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The oxidative-stress level determine patient's outcomes with a severe head injury at Sanglah General Hospital, Denpasar, Indonesia

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ABSTRACT

Background: In secondary brain injury, oxidative stress will occur due to a balance disorder between pro-oxidants with antioxidants. The antioxidant activity that is often used to assess oxidative stress, such as malondialdehyde (MDA), is superoxide dismutase (SOD) and glutathione peroxidase (GPx). This study aims to evaluate the level of oxidative stress, reflected by the MDA serum level and SOD level to determine the outcomes of patients with severe head injury.

Method: A cross-sectional analytic study was conducted among 40 patients with severe head injury within 24 hours post-trauma at Emergency Ward, Surgery Department, Sanglah General Hospital Denpasar from January - June 2017. MDA and SOD levels were assessed using ELISA at Clinical Pathology Laboratory, Sanglah General Hospital. Data were analyzed using SPSS ver. 16 software.

Results: This study found significant differences on elevated MDA levels (p-value < 0.05) in patients who died or had a persistent vegetative state, patients with severe disability and those with a good recovery/moderate disability. The statistical analysis also found a significant difference in MDA serum levels among patient with severe disability and patients with a good recovery/moderate disability (P=0,028). Meanwhile, there was no significant correlation between SOD serum levels and patients outcome (P>0.05).

Conclusion: Increased MDA serum levels is a significant factor in predicting outcomes of patients with severe head injury.

Keywords: severe head injury, oxidative stress, malondialdehyde, superoxide dismutase

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INTRODUCTION

Head injury is still a health and economic problem in Indonesia, also one of the leading cause of death and disabilities in productive age between 15 – 30 years old, especially in developing country. The number of death due to head injury in Indonesia in 2005 was 6,2% to 11,2% almost twice from the international standard literature that is around 3 – 8%.[1] The mean number of head injury incident in Sanglah General Hospital Denpasar is more than 2.000 cases per year where 30% is moderate and severe head injury.

Based on the pathophysiology, the head injury classified into primary head injury and secondary head injury. Secondary brain injury is a result of a complex process and happens a few moments after primary brain injury.[2] In secondary head injury will occur oxidative stress due to a balance disorder between pro-oxidants with antioxidants. Ischemia is caused by a decrease in cerebral blood flow (CBF), resulting in a change in aerobic metabolism to anaerobes resulting in a reduction of ATP reserves, lactic acid accumulation, and acidosis.[3-7] Loss of ATP reserves causes calcium pumps and membrane transporters including transporters for unworkable glutamate, resulting in intercellular Ca^{2+} accumulation, extracellular glutamate accumulation, and synaptic cleft.[7-9] Glutamate will stimulate glutamate receptors followed by Ca^{2+} influx. Ca^{2+} will activate the phospholipase A_2; phospholipase A_2 will release arachidonic acid from the cell membrane.[9,10] Arachidonic acid metabolism produces free radicals through several pathways, namely; pathway cyclo-oxygenase, lipooxygenase, and cytochrome monooxygenase P450[9,10] In addition to the enzyme pathway, arachidonic acid can produce oxygen-free radicals through autoxidation.[11] Ca^{2+} also activates proteases that convert enzyme Santin dehydrogenase into Santin oxidase then Santin oxidase converts hypoxanthine into Santin and reduces O_2 to superoxide radicals.[12,13]

Acidosis during ischemia will weaken the iron bond with the binding protein. In plasma, the iron metal is bound by a protein, i.e. transferrin, whereas in tissues, iron is stored in ferritin.[13] Both transferrin and ferritin have strong bonds with iron at neutral pH, effective in preventing iron as

catalytic in producing radical compounds.[13] In a state of trauma or ischemia, where the pH decreases, Fe escapes from the metalloprotein to free Fe.[14] Fe is free to participate in the formation of SOR through several stages.[15] The most likely mechanism is the autocesidation of Fe^{2+} by reducing O_2, producing superoxide radicals.[14,15]

SOR on oxidative stress will invade cell components such as DNA, protein, and lipids. The destruction of proteins by free radicals significantly affects mainly enzyme-forming proteins, receptors, and transporters.[16] Free radicals attack the SH group of proteins, so the enzyme, receptor, and transporter lose their function. Fat, especially the double-chain fatty acids, is particularly vulnerable to free radicals called lipid peroxidation. Brain and myelum are the most susceptible organs of SOR because they have a high concentration of fatty acid chains and high metabolic rates, and relatively low antioxidant enzyme activity compared to other organs.[16]

The degree of lipid peroxidation indicates the level of oxidative stress, one of the widely used lipid peroxidation products being malondialdehyde (MDA).[17] While the antioxidant activity often used to assess oxidative stress is superoxide dismutase (SOD) and glutathione peroxidase (GPx). SOD converts superoxide to hydrogen peroxide, then hydrogen peroxide by other radical endogenous antioxidants such as catalase and glutathione peroxidase (GPx) is converted to H_2O, so no hydroxyl radicals are formed. Based on the previously mentioned above, this study aims to evaluate the level of oxidative stress, reflected by the MDA serum level and SOD level to determine the outcomes of patients with severe head injury.

Table 1. Baseline characteristic of respondents

Variables	N(%)
Outcome	
Death/persistent vegetative	18 (70)
Severe disability	6 (15)
Good recovery/Moderate disability	6 (15)
Sex	
Males	31 (77.5)
Females	9 (22.5)
Smoking history	
Yes	21 (52.5)
No	19 (47.5)
Diabetes mellitus (DM) and hypertension	7 (17.5)
Diabetes mellitus (DM)	2 (5)
Hypertension	3 (7.5)
Comorbidities	
Yes	12 (30)
No	28 (70)
Surgery	
Yes	18 (45)
No	22 (55)

METHODS

A cross-sectional analytic study was conducted at IRA Surgical Installation Hospital of Sanglah Denpasar from January-June 2017 using consecutive sampling method among 40 samples. MDA and SOD levels were measured from venous blood samples taken 24 to 48 hours after trauma. Venous blood taken as much as 5 ml, centrifuged at 3000 rpm for 20 minutes with a temperature of 4° C. Separate serum of red blood cells is then used for examination of MDA and SOD levels. Serum MDA and SOD levels were examined with an assay kit using the ELISA method. After getting a decision to go outpatient or home by a doctor in charge of service, three months later assessed GOS-E.

Data on serum MDA and SOD levels in each group were first tested for normality of data using the Shapiro-Wilk test and homogeneity test by using the Levene test. Comparative test using one way ANOVA ($p < 0,05$) was assessed to determine the correlation between serum MDA and SOD levels with outcome death / persistent vegetative state, severe disability and good recovery/moderate disability. Then test independent t-test to see the relationship of serum MDA and serum level with outcome severe disability and good recovery/ moderate disability. Data were analyzed using SPSS ver. 16 software.

RESULT

In the data analysis, there were 28 patients (70%) outcome death / persistent vegetative state, severe disability 6 patients (15%), and good recovery / moderate disability 6 patients (15%). Based on gender, males were predominant by 31 patients (77.5%) compared with females 9 patients (22.5%). The study also found that 21 patients were smoking (52.5%), 19 patients no smoking (47.5%), 7 patients with history of hypertension and DM (17.5%), 2 patients with diabetes mellitus (DM) (5%), 3 patients hypertension (7.5%), without comorbidities 28 patients (70%), and the operation of 18 patients (45%), and 22 patients without surgery (55%) (Table 1).

In the normality test, serum MDA level obtained p-value 0,52 > 0,05 and serum SOD level with p-value 0,148 > 0,05 which mean normal distribution data. Mean serum MDA level was higher in death / persistent vegetative state outcome 4,68 (IK: 4,31-

Table 2. MDA and SOD Serum Concentration Variant between Treatment Groups

Groups	Mean Differences	P-value	CI 95%
MDA Levels			
Death/persistent vegetative state	1.41	0,000	0.93-1.88
Severe disability-death/persistent vegetative state	-1.41*	0,000	-1.88-(-.9336)
Good recovery-death/persistent vegetative state	-1.92*	0,000	-2.59-(-1.2537)
SOD Levels			
Death/persistent vegetative state	3,01	1,000	9,14-15,16
Severe disability-death/persistent vegetative state	-3,01	1,000	-15,16-9,14
Good recovery-death/persistent vegetative state	-8.34	0,280	-20,50-3,80

CI: Confidence interval; P: statistically significant if less than 0.05

Table 3. Mean differences between MDA and SOD serum concentration and patient with severe disability outcome and good recovery/moderate disability outcome

Examination	Outcome			Mean Differences	CI 95%	p-value
	Death/persistent vegetative state (X ± SD)	Severe disability (X ± SD)	Good recovery/ moderate disability X ± SD			
MDA	4,68 ± 0,95	3,27 ± 0,17	2.76 ± 0.45	0.51	0.06-0.95	0,028
SOD	36,67 ± 11,51	33,66 ± 9,49	28.32 ± 7.28	5.33	-5.54-16.22	0,300

CI: Confidence interval; P: statistically significant if less than 0.05; SD: Standard deviation; MDA: malondialdehyde; SOD: superoxide dismutase

5,06) compared with severe disability, mean 3,27 (IK: 3,09-3,45), and good recovery / moderate disability, mean 2.76 (IK: 2,28-3,24). Furthermore, the homogeneity test obtained p-value 0.001 < 0.05 which means the data is not homogeneous so that in the next test conducted Post Hoc test on the column Games-Howell obtained a significant difference between the average outcome death / persistent vegetative state with severe disability, severe disability with death / persistent vegetative state and good recovery / moderate disability with death / persistent vegetative state (Table 2).

In the statistical analysis, mean data of serum SOD level was higher in the outcome of death / persistent vegetative state 36,67 (IK: 32,2-41,13) compared with severe disability, mean 33,66 (IK: 23,67-43, 67), and good recovery / moderate disability, mean 28,32 (IK: 20,68-35,96). Furthermore, in Post Hoc test test because homogenous data with p-value 0.092 > 0.05 then in this test seen in Bonferroni column, which shows no significant difference between mean outcome death / persistent vegetative state with severe disability, severe disability with death / persistent vegetative state, and good recovery / moderate disability with death / persistent vegetative (Table 2).

Independent t-test to see the difference of mean serum MDA and SOD level in outcome severe disability with good recovery / moderate disability, found a significant difference from serum MDA level, while serum SOD level did not get significant difference (Table 3).

DISCUSSION

In this study, patients with outcome death / persistent vegetative state were 28 patients (70%), severe disability 6 patients (15%), and good recovery / moderate disability 6 patients (15%). Serum MDA levels were higher in the outcome of death / persistent vegetative state compared with severe disability, and good recovery / moderate disability, which also showed significant differences in outcomes.

In head injuries, damage to blood vessels and brain tissue leads to a cascade involving many substances. A head injury will lead to free radical production through several enzyme pathways and autoxidation in arachidonic acid metabolism.[16,17] The free radicals formed will increase the peroxidation of the lipid membrane and the release of arachidonic acid thus creating a destructive

cycle. The brain and myelum are the organs most susceptible to oxidative stress because they have both high fatty acid levels and high metabolic rates, and relatively lower antioxidant enzymes than other organs. This will lead to damage or death of tissue cells that escape the primary head injury, thus affecting outcomes in patients with severe head injury.[16,17]

In this study, it was found that mean serum SOD levels were higher in patients with outcome death / persistent vegetative state compared with severe disability, and good recovery / moderate disability, although from statistical analysis there was no significant difference from serum SOD level to the outcome. Shohami et al. in his 1991 study found no significant difference in SOD activity, the difference in serum-taking time may also have an effect on SOD measurements in patients with head injury. SOD may be increased as an adaptive compensation of oxidative pro processes, resulting in the absence of improvement of SOD which only slightly describes the prolonged oxidative stress process.[18]

In a study conducted by Priantono et al. in 2013 on levels of Manganese Superoxide Dismutase (MnSOD) in hypo-hypoxically induced rats, there was no significant difference in MnSOD levels in the kidneys between treatment groups, but there was a difference in MDA levels between various hypoxic exposure treatments. This is related to the duration of hypoxic hypoxia that affects the oxidative stress that occurs. Chronic hypoxia leads to an increase in ROS so that as compensation, MnSOD activity also increases, where this increase is a protective form of adaptation to the kidneys from structural damage. Various studies on the activity of MnSOD and MDA in rat renal after hypoxic treatment showed different results. Priantono et al. Also said that hypobaric hypoxic exposure has varied effects on various organs.[19]

Kucur et al. in 2005 found that SOD levels in mice induced by head trauma were no significant differences between treatment groups. However, there is an increasing trend of SOD and catalase proportional to the severity of head trauma, indicating the presence of SOD interactions and catalase as antioxidant enzymes with ROS for cell protection.[20]

Damage to brain tissue due to head trauma was also related to the demyelination process, which causes long-term cognitive deficits and sensorimotor. Oligodendrocytes as the primary cells responsible for producing and maintaining the myelin membranes under normal conditions and remyelination after axonal damage are highly sensitive to both ischemic and traumatic situations. The decreased oligodendrocyte is the determinant of demyelination after head trauma. Oligodendrocytes are very sensitive to the release of glutamate and various stimuli with higher amounts than astrocytes.[21,22] Lipid peroxidation inhibits membrane barrier function, inactivates the membrane enzyme, and increases the permeability of water and monovalent and divalent ions, leading to demyelinating myelin membranes. In this process, MDA is a marker that directly describes the intensity of lipid peroxidation.[21-23]

CONCLUSION

This study found significant differences in elevated MDA levels in patients who died or had a persistent vegetative state, patients with severe disability and those with a good recovery/moderate disability. However, there was no significant correlation between SOD serum levels and patients outcome.

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