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CHANGES OF ERYTHROPOIESIS IN CASE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND UROLITHIASIS COMORBIDITY

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Abstract. In comorbidity of chronic obstructive pulmonary disease and urolithiasis with oxalic acid stones changes of erythropoiesis were found in 78,3 % of patients, in particular – 52,2 % corresponded to mild iron-deficiency anemia and in 26,1 % of patients – chronic disease anemia or their combination. These changes were accompanied by likely reduction of blood erythropoietin within 2,9-3,5 times less ($p<0,05$).

Key words: oxalates, anemia, iron deficiency, erythropoiesis.

Introduction. In recent years disscussions about extrapulmonary manifestations of chronic obstructive pulmonary disease (COPD), which influence the severity of COPD course, especially if the comorbid pathology is present have been held [2, 3, 4, 5]. Extrapulmonary COPD manifestations form pathogenetic “vicious circle” of comorbid conditions resulting in bad therapeutic response on COPD therapy [1, 2, 8, 9]. According to the department of experimental research of the clinic of the University of Bologna (Italy), "systemic chronic inflammatory process” ("inflammaging") may be a risk factor of comorbid renal pathology of inflammatory genesis in patients with COPD and clinical manifestation of genenic deficiency peroxomal liver enzyme of alanine:glyoxylate aminotransferase (AGT / AGXT), as the formation of kidney stones (urolithiasis) of oxalic acid nature – oxalosis [7]. The course of oxalic urolithiasis is often accompanied by anemic syndrome [5, 9, 10]. The role of the pathogenic mechanisms of influence of erythropoiesis imbalance on development of anemic syndrome in COPD and urolithiasis of oxalic acid origin is still unknown. There is a thought that compensatory production of erythropoietin due to hypoxia in COPD by slowing apoptosis of red bone marrow stem cells, inhibits hepsidin secretion. Hepcidin is a disulfide peptide produced in the liver, macrophages and adipocytes and is a key regulator of iron metabolism. As a result of these changes, even enough erythropoietin production does not prevent anemic syndrome in patients with COPD and urolithiasis of oxalic origin [6, 7, 11]. Thus, the problem of research of the mechanisms and variation of anemic syndrome in COPD and urolithiasis of oxalate genesis, its impact on patients’quality of life is not studied and so defines the value of this study.

Objective. To determine pathogenetic links of erythropoietic disorders and clinical peculiarities of anemic syndrome in patients with comorbid COPD and urolithiasis of oxalate nature.

Material and methods. The study involved 60 patients who were divided into three groups. The first (I^{st}) group included 18 patients with urolithiasis of oxalic acid origin. The second (II^{nd}) group included 19 patients with COPD (2B, 3C GOLD). The third (III^{rd}) group included 23 patients with COPD (2B, 3C GOLD) with comorbid oxalate urolithiasis. The distribution of the patients by gender: males – 38 (63,2 %), females – 22 (36,7 %). The average age of the patients was (55,9±3,3).

The control group consisted of 20 practically healthy individuals: males - 13 (65,0 %), females – 7 (35,0 %), who, at the time of involving into research, didn’t have any acute or chronic diseases and no allergic history . Age of examined individuals in the control group was (53,5±3,2). Absence of significant age and sex differences between the control and study groups allowed to compare the data of these groups.

The degree of bronchial obstruction and the risk of worsening of COPD course was estimated by the Ministry of Health of Ukraine protocol № 555 (06.27.2013). Diagnosis of urolithiasis of oxalate origin was estimated according to the Ministry of Health of Ukraine protocol № 604 (06.12.2004).

Inclusion criteria for patients of the I^{st} group were symptoms according to the Ministry of Health of Ukraine protocol № 604 (06.12.2004): shadows of concrements during ultrasound, impaired urodynamics (renal pelvis dilatation), 24-h urinary excretion of oxalate. Inclusion criteria for patients of the II^{nd} group were the following: clinical symptoms according to the clinical protocol of COPD diagnosis of the Ministry of Health of Ukraine № 555 (06.27.2013), stage (2B, 3C GOLD): expiratory dyspnea, chronic cough, chronic sputum discharge; recurrent COPD course with frequent exacerbations. COPD was diagnosed by GOLD 2013 according to the computer spirography with post-bronchodilatator FEV_1. The severity of dyspnea was determined by a modified scale "Medical Research Council (mMRC) Dyspnea Scale". Pulmonary function test (PFT) was conducted on Spirometer"Microlab-3300" ("Sensor – Medics", Netherlands) with computer analysis of the results. To test the reversibility of bronchial obstruction we used inhalation of β-agonist (salbutamol). The criteria for selection of patients to the III^{rd} group was the presence of COPD (2B, 3C GOLD) with comorbid urolithiasis of oxalate genesis.

Exclusion criteria were the following diseases: diabetes, coronary artery disease, acute coronary syndrome, myocardial infarction, valvular heart disease, heart failure (II-III stage ; III-IV FC with left ventricular ejection fraction below 45 %), acute cer-

ebrovascular accident , rheumatic diseases (rheumatic fever, diffuse connective tissue diseases, etc.), oncology and infection; viral hepatitis B and C, cirrhosis of various etiologies, mental disorders, pregnancy or lactation, acute inflammation of any localization, other decompensated diseases or condition which may affect the results of the study.

State of erythropoiesis and iron metabolism parameters were evaluated on blood count: the number of red blood cells and hemoglobin, the color index (CI), relative content of reticulocytes in blood, serum iron, transferrin (mcmol/l), ferritin (mkg/l) and erythropoietin (mIU / ml) which were determined in the laboratory "Synevo" (Chernivtsi). Transferrin level was determined on analyzer Cobas 6000 (s501 module) of the test system Roche Diagnostics (Switzerland). Ferritin level was determined with immunochemical detection of (ECLIA) on the analyzer Cobas 6000 (e 601 module) and test system Roche Diagnostics (Switzerland). Erythropoietin level was determined by ELISA test system for the quantitative determination of erythropoietin in serum on Biomerica (USA) analyzer.

Statistical analysis of the material was performed by the methods of variation statistics with the definition of averages (M), the average error (m). By taking a probable difference parameters at p <0,05 [9]. Probability changes in variations of samples with normal distribution were determined by paired Student's test, using IBM SPSS Statistics 20 and Origin 8.0 programs.

Results and discussion. Analysis of the results of the study showed (table 1) that patients of the Ist and IIIrd groups had significantly lower hemoglobin content in the blood than the control group: 23,6 % and 31,1 % less respectively (p<0,05). Number of red blood cells in the peripheral blood in all groups was significantly reduced: in the Ist and IInd groups 34,0 % and 21,4 % less, with a maximum decrease in the IIIrd group 35,0 % less (p<0,05). The result of and IIIrd groups 21,3 % and 25,0 % (p<0,05) less respectively, compared with the data of the control group. The color index in all groups was in normal ranges.

Analysis of iron metabolism shows significant reduction in the Ist and IIIrd groups when compared with a control one, 2,2 and 2,4 times less respectively (p<0,05). Similar changes of ferritin levels: decrease in groups I and III, respectively 2,5 and 2,9 times less (p<0,05) and transferrin: 1,6 and 1,8 times less (p<0,05) respectively, indicate a deep iron deficiency. These changes of iron transport forms mean the presence of iron deficiency, sideropenic syndrome in particular. The reason of these changes is that patients with oxalic urolithiasis have a constant loss of red blood cells due to chronic microscopic hematuria.

At the same time, a significant reduction of erythropoietin in the blood was found in patients of the Ist and IIIrd groups, 1,6 and 2,7 times less(p<0,05) respectively, indicating the posthemorrhagic anemia with disorders of erythropoiesis regulation. Inhibition of erythropoietin production is present on the background of aseptic inflammation and incrustation of renal parenchyma with calcium oxalate crystals.

In patients of the IIIrd group signs of erythropoiesis disorders were found alongside with anemic syndrome. In order to indicate the kind of anemic syndrome within this group of patients cluster analysis was performed: cluster 1: COPD patients with oxalic calculi urolithiasis without anemia; cluster 2: COPD patients with oxalic urolithiasis with anemia of chronic disease; cluster 3: COPD patients with oxalic acid urolithiasis with iron deficiency anemia (table 2).

Cluster analysis of the group of patients with COPD and comorbid oxalate urolithiasis allowed to describe changes of erythropoiesis in more detailed manner. According to the blood count of these patients changes were the following: cluster 1were

Table 1

Indicators of erythropoiesis in group of patients with COPD and oxalic urolithiasis in comparison to groups of patients with isolated course of COPD and urolithiasis (M±m)

Indicators, measurements	Control group	Examined groups		
		I (urolithiasis) n=18	II (COPD) n=19	III (COPD and urolithiasis)n=23
Hb, g/l	125,0±9,7	95,4±4,7*	118,3±9,6	86,1±7,0*/***
Erythrocytes, 10^{12}/l	5,0±0,3	3,3±0,2*	3,9±0,2*	3,25±0,18*
Ht, %	45,1±2,7	35,4±1,8*	48,7±2,5**	33,32±1,9*/***
Color index	1,02±0,05	0,86±0,04	0,90±0,03	0,79±0,03
Serum iron, mcmol/l	20,5±1,6	9,3±0,6*	17,7±1,2**	8,5±0,4*/***
Ferritin, mkg/l	81,0±5,1	32,1±4,2*	74,1±3,5	28,2±1,9*/***
Transferrin, mcmol/l	3,6±0,1	2,3±0,2*	3,5±0,2	2,05±0,10*/***
Erythropoietin, mIU / ml	29,2±1,8	18,3±0,9 *	25,7±1,3	10,9±0,57*/**/***

Notes. *- changes are relevant in comparison with control group (p<0,05); ** - changes are relevant in comparison to group of patients with urolithiasis (p<0,05); *** - changes are relevant in comparison to group of patients with COPD (p<0,05)

Table 2

Indicators of erythropoiesis and iron metabolism in group of patients with COPD and oxalic urolithiasis after cluster analysis division

Indicators, measure-ments	Control group	Clusters of group of patients with COPD and urolithisis depending on changes in erythropoiesis		
		Cluster 1 (n=5)	Cluster 2 (n=6)	Cluster 3 (n=12)
Hb, g/l	125,0±9,7	120,1±6,2	80,7±4,2*	82,0±4,3 *
Erythrocytes10^{12}/l	5,0±0,3	3,8±0,2*	3,0±0,1*	3,1±0,1 *
Color index	1,02±0,05	0,92±0,04	0,81±0,04*	0,78±0,03*
Serum iron, mcmol/l	20,5±1,6	19,7±0,6	12,3±0,6*	9,3±0,4*
Ferritin, mkg/l	81,0±5,1	76,1±1,5	57,2±1,9*	10,7±0,5*
Transferrin, mcmol/l	3,6±0,1	3,6±0,2	3,9±0,2	2,0±0,1*
Erythropoietin, mIU / ml	29,2±1,8	27,8±1,1	10,3±0,9*	8,3±0,4*

Notes. *- changes are relevant in comparison with control group ($p<0,05$)

classified as patients without anemic syndrome (21,7 % of the patients of the IIIrdgroup). The 2nd cluster: patients with chronic disease anemia (26,1 % of the patients of the IIIrdgroup), the 3rd cluster involved patients with iron deficiency anemia (52,2 %). In patients of cluster 1the levels of hemoglobin, red blood cells and color index were within normal ranges. So in this subgroup anemic syndrome was not observed, although hemoglobin level was significantly lower than in the control group ($p<0,05$). Levels of serum iron, ferritin and erythropoietin indicate the normal state of erythropoiesis and iron stores in the body.

Patients of cluster 2 had hemoglobin levels 1.6 times less than in the control group ($p<0,05$). Red blood cells and color index levels were decreased 1,7 times and 20,6 % less respectively compared to the same indicators in the control group. The content of serum iron corresponded with lower range of this indicator for adult individuals of this age group, but was significantly lower than in the control group 1,7 times ($p<0,05$) less, indicating latent iron deficiency. These data were confirmed by normal levels of transferrin and erythropoietin with a tendency to decline (1,4 times less ($p<0,05$)) and (2,9 times less ($p<0.05$)) respectively. According to the diagnostic criteria such data correspond with chronic disease anemia due to present COPD and urolithiasis. Formation of this condition in patients with COPD is caused by prolonged hypoxia and chronic inflammation that burst reactive oxygen species and pro-inflammatory factors into blood circulation. That causes inhibition of hematopoietic function of bone marrow. In this group of patients high excretion of calcium oxalate crystals in urine was found, so we can suggest that probable deposition of oxalate crystals is also present in the bone marrow, which contributes erythropoesis inhibition. This is confirmed by significantly reduced levels of erythropoietin. So, in patients of the 2nd cluster due to COPD and oxalic urolithiasis mild chronic disease anemia is present.

In patients of cluster 3, decreased hemoglobin level (1,5 times less($p<0,05$) compared with the level of hemoglobin in the control group) was found. The level of red blood cells was reduced 1.6 times less ($p<0,05$) compared with the level of red blood cells in patients of the control group. The level of color index was 23,5 % less ($p<0,05$) than color index level of the control group. These changes indicate the presence of hypochromic anemia. Erythropoietin level was reduced 3,5 times less($p<0,05$) than in the control group. However, iron homeostasis in this cluster of patients indicates manifestation of sideropenic syndrome. In particular, reduced levels of serum iron (2,2 times less($p<0,05$)), transferrin 1,8 times less($p<0,05$), significantly reduced blood levels of ferritin (7,5 times less($p<0,05$)) than in the control group. According to the diagnostic criteria such data correspond with mild iron deficiency anemia.

Comorbidity of COPD and urolithiasis with oxalic acid crystals accumulation results in release of oxidative stress cytokines and free radicals into not only bronchopulmonary, but the systemic circulation with activation of leukocytes and redistribution of iron. All these processes lead to anemia. It should be noticed that at the initial period, markers of chronic inflammation do not appear in the blood. In patients with urolithiasis of oxalic genesis, calcium oxalate crystals deposit in the bone marrow, suppressing erythropoiesis and in this way develop anemic syndrome or chronic disease anemia [7]. Anemic syndrome is due to insufficient secretion of erythropoietin in kidneys caused by urolithiasis which inhibits production of erythropoietin through depositing calcium oxalate crystals in the renal parenchyma. Chronic loss of red blood cells by hematuria depletes iron pool.

Conclusion

In 78,3 % of patients in the group with comorbid chronic obstructive pulmonary disease and oxalate urolithiasis changes of erythropoiesis were

found. In particular: in 26,1 % of patients the chronic disease anemia was diagnosed, but mild iron deficdeficiency anemia prevailed in 52,2 % of the patients in the group. General decrease in erythropoietin level within ranges of 2,9-3,.5 times less ($p<0,05$) than in control group was found.

Prospect for further research is the develdevelopment of methods of adequate correction of anemia in patients with COPD and comorbid oxalate urolithiasis, taking into consideration the mechamechanisms of anemia pathogenesis.

References

1. Коваленко С.В. Зміни гемокоагуляційної ланки гемостазу у хворих на хронічні обструктивні захворювання легень / С.В. Коваленко, О.В. Андрусяк // Гал. лікар. вісник. – 2004. – № 4 (11). – С. 31-32.
2. Показатели крови и лейкоцитарного индекса интоксикации в оценке тяжести и определения прогноза при воспалительных, гнойных и гнойно-деструктивных заболеваниях / В.К. Островский, А.В. Маценко, Д.В. Янголенко [та ін.] // Клин. лаб. диагност. – 2006. – № 6. – С. 50-53.
3. Хухліна О.С. Дисметаболічні порушення обміну щавелевої кислоти як провокаційний фактор розвитку хронічного обструктивного захворювання легень / О.С. Хухліна, К.В. Вілігорська // Здобут. клін. та експерим. мед. – 2014. – № 1 (20). – С. 26-29.
4. Хухліна О.С. Зміни морфофункціональних властивостей еритроцитів та їх роль у патогенезі взаємообтяження бронхіальної астми та хронічного холециститу / О.С. Хухліна, Т.В. Дудка, І.В. Дудка // Вісн. ВДНЗУ "Укр. мед. стомат. академія". – 2013. – Т. 13, № 2 (42). – С. 95-98.
5. Хухліна О.С. Стан системи кровотворення у хворих на хронічне обструктивне захворювання легень та сечокам'яну хворобу / О.С. Хухліна, К.В. Вілігорська: матеріали наук.-практ. конф. [«Коморбідність у клініці внутрішньої медицини: фактори ризику, механізми розвитку та взаємообтяження, особливості фармакотерапії»], (Чернівці, 10-11 жовтня 2013 р.). – Чернівці: БДМУ, 2013. – С. 14-15.
6. An official American thoracic society / European respiratory society statement: update on limb muscle dysfunction in chronic obstructive pulmonary disease / F. Maltais, M. Decramer, R. Casaburi [et al.] // Am. J. Respir. Crit. Care Med. – 2014. – № 189. – P. 15-62.
7. Franceschi C. Chronic Inflammation (Inflammaging) and Its Potential contribution to Age-associated Diseases / C.Franceschi, J.Campisi // Advances in Geroscience: Impact on Healthspan and Chronic Disease Perspective. – 2014. – № 69. – P. 4-6.
8. Khukhlina O.S. Metabolic oxalic acid disorder as a provocative marker in the pathogenesis of chronic obstructive pulmonary disease / O.S. Khukhlina, K.V. Viligorska, A.A. Antoniv // Book of abstracts ISCOMS 2014: Pulmonary medicine and medical physiology. – Groningen, 2014. – P. 546.
9. Khukhlina O.S. Risk factors for pulmonary oxalosis and oxalate nephropathy / O.S. Khukhlina, K.V. Viligorska, A.V. Motrich // Abstract book of the 25th European students' conference [«Rethinking Medical Research»] (17-20th of September, 2014, Berlin, Germany). – Berlin, 2014. – P. 502.
10. Khukhlina O.S. The state of hematopoiesis in patients with comorbid course of chronic obstructive pulmonary disease and urolithiasis with anemic syndrome / O.S. Khukhlina, K.V. Viligorska, A.A. Antoniv // Abstract book of the 24th European students' conference [«Exploring the Unknown»] (4th-7th September, 2013, Berlin, Germany). – Berlin, 2013. – P. 395.
11. Montes de Oca M. Impact of chronic bronchitis on chronic obstructive pulmonary disease / Maria Montes de Oca // Lancet. – 2009. – № 3. – 10-15.

ИЗМЕНЕНИЯ СИСТЕМЫ ЭРИТРОПОЭЗА ПРИ КОМОРБИДНОМ ТЕЧЕНИИ ХРОНИЧЕСКОГО ОБСТРУКТИВНОГО ЗАБОЛЕВАНИЯ ЛЕГКИХ И МОЧЕКАМЕННОЙ БОЛЕЗНИ

О.С. Хухлина, К.В. Вилигорская

Резюме. При коморбидном течении хроничского обструктивного заболевания легких и мочекаменной болезни оксалатного генеза, у 78,3 % пациентов были установлены изменения в системе эритропоэза, которые у 52,2 % соответствовали железодефицитной анемии средней степени тяжести, а у 26,1 % пациентов - анемии хронического заболевания или их сочетанию, и сопровождались вероятным понижением уровня эритропоэтина в крови в пределах 2,9-3,5 раза ($p<0,05$).

Ключевые слова: оксалаты, ХОБЛ, МКБ, анемия, эритропоэз.

ЗМІНИ СИСТЕМИ ЕРИТРОПОЕЗУ ЗА КОМОРБІДНОГО ПЕРЕБІГУ ХРОНІЧНОГО ОБСТРУКТИВНОГО ЗАХВОРЮВАННЯ ЛЕГЕНЬ ТА СЕЧОКАМ'ЯНОЇ ХВОРОБИ

О.С. Хухліна, К.В. Вілігорська

Резюме. За коморбідного перебігу хронічного обструктивного захворювання легень та сечокам'яної хвороби оксалатного генезу в 78,3 % пацієнтів встановлено зміни еритропоезу, які в 52,2 % відповідають залізодефіцитній анемії середнього ступеня тяжкості, а у 26,1 % пацієнтів – анемії хронічного захворювання, або їх поєднанню, і супроводжуються вірогідним зниженням вмісту в крові еритропоетину у межах 2,9-3,5 раза ($p<0,05$).

Ключові слова: оксалати, ХОЗЛ, СКХ, анемія, еритропоез.

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