38

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BACKGROUND

Arterial hypertension is considered to be a major risk factor for cardiovascular incidents. Pathological mechanisms generating these complications are closely connected with the increase of systemic vascular resistance as well as with structural and functional endothelial damage. Impairment of endothelium's functions leads in turn to initiation of atherogenic lesions, which – according to the most recent theories – are of inflammatory origin [1,2]. In the course of these processes numerous substances are released, mainly proteins, whose role is to restore the homeostatic balance. This is called the acute-phase response and expresses the body's non-specific reaction to tissue injury.

Acute-phase proteins (APPs) form a heterogeneous group of proteins, produced mainly in hepatocytes. Expression of genes of some APPs can also be observed in other tissues and organs (eg, phagocytes in peripheral blood, kidneys, spleen, adipose tissue, brain or lungs). The production and release of APPs in hepatocytes is regulated by cytokines, particularly interleukin 1 and 6 and TNF α [3,4]. Earlier studies have provided evidence for the action of inflammatory agents in the pathogenesis of hypertension and obesity [5–7]. Approximately 10% to 35% of the body's interleukin-6 (IL-6) is produced in adipocytes. Visceral adipose tissue evidently produces significantly more IL-6 than does subcutaneous adipose tissue. Adipose tissue may also release TNF α , which in turn promotes IL-6 secretion. TNF α and IL-6 may be the missing links connecting visceral obesity, the acute-phase response and atherosclerosis [8–10]. Obesity and arterial hypertension are both components of metabolic syndrome; moreover, adipose tissue is considered to be a potent hypertensive agent.

The concentration of most APPs increases in response to inflammatory stimuli. This group consists of, among others, C-reactive protein (CRP), serum amyloid A protein (SAA), alpha-1 acid glycoprotein (AGP), alpha-1 antichymotrypsin (ACT), fibrinogen, ceruloplasmin and C3 complement. Serum level of alpha-1 antitrypsin (AT), albumin, and transferrin decreases in the course of the inflammatory response, which is why these particles are classified as "negative" APPs.

Changes in APPs' structure are an interesting new issue. When compared to quantitative changes, they present quite a complex problem [11,12]. Some of them are glycoproteins with a specific type of carbohydrate residues forming antennary structures. Depending on the method of glycosylation and the number of carbohydrate terminal chains (2 to 4), biantennary, triantennary and tetra-antennary chains are discerned, respectively. To evaluate the glycosylation profile of APPs, column affinity chromatography or affinity immunoelectrophoresis with lectins is commonly used. Lectins are proteins specifically binding single mono- or oligosaccharides. Among all tested lectins, best results were obtained using concanavalin A (ConA). Depending on the intensity of reaction with ConA, 4 variants of glycosylation can be discerned. In case of glycosylated APP, the reactivity coefficient (RC) is calculated; it is the sum of the area enclosed by conA – reactive variants divided by the area enclosed by conA – non-reactive variants.

The objective of this study was to determine the influence of hypertension, obesity and nicotine abuse on quantitative

and qualitative changes in acute-phase response, and to evaluate lipid profile and fasting serum glucose in patients with essential hypertension.

MATERIAL AND METHODS

Participants

Previously untreated patients with newly diagnosed grade 1 or 2 essential hypertension (systolic BP \geq 140 and $<$ 180 mm Hg, and/or diastolic BP \geq 90 and $<$ 110 mm Hg) [13], and with no signs of clinical cardiovascular disease, were eligible for inclusion in the study at the outpatient clinic of the Department of Internal Medicine, Metabolic Disorders and Hypertension, University of Poznan. As a control, normotensive healthy volunteers (office BP $<$ 140/90 mmHg) matched for demographic characteristics were used (Table 1).

The exclusion criteria were as follows: secondary hypertension, diabetes, chronic diseases (eg, renal failure), pulmonary disease, liver dysfunction, arteriosclerotic obliterans, sleep apnea syndrome, symptomatic cerebrovascular disease, abnormal thyroid function, and current use of any medication, including dietary supplements. Based on the patients' history, physical examination and laboratory findings, acute and/or chronic inflammatory process was excluded. We ruled out an inflammatory pathology within the respiratory, digestive and genitourinary tracts, as well as in the oral cavity, pharynx and paranasal sinuses. In the month preceding the study, patients from the studied groups showed no signs of infection.

The study was Approved by the Human Subjects Oversight Committee, University of Medical Sciences, Poznan, no. 938/01, and all participants provided informed consent.

Anthropometric and blood pressure measurements

Anthropometric measurements of individuals, wearing light clothing and no shoes, were conducted. Weight was measured to the nearest 0.1 kg and height was measured to the nearest 0.1 cm. BMI was calculated as weight divided by height squared (kg/m^2). Obesity was defined as BMI \geq 30 kg/m^2 . Waist circumference (cm) was measured at the level of the iliac crest at the end of normal expiration. Waist circumference was measured to the nearest 0.5 cm.

Resting seated blood pressure was measured 3 times and an average value was calculated according to guidelines of European Society of Hypertension [13]. Regular or large adult cuffs were used, depending on a patient's arm circumference. Hypertension was defined by measurement of arterial blood pressure as the average of 3 measurements obtained after 10 min of physical resting by the patients (2 times at 2 different visits within 1 month).

Laboratory measurements

Blood samples were taken after an overnight fast and after 30 minutes in the supine position. Serum levels of lipids, including total cholesterol (TCH), high-density lipoprotein cholesterol (HDL-C), and triglyceride were assayed in the central laboratory of the Poznan University Hospital by routine enzymatic method. Low-density lipoprotein cholesterol (LDL-C) was calculated from Friedewald's formula.

Table 1. Anthropometric and clinical characteristics of the participants.

Parameter	Study group	Control group	P
N	92	75	
Sex (male/female)	53/39	44/31	NS
Age (years)	42.3±12.3	40.1±9.9	NS
Number of smokers	43	34	NS
BMI (kg/m ²)	28.0±3.9	26.8±4.2	NS
Waist circumference (cm)	92.4±11.1	88.8±12.3	NS

BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; NS – not significant.

Table 2. Fasting plasma glucose and lipids profile in the study group and the control.

Parameter	Study group	Control group	P
TCH (mmol/L)	5.3±0.6	5.1±0.7	NS
LDL (mmol/L)	3.5±1.0	2.7±0.9	p=0.007
HDL (mmol/L)	1.1±0.3	1.7±0.3	p=0.001
TG (mmol/L)	1.9±0.9	1.1±0.9	p=0.005
Glucose (mmol/L)	5.3±0.6	5.0±0.7	NS

TCH – total cholesterol concentration; LDL – LDL cholesterol concentration; HDL – HDL cholesterol concentration; TG – triglycerides; NS – not significant.

Levels of blood glucose were determined by routine enzymatic method.

The following APPs were determined: high-sensitivity C-reactive protein, α 1-acid glycoprotein (AGP), AGP conA-reactive variants (AGP-R), AGP conA-nonreactive variants (AGP-NR), α 1-antichymotrypsin (ACT), α 1-antitrypsin (AT), transferrin, C3 and C4 complement.

In order to establish serum APP levels, rocket immunoelectrophoresis according to Laurell was performed (DAKO, Denmark). High sensitivity C-reactive protein was assayed by particle-enhanced immunonephelometry with reagents from Dade-Behring. Qualitative assessment of APPs (glycosylation profile) was done by means of affinity immunoelectrophoresis (according to Bøg-Hansen) with ConA as a ligand (Sigma, USA). C3 and C4 complement were determined using radial immunodiffusion according to Mancini (DAKO, Denmark). In case of AGP and ACT, the reactivity coefficient was calculated as a sum of the area enclosed by ConA – reactive variants divided by the area enclosed by ConA – non-reactive variants. Evaluation of serum IL-6 was performed by means of immunoenzymatic assay (R&D System Europe, Ltd.).

Statistical analysis

Detailed statistical analysis was performed by means of *Statistica for Windows*. Normality of the variables' distribution was verified using the Shapiro-Wilks test of normality. CRP was logarithmically transformed for statistical testing to improve its skewness. Mean values, standard deviation

and median were calculated. Significance of differences for independent samples was estimated with use of the Mann-Whitney method. To evaluate relations between variables, Pearson's correlation index and Spearman R were used. The result of each statistical test was considered as reliable when the P-value was below 0.05.

RESULTS

Ninety-two patients with hypertension and 75 healthy volunteers of comparable age were analyzed. There were no differences in BMI, waist circumference and smoking status. Characteristics of the hypertensive and control groups are shown in Table 1. Total cholesterol and fasting glucose concentration did not differ between groups (Table 2).

LDL cholesterol and triglycerides revealed significant elevation in the study group compared to the control group. HDL cholesterol showed significant reduction in the studied group compared to the control group (Table 2). Comparing APP serum levels between the study group and the control group, statistically significant differences in 4 quantities were found (Table 3). In serum levels of α 1-antitrypsin and transferrin, a decrease in the group of hypertensive patients were found (p<0.05 and p<0.01, respectively). An increase was observed in hsCRP levels, (2.56±3.59 vs. 0.89±2.83 mg/l; p<0.01). Multivariable regression analysis showed that these correlations were independent of age, sex, smoking status, BMI, waist circumference, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides concentration and fasting glucose level. Moreover, the study group presented values of AGP-RC higher than in the control group (p<0.05);

Table 3. Concentrations of serum acute phase proteins in the study group and the control group.

Parameter	Study group n=92	Control group n=75	P
hsCRP (mg/l)	2.56±3.59	0.89±2.83	<0.01
AGP (mg/l)	776±204	758±258	NS
AGP-R (mg/l)	469±125	449±152	NS
AGP-NR (mg/l)	307±106	302±115	NS
AGP-RC	1.62±0.46	1.43±0.31	<0.05
ACT (mg/l)	317±73	303±81	NS
α1-antitrypsin (mg/l)	1876±475	2135±728	<0.05
Transferrin (mg/l)	2328±623	3034±1028	<0.01
C3 complement (mg/l)	1300±273	1256±318	NS
C4 complement (mg/l)	252±77	264±86	NS

hsCRP – C-reactive protein; AGP – α1-acid glycoprotein; AGP-R – conA-reactive variants; AGP-NR – conA-nonreactive variants; AGP-RC – reactivity coefficient of AGP; ACT – α1-antichymotrypsin.

Table 4. Concentrations of serum acute phase proteins in subgroups of obese and non-obese hypertensive patients.

Parameter	Obese subjects n=51	Non-obese subjects n=41	P
Age (years)	41.6±12.3	43.1±12.4	NS
Number of smokers	23	20	NS
BMI (kg/m ²)	31.1±0.9	24.3±2.7	<0.0001
Waist circumference (cm)	100.1±6.5	83.1±7.8	<0.0001
SBP (mmHg)	153.7±7.2	150.8±7.7	NS
DBP (mmHg)	93.0±7.2	90.8±7.4	NS
hsCRP (mg/l)	3.10±3.96	1.99±3.12	NS
AGP (mg/l)	757±194	801±217	NS
AGP-R (mg/l)	448±145	485±120	NS
AGP-NR (mg/l)	300±70	316±118	NS
AGP-RC	1.65±0.42	1.53±0.49	NS
ACT (mg/l)	320 ± 70	315±81	NS
α1-antitrypsin (mg/l)	1857±509	1923±417	NS
Transferrin (mg/l)	2339±623	2304±644	NS
C3 complement (mg/l)	1361±264	1053±261	<0.05
C4 complement (mg/l)	255±80	248±78	NS

BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; hsCRP – C-reactive protein; AGP – α1-acid glycoprotein; AGP-R – conA-reactive variants; AGP-NR – conA-nonreactive variants; AGP-RC – reactivity coefficient of AGP; ACT – α1-antichymotrypsin.

it was estimated as 1.62 and its increase was similar to that found in an acute inflammatory state. No associations between APPs and BMI, waist circumference or lipid parameters were found.

In comparing subgroups of obese and non-obese hypertensive individuals, only serum levels of C3 complement were significantly higher in obese individuals ($p<0.05$) (Table 4). When other parameters were taken into consideration, obese

Table 5. Concentrations of serum acute phase proteins in subgroups of smoking and non-smoking hypertensive patients.

Parameter	Smoking subjects n=43	Non-smoking subjects n=49	P
Age (years)	40.7±12.3	43.7±12.3	NS
BMI (kg/m ²)	28.1±3.9	28.0±4.0	NS
Waist circumference (cm)	92.8±10.8	92.0.1±11.4	NS
SBP (mmHg)	152.3±7.8	152.4±7.5	NS
DBP (mmHg)	90.7±8.3	93.2±6.2	NS
hsCRP (mg/l)	2.35±4.66	2.64±4.36	NS
AGP (mg/l)	808±145	676±222	<0.05
AGP-R (mg/l)	486±93	382±134	<0.05
AGP-NR (mg/l)	321±95	294±105	NS
AGP-RC	1.64±0.45	1.56±0.29	<0.05
ACT (mg/l)	328±81	303±73	NS
α1-antitrypsin (mg/l)	1992±476	1808±640	NS
Transferrin (mg/l)	2201±341	2386±722	NS
C3 complement (mg/l)	1388±188	1398±200	NS
C4 complement (mg/l)	262±55	280±87	NS

BMI – body mass index; SBP – systolic blood pressure; DBP – diastolic blood pressure; AGP – α1-acid glycoprotein; AGP-R – conA-reactive variants; AGP-NR – conA-nonreactive variants; AGP-RC – reactivity coefficient of AGP; ACT – α1-antichymotrypsin.

hypertensive individuals were found to have higher LDL cholesterol (3.7±0.9 mmol/L *vs.* 3.2±1.0 mol/L) and triglycerides concentration (2.4±1.4 mmol/L *vs.* 1.8±0.9 mmol/L).

Furthermore, smoking hypertensive subjects, when compared to non-smokers, presented raised levels of AGP ($p<0.05$) as well as ConA-reactive variants of this protein ($p<0.05$) (Table 5). AGP-RC value was also significantly higher ($p<0.05$) and above normal range in this group, amounting to 1.64. Smokers showed statistically significant elevated levels of total cholesterol (5.9±1.1 mmol/L *vs.* 5.3±1.1 mmol/L) and triglycerides (2.4±1.2 mmol/L *vs.* 1.8±1.3 mmol/L) when compared to non-smokers.

DISCUSSION

It has been widely known that damage to the endothelium and vascular wall contribute to the development of complications observed in arterial hypertension. This evokes acute-phase response and consequently causes generalized atherosclerotic lesions [14]. In this context, assessment of APPs in patients with hypertension yields new approaches to understanding its pathogenesis, natural history and complications. Statistically significant quantitative changes of APPs that we observed in hypertensive patients seem to confirm the hypothesis that inflammatory processes are associated with blood pressure level. We showed that, as with any other non-specific inflammatory process, this chronic pathological state evokes acute-phase response, thus leading to an increase of positive and decrease of negative APPs.

C-reactive protein is probably the best-known acute-phase protein. Many recent experimental and clinical studies have demonstrated that elevated CRP is a marker of increased risk of atherothrombotic clinical events [15–17] and it provides laboratory evidence of the role of low-grade inflammatory process in pathogenesis of atherosclerosis. According to the literature, levels of CRP above 2.1 mg/l in Apparently healthy subjects correspond with high relative risk of CAD [18]. In our study, mean level of hsCRP in hypertensive patients was 2.56±3.59 mg/l and was significantly higher compared to the healthy controls independent of age, sex, smoking status, BMI, waist circumference, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides concentration and fasting glucose level. Measurement of CRP seems to be of great importance in primary prevention of CAD. Its relevant prognostic value results from the fact that CRP measurement is independent of other cardiovascular risk factors [19,20]. Recent studies have suggested that suppression of IL-6 release may inhibit acute-phase reaction and CRP production, and therefore decrease the risk of CAD. Such action is attributed to i.a. pentoxifylline, α-linolenic acid and other polyunsaturated fatty acids (PUFA), estrogens, statins and moderate consumption of ethanol [21–23].

Along with APPs, quantitative changes in inflammatory processes, changes in their glycosylation profile, dependent on stimulus evoking acute-phase response, have also been observed. The reactivity coefficient of AGP is 1.28 in normal conditions (normal range 1.03–1.53), is decreased in chronic inflammations, and increased in acute inflammatory states

(eg, myocardial infarction, bacterial infections, and acute pancreatitis) [11]. In our study, serum concentration of AGP ConA-reacting variants suggests that hypertension is a state provoking acute inflammatory reaction. Therefore, it seems probable that despite its obviously chronic character, arterial hypertension leads to an acute inflammatory state, probably due to a leap deformation of the vascular wall during an abrupt increase of blood pressure.

A growing body of evidence shows that obesity is associated with oxidative stress and chronic, low grade inflammatory responses. Data from recent studies show that adipocytes may be functioning as endocrine cells – they not only serve as a regular fat storage, but also secrete a variety of bioactive substances known as adipocytokines (eg, interleukin 6 [IL-6] and tumor necrosis factor alpha [TNF- α]), which creates an inflammatory environment [24,25]. Furthermore, accumulating evidence suggests that obesity influences acute-phase protein (APP) production [26,27]. Present research showed that C3 complement was the only APP whose concentration increased in the subgroup of obese hypertensive compared to non-obese individuals. It is a recognized regulator of humoral immune response and B-cell proliferation and it is also strongly involved in the control of lipid and glucose metabolism. In particular, C3a-des-Arg – the acylation-stimulating protein – was found to be the most potent recognized stimulant of triglyceride synthesis in adipose tissue [28]. Moreover, serum C3 is one of the most powerful independent predictors of myocardial infarction [29]. Studies have shown its presence in the human arterial wall, along with immunoglobulins, complement components C1q, C9, fibrinogen, CRP and other acute-phase reactants [30,31]. Levels of C3 complement correlate positively with the degree of atherosclerotic lesions. An alternative hypothesis concerning pathogenesis of atherosclerosis has been proposed, stating that atherosclerosis is a consequence of C3 complement elevation in the human arterial wall [32]. In our study, higher levels of C3 complement demonstrated in obese hypertensive individuals might confirm higher relative risk of coronary events in these patients.

The mechanism linking smoking and atherogenesis is complex and not fully understood. Proposed potential pathways by which smoking increases the risk of cardiovascular pathology include: endothelial dysfunction, systemic coagulation disturbances, lipid abnormalities, and induction of low-grade systemic inflammation. We proved that smoking is equivalent to acute inflammatory reaction, which is reflected by elevated values of AGP-RC. When compared to other APPs, CRP levels rise rapidly and to the largest extent when stimulated by nicotine. Tracy et al. [33] described a positive correlation between CRP and pack-years of smoking; this association was independent of cessation, suggesting that some smoking-related damage might be irreversible. They have also reported that smoking itself affects relations between CRP and other CAD risk factors. A large prospective study with 18-year follow-up revealed that all measured APP levels (fibrinogen, α 1-antitrypsin, haptoglobin, α 1-acid glycoprotein, and ceruloplasmin) increased significantly with increasing cigarette consumption in healthy men, independent of other known cardiovascular risk factors [34]. It was shown that there is an association between carotid wall thickness evaluated by ultrasound scan and lifetime exposure to cigarette smoke [35]. A dose-response relationship

between total tar consumption per day and myocardial infarction has also been observed [36]. Cigarette smoking can also promote oxidative stress, which plays an important role in coronary heart disease [37]. The role of smoking in inducing and maintaining an acute inflammatory condition in the body has been well established [38].

CONCLUSIONS

Arterial hypertension may evoke the acute-phase response in the human body. Quantitative and qualitative changes in acute-phase proteins that we observe in hypertension suggest that hypertension itself is an acute inflammatory condition. Markers of acute-phase response are particularly strongly expressed in smokers. Serum C3 complement, but not other APPs, is elevated in hypertension coexisting with obesity.

Conflict of interest

The authors indicate no potential conflicts of interest.

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