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VITAMIN-ANTIOXIDANT HOMEOSTASIS DATA IN PATIENTS WITH LONG-TERM CONSEQUENCES AFTER MILD TRAUMATIC BRAIN INJURY

Вітамінно-антиоксидантний гомеостаз у хворих із віддаленими наслідками легкої закритої черепно-мозкової травми

Abstract

Introduction. *Mild traumatic brain injury (mTBI) was reported to be the most frequent among other types of brain injuries and is the main reason for the disability in mid-life and middle-aged people. It's known that antioxidants can reduce oxidative stress, so, to prevent secondary brain injury modulating maintaining of long-term consequences after mTBI.*

Purpose of the study. *This work was to study the serum vitamin E, C and A levels in the patients with long-term consequences after mTBI to explore their potential pathogenetic influence.*

Materials and methods. *Sixty-seven patients with long-term consequences after mTBI were investigated with the mean age of 43,61 ± 8,24 years (18 women, 26,86% and 49 men, 73,14%) where the vitamin E, C and A contents were measured in sera by spectrophotometer method using standard protocols and reagents (Sigma, USA).*

Results. *In this work, it was found descending serum levels of all investigated vitamin-antioxidants in almost all patients with long-term consequences after mTBI where the content of vitamins A (M ± s: 1,63 ± 1,56 mkM/l) and E (25,41 ± 0,93 mkM/l) had a tendency to decreasing without significant differences compare to controls. It was found the statistically significant decreased of vitamin C levels in the serum samples of our investigated patients when compared to controls ($p < 0,05$, $t = 4,59$, 95% CI 98,81 to 55,68) where in the main patient group, the medians of total vitamin C level was 30,57 ± 5,38 mkM/l vs 36,91 ± 5,22 mkM/l*

Резюме

Вступ. *Закрита черепно-мозкова травма (ЗЧМТ) відноситься до найбільш поширених видів травматизму та є основною причиною інвалідизації серед осіб молодого та середнього віку. Відомо, що антиоксиданти сприяють зменшенню проявів оксидативного стресу та запобігають розвитку вторинного пошкодження головного мозку внаслідок ЗЧМТ та її наслідків.*

Мета дослідження. *Вивчення вмісту вітамінів Е, С та А у сироватці крові хворих із віддаленими наслідками легкої ЗЧМТ для вивчення їх потенційного патогенетичного впливу.*

Матеріали та методи. *Досліджено 67 хворих із віддаленими наслідками легкої ЗЧМТ середній вік котрих складав 43,61 ± 8,24 років (18 жінок, 26,86% та 49 чоловіків, 73,14%), де вміст вітамінів-антиоксидантів Е, С та А у сироватці крові було вимірюване спектрофотометричним методом згідно стандартного протоколу з використанням реактивів Sigma (USA).*

Результати. *У даній роботі було встановлено, що всі досліджувані вітаміни-антиоксиданти були знижені практично у всіх обстежених хворих. Однак при цьому, вміст вітамінів А (M ± s: 1,63 ± 1,56 mkM/l) та Е (25,41 ± 0,93 mkM/l) мав тенденцію до зниження без достовірних змін відносно контрольної групи. Було виявлено достовірне зниження рівню вітаміну С у зразках сироватки крові пацієнтів порівняно з контролем ($p < 0,05$, $t = 4,59$, 95% ДІ 98,81–55,68), при цьому у загальній групі пацієнтів медіана рівню вітаміну*

in controls. It was shown that the patients with long-term consequences after mild contusion in anamnesis (64,18%) had the prominent changes in the vitamin C content.

Conclusion. *The maintaining of long-term consequences of mTBI was accompanied by the vitamin-antioxidant dyshomeostasis such as decreasing of vitamin C serum level associated with a tendency to decreasing of vitamins A and E levels that may play the certain role in the pathogenesis. All these data are needed to be accounted into the consideration during the treatment of this patient category.*

Keywords: *long-term consequences of mild traumatic brain injury, vitamin-antioxidant homeostasis.*

С була 30,57 ± 5,38 мкМ/л щодо контролю, 36,91 ± 5,22 mkM/l. Було показано, що у пацієнтів із віддаленими наслідками після контузії легкого ступеню в анамнезі (64,18%) спостерігалися найбільш виразні зміни вмісту вітамінів.

Висновки. *Формування віддалених наслідків після легкої ЗЧМТ супроводжувалося розвитком вітамінно-антиоксидантного дисгомеостазу у вигляді зниження у сироватці крові хворих рівню вітаміну С та тенденції до зниження рівнів вітамінів А та Е, що може відігравати певну роль у їх патогенезі. Всі ці дані необхідно враховувати під час лікування даної категорії пацієнтів.*

Ключові слова: *віддалені наслідки легкої закритої черепно-мозкової травми, вітамінно-антиоксидантний гомеостаз.*

INTRODUCTION

Mild traumatic brain injury is considerable medical health problem that affects millions of people every year all over the world: around 2,6 million individuals receive mild traumatic brain injury (mTBI) annually and, for today, around of 1–2% of the population live with different chronic neurological or psychiatric impairments and long-term consequences due to mTBI [1, 2].

Concussion is induced by force transmitted to head resulting from direct or indirect injury to head, face, or elsewhere and presents with a range of clinical symptoms, including physical signs, behavioral changes, cognitive impairment, somatic symptoms, often presents with rapid onset and resolve spontaneously; the pathogenic mechanisms of long-term consequences after traumatic brain injuries are still unclear and combination of different alterations such as excitotoxicity, blood flow, free radical damage (oxidative stress), disrupted regional metabolic processes has been identified as major contributors to the secondary damage after mild brain injuries [3, 4, 5, 6]. Concussion results in pathological changes at ultrastructural level, which initiate neurochemical and neurometabolic cascades, including disruption of neurofilaments and hyperglycolysis associated with oxidative dysfunction [5, 7, 8]. Some authors have shown that secondary damage after mild brain injury leads to failure in adenosine triphosphate synthesis and increases of reactive oxygen in species [4]. In animal models it was shown that mTBI alters brain functions for hours to years in a variety of different pathological syndromes, including increase of glucose metabolism, which also may persist for up to four weeks after injury [3, 5, 7, 8, 9]. These dysmetabolic processes that follow concussion are associated with oxidative stress and are reversible in many cases of damaged brain cells, however, some cells may die [3, 6].

In many studies, various vitamins data have been explored with the regards to experimental brain injury [8, 10, 11]. Some authors have reported changes in vitamin status observed in the patients with mTBI [12]. Clinical studies supported the evidence that using of vitamins E and C could help in reducing neuropathology and cognitive deficits following brain trauma and a number of experimental studies have shown that vitamin dyshomeostasis may enhance neurodegeneration progression after neurotrauma and all these alterations in vitamin dysmetabolism have been associated with the increased in oxidative stress [9, 13].

Vitamin E is a lipid-soluble antioxidant presents with high concentrations in mammalian brain and has been shown neuroprotective effect in numerous experimental and clinical studies [9, 14–17]. In several animal models of brain injury administration of α-tocopherol has been shown to decrease oxidative stress and neuropathology [11]. Numerous clinical and experimental studies have shown that treatment with α-tocopherol is effective not only for some cancers, but it may prevent and repair cell tissue damage following radiation and trauma [9]. It was shown that the potential neuroprotective α-tocopherol effect was mediated by free radical propagation prevention [8, 10]. It was found in clinical trials that vitamin E supplementations was very useful because of neuroprotective properties in acquired brain injuries [10, 11, 13].

Vitamin C (ascorbic acid) is one of the most important endogenous radical scavengers having pronounced neuroprotective effect especially in reducing any kind of cell damages from excitotoxicity according to data from many experimental studies [9, 14, 16]. It has been shown that brain tissue levels of ascorbic acid highly reduced immediately after TBI and do not return to normal values until 72 hours in the post-injury period [3, 4, 6].

PURPOSE OF THE STUDY

With the respect to neuroprotection data reported in numerous experimental and clinical studies, we have suggested that vitamin homeostasis may have potential benefit on long-term effects from mTBI outcome. The aim of this work was to study the serum vitamin E, C and A levels in the patients with long-term consequences after mild traumatic brain injury.

MATERIALS AND METHODS

Sixty-seven patients with long-term consequences after mTBI (T90.5; T90.0) were enrolled into this study with the mean age of 43,61 ± 8,24 years (18 women, 26,86% and 49 men, 73.14%) and range aged between 24 and 61 years old (table 1). The definition of mTBI was consistent with the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10; 1992). Thirty healthy controls (M age ± s, 35,6 ± 9,21 years) without neurological and psychiatry diseases were included, as well. All patients were sporadic in nature and born in Ukraine; clinical, CT or MRI data were retrieved from the patients' history; physical, neurological and biochemistry examinations were performed under baseline conditions. Exclusion criteria were craniectomy in anamnesis, pregnancy, preexisting neurologic diseases, respiratory failure, acute or chronic liver and cardiovascular diseases.

The vitamin E, C and A contents were measured in sera by spectrophotometer method using the manufacture protocols. In this study, we used standard examples of vitamins from Sigma (USA). Concentrations of standards was 0,025–0,2 μM/per test; volume – 0.01 ml, electrophoresis 3 hours at E = 600 V in pyridine-acetate buffer. The method principle was based on releasing vitamin E from cells during alkaline hydrolysis and diethyl extraction by ether. After purification of the unsaponifiable extraction by the method of column chromatography on alumina, tocopherols were determined colorimetrically at color oxidation reaction with nitric acid, followed by the determination of direct spectrophotometry in the purified alcoholic extract at λmax = 92 nm. The vitamin C serum level was determined by titrometer method with dichlorphenolindophenol and the vitamin A serum level was determined by spectrophotometer method based on the reaction with three-chlorine antimony trichloride [18].

Controls and subject samples were performed, as usual, in the duplicate manner; all protocols were approved by the Health Research Ethics Committee and written informed consent was obtained from each patient before the investigation. Data were analyzed according to their distribution. Age, disease duration were compared between groups with the χ^2 test; parametric tests were used for normally distributed data; nonparametric tests were used for abnormal distributed data; Kruscall Wallis and Mann-Whitney U tests were applied in Prism, seeing differences between the groups, the multivariate analysis with considering covariates was performed; univariate analysis was performed to assess the relationships between various factors. All reported p values are two-tailed; p values ≤ 0,05 were considered statistically significant.

RESULTS AND DISCUSSION

All investigated patients were divided into two groups based on the character of the obtained trauma: the first clinical group was formed from the patients who had concussion (n = 24, 35,82%, group I) in their anamnesis and the second clinical group was consistent of the patients with long-term consequences after mild brain contusion (n = 43, 64,18%, group II) – table 1. Post-injury period of traumatic neuronal injury has led to different neurological and cognitive impairments: the most frequent focal neurological signs were horizontal nystagmus (14 patients from group I and 25 patients from group II), disturbances in coordinating sphere (11 patients, I and 21 patients, II accordingly), ataxia (8 patients, I and 16 patients, II), face asymmetry (9 patients, I and 14 patients, II), increase of tendon reflexes (15 patients, I and 29 patients, II), pathological foot reflexes (9 patients, I and 23 patients, II). Four patients from the gr. II had partial seizures with secondary generalization. One woman from first group had panic attacks and two patients from second group also had frequent panic attacks ranged till 3–5 per months.

Table 1

Demographic features of Ukrainian patients with long-term consequences after mild traumatic brain injury

Data	I group	II group	All patients
Number of patients, n (%)	24 (35,82%)	43 (64,18%)	67 (100%)
Male, n (%)	15 (62,5%)	34 (79,07%)	49 (73,14%)
Female, n (%)	9 (37,5%)	9 (20,93%)	18 (26,86%)
Mean patient age ± SD	31,63 ± 7,84	39,51 ± 11,65	43,61 ± 8,24
Mean onset ± SD	28,74 ± 8,1	42,68 ± 12,33	34,61 ± 9,73

CT or MRI scans has revealed areas of swollen brain tissue in frontal lobes (11 patients from gr. II, 25,58%), left-sided diffuse vascular injury (9 patients from gr. II, 20,93%), right-sided diffuse vascular injury (4 patients from gr. I, 16,67%; 7 patients from gr. II, 16,27%), hydrocephalus (8 patients from gr. I, 33,3% and 14 patients from gr. II, 32,55%), combination (2 patients from gr. I, 8,33%; 4 patients from gr. II, 9,3%).

Analyzing obtained biochemical data, the decreasing of targeting vitamins levels were found in almost all patients with long-term consequences after mTBI. At the same time, only the level of vitamin C decreased in 1,2 times and it was descended statistically significant compare to controls. The content of the remaining antioxidant vitamins had a tendency to a decrease without significant differences compare to the control group ($p > 0,05$).

It was found the decreased vitamin C levels in the serum samples of our investigated patients when compared to controls ($p < 0,05$, $t = 4,59$, 95% CI 98,81 to 55,68) where in the general patient group, the medians of total vitamin C (mean ± SD) level was 30,57 ± 5,38 mkM/l and 36,91 ±5,22 mkM/l, in controls (table 2).

Table 2

The vitamin-antioxidant contents in the patients with long-term consequences after mild traumatic brain injury and controls, M ± s

Data	All patients (n = 67)	I clinical group (n = 24)	II clinical group (n = 43)	Controls (n = 30)
Vitamin A, mkM/l	1,63 ± 1,56	1,65 ± 1,02	1,48 ± 1,2	1,89 ± 0,98
Vitamin E, mkM/l	25,41 ± 0,93	27,5 ± 1,06	23,62 ± 1,8	29,24 ± 1,94
Vitamin C, mkM/l	30,57 ± 5,38*	33,4 ± 7,61	28,96 ± 9,41	36,91 ± 5,22
*$p < 0,05$ – used for statistically significant results in the group versus controls				

Targeting lower serum levels of vitamin C in the patients in the post-injury period following mTBI we separated among the different TBI forms: the mean serum vitamin C level was 33,4 ± 7,61 mkM/l in the patients from I group (mean age 31,63 ± 7,84 years), whereas it was 28,96 ± 9,41 mkM/l in the patients with II group (mean age 39,51 ± 11,65 years); thus, we have observed the lower serum ascorbic acid levels in the patients with mild brain contusion as compared to patients after concussion, however without statistically significant difference between these groups (95% CI 109,2 to 68,7, $p > 0,05$, $t = 0,638$) (tabl. 2). Because the patients and controls were not gender matched, serum ascorbic acid levels were compared between men and women and the levels were not found to be comparable between male and female patients ($p = 0,18$) and controls. In the same subset of the samples, vitamin C data were not correlated with the age ($p = 0,61$) and other vitamins serum levels. Although, the patients from the group II were older we didn't find that our groups appreciably differ between them with the respect to the disease duration and vitamin C data.

Thereby, the patients with long-term consequences after mTBI showed the abnormal low serum vitamin C levels with the tendency to more decreasing of vitamin C levels especially in the patients from group II.

It's well-know that oxidative stress plays an important role in the mechanism of long-term consequences after TBI which is exacerbated by the impaired endogenous protection mechanisms such as vitamin-antioxidant homeostasis [12, 18].

There are previous experimental data that have been reported that ascorbic acid is a well-known antioxidant for controlling oxidative stress in rat brain and administration of ascorbic acid can reduce oxidative stress preventing neurological dysfunction in rats [14, 19]. Recent studies have demonstrated that some antioxidants can reduce oxidative stress, prevent secondary brain injury. Oxidative stress has been implicated in multiple models of TBI and is mainly induced by reactive oxidative species: free radicals induced by TBI have deleterious effects on the function and antioxidant vitamin levels of organs and systems including brain [19]. In the present study we focused on vitamin-antioxidant homeostasis that may have potential benefit on long-term consequences outcome in the patient with mTBI. Various metabolic conditions may challenge different strategies concerning vitamin data between neurons and astrocytes: decreased vitamin C level could be a marker of tissue hypoxia or brain hypoxia or different metabolic disturbances which could be associated with post-traumatic ischemia [13, 15–17].

Thus, in the pathogenesis of the formation of long-term consequences after mTBI vitamin-antioxidant dyshomeostasis may play the potential definite role. Along with the presented data, in the patients with long-term consequences after mTBI it could be necessary to conduct pathogenetic therapy including drugs that can improve their vitamin status.

Further studies are needed to examine exactly vitamin-antioxidant homeostasis along with the peculiarities of the functional state of brain enzymes systems involved into metabolism of neurotransmitters for the development of new differential diagnostic criteria and searching for the new treatment options for such patients category.

CONCLUSION

The study provides the novel data revealing vitamin-antioxidant dyshomeostasis accompanied by low ascorbic acid serum level data in the patients with long-term consequences after mild traumatic brain injury with a tendency to decrease of vitamin E and A serum levels in this patient category that may play the certain role in their pathogeneses.

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