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Higher exosomal tau, amyloid-beta 42 and IL-10 are associated with mild TBIs and chronic symptoms in military personnel

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Abstract

Objective: Identify biomarkers in peripheral blood that relate to chronic post-concussive and behavioural symptoms following traumatic brain injuries (TBIs) to ultimately improve clinical management.

Research design: We compared military personnel with mild TBIs (mTBIs) ($n = 42$) to those without TBIs ($n = 22$) in concentrations of tau, amyloid-beta ($A\beta_{42}$) and cytokines (tumour necrosis factor alpha (TNF α , interleukin (IL)-6 and -10) in neuronal-derived exosomes from the peripheral blood. We utilized nanosight technology coupled with ultra-sensitivity immunoassay methods. We also examined the impact of post-concussive and behavioural symptoms including depression and post-traumatic stress disorder (PTSD) on these neuronal-derived markers.

Results: We report that concentrations of exosomal tau ($F_{1, 62} = 10.50$), $A\beta_{42}$ ($F_{1, 61} = 5.32$) and IL-10 ($F_{1, 59} = 4.32$) were elevated in the mTBI group compared to the controls. Within the mTBI group, regression models show that post-concussive symptoms were most related to exosomal tau elevations, whereas exosomal IL-10 levels were related to PTSD symptoms.

Conclusions: These findings suggest that chronic post-concussive symptoms following an mTBI relate to altered exosomal activity, and that greater tau pathology may underlie chronic post-concussive symptoms that develop following mTBIs. It also suggests that central inflammatory

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Declaration of Interest

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activity contributes to PTSD symptoms following an mTBI, providing necessary insights into the role of inflammation in chronic PTSD symptoms.

Keywords

Exosomes; post-traumatic stress disorder; tau; traumatic brain injury (TBI); inflammation

Introduction

Traumatic brain injuries (TBIs), specifically mild TBIs (mTBIs), were the signature injuries of deployments in Operation Iraqi Freedom and Operation Enduring Freedom (1,2). Military personnel with a TBI have higher global disability, neurobehavioural impairment, post-traumatic stress disorder (PTSD) and depression, compared to non-injured service members (3,4). Previously, we have identified chronic post-concussive symptoms resulting from a TBI, to be associated with elevated plasma levels of tau and amyloid-beta (A β 42) (5,6). In civilians with moderate-to-severe TBIs, acute elevations of tau and A β 42 are linked to the onset of chronic symptoms (7). Post-mortem studies of athletes with repetitive head impacts have linked these exposures to chronic traumatic encephalopathy (CTE), a neurodegenerative disease defined by neuronal, astroglial and perivascular accumulations of phosphorylated tau (p-tau) at the depths of cortical sulci that form neurofibrillary tangles (8). A β deposition, both diffusely and in the form of neuritic plaques, in post-mortem CTE cases is associated with accelerated disease progression (9). Positron emission tomography (PET) imaging studies also demonstrate A β aggregates in athletes with sub-concussive hits and possible brain injuries (10); this is concerning, considering that A β accumulation following a severe TBI relate to the development of chronic symptoms (11). These findings raise questions regarding the role of tau and A β in the onset and progression of chronic post-concussive symptoms in military personnel with TBIs.

The role of inflammation following acute TBIs indicates that there is an initiation of inflammatory events (12–14), and when cytokines including interleukin (IL)-10, –8 and –6 (15–17) are elevated, patients are less likely to recover. Less is known regarding links between chronic post-concussive symptoms and inflammation following a TBI; however, behavioural symptoms, including PTSD, in military personnel both with and without TBIs relate to greater inflammation (18). Previously, we reported that in deployed military personnel, greater inflammation, as indicated by IL-6 elevations, related to higher PTSD symptoms, but did not relate to having a TBI (19). Other studies have shown associations between inflammatory activity and PTSD, in civilians (20,21), veterans and military personnel (22,23). A major limitation of this line of research is the inability to determine peripheral vs. central inflammation in studies using only blood-based biomarkers, resulting in a minimal understanding of the nature of inflammation in TBI and PTSD, and its role in chronic symptoms. Blood is the most clinically available source for biomarker discovery in clinical cohorts; therefore, advances in laboratory methods that provide new opportunities to link measures of central inflammation to behavioural symptoms using peripheral samples will likely advance this field of research.

Here, we have examined exosomes, which are extracellular vesicles (EVs) released by cells into extracellular spaces and contain cargo that reflects the content of the vesicle's cellular origin. Neuronal-derived exosomes can cross the blood–brain barrier and be detected in peripheral blood (24), providing a new avenue for probing neuropathological changes in TBIs. Exosomes are relevant to TBI since they are implicated in the removal of aggregated proteins in neurons and in cell-to-cell communication (25–27). A recent study reports that plasma and tau-positive exosomes were greater in retired National Football League (NFL) players compared to controls, and that these elevations were related to worse memory and psychomotor test performance (28). Exosomes carrying tau and A β (28,29), which are linked to both clinical Alzheimer's disease (AD) (30–33) and preclinical AD (30,33), suggest that the same neuropathology seen in CTE and AD may be observed in neuronal-derived exosomes in military personnel with mTBIs. Similarly, a recent study observed elevated levels of A β peptides and p-tau in neuronal-derived exosomes from patients with Down syndrome, perhaps indicating a preclinical AD phase in these individuals (34). Examining neural exosome protein markers of neuronal integrity and inflammation provides a minimally invasive window into the secondary injury mechanisms and pathophysiology following an mTBI. The purpose of this study was to determine the role of neuronal proteins (tau, A β 40 and A β 42) and inflammatory cytokines (IL-6, IL-10 and tumour necrosis factor alpha (TNF α)) within neuronal-derived exosomes and their role in chronic post-concussive and behavioural symptoms following a TBI in military personnel.

Materials and Methods

Clinical methods

This study is based on an observational assessment of US military personnel at the Madigan Army Medical Center who had been deployed within the previous 18 months (IRB approval: 145947). Participants included 42 military personnel who had sustained an mTBI and 22 healthy controls. Self-reported mTBI cases were identified using the Warrior Administered Retrospective Casualty Assessment Tool (WARCAT). Participants were classified as having an mTBI if they reported an event whereby they sustained a blow to the head (from: fragments, vehicular, blast, blow to the head or fall) and had a loss of consciousness (LOC) of 20 min or less (35). Participants were classified in the control group if they reported no events/injuries listed above on the WARCAT. Exclusion criteria for both cases and controls included: (1) history of drug or alcohol abuse; (2) a severe medical condition (e.g. cancer, diabetes, HIV or autoimmune disorders); (3) a severe psychiatric condition (i.e. schizophrenia or bipolar disorder); (4) a severe neurologic disorder (e.g. multiple sclerosis, seizure disorders, severe cognitive impairments suspected to be related to dementia or other progressive disease, and a history of stroke); and (5) a history of TBI that was classified as moderate, severe as indicated by a LOC greater than 20 min as determined by the WARCAT. Demographic information regarding age, gender, ethnicity, race, marital status, education level, service branch, number of deployments and time since deployment were obtained from each participant.

Determination of post-concussive PTSD and depression symptoms

The Neurobehavioural Symptom Inventory (NSI) was administered by trained research assistants to measure post-concussive symptom severity. The NSI is a 22-item tool which rates the presence/severity of each symptom on a 5-point scale (none, mild, moderate, severe, very severe). The NSI has a high internal consistency (total $\alpha = 0.95$; subscale $\alpha = 0.88$ – 0.92) and reliability ($r = 0.88$ – 0.93) (36). Symptoms of PTSD were assessed by the PTSD Checklist – Military Version (PCL-M), resulting in a score of 0–80 (37,38). The quick inventory of depressive symptomatology (QIDS) was used to measure total symptoms of depression. QIDS scores range from 0 to 27 (38).

Laboratory methods

Biological samples—Blood samples were collected between 0900 and 1100 h into plastic K₂EDTA tubes, inverted five times and placed on ice. Within 30 min of blood collection, samples were centrifuged at 4°C (for 15 min at 2000×g). Prior to isolation, samples were processed according to International Society of Extracellular Vesicles (ISEV) guidelines (39). Samples were stored at –80°C until further EV isolation.

EV isolation—EVs were isolated from 0.5 mL of frozen human plasma containing EDTA. After samples were thawed, 0.2 mL of Thromboplastin-D (Thermo-Fisher Scientific Inc., Rockford, IL, USA) was added and samples were incubated for 30 min at room temperature (RT). Thromboplastin-D was added to EDTA samples to remove fibrin and render the EV pellet easier to dissolve, according to instructions by the manufacturer of Exoquick® (https://www.systembio.com/downloads/PAC_EXOQ5TD-1.pdf) and guidelines by the ISEV (39). Next, samples received 0.3 mL of Dulbecco's calcium- and magnesium-free salt solution (Sigma-Aldrich, St. Louis, MO, USA) containing three times more than the suggested concentrations of protease inhibitor cocktail (Complete Tablets Easy pack; Roche Applied Sciences, Inc., Indianapolis, IN, USA) and phosphatase inhibitor cocktail (Pierce Halt; Thermo-Fisher Scientific, Inc., Rockford, IL, USA). After a 5-min incubation at RT, samples were centrifuged at 4°C (for 20 min at 4000×g). Supernatant fluid was transferred into fresh tubes and 252 µL of ExoQuick exosome solution (System Biosciences, Inc., Mountain View, CA, USA) was added. After mixing, the ExoQuick suspensions were incubated for 60 min at 4°C to precipitate total EVs and then centrifuged at 4°C (for 5 min at 1500×g). Resulting supernatant fluid was discarded and the pellet containing EVs was resuspended in 0.5 distilled mL of ultra-pure water (Invitrogen-Thermo-Fisher Scientific, Inc., Rockford, IL, USA) containing three times the suggested concentrations of the abovementioned protease and phosphatase inhibitors. To enrich for neuronal-derived EVs, suspensions were incubated for 1 h at 4°C with 4 µg of mouse-anti-human CD171 (L1CAM) biotinylated antibody (clone 5G3; eBioscience, San Diego, CA, USA) in 50 µL of 3% Bovine Serum Albumin (BSA) (1:3.33 dilution of blocker BSA in a 10% solution in PBS; Thermo-Fisher Scientific, Inc., Rockford, IL, USA) per tube. Next, 15 µL of Streptavidin-agarose Ultralink resin (Thermo-Fisher Scientific, Inc., Rockford, IL, USA) in 25 µL of 3% BSA was added to each tube. Samples were incubated with continuous mixing for 30 min at 4°C and then centrifuged at 4°C (for 10 min at 200×g). Again, resulting supernatant fluid was discarded. Each pellet was resuspended in 200 µL of 0.1 M glycine-HCl and centrifuged at 4°C (for 10 min at 4500×g). Supernatant fluid was then transferred to new tubes containing 25 µL of

10% BSA and 15 μ L of 1 M Tris-HCl and mixed. To lyse EVs, 260 μ L of mammalian protein extraction reagent (M-PER) was added to each tube (Thermo Scientific, Inc., Rockford, IL, USA). After EV isolation and lysis, the reconstituted diluents were stored at -80°C . Samples were thawed immediately prior to the quantification of each analyte; all samples went through the same amount of freeze/thaw cycles, which was not more than five, which is the earliest where the changes in analyte measurements can be observed (40,41). L1CAM+ EVs were isolated from an initial volume of 0.5 mL of plasma and the final volume of lysed EV diluents was also 0.5 mL. Therefore, all the expressed values refer to the original plasma volume.

Electrochemiluminescent immunoassays—General markers for EVs CD81, TSG101 and ALIX were quantified using custom electroluminescence assays developed in our laboratory using MSD GOLD Streptavidin (Meso Scale Diagnostics, Rockville, MD, USA).

Exosome counts—Each suspension of EVs (10 μ L of vesicles in 90 μ L PBS), collected after addition of glycine and before lysis with M-PER, was diluted to final dilution of 1:200. The Nanosight NS500 system was used in combination with a G532nm laser module and a nanoparticle tracking analysis (NTA) 3.1, nanoparticle tracking software (Malvern Instruments, Malvern, UK), to determine the mean diameter (nm) and concentration (particles/mL) of EVs. Camera settings were set as follows: gain 366; shutter 31.48; frame rate 24.9825 fps/s; five repeated 20-s video recordings were acquired. For each analyte, we examined a General Linear Model with and without co-varying EV concentrations determined by NTA, thereby adjusting for differential EV yield between samples. The EV size distributions were observed aligned with those previously reported by Saenz-Cuesta et al., showing similar EV size distribution when using similar isolation techniques (42).

Exosomal protein measurement—Concentrations of tau, A β 40, A β 42 and cytokines (IL-6, IL-10 and TNF α) in the cargo of neuronal-derived exosomes isolated from plasma samples were measured using digital array technology (Quanterix Corporation, Lexington, MA, USA); this method uses single molecule enzyme-linked immunoarrays (SimoaTM) (Quanterix, Boston, Mas, USA) (43). Both, the intra- and inter-assay coefficients of variation (CV) values were below 10%, with an average CV value of 7.09% for tau, 5.12% for A β 40, 5.67% for A β 42 and 4.38% for cytokines.

Data analysis

Data management and analyses were conducted using SPSS Statistics (IBM SPSS Inc., Chicago, IL, USA). Descriptive statistics were calculated for all demographic and clinical variables. TBI cases were compared to controls without TBIs. Group comparisons were made using chi-square (χ^2) for categorical variables and analysis of variance (ANOVA) for continuous variables. The *a priori* cut-off for statistical significance was $p < 0.05$ after applying the false discovery rate adjustment for multiple comparisons. To examine the role of post-concussive and behavioural symptoms following TBI, regression analyses were used in the TBI group to determine which symptoms most related to those proteins that significantly differed in the comparisons of TBI to non-TBI controls. A series of multivariable linear regressions were performed once the assumptions of regression models

were assured. Multicollinearity was examined to ensure that there were no significant intercorrelations that would have impacted regression results. Specifically, the variance inflation factor was calculated and determined to be indicative of collinearity if the value was equal or greater than 5. Correlations between exosomal tau, A β 40/A β 42 and exosomal cytokines (IL-6, IL-10 and TNF α) were calculated using Pearson correlation.

Results

Demographic and clinical characteristics

The sample included a total of 60 men and 4 women and the mean age of the participants was 31.3 (SD = 6.76) years. There were high rates of co-morbid depressive and PTSD symptoms in both groups (Table 1).

In the TBI group, the mean number of TBIs was 2.5 (SD = 1.6); notably, 30.0% of participants with a TBI reported four or more previous TBIs. The most common types of injuries were a blast exposure (52.5%), vehicular collision (37.5%), blow to the head (30.0%) and sports-related concussion (30.0%). Approximately, 38.5% of those with an mTBI reported losing consciousness for 1–20 min. The time since last TBI ranged from 3 months to over 3 years, with most participants reporting the TBI at least 18 months prior to the study. With respect to the total number and severity of symptoms reported using the NSI, the mTBI group had a higher ($p = 0.007$) mean score of 31.78 (SD = 16.86) compared to 17.77 (SD = 11.71) in the control group with no TBIs (Table 2).

Exosomal tau, A β 40, A β 42, cytokines and exosomal marker TSG10 in mTBI cases vs. controls

EV concentrations (particles/mL) among participants with no TBI and those with an mTBI were non-significant (Figure 1), and results for all analyses were similar without co-varying EV concentrations. Compared to controls without TBI, patients with mTBIs had elevated concentrations of exosomal tau ($F_{1, 62} = 10.50$, $p < 0.01$), A β 42 ($F_{1, 61} = 5.32$, $p < 0.01$) and IL-10 ($F_{1, 59} = 4.92$, $p < 0.05$) (Figure 2A–C).

A trend towards an increase in A β 40 ($F_{1, 59} = 3.18$, $p = 0.080$) was also observed in the mTBI group compared to controls (data not shown). There were no significant differences in TSG101, CD81 or ALIX marker levels (p 's > 0.40) (data not shown). Correlates between exosomal concentrations of tau, A β and cytokines were non-significant (p 's < 0.20).

Relationships between exosomal tau, A β 42 and IL-10, and post-concussive and behavioural symptoms

In the overall regression model of exosomal tau that included NSI, PTSD and depression symptoms, we observed a significant model ($r = 0.539$, $F_{3, 40} = 4.96$, $p < 0.01$). The only significant factor, however, was that of NSI ($B = 0.70$, $t = 2.95$, $p < 0.01$) and exosomal tau. PTSD only tended to relate to exosomal tau ($p = 0.08$) and depression did not relate to exosomal tau ($p = 0.12$). The IL-10 regression model that included NSI, PTSD and depression symptoms resulted in a significant model ($r = 0.651$, $F_{3, 40} = 5.72$, $p < 0.01$). In this model, PTSD was significantly related ($B = 0.8$, $t = 2.60$, $p < 0.01$) and depression

tended to be related ($B = 0.421$, $t = 1.41$, $p = 0.063$), but NSI did not relate to exosomal IL-10 ($p = 0.26$). The regression model for A β 42, NSI, PTSD and depression symptoms was not significant ($p = 0.15$). In all analyses, the variable inflation factor was below 5.

Discussion

Plasma exosomes enriched for neuronal origin had higher concentrations of tau, A β 42 and IL-10 in individuals with mTBIs and that elevations of tau related to post-concussive symptoms, whereas IL-10 related more to behavioural symptoms. This is the first report to link these exosomal markers to chronic symptoms following an mTBI. These findings support our previous studies that show higher peripheral tau in military personnel (5), civilians with moderate-to-severe TBIs (7), and other studies that link higher tau to adverse clinical outcomes 6 months after a severe TBI (44), indicating that peripheral tau in blood may underlie the risk for chronic post-concussive symptoms. These findings also suggest that chronic inflammation can be measured within neuronal-derived exosomes and that it relates to behavioural symptoms, supporting previous reports of higher inflammatory activity in peripheral blood of patients with PTSD and depression (18,20–23). Thus, both central and peripheral inflammation may relate to vulnerability for behavioural symptoms following an mTBI.

Our findings suggest that these neuronal-derived concentrations of tau and A β 42 may relate to the CTE pathology observed in military personnel and athletes who show deposition of tau and p-tau following repetitive head injuries, or even a single TBI (45–47). A β aggregates have also been observed in PET molecular imaging studies in athletes (10) and patients with severe TBIs (48). Therefore, it is likely that recovery from a TBI relates to the ability to clear both A β and tau, pointing towards the importance of exosomes. Previously, EVs have been implicated in both A β plaque formation and clearance (49,50). In the 5XFAD mouse model, inhibition of exosome biogenesis reduced A β 42 plaque load, whereas *in vitro*, exosomes were shown to interfere with A β clearance by astrocytes and microglia (51). Evidence of a protective function of EVs against A β comes from a PrPc knockout mouse model of neuroblastoma and SY5Y human neuroblastoma cell lines, which show that highly enriched exosomal PrPc to A β accelerates its fibrilization, thereby reducing neurotoxic effects caused by oligomeric extracellular A β (52). Sphingolipid-modulated exosome secretion promoted clearance of A β by microglia, again implicating a role for exosomes in A β activity (53). Thus, our observation of increased A β 42 in neuronal-derived exosomes may suggest that there are alterations in clearance of amyloid following mTBIs, increasing the susceptibility to chronic symptoms.

Neuronal exosomes are known to contain tau and there is an evidence to support that tau overexpression selectively recruits mitochondrial proteins implicated in neurodegeneration into exosomes (54). Thus, there is a novel link between tau exosome secretion and development of neurodegeneration following a TBI. In a study of ALZ17 mice, EVs containing P301L mutant tau accelerated pathological tau phosphorylation and oligomer formation (55). Since exosomes are extremely stable in peripheral circulation, molecules inside exosomes may serve as disease- and injury-specific diagnostic biomarkers for brain disorders, including TBI and CTE. Elevated exosomal tau is reported in symptomatic NFL

players compared to controls; however, in size-exclusion chromatography was used to determine the number of tau-containing exosomes (28) and no enrichment for neuronal origin was performed. Similar to our studies in AD (30–33), we used immunoprecipitation against L1CAM+ to isolate an EV population enriched for neuronal origin, rather than studying total plasma EVs. In doing so, we likely amplified the diagnostic signal-to-noise ratio and generated better-performing classifier biomarkers.

Our finding of higher IL-10 activity in patients with mTBI was not surprising, in that this elevation has been reported in TBI previously (16). Increased IL-10 levels have also been related to poor outcomes following a TBI (56). We were surprised to find that this elevation was related most to symptoms of PTSD, as previous studies measuring IL-10 in peripheral blood have not reported IL-10 to differ between patients with and without PTSD (19,57). This discrepancy may relate to our study measuring more central levels of inflammation, and suggesting that IL-10 may be a compensatory result of greater inflammation that has been reported in studies of patients with PTSD. In support of this, preclinical models that reduce neuronal inflammatory activity found that rats were protected against PTSD (58,59). Thus, additional studies are needed to better understand how inflammation in neuronal exosomes may relate to PTSD symptoms and the role of TBIs in these chronic symptoms.

This study had several limitations, including a relatively small sample size and a small number of demographic data missing for the participants in this study. The sample was further limited in that it consisted primarily of young males that varied in the time since the last TBI, necessitating more generalizable future studies. Furthermore, the control subjects used in this study were also military personnel, who may have had previous exposure to head trauma either through previous contact due to sports or combat training, even though they did not report a TBI. Nevertheless, our findings provide initial evidence that neuronal-derived EVs can be detected in peripheral blood even years following a TBI and relate to post-concussive symptoms. These findings suggest the potential for similar results in patients with various forms and acuity of TBI, where EVs may also constitute a causative factor in the progression of injury and thus, accumulation of cytotoxic proteins in neurons (60). Future prospective studies are needed to examine longitudinal changes in EVs and their content following TBI, as they may serve as prognostic indicators for chronic post-concussive symptoms, leading to improved identification of patients with TBI at high risk for chronic symptoms and perhaps CTE. These neuronal-specific biomarkers may also be useful in determining who is at differential risk for developing AD, CTE, chronic or progressive axonopathy.

Conclusion

This study is the first to examine the mechanisms underlying mTBIs and chronic symptoms using neuronal-derived exosomes and high-sensitivity detection of neurodegenerative and inflammatory biomarkers. We report exosomal levels of tau, A β 42 and IL-10 were significantly higher in military personnel with an mTBI, compared to individuals with no TBI, and that tau elevations were related to chronic post-concussive symptoms, whereas higher IL-10 levels were related to PTSD symptoms. These findings suggest that exosomes

may serve as clinically available, prognostic biomarkers to identify patients with mTBIs at risk for developing chronic symptoms

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Figure 1.
EV concentrations among participants.

Figure 2.

Neuronal-derived exosomal concentrations of tau, Ab42, and IL-10 (A-C) in military personnel with an mTBI, compared to those without mTBI.

Table 1.

Demographics and clinical characteristics of those with and without a TBI.

	No TBI (N = 22)	mTBI (N = 42)	Significance
Age, mean (SD), y	32.67 (8.58)	31.26 (5.60)	$F_{1, 63} = 1.588, p = 0.205$
Gender, No. (%)			$\chi^2 = 1.010, p = 0.12$
Male	20 (91%)	40 (95.23%)	
Female	2 (9%)	2 (4.67%)	
Ethnicity, No. (%)			$\chi^2 = 0.157, p = 0.692$
Hispanic	0 (0%)	7 (18%)	
Not Hispanic	22 (100%)	33 (83%)	
Race, No. (%)			$\chi^2 = 9.021, p = 0.182$
Caucasian	12 (55%)	21 (53%)	
African American	3 (14%)	5 (13%)	
Native American or Hawaiian	5 (23%)	4 (8%)	
Asian	1 (5%)	3 (9.52%)	
Mixed race	0 (0%)	3 (7.14%)	
Other/unknown	0 (0%)	6 (15%)	
Marital status, No. (%)			$\chi^2 = 2.01, p = 0.508$
Married	15 (68%)	25 (63%)	
Single	2 (9%)	9 (23%)	
Separated/divorced	4 (18%)	7 (16.67%)	
Education level, mean (SD), y	13.05 (1.77)	13.76 (1.89)	$F_{1, 663} = 0.010, p = 0.876$
Service branch, No. (%)			$\chi^2 = 1.20, p = 0.511$
Air Force	0 (0%)	1 (3%)	
Army	20 (91%)	36 (85.71%)	
Navy	0 (0%)	5.0 (2%)	
Number of deployments, mean (SD)	2.23 (1.10)	2.55 (1.60)	$F_{1, 62} = 1.016, p = 0.344$
Time since most recent deployment, No. (%)			$\chi^2 = 1.722, p = 0.622$
<3 months	3 (14%)	3 (8%)	
3–6 months	6 (27%)	10 (23.81%)	
6–12 months	6 (27%)	10 (25%)	
>12 months	5 (23%)	15 (38%)	
PCL-M score for PTSD, mean (SD)	34.01 (13.92)	39.02 (15.21)	$F_{2, 65} = 2.026, p = 0.1221$
QIDS score for depression, mean (SD)	9.02 (4.01)	11.30 (5.20)	$F_{2, 65} = 2.79, p = 0.1020$

Note: TBI, traumatic brain injury; SD, standard deviation; PTSD, post-traumatic stress disorder, PCL-M, PTSD Checklist – Military Version; QIDS, quick inventory of depression.

Table 2.

Comparison of NSI item symptom scores for those with and without an mTBI.

	NoTBI (N = 22)	mTBI (N = 42)	Significance
Total NSI score	17.77 (11.71)	31.78 (16.86)	$F_{1,60} = 8.017, p = 0.007$
Forgetfulness	0.86(1.28)	1.78 (1.37)	$F_{1,60} = 4.191, p = 0.021$
Concentration	1.09 (1.41)	1.60 (1.32)	$F_{1,60} = 1.986, p = 0.037$
Slowed thinking	0.72 (1.08)	1.48 (1.36)	$F_{1,60} = 4.942, p = 0.030$
Dizziness	1.09(1.02)	1.75 (1.28)	$F_{1,60} = 4.336, p = 0.042$
Headache	0.64 (0.85)	1.76 (1.10)	$F_{1,60} = 17.789, p = 0.000$
Hearing problems	0.86 (0.99)	1.55 (1.26)	$F_{1,60} = 4.863, p = 0.031$
Sleep difficulty	1.55 (1.63)	1.75 (1.51)	$F_{1,60} = 0.246, p = 0.622$
Nausea	0.64 (0.90)	1.60 (1.48)	$F_{1,60} = 7.701, p = 0.007$
Decision-making	0.45 (0.67)	1.56 (1.29)	$F_{1,60} = 8.206, p = 0.006$
Irritability	1.14(1.68)	1.67 (1.33)	$F_{1,60} = 2.646, p = 0.109$
Balance	0.91 (1.15)	1.83 (1.48)	$F_{1,60} = 6.289, p = 0.015$
Numbness	0.38 (0.67)	1.35 (1.17)	$F_{1,60} = 12.295, p = 0.001$
Coordination	1.09 (1.26)	1.58 (1.26)	$F_{1,60} = 13.753, p = 0.000$
Frustration	0.91 (1.06)	1.55 (1.28)	$F_{1,60} = 3.988, p = 0.050$
Light sensitivity	0.41 (0.80)	1.03 (1.27)	$F_{1,60} = 4.234, p = 0.044$
Taste/smell changes	0.14 (0.47)	0.50 (0.88)	$F_{1,60} = 3.255, p = 0.076$
Noise sensitivity	0.82 (1.22)	1.20 (1.30)	$F_{1,60} = 1.271, p = 0.264$
Vision problems	0.36 (0.58)	1.43 (1.22)	$F_{1,60} = 14.790, p = 0.000$
Anxiousness	1.36 (1.26)	1.63 (1.33)	$F_{1,60} = 0.568, p = 0.454$
Depression	1.00(0.93)	1.35 (1.39)	$F_{1,60} = 1.121, p = 0.294$
Appetite changes	0.68 (1.13)	1.18 (1.17)	$F_{1,60} = 2.572, p = 0.114$
Fatigue	1.09 (1.44)	1.48 (1.41)	$F_{1,60} = 1.032, p = 0.314$

Note: NSI, Neurobehavioural Symptoms Inventory; TBI, traumatic brain injury.