Higher levels of creatine phosphokinase in the patients with long-term consequences after traumatic brain injury

Abstract. Background. The secondary traumatic brain injury (TBI) consequences are associated with multiple cascades of biochemical reactions caused by initial neurotrauma. We assessed changes in radical oxidation and marker membrane-associated enzymes involved into various redox reactions reflecting basic metabolic processes by measuring creatine phosphokinase (CPK), CPK-BB, adenosine triphosphate (ATP), adenosine diphosphate (ADP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase (LDH) in the patients with long-term consequences after TBI. Materials and methods. Eighty-two patients (mean age (±SD) 39.04 ± 14.62 years), thirty controls (29.60 ± 4.73 years) were tested for serum CPK, CPK-BB, ATP, ADP measured using spectrophotometry methods according to standard manufacturer’s protocols. Serum AST, ALT, alkaline phosphatase, LDH levels were detected on gas-liquid chromatograph, by calorimetric methods. Results. We found elevated serum CPK level in patients with long-term consequences after TBI compared to controls (P < 0.001, t = 5.073, 95% CI –188.6 to –92.16) with the medians of total CPK of 163.5 ± 14.1 versus 92.6 ± 11.4 pg/ml in the basic and control groups, respectively. We found abnormal high serum CPK-BB level in the basic group with medians of total CPK-BB level of 24.7 ± 2.7 versus 16.14 ± 1.83 IU/L in controls (p < 0.05; t = 4.9). Metabolic changes in our patients were associated with higher LDH content being in direct proportion with disease duration (p < 0.05; r = +0.42); the median of total LDH level was 694.1 ± 16.3 and 381.9 IU/l in basic and control groups, respectively. The patients investigated were detected with deficiency of macroergic compounds: ATP and ADP being in direct proportion with disease duration over 15 years (p < 0.05; r = +0.67); the medians of total ATP level were 627.60 ± 12.38 and 735.48 ± 14.57 μmol/l (p < 0.05) in the basic and control groups; for ADP: 256.20 ± 14.21 versus 273.88 ± 11.42 (p < 0.05), respectively. Conclusions. Patients with long-term consequences after TBI showed higher serum CPK level associated with increased LDH level reflecting bioenergy dyshomeostasis and severity of secondary brain injuries. Keywords: creatine phosphokinase; long-term consequences after traumatic brain injury; bioenergy dyshomeostasis

Introduction

The secondary and delayed traumatic brain injury (TBI) consequences are associated with multiple and interacting cascades of different biochemical reactions caused by the initial neurotrauma [6, 7, 16, 18] whereas the pathogenetic mechanisms maintaining the long-term consequences after TBI are still unclear.

The previous experimental data have reported that in white or grey matter brain injuries, calcium influx is a key initiating element in molecular cascades resulting in both delayed cell death and/or their dysfunction, and delayed axonal disconnections [2, 4, 17, 18]. Many studies have demonstrated that in neurons calcium influx through NMDA channels results in excitotoxicity, generation of free radicals with the development of oxidative stress, mitochondrial dysfunction and postsynaptic receptor modifications [5, 6]. Other foreign reports have shown the presence of astrocytes proliferation that
is typical for central nervous system injury and dysfunction resulting in a reversal of glutamate uptake and neuronal depolarization through various excitotoxic neurobiochemical mechanisms and these mechanisms depend on both energy dysfunction in neurons and primary brain injury severity [7–9, 12]. Inflammatory cells also mediate this secondary brain injury via the release of many pro-inflammatory cytokines that contribute to activation of cell-death cascades or postsynaptic receptor modifications [18, 19]. Any change in bioenergy cell homeostasis is one of the important factors determining its pathological disturbances where some key enzymes are adenosine triphosphate (ATP) that provides energy to all biochemical processes and creatine phosphokinase (CPK) that catalyzes reversible reactions of creatine and ATP to phosphocreatine or adenosine diphosphate (ADP) in muscles, brain, retina and hair cells [11, 13]. Many conditions cause changes in ATP and CPK serum levels, first of all, heart and central nervous system diseases, kidney disease, rhabdomyolysis, certain medications uptake and others [1, 3, 10].

**The purpose of the study.** Given the data about the development of oxidative stress and bioenergy dyshomeostasis reported in the numerous experimental and clinical studies, we assessed the changes in radical oxidation and dynamics of some membrane-associated marker enzymes involved into various redox reactions and reflecting main metabolic processes in the organism by measuring complex compounds such as CPK, ATP, adenosine diphosphate, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase (LDH) in patients with long-term consequences after traumatic brain injury.

**Material and methods**

**Subjects.** Eighty-two patients with long-term consequences after TBI (mean age (±SD) 39.04 ± 14.62 years; mean disease duration (±SD) 9.51 ± 5.70 years) and thirty age-matched healthy controls (HC; 29.60 ± 1.83 IU/L in controls (Table 1). In normal value ranges, BB-isoform serum content usually is less than 1 % and during standard diagnostic tests, we could neglect it. However, at different pathological states, serious cell and intra- or extracellular protein damage was detected following cell disturbances with consequent enzyme activity loss [1, 3, 10, 15]. We found high serum CPK-BB level in the serum samples of all investigated patients with long-term consequences compared to controls (mean ± SD) level were 163.5 ± 14.1 and 92.6 ± 11.4 pg/ml in the basic and control groups, respectively; twenty-four patients (29.26 %) had the increased serum CPK levels up to 182 % of medium CPK value (Table 1).

We found the elevated creatine phosphokinase level in the serum samples of all investigated patients with long-term consequences after TBI compared to controls (Table 1, P < 0.001, t = 5.073, 95% CI –188.6 to –92.16); the medians of total creatine phosphokinase (mean ± SD) level were 163.5 ± 14.1 and 92.6 ± 11.4 pg/ml in the basic and control groups, respectively; twenty-four patients (29.26 %) had the increased serum CPK levels up to 182 % of medium CPK value (Table 1). We found high serum CPK-BB levels in the patients with long-term consequences after TBI that may play a certain role in the pathogenetic mechanisms of maintaining these long-term consequences.

To evaluate the clinical or prognostic value of CPK serum level changes, we further segregated among the groups with the different disease duration. When comparing the CPK content in the patients with different duration of the post-traumatic period, it was found that even the patients with the post-traumatic period less than 5 years (n = 36; 43.9 %) were characterized with up to 156.77 % increase of CPK serum levels (P < 0.05) from the upper normal mean value with the median of total CPK of 149.5 ± 16.3 pg/ml. In the group of patients with the disease duration 5–15
years (n = 31; 37.8 %), the CPK content increased to 165.14 % (p < 0.05) and in the patients with the disease duration over 15 years (n = 15; 18.29 %), the CPK level elevated up to 192.97 % (p < 0.01). In our opinion, this increase of serum CPK level was a marker of increasing of membrane destruction as a result of metabolic disturbances and was a poor prognostic sign.

The standard neurological examination has revealed the presence of focal neurological signs indicating the mesencephalic and brainstem structures lesions. Among the objective focal neurological symptoms, the most frequent were face asymmetry (12 patients from group I (33.3 %); 19 patients from group II (61.29 %) and 14 patients from group III (46.67 %)), visual disturbances were observed in 16.12 % patients from group II and 26.67 % patients from group III. Patients from the first (9.09 %) and second groups (12.9 %) complained of impaired sensitivity as numbness and feelings of insects crawling in the structure of nonspecific sensorimotor syndrome.

Table 1. The dynamics of the markers of membrane-associated enzymes and some biochemical compounds of the oxidative status in the patients with long-term consequences after traumatic brain injury and controls, M ± m

| Parameter              | All patients (basic group) | The post-traumatic period duration | Controls |
|------------------------|---------------------------|-----------------------------------|----------|
|                        | Up to 5 years             | 5–15 years                        | Over 15 years |       |
| ATP (µmol/l)           | 627.60 ± 12.38 p < 0.05   | 654.29 ± 18.09 p > 0.05           | 640.3 ± 14.4 p > 0.05 | 618.8 ± 9.2 p < 0.05 | 735.48 ± 14.57 |
| ADP (µmol/l)           | 256.20 ± 12.21 p < 0.05   | 249.90 ± 15.76 p > 0.05           | 251.80 ± 16.93 p > 0.05 | 249.40 ± 11.34 p < 0.05 | 273.88 ± 11.42 |
| Alkaline phosphatase   | 160.40 ± 18.83 p < 0.05   | 183.6 ± 16.3 p > 0.05             | 189.3 ± 19.5 p > 0.05 | 163.6 ± 14.7 p > 0.05 | 212.60 ± 9.63 |
| LDH (IU/L)             | 694.1 ± 16.3 p < 0.05     | 485.6 ± 32.7 p < 0.05             | 509.4 ± 36.2 p < 0.05 | 672.4 ± 25.2 p < 0.05 | 381.9 ± 28.1 |
| CPK (IU/L)             | 163.5 ± 14.1 p < 0.01     | 149.5 ± 16.3 p < 0.05             | 166.4 ± 12.8 p < 0.05 | 189.7 ± 15.2 p < 0.01 | 92.6 ± 11.4 |
| CPK-BB (IU/L)          | 24.7 ± 2.7 p < 0.05       | 20.65 ± 3.44 p < 0.05             | 22.31 ± 2.30 p < 0.05 | 26.48 ± 1.60 p < 0.01 | 16.14 ± 1.83 |
| AST (IU/L)             | 32.6 ± 6.1 p < 0.05       | 27.6 ± 3.5 p > 0.05               | 28.3 ± 3.2 p > 0.05 | 30.9 ± 6.4 p > 0.05 | 25.2 ± 1.9 |
| ALT (IU/L)             | 23.3 ± 4.6 p > 0.05       | 21.2 ± 3.9 p > 0.05               | 22.9 ± 3.8 p > 0.05 | 25.9 ± 4.1 p > 0.05 | 20.5 ± 1.4 |

Note: p is used for statistically significant results in the group versus HC.
of membrane-associated enzyme abnormalities could provide us with the useful clarification of a cause of the condition.

Our data have shown that the patients with long-term consequences after TBI presented with baseline bioenergy dyshomeostasis appearing as a deficiency of macroergic compounds — ATP and ADP, and this decrease was in a direct proportion with the disease progression in the patient with the disease duration over 15 years \( (p < 0.05; r = +0.67) \); the median of total ATP level was \( 627.60 \pm 12.38 \) and \( 735.48 \pm 14.57 \) umol/l \( (p < 0.05) \) in the basic group and in controls, respectively, and for ADP levels: \( 256.20 \pm 14.21 \) versus \( 273.88 \pm 11.42 \) (\( p < 0.05 \)), respectively.

These biochemical compounds partly reflect the appearance of oxidative stress reaction contributing to the development of oxidative dyshomeostasis in this category of the patients. According to the numerous literature data, TBI results in calcium dysregulation in mitochondria \([4–8, 20]\); the results of our study have also confirmed the presence of oxidative stress, which have to be accounted in the treatment options. Because the patients and controls were not gender-matched, CPK and LDH levels were compared between men and women. The levels were not found to be comparable between male and female patients with long-term consequences after TBI \( (P = 0.196 \) for CPK and \( P = 0.272 \) for LDH) and controls. In the same samples, biochemical data did not correlate with the age, onset age and ATP and ADP serum levels (for CPK: \( P_1 = 0.824 \) for age, \( P_2 = 0.673 \) for onset age, \( P_3 = 0.54 \) for ATP and \( P_4 = 0.59 \) for ADP and for LDH: \( P_1 = 0.72, P_2 = 0.591, P_3 = 0.43; P_4 = 0.57 \), respectively). Our results showed no significant relationships between LDH and CPK levels and bioenergy dyshomeostasis as in patients the serum ATP levels remained low during this time.

Thus, in this study, the effect of TBI oxidative stress parameters was assessed where a generation of reactive oxygen species in the pathogenesis of maintaining of long-term consequences after traumatic brain injury plays a definite role in neuronal and neurological dysfunction and where CPK levels may serve as a biochemical marker of severity of long-term consequences after TBI. Some authors have shown that possible mechanism for releasing of CPK was transient membrane rupture after trauma or inflammation; also, some experimental studies showed that another way to CPK release might be the possible cell necrosis leading to CPK release \([3, 4, 6, 15]\).

Long-term consequences after TBI is a pathological condition leading to progressive neuronal dysfunction where changes of some marker membrane-associated enzymes and various biochemical compounds of the oxidative status partly reflects the development of oxidative stress associated with bioenergy dyshomeostasis. Our study describes the detection of CPK as a brain biomarker to determine the severity of the secondary brain injury in patients with long-term consequences after TBI.

Conclusions

The patients with long-term consequences after TBI showed the abnormal high serum creatine phosphokinase levels associated with increased LDH levels reflecting the bioenergy dyshomeostasis and severity of the secondary brain injuries.

Conflicts of interests. Author declares the absence of any conflicts of interests and his own financial interest that might be construed to influence the results or interpretation of their manuscript.

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Підвищений рівень креатинфосфокінази у хворих із віддаленіми наслідками після перенесеної закритої черепно-мозкової травми

Резюме. Актуальність. Формування віддалених наслідків закритої черепно-мозкової травми (ЗЧМТ) супроводжується каскадом біохімічних реакцій, які ініціюються первинною нейротравмою. Мета роботи. Ми оцінювали стан і зміни радикального окислення та маркерних мембранасоційованих ферментів, що беруть участь у різних окислительно-восстановительних реакціях та відображають основні метаболічні процеси організму, що беруть участь у різних окислительно-восстановительних реакціях та відображають основні метаболічні процеси організму, що беруть участь у різних окислительно-восстановительних реакціях та відображають основні метаболічні процеси організму.

Мета роботи. Ми оцінювали стан і зміни радикального окислення та маркерних мембранасоційованих ферментів, що беруть участь у різних окислительно-восстановительних реакціях та відображають основні метаболічні процеси організму, що беруть участь у різних окислительно-восстановительних реакціях та відображають основні метаболічні процеси організму, що беруть участь у різних окислительно-восстановительних реакціях та відображають основні метаболічні процеси організму.

Методи. Вісімдесят два хворі (середній вік (±SD) 39,04 ± 14,62 року), тридцять здорових осіб (29,60 ± 4,73 року) були протестовані на рівень креатинфосфокінази (КФК), КФК-ВВ, аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznитрифосфат (АТФ), аденоznite...
у пацієнтів з отдаленними наслідками ЗЧМТ. Матеріали і методи. Восемь два болючих (середній вік (±SD) 39,04 ± 14,62 роки), тридцять здорових пацієнтів (29,60 ± 4,73 роки) були протестовані на рівень КФК, КФК-ВВ, АТФ, АДФ, які були визначені в сировинні крові спектрофотометричним методом в застосуванні з прилагодженнями стандартними інструкціями. АЛТ, АСТ, ще- лочна фосфатаза, ЛДГ були виміряні хроматографічним і колоріметричним методами. Результати. Було виявлено підвищений рівень КФК в сировинні крові пацієнтів з отдаленими наслідками ЗЧМТ порівняно з контролью (р < 0,001; t = 5,07), середній рівень КФК склав 163,5 ± 14,1 проти 92,6 ± 11,4 мкмоль/л у здорових людей. Ми були виявлені високий рівень КФК-ВВ в сировинні крові в обійняні пациентів, у яких середній рівень КФК-ВВ склав 24,7 ± 2,7 проти 16,14 ± 1,83 Е/л в контрольній групі (р < 0,05; t = 4,9). Метаболічні зміни у пацієнтів заключилися в більших показниках ЛДГ, які були прямо пропорційно зростанню тривалості посттравматичного періоду (р < 0,05; r = +0,42); в обійняні і контрольній групах середній рівень ЛДГ склав 694,1 ± 16,3 і 381,9 Е/л, відповідно (р < 0,05). У обслідуваних болючих було виявлено дефіцит макроелектрічних соєній, а іменого АТФ і АДФ, які були у відповідності з декількою змінами, особливо більші 15 років (р < 0,05; t = +0,67); в обійняні групі пацієнтів середній рівень АТФ склав 627,60 ± 12,38 мкмоль/л по споріянню з 735,48 ± 14,57 мкмоль/л в контрольній групі (р < 0,05); для АДФ: 256,20 ± 14,21 проти 273,88 ± 11,42 (р < 0,05) відповідно. Висновки. У пацієнтів з отдаленими наслідками ЗЧМТ було виявлено високий рівень об'ємної активності КФК в сировинні крові і підвищення рівня ЛДГ, що відображає біоенергетичний дисгомеостаз і тяжкість течії отдалених наслідків травми мозга. Ключові слова: креатинфосфокіназа; отдалені наслідки трави закритої черепно-мозової травми; біоенергетичний дисгомеостаз