

SOME METABOLIC PROCESSES IN THE PATIENTS WITH LONG-TERM CONSEQUENCES OF MILD TRAUMATIC BRAIN INJURY

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Background. Mild traumatic brain injury (mTBI) leads to disturbance of various metabolic processes significant in pathogenesis of the maintaining of long-term consequences after it.

The objective of the research was to analyse changes in the activity of some membrane-associated enzyme markers, which are involved in different redox reactions, reflecting main metabolic processes.

Methods. Forty-seven patients with long-term consequences of mTBI, thirty controls were enrolled. The levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase were evaluated in sera by gas-liquid chromatograph and calorimetric methods.

Results. The study revealed significant changes in metabolic processes observed for alkaline phosphatase and LDH, which were the indicators of membrane and redox processes disturbances, acidosis severity and impaired energy cell metabolism. The averages of LDH level was 662.7 versus 381.9 U/L, in the controls. The disease progression was followed by directly proportional LDH increase reaching very high values in the patients with disease duration more than 15 years (mean \pm SD 144.6 \pm 16.3 versus 82.6 \pm 8.4 U/L, controls $p < 0.05$). The long-term consequences of mTBI were characterized by statistically significant decrease of alkaline phosphatase and positive dependence ($p < 0.05$) of it ($r = +0.48$) on the disease duration with the averages of alkaline phosphatase level of 152.5 \pm 11.21 versus 212.6 \pm 9.63 U/L, controls ($p < 0.01$). The significance of changes in membrane-associated enzymes serum levels correlated with development of oxidative stress and metabolic processes dysfunction.

Conclusion. In the patients with long-term consequences of mTBI, dysregulation of enzymes activity was detected that might be a marker of nervous system energy impairment and membranes destruction.

KEY WORDS: long-term consequences of mTBI; membrane-associated enzymes; metabolic processes.

Introduction

Mild traumatic brain injury is a leading cause of disability in young people [2, 21]. Neurotrauma leads to disturbance of various types of metabolic processes that are significant in the pathogenesis of maintaining of long-term consequences after it [9, 12, 15]. It is established that metabolic disturbances are based on biochemical reactions proceeding with participation of enzymes located in various parts of the cell [3, 7, 22]. Most of these enzymes are inside the cell or mitochondria and appear in blood or cerebral spinal fluid only when cells are damaged, and this is of great diagnostic and prognostic importance. Thus, the changes in any parameters of membrane-associated

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enzymes evidence for disturbances of metabolic processes of biochemical redox reactions, cell or mitochondria destruction or development of membrane pathology [1, 4, 18, 20].

Enzymes are proteins, each type has a unique function facilitating catalyzation of routine and vital chemical reactions in the organisms [5, 17, 19]. Among the most sensitive and widely used membrane-associated enzymes are aspartate aminotransferase (AST) and alanine aminotransferase (ALT); in addition to them, alkaline phosphatase and lactate dehydrogenase (LDH) are the other interesting enzymes. LDH is not specific to the liver and can be elevated in many other diseases related to inflammation in tissues [16, 18]. Human studies have shown that serum levels of autoantibodies (anti-nuclear antibody, anti-smooth muscle antibody, and anti-liver and kidney microsomal antibody) are elevated in patients with autoimmune hepatitis and some rare muscle neurodegenerative disorders [6, 8, 10]. Symptoms of

long-term consequences after mild traumatic brain injury can or cannot be present in the patients with mild increase of metabolic enzymes [4, 13, 21] and symptoms of posttraumatic period are not specific, thus the membrane-associated enzyme abnormalities can sometimes provide us with the useful clarification of a cause of the condition.

According to these issues, **the objective of research** was to analyze the dynamics of changes in the activity of some indicators of membrane-associated enzymes, which are involved into certain redox reactions, reflecting the main metabolic processes in the patients with long-term consequences of mild traumatic brain injury.

Methods

Forty-seven patients with long-term consequences of mild traumatic brain injury (mTBI) of the average age of 47.41 ± 9.37 years old (16 women, 34.05% and 31 men, 65.95%) and the average disease duration (mean \pm SD, 12.67 ± 8.92 years old) were enrolled into the study. The definition of mTBI was consistent with the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD-10; 1992) [2]. Thirty healthy volunteers (Control; mean \pm SD, 35.6 ± 9.21 years) without neurological and psychiatry diseases were involved as well.

In the anamnesis, the investigated patients suffered from concussion ($n=18$; 38.29% among them there were eleven women, 61.11% and twenty men, 38.9%) and mild brain contusion ($n=29$; 61.71%, 17.24% women and 82.76% men). The patients were treated with symptomatic therapy. Inclusion criteria involved the patients of above 18 years of age with nonpenetrating head injury and initial Glasgow coma scale score more than 7 (mean \pm SD, 10.37 ± 2.14). Exclusion criteria were craniectomy and sepsis in the anamnesis, pregnancy, preexisting neurologic diseases, acute cardiovascular diseases, respiratory failure, any acute or chronic liver diseases.

Clinical data of each case were retrieved from the patients' history. Physical and neurological examinations were performed for all patients, CT and/or MRI, EEG recording were retrieved as well. Neurological examination revealed focal neurological signs, indicating mesencephalic and brainstem structures lesions; the most frequent were tendon reflexes increase in 23 patients (41.07%), coordination disturbances - 21 patients (37.5%), ataxia -

15 patients (26.78%), horizontal nystagmus - 11 patients (19.64%), pathological foot reflexes - 8 patients (14.28%) and rotator nystagmus - 5 patients (8.93%).

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, lactate dehydrogenase (LDH), creatine kinase and gamma-glutamyl transpeptidase (GGT) were detected in sera by the gas-liquid chromatography and the calorimetric methods according to the standard protocols provided by the manufacture using commercially available human ELISA kits (Clinical Biochemistry Department, Kharkiv National Medical University). Controls and individual sampling were performed in duplicate manner with a coefficient of variation of $<10\%$ (P values ≤ 0.05); all protocols were approved by the local Ethics Committee; written informed consents were obtained from all patients.

Age and disease duration were compared between the groups by the χ^2 test; parametric tests were used for normally distributed data; nonparametric tests were used for abnormal distributed data; Kruskal-Wallis and Mann-Whitney U tests were applied in Prism regarding the differences between groups, the multivariate analysis considering covariates was performed.

Results

The results of all patients examinations have revealed the significant changes in membrane-associated enzymes serum levels manifested as the increase of creatine kinase (CK) serum levels by 41.4%, LDH by 73.5% and decrease of alkaline phosphatase serum levels to 28.3% and a tendency of GGT serum level decrease to 18.7% in the general clinical group (Table 1).

Analysis of the dynamics of the membrane-associated enzymes markers in the patients with long-term consequences of mTBI proved that the most severe and statistically significant changes were observed for two enzymes: alkaline phosphatase and LDH, which are the indicators of cell or mitochondria membrane damage, acidosis severity, isolation of redox processes and phosphorylation, impaired energy metabolism in cells.

Metabolic changes in the examined patients evidenced that the content of serum LDH, a key glycolysis enzyme, was increased in almost all patients, and this increase was in a direct proportion with the disease progression or longer posttraumatic period ($p < 0.05$; $r = +0.36$). We found the elevated LDH level in serum

samples of the investigated patients compare to HC ($p=0.01$, $t=5.08$); in the general patient group the medians of total LDH level was 662.7 U/L and 381.9 U/L, in controls, respectively.

To evaluate the clinical prognostic value of LDH changes we further segregated among the different disease duration groups. When comparing the LDH serum level in the patients with different duration of the post-traumatic period after mTBI, it was proved that the patients with the disease duration no longer than 5 years ($n=14$; 29.78%) were characterized by increase of LDH serum levels by 27.8% ($p>0.05$). In the patient group with the disease duration from 5 to 15 years ($n=11$; 23.4%), the LDH was increased to 78.6% ($p=0.05$) with average level of 682.4 ± 24.8 U/L, and in the patients with the disease duration more than 15 years ($n=22$; 46.8%), the LDH was increased to 105.2% ($p=0.01$) with average level of 783.6 ± 31.4 U/L.

The revealed increase of LDH serum level, in our opinion, was a marker of increased membrane destruction as a result of metabolic disturbances. It should be noted that in the patients with long-term consequences for more than 15 years, LDH serum level was higher compare to HC and average group indicators, which, in our opinion, was a poor prognostic sign.

When comparing the alkaline phosphatase serum level in the patients with different dura-

tion of post-traumatic period after mTBI, it was found that the patients with the disease duration no longer than 5 years was characterized by 8.5% decreasing ($p>0.05$) of alkaline phosphatase serum level. In the patients with the disease duration between 5 and 15 years, the mean serum level of alkaline phosphatase was decreased by 24.1% ($p>0.05$); in the patients with the disease duration more than 15 years, alkaline phosphatase level was decreased by 27.6% ($p<0.01$) in sera.

At the same time, further disease progress or longer posttraumatic period was accompanied by directly proportional increase of CK serum levels: the values remained higher than the controls with an average of (mean \pm SD) 116.8 ± 19.4 U/L ($p<0.05$) in the general group and reached statistically significant values in the patients with the disease duration more than 15 years (mean \pm SD 144.6 ± 16.3 U/L versus 82.6 ± 8.4 U/L controls; $p<0.05$). However, long-term consequences of mTBI revealed quite significant increase in CK level by 50.3% compare to the patients of the subgroup with the duration of the posttraumatic period less than 5 years. This correlated positively with a higher frequency of various neurological syndromes ($p<0.05$; $r=+0.63$) for long-term consequences of mTBI and might be a biochemical marker of the latter neurological deficit.

Table 1. The dynamics of the markers of membrane-associated enzymes serum levels in the patients with long-term consequences after mild traumatic brain injury

Data	All patients (general group)	Dependence from the post-traumatic period duration		
		up to 5 years (stage 1)	from 5 to 15 years (stage 2)	more than 15 years (stage 3)
AST, (U/L)	29.6 ± 3.3 $p>0.05$	26.6 ± 2.9 $p>0.05$	27.3 ± 4.8 $p>0.05$	30.3 ± 7.1 $p>0.05$
Controls	$25,2\pm 1,9$			
ALT, (U/L)	22.8 ± 3.9 $p>0.05$	21.8 ± 4.2 $p>0.05$	21.4 ± 2.1 $p>0.05$	23.5 ± 3.7 $p>0.05$
Controls	$20,5\pm 1,4$			
Alkaline phosphatase, (U/L)	152.5 ± 11.21 $p<0.01$	194.6 ± 6.7 $p>0.05$	161.3 ± 15.3 $p>0.05$	153.9 ± 9.8 $p<0.01$
Controls	$212,6\pm 9,63$			
LDH, (U/L)	662.7 ± 21.9 $p=0.01$	488.3 ± 36.3 $p>0.05$	682.4 ± 23.1 $p=0.05$	783.6 ± 18.4 $p=0.01$
Controls	$381,9\pm 28,1$			
CK, (U/L)	116.8 ± 19.4 $p<0.05$	109.5 ± 27.1 $p>0.05$	126.4 ± 22.6 $p>0.05$	144.6 ± 16.3 $p<0.05$
Controls	$82,6\pm 8,4$			
GGT, (U/L)	14.8 ± 6.2 $p>0.05$	19.6 ± 3.5 $p>0.05$	15.9 ± 9.3 $p>0.05$	14.1 ± 4.2 $p>0.05$
Controls	$18,2\pm 1,4$			

Note. p – significance compared to the control group.

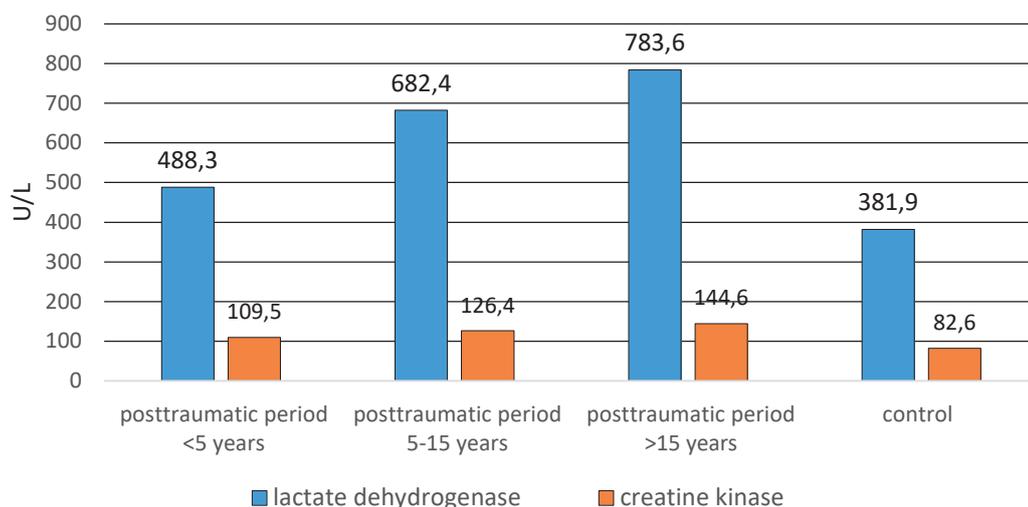


Figure 1. Analysis of the dynamics of lactate dehydrogenase and creatine kinase levels in the patients after traumatic brain injury with different duration of posttraumatic period.

The same dynamics was evidenced with the respect to GGT serum level but without statistically significant changes: the GGT serum level at stage 1 and stage 2 practically did not differ from the control. In the remaining group of the patients, there was a decrease of GGT serum levels compare to HC (mean \pm SD 14.1 \pm 4.2 versus 18.2 \pm 1.4 U/L) but also without any statistically significant changes ($p>0.05$).

Discussion

The attained data complied with the literature, in our opinion reflecting the effect of the increasing deficiency of oxidative reactions in the body on aminoacid metabolism in this category of patients [11, 12, 14]. The decrease in some markers of membrane-associate enzymes activity could be a marker of impaired metabolic processes following long-term consequences after mTBI. The revealed increase of membrane-associate markers enzymes in the patients with long-term consequences after mTBI was directly proportional to the severity of neurological deficit ($p<0.05$; $r=+0.63$) as a result of the development of membrane cell or mitochondrial membrane pathology and that might be a marker of the degree of nervous system damage. In turn, the destruction of cell membranes was pathogenetically associated with the development of oxidative stress [6, 15, 16, 18].

The long-term consequences after mTBI was characterized by the statistically significant decrease of alkaline phosphatase serum level and positive dependence of alkaline phosphatase ($p<0.05$; $r=+0.48$) on the disease duration reflecting the specificity of the revealed

changes in its activity in cases of cell energy deficiency in this category of the patients, which was an important diagnostic criterion. The decrease in alkaline phosphatase activity according to the previous literature [7, 10] might be a marker of impaired intracellular metabolism in long-term consequences of mTBI.

In the general patient group, there was a significant increase in CK serum level compare to controls with the maximal biochemical values in the third clinical group with long-term consequences of mTBI more than 15 years and combined in 18.2% of the patients ($n=4$) with partial epileptic seizures. In our opinion, the direct proportional increase in CK activity with the progression and increase of posttraumatic period was associated ($p<0.05$; $r=+0.63$) with development of destructive processes in cells as a result of oxidative stress and disturbance of intracellular bioenergetic processes. It was noteworthy that the CK increase in cases of posttraumatic encephalopathy in the patients with long-term consequences more than 15 years did not reach the same value (according to the literature) as in hereditary neuromuscular diseases and this might be a differential diagnostic criterion for posttraumatic encephalopathy. Slightly different dynamic was observed analysing the information about another enzyme involved into exchange of amino acids and peptides – GGT. At the first and second stages, a tendency of GGT serum level decrease was evidenced, which could not be significant in the patients with disease duration more than 15 years compare to the control. This data complied with the literature on GGT serum level

decrease in the patients, who suffered from severe TBI [2, 19] reflecting, in our opinion, the developing deficiency of oxidative reactions on amino acid metabolism in these patient groups and/or associated with long treatment with different painkillers and/or antiepileptic drugs.

At present, there is still no effective pharmacological treatment that stops the progression of secondary brain injury; the pathogenetic mechanisms of secondary traumatic brain injuries development are still unclear and urgent. Thereby, the further research on any other related markers and membrane-associated enzymes involved in internal redox reactions in the patients with long-term consequences of traumatic brain injury is topical.

Conclusion

Thus, in the patients with long-term consequences of mTBI, dysregulation of

enzymes activity was detected that might be a marker of nervous system energy processes impairment and membranes destruction. The severity of the changes in the serum levels of membrane-associated enzymes markers correlated with the development of oxidative stress and metabolic processes dysfunction. The revealed dysregulation of enzymes activity might be caused by dystrophic cell changes, their dysfunction and metabolic changes reflecting the severity of neurological symptoms.

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Conflict of Interests

The author declares no conflict of interest.

СТАН МЕТАБОЛІЧНИХ ПРОЦЕСІВ У ХВОРИХ З ВІДДАЛЕНИМИ НАСЛІДКАМИ ЛЕГКОЇ ЗАКРИТОЇ ЧЕРЕПНО-МОЗКОВОЇ ТРАВМИ

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ДУ "ІНСТИТУТ НЕВРОЛОГІЇ, ПСИХІАТРІЇ ТА НАРКОЛОГІЇ
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Вступ. Легка закрыта черепно-мозкова травма (ЗЧМТ) призводить до розвитку різноманітних порушень метаболічних процесів, що відіграють певну роль у розвитку її віддалених наслідків.

Метою роботи було вивчення аналізу активності деяких маркерних мембрано-зв'язаних ферментів, що приймають участь у різних окислювально-відновлювальних реакціях, метаболічних та енергетичних процесах.

Методи. У сорока семи хворих із віддаленими наслідками легкої ЗЧМТ та тридцяти здорових осіб були проведені обстеження та визначення активності аланінамінотрансферази, аспартатамінотрансферази, лужної фосфатази та лактатдегідрогенази (ЛД) у сироватці крові хроматографічним та колориметричним методами.

Результати. Дане дослідження виявило значущі зміни вмісту лужної фосфатази та ЛД, які є маркерами ушкодження клітинних мембран, важкості ацидозу, роз'єднання окислювально-відновлювальних процесів та порушення енергетичного обміну у клітинах. Середній рівень ЛД був 662,7 МО/л vs 381,9 МО/л у контролі. Виявлене збільшення рівню ЛД прямо пропорційно корелювало з прогресуванням захворювання, досягаючи найбільш значущих змін у групі хворих з тривалістю захворювання більш 15 років ($mean \pm SD$ 144,6 \pm 16,3 МО/л vs 82,6 \pm 8,4 МО/л; $p < 0,05$). Віддалені наслідки після перенесеної легкої ЗЧМТ також характеризувалися статистично значущим зниженням активності лужної фосфатази ($p < 0,05$) та позитивною кореляційною залежністю ($r = +0,48$) від тривалості посттравматичного періоду, при цьому загальний рівень лужної фосфатази був 152,5 \pm 11,21 МО/л vs 212,6 \pm 9,63 МО/л у здорових ($p < 0,01$). Ступінь вираженості змін активності мембрано-зв'язаних ферментів був пов'язаний з розвитком оксидативного стресу та дисметаболических процесів.

Висновки. У хворих з віддаленими наслідками легкої ЗЧМТ було виявлено дисрегуляцію ферментативних процесів, що є маркером порушення різних видів енергетичного обміну у нервової системі та деструкції клітинних мембран.

КЛЮЧОВІ СЛОВА: віддалені наслідки; легка закрыта черепно-мозкова травма; мембрано-зв'язані ферменти; метаболічні процеси.

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