ORIGINAL ARTICLE

Hypertonic saline solution reduces the oxidative stress responses in traumatic brain injury patients

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Background: Oxidative stress processes play an important role in the pathogenesis of secondary brain injury after traumatic brain injury (TBI). Hypertonic saline (HTS) has advantages as being preferred osmotic agent, but few studies investigated oxidant and antioxidant effects of HTS in TBI. This study was designed to compare two different regimens of HTS 5% with mannitol on TBI-induced oxidative stress. **Materials and Methods:** Thirty-three adult patients with TBI were recruited and have randomly received one of the three protocols: 125 cc of HTS 5% every 6 h as bolus, 500 cc of HTS 5%as infusion for 24 h or 1 g/kg mannitol of 20% as a bolus, repeated with a dose of 0.25-0.5 g/kg every 6 h based on patient's response for 3 days. Serum total antioxidant power (TAP), reactive oxygen species (ROS) and nitric oxide (NO) were measured at baseline and daily for 3 days. **Results:** Initial serum ROS and NO levels in patients were higher than control(6.86± [3.2] vs. 1.57± [0.5] picoM, $P = 0.001$, 14.6± [1.6] vs. 7.8± [3.9] mM, $P = 0.001$, respectively). Levels of ROS have decreased for all patients, but reduction was significantly after HTS infusion and mannitol (3. 08 [±3.1] to 1.07 [±1.6], $P = 0.001$, 5.6 [±3.4] to 2.5 [±1.8], $P = 0.003$ respectively). During study, NO levels significantly decreased in HTS infusion but significantly increased in mannitol. TAP Levels had decreased in all patients during study especially in mannitol ($P = 0.004$). **Conclusion:** Hypertonic saline 5% has significant effects on the oxidant responses compared to mannitol following TBI that makes HTS as a perfect therapeutic intervention for reducing unfavorable outcomes in TBI patients.

Key words: Hypertonic saline, mannitol, oxidative stress response, traumatic brain injury patients

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INTRODUCTION

Traumatic brain injury (TBI) is an important cause of morbidity and mortality throughout the world and is associated with expending a tremendous amount of resources in healthcare systems. The importance of the problem is more noticeable in low and middle income countries which carry a higher degree of risk factors for TBI and on the other hand their healthcare systems are inadequately equipped to deal with the associated health outcomes.[1]

Traumatic brain injury results in functional deficits due to both primary and secondary mechanisms. Now there is general agreement that there may be two kinds of injury during the pathophysiological course of head trauma: Primary and secondary. Primary injuries are the result of direct physical force to the brain and usually are not reversible whereas secondary brain injuries are potentially responsive to prevention or reversal.[2] This kind of brain injury is caused by a dynamic interplay between ischemic, inflammatory and cytotoxic processes.[3] The second stage of the pathophysiological cascade is marked by terminal membrane depolarization along with excessive release of excitatory neurotransmitters (i.e. glutamate, aspartate), activation of N-methyl-D-aspartate, a-amino-3-hydroxy-5-methyl-4 isoxazolpropionate, and voltage-dependent Ca^{2+} and Na^{+} channels. The resultant Ca^{2+} and Na^{+} influx initiate self-digesting intracellular processes. Ca^{2+} activates lipid peroxidases (Px), proteases, and phospholipases which then increase the intracellular concentration of free fatty acids and free radicals. These series of events ends in membrane destruction of vascular and cellular structures and finally cause necrosis or programmed cell death.[3-7]

The time between primary and secondary injury provides a window for therapeutic intervention

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to reduce unfavorable outcomes. In the past decades tremendous efforts have been made for illuminating mechanisms responsible for cerebral injuries happen after TBI. Unfortunately, these efforts did not translate into more successful therapeutic interventions for decreasing progressive damage after TBI in a considerable amount. This is in part due to complex and multi-factorial entity of these pathologic mechanisms. Furthermore, it seems that there are important gaps in our knowledge about pathogenesis of harmful events after TBI.[8]

Nonsurgical management of increased intracranial pressure (ICP) due to cerebral edema is mainly based on osmotic therapy. Mannitol and hypertonic saline (HTS) have been widely used and tested as preferred agents for hyperosmolar therapy.[9] Historically mannitol has been used more often as an agent of choice for this purpose. However in recent years, some studies provided more evidence supporting the superiority of HTS in decreasing ICP and brain edema both in animal models and humans.[10-12]

Evidences derived from laboratory investigations have shown a link between the effects of hypertonicity and the innate and adaptive immune responses, which could produce the dysfunctional inflammatory and oxidant responses, posttraumatic injury. These studies suggest that the immunomodulatory effects of hypertonic fluids may have an attenuating effect of organ injury and immune suppression seen after severe injury.[13] Still there is inadequate knowledge regarding safety, optimum duration and dose of HTS in TBI patients. Bolus administration of HTS resulted in the acute increase in serum sodium concentrations which were associated with the rapid reduction of ICP and increased in cerebral perfusion pressure.[14] However, continuous infusion of HTS have increased serum sodium gradually and maintained it for a large period.[15,16] Although to our knowledge, few studies investigated HTS and mannitol with respect to their antioxidant and antiinflammatory properties.[17] This prospective controlled study was designed to evaluate the oxidant and antioxidant effects of mannitol and two different regimens of HTS 5% in TBI patients.

METHODS

This open label randomized clinical trial (clinical registration ID: 201011055107N1), which conducted at three Intensive Care Units (ICUs) in Iran (between October 2009 and May 2011), was approved by the Ethic Committee of Tehran University of Medical Sciences and health services and all patients' relatives gave written informed consent before participating in the study.

Hundred patients were registered into the trial during the study only 39 subjects met the inclusion criteria. Subjects were included in the study if they were aged between 18 and 65 years and had Glasgow coma scale (GCS) ≤12, closed head trauma and evidence of brain edema on head computed tomography (CT) scan (e.g. sulci effacement, hypodensity surrounding discrete brain lesions in structures associated with consciousness, abnormal diffuse white matter lucency, lateral shift of midline structure), serum sodium 130-160 meq/L, serum osmolality <350 mOsmol/kg and not pregnant.

Patients who developed acute renal failure[18] during the study (defined as an abrupt (within 48 h) absolute increase in the serum creatinine concentration of ≥0.3 mg/dL from baseline, a percentage increase in the serum creatinine concentration of ≥50%, or oliguria of <0.5 mL/kg/h for >6 h), hepatic failure[19] (alanine transaminase, aspartate aminotransferase >5 upper limit normal or cirrhosis) before or during the study, heart failure[20] (ejection fraction <40%), shock[2] (mean arterial pressure [MAP] ≤60 mmHg), and pulmonary edema[2] (central venous pressure [CVP] >15 mmHg) were excluded from the study.

All patients were managed in the ICU based on the brain trauma foundation TBI guideline.[2] They were intubated and received mechanically ventilation with a head elevation of 300. Volume resuscitation was achieved with 0.9% normal saline for a target CVP of 8-12 mmHg. After adequate fluid resuscitation, MAP was kept above 90 mmHg. Sedation and analgesia were provided for all patients, using continuous infusion of midazolam and morphine to maintain good analgesic control and sedation. Insulin treatment was administered to maintain glucose at <200 mg/dL.[2]

Three milliliters venous blood samples were collected in heparinized tubes from central venous catheter at baseline and following 3 days of treatment on a certain time daily. Each sample was centrifuged (3500 × *g*) for 15 min then serum separated and stored at −80°C for further analysis.

Measurement of total antioxidant power (TAP)

Antioxidant power of plasma was determined by measuring its ability to reduce Fe^{3+} to Fe^{2+} established as the ferric-reducing antioxidant power (FRAP) test. The reagents included 300 mM acetate buffer (pH 3.6) with 16 mL acetic acid per liter of buffer solution, 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mMHCl and 20 mM $FeCl_3$.

Working FRAP reagent was prepared as required by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and 2.5 mL $FeCl_3$ solution. Ten micro liters of H_2O diluted sample was then added to 300 mL freshly prepared reagent warmed at 370°C. The complex between Fe_2 and TPTZ gives a blue color with absorbance at 593 nm.[21]

Measurement of reactive oxygen species

This assay was performed using methods as described by Mostafalou *et al*.,[22] with slight modifications. Levels of serum reactive oxygen species (ROS) were measured by using 2, 7- dichlorodihydrofluorescein-diacetate (DCFH). 50 µl Sample were mixed with 10 µl DCFH (final concentration 1 µM), then incubated at 37°C for 15 min in the dark. The rate of oxidation from DCFH to dichlorofluorescein is indicative of oxidant production that is read in the excitation wavelength of 488 nm and emission wavelength of 525 nm using a ELISA reader fluorescence spectrometer.

Measurement of nitric oxide

Serum nitric oxide (NO) was analyzed using commercially available Griess Reagent System (Promega, Madison, USA) according to the manufacturer instruction. This assay is based on NO concentration determination using sulfanilamide and N-1-napthylethylenediamine dihydrochloride under acidic (phosphoric acid) conditions which absorbed light was read at 550 nm with an ELISA reader.

Measurements

For each patient a set of variables included demographics (age, gender), admitting neurological diagnosis, initial GCS and Acute Physiology and Chronic Health Evaluation (APACHE) II[23] score and Sequential Organ Failure Assessment (SOFA) [24] score were collected on a standardized form.

The following parameters were assessed at baseline and daily for 3 days. Temperature, creatinine, blood urea nitrogen, glucose, hematocrit, hemoglobin, platelet, white blood cell count, MAP, CVP, heart rate, pupillary reaction (normal, unilaterally or bilaterally abnormal), blood osmolality, electrolytes, arterial blood gas, and PH. Serum sodium was checked every 6 h and the treatment was stopped if sodium reached above 155 meq/L. Serum osmolality was measured by osmomat 030 (Gonotec, Berlin, Germany). SOFA score and GCS were assessed daily for 3 days. APACHE II was assessed at the time of admission to the ICU.

Patients who met the inclusion criteria entered the study and randomized into 3 treatment groups using six block randomization.

In group A, mannitol 20% (Samen, Iran), 1 g/kg was administered over 20 min via central venous catheter and repeated with a dose of 0.25-0.5 g/kg every 6 h based on patient response (defined by GCS and CT improvement and serum osmolarity) for 3 days.[2,25]

Second group (B), received 125 cc HTS 5% (Samen, Iran), over an hour via central venous catheter every 6 h for 3 days. And in the third group (C) 500 cc HTS 5%was continuously infused over 24 h for 3 days.

A group of healthy volunteers (n = 30) without any psychiatric and neurologic disorders history were assessed for establishment of normal serum levels of ROS, total antioxidant power (TAP) and NO.

All data were assessed for normality by one sample Kolmogorov–Smirnov test. Qualitative variables were recorded by frequency and percentage and quantitative variables by mean ± standard deviation. Qualitative variables were compared by Fisher's exact test. When was appropriate, ANOVA and Kruskal–Wallis test were used for comparing quantitative variables in three groups, and Mann–Whitney U-test was used for comparing quantitative variables in two groups. Repeated measurement analysis was conducted for serial comparisons of biomarker concentration and quantitative variables and comparisons between groups in different times of treatment; pairwise comparison was applied by Scheffe. All statistical analysis were conducted using SPSS version 11.5 and 13 (SPSS Inc., Chicago, IL, USA) and $P < 0.05$ was considered as significant.

RESULTS

Thirty-nine consecutive patients with moderate and severe TBI were assessed, 6 of them were ineligible, and one in the mannitol group died after 3 doses. Among patients received HTS 5% one in bolus, and one in infusion group were misdiagnosed and 2 patients in bolus and one in infusion group were received 2 doses of mannitol instead of HTS. From 33 remaining patients, 10 of them received mannitol (group A), 11 patients received HTS as a bolus (group B) and 12 patients as a continuous infusion of HTS (group C) [Figure 1]. Baseline demographics and clinical characteristics are shown in Table 1. There was no significant difference between patient groups except for gender.

The mean serum concentration of ROS was 1.57 ± 0.5 picoM for the control group. As compared to the healthy control group, TBI patients had significantly higher initial serum levels of ROS at ICU admission (P = 0.001) [Table 2]. Following intervention, the levels of ROS decreased significantly in all groups (P = 0.01). The evaluation of each group showed that this reduction was significant for infusion part of HTS (P = 0.001) and mannitol (P = 0.003) [Table 2].

The mean serum concentration of NO was 7.8 ± 3.9 mM for the control group. As compared to the healthy control group, TBI patients had significantly higher initial serum levels of NO at ICU admission (P = 0.001) [Table 2]. During study period, the serum levels of NO significantly decreased in infusion part of HTS treatment groups but significantly increased in the mannitol group (respectively P = 0.002, P = 0.02) [Table 2].

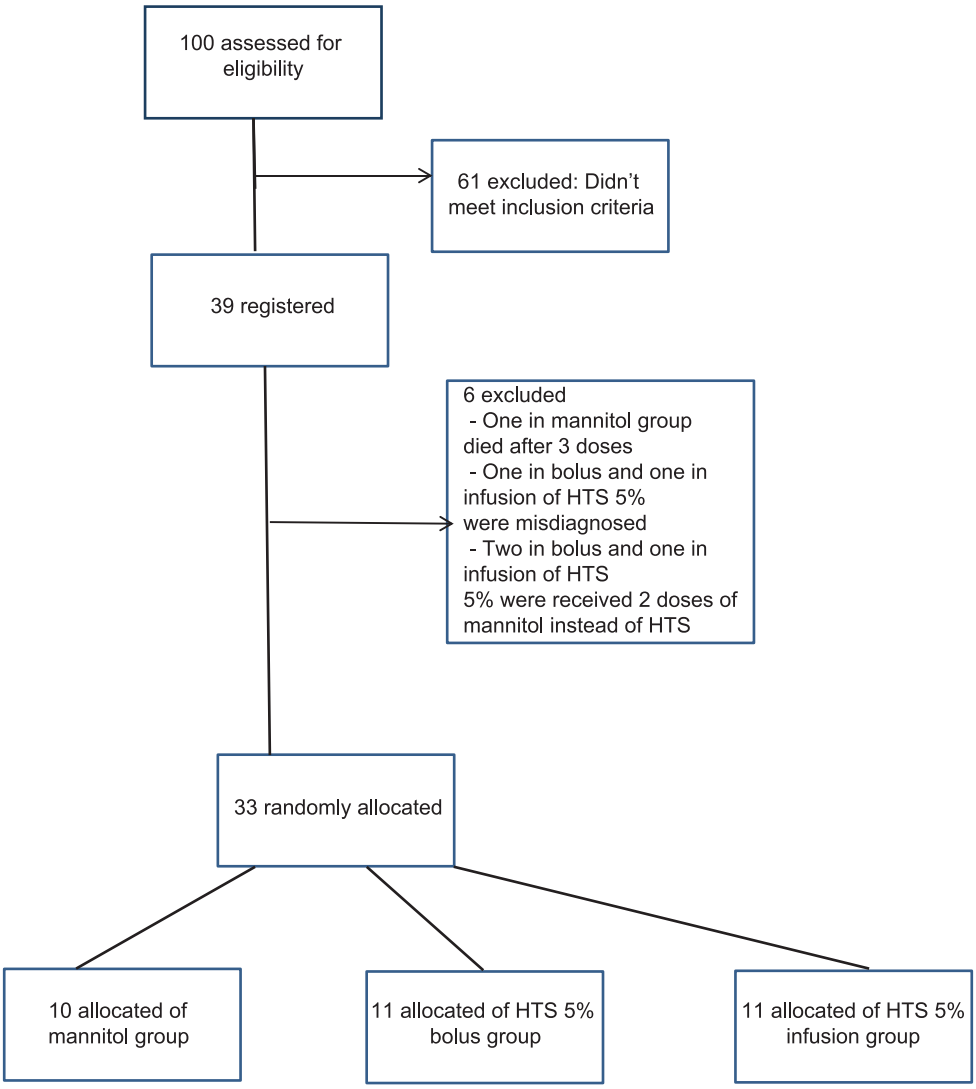

Figure 1: Trial flow diagram

The mean serum concentration of TAP was 236.2 ± 114.2 μM for the control group, and there was a significant difference between initial serum levels of TAP at ICU admission in TBI patients and the healthy control group ($P = 0.018$) [Table 2]. The serum levels of TAP had decreased in all treatment groups during study period ($P = 0.004$). The assessment of each group determined that serum TAP significantly decreased in mannitol group ($P = 0.004$) [Table 2].

DISCUSSION

In this study, the effects of mannitol and HTS 5% on oxidative stress due to TBI were compared. Using HTS 5% were accompanied by a higher degree of reduction in ROS and NO levels with respect to mannitol, which in turn could results in better cell protection against unfavorable effects due to oxidative stress. In addition, we observed that the serum level of TAP decreased in all treatment groups during study period. This could imply that following TBI, increased production of ROS and NO surpass the antioxidant power of the body. However, only in the mannitol group this reduction was significant.

Searching previous studies reveals that there is not enough number of well-controlled clinical trials that could provide evidence for the best concentration, administration interval and duration of therapy with HTS. Although numerous studies on TBI have been conducted, most of these investigations are observational or retrospective, which could not provide strong evidence for the quantitative evaluation of the effect of HTS on clinical outcomes.[26] To our knowledge, this was the first study which compared oxidant and antioxidant effects of both methods of administering HTS (bolus and continuous infusion) versus mannitol in TBI patients.

Posttraumatic cerebral inflammation is described by glial activation, leukocyte recruitment, and up-regulation and

Table 1: Baseline demographic and clinical characteristics of TBI patients

	Mannitol	Bolus of HTS	Infusion of HTS
Age (year)	34.2 (±9)	33.6 (±13.05)	40.58 (±16)
Gender, *n* (% male)[a,*]	6 (60)	11 (100)	11 (92)
Mechanism of injury, *n* (%)			
Car accident	4 (40)	5 (45.5)	5 (41.7)
Motor accident	5 (50)	4 (36.4)	3 (25)
Falling	1 (10)	1 (9.1)	4 (33.3)
Electricity insult	0 (0)	1 (9.1)	0 (0)
Initial GCS	6.5 (±3.3)	8.1 (±2.1)	6.4 (±1.5)
Initial SOFA	6.7 (±2.2)	6.5 (±1.5)	6.5 (±2.4)
Initial APACHE II	14.6 (±5.4)	12.18 (±5.9)	17.08 (±4.6)
Initial ROS (picoM)	5.6 (±3.4)	11.9 (±3.3)	3.08 (±3.1)
Initial NO (mM)	12.17 (±1.8)	14.03 (±1.7)	16.9 (±1.6)
Initial TAP (μM)[b,*]	331.8 (±30.07)	348.07 (±28.6)	216.5 (±27.4)
Initial serum Na^+ (mEq/l)	138 (±3.06)	141.55 (±7.6)	142.33 (±7.9)
Initial serum osmolality (mOsml/kg)	310 (±18.73)	307.82 (±16.8)	302.42± (±20.48)
Initial MAP (mmHg)	85 (±7.2)	85.45 (±14.5)	84.16 (±5.4)
Morbidity *n* (%)			
Sepsis	3 (30)	0 (0)	2 (17)
MOF	3 (30)	0 (0)	1 (8)
Seizure	1 (10)	0 (0)	0 (0)

[a,*]*P*=0.002, [b,*]*P*=0.01, *P*<0.05 considered significant, mean (±SD). TBI=Traumatic brain injury; HTS=Hypertonic saline; GCS=Glasgow coma scale; APACHE II=Acute physiologic and chronic health evaluation; SOFA=Sequential organ failure assessment; MOF=Multi organ failure; Na^+=Sodium; MAP=Mean arterial pressure; NO=Nitric oxide; ROS=Reactive oxygen species; TAP=Total antioxidant power; SD=Standard deviation

Table 2: Serum ROS, NO and TAP concentrations at baseline and during the study in treatment groups

	Mannitol	Bolus of HTS	Infusion of HTS
ROS (picomol/l)			
Baseline	5.6 (±3.4)	11.9 (±3.3)	3.08 (±3.1)
The 1th day	3.7 (±2.4)	7.3 (±2.3)	2.2 (±2.2)
The 2th day	3.1 (±1.9)	5.7 (±1.9)	1.5 (±1.8)
The 3th day	2.5 (±1.8)	4.9 (±1.7)	1.07 (±1.6)
P	0.003*	0.1	0.001*
NO (mmol/l)			
Baseline	12.17 (±1.8)	14.03 (±1.7)	16.9 (±1.6)
The 1th day	14.06 (±2.2)	18.6 (±2.1)	19.03 (±2.07)
The 2th day	24.9 (±2.05)	15.18 (±1.9)	12.9 (±1.8)
The 3th day	23.9 (±3.7)	11.2 (±3.5)	9 (±3.4)
P	0.02*	0.06	0.002*
TAP (μmol/l)			
Baseline	331.8 (±3.07)	348.07 (±28.6)	216.5 (±27.4)
The 1th day	302.06 (±41)	341.18 (±39.5)	231.4 (±37.8)
The 2th day	157.2 (±12.6)	175.05 (±12.09)	166.8 (±11.5)
The 3th day	308.5 (±39.9)	348.5 (±38.1)	220.06 (±36.4)
P	0.004*	0.09	0.6

*There is a significant difference between bolus and infusion group (*P*=0.02); *P*<0.05 considered significant, mean (±SD). ROS=Reactive oxygen species; TAP=Total antioxidant power; SD=Standard deviation; NO=Nitric oxide; HTS=Hypertonic saline

release of mediators such as cytokines and chemokines[13,27] Activated microglias let various neurotoxic substances, such as reactive oxygen, nitrogen species and glutamate, to be flow out which may further hasten neuronal death. While astrocyte reactivation, proliferation, and migration occurring after TBI seems to deleteriously affect axonal regrowth, the existence of these cells around the site of injury could positively supply a supporting environment via providing the tissue with neurotrophic factors that could possibly accelerate repair and neurogenesis.[27,28] Oxidative stress is precipitated by an imbalance between pro-oxidant and antioxidant substances synthesized as a consequence of excessive production of ROS. ROS could act as a double edged sword, which could play their role in normal physiological processes, or on the other hand participate in a number of disease processes, whereby they are involved into the injuries to cellular building blocks such as lipids, membranes, proteins, and DNA. It has been shown that the total antioxidant reservoir of brain homogenates and water-soluble antioxidant reservoir decreases after TBI.[29] Susceptibility of the brain tissue to oxidative damage could be understood in the light of the facts such as its extensive oxidative metabolic activity, enormous generation of reactive oxygen metabolites, partially low level of antioxidant capacity, low repair mechanism activity, nonregenerating nature of its neuronal cells, and the high membrane surface to cytoplasm ratio. The extensive amount of polyunsaturated fatty acids located in the membrane lipids of the brain is a major source for the decomposition reactions named "lipid peroxidation,"" in which a single initiating free radical can cause decomposition of the nearby molecules.[30]

In concordance with previous studies[4,5,8] TBI patients in our study had significantly higher initial serum levels of ROS at ICU admission with respect to a healthy control group. This result is in agreement with studies that postulated TBI could cause an uncontrolled increase in the amount of ROS, which could subsequently result in damages at cellular and molecular levels through different mechanisms. This rapid burst of increase in ROS level could be considered as a consequence of a sudden release of excitatory neurotransmitters parallel to limited antioxidant ability of the neuronal cells to overcome oxidative stress.

A number of therapeutic approaches, based on scavenging radicals and reducing oxidative stress have been tried in experimental models as well as in the clinical circumstances. N-acetylcysteine (NAC), a precursor of glutathione (GSH), a potent antioxidant, and free radical scavenger has been clearly shown to decrease oxidative stress and inflammation.[31] Animal model studies indicate that a single dose of NAC given 15 min after trauma could have some advantages in ameliorating lipid peroxidation, antioxidant enzyme activity and neuronal protection following closed head trauma.[32]

To our knowledge, few studies investigated HTS and mannitol with respect to their antioxidant and antiinflammatory properties.[17,32] Yilmaz *et al*. compared oxidant and antioxidant effects of mannitol and HTS 7.5% in rats exposed to TBI. They have found that mannitol is more effective than HTS on reduction of malondialdehyde, catalase and GSH-Px in the cellular level in TBI rats.[32]

In our study, using HTS were accompanied by a higher degree of reduction in ROS concentration in comparison with mannitol. This indicates that HTS could potentially provide more pronounced neuroprotection, which in turn decrease secondary brain insults and help to achieve a greater degree of neuronal and glial cell survival. Examination of NO (a rapid nitrogen species) also showed that TBI patients had significantly higher initial serum levels of NO at ICU admission compared to the healthy control group. With respect to this result, it could be concluded that TBI also increases concentration of rapid nitrogen species. Interestingly during study period, the serum levels of NO significantly decreased in HTS treatment groups especially in infusion group but significantly increased in the mannitol group. This finding is also in favor of HTS, confirming its ability in reducing damage due to NO, a rapid nitrogen species which in part could explain better clinical outcomes observed in the treatment with HTS as demonstrated by Hendoui *et al*.[10]

Studies, which have been done on the time course of oxidative stress, suggest that the production of free radicals happens shortly after TBI. On the other hand the extent of reactive species generated throughout the course of ischemia/reperfusion (I/R) of brain is determined by antioxidant defense mechanisms. The TAP of body fluid is a consequent of joint action between different antioxidants and is vital for the maximal prevention of free radical reactions in the extracellular compartments. Endogenous antioxidants have an indispensable role in coping with extensive increase in free radicals that are produced after I/R injury in the brain.[33] This defence against free radical insults is accomplished by enzymatic (catalase, superoxide dismutase, GSH-Px etc.) and nonenzymatic (GSH, Vitamin A, C, E, coenzyme Q, uric acid) free radical scavenging systems and metal chelators.

This study demonstrated that the serum level of TAP had decreased in all treatment groups during study period so it could be speculated that following TBI increased production of ROS and NO surpass the antioxidant power of the body which shows itself as a decrease in TAP at the time of ICU admission in our patients. Furthermore, the serum levels of TAP had decreased in all treatment groups but this reduction was significant in the mannitol group. Reduction of TAP during the time period ended to 2nd day after TBI and then its increase from the 2nd day to 3rd day is a valuable finding which could help us through designing proper antioxidant regimens that could be a potential treatment for preventing secondary damages in TBI patients. These findings are in concordance with previous studies that showed oxidative stress begins as early as 30 min after injury[29] and continues for at least 24 h after TBI.[34] In fact in all treatment groups the least measured TAP was in 2nd day of admission and after that TAP increased in all groups in 3rd day of admission up to about the amount of the baseline measure for each group. A possible explanation for this observation is that during pervasive brain I/R, the organism is trying to deal with the harmful effects of free radicals by rising the production of endogenous antioxidants (e.g. uric acid, ascorbic acid), a finding which is in agreement with the study of Sivonová *et al*.[35]

Our study had some limitations. limited number of TBI patients in each group caused a significant difference with respect to sex of the patients at baseline, as in mannitol group percentage of female patients were 40% that was significantly different from those of HTS bolus group (0%) and HTS infusion group (8%). Some of the previous studies provided evidence indicating a more pronounced neuroprotection in females rather than males. It has been proposed that this is due to direct and indirect sex hormone-mediated antioxidant mechanisms. Progesterone administration reduces brain levels of F (2)-isoprostane, a marker of lipid peroxidation, after experimental TBI in male rats and estrogen provide neuroprotection in experimental neurological injury.[36]

As a result, the greater percentage of female patients in the mannitol group could interfere with our judgment about different interventions, because it seems that female subjects may have a better prognosis even in the absence of any intervention. However, results of measurements of TAP indicated that in the mannitol group, the decrease in TAP was more significant than HTS groups. In other word, despite the fact of having more women in the mannitol group which could result in favor of mannitol for producing better outcomes, TAP reduced more prominently in this group which means that mannitol was less successful in ameliorating oxidative stress. More studies are necessaries with larger sample size and direct ICP monitoring in TBI patients under mannitol and two different regimens of HTS 5%.

CONCLUSION

Our data are suggesting that TBI could cause an uncontrolled increase in the amount of serum concentrations of ROS and NO which could subsequently result in damages at cellular and molecular levels through different mechanisms. HTS 5%

has significant effects on the oxidant responses compared with mannitol following TBI that makes HTS as a prefect therapeutic intervention for reducing unfavorable outcomes in TBI patients.

AUTHOR'S CONTRIBUTION

MM participated in designing the trial, evaluated the clinical data; he also participated in drafting the manuscript. AA, AM, MTB were involved in designing the study, and were responsible for recruiting patients. MA and FSh participated in designing the trial and performed tests; also participated in drafting the manuscript. ZKh reassessed the clinical data, reviewed the statistical analysis and interpreted them. Bk was involved in the original design of the proposal, and also in drafting and revising the manuscript.NH (the corresponding author) supervised the trial and was involved in designing the study as well as coordinating the project, drafting and revising the manuscript. All authors read and approved the final manuscript.

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