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Research Article | American Journal of Cardiovascular and Thoracic Surgery | Open Access

Inflammation and reactive oxygen species generation at the peripheral and coronary atherosclerosis, comparative evaluation of its intensity and grade

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Received: October 06, 2016; Accepted: October 14, 2016; Published: October 24, 2016

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Abstract

The inflammation and oxidative stress are a basis of pathogenesis of an atherosclerosis and vulnerability of the atherosclerotic plaque, triggered by risk factors, including the expression of adhesion molecules, the proliferation and migration of smooth muscle cells, the apoptosis of endothelial cells, the oxidation of lipids, the activation of metalloproteinase and the alteration of vasomotor activity. Heterogeneity of initiation of the system inflammatory answer at an atherosclerosis causes necessity of studying factors of an inflammation and its correlation with traditional metabolic disorders at an atherosclerosis of various localizations.

Materials and methods: C-reactive Protein (CRP), interleykin-6 (IL-6), tumor necrosis factor alpha (TNF-alpha), fibrinogen, general blood count, markers of Oxidative S tress (OS) – Malondialdehyde (MDA) and catalyze activity, marker of endothelial proliferation and migration – Vascular Endothelial Growth Factor (VEGF) were analyzed at 30 patients with a peripheral (in iliac-femoral arterial pool) atherosclerosis (PA) and at 43 patients with Coronary Atherosclerosis (CA). CRP and routine biochemical tests (lipidomic panel, total protein, albumin, glucose, uric acid) were made in automatic biochemical analyzer "VITROS-350" (Germany). IL-6, TNF-alpha, VEGFs levels were measured in the blood serum using enzyme-linked immunoassay method in analyzer ST-360, (China). General blood count was made by automatic hematological analyzer BC 5800 (Mindray, China), immature granulocytes were identified in blood smears by manual examination.

Results: It is established, that all patients with atherosclerosis had high level of CRP, IL-6, FNO-alpha and MDA in comparison with control group. There was increasing of WBC, neutrophils and the left shift at the PA patients. High CRP level was accompanied with increasing of immature granulocytes up to 6,9±0,4%, increasing of fibrinogen on 16%, decreasing of albumin on 25% concerning group of the control; high number of WBC correlated with CRP ($r=0,62$, $p<0,05$) at PA patients. In contrast, at CA patients increasing of CRP was associated with metabolic disorders such as increasing of glucose concentration up to 6,4 ± 0,5 mmol/L and body mass index (BMI) = 30,3±1,6 kg/m2. Proinflammatory cytokine IL-6 level was increased both at PA and CA patient in 12, 2 and 9,1 times in comparison with the control respectively. There was no significant difference in FNO-alpha concentration between PA and CA patients ($p > 0,05$), but FNO-a level was increased in 1,6 and 2,3 times respectively at PA at CA patients. There were correlative links between CRP and glucose ($r=0,9$, $p < 0,05$), CRP and triglycerides ($r=0,8$, $p < 0,05$), CRP and MDA ($r=0,6$, $p < 0,05$) at CA patients. Comparative analyses of PA and CA patients demonstrate that VEGF and IL-6 levels were increased at PA patients relatively to the CA patients in 2,2 and 2,7 times respectively. So, PA characterizes by increasing of pro-inflammatory cytokines IL-6 and high level of VEGF, which can stimulated collateral blood stream and endothelial proliferation. CA characterizes by low grade inflammation. This data suggests that inflammation at PA is acute, due to local inflammatory reaction of peripheral tissues after chronic ischemia. At CA inflammation is low grade, closely connected with metabolic disorders and Reactive Oxygen Species (ROS) generation.

Conclusion: Studying of factors of an inflammation and ROS generation at a peripheral and coronary atherosclerosis has allowed revealing characteristic distinctions. Prevalence of acute inflammation with WBS, LSI, CRP, fibrinogen increasing and partly compensated oxidative stress accompanied with VEGF increasing observed at PA patients. CA is associated with decompensate OS and low grade inflammatory reaction, correlating with metabolic disorders such as increasing of body mass index and glucose concentration.

Introduction

The inflammation is a pathogenesis basis of an atherosclerosis from the beginning up to a final stage – vulnerable atherosclerotic plaque formation [2, 23, 24]. The data of recent researches have proved an essential role of CRP, interleukins (IL)-6,-8,-1,-10, 12, tumor necrosis factor alpha (TNF-a) in progressing an atherosclerosis, in an estimation of risk of sudden death, development of acute coronary events and its complications [7,12,29]. In research GISSI among 11 324 patients with a acute heart attack of a myocardium and the contents of WBC less than 6000 mortality during 4 years has made 6,9 %, and at the

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contents of leukocytes more than 9000 – mortality has made 17,7 %. Thus prognostic value and the importance of WBC number did not depend on expressiveness of other factors of atherogenesis [15,19,22]. There is now considerable biochemical, physiological and pharmacological data to support a connection between free radical reactions and cardiovascular tissue injury that share common mechanisms of molecular and cellular damage [17]. Overproduction of reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide, and the hydroxyl radical leading to lipid peroxidation, denaturation of proteins or enzymes or mutagenic damage to nucleic acid and caused oxidative stress [11,14,17, 20]. As these mechanisms are elucidated, it can be possible to improve the techniques for clinical and pharmacological intervention. Ways of liquidation of inflammatory process and oxidative stress at an atherosclerosis have not found yet, probably, since of heterogeneity of trigger factors of the systemic inflammatory response and ROS generation. In this view, research of factors of an inflammation and oxidative stress and its correlation with traditional metabolic disorders is in the area of interest at an atherosclerosis of various localization, as was the purpose of this work.

Materials and methods

It is surveyed 73 patients with an atherosclerosis, middle age 60,1 ± 1,9; 16 women (22%) and 57 men (78%). There were 30 patients with an atherosclerosis in the iliac-femoral arterial pool - the Peripheral Atherosclerosis (PA), accompanying with chronic limb ischemia and 43 patients with coronary atherosclerosis (CA). The diagnosis established on the basis of clinical, ultrasound and Doppler data, multi scanning computer tomography, percutaneous coronary angiography. As a control group, we accessed 15 volunteers of same age (11 men, 3 women) without atherosclerosis. Laboratory tests included C-reactive protein (CRP) and routine biochemical tests (lipidomic panel, total protein, albumin, glucose, uric acid), which were made in automatic biochemical analyzer "VITROS-350" (Germany). Interleykin-6 (IL-6), tumor necrosis factor alpha (TNF-a), marker of endothelial proliferation and migration – vascular endothelial growth factor (VEGF) were measured in the blood serum using commercially available ELISA kits (VECTOR-BEST, Russia) in immunoassay analyzer ST-360, (China). General blood count was made by automatic hematological analyzer BC 5800 (Mindray, China), qualitative white cell-associated signs; such as immature granulocytes and left shift were identified by manual differential leukocyte count in blood smears. White blood cells (WBC), neutrophils (Neu), immature granulocytes (INeu), monocytes (Mon), Bazophils (Baz), Eozinophils (Eoz), lymphocytes (Lymph) were identified. Left Shift Index (LSI) was calculated as immature granulocytes and blasts to mature neutrophils ratio. Studies support a decision threshold of 0,069 for LSI.

Marker of oxidizing stress – malondialdehyde (MDA) was analyzed according to procedure of Ohkawa on reaction with thiobarbituric acid in Al-Gayyar's modifications [1, 21]. In brief, serum proteins are precipitated by the addition of trichloroacetic acid. Then, thiobarbituric acid reacts with MDA to form thiobarbituric acid-reactive substance that is measured at 532 nm. Catalase activity was determined by the speed of hydrogen peroxide degradation in the semi-automatic analyzer "Screen Master Plus" (Hospitex Diagnostics, Italy). The results are presented as the M ± m, independent student t-test was applied to find out the statistical significant difference ($p < 0,05$) between the groups, Pearson's correlation was used to figure out the correlation among the circulating biomarkers.

Results and discussion

The blood count and blood smear examination results in the patients with PA and CA were different. There was increasing of WBC, Neu and the left shift at the PA patients (Table 1).

There was no statistically significant difference between control group and CA patients in the all of blood count and blood smear parameters exclude WBC and LSI. WBC was increased in 1,4 times in compare with control at CA patients. Amount of Mon at PA patients was in reference value [5], but it was higher, than in control in 1,9 times. Our study supports a decision threshold of 0,069 and the reference value 0,050 - 0,070 for LSI. LSI characterizes the intensity of inflammation, the intensity of inflammation is middle if LSI is 0,080 – 0,099 and is high if LSI is more 0,100 [16]. Our data shows, that LSI was increased in 1,6 and 1,4 times concerning the control at PA and CA patients respectively. This data shows that intensity of inflammation, according to LSI, is middle at the CA patients and is high at the PA patients.

High LSI and WBC amount was accompanied with increasing of CRP in 2,5 times, fibrinogen on 16%, decreasing of albumin on 25% concerning the control at PA patients. At CA patients CRP was higher, then control in 2,3 times, and it was associated with metabolic disorders such as increasing of glucose concentration up to 6,4±0,5 mmol/L and Body Mass Index (BMI) up to 30,3 ± 1,6 kg/m^2 (Table 2).

Changes of plasma lipidomic profile were similar at the

Table1: The blood count and blood smear parameters at patients with peripheral and coronary atherosclerosis

Groups of patients	WBC, 10^9/L	Neu, %	INeu, %	Mon %	Baz, %	Eoz, %	Limph %	LSI
The control, n=15	4,9±0,4	56,7± 3,7	3,9± 0,4	3,5± 0,3	0,40± 0,01	1,6± 0,4	30,7± 0,6	0,068± 0,003
1 group - PA, n=30	8,2±0,4*	62,3±1,9*	6,9± 0,4*	6,7± 0,4*	0,50± 0,10	3,4± 0,7*	28,2± 2,0	0,112 ± 0,007*
2 group - CA, n=43	6,9±0,5 *, **	54,2± 2,9 **	5,3± 0,6 **	2,9± 0,6 **	0,52± 0,08	2,2± 0,3	32,2± 3,1	0,094± 0,005 *, **

** - significant difference at p < 0,05 concerning the control; ** - significant difference at p < 0,05 concerning 1 group.*

Table 2: Metabolic parameters, pro-inflammatory cytokines and markers of oxidative stress at patients with a peripheral and coronary atherosclerosis

Groups of patients	The control, n=15, P1	1 group - PA, n=30, P2	2 group -CA, n=43, P3	P 1:2	P 1:3	P 2:3
FNO-a, pg/ml	4,3±1,2	7,1±0,8	12,5±1,5	*p<0,05*	*p<0,05*	*p<0,05*
IL-6, pg/ml	2,9±0,3	35,5±13,1	26,5±5,6	*p<0,05*	*p<0,05*	***p>0,05***
CRP, mg /L	4,1±0,3	10,3±0,5	9,5±1,5	*p<0,05*	*p<0,05*	***p>0,05***
Fibrinogen, mg/L	3294±252	3829±199	3330±107	*p<0,05*	***p>0,05***	*p<0,05*
Albumin, g/L	44,2±1,2	35,5±0,7	42,2±1,5	*p<0,05*	***p>0,05***	*p<0,05*
MDA, nmol/mg protein*h	4,7±0,25	7,1±0,1	9,8±0,3	*p<0,05*	*p<0,05*	*p<0,05*
Catalase, U/L	19,2±1,8	23,0±1,3	29,5±2,1	*p<0,05*	*p<0,05*	*p<0,05*
Uric acid, mkmol/L	230±19	321±11	330±28	*p<0,05*	*p<0,05*	***p>0,05***
BMI, kg/m2	24,0±0,9	24,3±0,6	30,3±1,6	***p>0,05***	*p<0,05*	*p<0,05*
Glucose, mmol/L	4,2±0,4	4,8±0,4	6,4±0,5	***p>0,05***	*p<0,05*	*p<0,05*
Triglycerides, mmol/L	0,91±0,11	1,99±0,16	1,63±0,12	*p<0,05*	*p<0,05*	*p<0,05*
HDL, mmol/L	1,34±0,11	0,95±0,04	1,01±0,03	*p<0,05*	*p<0,05*	***p>0,05***
VEGF, pg/ml	112±15	199±23	89±11	*p<0,05*	***p>0,05***	*p<0,05*

PA and CA patients and included increasing of the triglycerides (TG) with decreasing of High Density Lipoproteins (HDL), but at PA patients TG concentration was significantly higher, than at CA patients ($p < 0,05$); glucose level was 1,3 time higher at CA patients in contrast to the PA patients ($p < 0,05$).

There were no significant difference in CRP and FNO-a levels between PA and CA groups ($p > 0,05$), but CRP was increased in contrast with control in 2,5 at PA and in 2,3 times at CA patients; FNO-a concentration was increased in 1,6 and 2,3 times respectively. Fibrinogen concentration was statistically significant increased at PA patients, while at CA patients it does not differ from the control. The high level of fibrinogen at PA patients specifies not only activation of an inflammation, but also predisposition for thrombosis and microcirculation disturbances [4, 13]. It has established, that a change of coagulation at the patients with PA includes a high level of fibrinogen with increasing of thrombin and fibrinolytical activities. All of this exhausts reserve capacity of the coagulation system and can lead thrombosis [2,3,13].

It is known that IL-6- is mediator of the cell damages, produced by monocytes, macrophages, endotheliocytes, its high level triggers syntheses of fibrinogen, CRP, haptoglobin, amiloid A and inhibits the FNO-alpha production. Also IL-6 can induce increasing of the glucose concentrations due to hypothalamic - pituitary stimulation [2, 12]. We assume, that high concentrations of CRP and fibrinogen at PA patients are linked with exactly increasing of IL-6, which triggered synthesis of those proinflammatory proteins in the liver. High level of IL-6 occurs together with high FNO-alpha concentration, hyperglycemia and BMI increasing at the CA patients. This allows to expect the contribution of visceral adipose tissue in IL-6 and FNO-a production. There is indeed evidence that obesity is associated with macrophage accumulation in adipose tissue and it is directly linked with inflammatory response. Obesity associated TNF- a is primarily secreted from macrophages, accumulated in adipose tissue, whereas the adipocytes, predominantly produce unsecreted, membrane-bound TNF-a [28]. Obesity leads insulin resistance too [24].

For searching of the typical particularities of metabolic disorders and inflammation at an atherosclerosis of various localization correlative analyses has done. There was established, that at CA patients correlative link between CRP and some of the components of metabolic syndrome is strong (CRP/Glucose, CRP/LDL, CRP/TG, CRP/BMI), correlative link between CRP and MDA is middle (Figure 1).

This data shows that at CA inflammation is closely connected with metabolic disorders and oxidative stress.

Markers of oxidative stress (OS) –MDA and catalase were changed greater at CA patients. There were high MDA level (increased in 2,1 times compare with control) with increasing of catalase activity in the blood on 52% ($p < 0,05$) at CA patients. At PA patients MDA level was increased in 1,5 time, catalase activity was comparable to the control, that specifies on compensated OS. Probably, the chronic ischemia / hypoxia of heart is accompanied by activation of generation of reactive oxygen species and accumulation of MDA in blood greater, rather than an ischemia of peripheral muscles at PA [14]. It may be caused by specific properties and differs of bloodstream and metabolism intensity, different value of drainage function of micro vascular system and activity of endogenous antioxidative capacity of the heart and skeletal muscles.

Concentration of the uric acid (UA) was increased concerning to the control at 1,4 times both at PA and CA patient, but it was in the reference value 360 mkmol/L, determined by EULAR [16].

Increasing of the UA level may be explained controversial. Several mechanisms have been postulated for explaining perceived endothelial abnormalities induced by UA. Incubation of vascular smooth muscle cells with UA has been found to

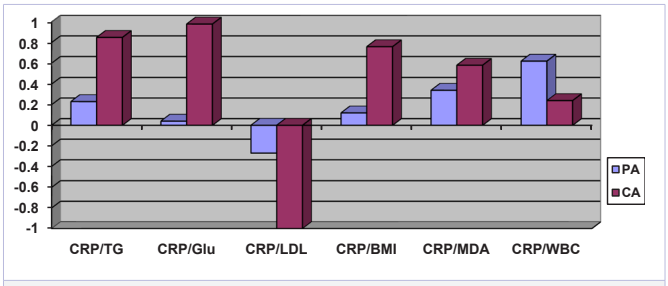

Figure 1: Coefficient of correlation r, p < 0,05 between factors of an inflammation and components of metabolic syndrome at patients with a peripheral and coronary atherosclerosis.

stimulate proliferation, angiotensin II production, and oxidative stress; human aortic smooth muscle cells exposed to different concentrations of UA experienced dose-dependent cell proliferation and phosphorylation-dependent endothelin-1 expression, along with an increased activity of NADPH-oxidase - mechanism of production of ROS. Interestingly, those effects were reversible after treatment with antioxidants, such as N-acetylcysteine [10]. On the other hand, UA has the highest concentration of any blood antioxidant and provides over half of the total antioxidant capacity of human serum at levels as high as 285 μmol/L; it does act against peroxynitrite, peroxides, and hypochlorous acid [9,26]. The effects of UA in atherosclerosis are still not well understood, with some studies linking higher levels of UA with increased mortality [27]. In the best way this contradiction has characterized by Proctor P.H.: "the well-established association between high urate levels and atherosclerosis could be a protective reaction (antioxidant) or a primary cause (pro-oxidant)" [25], so it might be due to uric acid being activated as a defense mechanism against oxidative stress, but instead acting as a pro-oxidant in cases where metabolic derangements shift its production well outside of normal levels. Our data show, that at PA patients UA level is higher and oxidative stress is less expressed in contrast with CA patients, probably due antioxidant properties of UA. We assume that extracellular antioxidant capacity and total antioxidant system activity is compensated at PA patients (catalase activity is normal) and strongly mobilized at the heart ischemia at CA patients. Oxidative stress is developed in atherosclerosis due to disturbance in the pro-oxidant / antioxidant balance and impairment of antioxidant mechanisms in the ischemic tissue [13,20]. The main sources of oxidative substances and ROS in atherosclerotic vessels are macrophages and smooth muscle cell. ROS production, in turn, induces endothelial dysfunction [28]. So, ROS generation, endothelial dysfunction and inflammation are closely connected at atherosclerosis.

Our data confirm that ischemia of peripheral muscles at acute inflammation at PA patients leads endothelial proliferation and collateral bloodstream, which are more intensive in contrast with CA patients due to VEGF concentration increasing. VEGF level was increased at PA patients at 2,2 times concerning the CA patients. This data can be used in therapeutic angiogenesis conception development, because high level of VEGF is associated with stimulation of collateral bloodstream and endothelial proliferation [4,6,8]. On different models has shown that administration of vascular endothelial growth factor induced dose-dependent collateral artery augmentation of persistent ischemia [18,30].

As have shown our researches, the peripheral atherosclerosis is closely accompanied by inflammatory reaction, and a coronary atherosclerosis is closely connected with metabolic disorders and oxidative stress. The certain contribution to it development can bring co morbidity, in particular presence of metabolic syndrome [24]. This data suggests that inflammation at PA is acute, due to local inflammatory reaction of peripheral tissues after chronic ischemia. At CA inflammation is low grade, closely connected with metabolic disorders and ROS generation.

Conclusion

Thus, studying of factors of an inflammation and ROS generation at a peripheral and coronary atherosclerosis has allowed revealing characteristic distinctions. Prevalence of acute inflammation with WBS, LSI, CRP, fibrinogen increasing and partly compensated oxidative stress accompanied with VEGF increasing observed at PA patients. CA is associated with decompensate OS and low grade inflammatory reaction, correlating with metabolic disorders such as increasing of body mass index and glucose concentration.

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