Oxidative stress in isolated blunt traumatic brain injury

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Traumatic brain injury is a common cause of death after trauma. The aim of this study was to investigate the relationship between oxidative stress parameters and outcomes and clinical findings in patients with isolated traumatic brain injuries. Fifty-four patients who were admitted into the emergency department of Ankara Ataturk Training and Research hospital with isolated blunt traumatic brain injuries and 33 healthy adults as control group, were included in this study. Serum oxidant status was evaluated by measuring Total Oxidant Status (TOS) levels in patients with traumatic brain injury and in healthy individuals. Serum antioxidant status was evaluated by measuring Total Antioxidant Status (TAS) levels. Then, also Oxidative Stress Index (OSI) was calculated. A total of 54 patients with isolated traumatic brain injuries (mean age 36.7 ± 18.3 years; 60.4% male, 39.6% female) were enrolled. TOS and OSI levels increased in patient group compared to the control group. High levels of OSI, TOS and TAS were observed in patients who finally became dead. A significant correlation between symptoms including nausea, vomitus, loss of consciousness, seizing and TOS, OSI levels of all patients have been observed. Moreover, there was a meaningful correlation between Glaskow Coma Scale (GCS) score, TOS and OSI levels of patients. The oxidative stress parameters may be valuable prognostic markers in traumatic brain injury patients. It can be concluded that oxidative stress parameters may be valuable in the assessment of clinical severity and in predicting outcome of traumatic brain injury patients.

Key words: Traumatic brain injury, oxidative stress, symptom, outcome, glaskow coma scale.

INTRODUCTION

Traumatic brain injury (TBI) is a leading cause of morbidity and mortality and represents a major public health burden for the previously healthy individuals under the age of 45 years old (Ates et al., 2007; Vink and Van Den Heuvel, 2004; Werner and Engelhard, 2007).

Two substantially different mechanisms determine the damage after TBI. Firstly, the primary insult occurring at the moment of impact. Next, secondary insult represents consecutive pathological processes such as oxidative stress, which is initiated at the moment of injury (Ferguson et al., 2010; Seifman et al., 2008; Werner and Engelhard, 2007; Yi and Hazell, 2005).

Imbalance between cellular production of free radicals and the ability of cells to defend against them is referred to as oxidative stress (Gilgun-Sherki et al., 2002). Oxidative stress begins immediately after TBI and initiates the events resulting in neuronal dysfunction and death. It has a significant role in secondary damage and responsible for morbidity and mortality following TBI. However, their underlying molecular mechanisms are complex and remain unclarified (Ates et al., 2007; Cook et al., 2010).

Oxidative stress due to excessive generation of reactive oxygen species with consequent impairment of endogenous antioxidant defence mechanisms plays a significant role in the secondary events leading to neuronal death (Eghwrudjakpor and Allison, 2010). Oxidative stress, an imbalance between oxidants and antioxidants, contributes to the pathogenesis of TBI (Ansari et al., 2008a).

Free radical production is a normal part of cellular physiology that is regulated by various antioxidant defense systems, including superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalases (Giza et al., 2009).

Measurement of TAS provides information about the antioxidant capacity of the organism (Rabus et al., 2008).
Table 1. TOS, TAS and OSI levels between patient and control groups (mean± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOS (μmol H₂O₂ equivalent/L)</td>
<td>Patient</td>
<td>18.07</td>
<td>15.32</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>8.07</td>
<td>2.83</td>
<td></td>
</tr>
<tr>
<td>TAS (μmol Trolox equivalent/L)</td>
<td>Patient</td>
<td>2317.50</td>
<td>386.28</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>2060.30</td>
<td>386.28</td>
<td></td>
</tr>
<tr>
<td>OSI (arbitrary unit)</td>
<td>Patient</td>
<td>0.76</td>
<td>0.61</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.39</td>
<td>0.10</td>
<td></td>
</tr>
</tbody>
</table>

TOS reflects the present oxidative status. The percent ratio TOS to TAS was accepted as the OSI, an indicator of the degree of oxidative stress (Sirmatel et al., 2007).

The aim of the present study was to determine relationship between oxidative stress and last status of TBI patients as well as clinical findings.

MATERIALS AND METHODS

Study population and protocol

Fifty-four patients who were admitted into the Emergency Department of Ankara Ataturk Training and Research hospital with TBI and 33 healthy adults as a control group, were included in this study. The patients with other system injuries and previously known illnesses were excluded.

The study was performed in 2010. It was approved by institution’s ethic committee. On admission to the emergency department, venous blood was drawn into blood tubes from TBI patients and serum was separated from the cells by centrifugation at 1500 g for 10 min, and the serum samples were stored at −80°C until the analyses. Serum antioxidative status was evaluated by measuring TAS levels in patients with TBI and in control group. Serum oxidative status was evaluated by measuring TOS. The percent ratio of TOS to TAS level was accepted as OSI. All these data were compared between TBI patients and healthy control individuals. Subsequently, TBI patients were divided into fatal and non fatal groups and similar comparisons were performed between them. Relationship between symptoms of patients and oxidative stress parameters were analysed. Also correlation between GCS score and these parameters was determined.

Determination of serum Total Antioxidant Status (TAS) levels

TAS levels were measured using commercially available kits (Rel assay, Turkey). The novel automated method is based on the bleaching of characteristic color of a more stable ABTS (2,2’-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/L (Erel, 2004).

Determination of serum Total Oxidant Status (TOS) levels

TOS levels were measured using commercially available kits (Rel assay, Turkey). In the new method, the oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H₂O₂ equivalent/L) (Erel, 2005).

Calculation of oxidative stress index (OSI)

The ratio of TOS to TAS was accepted as the OSI. For calculation, the resulting unit of TAS was converted to μmol/L, and the OSI value was calculated according to the following formula:

\[
\text{OSI (arbitrary unit)} = \frac{\text{TOS (μmol H}_2\text{O}_2 \text{equivalent/L)}}{\text{TAS (μmol Trolox equivalent/L)}}
\]

(Harma and Erel, 2003; Kosecik et al., 2005; Yumru et al., 2009).

Statistical analysis

The compliance with normal distribution of the permanent data (TOS, TAS, OSI) that were obtained by this study was examined using graph and Shapiro-Wilk test. As the data were normally distributed and independent, statistical analysis was performed using Student’s t-test when comparing groups. The results were given as the mean ± standard deviation (SD). Pearson correlation analysis was assessed for the relationships between oxidative stress parameters and the GCS scores. For statistical evaluation, we used SPSS for Win. Ver. 15.0 (SPSS Inc., Chicago, IL, USA). The value of \( p \leq 0.05 \) was accepted as statistically significant.

RESULTS

TOS, TAS and OSI levels in TBI patients and healthy control individuals are presented in Table 1 and also these parameters in fatal and non fatal groups are presented in Table 2.

Serum TOS levels

TOS levels which show oxidative status in TBI patients, were significantly higher than control group (18.07 ± 15.32 vs 8.07 ± 2.83 μmol H₂O₂ equivalent/L, \( p < 0.001 \)). On the other hand, TOS levels were increased in fatal
Table 2. TOS, TAS and OSI levels between fatal and nonfatal patients (mean ± SD).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>mean</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOS (μmol H₂O₂ equivalent/L)</td>
<td>Fatal</td>
<td>53.69</td>
<td>5.81</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Nonfatal</td>
<td>13.50</td>
<td>8.60</td>
<td></td>
</tr>
<tr>
<td>TAS (μmol Trolox equivalent/L)</td>
<td>Fatal</td>
<td>2674.00</td>
<td>495.05</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Nonfatal</td>
<td>2271.79</td>
<td>352.40</td>
<td></td>
</tr>
<tr>
<td>OSI (arbitrary unit)</td>
<td>Fatal</td>
<td>2.09</td>
<td>0.62</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Nonfatal</td>
<td>0.58</td>
<td>0.34</td>
<td></td>
</tr>
</tbody>
</table>

patient group with compared to nonfatal patient group (53.69 ± 5.81 vs 13.50 ± 8.60 μmol H₂O₂ equivalent/L, p < 0.001).

Serum TAS levels
TAS levels, which show antioxidant status in TBI patients, were significantly higher than control group (2317.50 ± 386.28 vs 2060.30 ± 386.28 mmol Trolox equivalent/L, p=0.002). On the other hand TAS levels were increased in fatal patient group compared to nonfatal patient group (2317.50 vs 2060.30 mmol Trolox equivalent/L, p = 0.02). TOS and OSI levels in patients with nausea and vomitus were higher than the patients who did not have these symptoms (relatively, 27.70 ± 19.23 vs 12.00 ± 7.87, p = 0.000 and 1.13 ± 0.78 vs 0.52 ± 0.30, p = 0.001) (Figure 1). On the other hand, TOS levels were increased in fatal patient group compared to nonfatal patient group (2.09 ± 0.62 vs 0.58 ± 0.34, p < 0.001) (Figure 2).

Correlation between GCS score and oxidative parameters
TOS levels were negatively correlated with GCS score (R = -0.774, p = 0.000). OSI levels were negatively correlated with GCS score (R = -0.696, p = 0.000).

DISCUSSION
In this study, we demonstrated that TOS, TAS and OSI levels in TBI patients were higher than healthy individuals. Secondary brain injury plays a pivotal role in the outcome of patients suffering from TBI, which produces a state of vulnerability that reduces the brain capacity (Wu et al., 2007; Yan et al., 2008). Although the mechanisms of secondary brain injury following TBI are complex and not completely understood, oxidative stress has generally been considered as one of the important factors in the TBI pathogenesis (Moriyama et al., 2010; Petronilho et al., 2009). The knowledge of the pathophysiology after TBI, is necessary for adequate and successful treatment. Thus, in this study, we investigated oxidative stress markers in patients with TBI. Because an imbalance between oxidants and antioxidants has been postulated to lead to oxidative damage in TBI, there is a close relationship between degree of oxidative stress and severity of brain insults (Ansari et al., 2008b). In line with mentioned literature, in our findings, in fatal group of TBI patients, TOS and OSI levels were significantly increased with respect to non fatal group.

Additionally, as a response to oxidative stress, TAS levels significantly increased in fatal group. Similarly, in patients with symptoms including nausea, vomitus, seizure and loss of consciousness, oxidative stress was higher than the ones without symptoms. In all results of the current study, as a whole, implies that there is a strong relationship between severity of TBI and oxidative stress. The present findings on admission provide that early oxidative changes can influence the clinical...
Figure 1. Differences of OSI levels between traumatic brain injury patients and healthy control individuals.

Figure 2. Differences of OSI levels between fatal and nonfatal traumatic brain injury patients.
outcomes of TBI patients. Consequentially, a therapeutic strategy for TBI may be to control oxidative stress.

The GCS score is the most common grading scale in neurotraumatology and is used to quantify the clinical severity of brain trauma. Its validity in providing strong predictive value to assess the functional outcome for TBI patients is well accepted in the Anglo-American literature (Nayak et al., 2007). In our study, the increased levels of TOS and OSI were observed in low level GCS score patients. So, we can say that there is a prognostic role for these parameters in TBI patients.

Oxidants and antioxidants have additive effects. Although the concentration of oxidant and antioxidant components can be measured individually, these measurements are time and cost consuming and require sophisticated systems. In addition, it may not accurately reflect the TAS and TOS (Horoz et al., 2005). For this reason, we measured oxidant status and antioxidant status totally and demonstrated that there is an immediate increase in oxidant status and antioxidant status after TBI.

The results of our study further contribute to the previous literature (Awasthi et al., 1997) supporting the severe oxidative stress hypothesis in TBI. The results reflect the necessity of designing antioxidant therapeutic strategies for these patients in the early posttraumatic period. This is because treatment with antioxidants may theoretically act to prevent propagation of tissue damage and improve both the survival and neurological outcome.

Conclusion

Finally, our results provide a better understanding of the early oxidative cascades following TBI and have important implications for antioxidant treatment.

REFERENCES


