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CHEMOTHERAPY EFFECT ON ARGININE / CITRULLINE CYCLE INDICATORS IN PATIENTS WITH ACUTE MYELOID LEUKEMIA AND CONCOMITANT OBESITY

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Ключові слова: *аргінін, цитрулін, орнітиндекарбоксілаза, аргіназа, гостра мієлобластна лейкемія, ожиріння*

Ключевые слова: *аргинин, цитруллин, орнитиндекарбоксілаза, аргиназа, острая миелобластная лейкемия, ожирение*

Abstract. Chemotherapy effect on arginine / citrulline cycle indicators in patients with acute myeloid leukemia and concomitant obesity. Maslova G.S., Skrypnyk I.M. *The aim of study was to investigate the changes in the arginine / citrulline cycle in patients with acute myeloid leukemia (AML) with concomitant obesity in dynamics of chemotherapy (CT). The study included 20 patients with newly diagnosed AML who had a body mass index (BMI) above 25. The average BMI was $29.35 \pm 0.93 \text{ kg/m}^2$. Patients have undergone a course of remission induction CT "7+3" or "5+2". All patients were examined twice: before CT and on the 28th day. Complete blood test was assessed. Arginine and citrulline content, arginase and ornithine decarboxylase (ODC) activity were measured in the blood serum. The study results showed a 3-fold decrease in arginine content in the blood serum of patients with AML, as well as a 3.9-fold increase in arginase activity, 1.9-fold – ODC, 7.6-fold higher citrulline concentration compared with control ($p < 0.05$). After CT, against the background of complete blood count normalization in AML patients with concomitant obesity, a 1.3-fold decrease in arginine concentration was noted, while the arginase activity increased by 2.2 times, ODC activity – by 1.8 times and citrulline content – by 2.4 times ($p < 0.05$). After CT a high direct correlation between BMI and arginine concentration in AML patients with concomitant obesity ($r = +0.63$; $p < 0.05$), as well as between the activity of ODC and arginine content ($r = +0.55$; $p < 0.05$) was revealed. Thus, in AML patients with obesity prior to CT the disorders in the arginine / citrulline cycle were present, which are caused by oncohematological disease. Excess weight and obesity affect serum arginine levels, which may contribute to chemotherapy resistance.*

Реферат. Влияние химиотерапии на показатели аргинин/цитруллинового цикла у больных острой миелобластной лейкемией с сопутствующим ожирением. Маслова А.С., Скрыпник И.Н. *Целью работы явилось исследование изменения показателей аргинин/цитруллинового цикла у больных острой миелобластной лейкемией (ОМЛ) с сопутствующим ожирением в динамике химиотерапии (ХТ). В исследование было включено 20 пациентов с впервые установленным диагнозом ОМЛ, которые имели индекс массы тела (ИМТ) выше 25. Среднее значение ИМТ составило $29,35 \pm 0,93 \text{ кг/м}^2$. Пациентам был проведен курс индукции ремиссии «7+3» или «5+2». Обследование проведено дважды: до ХТ и на 28-й день. Оценивали показатели общего анализа крови. В сыворотке крови определяли содержание аргинина, цитруллина, активность аргиназы, орнитин-декарбоксилазы (ОДК). По результатам исследования в сыворотке крови пациентов с ОМЛ выявлено снижение содержания аргинина в 3 раза, а также – увеличение активности аргиназы в 3,9 раза, ОДК – в 1,9 раза, концентрации цитруллина – в 7,6 раза в сравнении с контролем ($p < 0,05$). После проведения ХТ на фоне нормализации показателей общего анализа крови у больных ОМЛ с сопутствующим ожирением наблюдалось снижение концентрации аргинина в 1,3 раза, активности аргиназы – в 2,2 раза, ОДК – в 1,8 раза, содержания цитруллина – в 2,4 раза ($p < 0,05$). После ХТ выявлено наличие высокой прямой корреляционной связи между ИМТ и концентрацией аргинина у пациентов с ОМЛ с сопутствующим ожирением ($r = +0,63$; $p < 0,05$), а также между активностью ОДК и содержанием аргинина ($r = +0,55$; $p < 0,05$). Таким образом, у больных ОМЛ с сопутствующим ожирением до ХТ имело место наличие нарушений в аргинин/цитруллиновом цикле, которые обусловлены онкогематологическим заболеванием. Наличие избыточного веса и ожирения влияет на показатели сывороточного уровня аргинина, что может способствовать формированию резистентности к ХТ.*

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Acute myeloblastic leukemia (AML) accounts for up to 80% of all cases of acute leukemia [2, 6]. In Europe and the United States, there is a tendency to increase in the incidence of AML from 3.48 per 100,000 population in 1976 to 5.06 per 100,000 population in 2013 [6]. In recent decades new evidence as for the association between obesity and the risk of all cancers, including AML has emerged [11, 12].

The detailed picture of AML is accompanied by a number of disorders in the arginine/citrulline cycle, which are characterized by an increase in arginase activity due to its hyperproduction by blast cells [8]. High arginase activity leads to the formation of

arginine deficiency, which cannot be eliminated by resynthesis from citrulline in intestinal and renal cells [7, 8, 13]. This mechanism is aimed at creating an immunosuppressive microenvironment in the bone marrow, which promotes the proliferation of malignant cells [8]. The presence of a high activity of the enzyme arginine/citrulline cycle – ornithine decarboxylase (ODC) potentiates the occurrence and progression of tumors. [10]. The presence of obesity in patients with acute leukemia may contribute to the growth of arginine production as a substrate of arginine/citrulline cycle, which may contribute to the formation of resistance to specific chemotherapy (CT) [2, 11]. From this point of view, it may be of particular importance to study changes in arginine/citrulline cycle on the background of CT in patients with AML with concomitant obesity.

The aim of the study was to investigate changes in the arginine/citrulline cycle in patients with AML with concomitant obesity in the dynamics of standard CT.

MATERIALS AND METHODS OF RESEARCH

After obtaining written consent to conduct a comprehensive examination in accordance with the principles of the Helsinki Declaration of Human Rights, the Council of Europe Convention on Human Rights and Biomedicine and relevant laws of Ukraine, 20 patients with a newly diagnosed AML were included in the study by randomized trial. There were 10 (50%) men and 10 (50%) women, aged 18-77 (main group). Patients underwent examination and treatment in the hematology department of MI "Poltava Regional Clinical Hospital named after M.V. Sklifosovsky PRC" over the period 2010-2019.

The diagnosis of AML was established on the basis of a comprehensive clinical, laboratory and instrumental study in accordance with the requirements of modern medicine [6]. The general condition of patients according to ECOG corresponded to I-II and according to Karnowski's index – 60-80%. The criterion for inclusion in the study was the presence of overweight or obesity. Anthropometric indicators of patients – height and weight were determined. BMI was calculated by the formula: $BMI = \text{weight (kg)} / \text{height (m}^2\text{)}$. The study included patients with AML with a BMI greater than 25. The mean BMI of patients with BMI was $29.35 \pm 0.93 \text{ kg/m}^2$. Viral hepatitis B and C were excluded by screening markers. The control group consisted of 20 healthy individuals, including 9 (45%) women, 11 (55%) men aged 22-26 years.

Patients with AML received a course of induction of remission "7+3" and "5+2", including cytarabine and anthracycline antibiotic (doxorubicin, idarubicin), for variants M4 and M5 of acute myelomonoblastic and monoblastic leukemia respectively, remission courses "7+3+ etoposide" and "5+2+ etoposide" were used [6].

Examination of patients was performed twice: before CT and on the 28th day. The following indices of the general analysis of blood were analyzed: erythrocytes, hemoglobin, leukocytes, thrombocytes. The content of arginine [3], citrulline [9], arginase activity [4] and ODC [5] was studied in blood serum.

Statistical processing of the obtained results was performed using the statistical program GraphPad Prism version 5.00 (GraphPad Software, Inc., San Diego, CA, USA, license number U1048-12MC), which allows for parametric and nonparametric statistical analysis. With the normal distribution of data, the results were presented in the form of arithmetic means (M) and their error (m). Significance of differences was calculated using Student's t test (t). Paired nonparametric methods of Wilcoxon

rank criteria (W) were used for the distribution that differs from the normal one. The relationship between the studied indicators was evaluated using Pearson correlation analysis (r). Differences at $p < 0.05$ were considered statistically significant [1].

RESULTS AND DISCUSSION

In the onset of oncohematological disease, changes in the general analysis of blood of the examined patients corresponded to the full-scaled picture of AML, which were characterized by an increase in the level of leukocytes by 4.6 times ($p=0.04$ by t and W criteria) with a simultaneous decrease in erythrocytes by 1.8 times ($p=0.0001$ by t test), hemoglobin – by 1.7 times ($p=0.0001$ by t and W criteria), platelets – by 3.4 times ($p < 0.0001$ by t and W criteria) compared with almost healthy individuals (Table 1).

In the serum of patients with AML with concomitant obesity a decrease in arginine content by 3 times ($p < 0.0001$ according to t and W criteria) compared with normal was revealed. At the same time, the patients we examined showed an 3.9-fold increase in arginase activity compared with almost healthy ones ($p < 0.0001$ according to t and W criteria). Our results coincide with the results of other studies that prove a decrease in arginine levels on the background of oncohematological diseases, including AML [8, 13]. The decrease in the concentration of arginine in the serum of patients with AML may be due to the high level of arginase synthesis by blast cells [8].

In patients of the main group in the serum an increase in ODC activity by 1.9 times ($p=0.0007$ by t and W criteria) compared with control was revealed. Clinical studies have shown that against the background of various types of cancer, there is an increase in the activity of ODC as a factor that potentiates tumor progression [10]. In parallel, in the serum of patients with AML with concomitant obesity an increase in citrulline by 7.6 times ($p=0.0001$ by t and W criteria) was recorded, which is an intermediate product of nitric oxide (NO) in the reaction of arginine metabolism under the influence of synthase NO (NOS), preferably of inducible form. The arginase and NO-synthase pathways of arginine conversion are considered to be competitive [7]. A strong inverse correlation was found between the concentration of citrulline and the activity of ODC in the serum of patients with AML with concomitant obesity ($r = -0.61$; $p < 0.05$ Pearson). However, against the background of AML manifestation we found the activation of both pathways: arginase and NO-synthase. The latter causes the formation of oxidative stress due to excessive production of NO [13].

After the first course of induction of remission, there was a tendency to normalize the indices of the general blood test, which was characterized by an increase in erythrocytes by 1.2 times ($p=0.0002$ by t and W criteria), hemoglobin – by 1.3 times ($p=0.0001$ by t and W criteria), platelets – by 2.7

times ($p=0.0002$ by t and W criteria) compared with the initial examination (Table 1). CT in the main group of patients was accompanied by a 6.8-fold decrease in the level of leukocytes ($p=0.004$ by t and W criteria) (Table 2).

Table 1

Indices of general blood test in patients with AML in dynamics of CT, $M \pm m$

Indices	Practically healthy (n=20)	AML (n=20)	
		Before CT	After CT
Erythrocytes $10^{12}/l$	4.54±0.05	2.43±0.18*	2.95±0.07&
Hemoglobin, g/l	136.4±1.70	78.25±5.78*	99.8±1.74&
Leukocytes $10^9/l$	6.15±0.19	28.27±8.85*	4.16±0.34&
Platelets $10^9/l$	210.2±4.76	61.10±9.44*	162.8±20.35&

Notes: significant difference: * – $p<0.05$ –between indices of patients with AML before CT and practically healthy; & – $p<0.05$ – between indices of patients with AML before and after CT and practically healthy.

On the 28th day of treatment in the serum of patients with AML with concomitant obesity against the background of CT there was a decrease in arginine concentration by 1.3 times ($p<0.0001$ by t and W criteria), arginase activity – by 2.2 times ($p=0.0001$ by t and W criteria), ODC – by 1.8 times ($p<0.0001$ by t and W criteria), citrulline content – by 2.4 times ($p<0.0001$ by t and W criteria). Such changes can be explained by the effectiveness of CT and the reduction in tumor volume. After CT in patients with AML with concomitant obesity, there was a high direct correlation between BMI and arginine concentration ($r=+0.63$; $p<0.05$ according to Pearson). This fact may confirm the impact of obesity on the effectiveness of specific therapy, as

the growth of BMI is associated with higher rates of arginine as an important substrate for maintaining the viability of cells, including tumor cells [8, 11].

Thus, arginine/citrulline disorders were found in patients with AML with concomitant obesity, which were characterized by a decrease in serum arginine content, an increase in arginase and NO-synthase activity. CT performed led to normalization of hemogram of patients, which was associated with inhibition of arginase activity, ODC, as well as a decrease in the content of citrulline in the serum of patients. Overweight and obesity affected the serum levels of arginine in patients with AML, which may contribute to the development of resistance to CT.

Table 2

Indices of arginine, arginase, ODC, citrulline in the serum of patients with AML in the dynamics of CT, $M \pm m$

Indices	Practically healthy (n=20)	AML (n=20)	
		Before CT	After CT
Arginine, $\mu\text{Mol}/l$	93.65±3.61	30.87±1.55*	23.89±0.81&
Arginase, mMol/l	3.09±0.44	11.82±0.14*	5.38±0.26&
ODC, nkatal	1.48±0.27	2.86±0.91*	1.62±0.13&
Citrulline, $\mu\text{Mol}/l$	56.31±2.47	428.7±18.67*	178.6±8.13&

Notes: significant difference: * – $p<0.05$ –between indices of patients with AML before CT and practically healthy; & – $p<0.05$ – between indices of patients with AML before and after CT and practically healthy.



CONCLUSIONS

1. In patients with AML with concomitant obesity in the blood serum there was revealed an increase in arginine content by 3 times, arginase activity – by 3.9 times, ODC – by 1.9 times, citrulline concentration - by 7.6 times.

2. CT was accompanied by an increase in the level of erythrocytes by 1.2 times, hemoglobin – by 1.3 times, platelets – by 2.7 times with a simultaneous decrease in the number of leukocytes by 6.8 times compared with the initial examination.

3. After CT in patients with AML with concomitant obesity there was a decrease in the concentration of arginine by 1.3 times, arginase activity – by 2.2 times, ODC by – 1.8 times, citrulline content by 2.4 times.

Conflict of interest. The authors declare no conflict of interest.

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