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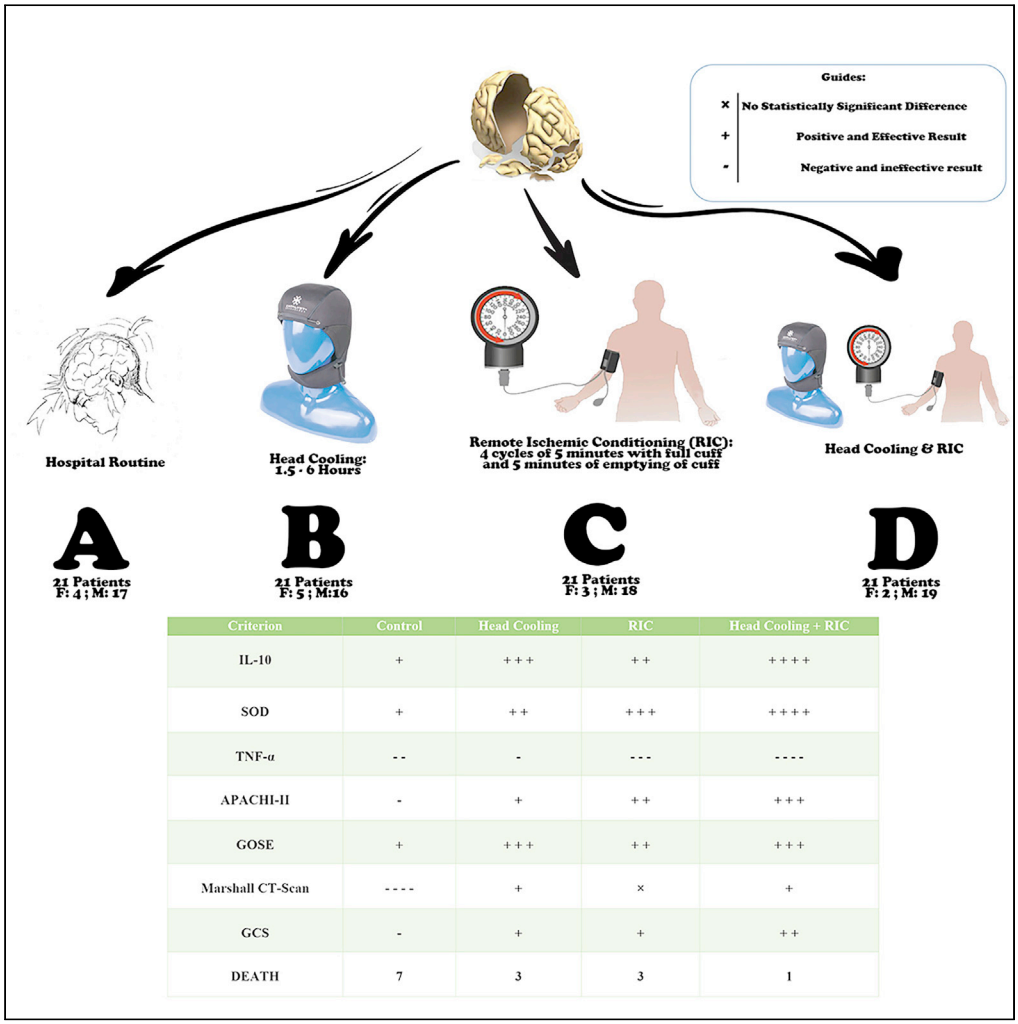

Criterion	Control	Head Cooling	RIC	Head Cooling + RIC
IL-10	+	+++	++	++++
SOD	+	++	+++	++++
TNF-α	--	-	---	----
APACHI-II	-	+	++	+++
GOSE	+	+++	++	+++
Marshall CT-Scan	----	+	×	+
GCS	-	+	+	++
DEATH	7	3	3	1

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Highlights

The effect of the head cooling method in controlling secondary injury in patients with TBI.

The effect of the RIC method in controlling secondary injury in patients with TBI.

Comparison of two interventions of head cooling and RIC.

Evaluation of clinical and paraclinical parameters.

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The effect of head cooling and remote ischemic conditioning on patients with traumatic brain injury

Fardin Hodoodi,[1] Mohammad Allah-Tavakoli,[1,2] Farzad Tajik,[3,4] Iman Fatemi,[5] and Amir Moghadam Ahmadi[4,6,7,*]

SUMMARY

Cerebral impairment caused by an external force to the head is known as traumatic brain injury (TBI). The aim of this study was to determine the role of local hypothermia and remote ischemic conditioning (RIC) on oxidative stress, inflammatory response after TBI, and other involved variables. The present study is a clinical trial on 84 patients with TBI who were divided into 4 groups. The head cooling for 1.5 to 6 hr was performed in the first three days after TBI. RIC intervention was performed within the golden time after TBI in the form of four 5-min cycles with full cuff and 5 min of emptying of cuff. The group receiving the head cooling technique recovered better than the group receiving the RIC technique. Generally, combination of the two interventions of head cooling and RIC techniques is more effective on the improvement of clinical status of patients than each separate technique.

INTRODUCTION

Brain disorder caused by an external force to the head is known as traumatic brain injury (TBI). Brain trauma is one of the 5 main causes of death and disability in the world, which results in about 5 million annual deaths or 16,000 daily deaths (Maas et al., 2008). TBIs are primary and secondary. Primary damage occurs immediately after major injury. Trauma involves bruising, damage to the blood vessels, and axonium cutting, in which the axons of the neurons are actually pulled or torn. Secondary injuries include damage to the blood-brain barrier (BBB) and release of factors that lead to inflammation, free radical overload, excessive glutamate neurotransmitter release, calcium and sodium ion entry into neurons, and mitochondrial dysfunction (Godoy et al., 2016). Some studies have shown that IL-10 levels are elevated in severe TBI conditions. Tumor necrosis factor alpha (TNF-α) levels increase in the cerebrospinal fluid (CSF) and serum of patients with TBI. The usual clinical goal in TBI and stroke is to reduce body temperature to the normal level (Dikmen et al., 2003; Csuka et al., 1999). Occasionally, the body temperature can be reduced to below normal levels to decrease intracranial hemorrhage, cerebral edema, intermittent tissue perfusion, brain hypoxia, ischemia, and reperfusion injury. Cooling methods can be divided into two general categories of general cooling of the body and the target organ—it is direct cooling of the brain here (Sinclair and Andrews, 2010). The prevention of secondary brain injury is a primary goal in treating patients with severe TBI. Secondary brain injury results from tissue ischemia induced by increased vascular resistance in the at-risk brain tissue due to compression by traumatic hematomas and development of cytotoxic and vasogenic tissue edema (Bouma et al., 1991). While traumatic hematomas may be managed surgically, cytotoxic and vasogenic edema with resulting perfusion impairment perpetuates brain ischemia and injury (Kitagawa et al., 1990). There is a new technique for protection against ischemic-reperfusion injury called the remote ischemic conditioning (RIC). RIC is a process where normal tissues are subjected to short cycles of ischemia and reperfusion. When your arm falls asleep from an irregular posture, blood rushes back into the limb and the nerves previously deprived of oxygen and electrolytes spontaneously regain function (Saxena et al., 2010). During this process, cellular and molecular processes within the ischemic limb may produce bioactive restorative and regenerative compounds. RIC is used to describe the process of transiently impeding blood flow to a limb. It has shown to provide protection against subsequent major ischemic events in the brain and other organs (Saxena et al., 2010). RIC can be achieved through a simple and cost-effective technique of applying a tourniquet to a limb for a pre-determined duration. Pre-clinical animal studies on RIC efficacy have been conducted in models of cardiac arrest and cerebral ischemia in which histopathological

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Table 1. Comparison of average levels of inflammatory cytokines at different times in the 4 studied groups

Group Variable	A (n = 21)	B (n = 21)	C (n = 21)	D (n = 21)	p value
IL-10 at admission	232.52 ± 14.36	237.43 ± 13.74	234.00 ± 12.03	233.29 ± 15.97	0.687
IL-10 72 hr after admission	514.00 ± 13.08[a]	1481.81 ± 16.24[b]	1596.14 ± 11.63[c]	1708.43 ± 15.49[d]	<0.001
IL-10 6 days after admission	414.72 ± 19.60[a]	1305.35 ± 18.14[b]	723.00 ± 36.02[c]	1435.33 ± 40.71[d]	<0.001
SOD at admission	343.76 ± 67.17	337.05 ± 54.54	330.29 ± 100.34	327.43 ± 64.12	0.891
SOD 72 hr after admission	309.14 ± 15.95[a]	450.10 ± 47.62[b]	567.14 ± 80.61[c]	777.29 ± 24.06[d]	<0.001
SOD 6 days after admission	464.22 ± 59.39[a]	620.30 ± 120.53[b]	688.70 ± 112.49[c]	737.67 ± 100.57[d]	<0.001
TNF-α at admission	17.98 ± 3.75	18.89 ± 3.70	18.92 ± 3.27	19.94 ± 3.23	0.325
TNF-α 72 hr after admission	13.88 ± 1.86[a]	22.84 ± 6.37[c]	15.69 ± 1.93[b]	10.69 ± 3.11[a]	<0.001
TNF-α 6 days after admission	14.16 ± 2.60[a]	14.39 ± 1.63[a]	12.95 ± 1.55[b]	9.44 ± 1.27[c]	<0.001

Data are presented as mean ± SD. Means are compared across groups using one-way ANOVA followed by Tukey's multiple comparison tests. Related to Figures 1–3.

[a-d] Differences between groups with different letters are statistically significant ($P < 0.05$).

and functional outcomes have been improved by RIC (Saxena et al., 2010). Despite having dissimilar primary pathologies, cerebral ischemia and TBI share common secondary injury processes, including inflammation, oxidative stress, and blood-brain barrier permeability (Chen et al., 1996; Wei et al., 2012; Wang et al., 2014b; Ren et al., 2011; Liu et al., 2014; Joseph et al., 2015; Di Battista et al., 2015). Based on the pathological processes shared by TBI and cerebral ischemia, in this article, we test the hypothesis that RIC restores function following diffuse TBI and examine the potential mechanism of RIC action through a class of small lipids termed specialized pro-resolving mediators. RIC has been shown to improve the outcomes after myocardial infarction, sepsis, transplantation, reimplantation, and elective neurologic surgery (Loukogeorgakis et al., 2005, 2008; Hu et al., 2010; Steiger and Hänggi, 2007; Konstantinov et al., 2004; Hausenloy and Lim, 2012). It is thought to work by releasing endogenous systemic anti-inflammatory mediators and humoral factors while using neural pathways, rendering global protection to the body against subsequent TBI insults in a remote area (Konstantinov et al., 2004; Steiger and Hänggi, 2007). This protection provided by RIC has two phases, an early (short) phase and a late (prolonged) phase, both of which have proven to be effective in improving survival. Multiple animal studies and a small number of randomized clinical trials have shown the protective effect of RIC in patients with TBI (Saxena et al., 2010). Based on these findings, it is hypothesized that RIC will exert beneficial effects on TBI in humans, thereby representing a new therapeutic strategy for severe TBI. Therefore, the purpose of this study was to determine the role of local brain hypothermia and the RIC method on oxidative stress and inflammatory responses after TBI and other involved variables.

RESULTS

In the present study, four groups of 21 subjects were investigated: control group (patients who only received routine hospital treatment), head cooling group (patients who received head cooling cap in addition to routine hospital treatment), RIC group (patients who received RIC technique in addition to routine hospital treatment), and group D (patients who received the two head cooling and RIC interventions in addition to routine hospital treatment). The patients were admitted to the emergency department and the intensive care unit of Farrokhi Hospital in Yazd. The interventions began at the time of admission. The total number of patients included in this study was 84.

Based on the one-way analysis of variance (ANOVA), there was no statistically significant difference between the 4 groups in the mean score of IL-10 at the time of admission ($p > 0/05$). However, there was a statistically significant difference between the 4 groups in terms of mean IL-10 score 72 hr after admission (Table 1). The differences between all four groups were investigated using Tukey's post-hoc test. The difference in group D was more than that in other groups. The difference in group C was more than that in groups A and B. The difference in group B was more than that in group A. Based on one-way ANOVA, there was a statistically significant difference between the 4 groups in the mean score of IL-10 on day 6 after admission (Table 1). The differences between all four groups were investigated using Tukey's post-hoc

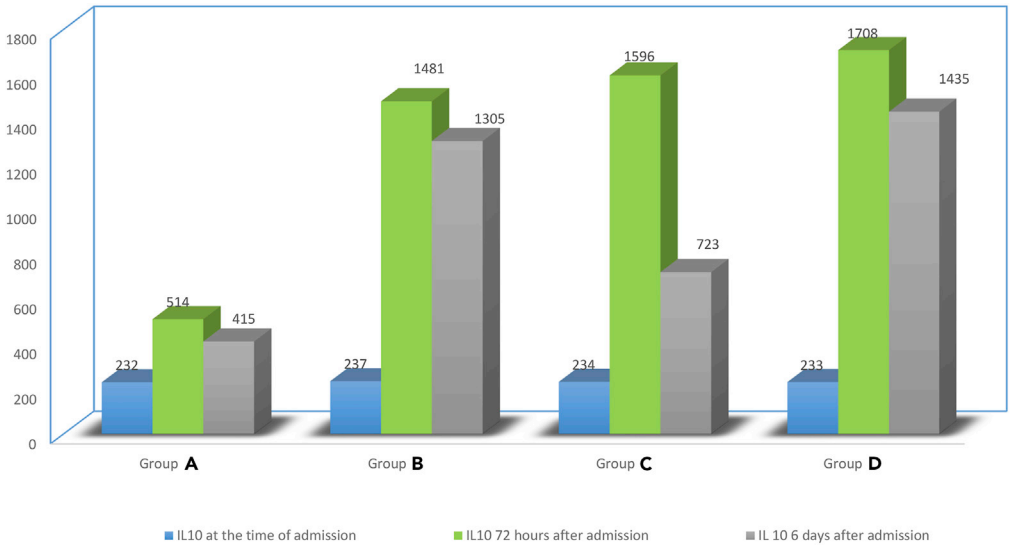

Figure 1. Compares average of IL-10 in 4 groups studied

test. The difference in group D was more than that in other groups. The difference in group B was more than that in groups A and C. The difference in group C was more than that in group A (Figure 1).

Based on one-way ANOVA, there was no statistically significant difference between the 4 groups in the mean score of superoxide dismutase (SOD) at the time of admission ($p > 0.05$). However, there was a statistically significant difference between the 4 groups in the mean score of SOD 72 hr after admission (Table 1). These differences were investigated using Tukey's post-hoc test. The difference in group D was more than that in other groups. The difference in group C is more than that in group B. However, the difference in group A is not significantly different from that at the time of admission, it even decreased slightly. Based on one-way ANOVA, there was a statistically significant difference between the 4 groups in the mean score of SOD on day 6 after admission (Table 1). The results of Tukey's post-hoc test showed that the difference in group D was more than that in other groups. In group C, it was more than that in groups A and B. Also, the difference in group B was more than that in group A (Figure 2).

Based on the one-way ANOVA, there was no statistically significant difference between the 4 groups in the mean score of TNF-α at the time of admission ($p > 0.05$). However, there was a statistically significant

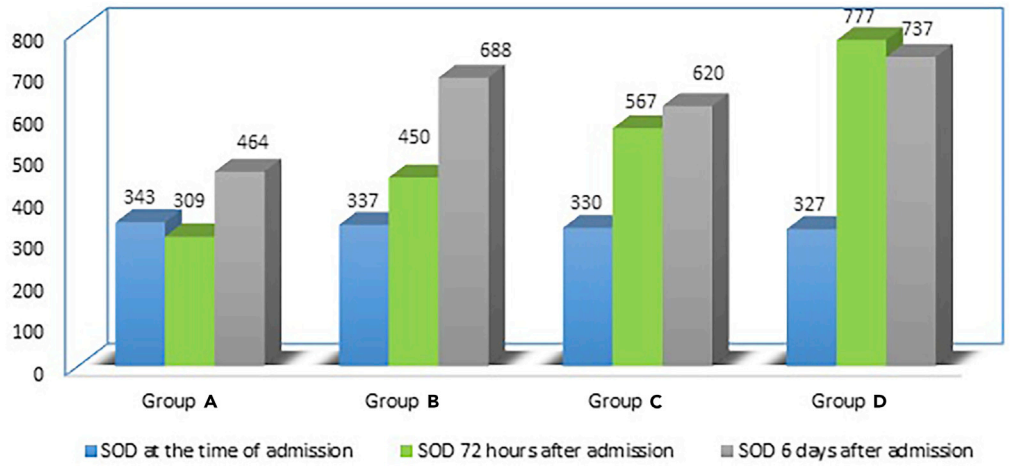

Figure 2. Compares average of SOD in 4 groups studied

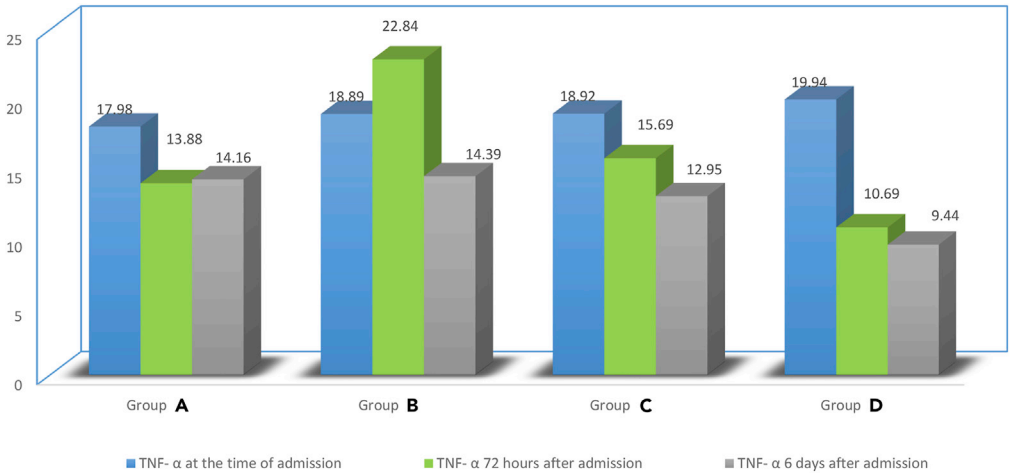

Figure 3. Compares average of TNF-α in 4 groups studied

difference between the 4 groups in the mean score of TNF-α 72 hr after admission (Table 1). The results of Tukey's post-hoc test showed that the difference in group D was more than that in other groups. The difference in group C decreased less than that in group A. In group B, unlike the other three groups, the level of TNF-α increased. One-way ANOVA results showed a statistically significant difference between the 4 groups in the mean score of TNF-α 6 days after admission (Table 1). The results of Tukey's post-hoc test showed that the difference in group D was more than that in other groups. The difference in group C was more than that in groups A and B. Also, groups B and A were almost the same as the time of admission (Figure 3).

The results of one-way ANOVA showed no statistically significant difference between 4 groups in the mean score of Glasgow Results Scale-Extended (GOSE) at the time of admission ($p > 0.05$). The same results showed no statistically significant difference between 3 groups (A, B, C) in the mean score of GOSE 72 hr after admission; a statistically significant difference was seen only in group D ($p > 46/0$) (Table 2). In group D, according to the GOSE, category 5, i.e., the "Lower moderate disability", improved. Based on one-way ANOVA, there was a statistically significant difference between 3 groups (A, B, D) in the mean score of GOSE 6 days after admission (Table 2).

The results of Tukey's post-hoc test showed that, on the sixth day, groups A and B had a GOSE score lower than that at the time of admission, the score of group C did not change, and group D received a higher score than that at the time of admission of patients, which means it is in a more improved category. Based on one-way ANOVA, there was a statistically significant difference between 4 groups in the mean score of GOSE 28 days after admission (Table 4). The results of Tukey post-hoc test showed that group A improved one degree in the GOSE category; groups B and D increased equally and were higher than group C (Figure 4).

Based on one-way ANOVA, there was no statistically significant difference between 4 groups in the mean score of Marshall computed tomography (CT) scan at the time of admission ($p > 0/05$). However, there was a

Table 2. Comparison of average GOSE score at different times in the 4 groups

Group Variable	A (n = 21)	B (n = 21)	C (n = 21)	D (n = 21)	p value
GOSE at admission	4.10 ± 1.55	4.14 ± 1.59	3.95 ± 1.39	3.95 ± 1.59	**0.086**
GOSE 72 hr after admission	3.71 ± 1.71	4.14 ± 1.91	3.86 ± 1.74	4.57 ± 2.04	**0.461**
GOSE 6 days after admission	3.28 ± 1.18	3.80 ± 1.82	3.80 ± 1.82	4.95 ± 1.70	**0.017**
GOSE 28 days after admission	4.64 ± 1.08[a]	6.61 ± 1.29[b]	5.72 ± 1.36[ab]	6.35 ± 1.59[b]	**0.001**

Data are presented as mean ± SD. Means are compared across groups using one-way ANOVA followed by Tukey's multiple comparison tests. Related to Figure 4.
[a-d] Differences between groups with different letters are statistically significant ($P < 0.05$).

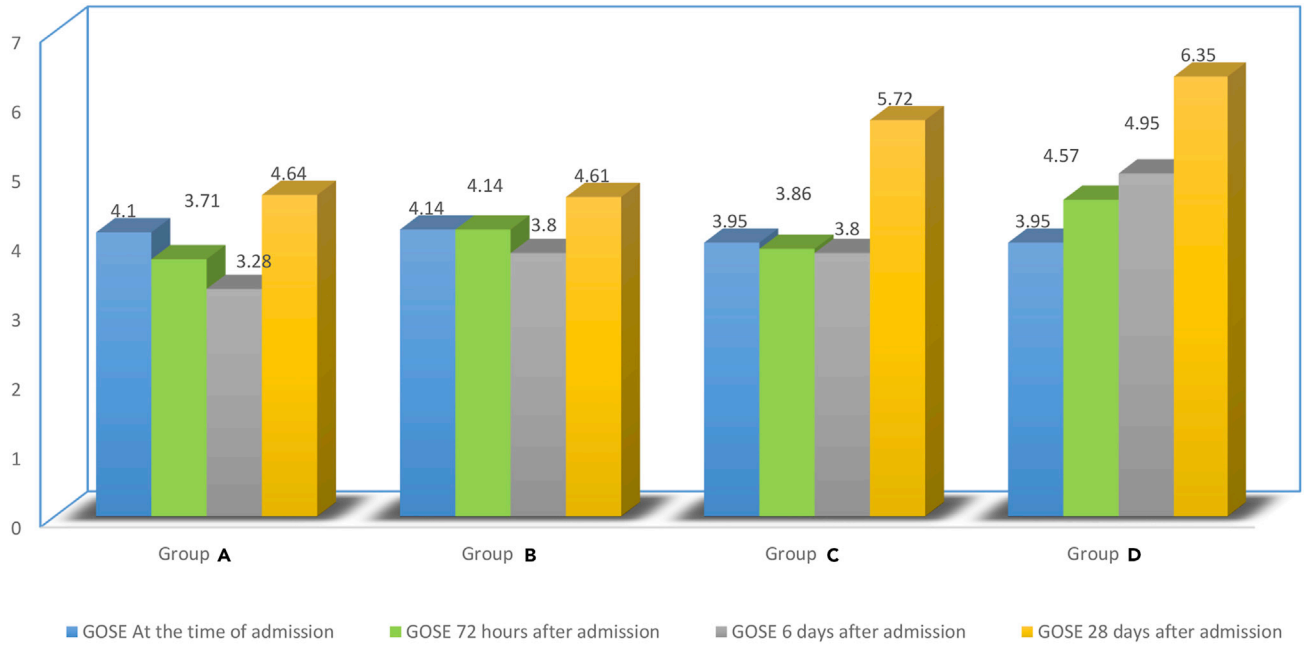

Figure 4. Compares average of GOSE in 4 groups studied

statistically significant difference between 2 groups (A, C) in terms of the average score of Marshall CT scan 72 hr after admission (Table 3). Tukey's post-hoc tests showed that groups A and C had higher scores than those at the admission time, both the score of group C was higher than that of group A. Groups B and D maintained almost the same status after 72 hr as that at the admission time. Based on one-way ANOVA, there was a statistically significant difference between 3 groups (A, B, C) in terms of the average score of Marshall CT scan 6 days after admission (Table 3). According to Tukey's post-hoc tests, groups A and B had the same increase in Marshall CT scan scores. This means that, in the Marshall CT scan classification, both groups are equally worse and go to the fourth class of Marshall CT scan classification. Group C had an increase in Marshall CT scan scores, but the increase was less than that for groups A and B. Group D maintained almost the same status as that at the time of admission on the sixth day. Based on one-way ANOVA, there was a statistically significant difference between 3 groups (A, B, D) in terms of the average score of Marshall CT scan 28 days after admission (Table 3). Tukey's post-hoc tests showed that group A had an increase in Marshall CT scores compared to the day of admission, pointing to a worse status in this group. Group C increased very little, but it remained in the same third category of Marshall CT scan classification. Group B and D had a decrease in scores compared to the day of admission; both groups improved almost one category. They went from category 3 to category 2, but, statistically, group D had a greater decrease than group B (Figure 5).

Based on one-way ANOVA, there was no statistically significant difference between the 4 groups in terms of Apache II score at the time of admission ($p > 0.05$). Also, there was no statistically significant difference

Table 3. Comparison of average score of Marshall CT scan at different times in the 4 groups

Group Variable	A (n = 21)	B (n = 21)	C (n = 21)	D (n = 21)	p value
Marshall CT scan at admission	3.57 ± 1.59	3.14 ± 1.53	2.95 ± 1.53	2.95 ± 1.59	**0.530**
Marshall CT scan 72 hr after admission	4.48 ± 0.93[a]	3.76 ± 1.38[a]	4.5 ± 1.53[a]	2.57 ± 1.03[b]	**<0.001**
Marshall CT scan 6 days after admission	4.28 ± 1.36[a]	4.30 ± 1.34[a]	3.80 ± 1.61[a]	2.62 ± 1.24[b]	**0.001**
Marshall CT scan 28 days after admission	4.36 ± 1.08[a]	2.61 ± 1.09[c]	3.06 ± 1.26[b]	2.40 ± 1.05[d]	**<0.001**

Data are presented as mean ± SD. Means are compared across groups using one-way ANOVA followed by Tukey's multiple comparison tests. Related to Figure 5.
. [a-d] Differences between groups with different letters are statistically significant ($P < 0.05$).

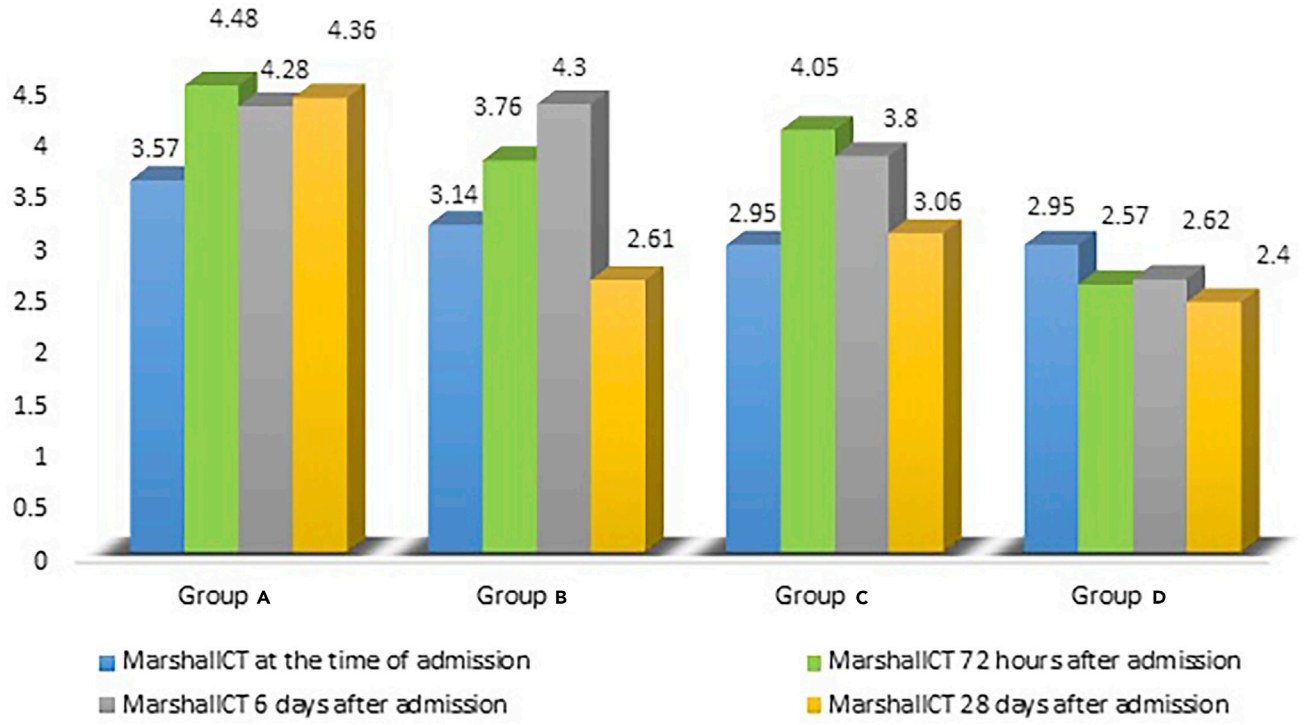

Figure 5. Compares average of CT scan Marshall in 4 groups studied

between 3 groups (A, B, C) 72 hr after admission ($p > 0.05$). Group D scored lower, indicating an improvement in physiological status. There was a statistically significant difference between the 4 groups in terms of Apache II score 6 days after admission (Table 4). In Tukey's post-hoc tests, group A scored higher, indicating a deterioration in their physiological condition. The B and C groups dropped almost as much, and their status improved almost as much since the day of admission. Group D scored much lower, indicating that they improved much more than the other groups. Based on one-way ANOVA, there was a statistically significant difference between 4 groups in terms of mean score of APACHE II 28 days after admission. In Tukey's post-hoc tests, group A scored higher, indicating a deterioration in their physiological condition. The condition of group B improved less than that on the day of admission compared to group C. Group D scored lower than the two groups B and C, indicating that they improved more than the other groups (Figure 6).

According to one-way ANOVA, there was no statistically significant difference between the 4 groups in terms of the mean Glasgow Consciousness Scale (GCS) score at the time of admission ($p > 0.05$). Also, no significant difference between the 4 groups was observed 72 hr after admission ($p > 0/05$). However, there was a statistically significant difference between the 4 groups in terms of the average score of GCS 6 days after admission (Table 5). In Tukey's post-hoc tests, groups A and B had approximately the

Table 4. Comparison of the average score of APACHE II at different times in the 4 groups

Group Variable	A (n = 21)	B (n = 21)	C (n = 21)	D (n = 21)	p value
APACHE II at admission	33.90 ± 14.73	41.05 ± 15.81	40 ± 14.09	44.10 ± 17.23	**0.192**
APACHE II 72 hr after admission	33.24 ± 13.55[a]	42.43 ± 16.69[a]	41.29 ± 17.65[a]	33.10 ± 12.29[b]	**0.081**
APACHE II 6 days after admission	40.22 ± 13.35[a]	35.40 ± 13.84[b]	35.26 ± 15.59[b]	26.95 ± 4.39[c]	**0.012**
APACHE II 28 days after admission	39 ± 16.67[a]	30.06 ± 11.18[b]	27.50 ± 15.99[c]	24.50 ± 12.59[d]	**0.034**

Data are presented as mean ± SD. Means are compared across groups using one-way ANOVA followed by Tukey's multiple comparison tests. Related to Figure 6.
[a-d] Differences between groups with different letters are statistically significant ($P < 0.05$).

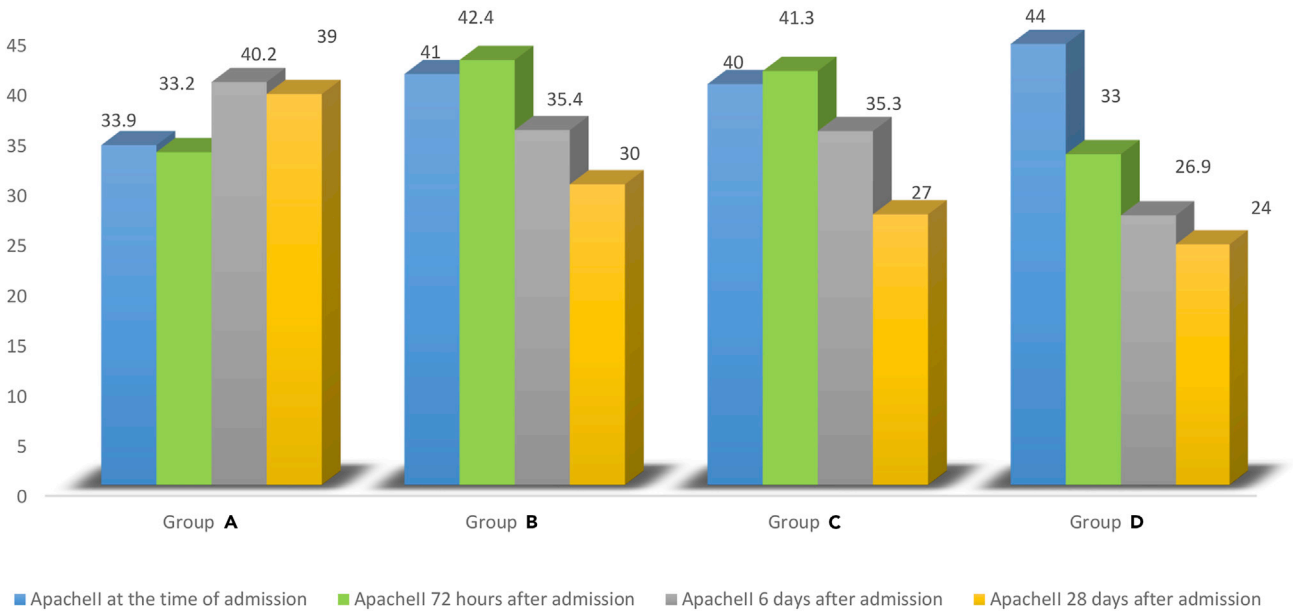

Figure 6. Compares average of APACHE II in 4 groups studied

same GCS score on the day of admission, group C scored lower than that on the day of admission, and group D scored higher. One-way ANOVA indicated a statistically significant difference between the 4 groups in terms of the average score of GCS 28 days after admission (Table 5). Tukey's post-hoc tests showed that group A scored lower, group B and C scored almost identically, and group D scored higher than groups B and C (Figure 7).

DISCUSSION

The purpose of this study was to compare the effect of head cooling and RIC on improvement of patients with TBI. IL-10 may have a protective role as it can limit tissue damage and reduce inflammation by inhibiting the synthesis of TNF-α. In this study, the mean serum level of IL-10 was studied among the studied groups—control (patients with TBI who received only routine hospital treatment), head cooling group (patients with TBI who received the cooling of the head), the RIC group (patients with TBI who received the RIC technique), group D (patients with TBI receiving both head cooling and RIC techniques). Seventy-two hours after admission, group C (RIC) had higher IL-10 levels than group B (head cooling), although they had lower IL-10 levels than group D (RIC and head cooling). On day 6 after admission, group C had lower IL-10 levels than group B and D. Group D had a higher IL-10 level than other groups. This result is consistent with the results of Morganti-Kossmann et al. (2002) and Tao Wang et al. (Morganti-Kossmann et al., 2002; Wang et al., 2014a)

In their study titled "Inflammatory response in acute traumatic brain injury: A double-edged sword", Morganti-Kossmann et al. (2002) showed that serum levels of TNF, IL-6, IFN-γ, lymphotoxin-α, IL-10, TGF-β, and

Table 5. Comparison of the average score of the GCS at different times in the 4 groups

Group Variable	A (n = 21)	B (n = 21)	C (n = 21)	D (n = 21)	p value
GCS at admission	8.34 ± 2.54	7.81 ± 2.75	7.95 ± 2.92	8.10 ± 2.59	**0.893**
GCS 72 hr after admission	8.10 ± 2.68[a]	8.14 ± 2.41[a]	7.48 ± 2.77[a]	9.24 ± 2.27[b]	**0.172**
GCS 6 days after admission	8.17 ± 2.62[a]	8.25 ± 2.27[a]	6.70 ± 2.78[b]	10.57 ± 2.66[c]	**<0.001**
GCS 28 days after admission	7.43 ± 3.25[a]	10.39 ± 2.68[b]	10.28 ± 2.56[b]	11.35 ± 2.48[c]	**0.001**

Data are presented as mean ± SD. Means are compared across groups using one-way ANOVA followed by Tukey's multiple comparison tests. Related to Figure 7.

[a-d] Differences between groups with different letters are statistically significant ($P < 0.05$).

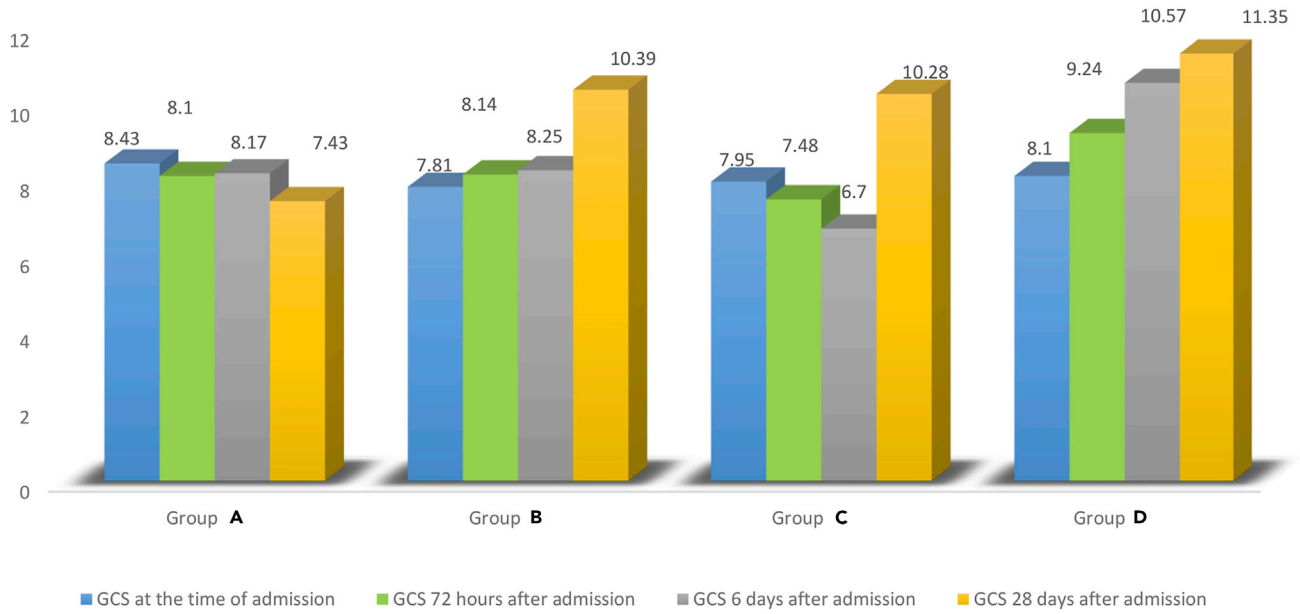

Figure 7. Compares average of GCS in 4 groups studied

ICAM-1 increased in patients with TBI (Morganti-Kossmann et al., 2002). IL-10 may have a protective role as it can limit tissue damage and reduce inflammation by inhibiting the synthesis of TNF-α (Ribbons et al., 1997; Fiorentino et al., 1991). The findings of this study are consistent with those of Morganti-Kossmann et al. (2002).

Dingtai Wei et al. (2012) showed that limb remote preconditioning improved mechanisms involved in inflammatory factors in rat stroke, which is consistent with the results of the present study (Wei et al., 2012). Changhong Ren et al. (2011) showed that remote post-conditioning significantly reduced brain edema and BBB leakage compared to the control groups. Their results are consistent with the results of the present study (Ren et al., 2011). Bellal Joseph et al. (2015) showed that RIC significantly decreased the standard biomarkers of acute brain injury in patients with severe TBI, which are consistent with the results of the present study. However, they measured S-100B and neuron-specific enolase, but we measured IL-10, SOD, and TBF-α (Joseph et al., 2015). Stavros P Loukogeorgakis et al. (2005) showed that remote ischemic preconditioning (RIPC) caused a decrease in the level of trimetaphan. This in turn reduced intracranial pressure and cerebral edema and its consequences. Our paraclinical findings of Marshall CT scan also showed that RIC has the same effect (Loukogeorgakis et al., 2005). Sheng Hu et al. (2010) showed that the recovery rate 7 days and 1 and 3 months after elective cervical decompression was higher in the RIPC group than in the control group. We also showed that the recovery rate 6 days and 28 days after TBI was higher in the RIC group than in the control group (Hu et al., 2010).

SOD is an enzyme that intermittently catalyzes radical superoxides into any conventional oxygen molecule or hydrogen peroxide (Packer, 2002). Seventy-two hours after admission, group C (RIC) had higher SOD levels than group B (head cooling), although they had lower IL-10 levels than group D (RIC and head cooling). On the sixth day after admission, group C still had higher SOD levels than group B, and group D had higher levels than other groups. The results of this study are consistent with those of Bidmon et al. who showed an increase in SOD levels after focal cortical photo-thrombotic injury (Bidmon et al., 1998). It was found that RIC also significantly induced a reduction in SOD activity compared with the RIC group (Wang et al., 2014a).

TNF-α is a signaling protein (cytokine) involved with systemic inflammation. It is one of the cytokines responsible for the development of acute phase of reaction (Rogler et al., 2015). TNF-α plays an important role in inflammatory processes through activation of neutrophils and endothelial cells and granulocytes (Hess et al., 2015). Seventy-two hours after admission, group B had higher TNF-α levels than group C

and D, and group C had higher TNF-α levels than group D. On the sixth day after admission, the conditions were more severe than 72 hr after admission. Following the TBI, increasing levels of TNF-α in the CSF and serum of patients are reported that is consistent with the above results (Dalgard et al., 2012; Csuka et al., 1999; Longhi et al., 2011). The increase in these cytokines leads to better function of the BBB that leads to a recovery.

The Glasgow Outcome Scale is a global scale for functional outcome that puts patient status into one of five categories: dead, vegetative state, severe disability, moderate disability, or good recovery (Jennett and Bond, 1975; Wilson et al., 1998, 2007). Seventy-two hours after admission, group C had a GOSE score lower than that of group B, and group D had a higher score than the other groups. On day 6 after admission, groups B and C scored the same, and group D scored higher than the other groups. On day 28 after admission, group C had a lower GOSE score than group D and group B had a higher score than the other groups.

Marshall CT scan identified six groups of patients with TBI based on morphological abnormalities on CT scans (Wikipedia, 2013). Seventy-two hours after admission, group C was in category 4 and 5 of Marshall CT scan, group B was in category 3 and 4, and group D was in category 2 and 3. On day 6 after admission, the conditions were the same as 72 hr after admission. On day 28 after admission, group C was placed in category 3 of Marshall CT scan, and groups B and D were placed in category 2.

Acute Physiology and Chronic Health Evaluation II (APACHE II) is a disease severity scoring system, which helps to assess the patient's outcome for objective evaluation by estimating the odds of their recovery (Wikipedia, 2013). The prognostic system can also help to estimate patient's physiologic instability upon admission (Wikipedia, 2013). Seventy-two hours after admission, group D scored lower than the other groups, followed by group C, and group B scored higher than the other groups. On days 6 and 28 after admission, the groups had the same conditions as those at 72 hr after admission but with more severity. The results of the present study are consistent with those of Bian et al., which showed that the high score of APACHE II was associated with a high mortality rate (Bian et al., 2015).

The GCS falls between 3 and 15 points, 3 indicating the worst situation and 15 indicating the best alertness status. The GOSE delivers the most trusted and measured outcomes following brain damage (Teasdale and Jennett, 1974). Seventy-two hours after admission, group C had lower GSC scores than group B, and group D scored higher than the other groups. On day 6 after admission, the conditions of the groups were the same as those at 72 hr after admission. On day 28 after admission, the B and C groups had the same GSC score and group D scored higher. The results of the experiment showed that brain hyperthermia can cause increased mortality and morbidity (Dietrich et al., 1996). The results of the present study are consistent with those of Marion et al., Joseph et al. (2015), and (Marion et al., 1997; Kuo et al., 2011; Joseph et al., 2015; Gonzalez et al., 2014).

CONCLUSIONS

According to the findings of the study on IL-10, SOD and TNF levels, the GOSE and APACHE-II scores, and the Marshall CT scan classification, it can be argued that the effect of the RIC technique was greater than that of the head cooling technique. On days 6 and 28 after admission, conditions changed and the group receiving the head cooling technique recovered better than the group receiving the RIC technique. Therefore, the RIC method should be performed in the same way as the head cooling method, in the first three days of admission. Generally, the combination of two interventions of head cooling and RIC techniques is more effective on the improvement of clinical status of patients than any of the techniques alone.

Limitations of the study

Time constraints, differences in the researcher's accent and race with the patients and their family, and lack of proper cooperation and limited budget allocated were among the limitations in the present research project.

METHODS

All methods can be found in the accompanying transparent methods supplemental file.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2021.102472.

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AUTHOR CONTRIBUTIONS

F.H. contributed in implementation of the project and is author of the article. M.A.-T. is the initial project designer and dissertation supervisor. F.T. designed the project implementation stages and is the first advisor of the dissertation. I.F. designed the project implementation stages and is the second advisor of the dissertation. A.M. is the corresponding author and first thesis supervisor and executive management of the entire process of data collection and data analysis.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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iScience, Volume *24*

Supplemental information

The effect of head cooling and remote ischemic conditioning on patients with traumatic brain injury

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Transparent Methods

ETHICAL CONSIDERATIONS

The present study was approved by the headquarters of the Ministry of Health and Medical Education with the code of ethics IR.RUMS.REC.1395.138. Oral and written consent was obtained from all patients or first-degree families.

THE WHO INTERNATIONAL CLINICAL TRIALS REGISTRY PLATFORM (ICTRP)

The Registration Reference (IRCT: Iranian Registry of Clinical Trials) is IRCT20210209050307N1.

RESTRICTED PLAN IMPLEMENTED

Criteria for patient exclusion from the plan are as follows:

- Mild TBI
- Other known inflammatory processes
- Other known infections
- History of neurology
- History of psychological disorders
- Drug or alcohol dependence
- Inability to maintain hemodynamic stability and respiratory stability
- Injuries from intrusive objects (bullet wounds and root injuries)
- CT scan signs of diffuse injury without mass effect
- Epidural hemorrhage (ie unrelated to intradoral mass lesions)
- Chronic subdural hemorrhage

METHOD DETAILS

Sample Collection

Patient`enrolment began on May 16, 2016. Emergency Department of Farrokhi Hospital in Yazd admitted the TBI patients where they were referred to the Radiology Department for fracture diagnosis. Blood samples were taken after admission in the hospital. The age of patients was between 14 and 65 years. Patients with a GCS score of less than 8 were transferred to the intensive care unit. Patients were constantly monitored to prevent hypoxia and low blood pressure. It was tried to maintain their blood flow adequately, i.e. at a systolic pressure of 90 mmHg and a brain perfusion pressure of at least 60 mmHg. The intervention took place within the first six hours after the injury. CT scan was performed by the hospital's Radiology Department after admission and taking blood samples. Then, upon the diagnosis of the neurologist, patients who needed surgery were immediately referred to the operating room. Mannitol and hypertonic saline were used to reduce intracranial pressure (ICP). At the beginning of the admission, the first sample was taken and encoded to protect the patient's identity. The sample was immediately

referred to the paraclinical section of the hospital to centrifuge the serum at 4°C and check the requested parameters.

In this study, the score of GOSE, APACHE II and Marshall CT Scan score and SOD, IL-10 and TNF-α were evaluated before and after the intervention. ELISA kits are used to measure IL-10, TNF-α and SOD in patient samples. The score of GOSE, APACHE II and Marshall CT Scan score were evaluated at the time of admission, 72 hours, 6 days and 28 days after admission. IL-10, TNF-α and SOD were evaluated at the time of admission, 72 hours and 6 days after admission.

The age range of patients is as follows:

The number of patients under the age of 20 years were 13; The number of patients in the age range of 21 to 29 years were 36; The number of patients in the age range of 30 to 39 years were 11; The number of patients in the age range of 40 to 49 years were 13; The number of patients in the age range of 50 to 59 years were 6; The number of patients in the age range of 60 to 65 years were 5.

Patients' sex is as follows:

The number of Female patients in groups A, B, C and D were 4, 5, 3 and 2 patients, respectively.

The number of Male patients in groups A, B, C and D were 17, 16, 18 and 19 patients, respectively.

The level of consciousness of patients included in the study is as follows:

The number of patients with GCS in range of 3 to 8 in groups A, B, C and D were 8, 10, 10 and 11 patients, respectively.

The number of patients with GCS in range of 9 to 12 in groups A, B, C and D were 13, 11, 11 and 10 patients, respectively.

The number of deaths in the current study after 28 days in groups A, B, C and D were 7, 3, 3 and 1 patients, respectively.

Blinding

The study was single blind. None of the participants, laboratory technicians, CT scan, clinical nurse, and statistical consultant knew the type of participants.

Randomized

The study was randomized as a random allocation rule. The groups were numbered sequentially and assigned to the next group before the sample entered the study. For example, the present sample was in group B and the next sample was accepted in group C before entering the hospital.

Studied groups

Four groups were included in this study:

1. Control group: Patients with TBI who did not take an intervention and received routine hospital treatment.

2. Head cooling group: Patients with TBI whose head were cooled down.

3. RIC technique group: Patients with TBI who underwent remote ischemic conditioning (RIC) technique.

4. Head cooling and RIC technique: Patients with TBI who received both head cooling and RIC techniques.

The Head Cooling Technique

Hypothermic caps are usually made of synthetic materials such as neoprene, silicon or polyurethane. They cool with a cooling agent such as ice and gel that can be cooled down to -25°C to -30°C before use. Ice caps are filled with a chillon gel that freezes up to -30°C for multiple usages. Since caps are heated during usage, several ice caps are prepared. The used cap has to be replaced every 20-30 minutes with a completely frozen ice cap. In groups that used an ice cap, treatment started immediately after the patient was admitted. The cap was used for the first three consecutive days after admission for 0 to 6 hours (an average of 4.5 hours), depending on the patient's condition and tolerance, to maintain the brain temperature at 33 to 34 degrees Celsius. The researcher measured the temperature of the brain with a tympanic thermometer (Harris, Andrews et al. 2012, Li and Jiang 2012, Papo 2014, Chen, Chen et al. 2017).

Remote ischemic conditioning (RIC)

RIC will be performed using a standard manual blood pressure cuff. The pressure in the blood pressure cuff will be maintained at 25 mm of Hg higher than the patient's systolic blood pressure. Each cycle consists of 5 min of controlled upper limb ischemia (cuff up) followed by 5 min of reperfusion (cuff down). The total duration of the treatment cycle will be 40 min (Saxena, Newman et al. 2010). This will be done simultaneously by placing an ice cap within the golden hours after injury (Munk, Andersen et al. 2010, Seino 2013). This was done simultaneously by putting the ice cap within the golden hours after injury. The effect of interventions was investigated on GCS, the response of pupils to light, hypotension, hypoxia, SAH, Epidural mass and hemoglobin at admission, 72 hours after admission, 6 days after admission, and 28 days after admission. ELISA kits were used for patient samples to measure serum levels of IL-10, TNF-α and SOD (Papo 2014).

The Glasgow score (GCS)

The Glasgow score (GCS) from 13 to 15 represents a mild TBI that did not enter the study. GCS 8 to 12 represents the medium TBI and the patients with this score range were included in the study. GCS 3 to 7 indicates intense TBI and the patients with this score range were included in the study. Patients with a GCS of 3, fixed and dilated bilateral pupil, and those who were injured to the extent that they were thought not to survive were excluded (Teasdale and Jennett 1974).

The Glasgow Outcome Scale (GOS)

The Glasgow Outcome Scale (GOS) is a global scale for functional outcome that puts patient's status into one of five categories: Dead, Vegetative State, Severe Disability, Moderate Disability or Good Recovery

(Jennett and Bond 1975, Wilson, Pettigrew et al. 1998, Wilson, Slieker et al. 2007). The Extended GOS (GOSE) provides more detailed categorization involving eight categories by subdividing the categories of severe disability, moderate disability and good recovery into a lower and upper category (Jennett and Bond 1975, Wilson, Pettigrew et al. 1998, Wilson, Slieker et al. 2007):

Marshall CT Scan

Marshall CT Scan is a classification system based on CT scan findings (Zhu, Wang et al. 2009). Its application is in the rapid diagnosis of intracerebral hypertension, which makes it possible for rapid intervention. The Marshall classification of traumatic brain injury is a CT scan derived metric using only a few features and has been shown to predict the outcome in TBI patients (Eisenberg, Gary et al. 1990, Marshall, Marshall et al. 1992, Saatman, Duhaime et al. 2008, Munakomi, Bhattarai et al. 2016). The Marshal system places patients into one of six categories (I to VI) of increasing severity on the basis of findings of non-contrast CT scan of the brain. Higher categories have worse prognosis and survival rate. It is primarily concerned with two features:

1. Degree of swelling, as determined by

- midline shift and/or
- compression of basal cisterns

2. Presence and size of contusions/hemorrhages referred to as “high or mixed density lesions” (Eisenberg, Gary et al. 1990, Marshall, Marshall et al. 1992, Saatman, Duhaime et al. 2008, Munakomi, Bhattarai et al. 2016).

Marshall CT Scan Classifications is as follows:

- Diffuse injury I (no visible pathology):

  No visible intracranial pathology

- Diffuse injury II:

  Midline shift of 0 to 5 mm

  Basal cisterns remain visible

  No high or mixed density lesions >25 cm3

- Diffuse injury III (swelling):

  Midline shift of 0 to 5 mm

  Basal cisterns compressed or completely effaced

  No high or mixed density lesions >25 cm3

- Diffuse injury IV (shift):

  Midline shift >5 mm

  No high or mixed density lesions >25 cm3

- Evacuated mass lesion V:

  Any lesion evacuated surgically

- Non-evacuated mass lesion VI:

  High or mixed density lesions >25 cm3

  Not surgically evacuated (Eisenberg, Gary et al. 1990, Marshall, Marshall et al. 1992, Saatman, Duhaime et al. 2008, Munakomi, Bhattarai et al. 2016).

APACHE II

APACHE II is a disease-categorizing system that relies on scoring based on the 12 initial values of routine physiological measurements, age, and previous health status. A high score (ranging from 0 to 71) is closely associated with the threat of hospital death (Knaus, Draper et al. 1985).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data analysis was performed using SPSS software version 22 and T-test and Anova tests.

T-test and Anova tests was carried out using SPSS software version 22 to calculate significance. p values are indicated on Tables. Results are expressed as mean ± standard error (SD).

RESOURCE AVAILABILITY

CONTACT FOR REAGENT AND RESOURCE SHARING:

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Dr. Amir Moghadam-Ahmadi; a.moghadamahmadi@gmail.com

MATERIALS AND DATA AVAILABILITY:

According to the researcher's commitment to the families of patients, patients' demographic data is encoded and can not be accessed in any way.

Raw data collected from tests and scales and CT scans of patients were recorded in the database of the Mendeley Data website, which is available to the public indefinitely. The study data is recorded on the Mendeley Data website at the following address:

Hodoodi, Fardin (2020), “TBI”, Mendeley Data, V2, doi: 10.17632/rr5xf4p4j7.2

http://dx.doi.org/10.17632/rr5xf4p4j7.2

https://data.mendeley.com/datasets/rr5xf4p4j7/2