The effect of mild hypothermia therapy in the level of MMP-9 protein and the Marshall CT score in high risk traumatic brain injury

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Abstract

The effect of mild hypothermia therapy (34°-36°C) and alterations of matrix metalloproteinase-9 (MMP-9) were examined in 20 patients with high risk traumatic brain injury (TBI). The neurologic status was assessed using the Full Outline of UnResponsiveness (FOUR) score and the outcome using the Marshall CT score. The objective of this study was to determine serum MMP-9 level and the Marshall CT score. This research used a prospective randomized controlled study and was conducted in RD Kandou Hospital Manado. Patients with high risk TBI (the FOUR score ≤7) were randomized into two groups, with and without mild hypothermia therapy, and were investigated within 24 and 72 hours. The MMP-9 protein levels were estimated using enzyme-linked immunosorbent assay (ELISA). Different levels of these variables were compared in the two groups. The results showed that the level of MMP-9 protein significantly decreased ($p<0.05$) in the hypothermia group; however, there was no significant improvement of the Marshall CT score ($p>0.05$) within 24-72 hours. The study concluded that mild hypothermia therapy had a significant influence on the alteration of biomarkers rather than the alteration of anatomical imaging in high risk TBI patients.

Key words: Mild hypothermia, MMP-9, Marshall CT score, TBI.

Introduction

Traumatic brain injury (TBI) is a changed brain function that manifests in decreased consciousness, seizures, coma, or neurologic deficits caused by an impact on the head. (1) In the USA, this type of injury caused 290,000 hospital admissions, 51,000 deaths, and 80,000 permanently disabled survivors. (2) In Indonesia, the prevalence of national injuries was 8.2% with the following details: 14.9% of head injury with a concussion of 0.4% where the victim of motorcyclist was 40.6% with the largest age group of 15-24 year-old (11.7%) and the prevalence of male by 10.1%. (3) TBI continues to be a significant cause of mortality, morbidity, and economic burden globally. (4)

Computerized tomography (CT) scan of the brain remains a standard diagnostic tool for assessing TBI, and it is also used for outcome prediction. In the acute stage of TBI, brain CT scan is the most frequently used neuroimaging method. The brain injury may be characterized based on its finding, e.g. presence of focal lesion, mass lesion, or diffuse brain injury. In order to systematize such pathological changes after TBI, imaging features have been combined into classification systems such as Marshall CT score. The Marshall CT score class I-IV comprises a diffuse injury severity rating scale and class V-VI reflects a mass lesion. (5-7)

The principle treatment of the TBI patients was to prevent the process of secondary brain injury. Biochemical changes and cellular metabolism lead to increased intra-cranial pressure, damage to blood-brain barrier (BBB), neuroinflammation, cerebral edema, brain hypoxia, ischemia, and neurodegeneration. (8)

In many types of brain pathologies, including TBI, matrix metalloproteinase-9 (MMP-9) is markedly upregulated 24-72 hours and this is thought to cause BBB disruption. (9) MMP-9 may be involved in the pathophysiology of neural damage

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after TBI. MMP-9 is a family of zinc dependent endopetidases enzyme, degrading various components of extracellular matrix. This enzyme causes endothelial lamina degradation, e.g. laminin, fibronectin, collagen, proteo glycans, zonula occludens-I (ZO-I), occludin and claudin. The degradation process will cause cytoskeletal damage, disrupting cellular homeostasis, ischemia, inflammation, tissue necrosis, and cell death or apoptosis. (10) The elevation of MMP-9 contributes to cerebral edema, neuronal death, and the associated severity of neurological deficits. The important effort in the treatment of secondary brain injury is to provide neuroprotectors, and the selected ones are able to inhibit many cascades with mild hypothermia. (11,12) Hypothermia is believed to reduce neuroinflammatory processes as an integral part of secondary brain injury. (13) Pardamean concluded that mild hypothermia therapy improved neurological outcome in patients with severe TBI. (14)

The aim of this study was to demonstrate the beneficial effect of the mild hypothermia therapy in high risk TBI patients. We performed evaluation based on anatomical imaging assessment using the Marshall CT score and the level of MMP-9 protein.

Materials and methods

The study covered a period of time, that was, from September to December 2016 in RD Kandou Hospital Manado. The samples were patients with high risk TBI who came to the Emergency Unit of RD Kandou Hospital Manado. All of the patients received a standard therapy, which is recommended by Brain Trauma Foundation Guidelines 2007. The protocol and consent procedures were approved by the Human Research Review Committee of RD Kandou Hospital. Written informed consents were obtained from patients' family members for inclusion in the study. All patients were with high risk isolated closed TBI (Full Outline of UnResponsiveness [FOUR] score ≤7) and confined the Marshall CT score (class I-III). They had fulfilled the inclusion criteria, and then were divided into 2 groups: the control and the hypothermia treatment, which was performed with the Marshall CT score and the level of MMP-9 protein. These patients were randomly put into the mild hypothermia therapy group (n=10) and the control group (n=10). After 24 and 72 hours, the Marshall CT score and the level of MMP-9 protein were examined.

The mild hypothermia therapy on patients with a high risk TBI, with a decrease in normal body temperature to 34-36°C, was carried out with an ice packed on the entire body of the patients (surface cooling). The mild hypothermia therapy consists of three phases: the induction, maintenance, and rewarming phase (0.5 to 1°C/hour); and the total overall time was 72 hours.

SPSS software V.20.0 (SPSS Inc., Chicago, IL, USA) was employed to input and analyze data. Univariate and bivariate analysis was performed on significant boundaries where p-values were considered when $p<0.05$. The tests of different changes in the level of MMP-9 and the Marshall CT score used independent t test or Mann-Whitney U and Wilcoxon rank test.

Results

Basic characteristics

Subject characteristics and group homogeneity can be seen in the analysis summary in **Table 1**. As shown in **Table 1**, there was no significant difference in characteristics ($p>0.05$) between the two groups. The two groups can be considered as homogenous data based on the characteristics of sex, age, and onset of hospitalization.

Mild hypothermia therapy and the serum MMP-9 level

Mild hypothermia therapy influenced the protein content of serum MMP-9 at the time of 24 and 72 hours, which can be seen in **Table 2** and **Figure 1**. **Table 2** shows that there was a significant increase in MMP-9 protein ($p<0.05$) of 98.10 pg/ml in the control group, whereas in the group of patients receiving mild hypothermia, there was no significant change ($p>0.05$) within 24 hours. There was a significant difference in the level of MMP-9 ($p<0.05$) between the two groups within 72 hours. The level of MMP-9 protein in the hypothermia group (309.98±226.84 pg/ml) was lower than in the control group (553.37±198.87 pg/ml) within 72 hours. **Figure 1** presents that within the period of 24-72 hours, the level of MMP-9 protein tended to decrease in the mild hypothermia therapy group and contrariwise in the control group. The obvious difference of level of MMP-9 protein occurred within 72 hours.

Mild hypothermia therapy and the Marshall CT score

The effect of mild hypothermia therapy on the alteration of the Marshall CT score can be seen in **Table 3** and **Figure 2**. **Table 3** shows that there was a slight improvement of the Marshall CT score. However, there was no significant difference ($p>0.05$) within 24 and 72 hours in the mild hypothermia therapy group and the control group. **Figure 2** presents that the Marshall CT score was

starting to coincide during the period of 24 hours in the mild hypothermia therapy group and the control group. It was clear that the Marshall CT score decreased in the mild hypothermia group within 72 hours.

Figure 3 shows the comparative alteration of each mean variable in both groups and there was a significant change in mean variable of the serum MMP-9 level ($p<0.05$), while the Marshall CT score mean variable did not change significantly ($p>0.05$).

Discussion

There was a significant increase in level of MMP-9 protein in the control group; whereas in the mild hypothermia therapy group, there was no significant decrease within 24-72 hours. There was a significant decrease in the level of MMP-9 protein in both control and mild hypothermia groups within 72 hours. This was in accordance with the nature of synthesis of the MMP-9 enzyme, which is temperature sensitive. By providing mild hypothermia therapy action, there will be an inhibition of MMP-9 synthesis and regulation. Hypothermia provokes down-regulation of extracellular signal-regulated kinase (ERK) phosphorylation. Degradation of ERK results in reduced nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) dependent proinflammatory gene expression in MMP-9 synthesis. (15-17) Hypothermia appeared to regulate MMP-9 expression in particular at the transcription level and suppressed its protease activity. (18,19)

In the acute period of TBI, brain CT is sensitive in detecting skull fractures, intracranial lesions, intracranial hematomas such as subarachnoid hemorrhages and subdural hematomas, and mass effect. (20) Detection of these macroscopic lesions in the acute setting of a trauma is crucial for patient prognosis and provides essential information for determining correct patient management. Intracranial hemorrhages as a result of trauma will appear hyperdense compared to gray manner in the acute setting, but transitions to isodense during the subacute and chronic periods. (21)

This study proves that the effect of mild hypothermia therapy improved the Marshall CT score; however, it was not significant. The results evaluate that the anatomical radiological use of the Marshall CT score did not indicate considerable improvement of the anatomical features due to the mild hypothermia within the periods of 24 and 72 hours. This possibility can be attributed to the improvement of pathological anatomical structures including bleeding, edema, and ischemia that require longer mild hypothermia therapy action time. Another possibility is that more than 72 hours of observation are needed to determine the improvement of the pathological process on CT scan. This is because the alteration of CT scan images in patients with TBI occurs within 3-5 days. (22,23) On the results of CT scan, it was found 35 to 60% of brain contusions picture. The brain contusions are a density mixture consisting of a picture of necrosis, bleeding, infraction, and ischemia that determine the degree of lightness of a trauma. Contusions are formed from areas of white color or hyperdensity that describe bleeding areas on CT scan images. Hemoglobin in the blood will lose oxygen and become deoxyhemoglobin within 10-15 days and will dissolve into methhemoglobin. (24)

Anatomical imaging and time processes are notable part of the secondary brain injury. The mechanism of secondary damage is an important point of neuroprotective treatments of TBI that should be extended up to 72 hours. A reason for using more long-term cooling is that cerebral swelling is often the greatest in 3-5 days after injury. (25) Peterson reported that reduction in risk mortality was the greatest and most favorable neurologic outcome, much more common when hypothermia was maintained for more than 48 hours. (26)

Conclusion

The mild hypothermia therapy in high risk TBI patients significantly decreased the level of MMP-9 protein and did not significantly improve the Marshall CT score within 72 hours.

Conflict of interest disclosure

The authors report no conflicts of interest. The authors have no personal, financial, or institutional interest in any of the drugs, materials or devices used in the study.

Table 1. Characteristics of subjects

Characteristics	Group		P
	Control (n=10)	Hypothermia (n=10)	
Gender (M/F)	6/4	7/3	0.500*
Age (years): mean (SD)/min-max	29.1 (8.5)/20-44	29.3 (8.4)/20-43	0.958**
Onset (min): mean (SD)/min-max	75.0 (21.2)/45-120	79.0 (22.8)/45-120	0.690**

Legend: M=male; F=female; SD=standard deviation; *=Fisher's exact; **=Independent t test.

Table 2. The changes in the level of MMP-9 protein in subgroups

Group	MMP-9 (pg/ml); mean±SD			P
	24 hours	72 hours	Δ	
Control	455.27±74.76[b]	553.37±198.87[a]	98.10[q]	0.037**
Hypothermia	460.57±62.00[b]	309.98±226.84[c]	-150.59[p]	0.203**

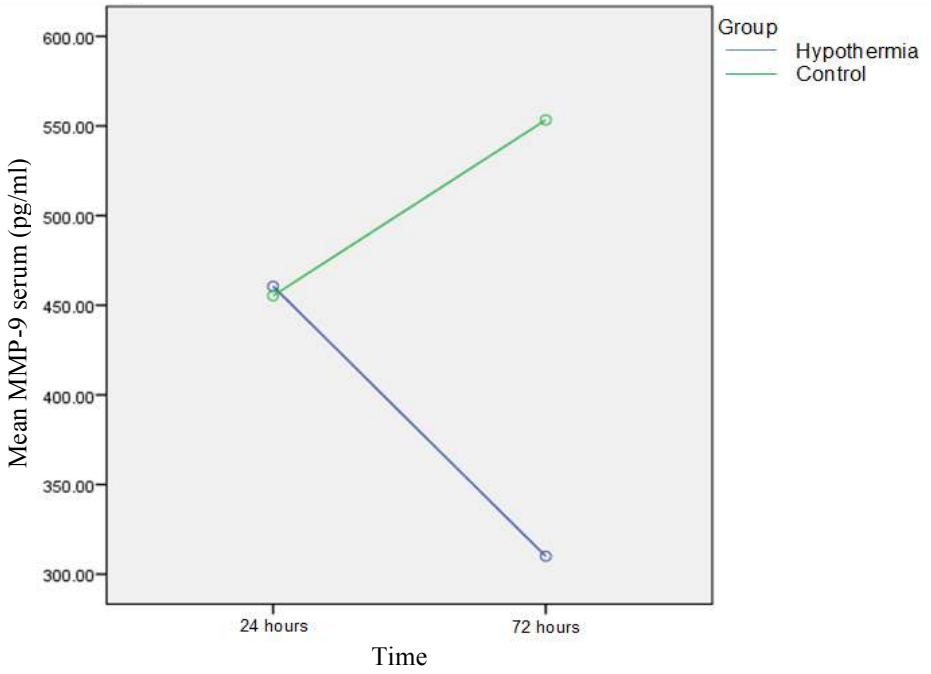
Legend: MMP-9=matrix metalloproteinase-9; SD=standard deviation; Δ=time differences 24 and 72 hours; [a,b,c,p,q]=same letter indicates not significant ($p>0.05$) and different letter indicates significant ($p<0.05$); **=Wilcoxon test.

Table 3. The changes in the Marshall CT score in subgroups

Group	Marshall CT score (mean±SD)			P
	24 hours	72 hours	Δ	
Control	2.7±0.5[a]	2.6±0.5[a]	-0.1[a]	0.317**
Hypothermia	2.7±0.5[a]	2.3±0.8[a]	-0.4[a]	0.102**

Legend: SD=standard deviation; Δ=time differences 24 and 72 hours; [a]=not significant; **=Wilcoxon test.

Figure 1. The changes in the level of MMP-9 protein serum in the two groups within 24-72 hours



Legend: MMP-9=matrix metalloproteinase-9.

Figure 2. The changes in the Marshall CT score in the two groups within 24-72 hours

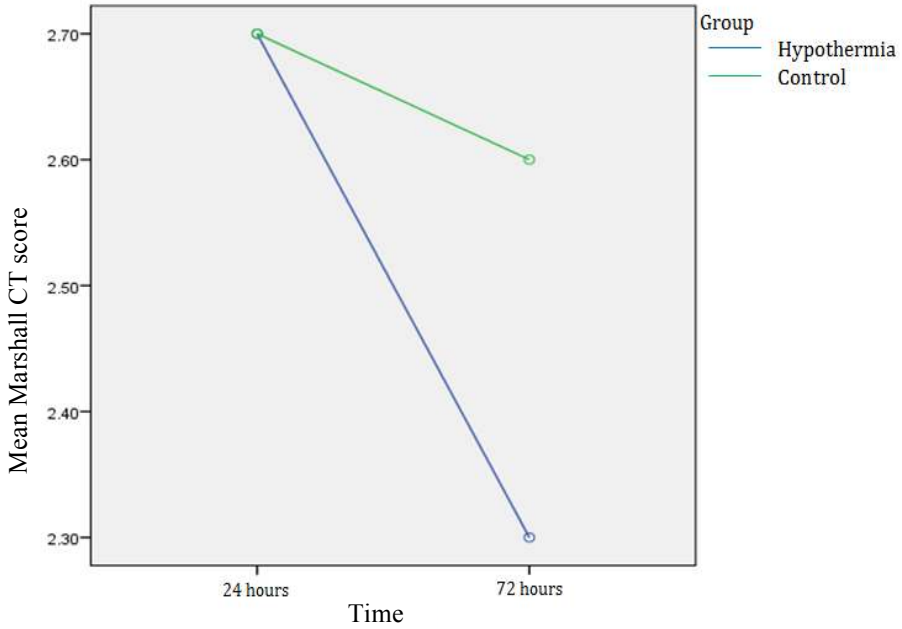
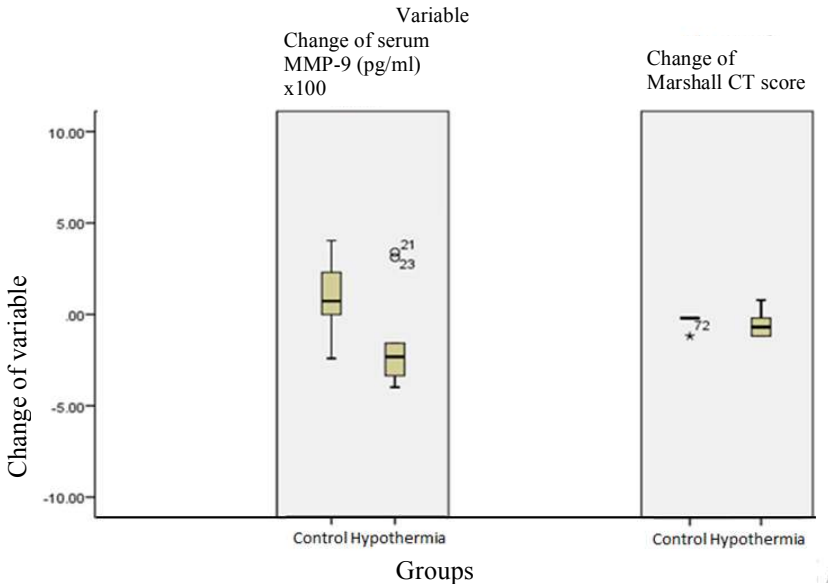


Figure 3. The Box-Whisker graph of changes in mean the level of MMP-9 protein and the Marshall CT score in the two groups within 24-72 h



Legend: MMP-9=matrix metalloproteinase-9.

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