Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta morphologica et anthropologica, 25 (3-4)
Sofia ● 2018

Protein YKL-40 in Cerebrospinal Fluid in Traumatic Brain Injury

Maria Kazakova[1], Georgi Pavlov[2,5], Dorian Dikov[3], Kiril Simitchiev[4], Valentin Dichev[1,6], Chavdar Stefanov[2,5], Victoria Sarafian[1,6]*

[1] *Department of Medical Biology, Medical Faculty, Medical University – Plovdiv*
[2] *Department of Anesthesiology, Emergency and Intensive Care Medicine, Medical Faculty, Medical University – Plovdiv*
[3] *Department of General and Clinical Pathology, Medical Faculty, Medical University – Plovdiv*
[4] *Department of Analytical Chemistry and Computer Chemistry, Faculty of Chemistry, University of Plovdiv*
[5] *University Hospital "St. George" Plovdiv*
[6] *Technological Center for Emergency Medicine, Plovdiv*

* Corresponding author e-mail: kazakova25@abv.bg

Traumatic brain injury (TBI) is one of the leading causes of non-natural death in younger adults. High levels of YKL-40 are discussed to serve as a biomarker in acute and chronic inflammatory diseases. The aim of our study was to examine the expression and dynamics of YKL-40 secretion in TBI patients. Cerebrospinal fluid (CSF) and plasma samples were collected from nineteen patients with TBI – on the 24th and 96th hour after injury. ELISA and immunocytochemical methods were performed.
We determined that the CSF levels of YKL-40 were significantly lower compared to plasma concentrations on the 24th and 96th hour. No significant change between CSF levels of YKL-40 on the 24th h after TBI compared to the control group was found. The presence of YKL-40 protein in the cytoplasm of polymorphonuclear leukocytes was detected as a strong diffuse reaction. We suggest that YKL-40 levels reflect the inflammatory process and could provide new information about its dynamics in patients with TBI.

Key words: traumatic brain injury, YKL-40, biomarker

Introduction

Traumatic brain injury (TBI) is a global health problem, characterized by high frequency of occurrence, high mortality rate, severe and long lasting disability in patients who have survived [12]. There is a lack of accurate and standardized panel of biomarkers for assessment of progression and disease outcome. Severety of TBI is defined by clinical prognostic models. Brain damage following head injury may occur as a direct physical effect at the moment of action of the traumatic agent (skull fractures, hematoma, cerebral

contusion). It is referred to primary brain injury and leads to deformation and damage of brain tissue due to exceeding its structural tolerance [19].

Complications after the trauma are defined as a secondary brain damage. They happen within hours to days and are performed as brain-barrier alteration, oxidative stress [5], exocytotoxicity [13], mitochondrial dysfunction [14]. Inflammation is also an accompanying process [10]. The inflammatory process is mediated by the activation of cells (astrocytes, glial cells, macrophages and lymphocytes) and production of pro- and anti-inflammatory cytokines and chemokines [1].

YKL-40, also known as CHI3L1, is an extracellular glycoprotein with a molecular weight of 40 kDa. It is a member of the glycosyl hydrolase family 18 and has no enzymatic activity [4]. Protein expression was detected in neutrophils, activated macrophages, T cells, chondrocytes, synovial cells, endothelial cells, stelate liver cells, activated astrocytes, oligodendrocytes [16]. There is evidence that YKL-40 is involved in the immune response by inhibiting oxidant injury, apoptosis and pyroptosis [7]. It was proved that YKL-40 was associated with T cell activation and proliferation [9]. Participation in neoangiogenesis, tissue remodeling, fibrosis, cell migration and proliferation was reported [11].

However, the particular mechanism of YKL-40 function remains unclear. Elevated YKL-40 in acute and chronic inflammatory diseases was determined [6, 8, 18]. There are few investigations focused on YKL-40 levels in TBI pathology which suggested that the protein expression correlated to the local neuroinflammation [3].

The aim of our study was to examine the expression and dynamics of YKL-40 production in TBI patients.

Material and Methods

Cerebrospinal fluid (CSF) and plasma samples were collected from nineteen patients with TBI – on the 24th and 96th hour after the trauma. The age distribution of the patients assessed as mean ± standard deviation was 50 ± 15 years. Seventeen of the patients were males and two were females. CSF samples were isolated also from forensic autopsies of 15 adult cadavers (healthy individuals before death) and served as an age-matched (63 ± 18 years) and gender-matched (13 males and 2 females) control group. Concentrations of YKL-40 in biological fluids were analyzed by ELISA using MicroVue™YKL-40 kit (QUIDEL, Cat. №8020) according to manufacturer's instructions.

Immunocytochemical staining of YKL-40 in lumbar punctuate was performed by the indirect immunoperoxidase method. The avidin-biotin system was applied (VectastainElite ABC Kit, Vector Laboratories, Burlingname,CA). The sections were incubated at 37 °C for 1 h with primary goat anti-human chitinase 3-like antibody (YKL-40) (R&D Systems, Minneapolis, MN) and anti-goat IgG secondary antibody. The final detection was performed by freshly prepared DAB as a chromogen. Nuclei were counterstained with haemotoxylin. A morphological analysis was performed by standard hematoxylin and eosin (H&E) staining.

The cytopathological examination was verified by two independent observers. The study was approved by the University Ethics Committee (Protocol№3/31.05.2018). Informed consent was signed by all examined individuals according to the Helsinki Declaration.

Statistical analysis. The Wilcoxon signed rank test was used to compare the concentrations of YKL-40 in plasma and CSF on the 24th and 96th hour after TBI. The levels of YKL-40 in the group of patients were compared with the ones in the control group by the Mann-Whitney test. Boxplot diagrams were used for graphical visualization of

YKL-40 concentrations - the criteria 1.5 of the interquartile range have been accepted to distinguish outliers. The significance level was set at 5%.

Results and Discussion

Inflammatory processes after TBI induce time-dependent cascades of acute phase response [15]. Proteins secreted into the systemic circulation and CSF are able to reflect the severity of the inflammation and the disease course [1]. We found no significant change between CSF levels of YKL-40 on the 24th h after TBI compared to the control group (mean ± standard deviation: 269±165 *vs* 153±51 ng/ml) (**Fig.1**). However, the interquartile range (25–75 percentiles) of YKL-40 concentrations for the control group was much narrower than the one of TBI patients (**Fig. 1**). Our results are in accordance with earlier investigations [3] suggesting that the level of the glycoprotein illustrates the inflammatory process.

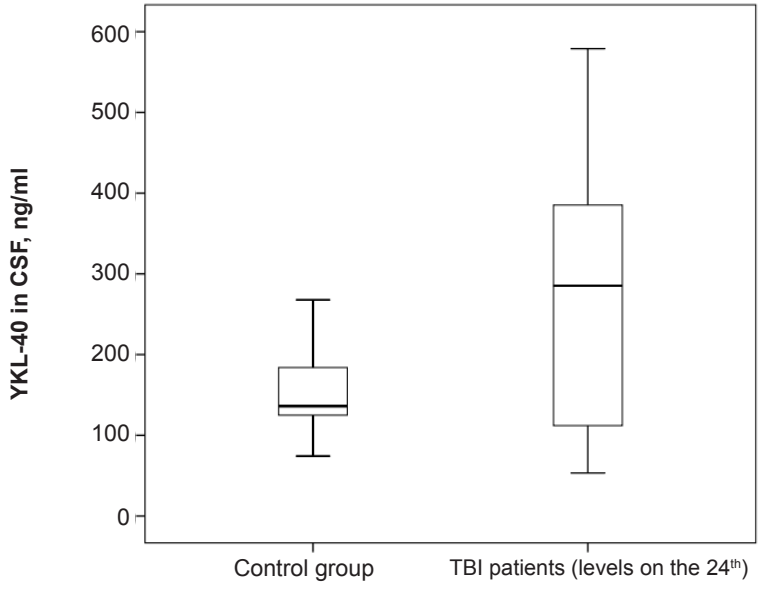


Fig. 1. YKL-40 concentrations in CSF of TBI patients and the control group.

We showed that CSF levels of YKL-40 were significantly lower compared to plasma concentrations on the 24th (p=0,027) and 96th hour (p=0,044) (**Fig. 2**). A recent study revealed increased CSF concentrations of YKL-40 in athletes with postconcussion syndrome due to repetitive concussive TBI. YKL-40 concentration correlated with lifetime concussion events. The authors claimed that head trauma was associated with biomarker evidence of astroglial activation but they did not report plasma YKL-40 values [17].

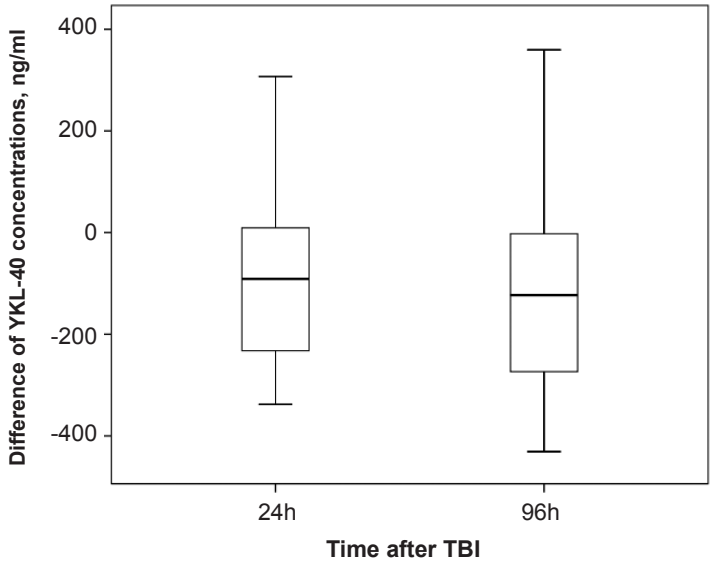
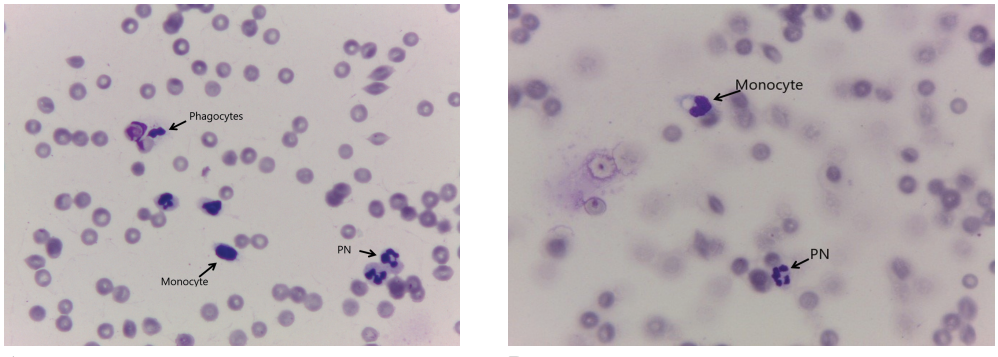


Fig. 2. Time-dependent differences of YKL-40 concentrations in CSF and plasma in TBI patients; the distributions of the differences $CSF_{concentration}$ – $Plasma_{concentration}$ calculated for each individual patient are visualized, ng/ml

Another clinical study following neuroinflammation and CSF YKL-40 levels in Huntington's disease showed that the concentration of the protein was not suitable as an early marker in this pathology but might have a possible role as a marker for glia activation [20].

Monocytes, phagocytes and polymorphonuclear cells were observed by morphological examination (**Fig. 3 A, B**). The results are in accordance with the findings of other researchers in CSF cytology who reported that lymphocytes and macrophages/monocytes were the cells frequently seen in nonspecific reactive conditions [2].



A **B**

Fig. 3. Cytology of CSF in TBI patients (hematoxylin and eosin staining).
Monocytes, phagocytes and polymorphonuclear (PN) cells are observed (3A, B). (magn × 630).

The immunocytochemical examination determined the presence of the YKL-40 protein in the cytoplasm of polymorphonuclear leukocytes. A strong diffuse staining was recorded (**Fig. 4**).

Another study suggested that YKL-40 expression at the perimeter of contusions might be an important feature of the astrocytic response to modulate neuroinflammation[21].

Therefore, CSF and plasma YKL-40 measurements are useful tools to analyze the course of inflammation and the time of lethal outcome in TBI. Higher plasma concentrations of the glycoprotein seem to be a part of the acute phase response as an immediate reaction after injury. The biomarker value and its dependency on trauma survival times are in prospect examination.

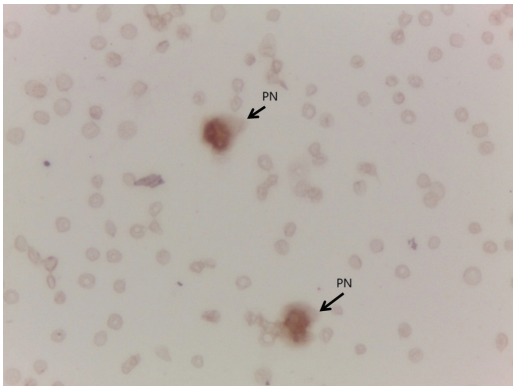


Fig. 4. Immunocytochemical expression of YKL-40 in a smear of CSF (indirect immunoperoxidase reaction). A strong diffuse cytoplasmic expression with perinuclear accentuation in two polymorphonuclear (PN) cells is observed (magn × 630).

Conclusion

Our study is the first to show time-dependent change of YKL-40 levels in TBI. We assume that plasma and CSF YKL-40 levels reflect the inflammatory process and would provide new information about its dynamics in TBI patients.

Acknowledgements: The financial support by the National Science Fund of Bulgaria (Contract DM 03/2 12.12.2016) is acknowledged.

References

1. **Alawieh, A.** Neuro-proteomics and neuro-systems biology in the quest of TBI biomarker discovery. – *Biomarkers of Brain Injury and Neurological Disorders* (Eds. K Kevin.), 2014, https://www.taylorfrancis.com/
2. **Bigner, S.** Cerebrospinal fluid (CSF) cytology: Current status and diagnostic applications. – *J. Neuropathol. Exp. Neurol*., **51,** 1992, 235-245.
3. **Bonneh-Barkay D., P. Zagadailov, H. Zou, C. Niyonkuru, M. Figley, A. Starkey, G. Wang, S., J. Bissel, C. A. Wiley, A. K. Wagner.** YKL-40 expression in traumatic brain injury: an initial analysis. – *J. Neurotrauma*., **27,** 2010, 1215–1223.
4. **Bussink, A. P., D. Speijer, M. F. Johannes, G. Aerts, R. G. Boot.** Evolution of mammalian chitinase (-like) members of family 18 glycosyl hydrolases. **–** *Genetics.,* **177,** 2007, 959–970.
5. **Cernak, I., L. J. Noble-Haeusslein.** Traumatic brain injury: an overview of pathobiology with emphasis on military populations. **–** *J. Cereb. Blood Flow Metab*., **30**, 2010, 255-266.
6. **Guan, J., Z. Liu, F. Li, J. S. Feng, H. J. Wang, J. G. Chu, Y. Z. Song, L. Xie, L. B. Ding.** Increased synovial fluid YKL-40 levels are linked with symptomatic severity in knee osteoarthritis patients. – *Clin. Lab.,***61,** 2015, 991-997.
7. **He, C. H., C. G. Lee, C. S. Dela Cruz, C. M. Lee, Y. Zhou, F. Ahangari, B. Ma, E. L. Herzog, S. A. Rosenberg, Y. Li, A. M. Nour, C. R. Parikh, I. Schmidt, Y. Modis, L. Cantley, J. A. Elias.** Chitinase 3-like 1 regulates cellular and tissue responses via IL-13 receptor α2. – *Cell. Rep.,* **29,** 2013, 830-41.

8. **Kazakova, M. H., A. Z. Batalov, N. G. Mateva, Z. G. Kolarov, V. S. Sarafian.** YKL-40 and cytokines a new diagnostic constellation in rheumatoid arthritis? – *Folia Med. (Plovdiv)*., **59,** 2017, 37-42.
9. **Kim, D. H., H. J. Park, S. Lim, J. H. Koo, H. G. Lee, J. O. Choi, J. H. Oh, S. J. Ha, M. J. Kang, C.M. Lee, C. G. Lee, J. A. Elias, J. M. Choi.** Regulation of chitinase-3-like-1 in T cell elicits Th1 and cytotoxic responses to inhibit lung metastasis. – *Nat. Commun.,* **9,** 2018, 503.
10. **Kokiko-Cochran, O. N., J. P. Godbout.** The inflammatory continuum of traumatic brain injury and Alzheimer's disease. – *Front. Immunol.,* **9,** 2018, 672.
11. **Lee, C. G., C. A. Da Silva, C. S. Dela Cruz, F. Ahangari, B. Ma, M. J. Kang, C. H. He, S. Takyar, J. A. Elias.** Role of Chitin and Chitinase/Chitinase-Like proteins in inflammation, tissue remodeling, and injury. **–** *Annu. Rey Physiol.,***73,** 2011, 479-501.
12. **Leibson, C. L., A. W. Brown, J. E. Ransom, N. N. Diehl, P. K. Perkins, J. Mandrekar, J. F. Malec.** Incidence of traumatic brain injury across the full disease spectrum: a population-based medical record review study. – *Epidemiology*, **22,** 2011, 836-844.
13. **Maas, A., N. Stocchetti, R. Bullock.** Moderate and severe traumatic brain injury in adults. – *Lancet Neurol*., **7,** 2008, 728-741.
14. **Mazzeo, A. T., A. Beat, A. Singh, M. R. Bullock.** The role of mitochondrial transition pore, and its modulation, in traumatic brain injury and delayed neurodegeneration after TBI. – *Exp. Neurol*., **8,** 2009, 363-370.
15. **Ondruschka, B., S. Schuch, D. Pohlers, H. Franke, J. Dreßler.** Acute phase response after fatal traumatic brain injury**.** – *Int. J. Legal Med.,* **132,** 2018, 531-539.
16. **Ringsholt, M., E. V. Høgdall, J. S. Johansen, P. A. Price, L. H. Christensen.** YKL-40 protein expression in normal adult human tissues-an immunohistochemical study. **–** *J. Mol. Histol.,* **38,** 2007, 33-43.
17. **Shahim, P.,Y. Tegner, N. Marklund, K. Höglund, E. Portelius, D. L. Brody, K. Blennow, H. Zetterberg.** Astroglial activation and altered amyloid metabolism in human repetitive concussion. – *Neurology,* **88,** 2017, 1400-1407.
18. **Spoorenberg, S. M. C., S. M. T. Vestjens, G. P. Voorn, C. H. M. van Moorsel, B. Meek, P. Zanen, G. T. Rijkers, W. J. W. Bos, J. C. Grutters.** Course of SP-D, YKL-40, CCL18 and CA 15-3 in adult patients hospitalised with community-acquired pneumonia and their association with disease severity and aetiology: A post-hoc analysis. – *PLoS One,* **13,** 2018, e0190575.
19. **Stergiou-Kita, M., S. Rappolt, D. Dawson.** Towards developing a guideline for vocational evaluation following traumatic brain injury: the qualitative synthesis of clients' perspectives. – *Disabil. Rehabil.,* **34,** 2012, 179-88.
20. **Vinther-Jensen, T., E. Budtz-Jørgensen, A. H. Simonsen, J. E. Nielsen, L. E. Hjermind.** YKL-40 in cerebrospinal fluid in Huntington's disease -a role in pathology or a nonspecific response to inflammation? – *Parkinsonism Relat. Disord*., **20,** 2014, 1301-1303.
21. **Wiley, C.A., D. Bonneh-Barkay, C. E. Dixon, A. Lesniak, G. Wang, S. J. Bissel, P. M. Kochanek.** Role for mammalian chitinase 3-like protein 1 in traumatic brain injury. – *Neuropathology*, **35**, 2015, 95-106.