Cell Free DNA as a Potential Biomarker of Blast-Wave Mild Traumatic Brain Injury and Posttraumatic Stress Disorder.

Gad Shaked (shakedg@bgu.ac.il)
Soroka University Medical Center: Soroka Medical Center https://orcid.org/0000-0002-4992-252X
Amitai Zuckerman
Ben-Gurion University of the Negev
Zeev Kaplan
Ben-Gurion University of the Negev
Oren Sadot
Ben-Gurion University of the Negev
Amos Douvdevani
Ben-Gurion University of the Negev
Hagit Cohen
Ben-Gurion University of the Negev

Research

Keywords: Cell free DNA, Potential Biomarker, Posttraumatic Stress Disorder, Brain Injury, Blast-Wave

Posted Date: November 10th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1019777/v1

Abstract

Background

Individuals being within non-lethal distance from explosion may present with blast induced mild traumatic brain injury, post traumatic stress disorder, or combination of the two conditions. Early diagnosis to enable interventions is important. This study tested the possible role of cell free DNA in the diagnosis of blast related head injuries in a rat model.

Methods

A rat controlled model of a blast. Cell free DNA concentrations were determined in the serum by a direct fluorescence method. Cognitive and behavioral tests were used to diagnose affected rats.

Results

Mean cell free DNA concentration increased significantly at 2 hours following the blast compared to baseline level and remained high throughout the follow-up period (665.43±159.15 ng/ml vs. 344.20±69.62 ng/ml, $p<0.0001$). The rate of affected rats among the blast exposed animals was 42.5%. A significant increase in mean cell free DNA concentration was found at 2 hours after exposure in the affected group (741.40±47.18 ng/ml) compared to both the baseline concentration (372.42±149.11 ng/ml), $p<0.0001$ and to the well-adapted group (517.47 ng/ml), $p<0.0045$.

Conclusion

This rat model of blast demonstrated that the incidence of mild brain injury and or PTSD is significant and that affected animals demonstrated increased serum concentrations of cell free DNA. Cell free DNA may potentially serve as a biomarker to diagnose brain psychopathology early in individuals exposed to blast.

Background

Blast injuries are common among military personnel involved in the modern battle fields. However, nowadays civilians all over the globe are also exposed to blast incidents due to terrorist acts involving suicide bombers, car bombs, rockets and other artillery attacks on civilian communities.

The American and NATO forces have gained a vast experience in modern warfare in Iraq and Afghanistan during the last two decades. This experience added a lot to our understanding of the nature of blast injuries.[1,2] According to these reports, blast exposure accounts for 78% of injuries, with an incident of blast traumatic brain injury (bTBI) being as high as 60% in this group of patients.[3]

Blast-related brain injury can be caused by several mechanisms and to present itself in a variety of pathologies. The blast-wave that hits the skull and is transferred to the brain, can force it to move inside

the skull and hit it, resulting in a concussion and hemorrhagic contusions. This movement can also tear superficial veins that connect the brain surface to the dural venous sinus to cause subdural hemorrhage. Shear and stress waves from the primary blast could lead to diffuse axonal injury, hemorrhages, and edema.[4]

While a blast can cause mild TBI (mTBI) in many victims who are exposed to explosion, the management of such patients can be delayed, or even worse, not delivered to undiagnosed patients. For many years there was no consensus over the definition of mTBI. We adopt the definition of the World Health Organization (WHO) task force on mTBI that is an acute brain injury resulting from mechanical energy to the head from external physical forces with one or more of the following clinical presentations: (i) confusion or disorientation, loss of consciousness for 30 minutes or less, post-traumatic amnesia for less than 24 hours, and/or other transient neurological abnormalities such as focal signs, seizure, and intracranial lesion not requiring surgery; (ii) Glasgow Coma Scale score of 13–15 after 30 minutes post-injury or later upon presentation for healthcare.[5]

Recently, more than 100 U.S. troops have been diagnosed with TBI following an Iranian missile attack on a military compound at al-Asad airbase in Iraq. This was a typical presentation of mild bTBI which started with an announcement of no wounded individuals in the attack. Later, more and more service personnel were diagnosed as having symptoms of mTBI. This incident demonstrated clearly the problematic situation of sorting out the affected individuals from hundreds of people who were at the vicinity of an explosion without a MRI machine available to accurately diagnose such victims.[6]

The diagnosis of TBI in the acute setting is based on neurological examination and neuro-imaging tools such as CT scan and MRI. However, CT scanning has low sensitivity to diffuse mild brain damage and exposes the patient to radiation. On the other hand, MRI can provide information on the extent of diffuse injuries but its widespread application is restricted by cost and the limited availability of MRI in many centers. Under the circumstances of military field hospitals that lack such imaging modalities and in mass casualty incidents the utility of these is very low or absent. The clinical symptoms of mTBI are less clear-cut than those of TBI and may overlap with those of post-traumatic stress disorder (PTSD) and other mental syndromes. The traumatizing events associated with explosive detonations during combat or terrorist attacks often make it difficult for clinicians to distinguish between PTSD and mTBI, but they are often comorbid. The impact of the comorbid condition on treatment and rehabilitation is vast.[7] It is reasonable to assume that a biomarker may serve as a rapid reliable diagnostic aid to diagnose TBI and especially mTBI. Despite extensive research which explored a wide range of biomarkers specific to brain injury, there is still no proved biomarker in routine clinical use.[8] One of the major drawbacks of the studied biomarkers is that they originate in specific neuroanatomical locations and so using one of them may lead to false negative diagnosis of TBI. In a previous study we determined the levels of cell free DNA (cfDNA) in the serum of isolated brain injury patients. We used a validated, simple, rapid and cheap direct fluorescence method which we developed in our laboratory. The results showed that severely injured patients had high cfDNA concentrations on admission compared to patients with mild injuries that had

low concentrations. In addition, patients who recovered fully from their injury had lower cfDNA concentrations compared to the high concentrations found in patients who were discharged with disabilities. Thus, the conclusion of the study was that cfDNA may be used as a marker to assess the severity of TBI and to predict the prognosis.[9]

This study aimed to test cfDNA as a potential marker for fast identification of blast-wave obscured injuries and the evaluation of the resultant psychopathology in an innovative rat model. The main goal of the study was to explore the changes of cfDNA concentrations in the serum of rats exposed to non-lethal blast wave. We evaluated the pattern and timing of cfDNA concentration changes after blast-wave exposure in different intensities. The secondary goal was to learn whether a relationship exists between the concentration of cfDNA and behavioral responses. We hypothesized that the low pressure blast-wave that causes psychopathology (mTBI, PTSD, or comorbid mTBI-PTSD phenotypes) but no other internal injuries would result in a detectable increase in cfDNA that may be useful in the future to detect early patients with blast induced mTBI.

Methods

Setup All procedures were performed under strict compliance with ethical principles and guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All treatment and testing procedures were approved by the Animal Care Committee of Ben-Gurion University of the Negev, Israel (IL-10-06-2014).

Animals A total of 97 male Sprague–Dawley rats (Envigo RMS Israel Ltd) weighing 150–200 g were habituated to housing conditions for at least 7 days prior to any experimental procedures. The animals were housed four per cage in a vivarium with a stable temperature and a 12 h light/dark cycle (lights on: 08:00 h) with unlimited access to food and water.

Experimental design The rats were exposed to 2 different intensities of blast-wave (see subsequent description). Fourty rats were exposed to a low-pressure blast-wave and 47 were exposed to a high-pressure blast-wave. The animals were awake through the entire procedure and received no drugs. All animals underwent neurological assessment using the Neurological Severity Score (NSS) which was performed 1.5–2 hours following the blast and daily thereafter. Behavior measures were conducted on day 7. The rats were initially assessed in the elevated plus maze (EPM) followed 1 h later by the acoustic startle response (ASR) paradigm. Spatial memory performance using the Morris water maze (MWM) test was assessed 8 days post-exposure for 7 consecutive days. The prevalence rate of rats exhibiting post traumatic stress disorder (PTSD) phenotype or mTBI phenotype responses (see details described subsequently) were calculated from this data and compared with a sham-exposed group (n = 5) (see details described subsequently) and with those of unexposed controls (n = 5). In all experiments, the rats were tested in batches of 10–15 individuals.

Blast-wave exposure

An exploding wire technique was used to generate small-scale cylindrical and spherical blast waves. This technique has been previously demonstrated to simulate the effects of air blast exposure under experimental conditions, and permits safe operation with high repeatability.[10] Pilot tests have shown that this model is non-lethal for the rats and does not result in any external, limbs, thoracic, or intra abdominal injuries (data not shown).

Exploding wires

The experimental system is based on the rapid discharge of high voltage through 0.4 mm thickness copper wire. When electrical currency passes through the copper wire, Joule heating is created. This heating vaporizes and liquefies the copper in a very short time (5 μsec) at 1084°C, with virtually no change in the wire volume. The high current continues to pass through the liquid copper and continues to heat it to the point at which it transitioned to a gaseous state beyond the temperature of 2927°C. As the copper gas begins to expand in space, it forms a blast-wave. This method of explosion produces a cylindrical blast-wave that simulates a blast-wave profile similar to that seen from an explosive device common to the battlefield. In an actual explosion, the blast-wave causes an acute, short duration elevation in pressure followed by a negative phase. The exploding wire system has been shown to be capable of duplicating this overpressure and negative pressure blast-wave profile.

Procedure Each rat was restrained in a custom flexible harness located on a tray, which was then placed in the blast-wave generator system at a distance of 20 cm from the wire (high-intensity blast group), or a distance of 35 cm (low-intensity blast group). Minimal movement of the animals was allowed during the blast exposure. Pressure values were recorded using a Kistler 211B3 piezoelectric pressure transducer mounted on a perpendicular wall. Rats (two at the same time) were subjected to a single blast-wave with the head facing the blast without any body shielding, resulting in a full body exposure to the blast wave. A high-speed video was taken during the exposure by a PHANTOM V12.1 high-speed camera. Following the blast rats were returned to their home cage.

Sham exposure (control) Sham-exposed animals were treated identically, except that they were not exposed to the blast. The rats were in the same room where the explosion occurred, but were placed 150 cm below the blast wave. This enabled them to experience the bright flash of light and smell of the explosion while shielding them from any potential physical injury. This enabled us to focus on the psychological components without the compounding effects of physical brain injuries.

Cell free DNA Blood samples were obtained at baseline (just before the blast) and 1 hour, 2 hours, 3 hours, 5 hours, 24 hours, day 10, and day 15 after the blast in Flex-Tube®, Eppendorf blood collection tubes. Blood samples were immediately centrifuged at 2000 G for 10 minutes at 4°C and serum was transferred to collection tubes and stored in −20°C. cfDNA levels were quantified by a direct rapid fluorometric assay, the fluorochrome SYBR Gold which does not require prior processing of samples, that is, DNA extraction and amplification. Briefly, SYBR Gold Nucleic Acid Gel Stain (Invitrogen Paisley, UK) was diluted 1 : 1000 in dimethyl sulphoxide and then 1 : 8 in phosphate-buffered saline. Ten microliters of serum or DNA standard was applied to a 96-well plate and forty microliters of diluted SYBR Gold was applied to each

well. Fluorescence was measured with a 96-well fluorometer (Spectrafluor Plus, Tecan, Durham, NC) at an emission wavelength of 535 nm and an excitation wavelength of 485 nm. The method was tested in comparison with the gold standard, QPCR, and was found to be in good correlation of R^2 0.9987 ($p<0.0001$) as previously described.[11]

Neurological Severity Score (NSS) To ensure that any damage to the central nervous system (CNS) caused by the blast-wave did not result in vast neurological deficits, we employed the NSS. The NSS was performed 1 h following the initial blast-wave exposure and served as a baseline for comparison with later evaluations throughout the study. The NSS assesses somatomotor and somatosensory function by evaluating the animals' activities as was described before.[12] An observer who was blind to the different treatment groups tested the animals.

Behavioral assessments All rats underwent a number of different behavioral assessments. All behavioral tests were performed in standardized conditions. Rats underwent more than one behavioral test; therefore, tests were performed with a break of at least 24 h between sessions. Also, no animals underwent the same test twice. All behavioral tests were video recorded for future analysis using the ETHO-VISION program (Noldus), by an investigator blinded to the experimental protocol. The behavioral tests included the EPM and ASR for anxiety phenotype/PTSD phenotype responses, and the MWM for cognitive performance.

Statistical analysis

Cell free DNA concentrations and behavioral data were analyzed using a one way analysis of variance (ANOVA) or two way ANOVA. In the event of a significant F ratio, a Bonferroni post-hoc test was used for multiple comparisons. Repeated measures ANOVA (RM-ANOVA) was used to analyze MWM data. The prevalences of the affected and well adapted rats are expressed in ratios and percentages. All data are reported as mean ± SE. An α level of $p < 0.05$ was used to determine statistical significance.

Results

Blast-wave details A 0.4 mm diameter 70 mm in length copper wire and charging voltageof 4.2 kV were used to generate the blast-wave. The discharge current was ~500 kA. The short pulse at t = 0 is associated with the electromagnetic pulse generated by the capacitor discharge. Animals subjected to the blast-wave experienced a mean peak overpressure of 95 kPa (13.77 psi) (rise time of 0.01 ms) sustained for duration of 0.189 ms. The peak pressure was equivalent to 193 dB SPL (sound pressure level). The exposure led to a peak impulse of 10.8 × 10-3 kPa. A negative pressure was sustained for more than 0.659 ms with a peak negative pressure of −40 kPa (−5.8 psi). The light intensity generated by the explosion is significant and measured to be ~5 Mlux. This light intensity is of the same order of magnitude as the M84 stun grenade at a distance of 1.5 m (3.1 Mlux). There was no mortality in any of the blast-exposed rats.

cfDNA: Mean cfDNA concentration increased significantly at 2 hours after the blast exposure (665.43±159.15 ng/ml) from mean baseline level (344.20±69.62 ng/ml) and remained high, excluding the exceptional time point of 5 hours, to the end of 15 day follow up (One way ANOVA: $F(7, 463)=17.4$, $p<0.0001$). Animals of the sham exposure and unexposed groups did not manifest such unique pattern of change in cfDNA concentrations. Furthermore, there was no difference between these two control groups, but at the 2 hour point there was a significant difference ($p<0.0001$) between the mean concentration of the exposed rats (665.43±159.15 ng/ml) and the concentrations of the control groups [273.80±80 ng/ml in the sham exposure, 286.66±73.03 ng/ml in the unexposed group (Figure 1A)]. Figure 1B demonstrates the distribution of all individual sample concentrations and means of cfDNA concentrations along the time axis of the study. The detailed mean±S.E cfDNA concentrations of the 3 groups are in Table 1. Positioning the animals in different distances from the exploding wire allowed us to expose them to two levels of pressure intensity defined as high-intensity pressure of 250 Kpa and low-intensity pressure of 75 Kpa. The exposure to the two levels of pressure resulted in the same pattern of surge of cfDNA concentrations at the time point of 2 hours after the blast, but no significant difference was found between the low and high pressure groups (Figure 2A). Figures 2B and 2C show the distribution of the individual sample concentrations and means of cfDNA concentrations of the two pressure intensity groups along the time axis of the study. The cfDNA concentrations at 2 hours were significantly higher than the concentrations at the other time points (Two way ANOVA: time: $F(7, 337)=27.5$, $p<0.0001$; pressure: NS; time x pressure interaction: NS). The detailed mean±S.E cfDNA concentrations of the 2 groups are shown in Table 2.

Table 1
The detailed mean±S.E cfDNA concentrations of the 3 study groups.

Time point	Blast exposure (mean±S.E ng/ml)	Sham exposure (mean±S.E ng/ml)	Unexposed (mean±S.E ng/ml)
BL	344.20±69.62	315.25±77.21	295.94±25.21
1 h	397.21±87.44	-	-
2 h	665.43±159.16	273.80±100.67	286.66±73.03
3 h	390.05±61.55	319.22±76.06	241.34±70.35
5 h	302.97±0	94.09±57.62	195.45±90.55
24 h	437.48±53.20	252.09±69.69	229.33±76.31
10 d	447.05±96.99	-	-
15 d	365.79±59.86	299.68±54.99	288.24±58.23

Table 2
The detailed mean±S.E cfDNA concentrations of the high and low pressure intensity groups.

Time point	Low pressure (mean±S.E ng/ml)	High pressure (mean±S.E ng/ml)
BL	338.38±89.48	350.21±69.62
1 h	400.31±156.20	381.17±87.44
2 h	766.98±330.23	816.53±566.09
3 h	409.49±120.94	356.27±61.55
5 h	274.63±138.55	317.97±118.60
24 h	448.76±162.68	497.61±210.55
10 d	427.09±97.00	484.56±271.71
15 d	337.22±59.86	358.80±139.60

NSS: There were no significant differences between the groups in reflex responses, motor coordination, motor strength, or sensory function (data not shown). These findings indicate that differences in behavioral and cognitive tasks were not related to abnormal motor function required of the animals to complete the behavioral tasks.

mTBI and PTSD: The majority, 50/87 rats (57.5%), of the sample exposed to the blast-wave were unaffected (well-adapted phenotype). In contrast, the prevalence of affected rats among the blast exposed rats was 37/87 (42.5%). Specifically, the prevalence of mTBI-like pattern among the blast exposed animals was 21/87 (24.1%), whereas the prevalence of PTSD-like phenotype was 4/87 (4.6%). The prevalence of PTSD+mTBI-like among the blast exposed animals was 12/87 (13.8%). None of the sham exposed and unexposed rats exhibited phenomena of mTBI or PTSD characteristics. Figure 3A shows the overlapping curves of cfDNA concentrations of the affected and well-adapted groups A significant increase of the mean concentration was found at 2 hours in the affected group (741.40±47.18 ng/ml) compared to the mean baseline concentration (372.42±149.11 ng/ml), $p<0.0001$. This 2 h mean concentration of 741.40±47.18 ng/ml is also higher than the parralel result of 517.47 ng/ml of the well-adapted group, $p<0.0045$. Figures 3B and 3C show the distribution of individual sample concentrations and means of cfDNA concentrations of the affected and well-adapted groups along the time axis of the study. A significant increase in cfDNA concentrations at the time point of 2h over the BL and other time points was observed in the affected group. Such an increase was not apparent in the well-adapted rats. (Two way ANOVA: time: $F(7,431)=11.85$, $p<0.0001$; Diagnosis: NS; time x diagnosis: $F(7,541)=3.6$, $p<0.001$). The detailed mean±S.E cfDNA concentrations of the 2 groups are shown in Table 3.

Table 3
The detailed mean±S.E cfDNA concentrations of the affected and well adapted groups.

Time point	Affected (mean±S.E ng/ml)	Well adapted (mean±S.E ng/ml)
BL	372.42±149.11	311.11±69.62
1 h	371.98±271.73	403.51±87.62
2 h	741.40±47.18	517.47±49.22
3 h	345.00±73.59	415.51±61.55
5 h	352.67±55.57	327.56±35.44
24 h	390.94±125.16	483.86±162.68
10 d	419.18±271.71	454.02±97.00
15 d	352.00±43.06	394.09±132.22

Discussion

The main findings of this study were the augmentation of cfDNA concentrations following blast-wave exposure, this rise peaked at 2 hour after the exposure and lasted for 15 days. Sham exposure and unexposed subjects did not demonstrate this alteration of cfDNA concentrations. No significant difference was found between the low and high pressure blast-wave groups in cfDNA concentrations. The exposure to the two levels of pressure resulted in the same pattern of surge of cfDNA concentrations in the time point of 2 hours after the blast exposure. Moreover, there was a striking association between the degree of behavioral disruption following blast exposure and the pattern of changes in the cfDNA concentrations 2 hours post exposure: animals whose behavior was extremely disrupted (mTBI-phenotype, PTSD-phenotype and comorbid mTBI-PTSD-phenotype) selectively displayed significantly higher cfDNA concentrations. In contrast, rats whose behavior was minimally affected or unaffected (well-adapted rats) displayed no cfDNA concentrations changes and were indistinguishable from sham exposed or unexposed controls.

The response patterns of the blast-wave exposure model employed in this study replicate our previous data [13,14] in that they demonstrate that exposure to a single low-pressure blast-wave can produce distinctive long-lasting psycho-neuro-behavioral responses which model PTSD, mTBI, and comorbid PTSD-mTBI sequelae in a proportion of animals. Nevertheless, the NSS was normal in all the animals. This set of tests was taken in order to ensure that any damage to the central nervous system caused by the blast-wave is mild and does not result in vast neurological deficits. NSS assesses somatomotor and somatosensory function by evaluating the animals' activities in motor, sensory, reflexes, beam walking, and beam balancing tasks. Taken together, these findings indicate that cfDNA concentrations may

provide a quick, reliable, and simple prognostic indicator of pathology after blast-wave exposure. It is not clear to us why the two intensities of blast resulted in non different cfDNA concentrations. A possible explanation may be that the disparity between the two intensities was not enough to be translated into a detectable significant different amount of brain injury. Another series of tests that use greater differences in blast intensities may also demonstrate differences in cfDNA levels.

Recent literature on the utility of imaging modalities such as CT scanning and even MRI empahasizes their low sensitivity to accurately diagnose mTBI.[15] This is in accordance with MRI examinations of rats that underwent a blast-wave exposure at exactly the same model conditions.[14] The brain MRI examinations revealed no lesions, edema, or hemorrhage in any of the rats. This was in agreement with the results of the brain tissue histo-pathological assessments. However, despite the lack of macro and micro tissue changes, brain cellular damage does occur in mTBI. There are multiple molecules that point to pathophysiological changes associated with mTBI including brain cell injury and disruption of the blood-brain barrier.[16] To name some of them, astrocyte injury is identified by the presence of the S100B, and glial fibrillary acidic protein (GFAP) proteins in the blood. Damaged neurons release neuron-specific proteins including neuron-specific enolase (NSE), ubiquitin carboxyl-terminal hydrolase isoenzyme L1 (UCHL1), and Cellular prion protein (PrP^{c}). Axonal injury is associated with increased concentrations of hyperphosphorylated tau protein (p-tau), and Neurofilaments (NFs). Very few studies have tested the value of nucleic acids for the diagnosis of TBI. Over-expression of a micro RNA (miRNA), miR-21, has been reported by two groups in severe TBI [17,18], and down-regulation of miR-425-5p and miR-502 in mTBI was reported in one of these studies.[18] We elected to use cfDNA in this study. cfDNA has been well studied for its potential use in the diagnosis, prognosis, and monitoring of a variety of conditions such as trauma, inflammation, infection and sepsis.[19–23] Previous reports have pointed out the utility of cfDNA in TBI.[9,24,25]

In our study on human TBI patients we used the same simple “mix and measure” technique, as described in the present study, to measure cfDNA. The study population was isolated head injury patients. The present study attempted to model the unique condition of mTBI caused by blast. The pattern of cfDNA augmentation after the trauma in our model resembles the description of Lam et al. on the early and late changes in plasma DNA in trauma patients.[26] In their study they observed the same early increase in DNA concentrations that peaked around 2 hours from injury and continued to be over baseline levels for about 3 weeks. Also, they reported higher DNA concentrations in patients with severe injuries and in those who had developed organ failure. This is in line with our observations in mild bTBI regarding affected subjects compared with well-adapted animals. The finding that only the 2 hour point in our study showed a significant peak is raising the concern that the test may be limited to a short period of time, although as is obvious from the plots there is a trend of increased cfDNA concentrations through the entire length of follow up. Future studies may give us more information regarding the cutoff concentration and the timing of testing before it is possible to introduce the test for routine use in human patients.

Limitations of the study are: from technical reasons not all subjects were sampled in each of the time points which can cause some bias in the results. In these experiments no attempt was made to rule out other possible sources for cfDNA except for the TBI. We relied upon earlier pilot experiments that showed no torso or extremities injuries. Other serum biomarkers have not been measured. It may be interesting to compare cf DNA to other biomarkers such as S100B, NSE, GFAP in future studies. Caution should be made in the translation of rodent experiments to human. Of special interest is to learn when the peak concentration of cfDNA happens, and for how long it remains significantly above baseline levels in humans.

Conclusion

cfDNA concentrations increase early after mild bTBI and remain high for at least two weeks following the exposure. Determination cf DNA by a simple, cheap, rapid, and reliable method as we use in our laboratory can be done in areas that lack full modern medical infrastructure, or in cases of mass casualty event. It can help clinicians sort out mTBI patients from other persons who are exposed to a blast especially when no other obvious injury is apparent. It can potentially reduce the number of patients who remain undiagnosed or get late to treatment.

Declarations

Author Contribution

Gad Shaked – writing the manuscript, Amitai Zuckerman – data collection, Zeev Kaplan – study design, Oren Sadot – explosion model design and performance, Amos Douvdevani – laboratory tests, Hagit Cohen – data analysis, data interpretation, critical revision.

Acknowledgment

We would like to thank Mrs. Tal Shaked for her language editing.

Conflict of interest

We declare that none of the authors has any conflict of interest in this manuscript.

We declare that this study did not receive any outside funding support.

References

1. Owens BD, Kragh JF Jr, Wenke JC, Macaitis J,Wade CE,Holcomb JB. Combat wounds in operation Iraqi freedom and operation enduring freedom. *J Trauma.* 2008;64:295-299.
2. Belmont PJ Jr, Goodman GP, Zacchilli M, Posner M, Evans C, Owens BD. Incidence and epidemiology of combat injuries sustained during "the surge" portion of operation Iraqi freedom by a U.S. army brigade combat team. *J Trauma.* 2010;68:204-210.

3. Warden D. Military TBI during the Iraq and Afghanistan wars. *J Head Trauma Rehabil.* 2006;21:398-402.
4. Taber KH, Warden DL, Hurley RA. Blast-related traumatic brain injury: What is known? *J Neuropsychiatry Clin Neurosci.* 2006;18:141-145.
5. Carroll LJ, Cassidy JD, Holm L, Kraus J, Coronado VG, WHO Collaborating Centre Task Force on Mild Traumatic Brain Injury. Methodological issues and research recommendations for mild traumatic brain injury: The WHO collaborating centre task force on mild traumatic brain injury. *J Rehabil Med.* 2004;(43 Suppl):113-25. doi:113-125.
6. Garamone J. Joint staff doctor explains TBI diagnosis procedures. *DOD news.* Feb. 24, 2020;.
7. Mattson EK, Nelson NW, Sponheim SR, Disner SG. The impact of PTSD and mTBI on the relationship between subjective and objective cognitive deficits in combat-exposed veterans. *Neuropsychology.* 2019;33:913-921.
8. Papa L, Edwards D, Ramia M. Exploring Serum Biomarkers for Mild Traumatic Brain Injury. In: Kobeissy FH, ed. *Brain Neurotrauma: Molecular, Neuropsychological, and Rehabilitation Aspects. DOI: NBK299199.* Boca Raton (FL): by Taylor & Francis Group, LLC; 2015:.
9. Shaked G, Douvdevani A, Yair S, Zlotnik A, Czeiger D. The role of cell-free DNA measured by a fluorescent test in the management of isolated traumatic head injuries. *Scand J Trauma Resusc Emerg Med.* 2014;22:21-7241-22-21.
10. Ram O SO. Implementation of the exploding wire technique to study blast-wave–structure interaction. *Experiments in fluids.* 2012;53:1335-1345.
11. Goldshtein H, Hausmann MJ, Douvdevani A. A rapid direct fluorescent assay for cell-free DNA quantification in biological fluids. *Ann Clin Biochem.* 2009;46:488-494.
12. Feldman Z, Gurevitch B, Artru AA, Oppenheim A, Shohami E, Reichenthal E, Shapira Y. Effect of magnesium given 1 hour after head trauma on brain edema and neurological outcome. *J Neurosurg.* 1996;85:131-137.
13. Zuckerman A, Ram O, Ifergane G, Matar MA, Kaplan Z, Hoffman JR, Sadot O, Cohen H. Role of endogenous and exogenous corticosterone on behavioral and cognitive responses to low-pressure blast wave exposure. *J Neurotrauma.* 2019;36:380-394.
14. Zuckerman A, Ram O, Ifergane G, Matar MA, Sagi R, Ostfeld I, Hoffman JR, Kaplan Z, Sadot O, Cohen H. Controlled low-pressure blast-wave exposure causes distinct behavioral and morphological responses modelling mild traumatic brain injury, post-traumatic stress disorder, and comorbid mild traumatic brain injury-post-traumatic stress disorder. *J Neurotrauma.* 2017;34:145-164.
15. Chiara Ricciardi M, Bokkers RP, Butman JA, Hammoud DA, Pham DL, Warach S, Latour LL. Trauma-specific brain abnormalities in suspected mild traumatic brain injury patients identified in the first 48 hours after injury: A blinded magnetic resonance imaging comparative study including suspected acute minor stroke patients. *J Neurotrauma.* 2017;34:23-30.
16. Kim HJ, Tsao JW, Stanfill AG. The current state of biomarkers of mild traumatic brain injury. *JCI Insight.* 2018;3:10.1172/jci.insight.97105. eCollection 2018 Jan 11.

17. Redell JB, Zhao J, Dash PK. Altered expression of miRNA-21 and its targets in the hippocampus after traumatic brain injury. *J Neurosci Res.* 2011;89:212-221.
18. Di Pietro V, Ragusa M, Davies D, Su Z, Hazeldine J, Lazzarino G, Hill LJ, Crombie N, Foster M, Purrello M, Logan A, Belli A. MicroRNAs as novel biomarkers for the diagnosis and prognosis of mild and severe traumatic brain injury. *J Neurotrauma.* 2017;34:1948-1956.
19. Rainer TH, Lam NY. Circulating nucleic acids and critical illness. *Ann N Y Acad Sci.* 2006;1075:271-277.
20. Lo YM, Rainer TH, Chan LY, Hjelm NM, Cocks RA. Plasma DNA as a prognostic marker in trauma patients. *Clin Chem.* 2000;46:319-323.
21. Rhodes A, Wort SJ, Thomas H, Collinson P, Bennett ED. Plasma DNA concentration as a predictor of mortality and sepsis in critically ill patients. *Crit Care.* 2006;10:R60.
22. Saukkonen K, Lakkisto P, Pettila V, Varpula M, Karlsson S, Ruokonen E, Pulkki K, Finnsepsis Study Group. Cell-free plasma DNA as a predictor of outcome in severe sepsis and septic shock. *Clin Chem.* 2008;54:1000-1007.
23. Saukkonen K, Lakkisto P, Varpula M, Varpula T, Voipio-Pulkki LM, Pettila V, Pulkki K. Association of cell-free plasma DNA with hospital mortality and organ dysfunction in intensive care unit patients. *Intensive Care Med.* 2007;33:1624-1627.
24. Campello Yurgel V, Ikuta N, Brondani da Rocha A, Lunge VR, Fett Schneider R, Kazantzi Fonseca AS, Grivicich I, Zanoni C, Regner A. Role of plasma DNA as a predictive marker of fatal outcome following severe head injury in males. *J Neurotrauma.* 2007;24:1172-1181.
25. Macher H, Egea-Guerrero JJ, Revuelto-Rey J, Gordillo-Escobar E, Enamorado-Enamorado J, Boza A, Rodriguez A, Molinero P, Guerrero JM, Dominguez-Roldan JM, Murillo-Cabezas F, Rubio A. Role of early cell-free DNA levels decrease as a predictive marker of fatal outcome after severe traumatic brain injury. *Clin Chim Acta.* 2012;414C:12-17.
26. Lam NY, Rainer TH, Chan LY, Joynt GM, Lo YM. Time course of early and late changes in plasma DNA in trauma patients. *Clin Chem.* 2003;49:1286-1291.

Figures

Fig. 1A

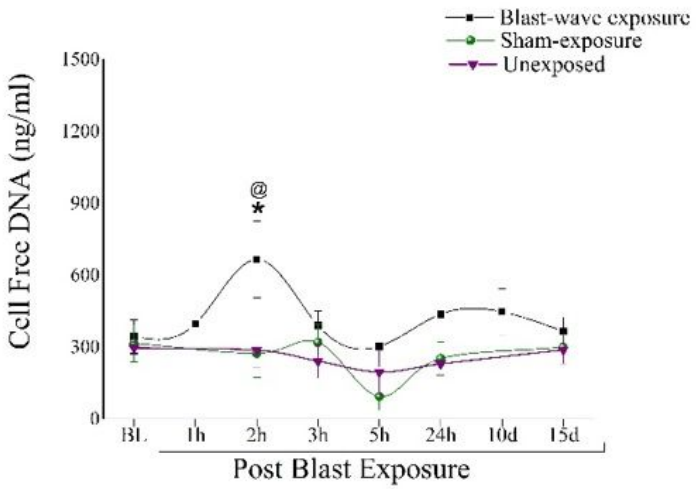

Fig. 1B

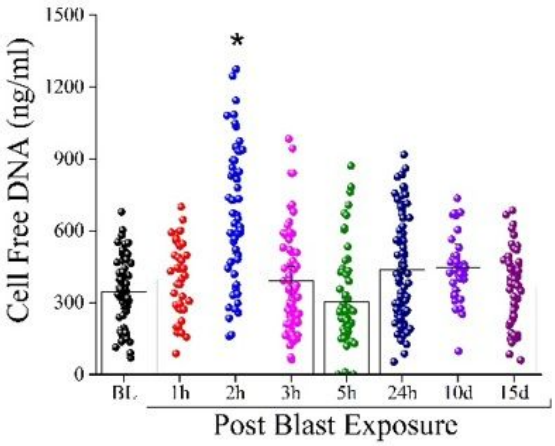

Figure 1

1A. cfDNA concentrations at baseline (BL) and in the follow up time points. Post hoc Bonferroni test demonstrated that the concentration at 2h was significantly different ($p<0.0001$) than at BL and in all the other time points (*). It was also significantly different ($p<0.0001$) compared to the 2h concentrations of the sham exposure and unexposed groups (@). 1B. Distribution of individual sample concentrations and means of cfDNA concentrations along the time axis of the study. Post hoc Bonferroni test demonstrated that the concentration at 2h was significantly different ($p<0.0001$) than at BL and in all the other time points (*).

Fig. 2A

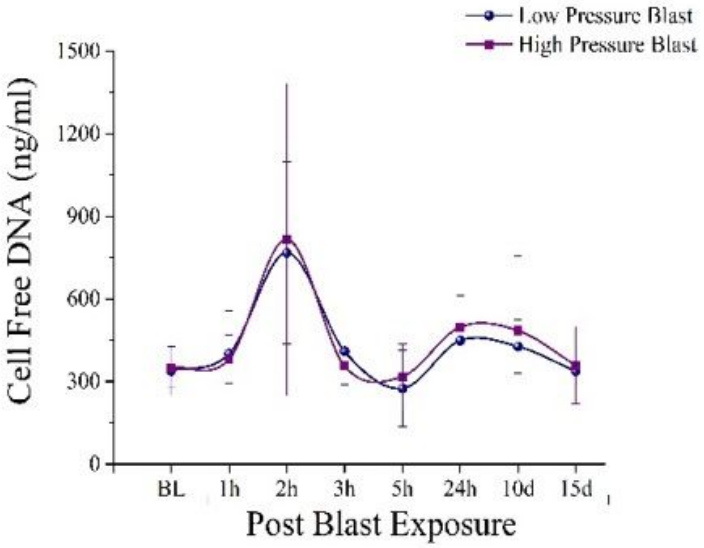

Fig. 2B

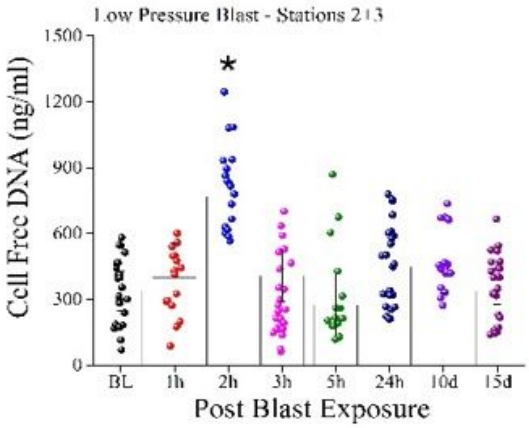

Fig. 2C

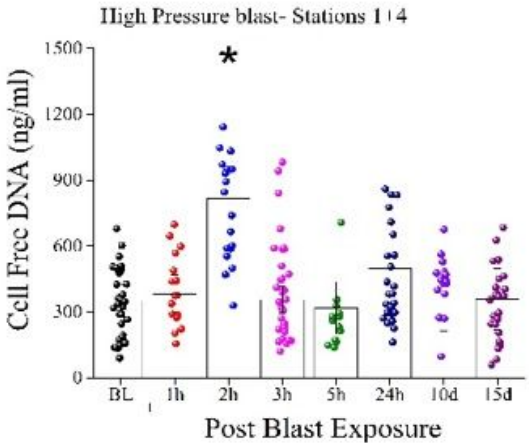

Figure 2

2A. High and low pressure intensity blast waves created the same pattern of cfDNA concentrations as is shown in the overlapping curves. No significant difference was demonstrated between the two groups. 2B. The distribution of the individual sample concentrations and means of cfDNA concentrations along the time axis of the study in the high intensity group. The mean cfDNA concentration at 2h (*) was significantly higher ($p<0.0001$) than the concentrations at BL and in all other time points. 2C. The distribution of the individual sample concentrations and means of cfDNA concentrations along the time axis of the study in the high intensity group. The mean cfDNA concentration at 2h (*) was significantly higher ($p<0.0001$) than the concentrations at BL and in all other time points.

Fig. 3A

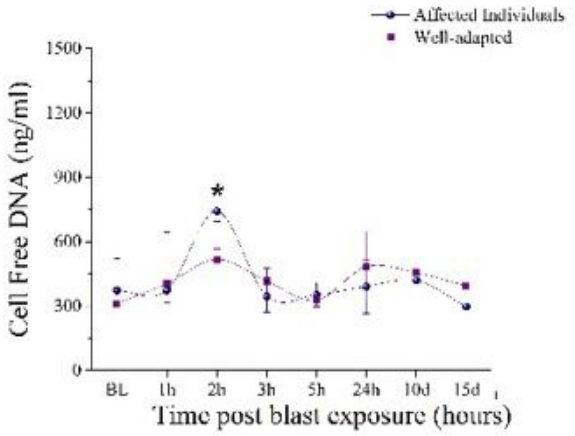

Fig. 3B

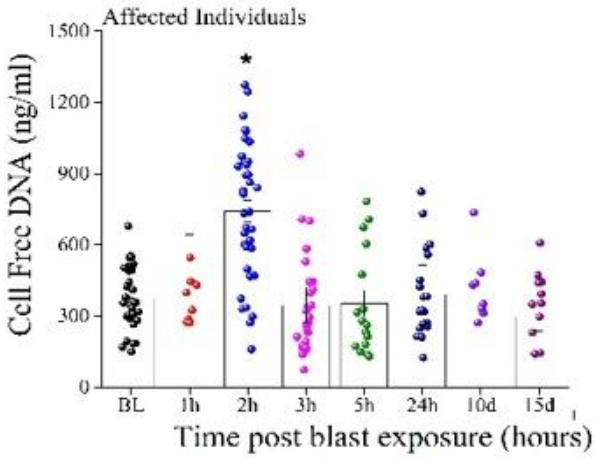

Fig. 3C

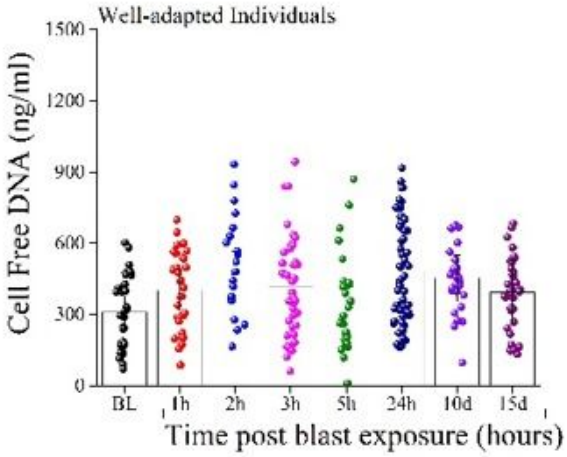

Figure 3

3A. The mean cfDNA concentration at 2h (*) was significantly higher than the concentrations at BL ($p<0.0001$) and than the mean conentration in the well-adapted rats ($p<0.0045$). 3B. The distribution of the individual sample concentrations and means of cfDNA concentrations along the time axis of the study in the affected rats group. * presents a significant difference ($p<0.0001$) of mean cfDNA concentration at 2 h compared to BL and other time points concentrations. 3C. The distribution of the individual sample concentrations and means of cfDNA concentrations along the time axis of the study in the well-adapted group.