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CHANGES OF SOME IMMUNE AND METABOLIC INDICES AS BURDENING CRITERION OF CHRONIC SYSTEMIC INFLAMMATION IN ESSENTIAL HYPERTENSION COMORBIDITY

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Abstract

Present article highlights the cytokine profile features, severity of oxidative stress, changes of endothelium function in patients with isolated course of essential hypertension and its combination with duodenal peptic ulcer. It describes evaluation of chronic systemic inflammation data: level of serum cytokines (tumor necrosis factor-α, interleukins -1β, -6, -10), content of intermediate and end products of lipid peroxidation in blood (conjugated dienes, malondialdehyde), activity of antioxidant defense enzymes (superoxide dismutase, catalase), change of endothelium function (level of stable metabolites of nitrogen oxide in blood serum and severity of ristomycin-induced platelet aggregation).

It was found that in the presence of tendency to increasing interleukin-10 ($p>0.05$), content of tumor necrosis factor-α, interleukins -1β and -6 in patients under the conditions of comorbidity significantly exceeded the reference data ($p<0.001$) and the data of patients with isolated course of essential hypertension. This predetermined significant increase of the ratio of pro-inflammatory cytokines to interleukin-10 that emphasized the presence of the highest pro-inflammatory activity of blood in patients with comorbid pathology. Later on the most significant manifestations of oxidative stress were identified in patients with comorbid pathology that was reflected in the highest level of conjugated dienes and malondialdehyde ($p<0.001$). It also turned out that the process of lipid peroxidation was proceeding under the conditions of superoxide dismutase and catalase activity deficit that caused the greatest decrease of integrated indicator of the antioxidant capacity of blood ($p<0.001$). A negative correlation was found between high pro-inflammatory activity and low antioxidant ability of blood, reflecting the commonality of immune and metabolic processes in the mechanisms of the comorbid pathology development. The latter was also confirmed by the presence of inverse relationship between low total content of stable metabolites of nitrogen oxide in blood, as a reflection of the severity of endothelium dysfunction, and high pro-inflammatory blood activity ($p<0.01$) that also had a direct relationship with high ristomycin-induced platelet aggregation ($p<0.001$), as a high thrombogenic risk indicator in this category of individuals.

Therefore, identifying the relationship between indicators of chronic systemic inflammation in patients with essential hypertension in combination with duodenal peptic ulcer should be considered as a manifestation of a single mechanism of comorbid pathology formation and most of their severity – as a burdening criterion of essential hypertension comorbidity.

Keywords: *essential hypertension, comorbidity, cytokines, lipid peroxidation, endothelium dysfunction.*

Introduction

Characteristic feature of the incidence modern structure is the considerable predominance of cardiovascular diseases, among those the most prevalent is essential hypertension. According to the available statistics prevalence of essential hypertension has risen three times during the last 25 years, and up to one third of grown-up population has increased level of arterial blood pressure [*Ipatov A et al., 2012; Kornatsky V et al., 2015; Radchenko G et al., 2015*]. In the course of a lifetime

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the share of population with arterial hypertension rises up to 80% in elderly and senile age, meanwhile, antihypertensive therapy effectiveness is observed only in one sixth of patients [*Zafar F, Naveed S, 2012; Yena L et al., 2013; Amosova K, Rudenko Yu, 2015*]. It is necessary to mention about high mortality rate caused by cardiovascular diseases, which accounts for two-thirds of all fatal outcomes, moreover, essential hypertension appears to be the reason in half of the clinical cases [*Sirenko Yu, 2014*]. Thus, essential hypertension is a significant problem not only from the medical, but also from the social point of view.

New problems had been revealed at all stages of longstanding studies of essential hypertension development and progression mechanisms that had led to diagnostics, treatment and life quality improvement. Appearance of the new terminology such as "endothelial dysfunction" and "chronic systemic inflammation" has designated a new round in the study of this disease, namely – to defining the role of the immune inflammatory mechanisms in the development and progression of vascular pathology [*Mishchenko L, 2012 a,b; Bespalova I et al., 2013; Bespalova I, 2014; Idris-Khodja N et al., 2014*].

It should be noted that the peculiarity of internal pathology at present time is its comorbidity, while in therapeutic practice, such a concept as the "syndrome of mutual burdening" appears more frequently [*Boyd C, 2005; Roach H, Aigner T, 2007; Puenpatom R, Victor T, 2009; Sharabichev J et al., 2014; Shirinsky V, Shirinsky I, 2014*]. It is pointed out that patients with essential hypertension are not exception in this case, and essential hypertension comorbidity worsens disease forecast on the whole [*Davila E, Hlaing W, 2008; Frostegård J, 2013*]. Not uncommon is combination of essential hypertension with digestive system pathology, particularly with duodenal peptic ulcer, which can complicate process of diagnostics process, modify clinical symptoms and worsen treatment quality [*Khlynova O et al., 2013*].

Taking into account the foregoing, as well as the data from a number of studies dedicated to the role of immune response and adaptation of the immune system in patients with essential hypertension, oxidative stress significance in arterial hypertension realization and endothelium functional changes in disease development and progression [*Kovalenko V et al., 2011; Kostenko V et al., 2014*], we supposed that under conditions of essential hypertension comorbidity the abovementioned phenomena could have certain peculiarities, as a result of which the present study was undertaken.

The study aimed to identify the cytokine profile features, to estimate the severity of oxidative stress, function of endothelium changes in patients with essential hypertension in combination with duodenal peptic ulcer and to define their role as a burdening criterion of comorbid pathology.

Material and methods

During this study totally 55 patients were examined (30 males and 20 females) with second stage of essential hypertension (medication control); 32 of them had isolated disease course (comparison group) and 33 patients (main group) had essential hypertension in combination with duodenal peptic ulcer (non-exacerbation period). The study population had a mean age of 44.3±2.9 years. Reference indicators were obtained while studying 23 practically healthy individuals, sex and age of whom did not differentiate with those of examined patients.

Blood test for pro- (TNFα, IL-1β, IL-6) and anti-inflammatory (IL-10) cytokine level was conducted by immunoenzyme method using "Protein Circuit" test system (St. Petersburg, RF).

Spectrophotometrical method was used to evaluate the level of intermediate (conjugated dienes) and end (malondialdehyde) products of lipid peroxidation. It was also used for defining activity of antioxidant defense system ferments (catalase and superoxide dismutase level in blood erythrocytes) and for the level of ultimate stable metabolites of nitrogen oxide (NO) – nitrites (NO_2) and nitrates (NO_3), and their total content (NO_x) in blood serum (Griss reagent, Thermo Fisher Scientific, USA). Primary hemostasis tissue component was evaluated spectrophotometrically according to ristomycin-induced platelet aggregation.

Statistical analysis was performed using the license software packages Microsoft Office 97, Microsoft Excel Stadia 6.1/prof and Statistica.

Results and discussion

During the examination of cytokine profile in patients with essential hypertension comorbidity (Table 1) an essentially increased level (in com-

TABLE 1

Cytokine profile indices of patients with isolated course of essential hypertension and with essential hypertension comorbidity

Index	Healthy patients (n=23)	Control group (n=33)	Comparison group (n=32)
TNFα, *pcg/ml*	20.3±1.4	32.9±2.1***	48.9±2.3$^{***)xxx}$
IL-1β, *pcg/ml*	11.4±1.3	19.1±2.2***	26.1±2.7$^{***)x}$
IL-6, *pcg/ml*	14.3±1.6	17.2±1.7	22.5±2.0$^{**)x}$
IL-10, *pcg/ml*	34.1±4.2	37.6±2.6	43.1±3.9*
TNFα/IL-10	0.58±0.03	0.87±0.04***	1.14±0.07$^{***)xx}$
IL-1β/IL-10	0.32±0.03	0.51±0.06***	0.61±0.06***
IL-6/IL-10	0.40±0.03	0.44±0.04	0.53±0.04**

Notes: *Significance of differences with reference data: * – p<0.05, ** – p<0.01, *** – p<0.001; significant differences between groups of patients: x – p<0.05, xx – p<0.01, xxx – p<0.001.*

parison with reference data) of pro-inflammatory cytokine was defined – TNFα (2.6 times higher, p<0.001), IL-1β (2.3 times higher; p<0.001), IL-6 (1.6 times higher; p<0.001), and anti-inflammatory cytokine IL-10 (1.3 times higher, p<0.05). It should be mentioned that increased absolute content of serum cytokine was also observed in patients with essential hypertension (control group), but certain differences in comparison with reference data were found only in the indices of TNF-α (1.6 times higher, p<0.001) and IL-1β (1.7 times higher, p<0.001). Noteworthy was that absolute value of all studied cytokines in patients with comorbid pathology had been higher than in the control group: TNF-α – 1.5 times higher (p<0.001), IL-1β – 1.4 times higher (p<0.05), IL-6 – 1.3 times higher (p<0.05) in the presence of tendency towards increasing IL-10 1.1 times higher (p>0.05). Increased level of pro-inflammatory cytokine in patients with essential hypertension was described in the research of D. Trott and D. Harrison (2014), where their participation in induction and maintenance of chronic low intensity inflammation process was pointed out [*Voznyuk L et al., 2012; Fushtey I et al., 2015*]. In this regard ratio (indices) of pro-inflammatory cytokines (TNFα, IL-1β, IL-6) to anti-inflammatory IL-10 turned out to be more indicative.

While comparing indices TNF-α/IL-10, IL-1β/IL-10 and IL-6/IL-10 among the study population it turned out that their most increased level was found in patients with essential hypertension comorbidity. At the same time, TNFα/IL-10 index (almost twice higher (p<0.001) than in the reference data) was also higher in the data of the control group (essential hypertension patients) – 1.3 times higher (p<0.01); IL-1β/IL-10 and IL-6/IL-10 indices, that exceeded reference data values 1.9 times higher (p<0.001) and 1.3 times higher (p<0.05) respectively, comparing with essential hypertension patients had a tendency to increase. However, it should be noted that patients with isolated course of essential hypertension had also sufficiently increased TNFα/IL-10 and IL-1β/IL-10 indices in comparison with reference data (p<0.001 in both cases), but there was no significant increase of IL-6/IL-10 unlike patients with essential hypertension comorbidity.

In research study O. Vlasenko (2015) mentioned about increased ratio indices of pro- to anti-inflammatory cytokines in patients with essential hypertension, pointing to predominant pro-inflammatory cytokine activity. During present study the most significant increase of TNF-α/IL-10, IL-1β/IL-10 and IL-6/IL-10 ratios was observed in patients with essential hypertension comorbidity, emphasizing that they had the highest level of blood serum proinflammatory activity.

Present study revealed that in comparison with reference data patients with essential hypertension comorbidity had the increased level of intermediate (conjugated dienes) and end (malondialdehyde)

products of lipid peroxidation – 1.9 and 2 times higher, respectively (p<0.001 in both cases) (Table 2). Furthermore, their level was more than 1.5 times higher in comparison with essential hypertension patients (control group; p<0.001 in both cases). Significance of oxidative stress in mechanism of arterial hypertension realization is described in the studies of P. Rajendran and co-authors (2013), I. Bespalova (2014) and O. Vlasenko (2015), at the same time it is pointed out that under the conditions of oxidative stress antioxidant defense system plays an important role in levelling the formation of active oxygen forms.

Conducted analysis for defining the level of antioxidant defense ferments in patients with essential hypertension comorbidity showed unreliable, but the most distinct among all study population decrease of superoxide dismutase and catalase activity (p>0.05 in both cases). Moreover, evaluation of integrated indicator of the antioxidant capacity of blood, defined as the ratio of producting multiple antioxidant defense key ferments (superoxide dismutase, catalase) to lipid peroxidation end product (malondialdehyde) showed that its most significant decrease was observed in patients with essential hypertension comorbidity – 2.5 times lower in comparison with reference data and 1.9 times lower than in the control group (p<0.001 in both cases).

Such distribution of patients by integrated indicator of the antioxidant capacity of blood was an evidence of the most decreased blood antioxidant capacity in patients with comorbid pathology, while prevalent oxidative stress activity occurred against the depletion of antioxidant defense key ferments, which was also specified by M. Beg and co-authors (2011), B. Agarwal and co-authors (2015).

Revealed negative correlations between integrated indicator of the antioxidant capacity of blood and TNF-α/IL-10 (r= -0.29; p<0.001) and IL-1β/IL-10 (r= -0.27; p<0.001) indices were more significant in patients with comorbid pathology than in patients with isolated course of essential hypertension (r= -0.25; p<0.01 and r= -0.23; p<0.01), that emphasized their commonality in mechanisms of comorbid pathology development.

By the level of stable nitrogen oxide metabolites in blood, as one of the indices reflecting endothelium functional state, it was determined that patients with essential hypertension comorbidity (compared to reference data) had 1.5 times decreased level of nitrites and 1.6 times decreased level of nitrates (p<0.001 in both cases) in blood, at the same time their summary content was decreased 1.6 times (p<0.001); no significant differences with control group indices (patients with isolated disease course) were found.

It should be noted, that in a number of research studies the presence of a link between cytokines

Table 2

Indices of lipid peroxidation, activity of antioxidant defense ferments, nitrogen oxide metabolites and ristomycin-induced platelet aggregation in patients

Index	Healthy patients (n=23)	Control group (n=33)	Comparison group (n=32)
Malondialdehyde (*U-mol/l*)	3.23±0.06	4.12±0.25*	6.55±0.28$^{***)xxx}$
Conjugated dienes (*U-mol/l*)	9.21±0.09	11.86±0.23*	17.89±0.31$^{***)xxx}$
Superoxide dismutase *(IU mg/Hв)*	28.6±1.3	27.7±1.4	25.4±1.7
Catalase *(IU mg/Hв)*	346.1±8.9	335.6±10.6	311.7±9.9
Integrated indicator of the antioxidant capacity of blood	3065.1±38.7	2257.4±25.6***	1209.6±21.4$^{***)xxx}$
NO_2 (*U-mol/l*)	11.8±0.5	7.5±0.7***	8.1±0.3***
NO_3 (*U-mol/l*)	14.4±0.8	7.8±0.6***	9.6±0.6$^{***)x}$
NO_x (*U-mol/l*)	26.3±1.1	15.4±1.7***	17.8±1.0***
Ristomycin-induced platelet aggregation (%)	94.06±2.11	123.3±2.61***	136.9±4.9$^{***)xx}$

Note: *Significant differences with reference data: * – p<0.05, ** – p<0.01, *** – p<0.001; significance of differences between patients with essential hypertension and patients with comorbid pathology: x – p<0.05, xx – p<0.01, xxx – p<0.001.*

and nytric oxyde was noted in patients with essential hypertension [*Ambrosova T, 2013; Bespalova I, 2014; Fushtey I et al., 2015*], emphasizing their commonality in mechanisms of arterial hypertension realization. It was defined that patients with essential hypertension comorbidity had negative correlations between NO_x and TNF-α (r= -0.30; p<0.01) as well as between NO_x and IL-1β (r= -0.28; p<0.01) and their severity was higher than in patients with isolated course of essential hypertension (r= -0.27 and r= -0.24, correspondingly; p<0.01 in both cases).

In patients with essential hypertension comorbidity more significant changes were also found in ristomycin-induced platelet aggregation index, as a thrombogenic risk indicator [*Voznyuk L et al., 2012; Gomellya M, 2014*], reflecting the state of tissue component of primary hemostasis: its value was 1.5 times higher in comparison with the reference data (p<0.001). Unlike patients with isolated course of essential hypertension, in essential hypertension comorbidity ristomycin-induced platelet aggregation index not only exceeded the data of the control group (p<0.05), but also the physiological threshold of this index on the whole. Direct correlation was found between ristomycin-induced platelet aggregation and TNF-α (r= +0.28; p<0.01) as well as between ristomycin-induced platelet aggregation and IL-1β (r= +0.26; p<0.01), thereat, they were more pronounced than in patients with isolated course of essential hypertension (r= +0.25 and r= +0.22, respectively; p<0.05 in both cases); the inverse correlation was found between ristomycin-induced platelet aggregation and NO_x (r= -0.27; p<0.01).

Thus, the study and analysis of serum cytokine profile, level of lipid peroxidation products, activity of antioxidant defense ferments (superoxide dismutase and catalase) and NO metabolites, as well as evaluation of tissue component of primary hemostasis in patients with essential hypertension in combination with duodenal peptic ulcer, allowed to define a number of certain features which are stated below.

Conclusion

Characteristic feature for cytokine profile in patients with essential hypertension comorbidity is the highest pro-inflammatory activity of blood serum (TNF-α/IL-10, IL-1β/IL-10, IL-6/IL-10), caused by high content of predominantly pro-inflammatory cytokines (TNFα, IL-1β, IL-6). These patients are characterized by a significant decrease in antioxidant potencies of blood (integrated indicator of the antioxidant capacity of blood), while the highest activity of lipid peroxidation (conjugated dienes, malondialdehyde) occurs on the background of significantly decreased activity of antioxidant defense key ferments (superoxide dismutase, catalase). Patients with essential hypertension comorbidity have had a decreased level of stable nitrogen oxide metabolites and the most evident increase of primary hemostasis tissue component (ristomycin-induced platelet aggregation) that reflects pronounced endothelial dysfunction and increased thrombogenic risk. Defined direct and inverse correlations in the analyzed indices point to their commonality in the mechanisms of comorbid pathology formation, while apparently their high severity should be considered as burdening criterion of essential hypertension comorbidity.

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