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Results of bone marrow immunocompetent function in critical patients

Abstract:

In body with critical state it is studied the puncture morphology of bone cerebral and is defined the consistence of nitric oxide and peroxide-radicals.

It is established that during the critical condition of body, in bone cerebrals together with morphology changes takes place appearance of nitric oxide and peroxide radicals.

Key-words; Bone marrow, immunocompetent, critical patients.

Introduction:

Bone cerebral is not studied during the critical states. Though, if we take into consideration, that critical state is often associated with hemorrhage, intoxication, inflammatory processes and with other pathology, consequently takes place quantitative and qualitative changes of myeloid elements, then existence of significant changes are not excluded.

Herewith, in critical patients various pathologic processes (global and local hippo-perfusion, endothelia injure, acidosis and etc) are related to oxygen and nitric reactive radicals predominance and irregular production, what possibly leaves significant footprint on critical conditions processes and helps in bone cerebral changes formation;

Material and methods:

There are examined adult patients of 27 years old (20 male and 7 female); critical condition was caused with hemorrhagic insult (10 patients), ischemic insult (8 patients) polio-trauma (4 patients); cranium heavy trauma (5 patients) all patients had after-effect contagion complications (trachea bronchitis, sepsis, pneumonia, cystitis). All patients were under lungs artificial ventilation. A treatment was conducted with water deficiency replenishment and correction of metabolism, parentheral and enthrals nutrition Antibiotic therapy and intensive therapy, other traditional arrangements.

Material for studies was taken from breast bone punctuate 2-3 breast bone spaces. Before taking the material there was conducted Kasirski's needle dehydration, because hydro fusion injures cellular elements. Taking into consideration that punctuate becomes ferment very fast, it is needed to dilute it very fast (to count cariosities) and for pattern preparation. Following treatment of punctuate pattern is done as it is done with blood pattern. First of all it is calculated the number of megakaryocytic. Punctuate dilution and calculation of megakaryocytic in bone cerebrals is done in Phuks-Rozentals calculation cell. Finally 1 MKL punctuate is multiplied on punctuate dilution (20) and divided on cell capacity.

Also it is conducted electronic-paramagnetic resonance analysis (EPR) nitric oxides definition was implemented by M.Gallegan and other methods. Cellular culture spectres registration was conducted on radio-spectre-meter PE -1307, which operates on frequency 9.77 Hr by modulation frequency

80 KHz liquid nitrogen temperature (-196 ºC). In order to define the free nitric oxidize is used spin-snare – natrium diethyl-dithyo-carbonic (DETC) – sigma. (dozed by 10 mg 0.6 X 106 on cell 0.5 ml in area) and Fe 2+ citrate (0.5 mg FeSO4. 6H2O+25mg citrate of atrium 10 mg 0.6 X106 on cell 0.5 ml in area) NO – Fe 2+ - (DETC) 2 complexes EPR spectres were defined with liquid nitric temperature on microwave frequency 20 mvt, concerning definition of peroxide-radicals (LOO) was used spin-snare-phenyl - tert – butyl neutron (PBN) (sigma) with doze 50g 0.6 X106 on cell 0.5 ml in area. The EPR spectre of LOO was defining on room temperature on microwave frequency 20 mvt.

Results and discussion:

From analysis it is evident that in the patients with critical condition bone cerebral cells in some medicine was revealed toxic graining in neytrophils. The results of the study is presented in tables N 1 and N 2 where it is seen , that in bone cerebral of patients with critical state, neytrophils myelocytis are in norm (8.4 ± 1.1) on the third day it decreases, (6.5 ± 1.8) but it still stays in the norm frames. It is marked the increase of the metalomietocitis (7.1 ± 0.5 – N 7.7 ± 0.7 P> 0.5) what is concerned on neytrophils segment nucleus, their amount6 is in critical patients is augmented. (34.7 ± 1.4 – N - 36.4 ± 2.1 P> 0.5) the amount of neytrophils bacillus nucleus increases (12.6 ± 0.5 – N – 13.5 ± 0.7 P> 0.5) neytrophils is in the norm frame (0.96 ± 0.1 – N -1.4 ± 0.2 P> 0.1)

Concerning lymphoid elements, lymphocytes number is augmented (9.0 ± 0.6– N - 10 ± 0.7 P> 0.5). The numbers of monocitus do not change (1.3 ± 0.1– N – 1.3 ± 0.2 P> 0.5).

Table I

#	Statistic figures	Neitrophilic myelocitus %	Neitrophilic Metamyelocitus %	Neitrophilic Bacillus-ucleus %	Neitrophilic Segment-Nucleus %	Neitrophilic Promielocitus %	Eozenophilic Segment – Nucleus %	Lymphocits %	Monocitus %	Bazophilic Normoblasts %
1	M ± m n	8.4±1.1 27	7.1±0.5 27	12.6±0.5 27	34.7±1.4 27	0.96±0.1 27	2.4±0.2 27	9.0±0.6 27	1.3±0.1 27	2.0±0.4 27
2	M ± m n t P 1/2	6.5±1.8 16 0.901<0.5	7.7±0.7 16 0.698<0.5	13.5±0.7 16 1.047<0.5	36.4±2.1 16 0.6742. 208<0.5	1.4±0.2 16 1.964<0.1	1.7±0.2 16 2.473<0.05	10.0±0.7 16 1.085<0.5	1.3±0.2 16 <0. 5	2.1±0.5 16 0.640<0. 5

Basophilic normoblasts are augmentatedin patients with critical conditions (2.0 ± 0.4– N – 2.1 ± 0.5 P> 0.5), it has to be remarked the significant decrease of policromathohilic normoblasts. (4.6 ± 0.96– N – 2.1 ± 0.6 P> 0.005) the number of oxiphilic normoblasts their quantity increases.

(6.9 ± 0.7– N – 9.0 ± 0.4 P> 0.5) plasma is in the norm frames (0.8 ± 0.1– N – 1.0 ± 0.1) and the number of blastic cells (0.6 ± 0.04– N – 0.5 ± 0.1).

As to the Electronic – paramagnetic resonance analysis outcome, it is given in the table N 3:
Data of Bone Cerebral Electronic – paramagnetic resonance analysis during the critical conditions:

Table II:

#	Statistic figures	Policromatophililic normoblastsn %	Oxophilic normoblasts%	Blastic cells %	Proeritroblasts %	Plazmocists %
1	M ± m N	4.6±0.96 27	6.9±0.7 27	0.6±0.04 27	0.4±0.2 27	0.8±0.1 27
2	M ± m n t P ½	2.1±0.6 16 2.208 <0.05	9.0±0.4 16 2.605>0.05	0.5±0.1 16 1.0>0.5	0.7±0.1 16 3.7<0.01	

Table III

#	Patients Category	Statistic figures	NO	LOO
1	Patients in critical condition, Day I	M ± m n	18.1 ± 5.4 10	4.0±0.3 10
2	Patients in critical condition, Day II	M ± m n t p	13.4 ± 1.1 4 0.853>0.5	1.3±0.4 4 5.4<0.01

As it is seen according to the table above during the critical condition takes place appearance of nitric oxides' and peroxide-radicals. (NO – 1.8 ± 5.4 and LOO – 4.0 ± 0.3) this similarly indicates existence of oxidation stress (peroxidation) in critical patients, what gives hand to the development of apoptosis. After treatment, on the third day is marked decrease of the nitric oxide radicals. (13.4 ± 1.1 P> 0.5) and also significantly decreases the level of peroxide-radicals (1.3 ± 0.4 P<0.01).

Materials and methods:

There were investigated 30 patsients (100 %) in critical condition. Clinical description of the patients is given in table # 1. female were 11. male-19 and age of patients was above 40 years. In 10 cases the critical condition was associated with an ischemic stroke. 7 - with hemorrhagic stroke. 3-with postreanimation disease. 4-with pneumonia.2-with polytrauma. 2-with cardiogenic shock and 2-with cranial trauma.

The management of critical condition was performing by state standard about treatment of critical condition (Z. khelidze 2002) which contains:the artificial ventilation of lungs. Recovering blood circulation. Correction of acid-base balance, water and electrolytic balance, analgesic and sedation antioxidant and antibacterial therapy and other medical avents.

The special methods of research are presented as studding a number of bone marrow and peripheral blood stem and immune competent cells. In time of research of stem and immune

Cells in the heparinzed bone mrrow and peripheral blood we were studying CD3,CD4,CD8,CD34,CD72.

In table #4-5 are given the data of changes of examined cells (CD3,CD4,CD8,CD34,CD72) after treatment by electrical impulses in bone marrow and peripheral blood.

The cytokines are the mediators of intercellular interaction and play the important role in regulation of the organisms reaction. Any inflammatory reaction in the organism is formed with the participation of cytokines. Its quantitative definition has a great importance in assessment of pathological process activity, in disease recognition and in its flow gravity as well as in monitoring of the treatment efficiency and in prognosis of the disease outcome.

48 patients were surveyed, who passed the course of treatment in the institute of critical medicine, age range – from 35 up to 82 years.

Including diseases:

- Ischemic stroke-14
- Hemorrhagic stroke-14
- Cardiac failure-14
- Sepsis-8

In all patients multiple organ failure was detected with additional diseases such as: diabetes, atherosclerosis, pneumonia, chronic cardiac insufficiency. All the patients were treated with traditional method. All of them were on the artificial respiration and parenteral nutrition. The drug treatment was administered according to the symptoms. The patients were examined on the first, sixth and seventh day after their hospitalization. It should be mentioned that the examination of cytokines concentration of cytokines is not determined almost at all.

The cytokines were determined by the method of immune-enzyme analysis! The results were calculated by immune-enzyme reader RAITO 2100. During the researches the immune-enzyme analysis test-systems Vector-Best manufactured by Russia.

Methods	groups	IL4	IL6 <30϶g/ml	IL8 30϶g/ml	IL10	TNF-α
bone mrrow	before treatment	2,5±0,2	75,6±4,4	76,4±15,3	10,4±1,0	12,0±1,6
	after	3,6±1,0	36,7±4,2	89,4±12,2	12,0±1,4	6,2±1,6
	treatment	>0,05	<0,001	>0,05	>0,05	<0,05

As it is seen from the table the profile of the cytokines are always changed in critical situations. At the same time the proinflammatory cytokines are increased especially before the treatment. In the process of treatment the concentration of cytokines (IL-6, IL8, TNF) in blood and in CFS in some cases is decreased, and the concentration of anti-inflammatory cytokines (IL4 and IL10) is increased.

In cases of 48 patients, 28 of them had a fatal outcome. The situation of 10 patients having perceptible increase of IL-10 in liquor and peripheral blood has been improved. The situation was improved in cases of those patients having the perceptible decrease of IL-6 after the treatment start. Thus, during our researches the perceptible increase of proinflammatory cytokines has been determined in critical patients. The increase of IL-10 after the treatment start is a hopeful forecast. The unchanged level of IL-4 and IL-8 is an indicator of situation aggravation and practically, of death. Our researches are the minimal part of explanation of death process mechanism. It is necessary to continue the researches in this direction that is being done at the basis of our institute. We hope that these researches will contribute to the issues of critical situation management.

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ძვლის ტვინის იმუნოკომპეტენტური ფუნქციის შესწავლილი შედეგები კრიტიკულ ავადმყოფებში

გამოკვლეული იყო ზრდასრული ასაკის 27 ავადმყოფი (20 მამაკაცი, 7 ქალი) კრიტიკული მდგომარეობა გამოწვეული იყო ჰემორაგიული ინსულტით. (10 ავადმყოფი), იშემიური ინსულტი (8 ავადმყოფი), პოლიტრავმით (4 ავადმყოფი) და ქალა – ტვინის მძიმე ტრავმით (5 ავადმყოფი). ყველა პაციენტს აღენიშნებოდა ინფექციური გართულებები (ტრაქეობრონქიტი, სეფსისი, პნევმონია, ცისტიტი და სხვა) იმუნური პასუხის კვლევასთან ერთად ჩატარებულია ძვლის ტვინის კვლევა, ელექტრულ-პარამაგნიტური რეზონანსის მეთოდით. გამოკვლევებიდან ჩანს, რომ კრიტიკულ მდგომარეობაში მყოფ ავადმყოფთა ძვლის ტვინის უჯრედებში ადგილი აქვს აზოტის ოქსიდისა და პეროქსირადიკალების გაჩენას. (NO-1,8±5,4 და LOO-4,0±0,3) ასევე ინახა იმუნოკომპეტენტურ უჯრედთა რაოდენობის მნიშვნელოვანი ცვლილებები. სახელდობრ; იმუნოკომპეტენტური უჯრედების ყველა მაჩვენებელი იცვლება. მომატებულია სტატისტიკურად სარწმუნოდ. კერძოდ, CD3-11%, CD4-12%, CD8-10%, CD34-90%, CD72-11%.