

**CHANGES OF SOME IMMUNE AND METABOLIC INDICES AS BURDENING
CRITERION OF CHRONIC SYSTEMIC INFLAMATION IN THE PRESENCE OF THE
ESSENTIAL HYPERTENSION COMORBIDITY**

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Abstract

The article highlights the cytokine profile features, severity of oxidative stress, changes of endothelium functions in patients with isolated course of essential hypertension and its combination with duodenal peptic ulcer. It describes evaluation of chronic systemic inflammation data: the 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 discovered that in the presence of tendency to increasing of interleukin-10 ($p > 0,05$), content of tumor necrosis factor α , interleukins 1β and 6 in patients under condition of comorbidity significantly exceeded the reference data ($p < 0,001$) and the data of patients with isolated course of essential hypertension. Thus was predetermined significant increase of 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 in patients with comorbid pathology were identified the most significant manifestations of oxidative stress 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 condition of superoxide dismutase and catalase activity deficit that caused the greatest decrease of integrated indicator of the antioxidant capacity of the blood ($p < 0,001$). It was discovered the negative relationship 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. This 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 ($p < 0,01$), as a high thrombogenic risk indicator in this category of persons.

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.

Key words: essential hypertension, comorbidity, cytokines, lipid peroxidation, endothelium dysfunction.

Introduction

Distinctive feature of the incidence modern structure is the considerable predominance of the cardiovascular diseases; among those the most prevalent is essential hypertension (EH). According to the available statistics prevalence of EH has risen three times during the last 25 years, also up to one third of grown-up population has the increased level of the arterial blood pressure [Ipatov AV et al., 2012; Kornatsky BM et al., 2015; Radchenko TD et al., 2015]. In the course of lifetime the stake 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 et al., 2012; Yena LM et al., 2013; Amosova KV et al., 2015]. It is necessary to mention about high mortality rate caused by cardiovascular diseases, which has caused two one third of all fatal outcomes, moreover EH appears to be the reason in half of the clinical cases[Sirenko YuM et al., 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. Appearing of the new terminology such as «endothelial dysfunction» and «chronic systemic inflammation» has designated a new coil in the study of this disease, namely – to defining the role of the immune inflammatory mechanisms in the development and progression of vascular pathology [Bespalova ID et al., 2013; Bespalova ID, 2014; Idris-Khodja N et al., 2014; Mischenko LA, 2012; Mischenko LA, 2012].

We should take into consideration the fact that at present time comorbidity has become the internal pathology distinctive feature, while in therapeutic practice more frequently appears such term as « syndrome of mutual burdening» [Boyd CM et al., 2005; Roach HI et al., 2007; Puenpatom RA et al., 2009; Sharabichev YuT et al., 2014; Shirinsky VS et al., 2014]. It is pointed out that patients with essential hypertension are not exception in this case, and EH comorbidity worsens disease forecast on the whole [Davila EP et al., 2008; Frostegård J, 2013]. Not uncommon is combination of EH with digestive system pathology, particularly with duodenal peptic ulcer (DPU) - such combination can complicate diagnostics process, modify clinical symptoms and to worsen of treatment quality [Khlynova OV et al., 2013].

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

The aim of the study: to educe the cytokine profile features, to estimate expressed 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.

Materials and Methods

During this study were examined 55 patients (30 of them were male and 20 female) with second stage of EH (medication control); 32 of them had isolated disease course (group of comparison) and 33 patients

(main group) had EH in combination with DPU (non exacerbation period). The study population had a mean age of $44,3 \pm 2,9$ years old. Reference indicators were got at research received of 23 practically healthy persons, sex and age of that did not differentiate with such investigated patients.

Blood test for pro- (TNF α , IL-1 β , IL-6) and anti-inflammatory (IL-10) cytokines (CtK) level was conducted by the immune fermentative method («Protein Circuit» test systems, (St. Petersburg, RF).

Spectrophotometrical method was used to evaluate the level of intermediate (conjugated dienes - CnD) and end (malondialdehyde - MDA) products of lipid peroxidation (LP). It was also used for defining antioxidant defense (AD) system ferments activity catalase (CT) level and superoxide dismutase (SOD) level in blood erythrocytes) and for defining level of nitrogen oxide (NO) ultimate stable metabolites (nitrites (NO₂) and nitrates (NO₃), and their sum in total (NO_x) in blood serum (Griss reagent). Spectrophonometrical method became a tool for evaluation of the primary hemostasis tissue component according to ristomycin-induced platelet aggregation (RIPA).

Statistical calculation was done by 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 EH comorbidity (Table 1) it was defined essentially increased (in comparison with reference data) level of pro-inflammatory CtK – TNF α (2,6 times higher, $p < 0,001$) IL-1 β (2,3 times higher; $p < 0,001$), IL-6 (1,6 times higher; $p < 0,001$), and anti-inflammatory CtK IL-10 (1,3 times higher, $p < 0,05$). It should be mentioned that increased absolute content of serum CtK was also observed in patients with EH (group of control), 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-1 β (1,7 times higher, $p < 0,001$). Noteworthy was that absolute value of all studied CtK in patients with comorbid pathology had been higher than in the group of control: TNF- α – 1,5 times higher ($p < 0,001$), IL-1 β – 1,4 times higher ($p < 0,05$), IL-6 – 1,3 times higher ($p < 0,05$) in the presence of tendency towards increasing of IL-10 – 1,1 times higher ($p > 0,05$). Increased level of pro-inflammatory CtK in patients with EH was described in research of Trott DW et al. (2014), where their participation in induction and maintenance of chronic low intensity inflammation was pointed out [Voznyuk LA et al., 2012; Fushyey IM et al., 2015]. In this regard ratio (indices) of pro-inflammatory CtK (TNF α , IL-1 β , IL-6) to anti-inflammatory IL-10 turned out to be more indicative.

As a result of comparison of indices TNF- α / IL-10, IL-1 β / IL-10 and IL-6/IL-10 it turned out to be that among the study population their most increased level had patients with EH comorbidity. At the same time, index TNF α /IL-10 (almost twice higher ($p < 0,001$) than in the reference data) was also higher in the data of the group of control (EH patients) – 1,3 times higher ($p < 0,01$); indices IL-1 β /IL-10 and IL-6/IL-10, that exceeded reference data values (1,9 times higher ($p < 0,001$) and 1,3 times higher ($p < 0,05$) accordingly), comparing with EH patients had a tendency to increasing. Still it should be mentioned that patients with isolated course of essential hypertension had also sufficiently increased indices TNF α /IL-10 and IL-1 β /IL-10 in comparison with reference data ($p < 0,001$ in both cases), but there was no significant increase of IL-6/IL-10 unlike in case of the patients with EH comorbidity.

Table 1

Cytokine profile indices of patients with isolated course of EH and with EH comorbidity

Index	Healthy patients (n = 23)	Patients with EH (n = 33)	Patients with EH in combination with DPU (n = 32)
TNF α , pcg/ml	20,3 \pm 1,4	32,9 \pm 2,1 ^{***}	48,9 \pm 2,3 ^{***)xxx}
IL-1 β , pcg/ml	11,4 \pm 1,3	19,1 \pm 2,2 ^{***}	26,1 \pm 2,7 ^{*)x*}
IL-6, pcg/ml	14,3 \pm 1,6	17,2 \pm 1,7	22,5 \pm 2,0 ^{*)x}
IL-10, pcg/ml	34,1 \pm 4,2	37,6 \pm 2,6	43,1 \pm 3,9 [*]
TNF α /IL-10	0,58 \pm 0,03	0,87 \pm 0,04 ^{***}	1,14 \pm 0,07 ^{***)xx}
IL-1 β /IL-10	0,32 \pm 0,03	0,51 \pm 0,06 ^{***}	0,61 \pm 0,06 ^{***}
IL-6/ IL-10	0,40 \pm 0,03	0,44 \pm 0,04	0,53 \pm 0,04 ^{**}

Notice: Significant 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.

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

During our study it was defined that in comparison with reference data patients with EH comorbidity had the increased level of intermediate (CnD) and end (MDA) products of lipid peroxidation (LP) (table 2) – 1,9 and 2 times higher accordingly (p<0,001 in both cases). Furthermore, their level was more than 1,5 times higher in comparison with EH patients (group of control; p<0,001 in both cases). Significance of oxidative stress in mechanism of arterial hypertension realization is described in the studies of Rajendran P et al., (2013), Bepalova ID et al. (2014) and Vlasevko EM (2015), at the same time it is pointed out that under condition of oxidative stress antioxidant defense (AOD) system plays an important role in the levelling of formation of active oxygen forms.

Conducted analysis for defining the level of AOD ferments in patients with EH comorbidity showed unreliable, but the most distinct among all study population decline of SOD and CT activity (p>0,05 in both cases). At the same time, evaluation of integrated indicator of the capacity antioxidant of the blood (IIACB), defined as ratio of product of multiple AOD key ferments (SOD x CT) to LP end product (MDA) showed that its most significant decline was observed in patients with EH comorbidity: 2,5 times lower in comparison with reference data and 1,9 times lower than in the group of control (p<0,001 in both cases).

Such distribution of patients according to the integral value IIACB was an evidence of the most decrease of blood anti-oxidizing ability in patients with comorbid pathology, while prevalent oxidative stress

activity took place against the background of AOD key ferments depletion, on what Beg M et al., (2011) and Agarwal BH et al. (2015) specified also.

Table 2

Indices of lipid peroxidation indices, AOD ferments activity, nitrogen oxide metabolites and RIPA in patients with isolated course of EH and in patients with EH comorbidity

Index	Healthy patients (n = 23)	Patients with EH (n = 33)	Patients with EH in combination with DPU (n = 32)
MDA, U-mol/l	3,23±0,06	4,12±0,25*	6,55±0,28***)xxx
CnD, U-mol/l	9,21±0,09	11,86±0,23*	17,89±0,31***)xxx
SOD, IU/mg Hb	28,6±1,3	27,7±1,4	25,4±1,7
CT, IU/mg Hb	346,1±8,9	335,6±10,6	311,7±9,9
IIACB	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***
RIPA, %	94,06±2,11	123,3±2,61***	136,9±4,9***)xx

Notice: Significance of differences with reference data: p<0,05, ** - p<0,01, *** - p<0,001;significance of differences between patients with EH and patients with comorbid pathology: ^x - p<0,05, ^{xx} - p<0,01, ^{xxx} - p<0,001.

Such distribution of patients according to the integral value IIACB was an evidence of the most decrease of blood anti-oxidizing ability in patients with comorbid pathology, while prevalent oxidative stress activity took place against the background of AOD key ferments depletion, on what Beg M et al., (2011) and Agarwal BH et al. (2015) specified also. Revealed negative correlation relationship between value IIBAA and indices TNF-α/ IL-10 (r = -0,27; p<0,001) and IL-1β/ IL-10 (r = -0,29; p<0,001) in patients with comorbid pathology were more significant than in patients with isolated course of EH (r = -0,25; p<0,01 and r = -0,23; p<0,01), that emphasized their commonality in mechanism of comorbid pathology development.

By the level of stable nitrogen oxide metabolites, as one of the indices of endothelium functional state reflection, it was defined that patients with EH comorbidity (comparing 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 ($p < 0,001$); reliable distinctions with indices of the group of control (patients with isolated disease course) was not induced.

It should be noted, that connection between CtK and NO in patients with EH was described in a number of research studies [Ambrosova TM et al., 2013; Bespalova ID, 2014; Fushtey IM et al., 2015], emphasizing their commonality in mechanisms of arterial hypertension realization. During our study it was defined that patients with EH comorbidity had negative correlation relationship between NO_x and $\text{TNF-}\alpha$ ($r = -0,30$; $p < 0,01$) as well as between NO_x and $\text{IL-1}\beta$ ($r = -0,28$; $p < 0,01$) and their severity was higher than in patients with isolated course of EH ($r = -0,27$ and $r = -0,24$ accordingly; $p < 0,01$ in both cases).

It was defined that patients with EH comorbidity had also more significant changes in RIPA index, as an indicator of thrombogenic risk [Voznyuk LA et al., 2012; Gomellya MV, 2014], that reflected state of tissue component of primary hemostasis: its value was 1,5 times higher comparing to reference data ($p < 0,001$). Patients with EH comorbidity (unlike patients with isolated course of EH) had increased RIPA index not only in comparison to the data of the group of control ($p < 0,05$), but also comparing to the physiological threshold of this index on the whole. It was revealed the direct correlation relationship between RIPA and $\text{TNF-}\alpha$ ($r = +0,28$; $p < 0,01$) as well as between RIPA and $\text{IL-1}\beta$ ($r = +0,26$; $p < 0,001$), thus the same time they were more distinct than in patients with isolated course of EH ($r = +0,25$ and $r = +0,22$ accordingly; $p < 0,05$ in both cases); also it was revealed the inverse correlation relationship between RIPA and NO ($r = -0,27$; $p < 0,01$).

Thus, studying and analysis of serum CtK profile, level of lipid peroxidation products, AOD ferments activity (SOD and CT) and NO metabolites, as well as evaluation of tissue component of primary hemostasis in patients with EH in combination with DPU, allowed us to define a number of certain features which are stated below.

Conclusion

Distinctive feature of cytokine profile in patients with EH comorbidity is the highest level of blood serum pro-inflammatory activity ($\text{TNF-}\alpha$ / IL-10 , $\text{IL-1}\beta$ / IL-10 , IL-6/IL-10), caused by high content of predominantly pro-inflammatory CtK ($\text{TNF}\alpha$, $\text{IL-1}\beta$, IL-6). These patients are characterized by considerable decrease antioxidant potencies of blood (IIACB), while the highest activity of lipid peroxidation (CnD, MDA) occurs against a background of considerable decline of antioxidant defense key ferments activity (SOD, CT). Patients with EH comorbidity have had a decreased level of stable nitrogen oxide metabolites and the most evident increase of primary hemostasis tissue component (RIPA) that reflects significant infringement of endothelium function and increase of thrombogenic risk. Defined direct and inverse correlation relationships in the analyzed indices are pointing to their commonality in mechanism of comorbid pathology formation, while apparently their high severity should be considered as burdening criterion of comorbidity essential hypertension.

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