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Reduced plasma BDNF concentration and cognitive decline in veterans with PTSD

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ABSTRACT

Post-traumatic stress disorder (PTSD) is a trauma and stress related disorder frequently associated with cognitive decline. War veterans with PTSD have a higher risk of developing dementia than healthy subjects. Brain derived neurotrophic factor (BDNF) is an important protein that modulates plasticity, memory consolidation and cognitive processes. Lower circulating BDNF levels were related to memory impairment and cognitive deterioration. The aim of this study was to evaluate cognitive deterioration and plasma BDNF concentration in 120 veterans with combat related PTSD, 120 healthy controls, 47 subjects with mild cognitive impairment (MCI) and 76 patients with Alzheimer's disease (AD), and to assess if plasma BDNF concentration might be used as biomarker of cognitive deterioration. Veterans with PTSD had significantly decreased plasma BDNF concentration and worse cognitive performances (assessed using the Mini Mental State Examination, Clock Drawing test and Montreal Cognitive Assessment scores/categories) than healthy subjects, and similarly reduced plasma BDNF and cognitive decline as MCI subjects. Reduced plasma BDNF was found in cognitively impaired subjects. These results suggest that veterans with PTSD should be closely monitored in order to early detect and predict cognitive worsening and promote interventions that might help restore blood BDNF levels and cognitive functions.

1. Introduction

Post-traumatic stress disorder (PTSD) is a trauma and stress related disorder (APA, 2013) that is frequently associated with significant cognitive decline (Hayes et al., 2012). Cognitive deterioration includes processing speed, concentration difficulties, deficits in learning, memory, attention, planning, problem solving and executive function (Hayes et al., 2012; Scott et al., 2015). It is assumed that PTSD is characterized by decreased hippocampal and frontal lobe volumes and lower total brain volume (Karl et al., 2006). Meta-analysis, that investigated emotions and cognition, and their interactions, reported that during emotional and cognitive processing tasks, individuals with PTSD showed significantly greater activation in the striatum compared to controls (Lee et al., 2021). Disruption in social cognition (Couette et al., 2020), and disturbances in visual spatial perception and short and long-term visual memory functions, evaluated using the Rey Osterrieth Complex Figure (ROCF) test (Havelka Mestrovic et al., 2020), were also reported for PTSD. Furthermore, PTSD was reported as a significant and important risk factor for all-cause dementia in a meta-analysis (Günak et al., 2020). Longitudinal follow up revealed that war veterans with PTSD have a 2-fold-higher risk of developing dementia compared to veterans without PTSD (Kang et al., 2019), and the results were controlled for the possible influence of demographic, medical and neuropsychiatric comorbidities (Yaffe et al., 2010). Although the

Abbreviations: PTSD, Post-Traumatic Stress Disorder; AD, Alzheimer's Disease; MCI, Mild Cognitive Impairment; HC, Healthy Controls; BDNF, Brain-Derived Neurotrophic Factor; MMSE, Mini-Mental State Examination; CDT, Clock Drawing Test; MoCA, Montreal Cognitive Assessment; ROCF, Rey Osterrieth Complex Figure.

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mechanisms involved are not clear, it is believed that there are common pathways in PTSD and dementia that might accelerate the development of neuropathological alterations in dementia, while PTSD symptoms (hypervigilance and trauma re-experiencing) induce social withdrawal associated with cognitive decline (Bryant, 2019; Desmarais et al., 2020). There is a bidirectional relationship between PTSD and dementia since PTSD increases the risk for late-onset dementia and vice versa (Desmarais et al., 2020). Alzheimer's disease (AD) is a most common cause of dementia characterized by progressive loss of memory and impairment of cognitive functions in older people, while mild cognitive impairment (MCI) is a state between normal aging and AD, assumed to represent a stage that proceeds the development of AD in most cases (Miranda et al., 2019). Subjects with PTSD frequently develop deterioration in concentration, learning and memory, problems in decision-making, and have increased incidence of MCI (Clouston et al., 2019).

Brain derived neurotrophic factor (BDNF) regulates synaptic plasticity and processes related to learning and memory consolidation (Miranda et al., 2019). Altered BDNF signaling and changes in its concentrations affect memory performance in different age-related and neuropsychiatric-related cognitive dysfunctions, such as AD and MCI (Miranda et al., 2019). In AD patients, lower serum BDNF levels were reported to predict faster cognitive decline, and baseline BDNF serum levels were significantly and independently associated with the rate of cognitive decline in AD (Laske et al., 2011). BDNF also regulates stress response, and has a complex role in vulnerability to stress related disorders, such as PTSD, since it is a part of the functional gene-regulatory network (Notaras and van den Buuse, 2020). Namely, it is involved in the pathways that regulate encoding of fear and might potentiate sensitivity to stress, but also in adaptive plasticity during extinction learning in order to suppress trauma related and PTSD-related fear responses (Notaras and van den Buuse, 2020). Many data reported the association of the BDNF Val66Met polymorphism (a functional polymorphism affecting the secretion of the mature BDNF protein, hippocampal activity and memory performance) with PTSD (Zhang et al., 2016) or cognition in PTSD (Havelka Mestrovic et al., 2020; Nedic Erjavec et al., 2021). However, there are no data on the association of plasma BDNF levels and cognitive decline in adult PTSD.

The aim of this study was to compare cognitive deterioration and plasma BDNF concentration in veterans with combat related PTSD, and compare these data with the findings in healthy controls and subjects with MCI or AD, and to assess if plasma BDNF concentration might be used as biomarker of cognitive deterioration. The hypothesis of this study was that cognitive performance and plasma BDNF concentration will be reduced in veterans with PTSD compared to control subjects and that this decline will be similar to the results obtained in subjects with MCI or AD.

2. Materials and methods

2.1. Subjects

The study included Caucasian male subjects: 120 veterans with combat related PTSD, 120 healthy control subjects, 47 subjects with MCI and 76 subjects with AD. All participants were recruited in the Clinics for Psychiatry Vrapce, Zagreb, Croatia.

2.2. Clinical evaluations

Diagnosis of PTSD was done with Structured clinical interview (SCID) according to the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) criteria (APA, 2013). Subjects with PTSD were exposed to the similar war related traumatic experiences lasting 3.0 ± 1.0 years, as they were all Croatian war veterans who participated in the Homeland war in Croatia from 1991 to 1995. They were active duty soldiers in the Croatian armed forces. They did not have a PTSD diagnosis before the combat experience. Age of onset for patients with PTSD was 40.5 ± 8.9 years, while the duration of disease was 16.1 ± 2.1 years. Inclusion criteria were in- and out-patients who signed informed written consent. Exclusion criteria for PTSD subjects were: drug abuse, alcohol dependence or pathophysiological changes in the liver, such as fibrosis, sclerosis, cirrhosis and malignant liver disease [alcoholic liver cirrhosis (K70.3), alcoholic liver fibrosis and sclerosis (K70.2) and hepatocellular carcinoma (C22.0), according to International Classification of Diseases, 10 revision /ICD-10/], major depressive disorder, schizophrenia, bipolar disorder, adult ADHD, AD or PTSD before the combat (according to DSM-5 criteria), current or recent (previous 3 months) use of psychotropic medication.

Control subjects were also evaluated in Clinics for Psychiatry Vrapce. They did not have any psychiatric disorder or pathophysiological changes in the liver, and were not treated with psychotropic medication.

The diagnosis of AD and MCI was done according to the criteria listed in DSM-5 (APA, 2013) and the criteria of the National Institute of Neurological and Communication Disorders and Stroke, which is part of the American National Institute of Health (NINCDS-ADRDA; National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's Disease and Related Disorders Association). Age of onset for patients with AD was 66.8 ± 9.9 years, and for MCI subjects 63.0 ± 10.6 years, while the duration of disease was 2.5 ± 1.8 years for AD and 2.1 ± 1.5 for MCI, respectively.

Cognitive status in all participants was tested using a Mini-Mental State Examination (MMSE) (Boban et al., 2012), and Clock Drawing test (CDT) (Shulman, 2000), while Montreal Cognitive Assessment (MoCA) was used to assess mild cognitive dysfunction in veterans with PTSD and healthy control subjects (Hobson, 2015). The MMSE is a gold standard, consists of 30-items, with scoring 0–30, and it is widely used screen for cognitive disorders. It assesses orientation to time and place, short-term memory, constructional capacities, and language use, with one point assigned to each successfully completed task. MMSE scores might differentiate subjects with normal cognitive functions (with 26–30 scores), mild cognitive deterioration (with 21–25 scores), moderate cognitive disturbances (with 10–20 scores) and severe loss of cognitive function (less than 10 scores). The CDT is easy to administer and short and accurate screening tool for dementia; with scoring of 1–5; and it assesses if numbers and hands are correctly placed (5 scores), mild visuo-spatial errors (4 scores); clear-cut errors in time given (3 scores), moderate visuospatial errors (2 scores), marked visuospatial errors (1 scores) and neither numbers nor hands are remotely correctly placed (0 scores) (Shulman, 2000). The MoCA is a rapid screening instrument used to assess visuospatial/executive, naming, memory, attention, language, abstraction, delayed recall and orientation (Hobson, 2015), with total possible score of 30 points; a score of 26 or above is considered normal.

The study was approved by the Ethics Committee of the Clinics for Psychiatry Vrapce, Zagreb, Croatia, and were carried out in line with the Helsinki Declaration (World Medical Association, 2013). All subjects have signed either informed consent prior to study procedures, or the consent forms were explained in details to the patients with AD or subjects with MCI and their caregivers.

2.3. Blood sample collection

Whole blood samples were collected at 8 a.m., following an overnight fast, in 8.5 ml yellow-top Vacutainer tubes with 1.5 ml of acid citrate dextrose anticoagulant. Sampling was performed during the routine laboratory visits. After a series of centrifugation of the whole blood, plasma was separated for BDNF analysis and stored at −20 °C.

2.4. Measurement of plasma BDNF concentration

BDNF concentration in plasma was determined using a commercial enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Quantikine ELISA, R&D Systems, Minneapolis, USA), as described before (Sagud et al., 2016). All samples were

measured in duplicates and plasma samples were diluted 1:2. The absorbance of each sample, standards and blanks was measured using a microplate reader set to 450 nm with wavelength correction set to 570 nm. The intra- and inter-assay coefficients of variations were less than 10%. The concentrations of samples in each plate were calculated based on a standard curve.

2.5. Statistical analysis

The results were evaluated with Sigma Stat 3.5 (Jandel Scientific Corp., San Jose, California, USA). Normality of data distribution was confirmed with the Kolmogorov-Smirnov test. Since the variables did not follow normal distribution, non-parametric tests were applied and the results were expressed as median and Q1-Q3 range. Kruskal-Wallis ANOVA by ranks was used to compare four groups, with the Dunn's multiple comparisons test for post-hoc comparisons. Age, MMSE, CDT and MoCA scores and BDNF concentrations were correlated using Spearman's correlation coefficient. All tests were two-tailed, and α was set at 0.05.

G*Power 3 Software (Faul et al., 2007) was used to calculate the needed sample size and statistical power. With expected moderate effect size = 0.25, and statistical power set to 0.800, the required sample size was $N = 180$ for Kruskal-Wallis ANOVA and $N = 84$ for correlation. Since the study included 363 participants, it had the needed sample size to detect differences between groups.

3. Results

Clinical variables for veterans with PTSD are presented in Table 1. Healthy control subjects and veterans with PTSD were matched for age.

Significant differences were found in plasma BDNF concentration between healthy controls, PTSD subjects, subjects diagnosed with MCI or AD (Fig. 1). These differences ($F = 40.22$; df=3; $p<0.001$) were the result of the higher plasma BDNF concentrations in healthy control subjects (1.740, 1.000–3.630) compared to PTSD subjects (0.785, 0.455–1.735), subjects diagnosed with MCI (0.800, 0.335–1.835) or AD (1.070, 0.575–2.180). Veterans with PTSD had significantly lower plasma BDNF concentration than healthy controls. The lowest BDNF plasma concentration was detected in PTSD subjects, even when compared to individuals with AD ($p \leq 0.050$; Dunn's post-hoc test).

When comparing cognitive status using the MMSE and CDT between healthy controls, veterans with PTSD and subjects with AD and MCI (Table 2), included participants differed significantly by age ($p<0.001$) since subjects diagnosed with MCI and AD were significantly older than healthy controls and PTSD patients. Veterans with PTSD did not differ in age vs. healthy controls. Subjects with PTSD, AD or MCI had significantly lower ($p \leq 0.050$; Dunn's post-hoc test) MMSE scores compared to scores in healthy control subjects. Patients with AD had the lowest MMSE scores when compared to other groups of subjects. When comparing CDT scores between groups, healthy control subjects had the highest CDT scores, while AD patients had the lowest CDT scores. Veterans with PTSD had significantly lower CDT scores compared to healthy controls and to subjects with MCI (Table 2).

Since four groups of participants differed by age, to evaluate possible effect of age on plasma BDNF levels, Spearman correlation was used. No significant correlation between age and peripheral BDNF concentration was detected in healthy control subjects, subjects with PTSD, subjects with MCI or subjects with AD (Table 3). However, no significant correlation was found between severity of cognitive symptoms, evaluated with MMSE and CDT, and BDNF plasma concentration in healthy subjects, veterans with PTSD, subjects with MCI and patients with AD (Table 3). In order to confirm the effect of diagnosis on BDNF plasma levels, and to correct for the possible effect of cognitive decline (MMSE and CDT scores) and age difference, multiple linear regression analysis (with diagnosis, age, MMSE scores, and CDT scores as independent variables) was used. Multiple linear regression analysis with plasma BDNF concentration as dependent variable revealed a significant model ($F(4358)$=2.80; $p = 0.026$; R^2_{adj}=0.019), due to the significant effect of diagnosis ($p = 0.014$) and age ($p = 0.034$), with no significant effects of MMSE ($p = 0.302$), and CDT ($p = 0.117$) scores on plasma BDNF concentration.

To further evaluate this lack of association/correlation between BDNF concentration and cognitive scores in individual groups, healthy control subjects and patients with PTSD were additionally subdivided into cognitive categories according to the MMSE, CDT and MoCA scores (Table 4). Within MMSE categories, subjects were subdivided into those with normal cognitive functions (with 26–30 MMSE scores), those with mild cognitive deterioration (with 21–25 MMSE scores) and those with moderate cognitive disturbances (with 10–20 MMSE scores). There were no subjects with MMSE scores lower than 10. Significantly lower plasma BDNF concentrations were found in subjects with moderate ($p = 0.006$; Dunn's post-hoc test) and mild ($p<0.001$; Dunn's post-hoc test) cognitive impairment, compared to subjects with normal cognitive functions (Table 4), assessed with the MMSE scores. Within CDT categories, subjects were subdivided into those with normal cognitive functions (with 5 CDT scores), and those with cognitive disturbances (with 0–4 CDT scores), Table 4. Plasma BDNF concentration differed significantly ($U = 9600.0$; $p<0.001$, Mann-Whitney U test) due to reduced plasma BDNF concentration in subjects with cognitive disturbances compared to values in subjects with normal cognitive functions evaluated by the CDT scores. In addition, healthy controls and PTSD subjects were also subdivided according to the MoCA categories (Table 4) into subjects with normal cognitive functions (with 0–25 MoCA scores) and subjects with cognitive disturbances (with 26–30 MoCA scores). Significant differences were found in plasma BDNF concentration ($U = 5564.0$; $p = 0.001$, Mann-Whitney U test), due to the significantly lower plasma BDNF concentration in subjects with cognitive disturbances ($p<0.001$) compared to subjects with normal cognitive functions, according to the MoCA scores (Table 4). There were significant correlations (Spearman coefficient of correlation) between BDNF concentration and cognitive scores evaluated using MMSE (r_s =0.350; $p = 0.001$), CDT (r_s=0.302; $p = 0.001$) and MoCA (r_s=0.369; $p = 0.001$) scores in healthy control subjects and patients with PTSD. All these results collectively suggested that BDNF concentration was significantly lower in cognitively impaired subjects.

Table 1
Demographic and clinical date of healthy control subjects (HC) and war veterans with PTSD.

	Subject groups		Mann-Whitney U test	
	HC($n = 120$)	PTSD($n = 120$)	*U*	*p*
Age (years)	59.0 (50.5–68.5)	59.0 (55.0–65.0)	7419.0	0.684
CAPS	NP	86 (78–90)	NP	NP
Age of onset	NP	40.5 ± 8.9 (years)	NP	NP
Duration of PTSD	NP	16.1 ± 2.1 (years)	NP	NP
CTQ	NP	61 (57–66)	NP	NP
PANSS	NP	63 (58–66)	NP	NP
Number of traumatic events	NP	8 (6–9)	NP	NP

CAPS=Clinician-administered PTSD scale for DSM-5; CTQ=Childhood trauma questionnaire; HC=Healthy control; *n*=number of subjects; NP=Not applicable; PANSS=Positive and negative syndrome scale; PTSD= Post-traumatic stress disorder.

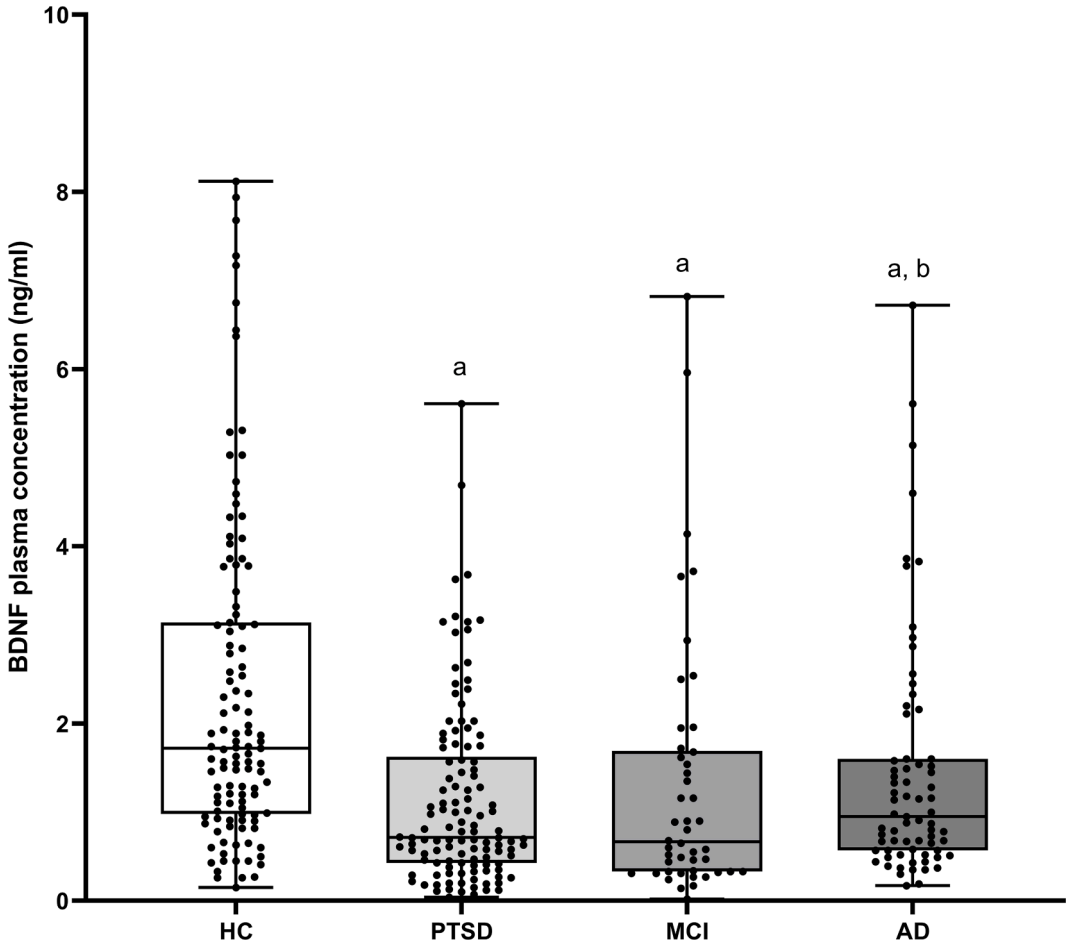

Fig. 1. BDNF plasma concentration in healthy controls (HC), veterans with PTSD, subjects with MCI and AD patients.
AD=Alzheimer's disease; BDNF=brain derived neurotrophic factor; HC=healthy controls; MCI=mild cognitive impairment; PTSD=posttraumatic stress disorder; data are shown as scatterplots; [a]<0.050 vs. HC (Dunn's post-hoc test); [b]<0.050 vs. PTSD (Dunn's post-hoc test).

Table 2
Comparison of age, MMSE scores and CDT scores between healthy controls (HC), veterans with PTSD, subjects with MCI and AD patients. Data are presented as median (25th - 75th percentile).

	Subject groups				Kruskal-Wallis ANOVA (*df*=3)	
	HC(n = 120)	**PTSD**(n = 120)	**MCI**(n = 47)	**AD**(n = 76)	***H***	***p***
Age (years)	59.0 (50.5–68.5)	59.0 (55.0–65.0)	72.0[a,b] (67.5–76.5)	75.0[a,b] (69.5–79.5)	141.29	<0.001
MMSE	30.0 [c] (30.0–30.0)	25.0[a,c] (22.0–25.0)	26.0[a,c] (22.5–27.0)	17.5[a] (13.0–21.0)	296.67	<0.001
CDT	5.0 (5.0–5.0)	3.0[a] (1.0–3.0)	4.0[a,b,c] (3.0–5.0)	1.0[a,b] (0.0–2.0)	200.29	<0.001

AD=Alzheimer's disease; CDT=Clock Drawing test; HC=healthy controls; MCI=mild cognitive impairment; MMSE=Mini Mental State Examination; n=number of subjects; PTSD=posttraumatic stress disorder;.
[a] p less than 0.050 vs. HC (Dunn's post-hoc test);
[b] p less than 0.050 vs. PTSD (Dunn's post-hoc test);
[c] p less than 0.050 vs. AD (Dunn's post-hoc test).

4. Discussion

This study is the first to evaluate the association between plasma BDNF concentration and cognition in veterans with PTSD, and compare these data with those in subjects with MCI, patients with AD and healthy control subjects. The results revealed that plasma BDNF concentration was significantly reduced in veterans with PTSD compared to age matched healthy subjects, and that veterans with PTSD, although younger than MCI subjects, had similarly decreased BDNF concentration and similar cognitive deterioration as subjects with MCI. Lower BDNF concentration in veterans with PTSD compared to healthy subjects is not consistent with previous results suggesting higher plasma and serum BDNF concentration in subjects with PTSD, compared to control subjects with or without PTSD (Mojtabavi et al., 2020). However, Mojtabavi and colleagues (2020) did not evaluate cognition in their PTSD sample. Therefore, disturbances in cognitive tasks in veterans with PTSD might explain the reduced plasma BDNF concentration found in our veterans with PTSD. In addition, differences might be due to the fact that these included participants had different clinical backgrounds (PTSD has developed antepartum, after earthquake, or after road traffic accident or

Table 3
Correlation between plasma BDNF concentration and age, MMSE scores and CDT scores in healthy controls (HC), veterans with PTSD, and in subjects with MCI or AD.

	Plasma BDNF (ng/ml)							
	HC ($n = 120$)		PTSD ($n = 120$)		MCI ($n = 47$)		AD ($n = 76$)	
	r_s	p	r_s	p	r_s	p	r_s	p
Age	−0.022	0.813	0.015	0.874	−0.037	0.804	0.091	0.436
MMSE	−0.110	0.232	−0.014	0.880	−0.183	0.219	−0.201	0.082
CDT	0.102	0.269	−0.017	0.850	0.025	0.866	−0.051	0.664

AD=Alzheimer's disease; BDNF=brain derived neurotrophic factor; CDT=Clock Drawing test; HC=healthy controls; MCI=mild cognitive impairment; MMSE=Mini Mental State Examination; n=number of subjects; PTSD=posttraumatic stress disorder; r_s=Spearman's correlation coefficient.

Table 4
Plasma BDNF concentration in healthy control subjects and war veterans with PTSD ($n = 240$) subdivided according to the severity of cognitive impairment into cognitive categories.

Cognitive test	Cognitive categories		
MMSE:	10≤MMSE≤20 ($n = 18$)	21≤MMSE≤25 ($n = 77$)	MMSE≥26 ($n = 145$)
Plasma BDNF (ng/ml)	0.701[a] (0.580–1.408)	0.828[a] (0.448–1.728)	1.660 (0.871–3.120)
Kruskal-Wallis ANOVA	$H = 23.60$; df=2; $p<0.001$		
CDT:	CDT<5 ($n = 100$)	CDT=5 ($n = 140$)	
Plasma BDNF (ng/ml)	0.817 (0.439–1.597)	1.715 (0.885–3.129)	
Mann-Whitney U test	$U = 9600.0$; $p<0.001$		
MoCA:	MoCA≤25 ($n = 49$)	MoCA≥25 ($n = 191$)	
Plasma BDNF (ng/ml)	0.711 (0.481–1.500)	1.464 (0.696–2.819)	
Mann-Whitney U test	$U = 5564.0$; $p = 0.001$		

CDT=Clock drawing test; MMSE=Mini mental state examination; MoCA=Montreal cognitive assessment; n=number of subjects;.
[a] p less than or equal to 0.050 vs. MMSE less than or equal to 26 (Dunn's post-hoc test).

combat), had other psychiatric disorders or hepatocellular carcinoma (Mojtabavi et al., 2020), while our study excluded liver diseases and included only combat related PTSD. Differences might also be due to inadequate statistical power or small sample size, ranging from 10 to 102 (Mojtabavi et al., 2020).

However, the unexpected result was that veterans with PTSD had even lower plasma BDNF concentration than patients with AD, who had reduced plasma BDNF concentration compared to healthy control subjects. This finding might be explained by the fact that higher BDNF levels are associated with the early stage of AD, when compared to patients with late stage of AD (Laske et al., 2006), suggesting that our patients diagnosed with AD are still in the early stages of the disease. In conformation, the median MMSE score was 17.5 for our AD patients, suggesting moderate dementia (13–20 scores). Therefore, either some compensatory effect, or treatment with donepezil (Leyhe et al., 2008), mood stabilizers (Ventriglia et al., 2013), vortioxetine (Dvojkovic et al., 2021; Sagud et al., 2016) or other antidepressant medication (Ng et al., 2019) that increase plasma BDNF concentration, might explain higher plasma BDNF levels in patients with AD.

In our study veterans with PTSD had similar BDNF concentration as subjects with MCI suggesting a high risk of cognitive deterioration (Fujiwara et al., 2021). There are inconsistent findings regarding blood BDNF levels in MCI, and meta-analysis reported similar (although slightly reduced) serum BDNF levels in subjects with MCI compared to healthy controls (Ng et al., 2019). In line with the effect of age on BDNF and on cognitive function (Miranda et al., 2019; Ng et al., 2019), multiple linear regression confirmed that diagnosis and age significantly affected plasma BDNF concentration in our study.

Altered BDNF levels were found in AD, indicating the lack of neurotrophic support in the physiopathology of AD and progression to neurodegeneration in older age or MCI (Diniz and Teixeira, 2011). In agreement, circulating BDNF levels were decreased in AD (Laske et al., 2006; Ng et al., 2019) and reduced peripheral BDNF levels were also detected in AD subjects with MMSE scores lower than 20, when compared to control subjects (Kim et al., 2017). Opposed to the findings of the meta-analyses (Kim et al., 2017; Ng et al., 2019), in the present and previous (Forlenza and Miller, 2006) studies, BDNF concentration was found to be lower in MCI subjects, revealing reduction in trophic support and contributing to progressive neurodegeneration. In line with our findings, similar blood BDNF levels were found among subjects with MCI and patients with AD when controlled for the effect of age, sex, and drug use (Kim et al., 2017). In our study lower plasma BDNF levels were associated with diagnosis and age, but not with MMSE or CDT scores, according to the multiple regression analysis, but BDNF was not correlated with age in individual groups. However, when healthy control subjects and patients with PTSD were additionally subdivided into cognitive categories according to the MMSE, CDT and MoCA scores, plasma BDNF concentration was significantly associated with cognitive deterioration. Namely, reduced plasma BDNF concentration was detected in groups with significant cognitive decline compared to groups with normal cognitive function, assessed using the MMSE, CDT and MoCA scores. In addition, plasma BDNF concentration was significantly correlated with MMSE, CDT and MoCA scores in these subjects. The MMSE scores are important moderators that might explain the heterogeneity of the reported findings of circulating BDNF levels in dementia (Ng et al., 2019). In our study all groups (veterans with PTSD, subjects with MCI and AD) had significantly lower plasma BDNF concentration and also significantly reduced MMSE and CDT scores compared to healthy subjects, showing cognitive decline. This reduction in BDNF might indicate a lack of neurotrophic support and consequent alterations in synaptic plasticity and memory process, including memory consolidation (Miranda et al., 2019). The association between reduced MoCA scores and plasma BDNF concentration agrees with associations between higher serum BDNF levels with substantially lower odds of cognitive decline in community-dwelling older adults (Fujiwara et al., 2021). Our results collectively suggest that decreased BDNF is associated with cognitive disturbances in these participants. The possible influence of sex was excluded since we included only male subjects, while possible influence of age and diagnosis was detected.

Regarding cognitive tasks, veterans with PTSD had reduced MMSE, CDT and MoCA scores compared to healthy control subjects who were matched for age, and similar MMSE scores as older individuals with MCI. In agreement with our findings, the MMSE scores were significantly reduced in subjects with PTSD compared to control subjects (Fayyazi Bordbar et al., 2012). However, the unexpected result was that veterans with PTSD had even lower CDT scores than subjects with MCI. These results suggest that cognitive deficits are more pronounced in veterans with PTSD than in control subjects of the general population, and that cognitive decline, evaluated with MMSE, is similar as in older subjects with MCI, while cognitive tasks of drawing clock are worse than in MCI individuals. In line with cognitive decline in PTSD, in our previous studies, other groups of Croatian veterans with PTSD had cognitive

deterioration evaluated using the ROCF test, showing deficits in visual spatial perception and short and long-term visual memory functions (Havelka Mestrovic et al., 2020), and using the PANSS cognition subscale scores (Nedic Erjavec et al., 2021). In a smaller Italian study, within 4 years follow-up, lower MMSE scores were detected in 17% of patients with PTSD suggesting matched criteria for dementia (Bonanni et al., 2018). After a longer follow up of 6–10 years, 13% of PTSD population developed semantic frontotemporal dementia, while 16% of patients with dementia had a history of PTSD (Bonanni et al., 2018). Since our study does not have a longitudinal design, we cannot demonstrate how many PTSD veterans have developed dementia. However, the MMSE scores for 120 PTSD subjects included in this study ranged from 22 to 25 and median was 25, suggesting a state between mild dementia (21–24 scores) and normal cognition. It is unclear whether cognitive deterioration develops prior to PTSD or is a complication of PTSD. Longitudinal assessment of veterans prior to combat and after the deployment might answer this question. Some studies have found that lower cognitive function before trauma increased the risk of developing PTSD (Brewin et al., 2000; Parslow and Jorm, 2007), while reduced pre-trauma visual immediate memory was related to higher post-deployment PTSD symptom severity in veterans (Marx et al., 2009).

In our study reduced plasma BDNF concentration was found in cognitively impaired subjects. Therefore, veterans with PTSD deserve to be closely monitored, to be able to early detect and predict cognitive worsening and to promote interventions that might increase blood BDNF levels and improve cognitive functions. These strategies include medication such as memantine and donepezil, but also exercise, diet, increased physical activity, intellectual stimulation and yoga (reviewed in Ng et al., 2019), interventions aiming to increase BDNF and to slow progression of dementia.

Limitations and strengths of the study should be acknowledged. Limitations are in the cross-sectional design, therefore due to lack of longitudinal follow up, we are not able to detect how many PTSD subjects will develop dementia; in the inclusion of only male combat veterans with PTSD, hence we could not generalize these findings to civilian PTSD subjects or to female subjects; and in the use of only three cognitive scales (MMSE, CDT and MoCA), as a more extensive cognitive battery could be more useful. Since the main aim was to evaluate BDNF levels and cognitive decline in veterans with PTSD, the inclusion of comparative groups of older MCI and AD subjects could not be omitted. Other limitations are that, although healthy control subjects and veterans with PTSD were medication free, subjects with MCI and AD were on their usual treatment regimen, and in the lack of appropriate control group of war veterans without PTSD to determine cognitive decline and BDNF concentration as predictors of progression to MCI and/or AD. Healthy controls and veterans with PTSD did not have depression; however, subjects with MCI and AD might have also developed depression, which was not considered in our study.

Strengths are in the inclusion of ethnically homogenous population for all groups, only male subjects, and for the comparator group for PTSD veterans we included age matched healthy control subjects. This study has adequate sample size ($N = 363$) and needed statistical power. In addition, as recently suggested, all BDNF determinations were done by researchers who were blind to diagnoses of subjects, and using and R&D System-Quantikine BDNF ELISA assays, reported to be specific for mature human BDNF (Ng et al., 2019).

Author statement

Nela Pivac developed the original idea. Matea Nikolac Perkovic and Gordana Nedic Erjavec and Dubravka Svob Strac collected blood samples, isolated plasma and platelets. Matea Nikolac Perkovic determined plasma BDNF concentration. Matea Nikolac Perkovic performed all the statistical analysis. Sandra Domitrovic Spudic, Suzana Uzun, Oliver Kozumplik and Ninoslav Mimica explained the research goals and described protocol in details to the patients; explained the inclusion/exclusion criteria, insured participant adherence for the participation in the study, motivated, selected, diagnosed, evaluated and sampled all patients and control subjects. Matea Nikolac Perkovic and Nela Pivac did the data analysis and interpretation. Nela Pivac and Matea Nikolac Perkovic wrote the final draft, and did the proof reading of the manuscript. All authors contributed to the final version of the manuscript.

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Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committees of the University Hospital Dubrava, Zagreb (protocol code: none; 20 February 2006) and University Psychiatric Hospital Vrapce, Zagreb, Croatia (protocol code 23–209-/4–19; 26 June 2019). Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical restrictions.

Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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