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CHARACTERISTICS OF ENDOTHELIAL DYSFUNCTION IN PATIENTS WITH GOUT COMORBID WITH ARTERIAL HYPERTENSION

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Abstract

Currently there is scarce data on endothelial dysfunction (ED) in patients with gout (GA), moreover there are no current studies of ED in gout comorbid with arterial hypertension (AH). The purpose of this study is to describe specific features of biochemical and instrumental markers of endothelial dysfunction in patients with GA comorbid with AH. We measured and compared the level of Von Willebrand factor (vWF), interleukin-1beta (IL-1), endothelin-1 (ET-1), plasma nitrites (NO_2^-) and nitrates (NO_3^-), the total activity of NO-synthase, endothelium-dependent (FMD) and endothelium-independent vasodilatation (NMD) in 26 patients with GA, 26 patients with AH and in 86 patients with GA+AH. The study showed that vWF concentration was highest in comorbidity group (92,9±29,0%) showing no significant difference from GA and AH (79,2% and 86,5% respectively). IL-1 was highest in GA+AH group (2,0 pg/ml) being significantly higher than in AH (p=0,01) but showing no difference from GA (p=1,0). ET-1 concentration was highest in the comorbid pathology group (3,07 fmol/ml) versus 1,58 fmol/ml in GA (p<0,0001) and 2,77 fmol/ml in AH (p=1,0). NO-synthase activity was greatest in GA and in comorbid pathology groups showing no significant intergroup difference; NO_2^- and NO_3^- concentrations, being similar in these two groups, were statistically higher that in AH. Greatest reduction of FMD and NMD was found in comorbid pathology group (5,80% and 10,35% respectively) and it was not significantly different from AH group (6,18%; p=0,83 for FMD and 13,59%; p=0,079 for NMD). FMD and NMD in gout (10,03% and 15,35% respectively) were similar to normal, being significantly different compared to GA+AH group (p=0,045 for FMD and p=0,018 for

NMD). This study shows that ED in gout is characterized by significantly higher concentration of IL-1, nitric oxide metabolites and intact FMD compared to hypertension. Hypertension is characterized by significantly higher concentrations of endothelin-1, more prominent increase in von Willebrand factor and significant FMD reduction. Endothelial dysfunction in comorbid pathology demonstrates "summation" of pathogenetic mechanisms of underlying disorders, expressed in significant increase of interleukin-1 and endothelin-1 concentrations, hyperactivation of NO synthesis together with decreased FMD and NMD.

Keywords: hypertension, gout, endothelium, comorbidity.

Introduction

Currently diseases are considered comorbid when they occur in the same patient and have pathogenesis connecting them with each other, which makes such a combination of diseases occur more frequently than their possible statistically random coincidence [2, 8].

Gout (GA), which is one of the most common rheumatic diseases, has become an actual problem in recent decades, but there are only a few works have studied the pathology of comorbidity with gout. Thus, in a retrospective study K. Joo et al. [9] found that in the Korean population the most common comorbid with gout conditions were hypertension (AH), type 2 diabetes mellitus, coronary artery disease, urolithiasis and chronic kidney disease.

The same argument has been confirmed in the Russian scientists, led by E.I. Markelova who showed that in Russian population the most frequent comorbid disorders in gout were hypertension (76%), nephrolithiasis (71%), obesity (63%), ischemic heart disease (23%), type 2 diabetes (17%) [13]. A retrospective study of the frequency of gout comorbidities in he UK and Germany gave slightly different results. So, the most frequently encountered were obesity (27,7%), hypertension (17,5%) and diabetes (8,3%) in the UK, and in Germany – type 2 diabetes mellitus (25,9%), hypertension (18,5 %) and obesity (13,3%) [1]. However, the general conclusion for both countries was the presence of hypertension in each 5-6th patient with gout.

Presented data shows that hypertension is one of the most frequent comorbid diseases with gout, which makes this combination of diseases clinically important and requires

identification of specific pathogenesis features, in particular, endothelial function, which is not investigated enough.

Interest in endothelial dysfunction (ED) in recent years increases due to the key role of this phenomenon in the development of vascular damage of various genesis. The major amount of studies traditionally is devoted to stable and unstable forms of coronary artery disease, where ED was found to appear as the preclinical manifestation of the disease and as secondary changes due to exposure to the major risk factors - smoking, hyperlipidemia, hyperglycemia, oxidative stress, etc.

Research on ED in hypertension includes large number of papers, studying various endothelial functions. For example, a 5-year study of 3500 patients with hypertension found a significant correlation between reduction of flow-mediated dilation (FMD) and the presence of hypertension, as well as with its severity. Interestingly, when evaluating FMD as a predictor of hypertension, no significant association was identified. Consequently, FMD is considered to be a marker, but not a predictor of hypertension [17]. Some authors have reported a decrease in concentrations of nitrate and nitrite in hypertension compared to the control group [16], which, in their opinion, may be due to a decrease in eNOS activity or accelerated destruction of NO and its free radicals capture. In contrast, a number of studies in patients with uncomplicated hypertension, as well as in the presence of left ventricular dilatation observed increase in the concentration of nitrates and nitrites [12], accompanied by a reduction in their functional efficiency, which was confirmed by a decrease in the level of the neurotransmitter cGMP. The authors attribute this phenomenon to the induced overproduction of NO-synthase, arising due to the impact of cytokines and oxidative stress factors. It is important to note that the increase in the level of NO leads to inhibition of the eNOS and promotes pro-inflammatory and toxic effects which further deepen endothelial dysfunction [11]. There is no consensus on the role of other ED marker, von Willebrand factor (vWF), in hypertension. For example, some studies show its increased concentrations in patients with essential hypertension relative to the control group (114 ± 20 IU/dl vs. 90 ± 47 IU/dl) [15], although there are studies that do not detect such changes in 1st degree hypertension [3].

Endothelial dysfunction in patients with rheumatologic disorders is even less studied: predominantly research is done on rheumatoid arthritis, lupus, ankylosing spondylitis and only single works are devoted to gouty arthritis. Rheumatological disorders were also found to have changes in markers of endothelial function, but the nature of the changes differed from those of cardiovascular disease. Most often, researchers have studied cytokine markers

of endothelial function (vWF interleukin-1, endothelin-1), NO-producing function (NO-synthase, NO_2^-, NO_3^-), vasomotor function (FMD, NMD), however, the data on changes of these parameters in patients with gouty arthritis presented by single works. It was found that in gout patients increased level of uric acid, which is identified in the nucleus of the atherosclerotic plaques [14] indicates the hyperactivation of xanthine oxidase, involved in the formation of peroxide oxygen compounds. The study by Cronstein B.N. supposed that in patients with gout, a key role in the chain of ED is given to interleukin-1which in turn causes the expression of adhesion molecules and selectins leading to chemotaxis of neutrophils into the inflammatory focus. [5] Information about changes in other markers of endothelial dysfunction in patients with gout is not found in the literature. Also, there are no in-depth study on ED in patients with a combination of gout and hypertension.

The aim of the study is to reveal features of biochemical and instrumental markers of endothelial dysfunction in patients with gout comorbid with arterial hypertension.

Materials and methods

The study included 138 patients with gout, hypertension and their combination, divided into the following groups: I group - 26 patients with gouty arthritis of varying severity (without hypertension), II group - 26 patients with hypertension of different severity (without gout), group III - 86 patients with gout and hypertension. Control group consisted of 10 healthy individuals. Clinical characteristics of studied groups is presented in Table 1.

Table 1

Clinical characteristics of studied groups

Item	**Group I (GA, n=26)**	**Group II (AH, n=26)**	**Group III (GA+AH, n=86)**
Age, y	60,8±12,2	58,3±12,0	59,9±12,8
Sex, m/f	25 (96,2%) / 1 (3,8%)	24 (92,3%) / 2 (7,7%)	84 (97,7%) / 2 (2,3%)
Body weight, kg	92,8 [84,0 – 96,0]	90,0 [82,0 – 96,0]	95,1 [84,0 – 103,0]
Hypertension duration, y		9,0 [5,0 – 11,0]	9,2 [4,0 – 12,0]
Gout duration, y	7,0 [3,0 – 10,0]		7,2 [4,0 – 9,0]
Hypertension grade:			
1		6 (23,1%)	22 (25,6%)
2		15 (57,7%)	40 (46,5%)
3		5 (19,2%)	24 (27,9%)
Gout severity			
Mild	3 (11,5%)		16 (18,6%)
Moderate	14 (53,9%)		40 (46,5%)
Severe	9 (34,6%)		30 (34,9%)
Diabetes mellitus type 2	4 (15,4%)	5 (19,2%)	15 (17,4%)
Coronary artery disease	8 (30,8%)	9 (34,6%)	28 (32,6%)

All the figures except for age, had a normal distribution and showed no statistically significant difference ($p > 0,05$).

Von Willebrand factor was determined using biochemical kit "vWF: Ag ELISA" (Technoclone, Austria); the level of interleukin-1beta - with the set "Interleukin-1beta-IFA-BEST" (Vector-Best, Russian Federation), and endothelin-1 was determined using biochemical kit "Endothelin 1-21" (Biomedica, Austria), the level of plasma nitrite (NO_2^-, mmol/l) was determined by the photometric method using reaction with Griess reagent, the level of nitrates (NO_3^-, mmol/l) with cadmium metal recovery method [11, 19], the total activity of NO-synthase (NOSx, nmol/min*mg) was determined by evaluating the $NADPH_2$ oxidation reaction by V. Sumbaev [18]. Determination of endothelium-dependent (FMD) and endothelium-independent vasodilatation (NMD) was performed on Aloka SSD-1100 with a

7.5 MHz linear probe according to M.C. Corretti modification of standard measurement protocol [4].

The results of descriptive statistics are presented as mean ± standard deviation (for data with a normal distribution), and the median [25 - 75 quartiles] (for data with a distribution different from the normal). Testing of all samples for normality was done using the Shapiro-Wilk test. Evaluation of the differences between the several groups was performed using a parametric Newman-Keuls test or nonparametric Kruskal-Wallis test with further comparison using Dunn criterion, qualitative characteristics were compared using the chi-squared method. A value of $p \leq 0,05$ was considered statistically significant.

Results and discussion

Comparative analysis of ED parameters in patients of the three study groups identified specific characteristics for each of them (see Table 2). Thus, the concentration of the vWF, not statistically different between groups ($p>0,05$) was significantly higher than normal in all groups: in patients with GA – 1,5 times higher than normal, in AH – 1,8 times higher, and in patients with comorbid pathology - two times higher than the normal values. These changes indicate the degree of damage of the vascular endothelium maximally expressed when combined damaging factors of GA and hypertension are present, which, in turn, leads to high protrombogenic activity of endothelium in this group of patients. This, in our view, may explain the higher frequency of severe coronary artery disease, angioplasty and CABG, found in an observational study by I.L. Yakimenko in patients with gout comorbid with hypertension compared with patients with isolated AH [7].

Table 2

Markers of endothelial function in patients with gout, hypertension and comorbid pathology

Item	Control group (n=10)	Group I (GA, n=26)	Group II (AH, n=26)	Group III (GA+AH, n=86)	p I-II	p II-III	p I-III
vWF, %	47,7 ± 17,7	79,2 ± 35,7	86,5 ± 41,5	92,9 ± 29,0	0,36	0,42	0,2
Interleukin-1β, pg/ml	0,2 [0,14 – 0,29]	1,58 [0,82 – 2,55]	0,55 [0,21 – 2,09]	2,0 [0,66 – 5,61]	0,21	0,01	1,0
Endothelin-1, fmol/ml	0,339 [0,13 – 0,45]	1,58 [1,28 – 2,02]	2,77 [2,11 – 5,03]	3,07 [2,11 – 4,32]	0,0003	1,0	<0,0001
NO-synthase, nmol/min*mg	1,4 [0,9 – 1,9]	6,0 [3,3 – 10,5]	3,8 [1,2 – 5,6]	5,7 [3,0 – 9,3]	0,10	0,11	1,0
NO_2^-, mmol/l	4,15 [2,94 – 4,96]	10,9 [7,9 – 21,7]	6,4 [4,3 – 11,3]	9,9 [7,5 – 26,2]	0,009	0,005	1,0
NO_3^-, mmol/l	6,93 [5,62 – 8,29]	17,5 [13,3 – 26,6]	11,0 [5,6 – 17,6]	22,1 [13,5 – 26,0]	0,03	0,006	1,0
Brachial artery diameter, mm	3,97 ± 0,28	4,43 ± 0,52	4,54 ± 0,62	4,67 ± 0,55	0,44	0,33	0,19
FMD, %	11,28 ± 1,98	10,03 ± 6,25	6,18 ± 6,29	5,80 ± 7,83	0,03	0,83	0,045
NMD, %	20,98 ± 6,01	15,35 ± 7,96	13,59 ± 8,21	10,35 ± 7,26	0,34	0,079	0,018

Analysis of IL-1 concentration in patients of all groups revealed its greatest concentration in patients with comorbid pathology. It should be noted that being more significantly increased in group with GA (1,58 [0,82 – 2,55] pg/ml) versus AH (0,55 [0,21 – 2,09] pg/ml) it did not show sufficient statistical difference between them (p_{I-II} = 0,21). Moreover, IL-1 levels in hypertension patients were significantly lower compared to patients with comorbid disorders (2,0 [0,66 – 5,61] pg/ml, p=0,01), at the same time there was no statistical difference in IL-1 levels between GA and comorbid pathology groups (p=1,0). This finding can indicate a much larger "contribution" of GA in increased IL-1 concentrations in comorbid pathology compared to the "contribution" of hypertension.

Different results were obtained analyzing ET-1 in the groups studied. Despite its significant increase in all groups, patients with hypertension had statistically higher results than the GA group (p=0,0003). The maximum change in this index was observed in the comorbid pathology group (3,07 fmol/ml versus 1,58 fmol/ml in GA and 2,77 fmol/ml in AH), which lead to its significant difference from the GA group (p_{I-III}<0,0001). Similar data was obtained in N.N. Kushnarenko and A.V. Govorina study, which revealed almost two-fold significant increaseinET-1 in patients with GA+ AH compared with isolated GA (1,36 fmol/ml and 0,88 fmol/ml respectively) [10]. This phenomenon, in our opinion, is related to the combined damaging effect of arterial hypertension, elevated uric acid and increased IL-1 concentration. It should be emphasized that the lack of significant difference in ET-1 levels between hypertension and comorbid pathology groups (p_{II-III}=1,0) implies a more significant contribution of hypertension than gout.

Comparison of NO-producing function showed a significant but varying degrees of increase in all groups studied. Thus, NO-synthase activity was greatest in GA and, accordingly, with comorbid pathology without expressing significant difference between the groups (p_{I-III}=1,0). At the same time increase in its activity in hypertension was not high enough to be statistically different from other groups (p_{I-II}=0,10; p_{II-III}= 0,11). Similarly, the least increase in concentration of NO_2^- and NO_3^- was found in hypertension, only showing a tendency to exceed the reference values, and was significantly lower than those in the GA group (p_{I-II}=0,009 and p_{I-II}=0,03 for NO_2^- and NO_3^- respectively) and comorbid pathology group (p_{II-III}=0,005 and p_{II-III}=0,006 for NO_2^- and NO_3^-, respectively). Importantly, the concentration of NO metabolites in GA and GA+AH groups showed no significant difference (p_{I-III}=1,0 for NO_2^- and NO_3^-), which indicates that gout is a possible cause of iNOS hyperactivation and nitrous oxide overproduction and introduces these changes in comorbid pathology.

Artery diameter exceeded normal values in all groups (p=0,01 for groups I and II; p=0,001 for group III) and it was the lowest in patients with GA (4,43±0,52 mm), bigger in AH group (4,54±0,62 mm), and the highest in GA+AH group (4,67±0,55 mm) with no significant intergroup difference (p_{I-II}, p_{II-III}, p_{I-III}>0,05). In our opinion this can be due to the greater age category of patients studied (group I – 60,8±12,2 years, group II – 58,3±12,0 years, group III – 59,9±12,8 years) compared to the age of the reference group (36,0±12,1 years). Such age-related difference was previously described in other studies and cannot be considered specific or diagnostically valuable [4, 6].

Comparative analysis of vasomotor endothelial function also has revealed some specific features. So, greatest reduction of FMD and NMD was found in comorbid pathology patients and it was not significantly different from the results of AH group (p_{II-III}=0,83 for FMD and p_{II-III}=0,079 for NMD). The least changes in vasomotor function were found in GA group, where FMD did not significantly differ from control values (p=0,54) and NMD was less by 27% only approaching the level of statistical significance (p=0,054). Such a small reduction in vascular reactivity in the GA group led to the presence of significant differences compared to GA+AH group (p_{I-III}=0,045 for FMD and p_{I-III}=0,018 for NMD) and can be regarded as a specific attribute of comorbidity due to a pronounced increase in ET-1 concentrations in the background of the lack of overproduction NO in patients with hypertension.

Conclusions

The data shows that endothelial dysfunction in gout, hypertension and their combination has its own characteristics. Thus, gout is characterized by significantly higher concentration of IL-1, nitric oxide metabolites and intact FMD compared to hypertension.

In its turn hypertension compared to gout is accompanied by significantly higher concentrations of endothelin-1, a somewhat larger increase in von Willebrand factor and the downward trend of NO-synthase activity in the background of slightly increased NO_2^- and of NO_3^- concentrations, which leads eventually to a significant FMD reduction.

Endothelial dysfunction in comorbid pathology demonstrates "summation" of pathogenetic mechanisms of its formation, expressed in significant increase of IL-1 and endothelin-1 concentrations, decreased FMD and NMD, and, consequently, impaired vasodilation.

The findings necessitate further research on endothelial dysfunction and its correction at each of the presented pathologies especially the comorbid one where it is most pronounced.

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