BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023387
Article Type:	Research
Date Submitted by the Author:	05-Apr-2018
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Keywords:	tbi, hbot, traumatic brain injury, hyperbaric oxygen, cognitive

Hyperbaric oxygen improves functioning of patients suffering from chronic neurocognitive delicts due to traumatic brain injury- correlations between cognitive functions and brain metabolic imaging

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Author Disclosure Statement

No competing financial interests exist.

No funding received for this work.

Contributorship statement

AH: concept, data collection, data analysis, manuscript draft, manuscript review

SA: data collection, data analysis

GS: data collection, manuscript review

YB: data collection, manuscript review

SE: concept, data analysis, manuscript draft, manuscript review

Data sharing statement

Extra data is available by emailing amir.had@gmail.com

Running title: HBOT improves cognitive function in TBI

Number of references: 40

Word count: 3331

Tables: 3

Figures: 6

No conflict of interests exist.

Abstract

Objectives

The aim of the study is to evaluate the effect of HBOT in participants suffering from chronic neurological deficits of all severities due to TBI in the largest cohort evaluated so far with objective cognitive function and metabolic brain imaging.

Methods

A retrospective analysis was conducted of 154 patients suffering from chronic neurocognitive damage due to TBI, and had pre- and post-HBOT treatment computerized cognitive evaluations.

Results

The average age was 42.7±14.6 years and 58.4% were males. All patients had documented traumatic brain injury 0.3-33 years (mean 4.6±5.8, median 2.75 years) prior to HBOT. HBOT induced significant improvement in all of the cognitive domains where the mean change in global cognitive scores was 4.6±8.5 ($p<0.00001$). The most prominent improvement was in memory index and attention with mean changes of 8.1±16.9 ($p<0.00001$) and 6.8±16.5 ($p<0.0001$), respectively. The most striking changes in brain SPECTs were in the anterior cingulate and the post-central cortex, the prefrontal areas and temporal areas.

Conclusions

HBOT induced significant cognitive improvements in patients suffering from chronic deficits in TBI of all severities. The clinical improvement was well correlated with increased activity in the relevant brain areas.

Keywords: hyperbaric oxygen, HBOT, post concussion, PCS, TBI, traumatic brain injury

Article Summary

- This is a retrospective analysis of the largest cohort of patients suffering from TBI related chronic neurocognitive damage, treated by HBOT. Patients suffering any degree of TBI injury (mild to severe) were included and evaluated with objective computerized cognitive tests.
- HBOT induced significant cognitive improvements in patients who suffer from chronic neurocognitive deficits due to mild, moderate and severe TBI.
- The improvement in memory correlated with activation of the perirhinal cortex, improvement of executive functions correlated with activation of the inferior frontal gyrus and improvement in attention correlated with activation of the anterior cingulate gyrus.

Strengths and limitations of the study

- The major limitation relates to its retrospective methodology. This limitation should be considered even thought this large cohort of patients was treated at late chronic stages. The findings presented here are in agreement with the findings from previous prospective controlled trials.
- In regards to strengths, objective cognitive assessments using computerized tests were performed to each patient both pre- and post-treatment. Objective measures are significantly superior to PCS questionnaires which are inaccurate, variable and contain various confounders. Second, most of the patients in the study underwent an objective ancillary brain SPECT to confirm PCS diagnosis prior to HBOT. This practice is crucial when considering the

differential diagnosis following TBI (PTSD, depression, etc.). Third, the study cohort included a civilian population that does not have any potential secondary gain (such as financial compensation by reporting sick).

Introduction

Traumatic brain injury (TBI) is one of the leading causes of death and disability in the general population.(1) Following TBI, patients may experience a set of symptoms known as post-concussion syndrome (PCS). PCS symptoms include headaches, dizziness, neuropsychiatric symptoms, and cognitive impairments.(2) PCS can continue for weeks or months, and up to 25% of all patients experience prolonged PCS (PPCS) in which the symptoms last for over six months.(3)

In the past years there is growing clinical evidence regarding the effect of hyperbaric oxygen therapy (HBOT) on PCS.(4, 5) Unfortunately, the clinical data gathered from those studies can be conflicting due to several inherent procedural issues such as the use of non-objective end points, the lack of appropriate brain imaging as part of the inclusion criteria, the inappropriate placebo of a hyperbaric environment and the inclusion of patients that may gain secondary benefits from reporting sick.(4, 5) The current study represents the largest cohort evaluated till now of civilian participants suffering from PCS treated by HBOT who had objective metabolic brain imaging and a computerized neurocognitive test battery before and after the treatment.

Pathophysiology of PCS and HBOT

The most common pathological mechanism in TBI is diffuse shearing of axonal pathways and small blood vessels, also known as diffuse axonal injury.(6) The secondary pathological mechanisms of TBI include ischemia, mild edema, and other biochemical and inflammatory processes culminating in impaired regenerative and/or healing processes resulting from increasing tissue hypoxia.(7) Due to the diffuse nature of injury, affecting multiple brain areas,(8, 9) cognitive impairments are usually the predominant symptoms.

Global brain hypoperfusion, and its related tissue ischemia, detected in patients suffering from TBI, serves as a rate-limiting factor for any regenerative process.(10-12) By increasing the oxygen level in blood and body tissues, HBOT can augment the repair mechanisms.(13) Various models have strongly suggested that HBOT can induce angiogenesis, improve brain plasticity, enhance neurogenesis and synaptogenesis and foster functional recovery.(14, 15)

Conflicting clinical HBOT data and objective measurements in PCS

Some of the previous studies which evaluated the effect of HBOT on chronic neurological and cognitive impairments due to TBI, mainly used self-assessment questionnaires as their primary endpoints.(16-18) Such endpoints have several inherent disadvantages. First, they lack an objective evaluation that is not biased by the patients' perspectives. Second, self-administrated questionnaires are exposed to various confounding variables such as litigation and compensation (19). Unlike the questionnaires, standardized cognitive tests with high test-retest reliability can and should be used as objective evaluations of neurocognitive impairments.(20) In addition, novel brain imaging techniques such as single photon emission computed tomography (SPECT) and perfusion sequences in magnetic resonance imaging (MRI), which evaluate cerebral blood flow and brain metabolism, can shed new light in PCS diagnosis and in evaluating therapeutic interventions.(20) In clinical studies which utilized objective cognitive assessments, HBOT induced significant improvements in patients suffering from PCS due to mild TBI. (13, 15, 21) However, to the best of our knowledge, the objective effect of HBOT on chronic neurocognitive impairments stemming from moderate or severe TBI (in addition to mild) has not been investigated.

In addition to objective evaluations, there are inherent ethical and logistic difficulties in handling the sham-control in HBOT trials.(4, 5, 20, 22) HBOT includes two active ingredients: pressure and oxygen. Pressure is needed to increase plasma oxygen, but the pressure change alone may also have significant cellular effects.(5) Additionally, the greatest effect of pressure is in human tissues that are under tight autoregulation pressure control, such as the brain, where the intracranial pressure is normally 0.0092-0.0197atm.(23, 24) To generate a pressure sensation, the chamber pressure must be 1.2ATA or higher. However, such a significant change in environmental pressure and subsequent tissue oxygenation (with an increase of tissue oxygenation by at least 50%) cannot be referred as a sham-control but rather as a lower dose of the active ingredient.(4, 20)

Any increase in pressure, even with reduced oxygen percentage, cannot serve as a true placebo, but rather as a low dosage of the active ingredient, further supporting the need for objective data gathered from large cohorts of patients suffering from PCS and treated by HBOT.

The aim of the current study was to evaluate the objective effects of HBOT on TBI patients suffering from chronic neurological deficits stemming from mild, moderate, and severe TBI in the largest cohort evaluated till now. Since all the patients had metabolic brain imaging and a computerized neurocognitive test battery before and after HBOT, correlations between specific cognitive indexes and their related brain region activity were also evaluated.

Materials and Methods

Participants

A retrospective analysis was conducted on patients suffering from TBI related chronic neurocognitive damage, treated by HBOT between January 2008 and January 2017 at the Sagol Center for Hyperbaric Medicine and Research at the Assaf Harofeh Medical Center, Israel. Patients were included if they had pre- and post-HBOT computerized cognitive evaluations. Patients with a history of potential additional brain insults such as spontaneous subarachnoid hemorrhage, anoxic brain injury or history of prior cognitive impairment, were excluded (Figure-1).

The study was approved by the Asaf Harofeh Medical Center's Institutional Review Board.

Patients and public involvement

Patients and public weren't involved in the study due to its retrospective nature.

TBI severity

TBI severities were rated according to the TBI admission documents. Mild TBI was defined as loss of consciousness (LOC) duration of 0–30 minutes, post traumatic amnesia (PTA) duration of less than a day and a Glasgow coma scale (GCS) grade of 13–15. (25) Moderate TBI was defined as LOC duration of more than 30 minutes up to 24 hours, PTA duration of 1-7 days and GCS grade of 9-12. Severe TBI was defined as LOC duration more than 24 hours, PTA duration more than seven days and GCS less than 9. In addition if there was imaging evidence of an injury such as a hematoma, contusion or hemorrhage, then the TBI was classified as moderate to severe. (25)

Hyperbaric oxygen treatment

Patients were treated with 40-70 daily hyperbaric sessions, 5 days a week. Each session consisted of 60 minutes of exposure to 100% oxygen at 1.5-2 ATA.

Cognitive assessment

The patients' cognitive functions were assessed by NeuroTrax computerized cognitive tests (NeuroTrax Corp., TX).(26) The NeuroTrax tests evaluate various aspects of brain functions and include verbal memory (immediate and delayed recognition), non-verbal memory (immediate and delayed recognition), go-no-go response inhibition, problem solving, Stroop interference, finger tapping, catch game, staged information processing speed (single digit, two-digit and three-digit arithmetic), verbal function and visual spatial processing. Cognitive index scores were computed from the normalized outcome parameters for memory, executive function, attention, information processing speed, visual spatial, verbal function and motor skills domains.(27) A global cognitive score was computed as the average of all index scores for each individual.

After administration, the NeuroTrax data were uploaded to the NeuroTrax central server, and outcome parameters were automatically calculated using software blind to diagnosis or testing site. To account for the well-known effects of age and education on cognitive performance, each outcome parameter was normalized and fit to an IQ-like scale (mean=100, S.D.=15) according to the patient's age and education. The normative data used by NeuroTrax consist of test data from cognitively healthy individuals in controlled research studies at more than 10 sites. (28)

Specifically, the patients were given two different versions of the NeuroTrax test battery before and after HBOT, to allow repeated administrations with minimal learning effects. Test-retest reliability for these versions was evaluated and found to

be high, with no significant learning effect.(29, 30) Regarding the current study cohort, in a previous randomized controlled trial in patients suffering from TBI, the NeuroTrax scores were found to be stable in the retest of the control group.(21)

Brain SPECT imaging

Brain activity was assessed using single photon emission computed tomography (SPECT) 1-2 weeks prior to and after the HBOT period. The imaging was conducted using 925–1,110 MBq (25–30 mCi) of a technetium-99m-methyl-cysteinate-dimmer (Tc-99m-ECD) at 40–60 min post injection using a dual detector gamma camera (ECAM or Symbia T, Siemens Medical Systems) equipped with high resolution collimators. Data was acquired in 3-degree steps and reconstructed iteratively using the Chang method of ($\mu = 0.12$/cm) attenuation correction.(31)

Both SPECT images were normalized to the median maximal brain activity in the entire brain and were then reoriented into Talairach space using NeuroGam (Segami Corporation) to identify Brodmann cortical areas and to compute the mean perfusion in each Brodmann area (BA). In addition, volume rendered brain perfusion images were reconstructed and normalized to the entire brain median maximal activity. All SPECT analyses were done by study team members who were blinded to the laboratory and clinical data. SPECT scans were performed late morning to midday. On the day of the SPECT scan, patients were treated with only their chronic medications and were instructed not to smoke. Changes in perfusion in all Brodmann areas for each subject were determined by calculating the percentage of the difference of the normalized activity values between post-period and pre-period divided by the pre-period value.

Statistical Analysis

Continuous data were expressed as means ± standard deviations. The normal distribution for all variables was tested using the Kolmogorov-Smirnov test. The mean differences between cognitive index scores before and after HBOT were analyzed using one-way ANOVA with post-hoc Bonferoni tests. Multiple linear regression models and multivariate logistic regression models were performed to control for potential confounders and to determine independent predictors for clinical outcome. The alpha level was set to 0.05. Data were statistically analyzed using SPSS software (version 22.0).

Results

Patient profiles

Of the 242 patients suffering from neurocognitive impairment due to TBI treated by HBOT between January 2008 and January 2017, 25 patients had potential additional brain insults and 63 did not have repeat computerized neurocognitive evaluations. Therefore, 154 patients were included in the final analysis, of whom 100 patients completed pre- and post-HBOT SPECT imaging (Figure-1).

The patients' baseline characteristics are summarized in Table 1. The average age was 42.7±14.6 years and 58.4% were males. All patients had documented traumatic brain injury 3 months to 33 years (mean 4.6±5.8, median 2.75 years) prior to HBOT. Sixty-nine (44.8%) had neurocognitive impairments due to mild TBI, 24 (15.6%) from moderate TBI and 61 (39.6%) from severe TBI. Most of the patients (86.2%) complained of cognitive impairment as their main symptom (Table 1).

Patients were treated with 40-70 (mean 52.0±9.9) sessions of hyperbaric oxygen at 1.5-2 ATA with 18 (12%) reporting adverse events which included mild barotrauma of the ears and palpitations/dyspnea while in the chamber.

Severity of TBI

Patients who suffered severe TBI were younger with higher proportion of males than in the mild and moderate TBI groups (P<0.0001, P=0.002 respectively, Table 1). As

expected, the severe group had significantly higher proportions of cognitive impairment and motor deficits (p=0.004 and p<0.0001 respectively, Table 1). Mild and moderate TBI had higher percentages of tinnitus and/or dizziness (p<0.0001, p=0.002 respectively, Table 1).

Severe TBI

Sixty-one patients had severe TBI. The main imaging findings at their admission are summarized in Figure-2. Of those 61 patients, 36 (59%) had surgical intervention during the acute event.

Neuro-cognitive evaluation

The effect of HBOT on the patients' cognitive functions, as assessed by the eight cognitive summary scores, is summarized in Table 2 and Figure-3. As can be seen, HBOT induced significant improvements in all of the cognitive domains with a mean change of 4.6±8.5 (p<0.00001). The most prominent improvement was in memory index, with 8.1 ±16.9 (p<0.00001) and attention with 6.8 ±16.5 (p<0.0001) (Table 2, Figure-3).

The mild TBI subgroup had the largest improvement in attention (8.8±2.1) followed by memory (7.9±2.3). Patients following moderate had noticeable improvements in memory (11.1±3.1) followed by information processing speed (6.6±3.5) (Figure-4).

The magnitude of the change in a cognitive score has different implications for patients at low or high baseline levels. Therefore, we further inspected the effect of HBOT on the relative changes, i.e. the changes relative to the baseline value, in each of the cognitive measured indexes. Marked improvement defined as >10% increase

compared to baseline cognitive index were found with different percentages in all study groups as summarized in Table 3.

Confounders

Relative change higher than 10% from baseline was considered a significant clinical improvement. Age, gender, education level, TBI severity, the time from injury to HBOT, HBOT protocol and number of sessions had no significant effect on the clinical improvement in both general, memory, attention, information processing speed and executive functions domains ($p>0.05$).

Metabolic imaging of the brain-SPECT

One hundred patients had brain SPECT evaluations before and after HBOT. When calculating the mean relative change in each cortical Broadmann's area for the entire cohorot, the largest changes were in the anterior temporal tip areas (BA 38, 28, 20) and prefrontal cortex (BA 10) (Figure-5). However, these change were minor, in the scale of 3-4% relative change. Further analysis per TBI severity group revealed several differences in Broadmann areas with involvement of the perirhinal cortex (BA 36) and the primary visual cortex (BA 18) as seen in the Figure-6.

To correlate SPECT imaging and the cognitive changes, analysis was performed on the top twenty patients who had the largest cognitive improvement (>10% relative increase from baseline). There was a significantly larger magnitude of metabolism increase (5-8%), compared to the entire cohort average increase (2-4%) ($p<0.05$). The most striking changes were found in the anterior cingulate (BA 24) and the post-central cortex (BA 5), the prefrontal areas (Ba 10,11, 46) and temporal areas (BA 20, BA 38, 36).

Discussion

The present study demonstrates the neurotherapeutic effects of HBOT for chronic TBI of all severities. Even though treatment started during late chronic stages (mean 4.6±5.8 years, median 2.75 years) after the acute insult, HBOT was found to be effective regardless of the TBI severity. The clinical improvements seen in all cognitive domains were well documented by objective computerized neurocognitive tests. The most significant measurable improvements were in memory, attention and executive function. We found the clinical improvement to be well correlated with increased brain activity in relevant brain areas, with significantly higher increases in patients with better cognitive improvements.

Numerous mechanisms of cellular and vascular repair by HBOT have been suggested in addition to tissue oxygenation.(5) These include improved mitochondrial function and cellular metabolism, improved BBB and inflammatory reactions, reduced apoptosis, alleviation of oxidative stress, increased levels of neurotrophins and nitric oxide, and upregulation of axonal guidance agents.(5, 13, 32) Moreover, the effects of HBOT on neurons can be mediated indirectly by glial cells. HBOT may also promote neurogenesis of endogenous neural stem cells.(13, 32) The common denominator underlying all these mechanisms is that they are oxygen-dependent. HBOT may enable the metabolic change simply by supplying the missing energy/oxygen needed for these regeneration processes (32). The induction of angiogenesis and improved brain metabolism, as demonstrated in this study, may

serve as the infrastructure that enables the regenerative process and the preservation of newly generated neuronal functioning. (14, 33)

The correlation between specific cognitive function improvements with the metabolic brain imaging changes gives further strength to the study results and serves as an excellent tool for gaining better understanding of brain functionality (Figure-6):

- The perirhinal cortex activation after HBOT was most prominent in patients that had significant memory improvement. The perirhinal cortex has a critical role in object recognition memory while interacting with the hippocampus.(34) Since the memory assessments in the cognitive tests were indeed recognition tasks, this area is expected to be involved.

-The pre-frontal cortex (BA 10, 11) and more specifically, the inferior frontal gyrus (BA 46, 47) activation after HBOT were prominent in all patients with significant executive function improvements. The right frontal gyrus is known to mediate a go/no go task,(35) which was among the executive function tests used in the present study. The prefrontal gyrus is presumed to act as a filtering system that enhances goal directed activities and inhibits irrelevant activations. This filtering mechanism enables executive control.(36)

-The anterior cingulate gyrus (BA 24) activation after HBOT was seen in the subjects with attention improvement. The anterior cingulate gyrus is presumed to be involved in error detection, especially in a Stroop task,(37) which was used in the attention tests. Lesions in this area can cause inattention to akinetic mutism.(37)

This study has several limitations. The major one relates to its retrospective methodology. This limitation should be considered even thought this large cohort of patients was treated at late chronic stages. The findings presented here are in agreement and reinforce the findings from previous prospective controlled trials in which the neuroplasticity effects of HBOT were demonstrated in chronic stages of different types of brain injuries.(15, 21, 38, 39) Moreover, the correlation between the changes in cognitive function and the metabolic brain imaging gives further strength to the results.

Another important limitation relates to the HBOT protocol which was inconsistent in the cohort. Although significant neurotherapeutic effects were seen with 60 minutes of 1.5 ATA, the optimal protocol needed to induce maximal neuroplasticity for the specific individual with minimal side effects has not been specified.

The strengths of the study are worth mentioning. First, objective cognitive assessments using computerized tests were performed to each patient both pre- and post-treatment. Objective measures are significantly superior to PCS questionnaires which are inaccurate, variable and contain various confounders rather than reflect the true PCS state.(40) Second, most of the patients in the study underwent an objective ancillary brain SPECT to confirm PCS diagnosis prior to HBOT. This practice is crucial when considering the differential diagnosis following TBI (PTSD, depression, etc.). Moreover, post-treatment brain SPECTs enabled an anatomical-functional correlation in regards to HBOT's effect in brain neuroplasticity. Third, the study cohort included a civilian population that does not have any potential secondary gain (such as financial compensation by reporting sick).

Conclusions

HBOT induced significant cognitive improvements in patients who suffer from chronic neurocognitive deficits due to mild, moderate and severe TBI. The improvement in memory correlated with activation of the perirhinal cortex, improvement of executive functions correlated with activation of the inferior frontal gyrus and improvement in attention correlated with activation of the anterior cingulate gyrus.

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Table 1: Baseline patient characteristics. 561

Characteristics		Total	Mild TBI	Moderate TBI	Severe TBI	Significance
Number of patients		154 (100%)	69 (44.8%)	24 (15.6%)	61 (39.6%)	
Age (years)		42.7±14.6	48.8±12.0	41.7±12.7	36.2±15.3	**P<0.0001**
Sex						
	Males	90 (58.4%)	31 (44.9%)	13 (54.2%)	46 (75.4%)	**P=0.002**
	Females	64 (41.6%)	38 (55.1%)	11 (45.8%)	15 (24.6%)	
Education (years)		14.8±3.3	14.9±3.6	14.9±3.3	14.6±3.1	P=0.895
Traumatic event						

	Motor Vehicle Accident	116 (75.3%)	56 (81.2%)	17 (70.8%)	43 (70.5%)	**P=0.048**
	Fall	21 (13.6%)	8 (11.6%)	1 (4.2%)	12 (57.1%)	
	Blow	12 (7.8%)	5 (7.2%)	5 (20.8%)	2 (3.3%)	
	Blast	4 (2.6%)	0	1 (4.2%)	3 (4.9%)	
	Penetrating	1 (0.6%)	0	0	1 (0.6%)	
Time from trauma (years)		4.6±5.8	4.4±5.9	5.0±5.8	4.6±5.7	P=0.923

Table 1: Patient characteristics (continued). 564

Characteristics		Total	Mild TBI	Moderate TBI	Severe TBI	Significance
Symptoms						
	Cognitive	100 (86.2%)	38 (74.5%)	19 (90.5%)	43 (97.7%)	**P=0.004**
	Motor	22 (19.0%)	1 (4.8%)	1 (4.8%)	20 (45.5%)	**P<0.0001**
	Sensory	32 (27.6%)	12 (23.5%)	7 (33.3%)	13 (29.5%)	P=0.653
	Dizziness/Vertigo	17 (14.7%)	14 (27.5%)	2 (9.5%)	1 (2.3%)	**P=0.002**
	Tinnitus	30 (25.9%)	26 (51.0%)	2 (9.5%)	2 (4.5%)	**P<0.0001**
	Headaches	20 (17.2%)	12 (23.5%)	3 (14.3%)	5 (11.4%)	P=0.272
HBO sessions		52.0±9.9	49.4±10.1	49.0±10.3	56.1±8.2	**P=0.0001**

HBO protocol (ATA)	1.5	106 (69.3%)	46 (67.6%)	18 (75.0%)	42 (68.9%)	P=0.795
	2	47 (30.7%)	22 (32.4%)	6 (25.0%)	19 (31.1%)	
Adverse Events		18 (12.0%)	10 (15.2%)	3 (12.5%)	5 (8.3%)	P=0.499

Table 2: Cognitive indices pre- and post-HBOT of the whole study cohort

	Baseline	Post HBOT	Mean Change	P-Value
General	88.3±15.2	92.9±14.2	4.6±8.5	**P<0.0001**
Memory	81.7±23.2	89.9±21.9	8.1±16.9	**P<0.0001**
Executive Functions	88.3±16.6	94.2±15.1	5.9±12.0	**P<0.0001**
Attention	84.3±20.5	91.1±18.4	6.8±16.5	**P<0.0001**
IPS	87.5±17.0	92.4±15.7	4.9±13.1	**P<0.0001**

VSP	95.0±18.0	98.5±18.0	3.4±14.6	**P=0.005**
Motor skills	92.3±17.3	96.2±14.5	3.9±11.7	**P<0.0001**

Table 3: Large significant increases (>10% change) in cognitive indices proportions across TBI subgroups.

	Total	Mild TBI	Moderate TBI	Severe TBI	
General	36 (23.4%)	15 (21.7%)	7(29.2%)	14 (23.0%)	P=0.756
Memory	64 (41.6%)	28 (40.6%)	9 (37.5%)	27 (44.3%)	P=0.830
Executive functions	51 (33.1%)	23 (33.3%)	7 (29.2%)	21 (34.9%)	P=0.897
Attention	62 (40.3%)	27 (39.1%)	8 (33.3%)	27 (44.3%)	P=0.631
Information processing speed	48 (31.2%)	23 (33.3%)	12 (50%)	13 (21.3%)	**P=0.032**

Figures legends

Figure-1**:** Patients flowchart.

Figure-2: Imaging findings in the severe TBI subgroup

Figure-3**:** Mean changes of post- compared to pre-HBOT for the entire cohort.

After HBOT, all cognitive domains improved significantly, where the most striking changes were seen in memory and attention.

*p<0.0001, **p=0.005, IPS=information processing speed

Figure-4**:** Mean changes of post- compared to pre-HBOT across the different TBI severities.

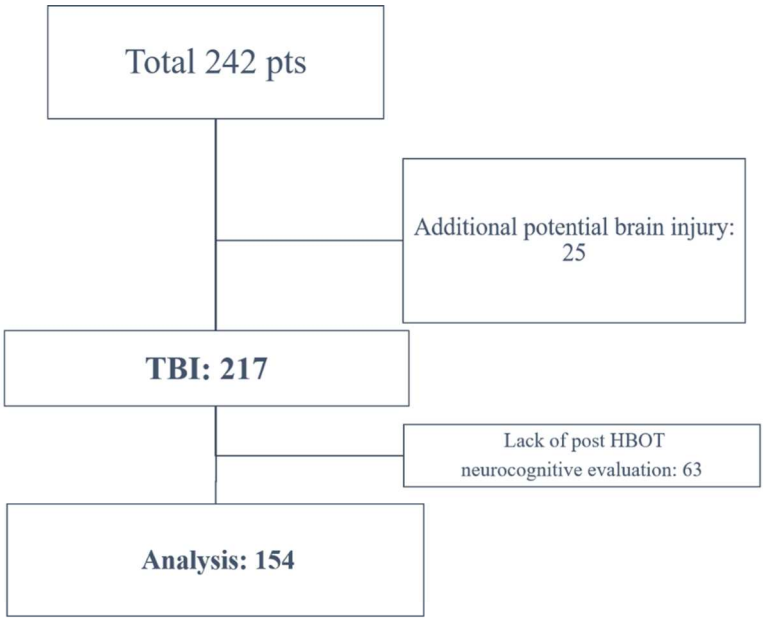
Both patients who suffered mild and severe TBI had improvements in general, memory, attention, information processing speed and motor skills scores whereas patients who suffered moderate TBI had significant improvement in memory.

*p<0.05, IPS =information processing speed

Figure-5**:** The mean relative change in broadmann areas post HBOT for the entire study cohort.

Figure-6**:** Cognitive functions correlated with Broadmann areas.

Each of the TBI severities (mild, moderate and severe) had perfusion/metabolism increase in specific Broadmann areas correlated with improved cognitive function.



Patients flowchart

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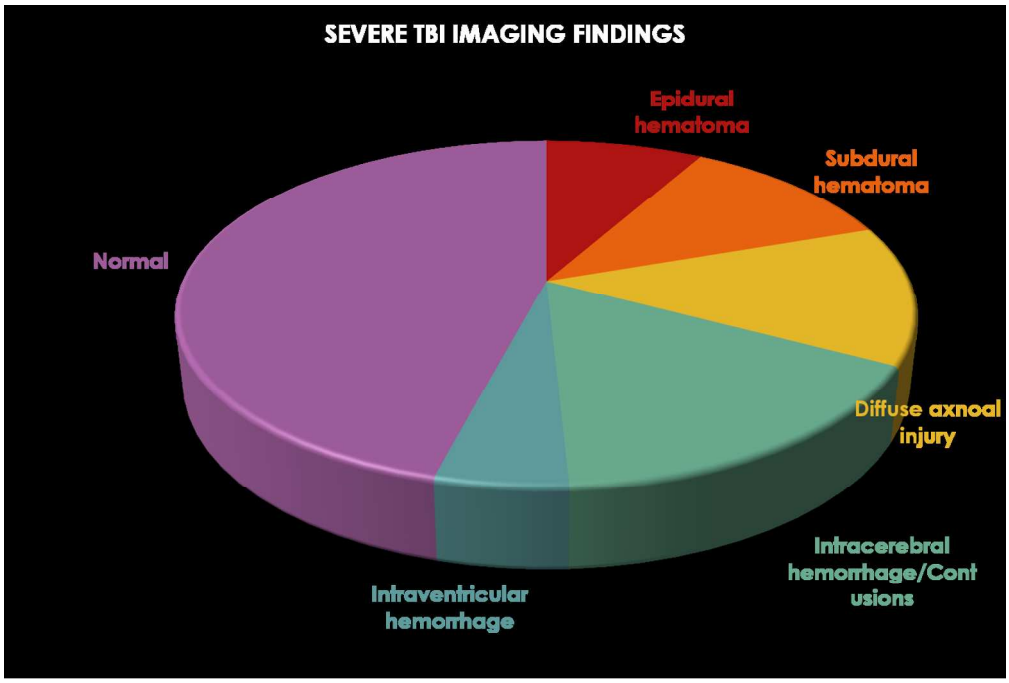

Imaging findings in the severe TBI subgroup

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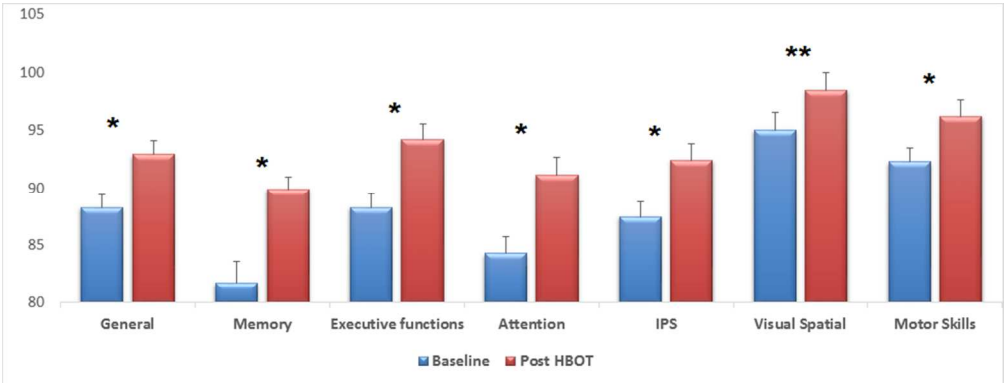
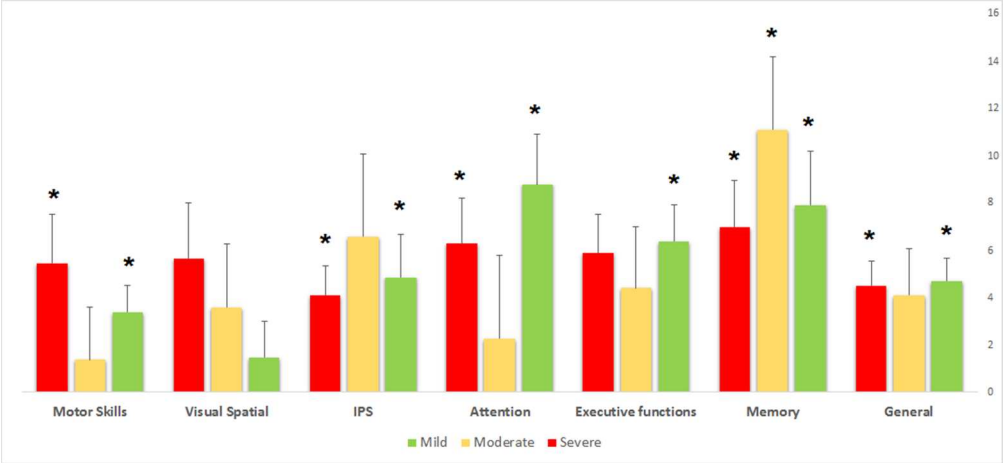


Mean changes of post- compared to pre-HBOT for the entire cohort.
After HBOT, all cognitive domains improved significantly, where the most striking changes were seen in memory and attention.
*p<0.0001, **p=0.005, IPS=information processing speed

204x77mm (300 x 300 DPI)



Mean changes of post- compared to pre-HBOT across the different TBI severities.
Both patients who suffered mild and severe TBI had improvements in general, memory, attention, information processing speed and motor skills scores whereas patients who suffered moderate TBI had significant improvement in memory.
*p<0.05, IPS =information processing speed

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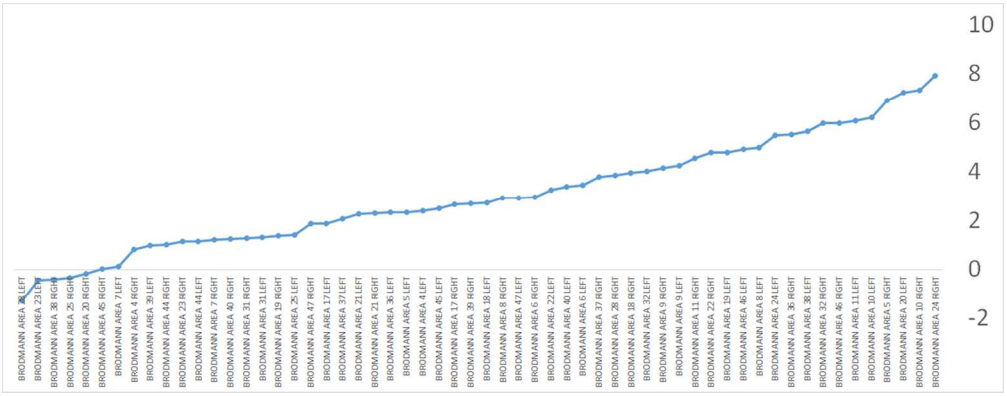

The mean relative change in broadmann areas post HBOT for the entire study cohort

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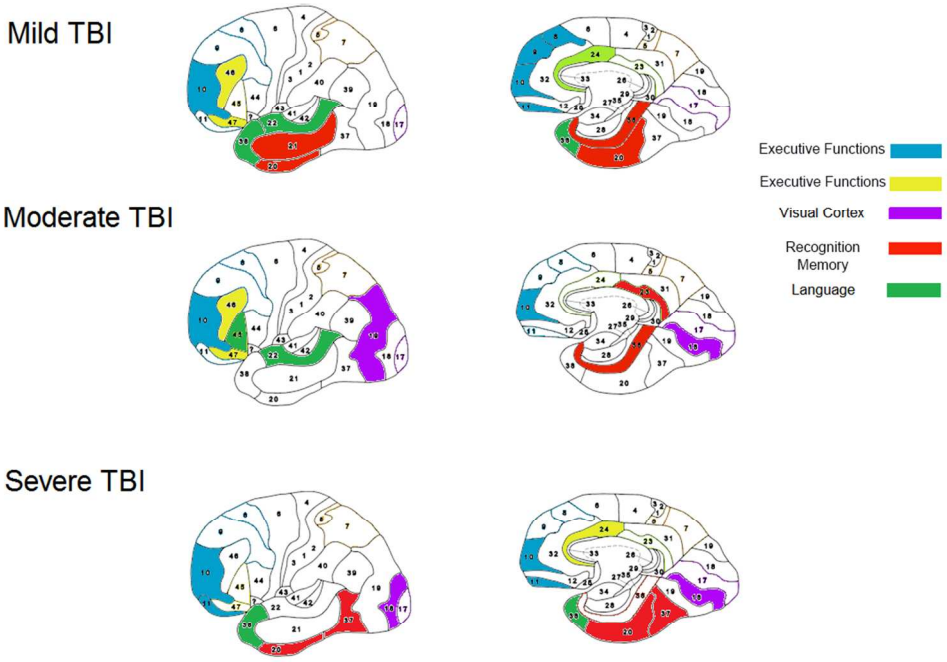

Cognitive functions correlated with Broadmann areas.
Each of the TBI severities (mild, moderate and severe) had perfusion/metabolism increase in specific Broadmann areas correlated with improved cognitive function

240x173mm (300 x 300 DPI)

BMJ Open

The effect of hyperbaric oxygen therapy on chronic neurocognitive function of deficits post traumatic brain injury patients – retrospective analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023387.R1
Article Type:	Research
Date Submitted by the Author:	31-May-2018
Complete List of Authors:	Hadanny, Amir; Galilee Medical Center, Neuosurgery; Assaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Abbott, Stefanie; Asaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Suzin, Gil; Asaf Harfoeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Bechor, Yair; Asaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Efrati, Shai; Assaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research; Tel Aviv University, Sackler School of Medicine
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Rehabilitation medicine
Keywords:	tbi, hbot, traumatic brain injury, hyperbaric oxygen, cognitive

The effect of hyperbaric oxygen therapy on chronic neurocognitive function of deficits post traumatic brain injury patients – retrospective analysis

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Author Disclosure Statement

No competing financial interests exist.

No funding received for this work.

No conflict of interests exists.

Contributorship statement

AH: concept, data collection, data analysis, manuscript draft, manuscript review

SA: data collection, data analysis

GS: data collection, manuscript review

YB: data collection, manuscript review

SE: concept, data analysis, manuscript draft, manuscript review

Data sharing statement

Extra data is available by emailing amir.had@gmail.com

Running title: HBOT improves cognitive function in TBI

Number of references: 43

Word count: 3615

Tables: 3

Figures: 6

Abstract

Objectives

The aim of the study is to evaluate the effect of hyperbaric oxygen therapy (HBOT) in participants suffering from chronic neurological deficits due to traumatic brain injury (TBI) of all severities in the largest cohort evaluated so far with objective cognitive function tests and metabolic brain imaging.

Methods

A retrospective analysis was conducted of 154 patients suffering from chronic neurocognitive damage due to TBI, who had undergone computerized cognitive evaluations pre- and post-HBOT treatment.

Results

The average age was 42.7±14.6 years and 58.4% were males. All patients had documented traumatic brain injury 0.3-33 years (mean 4.6±5.8, median 2.75 years) prior to HBOT. HBOT induced significant improvement in all of the cognitive domains, with mean change in global cognitive scores of 4.6±8.5 ($p<0.00001$). The most prominent improvements were in memory index and attention, with mean changes of 8.1±16.9 ($p<0.00001$) and 6.8±16.5 ($p<0.0001$), respectively. The most striking changes observed in brain single photon emission computed tomography (SPECT) images were in the anterior cingulate and the post-central cortex, in the prefrontal areas and in the temporal areas.

Conclusions

In the largest published cohort of patients suffering from chronic deficits post TBI of all severities, HBOT induced significant cognitive improvements. The clinical improvements were well correlated with increased activity in the relevant brain areas.

Keywords: hyperbaric oxygen, HBOT, post-concussion, PCS, TBI, traumatic brain injury

Article Summary

Strengths and limitations of the study

- The major limitation relates to its retrospective methodology, however this limitation is diminished by the fact that all patients of the study large cohort were treated at late chronic stages.
- In regards to strengths, objective cognitive assessments using computerized tests (which are superior to any clinical questionnaire), were performed on each patient both pre- and post-treatment.
- The study cohort consisted of a civilian population that does not have any potential secondary gain (such as financial compensation by reporting sick).

Introduction

Traumatic brain injury (TBI) is one of the leading causes of death and disability in the general population.(1) Following TBI, patients may experience a set of symptoms known as post-concussion syndrome (PCS). PCS symptoms include headaches, dizziness, neuropsychiatric symptoms, and cognitive impairments.(2) PCS can continue for weeks or months, and up to 25% of all patients experience prolonged PCS (PPCS) in which the symptoms last for over six months.(3)

In the past years there is growing clinical evidence regarding the effect of hyperbaric oxygen therapy (HBOT) on PCS.(4, 5) Unfortunately, the clinical data gathered from those studies can be conflicting due to several inherent procedural issues, such as the use of non-objective end points, the lack of appropriate brain imaging as part of the inclusion criteria, the inappropriate placebo of a hyperbaric environment, and the inclusion of patients that may gain secondary benefits from reporting sick.(4, 5) The current study represents the largest cohort evaluated until now of civilian participants suffering from PCS treated by HBOT, who had undergone objective metabolic brain imaging and a computerized neurocognitive test battery before and after the treatment.

Pathophysiology of PCS and HBOT

The most common pathological mechanism in TBI is diffuse shearing of axonal pathways and small blood vessels, also known as diffuse axonal injury.(6) Secondary pathological mechanisms of TBI include ischemia, mild edema, and other biochemical and inflammatory processes culminating in impaired regenerative and/or healing processes resulting from increasing tissue hypoxia.(7) Due to the diffuse nature of injury, affecting multiple brain areas,(8, 9) cognitive impairments are usually the predominant symptoms.

Global brain hypoperfusion, and its related tissue ischemia, detected in patients suffering from TBI, serves as a rate-limiting factor for any regenerative process.(10-12) By increasing the oxygen level in blood and body tissues, HBOT can augment the repair mechanisms.(13) Various models have strongly suggested that HBOT can induce angiogenesis, improve brain plasticity, enhance neurogenesis and synaptogenesis and foster functional recovery.(14, 15)

Conflicting clinical HBOT data and objective measurements in PCS

Some of the previous studies which evaluated the effect of HBOT on chronic neurological and cognitive impairments due to TBI, mainly used self-assessment questionnaires as their primary endpoints.(16-18) Such endpoints have several inherent disadvantages. First, they lack an objective evaluation that is not biased by the patients' perspectives. Second, self-administrated questionnaires are exposed to various confounding variables such as litigation and compensation.(19). Unlike the questionnaires, standardized cognitive tests with high test-retest reliability can and should be used as objective evaluations of neurocognitive impairments.(20) In addition, novel brain imaging techniques such as single photon emission computed tomography (SPECT) and perfusion sequences in magnetic resonance imaging (MRI), which evaluate cerebral blood flow and brain metabolism, can shed new light in PCS diagnosis and in evaluating therapeutic interventions.(20) In clinical studies which utilized objective cognitive assessments, HBOT was found to induce significant improvements in patients suffering from PCS due to mild TBI. (13, 15, 21) However, to the best of our knowledge, the objective effect of HBOT on chronic neurocognitive impairments stemming from moderate to severe TBI (in addition to mild) has not been investigated.

In addition to objective evaluations, there are inherent ethical and logistic difficulties in handling the sham control in HBOT trials.(4, 5, 20, 22) HBOT includes two active ingredients: pressure and oxygen. Pressure is needed to increase plasma oxygen, but the pressure change alone may also have significant cellular effects.(5) Additionally, the greatest effect of pressure is in human tissues that are under tight autoregulation pressure control, such as the brain, where the intracranial pressure is normally 0.0092-0.0197atm.(23, 24) To generate a pressure sensation, the chamber pressure must be 1.2ATA or higher. However, such a change in environmental pressure (from 1ATA to 1.2 ATA) and subsequent tissue oxygenation (with an increase of tissue oxygenation by at least 50%) has a significant biologic effect (25, 26) Thus, sham therapy in previous studies using 1.2 ATA on 21% inhaled oxygen (i.e., air) cannot be regarded as an inert or sham control but rather as a lower dose of the active ingredient.(4, 20) In regards to a possible effect of vasoconstriction of the large blood vessels induced by hyperbaric oxygen - it has been well established that the tissues are saturated by hyperoxia and do not suffer from hypoxia, as the vasoconstriction effect is compensated by increased plasma oxygen content and microvascular blood flow. (27)

Any increase in pressure, even with reduced oxygen percentage, cannot serve as a true placebo, but rather as a low dosage of the active ingredient, further supporting the need for objective data gathered from large cohorts of patients suffering from PCS and treated by HBOT.

The aim of the current study was to evaluate the objective effects of HBOT on TBI patients suffering from chronic neurological deficits stemming from mild, moderate, and severe TBI, in the largest cohort evaluated until now. Since all the patients had metabolic brain imaging and a computerized neurocognitive test battery before and

after HBOT, correlations between specific cognitive indexes and their related brain regions activity were also evaluated.

Materials and Methods

Participants

A retrospective analysis was conducted on patients suffering from TBI-related chronic neurocognitive damage (more than 3 months from injury), treated by HBOT between January 2008 and January 2017 at the Sagol Center for Hyperbaric Medicine and Research,Assaf Harofeh Medical Center, Israel. Patients were included if they had pre- and post-HBOT computerized cognitive evaluations. Patients with a history of potential additional brain insults, such as spontaneous subarachnoid hemorrhage, anoxic brain injury, or history of prior cognitive impairment, were excluded (Figure-1).

The study was approved by the Institutional Review Board of Asaf Harfoeh Medical Center.

Patients and public involvement

Patients and public weren't involved in the study due to its retrospective nature.

TBI severity

TBI severities were rated according to the TBI admission documents. Mild TBI was defined as loss of consciousness (LOC) with duration of 0–30 minutes, post traumatic amnesia (PTA) with duration of less than a day and a Glasgow coma scale (GCS) grade of 13–15. (28) Moderate TBI was defined as LOC with duration of more than 30 minutes and up to 24 hours, PTA with duration of 1-7 days and GCS grade of 9-12. Severe TBI was defined as LOC with duration of more than 24 hours, PTA with duration of more than seven days, and GCS less than 9. In addition, if there was imaging evidence of an injury such as a hematoma, contusion or hemorrhage, then the TBI was classified as moderate to severe. (28)

Hyperbaric oxygen treatment

Patients were treated with 40-70 daily hyperbaric sessions, 5 days a week. Each session consisted of 60 minutes of exposure to 100% oxygen at 1.5-2 ATA.

Cognitive assessment

The patients' cognitive functions were assessed by NeuroTrax computerized cognitive tests (NeuroTrax Corp., NY).(29) The NeuroTrax tests evaluate various aspects of brain functions and include verbal memory (immediate and delayed recognition), non-verbal memory (immediate and delayed recognition), go/no go response inhibition, problem solving, Stroop interference, finger tapping, catch game, staged information processing speed (single digit, two-digit and three-digit arithmetic), verbal function and visual spatial processing. Cognitive index scores were computed from the normalized outcome parameters for memory, executive function, attention, information processing speed, visual spatial, verbal function and motor skills domains.(30) A global cognitive score was computed as the average of all index scores for each individual.

After administration, the NeuroTrax data were uploaded to the NeuroTrax central server, and outcome parameters were automatically calculated using software blind to diagnosis or testing site. To account for the well-known effects of age and education on cognitive performance, each outcome parameter was normalized and fit to an IQ-like scale (mean=100, S.D.=15) according to the patient's age and education. The normative data used by NeuroTrax consist of test data from cognitively healthy individuals in controlled research studies at more than 10 sites. (31)

Specifically, the patients were given two different versions of the NeuroTrax test battery before and after HBOT, to allow repeated administrations with minimal learning effects. Test-retest reliability for these versions was evaluated and found to

be high, with no significant learning effect.(32, 33) Regarding the current study cohort, in a previous randomized controlled trial in patients suffering from TBI, the NeuroTrax scores were found to be stable in the retest of the control group.(21)

Brain SPECT imaging

Brain activity was assessed using single photon emission computed tomography (SPECT) 1-2 weeks prior to and after the HBOT period. The SPECT method was selected for evaluation due to its known normal range and test/retest established validity. The imaging was conducted using 925–1,110 MBq (25–30 mCi) of a technetium-99-methyl-cysteinate-dimmer (Tc-99m-ECD) at 40–60 min post injection, using a dual detector gamma camera (ECAM or Symbia T, Siemens Medical Systems) equipped with high resolution collimators. Data was acquired in 3-degree steps and reconstructed iteratively using the Chang method of attenuation correction ($\mu = 0.12$/cm).(34)

Both pre- and post-treatment SPECT images were normalized to the median maximal brain activity in the entire brain, and were then reoriented into Talairach space using NeuroGam software (Segami Corporation) to identify Brodmann cortical areas and to compute the mean perfusion in each Brodmann area (BA). In addition, volume-rendered brain perfusion images were reconstructed and normalized to the entire brain median maximal activity. All SPECT analyses were done by study team members who were blinded to the laboratory and clinical data. SPECT scans were performed late morning to midday. On the day of the SPECT scan, patients were treated with only their chronic medications and were instructed not to smoke. Changes in perfusion in all Brodmann areas for each subject were determined by calculating the

percentage of the difference of the normalized activity values between post-treatment and pre-treatment divided by the pre-treatment value.

Statistical Analysis

Continuous data were expressed as means ± standard deviations. The normal distribution for all variables was tested using the Kolmogorov-Smirnov test. The mean differences between cognitive index scores before and after HBOT were analyzed using one-way ANOVA with post-hoc Bonferroni tests. Multiple linear regression models and multivariate logistic regression models were performed to control for potential confounders and to determine independent predictors for clinical outcome. The alpha level was set to 0.05. Data were statistically analyzed using SPSS software (version 22.0).

Results

Patient profiles

Of the 242 patients suffering from neurocognitive impairment due to TBI treated by HBOT between January 2008 and January 2017, 25 patients had potential additional brain insults and 63 did not have repeat computerized neurocognitive evaluations. Therefore, 154 patients were included in the final analysis, of whom 100 patients completed pre- and post-HBOT SPECT imaging (Figure-1).

The patients' baseline characteristics are summarized in Table 1. The average age was 42.7±14.6 years and 58.4% were males. All patients had documented traumatic brain injury 3 months to 33 years (mean 4.6±5.8, median 2.75 years) prior to HBOT. Sixty-nine (44.8%) had neurocognitive impairments due to mild TBI, 24 (15.6%) moderate TBI and 61 (39.6%) severe TBI. Most of the patients (86.2%) complained of cognitive impairment as their main symptom (Table 1).

Patients were treated with 40-70 (mean 52.0±9.9) sessions of hyperbaric oxygen at 1.5-2 ATA. 18 (12%) patients reported adverse events, which included mild barotrauma of the ears and palpitations/dyspnea, while in the chamber.

Severity of TBI

In our cohort, patients who suffered severe TBI were found to be younger with higher proportion of males than in the mild and moderate TBI groups (P<0.0001, P=0.002 respectively, Table 1). As expected, the severe TBI group had significantly higher proportions of cognitive impairment and motor deficits (p=0.004 and p<0.0001 respectively, Table 1). The mild and moderate TBI groups had higher percentages of tinnitus and/or dizziness (p<0.0001, p=0.002 respectively, Table 1).

Severe TBI

Sixty-one patients had severe TBI. The main imaging findings at their admission are summarized in Figure-2. Of those 61 patients, 36 (59%) had surgical intervention during the acute event.

Neurocognitive evaluation

The effect of HBOT on the patients' cognitive functions, as assessed by the eight cognitive summary scores, is summarized in Table 2 and Figure-3. As can be seen, HBOT induced significant improvements in all of the cognitive domains with a mean change of 4.6±8.5 (p<0.00001). The most prominent improvement was in the memory index, with 8.1 ±16.9 (p<0.00001), and in attention, with 6.8 ±16.5 (p<0.0001) (Table 2, Figure-3).

The mild TBI group had the largest improvement in attention (8.8±2.1), followed by memory (7.9±2.3). Patients in the moderate TBI group had noticeable improvements in memory (11.1±3.1), followed by information processing speed (6.6±3.5). Lastly, the severe TBI group had the largest improvement in memory (7.0±2.0), followed by attention (6.3±1.9) (Figure-4). Using ANOVA analysis for repeated measures, there

were no significant differences in all cognitive domains improvements between the different TBI severity groups.

The magnitude of the change in a cognitive score has different implications for patients at low or high baseline levels. Therefore, we further inspected the effect of HBOT on the relative changes, i.e., the changes relative to the baseline value, in each of the cognitive measured indexes. Marked improvements defined as >10% increase compared to baseline cognitive index were found, with different percentages, in all three study groups as summarized in Table 3.

Confounders

Relative change higher than 10% from baseline was considered a significant clinical improvement. Age, gender, education level, TBI severity, the time from injury to HBOT, HBOT protocol and number of sessions had no significant effect on the clinical improvement in the general, memory, attention, information processing speed and executive functions domains ($p>0.05$).

Metabolic imaging of the brain usingSPECT

One hundred patients had brain SPECT evaluations before and after HBOT. When calculating the mean relative change in each cortical Brodmann area for the entire cohort, the largest changes were in the anterior temporal tip areas (BA 38,BA 28, BA 20) and in the prefrontal cortex (BA 10) (Figure-5). However, these changes were minor, in the range of 3-4% relative change. Further analysis per TBI severity group revealed several differences in Brodmann areas with involvement of the perirhinal cortex (BA 36) and the primary visual cortex (BA 18), as seen in Figure-6.

To correlate SPECT imaging and cognitive changes, analysis was performed on the top twenty patients who had the largest cognitive improvement (>10% relative increase from baseline). There was a significantly larger magnitude of metabolism increase (5-8%), compared to the entire cohort average increase (2-4%) ($p<0.05$). The most striking changes were found in the anterior cingulate (BA 24, ($p=0.01$)) and the post-central cortex (BA 5, ($p=0.04$)), in the prefrontal areas (BA 10 ($p=0.04$), BA 11 ($p=0.07$), BA 46 ($p=0.07$) and in the temporal areas (BA 20 ($p=0.02$), BA 38 ($p=0.07$), BA 36 ($p=0.1$)).

Discussion

The present study demonstrates the neurotherapeutic effects of HBOT for chronic TBI of all severities. Even though treatment started during late chronic stages (mean 4.6±5.8 years, median 2.75 years) after the acute insult, HBOT was still found to be effective regardless of the TBI severity. The clinical improvements seen in all cognitive domains were well documented by objective computerized neurocognitive tests. The most significant measurable improvements were in memory, attention and executive function. We found the clinical improvement to be well correlated with increased brain activity in relevant brain areas, with significantly higher increases in patients with better cognitive improvements.

In addition to tissue oxygenation, numerous mechanisms of cellular and vascular repair by HBOT have been suggested in addition to tissue oxygenation.(5) These include improved mitochondrial function and cellular metabolism, improved blood-

brain barrier and inflammatory reactions, reduced apoptosis, alleviation of oxidative stress, increased levels of neurotrophins and nitric oxide, and upregulation of axonal guidance agents.(5, 13, 35) Moreover, the effects of HBOT on neurons can be mediated indirectly by glial cells. HBOT may also promote neurogenesis of endogenous neural stem cells.(13, 35) The common denominator underlying all these mechanisms is that they are oxygen-dependent. HBOT may enable the metabolic change simply by supplying the missing energy/oxygen needed for these regeneration processes. (35) The induction of angiogenesis and improved brain metabolism, as demonstrated in this study, may serve as the infrastructure that enables the regenerative process and the preservation of newly generated neuronal functioning. (14, 36)

The correlation between specific cognitive function improvements with the metabolic brain imaging changes gives further strength to the study results and serves as an excellent tool for gaining better understanding of brain functionality (Figure-6):

- The perirhinal cortex activation after HBOT was most prominent in patients who had significant memory improvement. The perirhinal cortex has a critical role in object recognition memory while interacting with the hippocampus.(37) Since the memory assessments in the cognitive tests were indeed recognition tasks, this area is expected to be involved.
- The pre-frontal cortex (BA 10, BA 11) and more specifically, the inferior frontal gyrus (BA 46, BA 47) activations after HBOT were prominent in all patients with significant executive function improvements. The right frontal gyrus is known to mediate a go/no go

task, (38) which was among the executive function tests used in the present study. The prefrontal gyrus is presumed to act as a filtering system that enhances goal directed activities and inhibits irrelevant activations. This filtering mechanism enables executive control.(39)

- The anterior cingulate gyrus (BA 24) activation after HBOT was seen in the subjects with attention improvement. The anterior cingulate gyrus is presumed to be involved in error detection, especially in a Stroop task,(40) which was used in the attention tests. Lesions in this area can cause inattention to akinetic mutism.(40)

This study has several limitations. The major one relates to its retrospective methodology. This limitation is diminished when considering that this large cohort of patients was treated at late chronic stages. The findings presented here are in agreement and reinforce the findings from previous prospective controlled trials in which the neuroplasticity effects of HBOT were demonstrated in chronic stages of different types of brain injuries.(15, 21, 41, 42) Moreover, the correlation between the changes in cognitive function and the metabolic brain imaging gives further strength to the results.

Another important limitation relates to the HBOT protocol which was inconsistent across the cohort. Although significant neurotherapeutic effects were seen with 60 minutes of 1.5 ATA, the optimal protocol needed to induce maximal neuroplasticity for the specific individual, with minimal side effects has not been investigated.

The strengths of the study are worth mentioning. First, objective cognitive assessments using computerized tests were performed on each patient both pre- and

post-treatment. Objective measures are significantly superior to PCS questionnaires which are inaccurate, variable and contain various confounders rather than reflect the true PCS state.(43) Second, most of the patients in the study underwent an objective ancillary brain SPECT to confirm PCS diagnosis prior to HBOT. This practice is crucial when considering the differential diagnosis following TBI (PTSD, depression, etc.).

Moreover, post-treatment brain SPECTs revealed an anatomical-functional correlation in regards to HBOT's effect in brain neuroplasticity. Third, the study cohort consisted of a civilian population that does not have any potential secondary gain (such as financial compensation) by reporting sick.

Previous studies included post-concussion syndrome patients who suffered mild TBI injury. Considering its strengths and limitations, the current study implies that the cognitive function of patients post TBI, can be improved significantly, irrespectively of whether the primary brain injury was classified as mild, moderate or severe. Although long term data is still lacking, considering the high safety profile of the treatment, these results are promising and should encourage rehabilitation centers to consider HBOT for patients with chronic neurocognitive deficits following TBI. Future studies should monitor these patients in the long term (6 months, 12 months) as well as their return to activities of daily living.

Conclusions

HBOT induced significant cognitive improvements in patients who suffer from chronic neurocognitive deficits due to mild, moderate and severe TBI. Improvement in memory correlated with activation of the perirhinal cortex, improvement of

executive functions correlated with activation of the inferior frontal gyrus, and improvement in attention correlated with activation of the anterior cingulate gyrus.

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Table 1: Baseline patient characteristics. 587

Characteristics		Total	Mild TBI	Moderate TBI	Severe TBI	Significance
Number of patients		154 (100%)	69 (44.8%)	24 (15.6%)	61 (39.6%)	
Age (years)		42.7±14.6	48.8±12.0	41.7±12.7	36.2±15.3	**P<0.0001**
Sex						
	Males	90 (58.4%)	31 (44.9%)	13 (54.2%)	46 (75.4%)	**P=0.002**
	Females	64 (41.6%)	38 (55.1%)	11 (45.8%)	15 (24.6%)	
Education (years)		14.8±3.3	14.9±3.6	14.9±3.3	14.6±3.1	P=0.895
Traumatic event						

	Motor Vehicle Accident	116 (75.3%)	56 (81.2%)	17 (70.8%)	43 (70.5%)	**P=0.048**
	Fall	21 (13.6%)	8 (11.6%)	1 (4.2%)	12 (57.1%)	
	Blow	12 (7.8%)	5 (7.2%)	5 (20.8%)	2 (3.3%)	
	Blast	4 (2.6%)	0	1 (4.2%)	3 (4.9%)	
	Penetrating	1 (0.6%)	0	0	1 (0.6%)	
Time from trauma (years)		4.6±5.8	4.4±5.9	5.0±5.8	4.6±5.7	P=0.923

Table 1: Baseline patient characteristics (continued).

Characteristics		Total	Mild TBI	Moderate TBI	Severe TBI	Significance
Symptoms						
	Cognitive	100 (86.2%)	38 (74.5%)	19 (90.5%)	43 (97.7%)	**P=0.004**
	Motor	22 (19.0%)	1 (4.8%)	1 (4.8%)	20 (45.5%)	**P<0.0001**
	Sensory	32 (27.6%)	12 (23.5%)	7 (33.3%)	13 (29.5%)	P=0.653
	Dizziness/Vertigo	17 (14.7%)	14 (27.5%)	2 (9.5%)	1 (2.3%)	**P=0.002**
	Tinnitus	30 (25.9%)	26 (51.0%)	2 (9.5%)	2 (4.5%)	**P<0.0001**
	Headaches	20 (17.2%)	12 (23.5%)	3 (14.3%)	5 (11.4%)	P=0.272
HBO sessions		52.0±9.9	49.4±10.1	49.0±10.3	56.1±8.2	**P=0.0001**

HBO protocol (ATA)	1.5	106 (69.3%)	46 (67.6%)	18 (75.0%)	42 (68.9%)	P=0.795
	2	47 (30.7%)	22 (32.4%)	6 (25.0%)	19 (31.1%)	
Adverse Events		18 (12.0%)	10 (15.2%)	3 (12.5%)	5 (8.3%)	P=0.499

Table 2: Cognitive indices pre- and post-HBOT of the entire study cohort

	Baseline	Post HBOT	Mean Change	P-Value
General	88.3±15.2	92.9±14.2	4.6±8.5	**P<0.0001**
Memory	81.7±23.2	89.9±21.9	8.1±16.9	**P<0.0001**
Executive Functions	88.3±16.6	94.2±15.1	5.9±12.0	**P<0.0001**
Attention	84.3±20.5	91.1±18.4	6.8±16.5	**P<0.0001**
IPS	87.5±17.0	92.4±15.7	4.9±13.1	**P<0.0001**

VSP	95.0±18.0	98.5±18.0	3.4±14.6	**P=0.005**
Motor skills	92.3±17.3	96.2±14.5	3.9±11.7	**P<0.0001**

IPS=information processing speed, VSP=visual spatial processing

Table 3: Large significant increases (>10% change) in cognitive indices proportions across TBI groups.

	Total	Mild TBI	Moderate TBI	Severe TBI	
General	36 (23.4%)	15 (21.7%)	7(29.2%)	14 (23.0%)	P=0.756
Memory	64 (41.6%)	28 (40.6%)	9 (37.5%)	27 (44.3%)	P=0.830
Executive functions	51 (33.1%)	23 (33.3%)	7 (29.2%)	21 (34.9%)	P=0.897
Attention	62 (40.3%)	27 (39.1%)	8 (33.3%)	27 (44.3%)	P=0.631
Information processing speed	48 (31.2%)	23 (33.3%)	12 (50%)	13 (21.3%)	**P=0.032**

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Figures legends

Figure-1**:** Patients flowchart.

Figure-2: Imaging findings in the severe TBI group

Figure-3**:** Mean changes of post- compared to pre-HBOT for the entire cohort.

After HBOT, all cognitive domains improved significantly, with the most striking changes seen in memory and attention.

*p<0.0001, **p=0.005, IPS=information processing speed

Figure-4**:** Mean changes of post- compared to pre-HBOT across the different TBI severities.

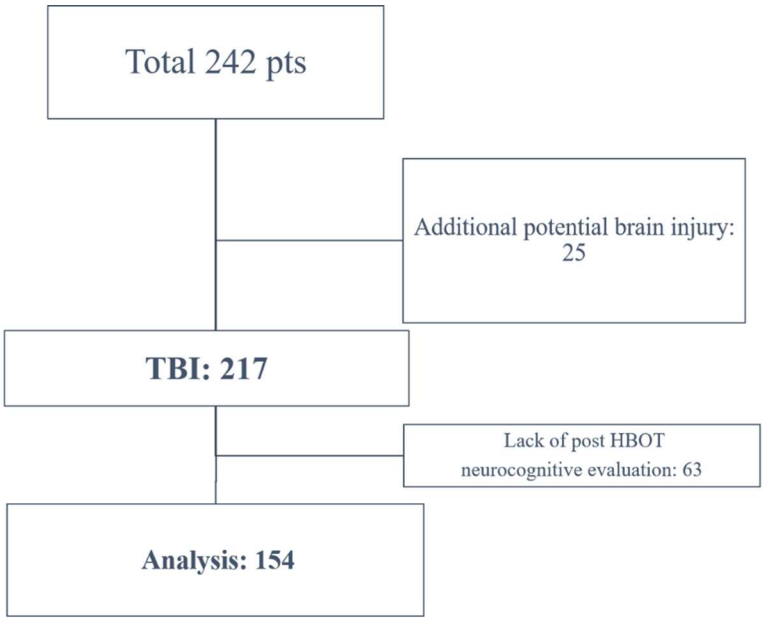
Both patients who suffered mild and severe TBI groups had improvements in general, memory, attention, information processing speed and motor skills scores, whereas patients who suffered moderate TBI had significant improvement in memory.

*p<0.05, IPS =information processing speed

Figure-5**:** The mean relative change in Broadmann areas post HBOT for the entire study cohort.

Figure-6**:** Cognitive functions correlated with Brodmann areas.

Each of the TBI groups(mild, moderate and severe) had perfusion/metabolism increase in specific Brodmann areas correlated with improved cognitive function.



Patients flowchart

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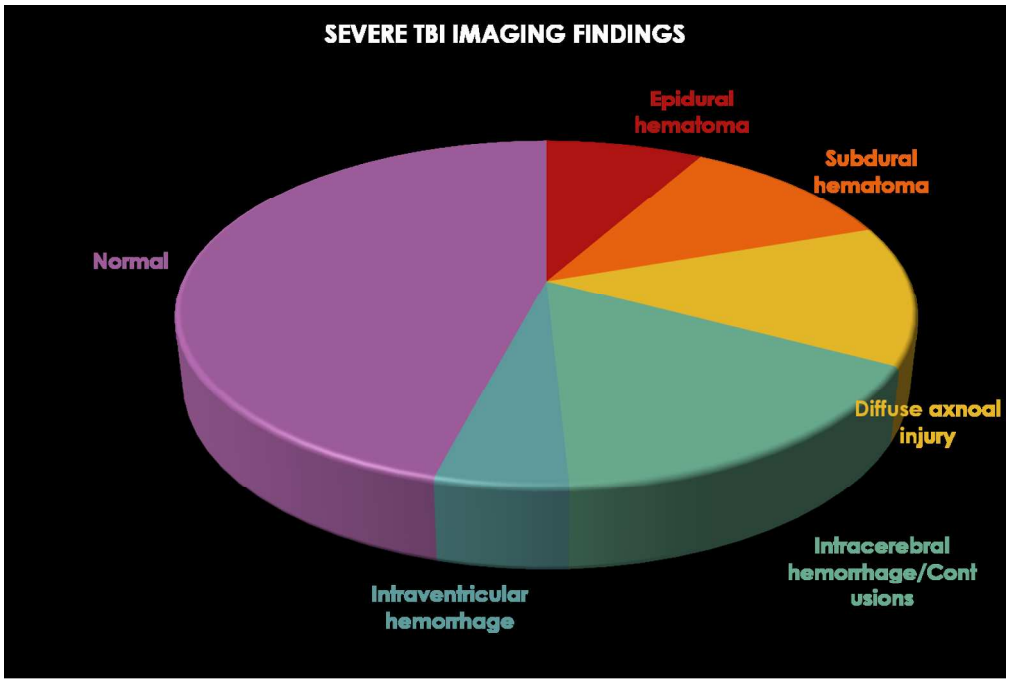

Imaging findings in the severe TBI subgroup

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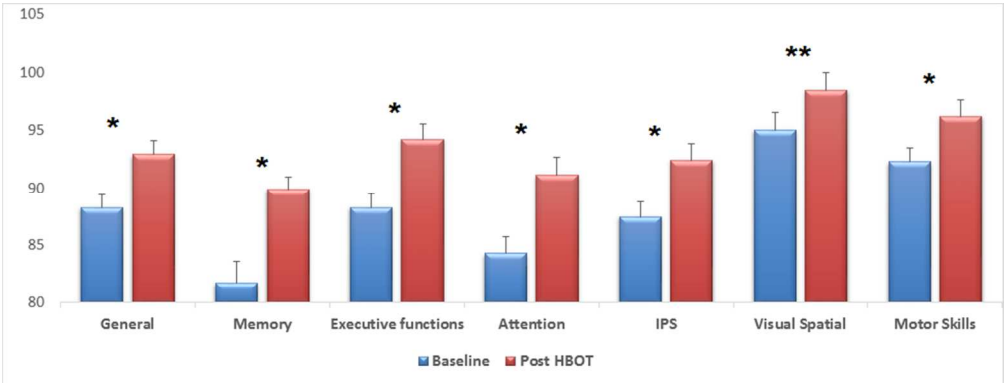

Mean changes of post- compared to pre-HBOT for the entire cohort.
After HBOT, all cognitive domains improved significantly, where the most striking changes were seen in memory and attention.
*p<0.0001, **p=0.005, IPS=information processing speed

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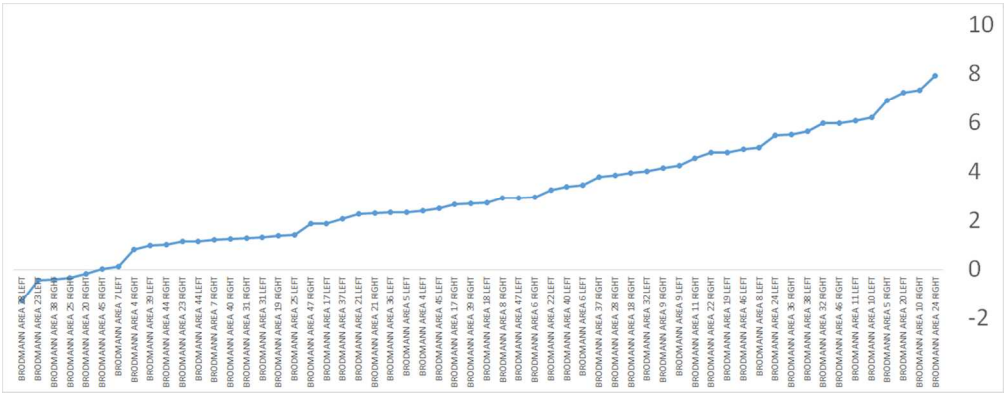

The mean relative change in broadmann areas post HBOT for the entire study cohort

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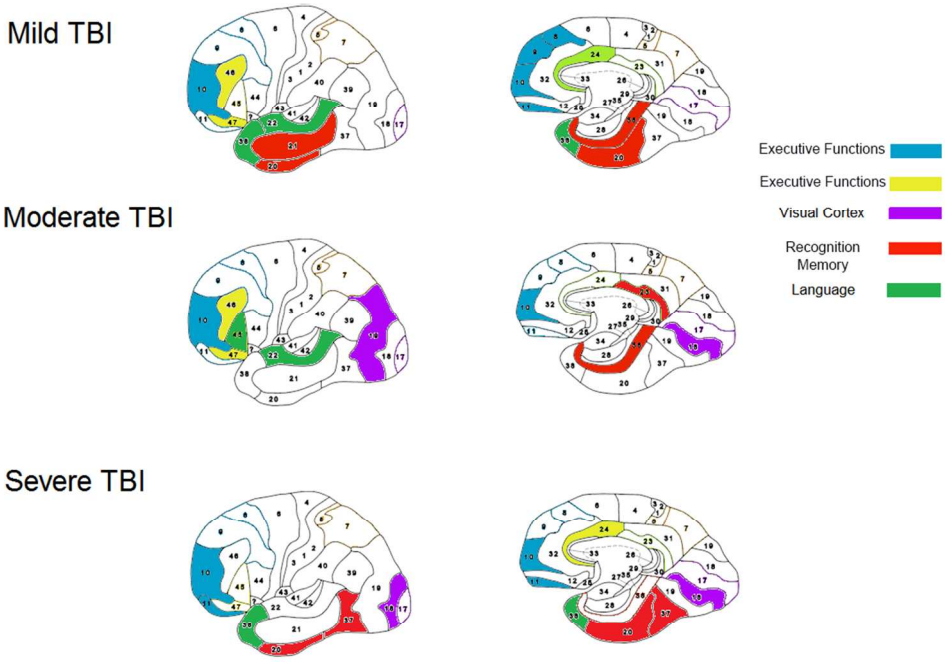

Cognitive functions correlated with Broadmann areas.
Each of the TBI severities (mild, moderate and severe) had perfusion/metabolism increase in specific Broadmann areas correlated with improved cognitive function

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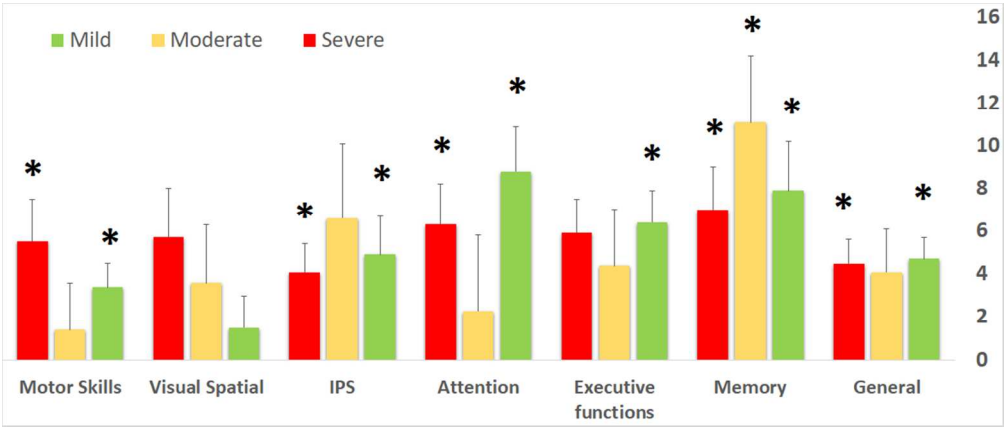

Mean changes of post- compared to pre-HBOT across the different TBI severities.
Both patients who suffered mild and severe TBI groups had improvements in general, memory, attention, information processing speed and motor skills scores, whereas patients who suffered moderate TBI had significant improvement in memory.
*p<0.05, IPS =information processing speed

247x104mm (120 x 120 DPI)

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study Design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key Results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

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BMJ Open

The effect of hyperbaric oxygen therapy on chronic neurocognitive function of deficits post traumatic brain injury patients – retrospective analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-023387.R2
Article Type:	Research
Date Submitted by the Author:	27-Jun-2018
Complete List of Authors:	Hadanny, Amir; Galilee Medical Center, Neuosurgery; Assaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Abbott, Stefanie; Asaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Suzin, Gil; Asaf Harfoeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Bechor, Yair; Asaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research Efrati, Shai; Assaf Harofeh Medical Center, Sagol Center for Hyperbaric Medicine and Research; Tel Aviv University, Sackler School of Medicine
Primary Subject Heading:	Neurology
Secondary Subject Heading:	Rehabilitation medicine
Keywords:	tbi, hbot, traumatic brain injury, hyperbaric oxygen, cognitive

The effect of hyperbaric oxygen therapy on chronic neurocognitive function of deficits post traumatic brain injury patients – retrospective analysis

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Author Disclosure Statement

No competing financial interests exist.

No funding received for this work.

No conflict of interests exists.

Contributorship statement

AH: concept, data collection, data analysis, manuscript draft, manuscript review

SA: data collection, data analysis

GS: data collection, manuscript review

YB: data collection, manuscript review

SE: concept, data analysis, manuscript draft, manuscript review

Data sharing statement

Extra data is available by emailing amir.had@gmail.com

Running title: HBOT improves cognitive function in TBI

Number of references: 43

Word count: 3615

Tables: 3

Figures: 6

Abstract

Objectives

The aim of the study is to evaluate the effect of hyperbaric oxygen therapy (HBOT) in participants suffering from chronic neurological deficits due to traumatic brain injury (TBI) of all severities in the largest cohort evaluated so far with objective cognitive function tests and metabolic brain imaging.

Methods

A retrospective analysis was conducted of 154 patients suffering from chronic neurocognitive damage due to TBI, who had undergone computerized cognitive evaluations pre- and post-HBOT treatment.

Results

The average age was 42.7±14.6 years and 58.4% were males. All patients had documented traumatic brain injury 0.3-33 years (mean 4.6±5.8, median 2.75 years) prior to HBOT. HBOT was associated with significant improvement in all of the cognitive domains, with mean change in global cognitive scores of 4.6±8.5 ($p<0.00001$). The most prominent improvements were in memory index and attention, with mean changes of 8.1±16.9 ($p<0.00001$) and 6.8±16.5 ($p<0.0001$), respectively. The most striking changes observed in brain single photon emission computed tomography (SPECT) images were in the anterior cingulate and the post-central cortex, in the prefrontal areas and in the temporal areas.

Conclusions

In the largest published cohort of patients suffering from chronic deficits post TBI of all severities, HBOT was associated with significant cognitive improvements. The clinical improvements were well correlated with increased activity in the relevant brain areas.

Keywords: hyperbaric oxygen, HBOT, post-concussion, PCS, TBI, traumatic brain injury

Article Summary

Strengths and limitations of the study

- The major limitation relates to its retrospective methodology, however this limitation is diminished by the fact that all patients of the study large cohort were treated at late chronic stages.
- In regards to strengths, objective cognitive assessments using computerized tests (which are superior to any clinical questionnaire), were performed on each patient both pre- and post-treatment.
- The study cohort consisted of a civilian population that does not have any potential secondary gain (such as financial compensation by reporting sick).

Introduction

Traumatic brain injury (TBI) is one of the leading causes of death and disability in the general population.(1) Following TBI, patients may experience a set of symptoms known as post-concussion syndrome (PCS). PCS symptoms include headaches, dizziness, neuropsychiatric symptoms, and cognitive impairments.(2) PCS can continue for weeks or months, and up to 25% of all patients experience prolonged PCS (PPCS) in which the symptoms last for over six months.(3)

In the past years there is growing clinical evidence regarding the effect of hyperbaric oxygen therapy (HBOT) on PCS.(4-6) Unfortunately, the clinical data gathered from those studies can be conflicting due to several inherent procedural issues, such as the use of non-objective end points, the lack of appropriate brain imaging as part of the inclusion criteria, the inappropriate placebo of a hyperbaric environment, and the inclusion of patients that may gain secondary benefits from reporting sick.(4, 5) The current study represents the largest cohort evaluated until now of civilian participants suffering from PCS treated by HBOT, who had undergone objective metabolic brain imaging and a computerized neurocognitive test battery before and after the treatment.

Pathophysiology of PCS and HBOT

The most common pathological mechanism in TBI is diffuse shearing of axonal pathways and small blood vessels, also known as diffuse axonal injury.(7) Secondary pathological mechanisms of TBI include ischemia, mild edema, and other biochemical and inflammatory processes culminating in impaired regenerative and/or healing processes resulting from increasing tissue hypoxia.(8) Due to the diffuse nature of injury, affecting multiple brain areas,(9, 10) cognitive impairments are usually the predominant symptoms.

Global brain hypoperfusion, and its related tissue ischemia, detected in patients suffering from TBI, serves as a rate-limiting factor for any regenerative process.(11-13) By increasing the oxygen level in blood and body tissues, HBOT can augment the repair mechanisms.(14) Various models have strongly suggested that HBOT can induce angiogenesis, improve brain plasticity, enhance neurogenesis and synaptogenesis and foster functional recovery.(15, 16)

Conflicting clinical HBOT data and objective measurements in PCS

Some of the previous studies which evaluated the effect of HBOT on chronic neurological and cognitive impairments due to TBI, mainly used self-assessment questionnaires as their primary endpoints.(17-19) Such endpoints have several inherent disadvantages. First, they lack an objective evaluation that is not biased by the patients' perspectives. Second, self-administrated questionnaires are exposed to various confounding variables such as litigation and compensation.(20). Unlike the questionnaires, standardized cognitive tests with high test-retest reliability can and should be used as objective evaluations of neurocognitive impairments.(21) In addition, novel brain imaging techniques such as single photon emission computed tomography (SPECT) and perfusion sequences in magnetic resonance imaging (MRI), which evaluate cerebral blood flow and brain metabolism, can shed new light in PCS diagnosis and in evaluating therapeutic interventions.(21) In clinical studies which utilized objective cognitive assessments, HBOT was found to induce significant improvements in patients suffering from PCS due to mild TBI. (6, 14, 16, 22) However, to the best of our knowledge, the objective effect of HBOT on chronic neurocognitive impairments stemming from moderate to severe TBI (in addition to mild) has not been investigated.

In addition to objective evaluations, there are inherent ethical and logistic difficulties in handling the sham control in HBOT trials.(4, 5, 21, 23) HBOT includes two active ingredients: pressure and oxygen. Pressure is needed to increase plasma oxygen, but the pressure change alone may also have significant cellular effects.(5) Additionally, the greatest effect of pressure is in human tissues that are under tight autoregulation pressure control, such as the brain, where the intracranial pressure is normally 0.0092-0.0197atm.(24, 25) To generate a pressure sensation, the chamber pressure must be 1.2ATA or higher. However, such a change in environmental pressure (from 1ATA to 1.2 ATA) and subsequent tissue oxygenation (with an increase of tissue oxygenation by at least 50%) has a significant biologic effect (26, 27) Thus, sham therapy in previous studies using 1.2 ATA on 21% inhaled oxygen (i.e., air) cannot be regarded as an inert or sham control but rather as a lower dose of the active ingredient.(4, 21) In regards to a possible effect of vasoconstriction of the large blood vessels induced by hyperbaric oxygen - it has been well established that the tissues are saturated by hyperoxia and do not suffer from hypoxia, as the vasoconstriction effect is compensated by increased plasma oxygen content and microvascular blood flow. (28)

Any increase in pressure, even with reduced oxygen percentage, cannot serve as a true placebo, but rather as a low dosage of the active ingredient, further supporting the need for objective data gathered from large cohorts of patients suffering from PCS and treated by HBOT.

The aim of the current study was to evaluate the objective effects of HBOT on TBI patients suffering from chronic neurological deficits stemming from mild, moderate, and severe TBI, in the largest cohort evaluated until now. Since all the patients had metabolic brain imaging and a computerized neurocognitive test battery before and

after HBOT, correlations between specific cognitive indexes and their related brain regions activity were also evaluated.

Materials and Methods

Participants

A retrospective analysis was conducted on patients suffering from TBI-related chronic neurocognitive damage (more than 3 months from injury), treated by HBOT between January 2008 and January 2017 at the Sagol Center for Hyperbaric Medicine and Research,Assaf Harofeh Medical Center, Israel. Patients were included if they had pre- and post-HBOT computerized cognitive evaluations. Patients with a history of potential additional brain insults, such as spontaneous subarachnoid hemorrhage, anoxic brain injury, or history of prior cognitive impairment, were excluded (Figure-1).

The study was approved by the Institutional Review Board of Asaf Harfoeh Medical Center.

Patients and public involvement

Patients and public weren't involved in the study due to its retrospective nature.

TBI severity

TBI severities were rated according to the TBI admission documents. Mild TBI was defined as loss of consciousness (LOC) with duration of 0–30 minutes, post traumatic amnesia (PTA) with duration of less than a day and a Glasgow coma scale (GCS) grade of 13–15. (29) Moderate TBI was defined as LOC with duration of more than 30 minutes and up to 24 hours, PTA with duration of 1-7 days and GCS grade of 9-12. Severe TBI was defined as LOC with duration of more than 24 hours, PTA with duration of more than seven days, and GCS less than 9. In addition, if there was imaging evidence of an injury such as a hematoma, contusion or hemorrhage, then the TBI was classified as moderate to severe. (29)

Hyperbaric oxygen treatment

Patients were treated with 40-70 daily hyperbaric sessions, 5 days a week. Each session consisted of 60 minutes of exposure to 100% oxygen at 1.5-2 ATA.

Cognitive assessment

The patients' cognitive functions were assessed by NeuroTrax computerized cognitive tests (NeuroTrax Corp., NY).(30) The NeuroTrax tests evaluate various aspects of brain functions and include verbal memory (immediate and delayed recognition), non-verbal memory (immediate and delayed recognition), go/no go response inhibition, problem solving, Stroop interference, finger tapping, catch game, staged information processing speed (single digit, two-digit and three-digit arithmetic), verbal function and visual spatial processing. Cognitive index scores were computed from the normalized outcome parameters for memory, executive function, attention, information processing speed, visual spatial, verbal function and motor skills domains.(31) A global cognitive score was computed as the average of all index scores for each individual.

After administration, the NeuroTrax data were uploaded to the NeuroTrax central server, and outcome parameters were automatically calculated using software blind to diagnosis or testing site. To account for the well-known effects of age and education on cognitive performance, each outcome parameter was normalized and fit to an IQ-like scale (mean=100, S.D.=15) according to the patient's age and education. The normative data used by NeuroTrax consist of test data from cognitively healthy individuals in controlled research studies at more than 10 sites. (32)

Specifically, the patients were given two different versions of the NeuroTrax test battery before and after HBOT, to allow repeated administrations with minimal learning effects. Test-retest reliability for these versions was evaluated and found to

be high, with no significant learning effect.(33, 34) Regarding the current study cohort, in a previous randomized controlled trial in patients suffering from TBI, the NeuroTrax scores were found to be stable in the retest of the control group.(22)

Brain SPECT imaging

Brain activity was assessed using single photon emission computed tomography (SPECT) 1-2 weeks prior to and after the HBOT period. The SPECT method was selected for evaluation due to its known normal range and test/retest established validity. The imaging was conducted using 925–1,110 MBq (25–30 mCi) of a technetium-99-methyl-cysteinate-dimmer (Tc-99m-ECD) at 40–60 min post injection, using a dual detector gamma camera (ECAM or Symbia T, Siemens Medical Systems) equipped with high resolution collimators. Data was acquired in 3-degree steps and reconstructed iteratively using the Chang method of attenuation correction ($\mu = 0.12$/cm).(35)

Both pre- and post-treatment SPECT images were normalized to the median maximal brain activity in the entire brain, and were then reoriented into Talairach space using NeuroGam software (Segami Corporation) to identify Brodmann cortical areas and to compute the mean perfusion in each Brodmann area (BA). In addition, volume-rendered brain perfusion images were reconstructed and normalized to the entire brain median maximal activity. All SPECT analyses were done by study team members who were blinded to the laboratory and clinical data. SPECT scans were performed late morning to midday. On the day of the SPECT scan, patients were treated with only their chronic medications and were instructed not to smoke. Changes in perfusion in all Brodmann areas for each subject were determined by calculating the

percentage of the difference of the normalized activity values between post-treatment and pre-treatment divided by the pre-treatment value.

Statistical Analysis

Continuous data were expressed as means ± standard deviations. The normal distribution for all variables was tested using the Kolmogorov-Smirnov test. The mean differences between cognitive index scores before and after HBOT were analyzed using one-way ANOVA with post-hoc Bonferroni tests. Multiple linear regression models and multivariate logistic regression models were performed to control for potential confounders and to determine independent predictors for clinical outcome. The alpha level was set to 0.05. Data were statistically analyzed using SPSS software (version 22.0).

Results

Patient profiles

Of the 242 patients suffering from neurocognitive impairment due to TBI treated by HBOT between January 2008 and January 2017, 25 patients had potential additional brain insults and 63 did not have repeat computerized neurocognitive evaluations. Therefore, 154 patients were included in the final analysis, of whom 100 patients completed pre- and post-HBOT SPECT imaging (Figure-1).

The patients' baseline characteristics are summarized in Table 1. The average age was 42.7±14.6 years and 58.4% were males. All patients had documented traumatic brain injury 3 months to 33 years (mean 4.6±5.8, median 2.75 years) prior to HBOT. Sixty-nine (44.8%) had neurocognitive impairments due to mild TBI, 24 (15.6%) moderate TBI and 61 (39.6%) severe TBI. Most of the patients (86.2%) complained of cognitive impairment as their main symptom (Table 1).

Patients were treated with 40-70 (mean 52.0±9.9) sessions of hyperbaric oxygen at 1.5-2 ATA. 18 (12%) patients reported adverse events, which included mild barotrauma of the ears and palpitations/dyspnea, while in the chamber.

Severity of TBI

In our cohort, patients who suffered severe TBI were found to be younger with higher proportion of males than in the mild and moderate TBI groups (P<0.0001, P=0.002 respectively, Table 1). As expected, the severe TBI group had significantly higher proportions of cognitive impairment and motor deficits (p=0.004 and p<0.0001 respectively, Table 1). The mild and moderate TBI groups had higher percentages of tinnitus and/or dizziness (p<0.0001, p=0.002 respectively, Table 1).

Severe TBI

Sixty-one patients had severe TBI. The main imaging findings at their admission are summarized in Figure-2. Of those 61 patients, 36 (59%) had surgical intervention during the acute event.

Neurocognitive evaluation

The effect of HBOT on the patients' cognitive functions, as assessed by the eight cognitive summary scores, is summarized in Table 2 and Figure-3. As can be seen, HBOT induced significant improvements in all of the cognitive domains with a mean change of 4.6±8.5 (p<0.00001). The most prominent improvement was in the memory index, with 8.1 ±16.9 (p<0.00001), and in attention, with 6.8 ±16.5 (p<0.0001) (Table 2, Figure-3).

The mild TBI group had the largest improvement in attention (8.8±2.1), followed by memory (7.9±2.3). Patients in the moderate TBI group had noticeable improvements in memory (11.1±3.1), followed by information processing speed (6.6±3.5). Lastly, the severe TBI group had the largest improvement in memory (7.0±2.0), followed by attention (6.3±1.9) (Figure-4). Using ANOVA analysis for repeated measures, there

were no significant differences in all cognitive domains improvements between the different TBI severity groups.

The magnitude of the change in a cognitive score has different implications for patients at low or high baseline levels. Therefore, we further inspected the effect of HBOT on the relative changes, i.e., the changes relative to the baseline value, in each of the cognitive measured indexes. Marked improvements defined as >10% increase compared to baseline cognitive index were found, with different percentages, in all three study groups as summarized in Table 3.

Confounders

Relative change higher than 10% from baseline was considered a significant clinical improvement. Age, gender, education level, TBI severity, the time from injury to HBOT, HBOT protocol and number of sessions had no significant effect on the clinical improvement in the general, memory, attention, information processing speed and executive functions domains ($p>0.05$).

Metabolic imaging of the brain usingSPECT

One hundred patients had brain SPECT evaluations before and after HBOT. When calculating the mean relative change in each cortical Brodmann area for the entire cohort, the largest changes were in the anterior temporal tip areas (BA 38,BA 28, BA 20) and in the prefrontal cortex (BA 10) (Figure-5). However, these changes were minor, in the range of 3-4% relative change. Further analysis per TBI severity group revealed several differences in Brodmann areas with involvement of the perirhinal cortex (BA 36) and the primary visual cortex (BA 18), as seen in Figure-6.

To correlate SPECT imaging and cognitive changes, analysis was performed on the top twenty patients who had the largest cognitive improvement (>10% relative increase from baseline). There was a significantly larger magnitude of metabolism increase (5-8%), compared to the entire cohort average increase (2-4%) ($p<0.05$). The most striking changes were found in the anterior cingulate (BA 24, ($p=0.01$)) and the post-central cortex (BA 5, ($p=0.04$)), in the prefrontal areas (BA 10 ($p=0.04$), BA 11 ($p=0.07$), BA 46 ($p=0.07$) and in the temporal areas (BA 20 ($p=0.02$), BA 38 ($p=0.07$), BA 36 ($p=0.1$)).

Discussion

The present study demonstrates the neurotherapeutic effects of HBOT for chronic TBI of all severities. Even though treatment started during late chronic stages (mean 4.6±5.8 years, median 2.75 years) after the acute insult, HBOT was still found to be effective regardless of the TBI severity. The clinical improvements seen in all cognitive domains were well documented by objective computerized neurocognitive tests. The most significant measurable improvements were in memory, attention and executive function. We found the clinical improvement to be well correlated with increased brain activity in relevant brain areas, with significantly higher increases in patients with better cognitive improvements.

In addition to tissue oxygenation, numerous mechanisms of cellular and vascular repair by HBOT have been suggested in addition to tissue oxygenation.(5, 36) These include improved mitochondrial function and cellular metabolism, improved blood-

brain barrier and inflammatory reactions, reduced apoptosis, alleviation of oxidative stress, increased levels of neurotrophins and nitric oxide, and upregulation of axonal guidance agents.(5, 14, 37) Moreover, the effects of HBOT on neurons can be mediated indirectly by glial cells. HBOT may also promote neurogenesis of endogenous neural stem cells.(14, 37) The common denominator underlying all these mechanisms is that they are oxygen-dependent. HBOT may enable the metabolic change simply by supplying the missing energy/oxygen needed for these regeneration processes. (37) The induction of angiogenesis and improved brain metabolism, as demonstrated in this study, may serve as the infrastructure that enables the regenerative process and the preservation of newly generated neuronal functioning. (15, 36, 38)

The correlation between specific cognitive function improvements with the metabolic brain imaging changes gives further strength to the study results and serves as an excellent tool for gaining better understanding of brain functionality (Figure-6):

- The perirhinal cortex activation after HBOT was most prominent in patients who had significant memory improvement. The perirhinal cortex has a critical role in object recognition memory while interacting with the hippocampus.(39) Since the memory assessments in the cognitive tests were indeed recognition tasks, this area is expected to be involved.
- The pre-frontal cortex (BA 10, BA 11) and more specifically, the inferior frontal gyrus (BA 46, BA 47) activations after HBOT were prominent in all patients with significant executive function improvements. The right frontal gyrus is known to mediate a go/no go

task, (40) which was among the executive function tests used in the present study. The prefrontal gyrus is presumed to act as a filtering system that enhances goal directed activities and inhibits irrelevant activations. This filtering mechanism enables executive control.(41)

- The anterior cingulate gyrus (BA 24) activation after HBOT was seen in the subjects with attention improvement. The anterior cingulate gyrus is presumed to be involved in error detection, especially in a Stroop task,(42) which was used in the attention tests. Lesions in this area can cause inattention to akinetic mutism.(42)

This study has several limitations. The major one relates to its retrospective methodology. This limitation is diminished when considering that this large cohort of patients was treated at late chronic stages. The findings presented here are in agreement and reinforce the findings from previous prospective controlled trials in which the neuroplasticity effects of HBOT were demonstrated in chronic stages of different types of brain injuries.(16, 22, 43, 44) Moreover, the correlation between the changes in cognitive function and the metabolic brain imaging gives further strength to the results.

Another important limitation relates to the HBOT protocol which was inconsistent across the cohort. Although significant neurotherapeutic effects were seen with 60 minutes of 1.5 ATA, the optimal protocol needed to induce maximal neuroplasticity for the specific individual, with minimal side effects has not been investigated.

The strengths of the study are worth mentioning. First, objective cognitive assessments using computerized tests were performed on each patient both pre- and

post-treatment. Objective measures are significantly superior to PCS questionnaires which are inaccurate, variable and contain various confounders rather than reflect the true PCS state.(45) Second, most of the patients in the study underwent an objective ancillary brain SPECT to confirm PCS diagnosis prior to HBOT. This practice is crucial when considering the differential diagnosis following TBI (PTSD, depression, etc.).

Moreover, post-treatment brain SPECTs revealed an anatomical-functional correlation in regards to HBOT's effect in brain neuroplasticity. Third, the study cohort consisted of a civilian population that does not have any potential secondary gain (such as financial compensation) by reporting sick.

Previous studies included post-concussion syndrome patients who suffered mild TBI injury. Considering its strengths and limitations, the current study implies that the cognitive function of patients post TBI, can be improved significantly, irrespectively of whether the primary brain injury was classified as mild, moderate or severe. Although long term data is still lacking, considering the high safety profile of the treatment, these results are promising and should encourage rehabilitation centers to consider HBOT for patients with chronic neurocognitive deficits following TBI. Future studies should monitor these patients in the long term (6 months, 12 months) as well as their return to activities of daily living.

Conclusions

HBOT was associated with significant cognitive improvements in patients who suffer from chronic neurocognitive deficits due to mild, moderate and severe TBI. Improvement in memory correlated with activation of the perirhinal cortex, improvement of executive functions correlated with activation of the inferior frontal

gyrus, and improvement in attention correlated with activation of the anterior cingulate gyrus.

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Table 1: Baseline patient characteristics. 595

Characteristics		Total	Mild TBI	Moderate TBI	Severe TBI	Significance
Number of patients		154 (100%)	69 (44.8%)	24 (15.6%)	61 (39.6%)	
Age (years)		42.7±14.6	48.8±12.0	41.7±12.7	36.2±15.3	**P<0.0001**
Sex						
	Males	90 (58.4%)	31 (44.9%)	13 (54.2%)	46 (75.4%)	**P=0.002**
	Females	64 (41.6%)	38 (55.1%)	11 (45.8%)	15 (24.6%)	
Education (years)		14.8±3.3	14.9±3.6	14.9±3.3	14.6±3.1	P=0.895
Traumatic event						

	Motor Vehicle Accident	116 (75.3%)	56 (81.2%)	17 (70.8%)	43 (70.5%)	**P=0.048**
	Fall	21 (13.6%)	8 (11.6%)	1 (4.2%)	12 (57.1%)	
	Blow	12 (7.8%)	5 (7.2%)	5 (20.8%)	2 (3.3%)	
	Blast	4 (2.6%)	0	1 (4.2%)	3 (4.9%)	
	Penetrating	1 (0.6%)	0	0	1 (0.6%)	
Time from trauma (years)		4.6±5.8	4.4±5.9	5.0±5.8	4.6±5.7	P=0.923

Table 1: Baseline patient characteristics (continued).

Characteristics		Total	Mild TBI	Moderate TBI	Severe TBI	Significance
Symptoms						
	Cognitive	100 (86.2%)	38 (74.5%)	19 (90.5%)	43 (97.7%)	**P=0.004**
	Motor	22 (19.0%)	1 (4.8%)	1 (4.8%)	20 (45.5%)	**P<0.0001**
	Sensory	32 (27.6%)	12 (23.5%)	7 (33.3%)	13 (29.5%)	P=0.653
	Dizziness/Vertigo	17 (14.7%)	14 (27.5%)	2 (9.5%)	1 (2.3%)	**P=0.002**
	Tinnitus	30 (25.9%)	26 (51.0%)	2 (9.5%)	2 (4.5%)	**P<0.0001**
	Headaches	20 (17.2%)	12 (23.5%)	3 (14.3%)	5 (11.4%)	P=0.272
HBO sessions		52.0±9.9	49.4±10.1	49.0±10.3	56.1±8.2	**P=0.0001**

HBO protocol (ATA)	1.5	106 (69.3%)	46 (67.6%)	18 (75.0%)	42 (68.9%)	P=0.795
	2	47 (30.7%)	22 (32.4%)	6 (25.0%)	19 (31.1%)	
Adverse Events		18 (12.0%)	10 (15.2%)	3 (12.5%)	5 (8.3%)	P=0.499

Table 2: Cognitive indices pre- and post-HBOT of the entire study cohort

	Baseline	Post HBOT	Mean Change	P-Value
General	88.3±15.2	92.9±14.2	4.6±8.5	**P<0.0001**
Memory	81.7±23.2	89.9±21.9	8.1±16.9	**P<0.0001**
Executive Functions	88.3±16.6	94.2±15.1	5.9±12.0	**P<0.0001**
Attention	84.3±20.5	91.1±18.4	6.8±16.5	**P<0.0001**
IPS	87.5±17.0	92.4±15.7	4.9±13.1	**P<0.0001**

VSP	95.0±18.0	98.5±18.0	3.4±14.6	**P=0.005**
Motor skills	92.3±17.3	96.2±14.5	3.9±11.7	**P<0.0001**

IPS=information processing speed, VSP=visual spatial processing

Table 3: Large significant increases (>10% change) in cognitive indices proportions across TBI groups.

	Total	Mild TBI	Moderate TBI	Severe TBI	
General	36 (23.4%)	15 (21.7%)	7(29.2%)	14 (23.0%)	P=0.756
Memory	64 (41.6%)	28 (40.6%)	9 (37.5%)	27 (44.3%)	P=0.830
Executive functions	51 (33.1%)	23 (33.3%)	7 (29.2%)	21 (34.9%)	P=0.897
Attention	62 (40.3%)	27 (39.1%)	8 (33.3%)	27 (44.3%)	P=0.631
Information processing speed	48 (31.2%)	23 (33.3%)	12 (50%)	13 (21.3%)	**P=0.032**

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Figures legends

Figure-1**:** Patients flowchart.

Figure-2: Imaging findings in the severe TBI group

Figure-3**:** Mean changes of post- compared to pre-HBOT for the entire cohort.

After HBOT, all cognitive domains improved significantly, with the most striking changes seen in memory and attention.

*p<0.0001, **p=0.005, IPS=information processing speed

Figure-4**:** Mean changes of post- compared to pre-HBOT across the different TBI severities.

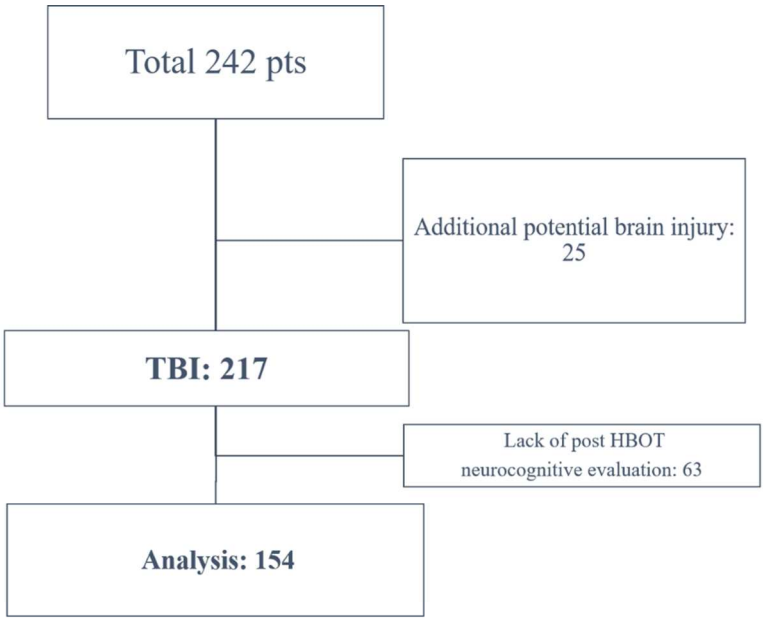
Both patients who suffered mild and severe TBI groups had improvements in general, memory, attention, information processing speed and motor skills scores, whereas patients who suffered moderate TBI had significant improvement in memory.

*p<0.05, IPS =information processing speed

Figure-5**:** The mean relative change in Broadmann areas post HBOT for the entire study cohort.

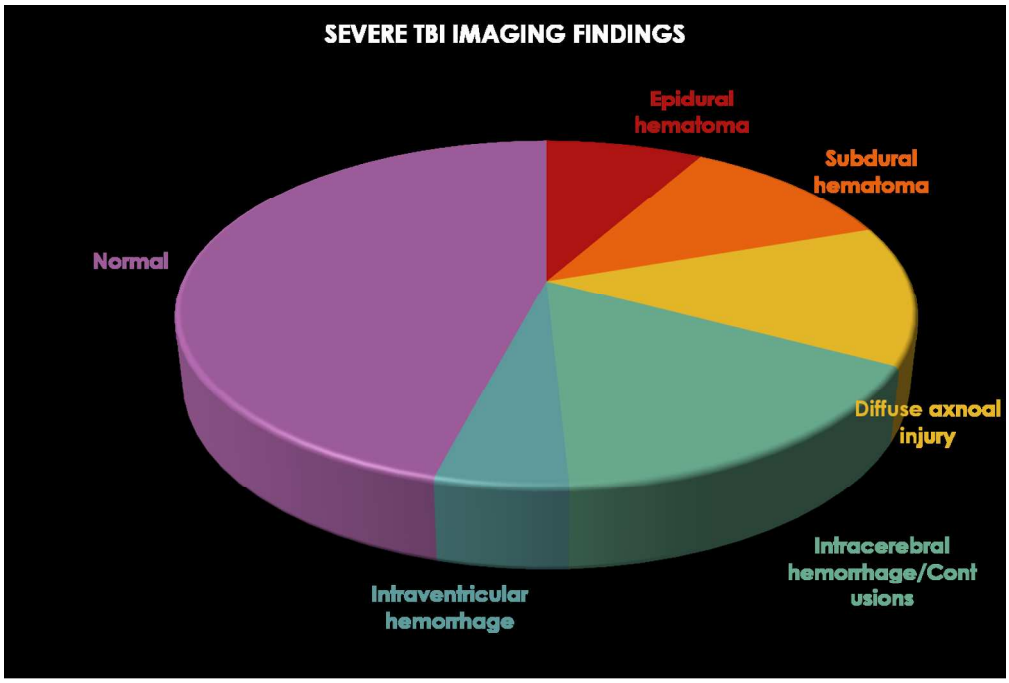
Figure-6**:** Cognitive functions correlated with Brodmann areas.

Each of the TBI groups(mild, moderate and severe) had perfusion/metabolism increase in specific Brodmann areas correlated with improved cognitive function.



Patients flowchart

334x232mm (300 x 300 DPI)



Imaging findings in the severe TBI subgroup

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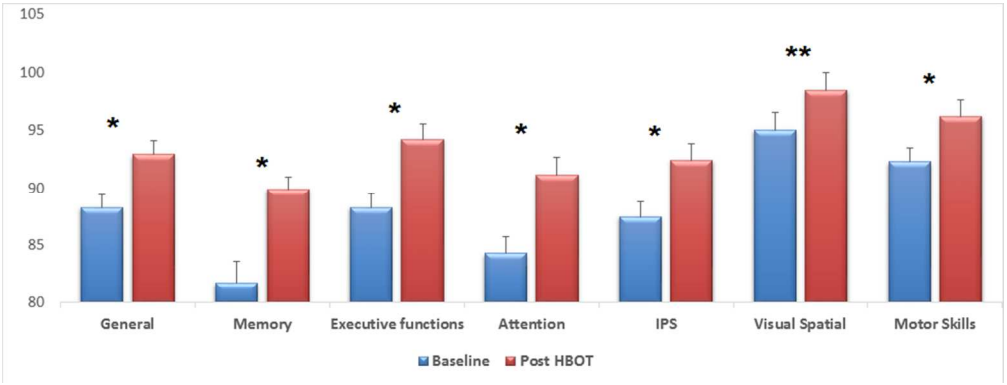
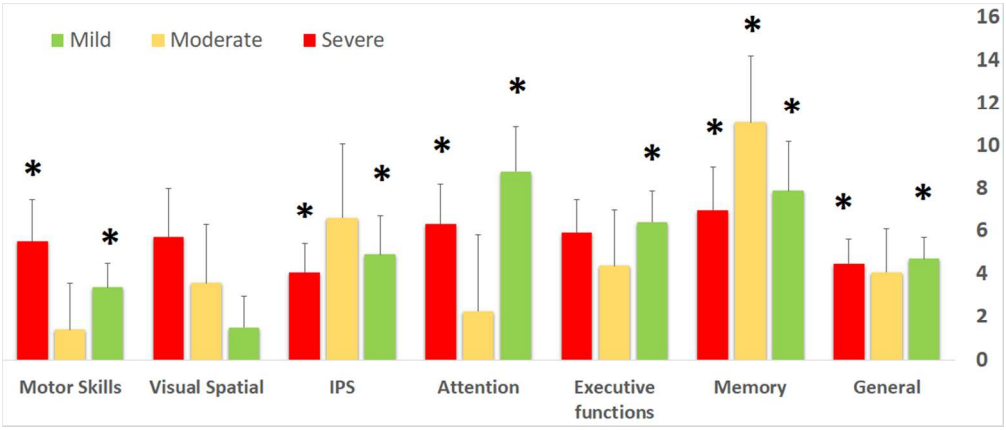


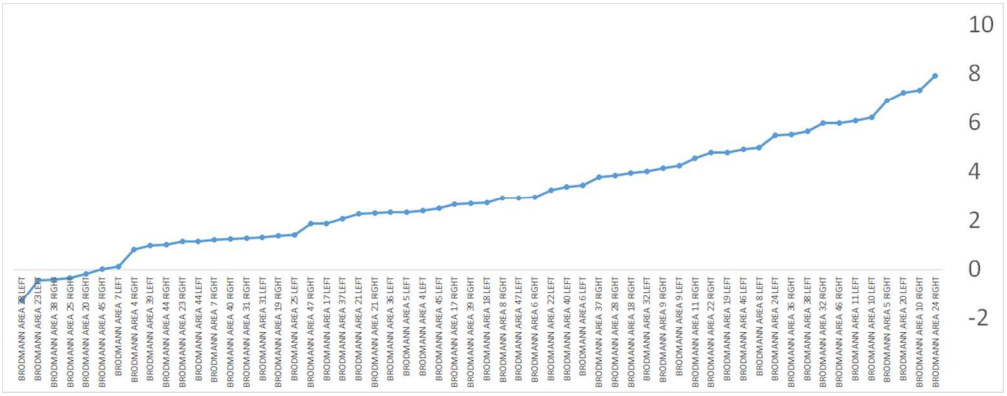
Mean changes of post- compared to pre-HBOT for the entire cohort.
After HBOT, all cognitive domains improved significantly, where the most striking changes were seen in memory and attention.
*p<0.0001, **p=0.005, IPS=information processing speed

204x77mm (300 x 300 DPI)



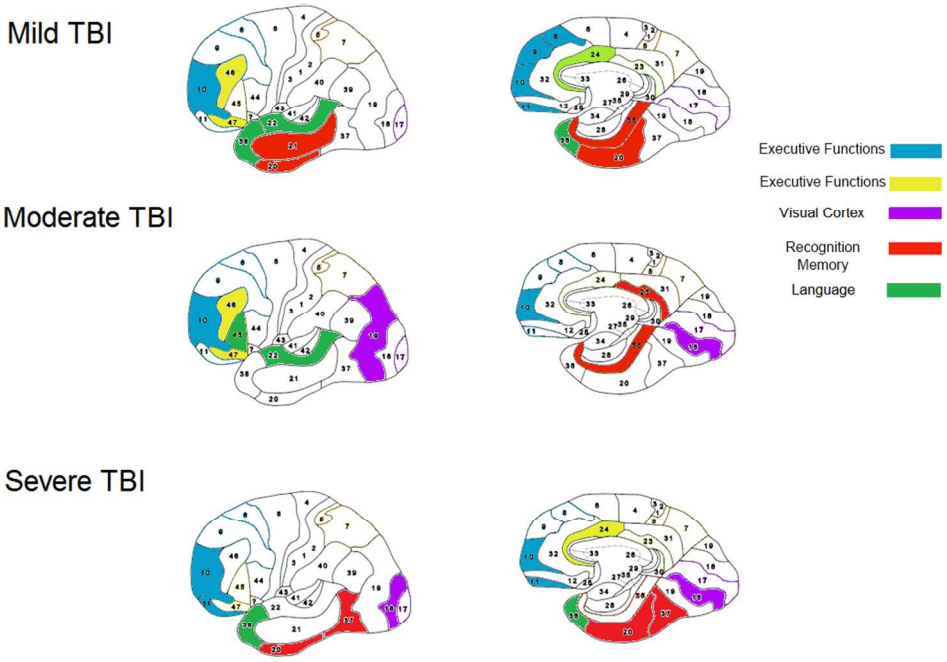
Mean changes of post- compared to pre-HBOT across the different TBI severities.
Both patients who suffered mild and severe TBI groups had improvements in general, memory, attention, information processing speed and motor skills scores, whereas patients who suffered moderate TBI had significant improvement in memory.
*p<0.05, IPS =information processing speed

247x104mm (120 x 120 DPI)



The mean relative change in broadmann areas post HBOT for the entire study cohort

261x101mm (300 x 300 DPI)



Cognitive functions correlated with Broadmann areas.
Each of the TBI severities (mild, moderate and severe) had perfusion/metabolism increase in specific Broadmann areas correlated with improved cognitive function

240x173mm (300 x 300 DPI)

STROBE (Strengthening The Reporting of OBservational Studies in Epidemiology) Checklist

A checklist of items that should be included in reports of observational studies. You must report the page number in your manuscript where you consider each of the items listed in this checklist. If you have not included this information, either revise your manuscript accordingly before submitting or note N/A.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Section and Item	Item No.	Recommendation	Reported on Page No.
Title and Abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	
Introduction			
Background/Rationale	2	Explain the scientific background and rationale for the investigation being reported	
Objectives	3	State specific objectives, including any prespecified hypotheses	
Methods			
Study Design	4	Present key elements of study design early in the paper	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	

Section and Item	Item No.	Recommendation	Reported on Page No.
Data Sources/ Measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	
Bias	9	Describe any efforts to address potential sources of bias	
Study Size	10	Explain how the study size was arrived at	
Quantitative Variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical Methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive Data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome Data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	

Section and Item	Item No.	Recommendation	Reported on Page No.
Main Results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other Analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	
Discussion			
Key Results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other Information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Once you have completed this checklist, please save a copy and upload it as part of your submission. DO NOT include this checklist as part of the main manuscript document. It must be uploaded as a separate file.