IRON METABOLISM, ERYTHROPOIESIS AND IMMUNE INFLAMMATION IN ANEMIC PATIENTS WITH CHRONIC HEART FAILURE IN THE PRESENCE OF DIABETIC NEPHROPATHY*

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Diabetes mellitus (DM) 2 type is a common disease that affects about 5 % of Europe's population [1]. A typical complication of DM 2 type is nephropathy. In industrialized countries, diabetic nephropathy (DN) has become the leading cause of end-stage chronic kidney disease (CKD) [2]. About half of patients with CKD suffer from anemia [3]. So DM 2 type is a major cause of renal anemia. Anemia in DN patients develops earlier and usually more severe than in patients with kidney disease of another cause. For example, according to epidemiological study NHANES III, the frequency of anemia in patients with CKD stages III — IV and DM 2 type was twice higher than comparable according to impaired renal function patients who do not suffer from DM [4].

Anemia, which significantly reduces the survival of patients, is an independent predictor of risk of death. These data confirmed the result of research ARIC, where anemia was closely associated with increased risk of cardiovascular complications [5]. According to numerous studies of anemia is seen as cause and result of CHF [6–8]. Vicious circle of pathophysiological relationships between kidney damage, anemia and heart failure was called «cardiorenal anemic syndrome» (CRAS) [7]. According to results of D.Ṡ. Silverberg. heart failure was identified in 80 % of patients suffering from chronic renal failure (CRF) and anemia, 50 % of them have got DM 2 type [8].

A study conducted by M. C. Thomas and co-authors was found that in patients with DM 2 type was defined significant hemoglobin (Hb) level decreasing in parallel with increasing of heart failure functional class (FC) severity (New York Heart Association (NYHA) classification was used). Anemia has a negative impact on the quality of life, causing decreased performance and exercise tolerance [9]. P. Vlagopoulos and collaborators studied the prognostic value of anemia in 3015 patients with DM 2 type. In patients with nephropathy anemia was associated with an increased risk of myocardial infarction and death from coronary heart disease in 1.64 times, stroke — in 1.81 times and death from any cause — in 1.88 times [10].

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Due to the increasing number of patients with DM 2 type, chronic renal failure and cardiovascular disease, including heart failure, the problem of relations within CRAS at the moment is one of the urgent and intensely studied [11, 12].

Mechanisms of anemia syndrome (AS) on a background of cardiorenal pathology are multifactoral. An important role belongs to interstitial volume increase, the extent of which is inversely proportional to the creatinine clearance, resulting in abnormal central regulator of erythropoiesis erythropoietin (EPO) synthesis in patients with DM 2 type [13]. A significant contribution to the formation of AS is given by the presence of high immunoinflammatory activity, which causes changes in the central regulator of iron metabolism (Fe) — hepcidin [14]. However, the question about activity of EPO and the status of functional iron fund (Fe) in patients with anemia, which developed on the background of chronic heart failure and DN remains debated.

Purpose of the study is to analysis the availability and character of the relationships between hemogram indicators, ferrokinetic parameters (transferrin saturation (TSAT), transferrin (Tf), ferritin, soluble transferrin receptor (sTf) activity — as a marker of erythropoiesis — and EPO), renal function (according to the glomerular filtration rate (GFR) and urinary albumin level) and immunoinflammatory markers (interleukin (IL) IL-6, IL-10) in patients with AS which was formatted on a background of chronic heart failure in the presence of diabetic nephropathy.

MATERIALS AND METHODS

The study involved 140 patients with chronic heart failure of II–IV FC due to coronary heart disease (CHD) with concomitant DN who were treated in the cardiological department of the Kharkiv City Clinical Hospital № 27 (average age 71.42 ± 8.66 years). From the study were excluded patients with acute coronary syndrome, acute myocardial infarction, a disease that could cause anemia, disorders of the gastrointestinal tract, cancer, bleeding that was diagnosed the day before admission or during hospitalization. The diagnosis was made in accordance with the valid orders of Ministry of Health of Ukraine.

FC established according to the NYHA classification of CHF. The diagnosis of anemia was put according to criteria of Hematology Standards Medical Committee (ICST, 1989): reducing the concentration of Hb in venous blood less than 120 g/l for women and less than 130 g/l for men. All patients fulfilled clinical and biochemical blood tests. Renal function was assessed by glomerular filtration rate (GFR) calculated using the Cockroft-Gault formula. Patients completed instrumental studies: ECG, echocardiography with Doppler-mode, ultrasound liver and kidney, fibrogastroduodenoscopy were done if necessary. the Concentrations of IL-6, IL-10 were determined by ELISA (set of «Interleukin-6 ELISA-BEST», «Interleukin-10 ELISA-BEST», «Vector-Best», Russia), sTf («Soluble transferrin receptor-IFA-BEST», «Vector-Best», Russia), hepcidin («Peptide Enzyme Immunoassay (EIA) Protocols» (Peninsula Laboratories, LLC, USA), EPO («Peptide Enzyme Immunoassay (EIA-3646)», DRG, USA), ferritin («Ferritin ELISA», DAI, USA), Tf («TRANSFERRIN», DIALAB, Austria), albumin urine («Albumin-IFA», LLC «Granum», Ukraine). The presence of albumin urine in the range of 20–200 mg/l considered as microalbuminuria (MAU) criteria. Study showed presence of MAU in all patients with anemia formatted on background of CHF and DN. TSAT, which was defined as the ratio of serum total Fe to the level of serum iron capacity, less 20 % regarded as a criterion for iron deficiency [6]. For availability and iron deficiency character diagnostics criteria recommended by FAIR-HF research were used [6]. Absolute Fe deficiency was identified at ferritin level < 100 ng/ml, TSAT < 20 %; functional Fe deficiency — at ferritin level $\geqslant 100$ ng/mL, TSAT < 20 %. Ferritin shows the amount of deposited Fe. However, the level of ferritin is directly dependent on the severity of the inflammatory process. The presence of high concentrations of

proinflammatory cytokines in patients with CHF is confirmed by many studies, so that the use of this indicator for the diagnosis of disorders in Fe metabolism in patients with symptoms of cardiac decompensation is unacceptable [15]. Using two markers — TSAT and ferritin — most adequately reflects the state of Fe metabolism in patients with a high immunological activity in accordance with the recommendations of multicenter studies and appropriate for the differential diagnosis between the absolute and functional deficiency of Fe [8, 16].

Statistical analysis of the results of research carried out using correlation analysis (Spearman correlation (r)) using software packages BIOSTAT version 3.4 and STATISTICA version 6.1. Differences between groups were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

In patients with anemia on the background of CHF and DN direct correlations were found between GFR and Hb ($r = 0.31$; $p < 0.05$), Tf ($r = 0.39$; $p < 0.05$), sTfR ($r = 0.58$; $p < 0.05$), IL-10 ($r = 0.48$; $p < 0.05$), EPO ($r = 0.70$; $p < 0.05$) and negative correlations between GFR and IL-6 ($r = –0.31$; $p < 0.05$), hepcidin ($r = –0.45$; $p < 0.05$). The positive correlation between EPO and sTfR levels ($r = 0.54$; $p < 0.05$). Thus renal dysfunction increasing is accompanied by high activity of proinflammatory IL-6, decrease of antiinflammatory IL-10, increasing of the central regulator of Fe metabolism hepcidin, disturbance of the Fe transport fund, reduced synthesis of EPO and, consequently falling erytropoietyc activity of the red bone marrow (according to sTfR), formation anemia. The presence of correlation between hepcidin and IL-6 ($r = –0.54$; $p < 0.05$) indicates cytokine induced hepcidin activity, resulting in functional Fe deficiency ($r = –0.29$; $p < 0.05$ between TSAT and hepcidin) with blocked Fe in deposite fund ($r = 0.36$; $p < 0.05$ between ferritin and hepcidin) and anemia developing ($r = –0.28$; $p < 0.05$ between hepcidin and Hb). In addition, proinflammatory IL-6 can have unmediated impact on the formation of relative iron deficiency, which maintains a negative relationship between IL-6 and TSAT ($r = –0.27$; $p < 0.05$). The presence of imbalance in the cytokine system excessively increasing activity of proinflammatory IL-6 and depletion of anti-inflammatory IL-10 ($r = –0.59$; $p < 0.05$ between IL-6 and IL-10), assists malfunction of Fe transport fund ($r = –0.86$; $p < 0.05$ between IL-6 and Tf), ($r = 0.47$; $p < 0.05$ between IL-10 and Tf). Inflammatory markers also have an impact on the activity of erythropoiesis (indirect links between sTfR and IL-10 ($r = –0.83$; $p < 0.05$), IL-6 ($r = 0.52$; $p < 0.05$), the increasing of MAU (MAU and IL-6 ($r = 0.32$; $p < 0.05$), IL-10 ($r = –0.47$; $p < 0.05$)). Links between the MAU and sTfR ($r = –0.21$; $p < 0.05$), Tf ($r = –0.23$; $p < 0.05$), EPO ($r = –0.27$; $p < 0.05$) may indicate loss of parameters as a part of protein's pool in the presence of MAU. These data are consistent with L. I. Mazur, according to research results in patients with CKD observed loss of EPO in urine as a part of protein's pool [17]. In patients present a paradoxical reaction to the decreasing Hb level in the form of lowering erythropoietyc activity (direct links between sTfR and Hb ($r = 0.21$; $p < 0.05$)), and reduced EPO (direct links between the EPO and Hb ($r = 0.46$; $p < 0.05$)), indicating the loss of an adequate compensatory response aimed to normalization of Hb, which is observed in healthy individuals. Thus patients with DN appears inadequate production of EPO by the kidneys even against moderate decline function of nitrogen excreation, as evidenced by the level of GFR (50.39 ± 2.86 ml/min/1.73 m^2). In healthy people Hb reduce accompanied by increased renal production of EPO, but in patients with DM 2 type, this relationship is disrupted due to the fact that EPO-synthesizing cell of interstices damaged and destroyed earlier than nondiabetyc nephropathy, lose adequate reverse reaction — increased production of EPO response to anemia.

Thus, in patients with anemia, which developed on the background of chronic heart

failure in the presence of DN found violation of the synthesis of the central regulator of erythropoiesis EPO, growth of hepcidin activity that causes development of iron metabolism disorders due to depletion of functional fund on the background of normal levels deposite fund of Fe, which can be considered as a factor in the formation of anemia in this cohort of patients.

CONCLUSIONS

1. The development of anemia in patients with chronic heart failure by the presence of DN is characterized by erythropoietyc activity fall due to the low level of erythropoietin and functional iron deficiency, as a result of influence of proinflammatory cytokine level on hepcidin activity, resulting in the disruption of the functional fund Fe, deposited block Fe.
2. The presence of MAU can be considered as a factor in formation of anemic syndrome due to loss of EPO, Tf, sTfR as a part of protein's pool in patients with chronic heart failure and DN.
3. The presence of anemia in patients with chronic heart failure and DN is associated with inadequate low central regulator of erythropoiesis level in response to low Hb concentrations on background of moderate decrease in GFR.

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МЕТАБОЛІЗМ ЗАЛІЗА, ЕРИТРОПОЕЗ ТА ІМУННЕ ЗАПАЛЕННЯ У АНЕМІЧНИХ ХВОРИХ З ХРОНІЧНОЮ СЕРЦЕВОЮ НЕДОСТАТНІСТЮ ЗА НАЯВНОСТІ ДІАБЕТИЧНОЇ НЕФРОПАТІЇ

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Розвиток анемії у пацієнтів з хронічною серцевою недостатністю за наявності діабетичної нефропатії характеризується падінням еритропоетичної активності внаслідок низького рівня еритропоетину і функціонального дефіциту заліза, пов'язаного з впливом прозапальних цитокінів на активність гепсидіна, в результаті чого порушується функціональний фонд заліза,

формується депозитний блок заліза. Наявність мікроальбумінурії можна розглядати як фактор формування анемічного синдрому через втрату еритропоетину, трансферину, розчинного рецептора трансферину у складі пулу білків у пацієнтів з хронічною серцевою недостатністю і діабетичною нефропатією.Наявність анемії у хворих з хронічною серцевою недостатністю і діабетичною нефропатією асоціюється з низькою активністю центрального регулятора еритропоезу у відповідь на низькі концентрації гемоглобіну на тлі помірного зниження швидкості клубочкової фільтрації.

К л ю ч о в і с л о в а: хронічна серцева недостатність, анемія, діабетична нефропатія, метаболізм заліза, імунне запалення.

МЕТАБОЛИЗМ ЖЕЛЕЗА, ЭРИТРОПОЭЗ И ИММУННОЕ ВОСПАЛЕНИЕ У АНЕМИЧНЫХ БОЛЬНЫХ С ХРОНИЧЕСКОЙ СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТЬЮ ПРИ НАЛИЧИИ ДИАБЕТИЧЕСКОЙ НЕФРОПАТИИ

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Развитие анемии у пациентов с хронической сердечной недостаточностью при наличии диабетической нефропатии характеризуется падением эритропоэтической активности вследствие низкого уровня эритропоэтина и функционального дефицита железа, связанного с влиянием провоспалительных цитокинов на активность гепсидина, в результате чего нарушается функциональный фонд железа, формируется депозитный блок железа. Наличие микроальбуминурии можно рассматривать как фактор формирования анемического синдрома вследствие потери эритропоэтина, трансферрина, растворимого рецептора трансферрина в составе пула белков у пациентов с хронической сердечной недостаточностью и диабетической нефропатией. Наличие анемии у больных с хронической сердечной недостаточностью и диабетической нефропатией ассоциируется с низкой активностью центрального регулятора эритропоэза в ответ на низкие концентрации гемоглобина на фоне умеренного снижения скорости клубочковой фильтрации.

К л ю ч е в ы е с л о в а: хроническая сердечная недостаточность, анемия, диабетическая нефропатия, метаболизм железа, иммунное воспаление.

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K e y w o r d s: chronic heart failure, anemia, diabetic nephropathy, iron metabolism, immune inflamation.