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Correlation of Antioxidant Levels with the Severity of Traumatic Brain Injury - A Systematic Analysis

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Abstract: Numerous experimental and clinical analyses of biomechanical injury have served to elucidate the pathophysiologic events involved in Traumatic Brain Injury(TBI).Secondary insults activate the release of cellular mediators including proinflammatory cytokines,prostaglandins, free radicals,and complement.The resulting cascade of events avalanche into free radical induced oxidative damage to the brain.The present study was carried out to determine the level of antioxidant enzymes like Superoxide Dismutase (SOD),Glutathione Peroxidase (GPx) and Malondialdehyde (MDA) as markers of lipid peroxidation.An increase in antioxidant levels was taken as a composite marker of defense against oxidative tissue damage. Comparison of mean levels of SOD, GPx and MDA levels between controls (Group1), moderate TBI (Group2) and severe TBI patients (Group3) was done using one way ANOVA to calculate the P value.The mean levels of SOD, GPx and MDA of group 2 were compared with that of group 1.The values were significantly higher in group2 than in group1 ($P < 0.001$). Similarly the mean level of SOD, GPx and MDA of group 3 was compared with that of group 2.The values were significantly higher in group 3 than in group 2 ($P < 0.001$). Our observation revealed that changes in antioxidant enzyme levels run parallel to the severity of head trauma.Thus, these enzyme levels could be used as potential biomarkers to correlate severe head trauma and thereby aid management plans.

Abbreviations: Reactive Oxygen Species (ROS) · Glasgow Coma Scale (GCS) · Traumatic Brain Injury (TBI) · Superoxide Dismutase (SOD) · Glutathione Peroxidase (Gpx) · Malondialdehyde (MDA) · Polyunsaturated Fatty Acids (PUFA) · Adenosine Triphosphate Phosphate (ATP)

Key words: Traumatic Brain Injury (TBI) · Superoxide Dismutase (SOD) · Glutathione Peroxidase (Gpx) · Malondialdehyde (MDA)

INTRODUCTION

Traumatic brain injury (TBI) represents one of the major causes of worldwide morbidity and mortality among middle aged individuals[1].Numerous experimental and clinical analyses of biomechanical injury have served to elucidate the pathophysiologic events involved in TBI. The results of these studies potentially serve as the basis to define new or refine established treatment strategies. The outcome of head injury is determined by the primary insult occurring at the moment of impact. In treatment terms, this type of injury is exclusively sensitive to preventive but not therapeutic measures.The secondary insult that follows represents consecutive pathological processes initiated after 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 [2].

Oxidative stress plays a major role in secondary brain damage which influences clinical outcome of TBI [3]. Secondary insults activate the release of cellular mediators including pro inflammatory cytokines, prostaglandins, free radicals and complement [4]. Oxidative stress occurs when the rate of Reactive Oxygen Species production exceeds that of their removal by cellular defense mechanisms.These defense mechanisms include a number of enzymes and antioxidants which

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neutralize the ROS [5]. The first stages of cerebral injury after TBI are characterized by direct tissue damage and impaired regulation of cerebral blood flow and metabolism.This 'ischemia-like' pattern leads to accumulation of lactic acid due to anaerobic glycolysis,increased membrane permeability and consequent edema formation.Since the anaerobic metabolism is inadequate to maintain cellular energy states,the ATP stores deplete and failure of energy dependent membrane ion pumps occur. The excessive production of ROS induces lipid peroxidation of cellular and vascular structures [6]. In addition to these mechanisms, oxidative stress induced early or late apoptotic programmes orchesters the cell death process [7]. The high utilization of oxygen is not the only reason why brain suffers more than its share of oxidative destruction. The brain tissue is highly enriched with polyunsaturated fatty acids (PUFA).The unsaturated bonds in these molecules render them susceptible to oxidative damage by free radicals [8].

MATERIALS AND METHODS

This prospective observational study was done during the period from February 2009 to June 2009. It was carried out in two groups namely, apparently healthy volunteers in the age group of 25 to 45years and age matched head injury patients within 24 hours of trauma.

Controls (group1) comprised of 45 apparently healthy subjects attending the outpatient department of Madras Medical College, Chennai for Master Health check up.These subjects had no significant medical illness like Diabetes Mellitus, Coronary Artery Disease, Hypertension and Liver disorders.

Test group comprised of 45 moderate TBI (group2) and 45 severe TBI (group3) patients diagnosed by Computerized tomography,being managed by the Department of Neurosurgery, Madras Medical College. CT scan was done at the time of admission to confirm the diagnosis and evaluate the extent of brain damage.Patients were divided into groups according to severity of TBI using Glasgow Coma Scale scores recorded during admission, as follows - Severe TBI (GCS score 8 or less) and moderate TBI (GCS score 9-12). Individuals with Diabetes Mellitus, Hypertension, Coronary Artery Disease, Liver disease, Space Occupying Lesion in brain, Cerebrovascular Accidents and Polytrauma were excluded.

Biochemical Analysis: About 5mL of venous blood was collected from the antecubital vein into grey topped tubes which contained Potassium Fluoride and Disodium EDTA.Malondialdehyde reacts with thiobarbituric acid forming MDA - TBA2 adduct with pale pink colour that absorbs light strongly at 532 nm [9]. SOD was estimated by Xanthine Oxidase Enzymatic method [10] and GPx by UV method [11].

Ethical Issues: The study was approved by the Institutional Ethical Committee of Madras Medical College. Periodic reports of the study progress were submitted and discussed at the review meetings of IEC.All participants gave informed,written consent to be enrolled in the study. For those participants who were in an inebriated status, their legal guardian gave the consent.

Statistical Analysis: In all the groups, one way ANOVA followed by Bonferroni's post hoc test were used to calculate the P value. The Pearson correlation was applied to correlate the changes in antioxidant enzymes activities and MDA levels between group1 (controls), group2 (moderate TBI) and group3 (severe TBI).All the values were expressed as mean (n) ± standard deviation (SD) and a 'P' value of $\leq$ 0.05 was considered statistically significant.

RESULTS

The mean level of SOD in the three groups was as follow; Group 1 - 1117.22+/-190. [Mean+/-SD], Group 2- 2286.82+/-700.47 and Group 3- 4321.04+/-468.68μ/g Hb.Comparison using Bonferroni's t test between the three Groups showed a p value of 0.001and F=465.10.Likewise, the mean level of GPx in the three Groups was; Group 1- 60.72+/- 60.40, Group 2 -92.80+/- 18.37 and Group 3 -122.86+/- 11.49μ/g Hb.Comparison between the three Groups gave a p value of 0.001 and F= 31.28. The mean of MDA levels in the three Groups was;Group 1 - 2.34+/- 0.22,Group 2 - 4.29+/- 0.87 and Group 3 - 6.69 +/- 0.37 nmol/ml. Comparing 3 Groups using Bonferroni's t test yielded a p value of 0.001.

The mean levels of SOD, GPx and MDA of group 2 were compared with that of group 1. The values were significantly higher in group2 than in group1 ($P < 0.001$). Similarly the mean of the three parameters were compared between groups 3 and 2.The values were significantly higher in group3 than in group2 ($P < 0.001$).

Table 1: Comparison of mean±SD levels of SOD, GP_X activity and MDA between controls (group1), moderate TBI (group2) and severe TBI (group3) patients.

Parameters	Group1 (controls=45)	Group2 (moderate cases=45)	Group3 (severe cases=45)	One way ANOVA P	Multiple comparison using Bonferroni's t-test
SOD activity Mean ± SD in U/g Hb	1117.22 ± 190.86	2286.82 ± 700.47	4321.04 ± 468.68	F=465.1 P=0.001	1 Vs 2,3
Gpx activity Mean ± SD in U/g Hb	60.72 ± 60.40	92.80 ± 18.37	122.86 ± 11.49	F=31.28 P=0.001	2 vs 1,3
MDA levels Mean ± SD in nmol /ml	2.34 ± .22	4.29 ± .87	6.69 ± .37	P=0.001	3 Vs1,2

DISCUSSION

Oxidative stress is stated to be an intrinsic component of the neurological sequel of traumatic head injury [12]. ROS generation and their appearance in the brain extracellular space during brain injury is well established with experimental evidence [13]. Oxidative stress has been found to cause breach of the Blood Brain Barrier.Previous experiments have shown that ROS, like superoxide, traverse the erythrocyte membrane with ease [14] and that the RBC also act as mobile ROS scavengers providing antioxidant protection to other tissues and organs.

Earlier studies have documented increased oxidative stress during TBI [15]. Several previous investigators have measured Lipid Peroxidation in plasma and erythrocyte membranes of head injury patients as a measure of oxidant stress.A reduction in erythrocyte Lipid Peroxidation levels [reflecting adaptation to chronic oxidative stress] in post traumatic patients has been associated with a significant trend towards clinical recovery in earlier studies.The current study hence attempted to evaluate the changes in the erythrocyte antioxidant enzyme activity in severe TBI patients in the post traumatic period.

Erythrocytes are very susceptible to oxidative damage. This is attributed to their high concentration of polyunsaturated fatty acids, intracellular oxygen and hemoglobin [whose redox chemistry is known to produce oxy radicals] [16]. Hence by evolution they have become highly specialized cells to handle the threat of ROS at all times with high activities of antioxidant enzymes SOD,GSH-Px and catalase compared to other cells of the body.They also have a rich pool of the nonenzymatic antioxidant Glutathione, which is preserved in its reduced state by the activity of Glutathione Reductase using NADPH.Basal level of antioxidant activity is maintained at all times,yet cells are said to have ways to amplify these activities to counter sudden increases in ROS [17]. Erythrocyte antioxidant enzyme activities in New Zealand white rabbits were significantly increased on chronic exposure to ROS and lipid peroxides [18].

SOD is said to be a substrate-inducible enzyme,and its increase is indicative of increased generation of superoxide radicals.Comhair *et al*. [19] have provided in vitro experimental evidence for an eightfold increase in GSH-Px mRNA in bronchial epithelial cells after exposure to ROS and attributed this to the gene expression. GSH-Px is an 'oxidative stress'-inducible enzyme which plays a crucial role in the peroxyl-scavenging and thereby maintains the functional integrity of the cell membranes [20].

Our observation revealed that changes in antioxidant enzyme levels run parallel to the severity of head trauma. Thus, these enzyme levels could be used as potential biomarkers to correlate severe head trauma and thereby aid management plans. Further multi centric, large scale prospective studies on this subject is the need of the hour to enhance our knowledge and device lifesaving therapeutic strategies for these patients.

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