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PLASMA LEVELS OF PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE AND BRAIN-DERIVED NEUROTROPHIC FACTOR ARE NOT SIGNIFICANTLY ALTERED IN POSTTRAUMATIC STRESS DISORDER AND TRAUMATIC BRAIN INJURY

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Posttraumatic stress disorder (PTSD) and traumatic brain injury (TBI) are common consequences of combat exposure, significantly affecting both mental and physical health of military personnel. Identifying reliable biomarkers for these conditions is critical for timely diagnosis, monitoring of treatment effectiveness, and prediction of the disease course. The results of recent studies suggesting potential role of PACAP and BDNF in peripheral blood as biomarkers for PTSD have been yet unconclusive. In this study we analyzed the level of pituitary adenylate cyclase-activating polypeptide and brain-derived neurotrophic factor in the blood plasma of 450 soldiers of the Armed Forces of Ukraine divided into six groups based on their diagnosis: controls, PTSD, PTSD with TBI, TBI, complex PTSD (CPTSD), CPTSD with TBI. PTSD and CPTSD were diagnosed using the International Trauma Interview, and TBI using the Ohio State University TBI Identification Method. PACAP and BDNF levels were analyzed in the the fasting blood samples using ELISA. PACAP and BDNF levels in blood plasma in control group were 2.10 ± 0.26 and 5.13 ± 0.50 ng/ml, respectively. The study findings revealed no statistically significant differences in PACAP and BDNF levels among the study groups. The results obtained indicate potential limitations of using these biomarkers for stress disorders and TBI diagnosing in combat-exposed military populations and highlight the need for further research to identify reliable biomarkers with objective diagnostic value and prognostic significance.

K e y w o r d s: posttraumatic stress disorder, traumatic brain injury, biomarkers, adenylate cyclase-activating polypeptide, brain-derived neurotrophic factor.

Post-traumatic stress disorder (PTSD) is a mental disorder that occurs as a result of a single or series of horrific traumatic events and is characterized by symptoms of re-experiencing (intrusion), avoidance and hyperarousal. Combat-exposed active-duty soldiers are among the most vulnerable populations to chronic psychological trauma, often leading to the development of PTSD. Additionally, the increasing use of artillery, bombardments, and first-person view drones has resulted in a rise in blast-related trauma and traumatic brain injury (TBI). Proper diagnosis of these conditions is time-consuming and requires highly trained medical personnel, posing significant challenges in resource-limited settings. These factors underscore the urgent need to identify reliable biomarkers for ICD-11 PTSD and complex PTSD (CPTSD), TBI, and their comorbidity not only to enable early diagnosis but also to monitor treatment efficacy.

Recent research suggests potential roles for pituitary adenylate cyclase-activating polypeptide (PACAP) and brain-derived neurotrophic factor (BDNF) as biomarkers for PTSD. PACAP plays a key regulatory role in neuroendocrine stress cir-

cuits and has been strongly implicated in PTSD pathophysiology [1]. Particularly, elevated plasma PACAP levels have been associated with PTSD diagnosis and symptom severity, but only in females, indicating a sex-specific involvement of the PACAP/PAC1 receptor pathway in abnormal stress responses [2]. Other studies suggest that alterations in BDNF expression or genetic variations have been associated with increased risk for various psychiatric disorders, including PTSD [3]. Hence, the studies investigating the association between BDNF levels in peripheral blood and PTSD diagnosis give mixed results, reporting both elevated and decreased levels of BDNF in PTSD subjects compared to controls.

Given the ambiguity of the data and recent changes in ICD-11 classification of stress disorder involving the new diagnosis of CPTSD, we aimed to compare the levels of PACAP and BDNF in peripheral blood between combat-exposed soldiers with PTSD, CPTSD, TBI, PTSD and TBI, CPTSD and TBI, and controls. We hypothesized that the presence of ICD-11 stress disorders, TBI and their comorbidities will be associated with increased level of PACAP and BDNF in peripheral blood.

Materials and Methods

The study used data collected from the Ukrainian National Initiative for Trauma studY (UNITY), a project focused on the consequences of the Russo-Ukrainian war on the physical and mental health of Ukrainian soldiers and war veterans. The study used convenient sampling, involving 450 active-duty military from the Armed Forces of Ukraine recruited from military hospitals and rehabilitation centers in a period between February, 2024 and May, 2024. Ethical approval was provided by the Ethics Commission at the Scientific Medical Center "DOSLID", Kyiv, Ukraine. Additionally, the protocol of the study was approved by local ethical committees of the involved military hospitals and rehabilitation centers (Protocol No. 2 dated August 14, 2023). The research adhered to the ethical standards outlined in the Declaration of Helsinki and Ukrainian national regulations on human research. All participants were contacted by the research team members and provided with full information about the study before giving informed consent to participate.

The sociodemographic characteristics of the sample are presented in Table.

Soldiers who met diagnostic criteria for severe medical conditions not related to PTSD or TBI, including cardiovascular conditions, liver, lung, renal diseases, autoimmune conditions, human immunodeficiency virus (HIV), cancer, tuberculosis, non-recovered bullet and shrapnel wounds, and severe mental health disorders either current or past episodes (e.g., psychosis, bipolar disorder, drug or alcohol abuse, suicide or homicide) were excluded from the study. Participants eligible for the study were further assessed for the presence of ICD-11 PTSD and CPTSD, TBI, and depression, followed by the blood collection procedure.

Clinical assessments. All interviews were conducted by psychiatrists/psychologists who were previously trained in using diagnostic instruments by a team of national and international experts qualified to work with stress disorders. All instruments in the study were translated using the standardized forward-backward translation procedure.

The mini-international nuropsychiatric interview (MINI) is a brief clinician-administered structured interview for diagnosing mental disorders according to DSM-IV [4]. In the current study, the instrument was used for diagnosing depression, mania, alcohol abuse, substance abuse, and psychotic disorders.

The international trauma interview (ITI) is a clinician-administered, semi-structured interview used to diagnose PTSD or CPTSD based on ICD-11 criteria [5]. The interview was previously validated and tested in a set of populations, reporting good internal consistency [6-8].

The Ohio State University Traumatic Brain Injury (TBI) Identification Method (OSU TBI-ID) is a short, structured interview indication of a lifetime history of TBI [9]. In the current study, only patients with a history of loss of consciousness as the result of blast-exposure were diagnosed with TBI.

Blood collection procedure. The fasting venous blood was collected from all enrolled subjects between 09:00 and 10:00 am. Prior to collection, all subjects were asked to rest in a supine position for 30 min. After collection, samples were immediately delivered to the clinical lab for analysis.

ELISA. We examined blood plasma levels of PACAP and BDNF using Biotek EL800 Plate Reader (The Lab World Group, Hudson, MA, USA) and reagent kits Fine Test Human ADCYAP1 Catalog No.: EH1515; Human BDNF Catalog No.: EH0043, Fine Test, Wuhan Fine Biotech Co.

Statistical plan. Statistical analysis was performed using R statistical software (ver. 4.0, r-pro-

T a b l e . Sociodemographic characteristics of the sample

Characte-ristics	Controls, *n* = 98	PTSD, *n* = 111	PTSD +TBI, *n* = 74	TBI, *n* = 55	CPTSD, *n* = 47	CPTCD+TBI, *n* = 65
	M±SD/n(%)	M±SD /n(%)	M±SD /n(%)	M±SD /n(%)	M±SD /n(%)	M±SD /n(%)
Age, years	34.93 ± 9.9	35.14 ± 7.92	37.0 ± 9.4	37.11 ± 9.17	37.13 ± 11.05	36.94 ± 9.18
Males	98 (100)	111 (100)	74 (100)	55 (100)	47 (100)	65 (100)
BMI	26.72 ± 4.05	25.36 ± 3.52	26.25 ± 4.26	26.57 ± 4.62	25.41 ± 3.34	25.48 ± 4.76
Ethnicity						
European	98 (100)	111 (100)	74 (100)	55 (100)	47 (100)	65 (100)
Education:						
• university degree	38 (38.8)	31 (27.9)	26 (35.1)	21 (38.2)	19 (40.4)	11 (16.9)
• below university degree	60 (61.2)	80 (72.1)	48 (64.9)	34 (61.8)	28 (59.6)	54 (83.1)
Marital status:						
• married	50 (51.0)	57 (51.4)	38 (51.4)	31 (56.4)	24 (51.1)	31 (47.7)
• not married	48 (49.0)	54 (48.6)	36 (48.6)	24 (43.6)	23 (48.9)	34 (53.3)
Military status:						
• soldier	91 (92.9)	99 (89.2)	67 (90.5)	47 (85.5)	32 (68.1)	49 (75.4)
• officer	6 (6.1)	11 (9.9)	5 (6.8)	6 (10.9)	14 (29.8)	10 (15.4)
• other	1 (1.0)	1 (0.9)	2 (2.7)	2 (3.6)	1 (2.1)	6 (9.2)
Being in captivity:						
• yes	7 (7.1)	4 (3.6)	4 (5.4)	2 (3.6)	2 (4.3)	3 (4.6)
• no	91 (92.9)	107 (96.4)	70 (94.6)	53 (96.4)	45 (95.7)	62 (95.4)
Smoking:						
• yes	63 (64.3)	76 (68.5)	56 (75.7)	39 (70.9)	28 (59.6)	47 (72.3)
• no	35 (35.7)	35 (31.5)	18 (24.3)	16 (29.1)	19 (40.4)	18 (27.7)
Alcohol use (past month):						
• yes	14 (14.3)	36 (32.4)	17 (23.0)	9 (16.4)	2 (4.3)	2 (3.0)
• no	84 (85.7)	75 (67.6)	57 (77.0)	46 (83.6)	45 (95.7)	63 (97.0)

Note. PTSD – posttraumatic stress disorder; CPTSD – complex posttraumatic stress disorder; TBI – traumatic brain injury; BMI – body-mass index

ject.org). ANOVA tests with trimmed means (F-trimmed means) followed by Yuen's post-hoc test and False Discovery Rate correction were used to identify the differences in the biomarker's concentration within groups. Results reported Fisher's statistical criteria (with degrees of freedom), effect size xi (ξ) and 95% confidence intervals (95% CI).

Results and Discussion

We found that the PACAP levels in blood plasma in the control group, ICD-11 PTSD, PTSD and TBI, TBI, CPTSD, CPTSD and TBI were 2.10 ± 0.26, 2.47 ± 0.37, 1.78 ± 0.25, 2.83 ± 0.44, 1.78 ± 0.38 and 1.75 ± 0.21, respectively. The levels

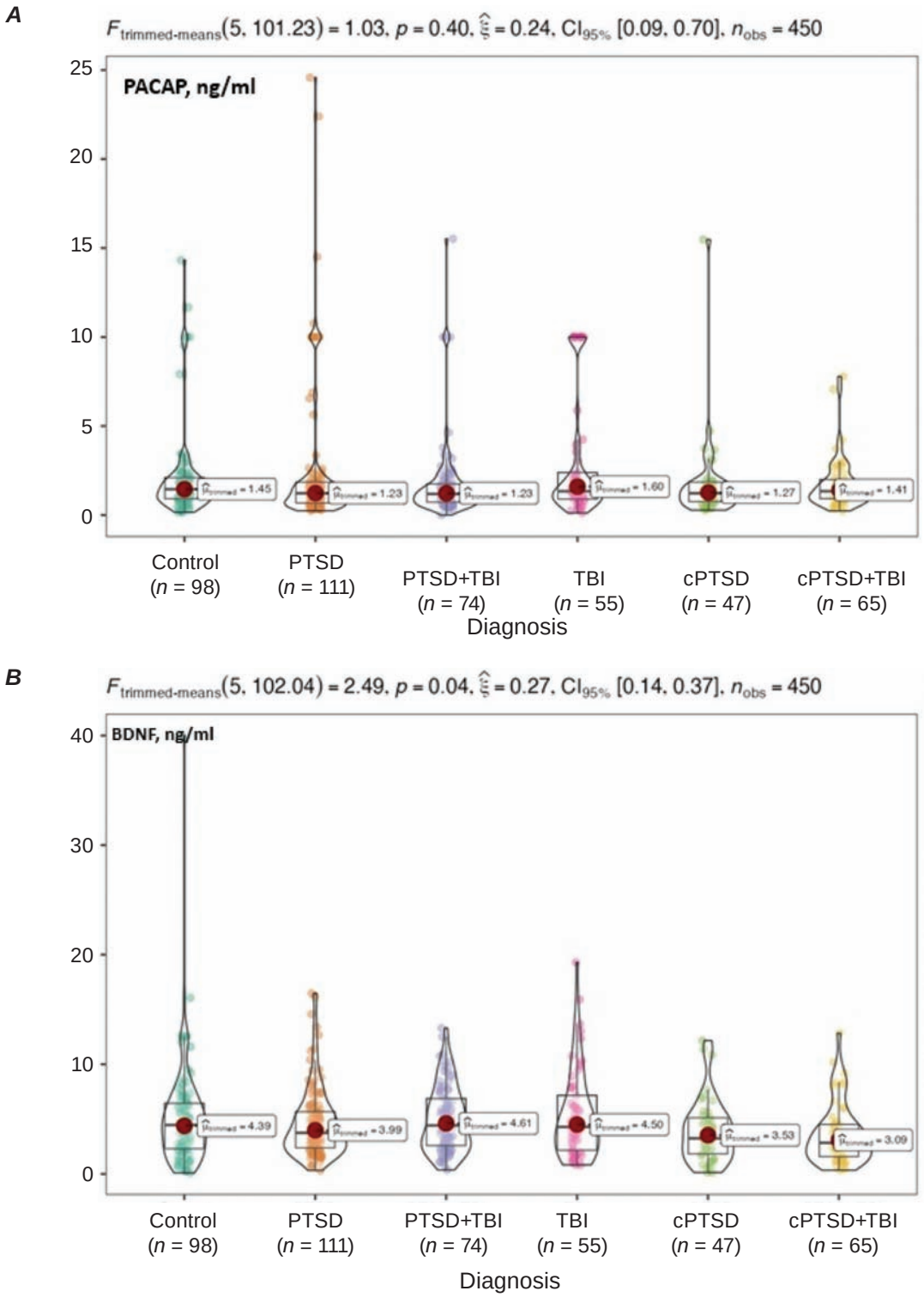

*Fig. Plasma levels of PACAP (**A**) and BDNF (**B**) in healthy subjects, patients with posttraumatic stress disorder, posttraumatic stress disorder with traumatic brain injury, patients with traumatic brain injury, patients with complex posttraumatic stress disorder and complex posttraumatic stress disorder with traumatic brain injury. Pairwise test: Yuen's trimmed means. Bars shown: significant*

of BDNF in blood plasma in the same groups were 5.13 ± 0.50, 4.64 ± 0.29, 4.99 ± 0.31, 5.37 ± 0.54, 3.96 ± 0.46 and 3.71 ± 0.39, respectively. The information on the features' means (μ trimmed) and their standard deviations within the comparison groups is presented in Fig.

In contrast to previous studies, we found no significant differences in PACAP and BDNF levels between study groups. Thus, we were not able to identify any significant changes, in contrast to the data reported in [10]. How can this be explained? With PACAP, this can be simply explained by the absence of women in our studied groups, who have previously been shown to have differences. Secondly, studies reporting higher concentrations of PACAP in cerebrospinal fluid and plasma in patients with TBI observed its markedly increased level in a period of 24-48 h after the trauma [11]. In our patients, the period between traumatic exposure and admission to the rehabilitation center and blood collection was much longer than 2 days. This may also explain the fact that the level of this peptide was not higher in TBI patients than in the other groups.

Thirdly, such changes may be a result of long-lasting changes due to chronic conditions. Speculatively, this can be compared with the findings of Pham et al. (2022), reporting a significant decrease in PACAP levels in Parkinson's disease patients [12]. Despite all three conditions being characterized by neuroinflammation, the time from onset of posttraumatic stress disorder and TBI was relatively short to result in significant changes. Thus, it also explains the lack of changes in BDNF levels, as most of the previous research were related to the study of its level in the blood of veterans [13] or patients with a long-term course of these disorders [10].

In our study, despite the absence of statistically significant differences in PACAP and BDNF levels between the groups, interesting patterns that deserve more detailed discussion were found. The highest mean concentration of PACAP was observed in the group with TBI alone (2.83 ± 0.44 ng/ml), while the lowest level was observed in the group with CPTSD with TBI (1.75 ± 0.21 ng/ml). Interestingly, individuals with PTSD alone had higher PACAP levels (2.47 ± 0.37 ng/ml) compared to those with comorbidity of PTSD and TBI (1.78 ± 0.25 ng/ml). A similar trend was observed in the group with CPTSD.

Regarding BDNF levels: the group with TBI alone had the highest BDNF level (5.37 ± 0.54 ng/ml), while the group with CPTSD and TBI had the lowest level (3.71 ± 0.39 ng/ml). It is noteworthy that BDNF levels were reduced in both the PTSD group and the PTSD and TBI group compared to the control group. A similar trend is observed in the PTSD group.

These patterns suggest that comorbidity with TBI may attenuate the levels of PACAP and BDNF, potentially indicating a compounded effect on neurobiological systems.

Conclusions. 1. *Plasma levels of PACAP and BDNF.* The analysis of the levels of pituitary adenylate cyclase-activating polypeptide (PACAP) and brain-derived neurotrophic factor (BDNF) in the blood plasma of Ukrainian military with posttraumatic stress disorder (PTSD), traumatic brain injury (TBI), and their comorbidity did not reveal statistically significant differences between the study groups. This suggests that the use of PACAP and BDNF as separate biomarkers for the diagnosis of PTSD and TBI in combat stress is limited.

2. *Possible influencing factors.* The absence of significant differences may be due to the multifactorial impact of combat stress, individual differences in stress response, and the complexity of the pathophysiological mechanisms of PTSD and TBI. It is also necessary to take into account the possible influence of gender differences, which has been documented in previous studies.

3. *Need for further research:*

The results emphasize the need for further research to identify reliable biomarkers of PTSD and TBI. Future research should include:

- Investigating the relationship between PACAP, BDNF with other neuropeptides and neurotrophins.

- Assessing possible genetic variations that may affect the expression of these biomarkers.

- Analyzing the influence of other factors, such as gender differences, duration of combat stress, and comorbidity with other mental disorders.

4. *Practical implications.* Although PACAP and BDNF did not demonstrate sufficient sensitivity and specificity for the diagnosis of PTSD and TBI, further research may help to understand the pathophysiology of these disorders better and develop a comprehensive approach to the diagnosis and treatment of posttraumatic conditions in the military.

Conflict of interest. The authors have completed the Unified Conflicts of Interest form at http://ukrbiochemjournal.org/wp-content/uploads/2018/12/coi_disclosure.pdf and declare no conflict of interest.

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РІВЕНЬ В ПЛАЗМІ КРОВІ ПОЛІПЕПТИДУ, ЩО АКТИВУЄ АДЕНІЛАТЦИКЛАЗУ ГІПОФІЗУ, ТА НЕЙРОТРОФІЧНОГО ФАКТОРА, ЩО ВИРОБЛЯЄТЬСЯ МОЗКОМ, НЕ ЗАЗНАЄ ІСТОТНИХ ЗМІН ПРИ ПОСТТРАВМАТИЧНОМУ СТРЕСОВОМУ РОЗЛАДІ ТА ТРАВМАТИЧНОМУ УРАЖЕННІ МОЗКУ

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Посттравматичний стресовий розлад (ПТСР) та черепно-мозкова травма (ЧМТ) є поширеними наслідками бойових дій, що значно впливають як на психічне, так і на фізичне здоров'я військовослужбовців. Визначення надійних біомаркерів цих станів є критично важливим для своєчасної діагностики, моніторингу ефективності лікування та прогнозування перебігу захворювання. Результати останніх досліджень, що вказують на потенційну роль PACAP та BDNF у периферичній крові як біомаркерів ПТСР, поки що не є остаточними. У цьому дослідженні ми проаналізували рівень поліпептиду, що активує аденілатциклазу гіпофізу (PACAP), та нейротрофічного фактора (BDNF), що походить з мозку, у плазмі крові 450 військовослужбовців Збройних сил України, розділених на шість груп залежно від їхнього діагнозу: контрольна група, ПТСР, ПТСР з ЧМТ, ЧМТ, комплексний ПТСР (КПТСР), КПТСР з ЧМТ. ПТСР та КПТСР були діагностувані за допомогою Міжнародного інтерв'ю з травм, а ЧМТ – за допомогою методу ідентифікації ЧМТ Університету штату Огайо. Рівні PACAP і BDNF аналізували в зразках крові натщесерце за допомогою ELISA. Рівні PACAP і BDNF у плазмі крові в контрольній групі становили 2,10 ± 0,26 і 5,13 ± 0,50 нг/мл відповідно. Результати дослідження не виявили статистично значущих відмінностей у рівнях PACAP та BDNF між досліджуваними групами. Отримані результати вказують на потенційні обмеження використання цих біомаркерів для діагностики стресових розладів та ЧМТ у військовослужбовців, які зазнали бойових дій, та підкреслюють необхідність подальших досліджень для виявлення надійних біомаркерів з об'єктивною діагностичною цінністю та прогностичним значенням.

Ключові слова: посттравматичний стресовий розлад, черепно-мозкова травма, біомаркери, поліпептид, що активує аденілатциклазу, нейротрофічний фактор, що походить з мозку.

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