Performance of Serum Superoxide Dismutase as a Prognostic Marker in Acute Traumatic Brain Injury Patients from Northern India and Its Clinical Outcome

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Abstract:

Traumatic brain injury (TBI) is primarily based on a clinical assessment, neurological examination and radiological imaging. Tools for diagnosis and risk stratification of intracranial injury are limited in emergency department. In emergency setting, main stay of treatment begin with clinical evaluation which include symptom, Glasgow Coma Scale (GCS), neurologic examination along with the radiological assessment by Computed Tomography Scanning and Magnetic Resonance Imaging. Long term neuropsychological dysfunction is known to occur in majorityof patients with intracranial injury even without radiological evidence. This fuels the need to identify tests which may guide in reducing the sequelae of TBI. Such test may play an important role in under diagnose case with mild TBI, in severe TBI with risk of secondary brain insult, and assessing patient status at peripheral center where immediate neurosurgical facilities are not available. In this study we investigated the role of serum superoxide dismutase as a prognostic markerand outcome of acute traumatic brain injury. The SOD levels were correlated with outcome at discharge in both the mild and severe TBI. Good outcome was defined as Glasgow Outcome Scale (GOS) score 4 and 5, and poor outcome group with GOS score 1, 2, 3. In the mild TBI group serum SOD protein levels in the good outcome group were not significantly higher compared to the poor-outcome group. Thus SOD may prove to be a significant prognostic marker in severe TBI.

Keywords: Serum Superoxide Dismutase; Traumatic brain injury; Glasgow Coma Scale; Computed Tomography Scanning; Magnetic Resonance Imaging
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Introduction

According to the World Health Organization (WHO), traumatic brain injury (TBI) is the leading cause of death among young adults worldwide. TBI affects approximately 50 million individuals annually (Ghaith et al.,

2022). The incidence of TBI is estimated to be 939 in 100,000 worldwide with the major causes being falls, vehicle accidents, wars, and sports (Ismail et al., 2020; Capizzi et al., 2020; Wang et al., 2016).These visible features can cause internal hemorrhage, bruises, lacerations, focal and diffuse injuries, hypoxia, along with interference in axonal connectivity (Williams et al., 2018). TBI further more contributes in the emergence of several neurodegenerative disorders and enhances survivor's possibility of developing chronic behavioral and neurological destructions that influence their quality of life. The mortality rate of TBI worldwide is estimated to be between 7% and 23% with 90% of TBI-related deaths occurring in developing countries (Ismail et al., 2020; Pavlovic et al 2019). Additionally, TBI imposes an economic burden on societies where its annual global cost reaches 400 billion dollars (Ismail et al., 2020; Cornelius et al., 2013).Traumatic brain injuries (TBIs) have high rates of mortality in India, claiming more than 165,000 lives and injuring almost 500,000 people annually (GOI, 2022)

The principal mechanisms of TBI are classified as (a) focal brain damage due to contact injury types resulting in contusion, laceration, and intracranial hemorrhage or (b) diffuse brain damage due to acceleration/deceleration injury types resulting in diffuse axonal injury or brain swelling (Baethman et al. 1998). Outcome,arising out of head injury can beverified through two significantlydiverse methods: (a) the major assault (primary injury, mechanical damage) happening at the time of collision. In terms of treatment, this type of damage is completely impressionable toward preventive but not therapeutic measures. (b) The secondary insult (secondary damage, delayed nonmechanical damage) represents consecutive pathological processes initiated at the moment of injury with delayed clinical presentation. Cerebral ischemia and intracranial hypertension refer to secondary insults and, in treatment terms, these types of injury are sensitive to therapeutic interventions (Werner et al. 2007). The difficulty of measuring the effects of the injury contributes to the challenge of assessing outcomes of TBI patients (Formisano et al. 2004). Early predictors of long-term morbidity in patients with closed traumatic brain injury (TBI) could potentially play an important role in identifying patient at risk for lasting sequel and in targeting costly surveillance efforts and preventive intervention strategies to enhance their functional recovery.

A biomarker is an indicator of a specific biological or disease state that can be measured from sample taken either from the affected tissue or peripheral body fluid. They can be product of alter enzymatic activity, changes in protein expression, or post-translation modification, alter gene expression, protein or lipid metabolites, or a combination of these. Clinically used TBI biomarkers provide information on the

diagnosis, prognosis, and treatment efficiency of TBIs(Zwirner et al., 2022; Dash et al. 2010).No specifically designed test to diagnose TBI is available commercially except for research purpose. We chose to study superoxide dismutase as source of this Manganese superoxide dismutase (MnSOD) provide the first line of deference against superoxide generation in mitochondria. Human MnSOD is a nuclear encoded tetrameric protein consisting of identical 24-kDa subunits, and an N-terminal 24 amino acid sequence signals the mitochondrial compartmentalization (MacMillan-Crow et al., 2001). Analyzing the predictive value of SOD for outcome in TBI is of utmost importance before putting in practice. However, such studies are limited in India.

Materials and Methods

Design

This study was conducted on patients with traumatic brain injury presenting to the Trauma Centre of Institute of Medical Sciences from January 2016 to July 2017.

Inclusion criteria

1. Age >18 years
2. Closed head injury presenting within 6 hrs.
3. No sever coagulopathy which was define as clinical evidence of excessive bleeding, platelet count < 1,00,000, International Normalized Ratio >1.4, or partial thromboplastin time >50.
4. No clinical indication for future anticoagulation e.g., life – threatening deep vein thrombi, pulmonary embolism, cardiac lesions.
5. Family or next of kin available to provide written informed consent.

Exclusion criteria

1) Age < 18
2) Lack of informed consent
3) Had evidence of alcoholism or any other addictive disorders, or known affective or other psychiatric diseases requiring sedatives or neuroleptics.
4) Had a central nervous infection during hospitalization.
5) Major systemic disease such as end-stage renal disease, liver cirrhosis, or congestive heart failure.
6) Pregnancy.

Study protocol

A total of 80 patients were enrolled in the study, together with 40 healthy volunteers who will serve as the control group. Patients presenting within 6 hours of injury were in study. Patient's demographic information was recorded at the time of enrollment. The GCS score was calculated at the time of admission, on 3rd and 7th day. The GCS measured verbal performance, motor response and eye opening on a 3 to 15 point scale with highest score reflecting normal performance. Time of injury, associated injuries and time of patient arrival at the hospital emergency and cranial CT finding were recorded. Clinical outcome was assessed with the Glasgow Outcome

Scale (GOS).This scale has five levels (1 to 5) to rate the individual on the basis of their degree of function and independence: good recovery, moderate disability, severe disability, persistent vegetative state and death. The mechanism of injury was categorized as fall from height, road traffic accident, assaults, fall of object on head or any other mode depending on case. Patients were called back for follow-up after six months. Venous blood samples from enrolled patients were collected at time of presentation into 10ml serum separator tube (SST). After collection, the sample were allowed to clot for two hours at room temperature or overnight at 4 °C and then centrifuged at 3000rpm for 15 minutes. The serum samples will be frozen at -70 °C and later assayed for serum super oxide dismutase (SOD) using ELISA technique.

Performance Kit

The pre-coated, 96 wells kit (assay range:6.25ng/ml- 400ng/ml) uses a double-antibody sandwich enzyme-linked immunosorbent one-step process assay (ELISA)(QAYEE-BIO for Life Science, China). This kit has no cross-reaction with other soluble structural similar object. The plate coefficient of variation is less than 15%. The complete experiment was performed within two hours. Contrary to traditional ELISA methods, only a single incubation and wash step is required, resulting in fewer handling steps, which reduce errors and deliver more consistent results. Thorough and regular tests of the system guarantee the stability and reliability of the kit.The quantity of the strips depends on the quantity to-be-tested samples and the standards. Set blank, standard, and test sample wells respectively: (1) Blank well: do not add samples and horseradish peroxidase (HRP), other operations are the same. (2) Standard wells: add standard 50µl to Standard wells. Test sample wells: Add 40µl of Special diluents and add 10µl of sample. Add 50µl of horseradish peroxidase (HRP) into each well, except blank well. Seal the plate, shake gently, and incubate for 60 minutes at 37 °C. Discard excess liquid, dry, fill each well with diluted washing liquid, mix and shake for 30 seconds, discard the washing liquid and tap the plate into absorbent papers to dry. Repeat five times, and then pat dry. Add 50µl of chromogen solution A and B one after the other. Gently shake and incubate for 10 minutes at 37 °C away from light. Add Stop Solution 50µl in each well to stop the reaction (the blue changesinto yellow immediately**).** Set blank well zero, measure the optical density (OD) at 450 nm which should be carried out within 15 minutes after adding the stop solution. According to standardconcentration and the corresponding OD values, calculate out the standard curve linear regression equation, and then apply the OD values of the sample on the regression equation to calculate the corresponding sample's concentration. It is acceptable to use

a variety of software to make calculations. Experiments were performed in duplicate.

Statistical analysis

The means and standard deviations (SD) of the measurements per group were used for statistical analysis using the software SPSS (version 24.0; SPSS Inc, Chicago, USA).

A p value of <0.05 was considered statistically significant.

Results

In mild TBI group the mean age was 33.60±13.32 years. Minimum and maximum ages were 18 and 72 years respectively. In the severe TBI group mean age was 37.60±15.90 years,where minimum age was 18 and maximum being 64 years. In control group, the mean age was 30.55±9.30 years, minimum age 18 and maximum age 50 years.The mild TBI group, comprised of 27 male (67.50%) and 13 female (32.25%) (n=40) and the male to female ratio was 2.07:1. 31 male (75.50%) and 9 female (22.5%) comprised the severe TBI group (n=40) and the male to female ratio was 3.34:1.

Combining the two study groups, the most common cause of TBI was road traffic accident (RTA) 63.75% (n=51) followed by fall from height 20.00% (n=16). Other modes of injury were in following order of frequency: assault 7.50% (n=6), sports injury 3.75% (n=3), fall of heavy object on head 2.50% (n= 2) and animal injury 2.50 % (n= 2), figure 1.

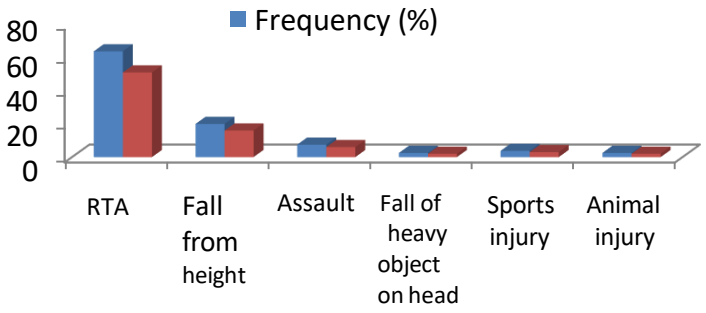


Figure 1: Bar diagram showing mode of injury

Radiological findings

All the TBI patients underwent routine investigation along with Non Contrast Computed Tomography of head. CT findings were found negative in 11.25% patients (n=9), where majority of patients (88.75%) had positive findings namely, Contusion/intraparenchymal hematomas in 58.75% (n=47), skull bone fracture (linear and depressed) in 8.75% (n=7), subdural hematoma (SDH) 13.75 % (n=11), epidural hematoma (EDH) 6.25% (n=5) and intraventricular hemorrhage (IVH) 1.25% (n=1) as shown infigure 2.Nearlyeighty three percent patients (n=33) in the mild TBI group and 55.00%(n=22) patients in severe TBI group were managed conservatively. However 17.5 % patients (n=7) in mild TBI along with 45% (n=18) patients with severe TBI underwent surgical intervention depending on the type of injury.

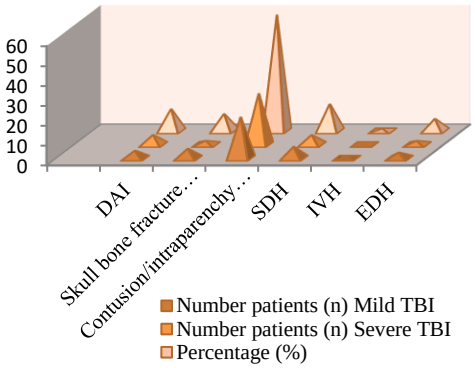
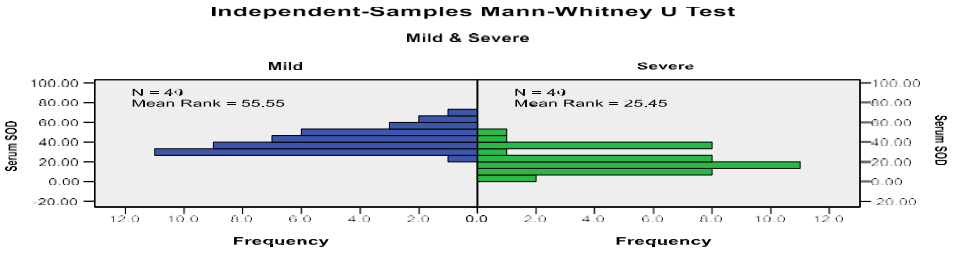


Figure 2: Bar diagram showing types of injury on CT scan

Serum SOD levels in mild TBI

Within the mild TBI group, the mean serum SOD levels were found to be 34.62±12.53 ng/ml in males while the range was 19-62. The mean serum SOD level in female was 31.46±15.14ng/ml, while the range was 18-64. There was no statistically significant difference in male and female with reference to serum SOD levels (p=0.264). Where as among severe TBI group, the mean serum SOD levels in males were 35.48±15.29 and range was 18-70. It was 44.88± 16.71 in female and range was 20-72, with the difference being statistically significant (p=0.012). The mean serum SOD level in mild TBI group was 41.74±11.00 ng/ml (range 25-70), 95% C.I. (38.22-45.26) where as in severe TBI group mean serum SOD level was 23.18±11.55, and 95% C.I. (19.49-26.88).

This difference was found to be statistically significant (p<0.001) as shown in figure 3**.** The mean serum SOD levels in control was 137.86 (range 75-220), 95% C.I. (127.22-148.5). However, compared to controls, this difference in mean serum SOD was statistically significant in mild TBI (p<0.001) and severe TBI (p<0.001)**.**



Total N	80
Mann-Whitney U	198.000
Wilcoxon W	1,018.000
Test Statistic	198.000
Standard Error	193.740
Standardized Test Statistic	-5.803
Asymptotic Sig. (2-sided test)	.000

Figure 3: Comparison of serum SOD in mild and severe TBI (P<0.001).

The mean serum SOD level in patients was 40.37±9.54ng/ml, 44.53±13.4, 70.00±11.00, with GCS 13, 14 and 15 respectively on day 0**.** Patients with GCS 13 had no significant difference in SOD levels compared to patients With GCS 14 (p=0.148). However serum SOD levels were significantly higher in patients with GCS 15 compared to patients with GCS 14 (p<0.001) and GCS 13 (p<0.001). Mean GCS in severe TBI group was 6.4, 10.18and 12.28

on day 0, 3 and 7 respectively.The mean serum SOD levels in patients with GCS 3, 4, 5, 6, 7, and 8 were 6.25±3.8 ng/ml, 9.37±4.4ng/ml, 20.10±12.14, 19.77±10.04, 26.95±8.62, 33.75±13.05 respectively**.** The distribution of SOD was in severe TBI group varied in all categories of GCS at day 0 (p=0.025). Paired comparison of GCS score with reference to serum SOD levels showed varied results. Difference in serum SOD levels was found to be statistically significant with GCS 4 and 7, GCS 4 and 8, GCS 6 and 8.

Serum SOD protein levelsand Mode of Injury

In mild TBI, the mean SOD level in RTA was 42.26±11.43, while it was 44.07±10.84, 43.05±12.40, 40.00, in fall from height, assaults, fall of object on head respectively (p=0.52) The mean SOD levels in RTA case was 21.83±10.38, while it was 17.25±8.67 in fall from height, 28.75±12.37 in assault, and 50.00 in sports injury within severe TBI group (p=0.09).**Computed tomography findings**

CT scan demonstrated intracranial injury in 36 patients of mild TBI, where as 35 patients in severe TBI. In mild TBI, the difference in the serum SOD values of patients with normal CT scan (39.37±6.57) and those with evidence of injury on CT(39.94±11.62) was not statistically significant (p=0.50**).** Similar were the results with severe TBI, where statistically lower serum SOD levels were found in CT positive group (25.42±10.86) compared to the CT scan negative group (33.20±8.69) (p=0.008),figure 4.

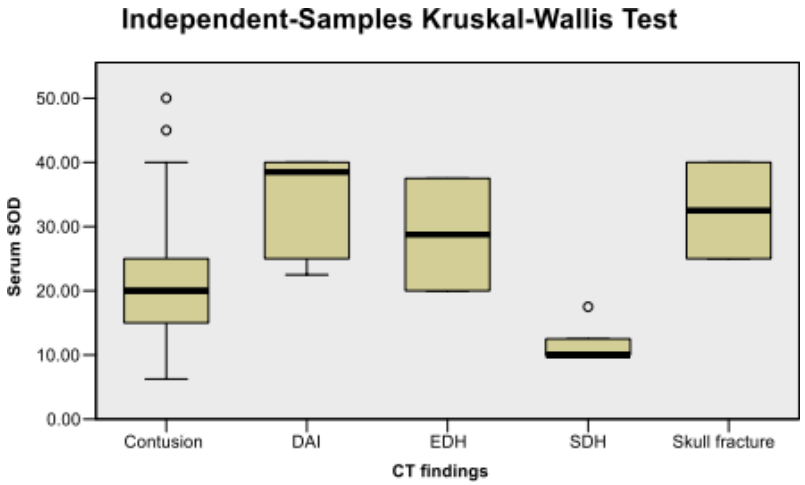


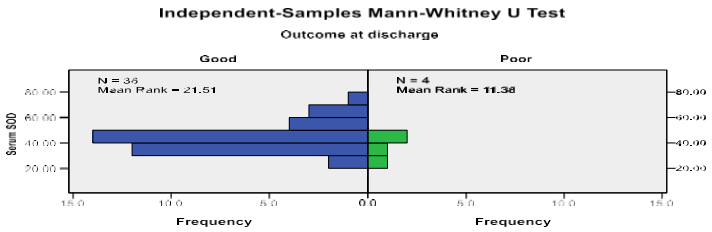
Figure 4: Box plot showing comparison between serum SOD levels of patients and CT findings in severe TBI (p=0.008).

Glasgow Outcome Score (GOS) at discharge and serum SOD levels

With reference to outcome of the patients at discharge, serum SOD levels within 12 hrs of injury were correlated with good outcome group and poor outcome group in both the mild and severe TBI. Good outcome was defined as GOS score IV and V and poor outcome group with GOS score I, II III. Thirty six patients in the mild TBI group had good outcome and 4 had poor outcome. In severe head injury 28 patients had good outcome where 12 had poor outcome (including six deaths**)** table 1**.**In the mild TBI group serum SOD protein levels in the good outcome group weresignificantly higher 44.29±10.91ng/ml, range 28.50-70.00, 95% C.I. (39.88-48.70) compared to the poor-outcome group 37.00±9.83ng/ml, range 25-60, 95% C.I. (31.32-42.67) (p=0.101**)** as showninfigure 5.In severe TBI, serum SOD protein levels in the good outcome group were 28.52±9.76,range 12.5-45, 95% C.I. (23.67-33.38) and 18.81±11.23, range 6.25-50, 95% C.I. (13.83-23.79) in the poor-outcome group demonstrating significant difference statistically (p<0.001) figure 6.

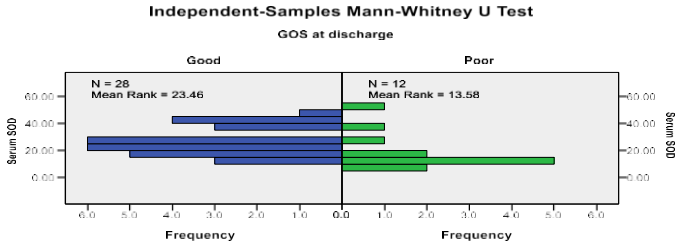
Table 1:Glasgow Outcome Score (GOS score) at the time of discharge.

Glasgow outcome score (GOS)	Glasgow outcome score at discharge		Total patients (n)
	Mild Head Injury	Severe Head Injury	
Good (V)	30	18	48
Moderate disability (IV)	6	10	16
Severe disability (III)	3	3	6
Persistent vegetative (II)	1	3	4
Death (I)	0	6	6



Total N	40
Mann-Whitney U	35.500
Wilcoxon W	45.500
Test Statistic	35.500
Standard Error	22.095
Standardized Test Statistic	-1.652
Asymptotic Sig. (2-sided test)	.099
Exact Sig. (2-sided test)	.101

Figure 5: Comparison of serum SOD levels in good and poor outcome patients in mild TBI (p=0.101)



Total N	40
Mann-Whitney U	85.000
Wilcoxon W	163.000
Test Statistic	85.000
Standard Error	33.734
Standardized Test Statistic	-2.460
Asymptotic Sig. (2-sided test)	.014
Exact Sig. (2-sided test)	.013

Figure 6: Comparison of serum SOD levels in good and poor outcome patients in severe TBI (p=0.013)

Discussion

In the present study, cases were divided into two groups – mild and severe TBI. Wang et al (2016) in his study found that 88 patients having acute TBI,having age ranging from 18-69,with mean age of 32 years. This study including our study reflected that TBI was more often seen in the most productive age

group which comprised a larger part of population and directly had an impact on countries economy, as they were more involved and exposed to the daily outdoor activities. This demands more sincere efforts to be directed in research to find out diagnostic molecules in TBI to reduce the mortality and morbidity.

In the present study, maximum patients were male in both the mild TBI (Male: Female 2.07:1) and sever TBI group (Male: Female ratio 3.4:1).Wang et al (2016) in his study reported that 88 patients with acute TBI included 55 men and 33 women (Male: Female 1.66). The mean serum super oxide dismutase (SOD) level in male was 34.62±12.53ng/ml in female it was 31.46±15.14ng/ml in mild TBI group. There was no statistically significant difference in male and female with reference to serum SOD levels (p=0.264) in our study. In the severe TBI group, the mean SOD levels in males and female were 35.48±15.29 ng/ml and 44.88±16.71 ng/ml respectively, with the difference being statistically significant (p=0.012). We found no correlation of serum SOD with gender in mild TBI. Severe TBI male patients have lower SOD then female patients in this study. A study in mice done by Igarashi et al (2001) reported that the gender differences in cortical and subcortical neuronal loss after TBI over expressed CuZn SOD. Study showed that lesion volume of the cortex was significantly reduced in the male transgenic mice (Tg-M) compared to the male non transgenic mice (nTg-M). However, similar protection was not observed in the female transgenic mice (Tg-F) compared to the female non transgenic mice (nTg-F). It has been hypothesized that the ischemic female brain benefits from the antioxidant properties of estrogen. A similar beneficial effect may account for the neuroprotection in the traumatized female brain. These finding are consistent with those by Hall and Sutter(1998) who studied gender difference related to infarct size among mice after permanent focal ischemia and with the protective effect of Cu/Zn superoxide dismutase over- expression. In our study, most common mode of injury among patients of mild TBI was road traffic accident followed by fall from height, assault, fall of heavy object on head, and sports injury. In mild head injury case, mode of injury showed no significant difference in levels of serum SOD in our study. Thus serum SOD levels showed no correlation to the mode of injury. Among severe TBI group, most common mode of injury was RTA followed by fall from height, assault, sports injury and animal injury. However, in sever TBI the serum SOD values were statistically significantly with reference to mode of injury. In an epidemiological study by National Institute of Mental Health and Neurosciences (NIMHANS), the most common cause of TBI was RTA (60%) followed by falls (25%), assaults (10%), and fall of objects (5%) (Agrawal et al., 2016; Gururaj et al., 2005).GOI-2022; Agrawal et al., (2016) conducted a review of RTAs and found that in developing nations, pedestrians, motorcyclists and bicyclists together were at high risk of sustaining head injuries.This fits well to India context also but not in Western countries where motor vehicle occupants are at a greater risk compared to motorcyclist and bicyclists. In our study serum SOD level in mild TBI was lower than control and this difference was statistically significant p<0.001. This support that mild TBI serum SOD levels do truly reflect the internal pathological change in brain matter post trauma and for serum SOD to be increase in serum trauma should be significant in intensity, volume, with axonal involvement. Cernak et al. (2000) investigatedthat plasma SOD activity of patients with mild TBI (GCS 13-15) initially decreased significantly but then increased 1-3 days after injury, before declining from day 5 to 7. Wang et al (2016) found that among 36 patients of mild TBI and 47 patients of control group,mean serum SOD level was 1947 U/g Hb (1549-2336) and 2849 U/g Hb (2180-3134) respectively. In above study there was decrease in serum SOD in mild TBI as compared to controlled group was statistically significant (p<0.001). Cernak et al. (2000) investigated the plasma SOD activity of patients with severe TBI an initial significant increase in plasma SOD activity was followed by a significant reduction in activity by the

end of the posttraumatic period. In above study there was decrease in serum SOD in severe TBI as compared to controlled group and was statistical significance ($p<0.05$).In severe TBI group, serum SOD was (23.187±11.550 ng/ml) lower than the mild TBI group (41.742±11.002 ng/ml) was statistical significance ($p<0.001$). In our study there was decrease in serum SOD in mid as well as severe TBI group but decrease in severe group was more than mild TBI group was statistical significance ($p <0.026$). Patients with GCS 13 had no significant difference in SOD levels compared to patients with GCS 14 ($p=0.98$). However serum SOD levels were significantly higher in patients with GCS 15 compared to patients with GCS 14 ($p<0.001$) and GCS 13 ($p<0.001$).

The distribution of serum SOD was in severe TBI group varied in all categories of GCS at zero day ($p=0.025$). Paired comparison of different GCS scores in reference to serum SOD levels showed varied results. Difference in serum SOD levels was found to be statistically significant with GCS 4 and 7, GCS 4 and 8, GCS 6 and 8. This shows that with the change in GCS, level of serum SOD varies. In our study, patients with lower GCS had significantly lower level of serum SOD level which was associated with poor outcome. This difference in levels may be studied in further detail with many large studies focused on timely serum SOD measurements alone with GCS scores are undertaken. Correlating the serum SOD levels and the GCS at which it is significant may help us to guide further in qualitatively analyzing the significant serum SOD levels and may shed some light on its role in projecting TBI prognosis. Wang et al (2016) found that the GCS score and the serum SOD level was significantly associated with clinical outcome in their study. Cernak et al. (2000) found plasma magnesium SOD (MnSOD) and oxidative status were investigated in 31 male casualties with traumatic brain injury (TBI) during a seven day posttraumatic period. The study group consisted of eight patients with mild closed head injury (GCS) of 13-15, 10 patients with extensive penetrating head injury (GCS 4-6), and 13 patients with blast injuries but without direct head trauma. The last group was selectedbecauseearlierinvestigational and clinical records have established the growth of indirect brain trauma in patients having blast injuries. Patients with multiple injuries were not included. Significant declines in plasma divalent cations i.e., MnSOD were found in GCS 4-6 patients immediately after TBI and persisting for the entire 7-day study period. A negative correlation between magnesium (MnSOD) balance and oxidative stress was observed in all patients immediately after injury persisting in GCS 4-6 patients to the end of the observation period. We attempted to correlate serum SOD levels in both the groups with and without any radiological findings on CT scan. In mild TBI, mean serum SOD level in CT positive group were not significantly lower compared to CT negative group ($p=0.635$). However the findings in the severe TBI group, where CT negative group had significantly higher mean serum SOD levels than CT positive group ($p<0.001$). These results signify that this decrease in serum SOD level may be used to discriminate between patients with intracranial lesions and those without intracranial lesions, in severe TBI. This finding may prove to be of highly diagnostic and prognostic importance in severe TBI where finding may be absent in CT but intracranial injury is present. This may be of immense help in primary center in identifying patients who will definitively require tertiary health care using a safe and simple blood test and at a comparative lesser cost. Besides this, it may have an important role in medico legal case in identifying presence or absence of head injury. In the study by Kasprzak et al. (2001) enhanced lipid peroxidation, as assessed by cerebrospinal fluid (CSF), in 30 patients with brain contusion and 37 control patients was correlated with the severity of head injury in adults with contusion. Compared with controls, during the 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, especially in mild TBI. Serum SOD lever were correlated with outcome at discharge in both the mild and severe TBI. Good outcome was defined as Glasgow Outcome Scale (GOS) score IV and V and poor outcome group with GOS score I, II, III. We found that patients with low serum SOD levels were found to be significantly associated with poor outcome. In mild TBI group, serum SOD levels in good outcome group were not significantly higher i.e. 44.29±10.91 ng/ml compared to the poor-outcome group 37.00±9.83 ng/ml (p=0.101). In severe TBI, serum SOD in good outcome group were 28.52±9.76 ng/ml and 18.81±11.23 ng/ml in poor outcome group demonstrating significant difference statistically (p= 0.013). Wang et al (2016) study compared with controls, patients with TBI had significantly decreased erythrocyte superoxide dismutase levels (SOD). Outcome was assessed on discharge using the Glasgow Outcome Scale erythrocyte superoxide dismutase levels were significantly higher in the good outcome group than in the poor outcome group on day one(median, 2021 U/g Hb vs. 1542 U/g Hb, (P =0.026). Muizelaar et al., (1993) done work in improving the outcome of severe head injury with the oxygen radical scavenger polyethylene glycol-conjugated superoxide dismutase (PEG-SOD). Outcome was assessed using the Glasgow Outcome Scale at three and six 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). Differences in outcome between the placebo group and either of the other two dosage groups were not statistically significant. It is concluded that PEG-SOD was generally well tolerated and appears promising in improving outcome after severe head injury. The sample size in our study was too small to reach a definitive conclusion. Further, no measurement of CSF SOD was done since CSF sampling was an invasive procedure. Our study lacked in temporal serum and CSF SOD measurements and its correlation which would be very beneficial in understanding the disease pathogenesis. Follow up forms an essential part of any clinical study. Loss of patients on follow up was one of the important limitations of this study. We found most of the lost follow up patients had good outcome at discharge. As the patients start feeling better, their willingness to visit treatment center again get reduced. Currently only handful of studies focused on SOD are available and are quite insufficient. The need of the hour is to plan studies focused on a larger population, with more consistent injury pattern, taking into consideration all the possible confounding variables (age, race, sex etc.), periodical sample collections, correlating CSF and serum levels, follow up, prospectively determined clinically relevant cut off values, combined use of clinical outcome predictors. Welling et al. (2012) also emphasized that the aim to find an ideal biomarker has proved difficult for several reasons. Human brain is a complex organ and is protected by a selective blood brain barrier. Its functions are both qualitative and quantitative, while most biomarkers are purely quantitative. Site of injury affected outcome for example involvement of brainstem may be more disastrous compared to trauma to the eloquent parts of cerebral cortex. Study done by Prins et al. (2013) each case of TBI is unique and affected individuals display different degree of injury. Papa et al. (2008) study was an attempt to study serum SOD and its correlation with both mild and severe TBI. The challenge of determining prognosis in mild TBI is the lack of all other objective measures of injury. Therefore, including case with no significant radiological finding, a biomarker that could predict worsening neurological status or long-term disability would be of immense clinical utility. Serum SOD need to be researched in greater depths as it my act as a promising diagnostic tool and prognostic marker in patients with traumatic brain injury especially in mild TBI, which comprise most of head injuries.

Conclusion and future prospects

In the present study, SOD levels were correlated with outcome at discharge in both the mild and severe TBI. Good outcome was defined as Glasgow Outcome Scale (GOS) score 4 and 5, and poor outcome group with GOS score 1, 2, 3. In the mild TBI group serum SOD protein levels in the good outcome group were not significantly higher compared to the poor-outcome group. Thus, SOD may prove to be a significant prognostic marker in severe TBI.

No correlation of SOD protein as a diagnostic marker was found in mild TBI whereas significant association was seen in severe TBI. Results obtained in our study are preliminary in nature and there is a need to undertake larger prospective studies to reach a definitive conclusion. Currently, only handful of studies focused on protein degradation products are available and are quite insufficient. Few animal studies have tried to study SOD but it does not truly reflect the human aspects like variability in severity of trauma, patient population, follow up. To conclude, more studies are required especially on mild TBI and in children as limited data is available on role of biomarkers in TBI.

A Serum test measuring one or more markers to determine the status of brain damage would no doubt be welcome in the setting of severe TBI. Such markers would be useful indeed in guiding critical care and evaluating the prognosis of patients with TBI.

Conflicts of interest

There are no conflicts of interests.

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