Research Article

Serum Superoxide Dismutase as A Biomarker in Severe Traumatic Brain Injury: A Case-Control Study

Running Title: Serum Superoxide Dismutase as Prognostic Biomarker

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HIGHLIGHTS

- Serum super-oxide dismutase (SOD) level were evaluated as potential biomarkers in Traumatic Brain injury (TBI) patients
- Significant differences were found between TBI patients and healthy controls
- Findings support further research in larger population and in Cerebrospinal fluid to validate the role of SOD in TBI

PLAIN LANGUAGE SUMMARY

Traumatic brain injury (TBI) is a serious condition that occurs when a sudden impact or injury to the head damages the brain. It can lead to long term physical and mental challenges, and identifying how severe the injury is can be difficult. Biomarkers are substances found in blood or other body fluids, can help doctors assess the extent of brain injury and predict a patient's recovery. One such biomarker is an enzyme called Super-oxide dismutase (SOD), which helps protect cells from damage caused by harmful molecules known as free radicals.

In this study, we measured the levels of SOD in the blood of patients with TBi to explore if these levels could provide useful information about their condition. The study was conducted at a tertiary care hospital in New Delhi, India, where blood samples were collected from TBI patients and compared to healthy individuals. We aimed to see whether the SOD levels in TBI patients were significantly different from the healthy group, and if this difference could be linked to the severity of the injury.

Abstract

Background and Aim: Traumatic Brain Injury (TBI) is often described as a "hidden epidemic." Various biochemical markers reflecting cerebral damage can be used to correlate the patient's prognosis and the development of secondary lesions. We studied human Superoxide dismutase (SOD) in the serum to evaluate its role in the outcome of TBI.

Methods and Materials/Patients:

This observational study was conducted in patients with severe TBI who presented to a tertiary care hospital in India. A total of 40 patients with severe TBI were enrolled, with 40 healthy people taken as controls. Serum samples were assayed for serum SOD using the ELISA technique.

Results:

The most common age group was 28-37 years. Of 40 patients, 28 were male (70%), and 12 were female (30 %). The most common mode of injury was road traffic accident (70%), followed by fall from height (12.5%), physical assault (12.5%) and sports injury (5%). The most common CT finding was intra-cerebral haematoma (55%), followed by sub-arachnoid haemorrhage (SAH) (10 %), skull fracture (10 %), subdural haemorrhage (SDH) (7.5%) and extradural haemorrhage (EDH) (5%). The mean serum SOD value in the severe TBI group was 23.23 U/ml, and in the control group, it was 135.93 U/ml, which was statistically significant. Out of 40 patients, 24 (60%) had a good Glasgow Outcome Scale (GOS) at the time of discharge, and 16 (40%) had poor outcomes. There was a significant improvement in outcome after six months compared to GOS at discharge.

Conclusion:

The results obtained in the study are preliminary, and more extensive prospective studies are needed to reach a definitive conclusion. A handful of studies on protein degradation products are available and need to be more comprehensive. In conclusion, with more extensive studies and continued exploration SOD can become a reliable tool in TBI and can be integrated into standard care protocols.

Keywords: Traumatic Brain Injury (TBI), SOD (Superoxide dismutase), biomarker, GOS.

INTRODUCTION

Measuring tissue or body fluid biomarkers can determine specific biological or disease states, with changes in enzyme activity, protein expression, gene expression, and metabolites playing key roles. Traumatic brain Injury (TBI), often called a “hidden epidemic”, is a significant global health and socio-economic challenge(2), leading to high rates of death and disability. Quantifying TBI is complex due to varying definitions and underreporting, but Asia, particularly India(8), has seen a rise in incidence, with many cases going unreported or untreated.

Biochemical markers correlating with cerebral damage severity and prognosis are crucial in identifying high-risk patients and guiding preventive measures. Brain-specific markers measurable in serum are particularly promising for patient care, with limited studies examining cerebrospinal fluid. Recent research suggests that Human Super oxide dismutase (SOD) may serve as a serum marker for TBI(3), becoming inactivated post-injury, with increased microvascular superoxide radical production following TBI.

AIMS AND OBJECTIVES

- To estimate the level of serum superoxide dismutase in patients with severe traumatic brain injury.
- To compare the levels of serum superoxide dismutase in severe TBI cases with a control population.

Methods and Materials/Patients:

This case-control study was conducted on patients with traumatic brain injury (TBI) presenting to a tertiary care Hospital in New Delhi, India from 1st December 2019 to 15th January 2021.

The Institutional Ethical Committee ABVIMS and Dr RML Hospital gave ethical approval for this research vide order no TP(DM/MCH) (30/2019) / IEC/ABVIMS/RMLH/863.

INCLUSION CRITERIA

Patients with Age > 18 years, a Closed head injury presenting up to 6 hours from injury, and a Glasgow coma scale (GCS) at the time of presentation less than 8 and Family or next of kin available to provide written informed consent were included in the study.

EXCLUSION CRITERIA

Patients with less than 18 yrs. Age, severe coagulopathy like excessive bleeding, platelet count < 1,00,000, International Normalized Ratio >1.4, or partial thromboplastin time >50, clinical indication for long-term anticoagulant therapy like life-threatening deep vein thrombus, pulmonary embolism, cardiac lesions. Lack of informed consent, history of chronic alcohol abuse or any other substance addiction, or known psychiatric

illness requiring sedatives or neuroleptics, any central nervous infection during hospitalisation and pregnant females were excluded from the study.

SAMPLE SIZE

Sample size was calculated using the formula $n = Z^2[P(1-P)]/D^2$ (daniel 1999) where in minimum sample size came to be 72 with a prevalence of 20% and margin of error of 10%

STUDY PROTOCOL

Patients who presented within 6 hours of injury were included in this study. The patient's demographic information was recorded at the time of enrolment. The GCS score was calculated at the time of admission, on the 3rd and 7th day.

The time of injury, associated injuries, and arrival of the patient at the hospital emergency and cranial CT imaging findings were recorded. The clinical outcome was assessed using the GOS.

The mechanism of injury was categorised as a fall from height, road traffic accidents, assaults, a fall of an object on the head, or any other mode, depending on the case. The patients were called back for follow-up after six months.

Venous blood samples of enrolled patients were collected in a 10 ml serum separator tube (SST) at the time of presentation. After collection, the sample was allowed to clot at room temperature for 10 to 20 minutes or to sleep at 4 degrees and then centrifuged at 3000 rpm for 10 minutes. If sedimentation occurs during storage, centrifugation is repeated. The serum samples were frozen and later assayed for serum superoxide dismutase (SOD) using the ELISA technique.

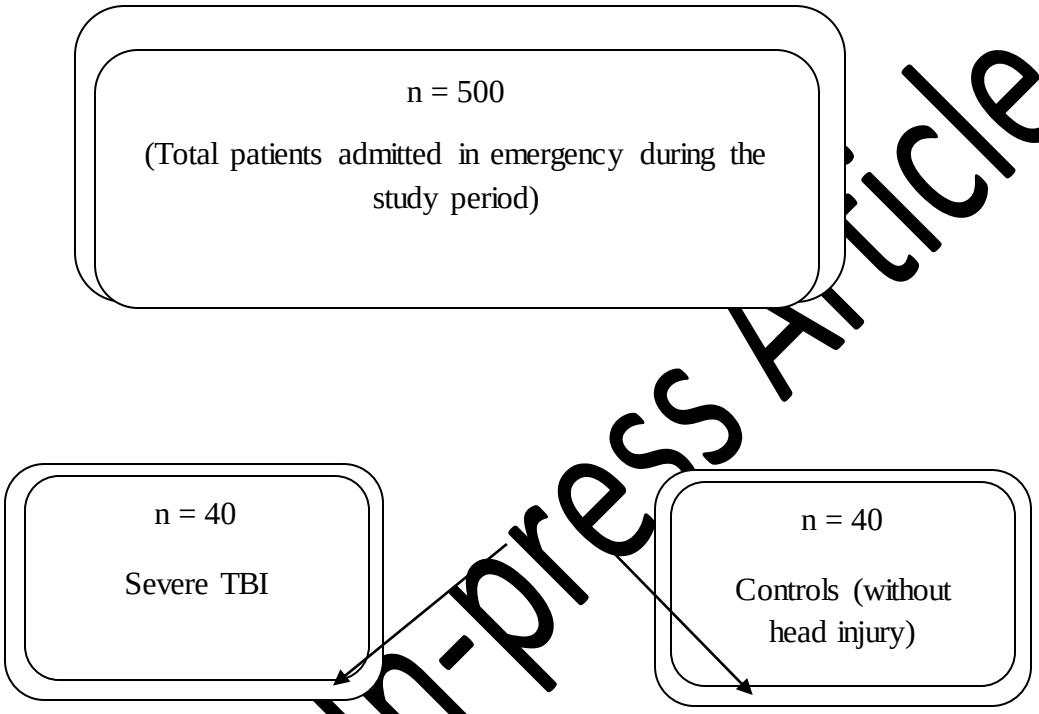
Statistical Analysis

Data was entered into MS Excel and analysed using SPSS Version 16.0. Continuous parametric data was reported as Means and standard deviation, while continuous non-parametric data was reported as median and interquartile range. Categorical data was reported in percentages. A comparison of categorical data between the groups was done using the Chi-square test. Comparison of continuous data between two groups was performed using an independent t-test, and between three groups was performed using Friedman Test (Figure 3).

OBSERVATION AND RESULTS

In this study, 500 patients were admitted to the emergency department of neurosurgery. Fulfilling the selection criteria, 40 patients with severe head injuries were finally included in the study. The control group also consisted of 40 patients.

A flow chart shows patients screened and included in the study group for severe TBI.



The most common age group in this study was 28-37 years (33.2%), followed by 18-27 years and 38-47 years (22.5%) in each group). Out of 40 patients with severe head injuries, 28 patients were male (70%), and 12 patients were female (30 %).

The most common mode of injury in this study was RTA (70%), followed by height fall (12.5%), physical assault (12.5 %) and sports injury (5%). [Table 1]

The most common CT finding in the study was intracranial haematoma (55%), followed by SAH (10 %), skull fracture (10 %), SDH (7.5%), and EDH (5%) [Table 2].

The mean serum SOD value in the severe head injury group was 23.23 U/ml, and in the control group was 135.93 U/ml. The difference was statistically significant, with a p-value of 0.001[Table 3].

The mean GCS score on day 0 was 6; on day three, it was 9.8; and on day seven, it was 10.08. [Table 4, figure 1]

A pairwise comparison of GCS differences on day 0, day 3 and day 7 showed significant improvements in GCS from day 0 to day 7 and from day 0 to day 3. But no significant difference in GCS was noted between day 3 and day 7 (Table 5, figure 2)

Out of 40 patients, 24 (60%) had good GOS at discharge, and 16 (40%) had poor outcomes during discharge. At six months of follow-up, 20 patients had good GOS, and four had poor GOS. The rest of the patients were lost in follow-up. There was a significant improvement in outcome after six months compared to GOS at discharge (Table 6).

DISCUSSION

HulyaBayiretal (1) reported in their study that the age range in patients with TBI was 21 to 62, compared to 16 to 77 in control groups. In this study, we observed that severe traumatic brain injury (TBI) predominantly affected individuals in the prime of their lives, with the most common age group being 28-37 years. This finding is consistent with previous research, highlighting that TBI disproportionately impacts younger adults who are more actively engaged in outdoor activities, thereby posing significant socio-economic challenges. The male predominance in TBI cases aligns with global data, emphasizing the need for targeted interventions to reduce risk in this demographic.

A study in mice by Takuji et al. (11) reported gender differences in the loss of cortical and subcortical neurons after TBI in animals with higher CuZn SOD. The study showed that male transgenic mice (Tg-M) significantly reduced the volume of the cortex lesion compared to male non-transgenic mice (nTg-M). However, compared to non-transgenic female mice (nTg-F), there was no similar protection in female transgenic mice (Tg-F). It is hypothesised that the female ischemia brain benefits from the antioxidant properties of estrogen. A similar beneficial effect can explain the neuroprotection in traumatised female brains.

In our study, in the severe TBI group, the most common mode of injury was RTA, followed by fall from height, assault and sports injury. The mean serum SOD levels in

RTA cases was 22.65±11.02 ng/ml, fall from height 24.00±15.29 ng/ml, assault 22.28±11.38 ng/ml, sports injury 31.77±9.57 ng/ml (p=0.750).[Table 1] In severe TBI, the serum SOD values did not show statistical significance in variation with the mode of injury.

In an epidemiological study by NIMHANS, the most common cause of TBI was falls (20%), assaults (10%), and falls of objects. Gururaj G et al. 1999.Odero et al. 1997 (Gururaj 2002) conducted a review of Road traffic accidents (RTA) and found that in developing nations, pedestrians, motorcyclists, and bicyclists were at high risk of sustaining head injuries. This also fits well in the Indian context but not in Western countries, where motor vehicle occupants are at a greater risk than motorcyclists and bicyclists.

In severe TBI patients, serum SOD was (23.22±11.31 ng/ml) lower than the control group (135.93±33.27 ng/ml), which was statistically significance (p<0.001) in our study. [Table 3]

A study by Cernak et al. (5) aimed to investigate the plasma SOD activity of patients with severe TBI reported an initial significant increase in plasma SOD activity followed by a substantial reduction in activity by the end of the posttraumatic period.

A study by Chandrika D Nayak et al. (16) to evaluate and compare the oxidative changes in patients with varying severity of TBI in the early posttraumatic period using erythrocyte indicators reported that the SOD activity was significantly increased only in SHI patients and remained unchanged in MHI patients as compared to controls. They concluded that early oxidative changes might reflect the severity of neurological insult and provide an early indication of patient outcome in TBI.

A study by Adolfo Paolin et al. (18) to establish the time course of reactive oxygen species after reported SOD activity increased significantly at 12 and 24 hrs post-trauma as compared with the time of onset of head injury. They came to the conclusion that Reactive oxygen species-mediated oxidative damage can play an essential role in determining the prognosis of severe brain injury in humans.

A study by E I Lvovskaya et al. (15) on the Prognostic value of the parameters of free radical oxidation in traumatic brain injury reported an increase in the level of total antioxidant activity, accompanied by the growth of glutathione peroxidases and catalase activity against the background of a decrease in SOD activity from 1 to 7-day post trauma.

In our study, the mean GCS was 6.00 on day 0, 9.80 on the 3rd day, and 10.08 on the 7th day.[Table 4] The distribution of serum SOD in the severe TBI group varied in all categories of GCS at zero days (p=0.721). The mean serum SOD levels in patients with GCS 3, 4, 5, 6, 7, and 8 were 16.11±5.49 ng/ml, 18.29±10.91, 22.72±14.98 ng/ml, 25.01±7.21 ng/ml, 24.15±13.88 ng/ml and 26.64±13.83 ng/ml respectively. Paired comparison of different GCS scores about serum SOD levels showed varied results.

This shows that with the changes in GCS, the level of serum SOD varies. In our study, patients with lower GCS had significantly lower levels of serum SOD level which was further associated with poorer outcomes. This difference in levels may be studied in further detail with a large sample size focused on temporal serum SOD measurements along with GCS scores. Correlating the serum SOD levels and the GCS at which it is significant may help us to guide further in qualitatively analysing the significant serum SOD levels and may shed some light on its role in projecting TBI prognosis.

In the study by Kasprzak et al. (13), enhanced lipid peroxidation, as assessed by CSF, in 30 patients with brain contusion was correlated with the severity of head injury in adults with a contusion. Compared with controls, during the 10-day follow-up, patients with brain contusion had significantly increased erythrocyte SOD activity. The highest CSF SOD concentrations were observed in 5 patients who died 2, 7, or 8 days after the head injury. However, although CSF samples may more directly reflect changes in brain injury, in clinical practice, CSF sampling is not easy.

In a study by Nwachuku E et al. (17) on CSF biomarkers in TBI, CSF concentrations of inflammatory biomarkers had a significant association with 6-month neurological outcome (p-values<0.05 for each marker), with the favourable outcome group having lower concentrations of these biomarkers on average, in comparison to the poor neurologic outcome group over the first five days after TBI.

In the current study, serum SOD levels were correlated with the outcome at discharge, but no significant difference was obtained (p= 0.991)[Table 6]

Hamm RJ et al. 1993 (10) reported the outcome of severe head injury with the oxygen radical scavenger polyethene glycol-conjugated superoxide dismutase (PEG-SOD) using the Glasgow Outcome Scale at 3 and 6 months post-injury in 91 and 93 patients, respectively, by blinded observers not involved in the clinical management of the patients. At three months, 44% of patients in the placebo group were vegetative or had died, while only 20% of patients in the group receiving 10,000 U/kg of PEG-SOD were in these outcome categories (p<0.03, multiple logistic regression test); at six months,

these figures were 36% and 21%, respectively (p=0.04). Though differences in outcome between the placebo group and either of the other two groups were not statistically significant, it was concluded that PEG-SOD was generally well tolerated and appeared promising in improving outcomes after severe head injury.

CONCLUSION

Based on the findings of this study, serum superoxide dismutase levels appear to be significantly lower in patients with severe traumatic brain injury compared to healthy controls. This suggests that serum SOD may serve as a valuable biomarker for assessing the severity of TBI and potentially guiding clinical management. Further research with larger cohorts and more comprehensive follow-up is required to fully understand the role of SOD and other biomarkers in TBI prognosis and treatment.

Limitations

The study has a small sample size, limiting the findings' generalizability. It also did not include CSF measurements of SOD which might have provided additional insights into disease pathogenesis. The study also lacked repeated measurements which could have helped us understand the dynamics of SOD levels over time and their correlation with the progression of TBI. A significant number of patients were lost to follow-up due to decreased willingness to return for a visit, especially as they started feeling better. These limitations highlight the need for further research with larger, more diverse populations and more comprehensive data collection methods to validate and expand upon the findings of the study.

Ethical Considerations

Compliance with ethical guidelines:

Funding:

Authors' contributions

Conception and design: ?

Data Collection:?

Data Analysis and Interpretation:? Drafting the article:? Critically revising the article: ? Reviewing submitted version of manuscript:? Approving the final version of the manuscript: All authors.
Conflict of interest: there is no conflict of interest.
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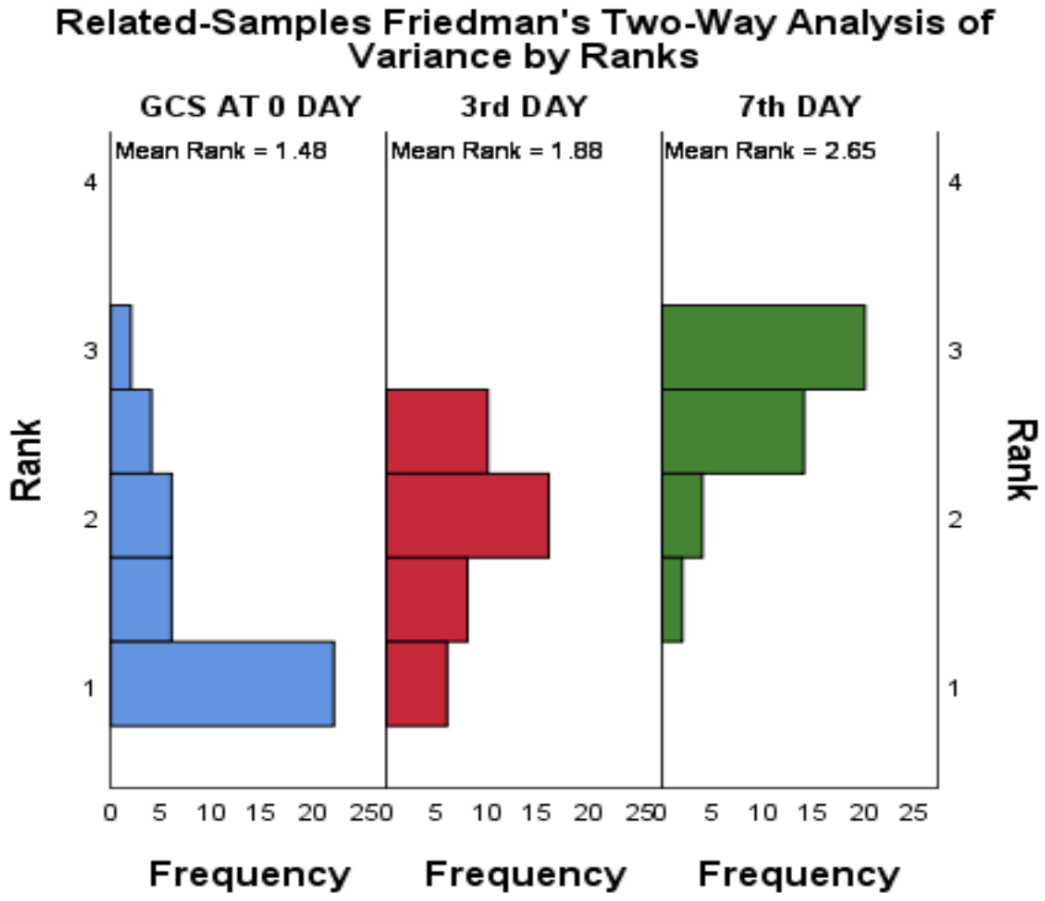
Figure Legends:

Figure 1 Flow chart showing the number of patients screened and total number of patients included in the study

Figure 2 GCS distribution on days 0,3 and 7 in severe head injury.

Figure 3 Pairwise comparison of GCS distribution on days 0,3,7

Figure 4 Related- samples Friedman's two way ANOVA test for GCS at 0,3 and 7th day in severe head injury


Related-Samples Friedman's Two-Way Analysis of Variance by Ranks
GCS AT 0 DAY
3rd DAY
7th DAY
Mean Rank = 1.48
Mean Rank = 1.88
Mean Rank = 2.65
Rank
Frequency
0 5 10 15 20 25
5 10 15 20 25
5 10 15 20 25