Relation between both oxidative and metabolic-osmotic cell damages and initial injury severity in bombing casualties

Zavisnost oksidativnih i metaboličko-osmotskih oštećenja ćelija od inicijalne težine povrede kod ljudi povređenih u bombardovanju

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Abstract

Background/Aim. We have recently reported the development of oxidative cell damages in bombing casualties within a very early period after the initial injury. The aim of this study, was to investigate malondialdehyde (MDA), as an indicator of lipid peroxidation, and osmolar gap (OG), as a good indicator of metabolic cell damages and to assess their relationship with the initial severity of the injury in bombing casualties. Methods. The study included the males (n = 52), injured during the bombing with the Injury Severity Score (ISS) ranging from 3 to 66. The whole group of casualties was divided into a group of less severely (ISS < 25, n = 24) and a group of severely (ISS ≥ 26, n = 28) injured males. The uninjured volunteers (n = 10) were the controls. Osmolality, MDA, sodium, glucose, urea, creatinine, total bilirubin and total protein levels were measured in the venous blood, sampled daily, within a ten-day period. Results. In both groups of casualties, MDA and OG levels increased, total protein levels decreased, while other parameters were within the control limits. MDA alterations correlated with ISS (r = 0.414, p < 0.01), while a statistically significant correlation between OG and ISS was not obtained. Interestingly, in spite of some differences in MDA and OG trends, at the end of the examined period they were at the similar level in both groups. Conclusion. The initial oxidative damages of the cellular membrane with intracellular metabolic disorders contributed to the gradual development of metabolic-osmotic damages of cells, which, consequently caused the OG increase. In the bomb ing casualties, oxidative cell damages were dependent on the initial injury severity, while metabolic-osmotic cell damages were not.

Key words: oxidative stress; malondialdehyde; osmotic pressure; lipid peroxidation; injury severity score; blast injuries; cells.

Apstrakt

Uvod/Cilj. U ranijim ispitivanjima pokazali smo da se kod ranjenika povređenih u bombardovanju, vrlo rano razvijaju oksidativna oštećenja ćelija. U ovom radu je prikazano određivanje malondialdehida (MDA) kao indikatora lipidne peroksidacije i osmolarnog „gap”-a (OG) kao dobrog pokazatelja metaboličkih oštećenja ćelija kod ranjenika povređenih u bombardovanju i ispitivanje njihove zavisnosti od inicijalne težine povrede. Metode. Ispitivani su muškarci (n = 52), povređeni za vreme bombardovanja, kod kojih je težina povrede, procenjena na osnovu Injury Severity Score (ISS), iznosila od 3 do 66. Ranjenici su podeljeni na grupu lakše (ISS < 25, n = 24) i teže povređenih (ISS ≥ 26, n = 28). Nepovređeni dobrovoljci (n = 10) su činili kontrolnu grupu. Iz venske krvi, koja je uzimana jednom dnevno u toku deset dana, određivani su osmolalnost, MDA, natrijum, glukoza, urea, kreatinin, ukupni bilirubin kao i ukupni proteini. Rezultati. U obe grupe ranjenika dobijene su povećane vrednosti MDA i OG, snižene vrednosti ukupnih proteina, dok su ostali parametri bili u okvirima kontrolnih vrednosti. Promene nivoa MDA su bile u korelaciji sa ISS (r = 0,414, p < 0,01), dok između ISS i OG nije dobijena statistički značajna korelacija. Interesantno je, da su i pored postojanja izvesne razlike u trendu srednjih vrednosti MDA i OG, one na kraju ispitivanog perioda dosežile skoro isti nivo u obe grupe. Zaključak. Početna oksidativna oštećenja ćelijaste membrane uz intraćeljske metabolicke poremećaje doprinos postepenom razvoju metaboličko-osmotskih oštećenja ćelija, koja sledstveno dovode do povećanja OG. Kod ranjenika povređenih u bombardovanju, oksidativna oštećenja ćelija su bila zavisna od inicijalne težine povrede, dok metaboličko-osmotska oštećenja nisu.

Ključne reči: stres, oksidativni; malondialdehid; osmotski pritisak; lipid, peroksidacija; povrede, indeksi težine; povrede, blast; ćelije.

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**Introduction**

Blast caused by an explosion is the main traumagenic factor in bombing casualties. Injuries, inflicted by a complex pressure wave of an explosion, known as blast injuries, represent a complex clinical syndrome. Their pathogenesis is still a challenging issue, and the treatment of casualties is not fully elucidated, since its early diagnosis is difficult, due to the lack of specific sings. Increased free oxygen radical formation, and also an early nitric oxide overproduction following pulmonary blast injury in an animal model have already been documented. Also, in our previous investigation we reported that in blast casualties a very early increase of nitric oxide and malondialdehyde (MDA) formation are associated with the reduction of tissue oxygenation.

The increase of plasma MDA is a good marker of oxidative cell damages under different conditions. On the other hand, there is an evidence that the increase of osmol gap (OG) could indicate the "sick cell syndrome". It is known that OG represents the difference between the measured (OSM) and the calculated (OPP) osmolality of plasma/serum. Other authors emphasize that this parameter can be a good indicator of metabolic damages of cells in a trauma. Also, it has been shown that OG can be a good indicator of an organ dysfunction under different conditions. In 1973, Flear et al. claimed that there was a significant correlation between OG and the hepatic cell function tests, indicating that the acute increase of OG following trauma was caused by the abnormal loss of intracellular fluid from sick cells. In addition, a significant increase of OG has been found in patients with a failure of one or more organs. Also, a mild increase in OG has been found in patients with a failure of one or more organs.

Thus, we hypothesized that the levels of OG and MDA in venous circulation could be useful for monitoring metabolic disturbances and cells damages in blast injury. The aim of the present study was to investigate the time course alterations of both OG and MDA levels within ten days after the injury. Also we assessed the relationship between these parameters and the initial severity of the injury.

**Methods**

This investigation was a comparative cross-sectional study. The study was approved by the Ethics Committe of the Military Medical Academy (MMA), Belgrade. The males (n = 52), at the age from 20–50 years (mean age, 26.3 ±1.14 years), injured during the bombing of Serbia and Montenegro within the period from May to June 1999, and treated at the MMA were included in this study. The control group comprised of 10 uninjured volunteers of the similar age (mean age, 32.9 ±2.84 years). The severity of injuries was estimated on admission by the Injury Severity Score (ISS). After an adequate resuscitation and the surgery, the patients were treated in the Intensive Care Unit, and mechanically ventilated with the Intermittent Positive Pressure Ventilation + Positive Endexpiratory Pressure (IPPV+PEEP) between 3 and 7 cm H2O; ventilators Drager Evita 4). If necessary, crystalloid solution (Ringer’s solution) and colloids (Haemmacell and Dextran 70) with the Central Venous Pressure (CVP) range of 5–7 cm H2O were also administrated for the blood volume replacement. Transfusion and red blood cells were indicated in the patients with hemoglobin < 100 g/l. Antibiotic prophylaxis was achieved by cephalosporins, metronidazole and aminoglycosides.

In order to prevent the effects of hepatic and kidney disorders, only the patients with the total serum bilirubin level below 30 μmol/l and serum creatinine below 180 μmol/l, were examined. Blood was sampled once daily throughout ten days after the injury, and the results were presented as: A (1st and 2nd day), B (3rd and 4th day), C (5th and 6th day), D (7th–10th day) terms. According to the ISS, patients were devided into two groups: group I with ISS ≤ 26 (less severe injuries), and group II with ISS ≥ 26 (severe injuries).

Considering the fact that in the critically ill patients, the serum water content changed due to the total protein and lipid concentration alterations, we calculated the serum water content according to the Waugh’s equation:

\[
F = (991−0.91M)\times1000
\]

\[
F – \text{water content (kg/l)}, L – \text{total lipids (g/l)}, P – \text{total protein (g/l)}, \text{atmosphere of gases as a constant at 6%/l level}
\]

An osmolar gap was calculated by subtracting the calculated (OPP) from the measured (OSM) plasma osmolality. Plasma osmolality was measured by the cryoscopy method (Osmometer Knauer). OPP was calculated by the formula:

\[
OPP = 1.86 \times \text{sodium (mmol/l)} + \text{glucose (mmol/l)} + \text{urea (mmol/l)} + 0.73P/1000
\]

The serum concentrations of sodium, glucose, urea, total protein, bilirubin and creatinine were determined on an analyzer SMAC II (Technicon, USA) with Randox reagents, while plasma MDA was measured by the colorimetric method with thiobarbituric acid. The investigation was performed mostly in fresh serum/plasma, or in frozen samples stored at -70 °C, for not more than a week.

The values were expressed as a mean ± standard error. The statistical tests were performed by the Microsoft Excel and by the statistical package, Statistic for Windows (Stat for Windows, R. 4.5, USA). The Student’s t test, analysis of variance and Tukey HSD test were used for the statistical analyses. The correlation between the measured values was analyzed by the linear regression and Spearman’s test. The values of p < 0.05 were taken as statistically significant.

**Results**

The ISS ranged from 3–66 units, average 27.04±2.53 (n = 52). The average value of ISS in the less severe injured group (group I) was 9.87±1.42 units (n = 24), and 41.7±1.42 units in the severely injured (group II) (n = 28), respectively. The difference in ISS between the examined groups was statistically significant (p < 0.001).

Within the first days after the injury, in the whole group of casualties the serum water content was significantly ($p < 0.001$) above the control limits, while at the end of the period of investigation, it entered the limits (Table 1). However, during the very early period it was significantly greater ($p < 0.001$) in the group II than in the group I (Figure 1A). In addition, the serum water content significantly correlated with the ISS ($r = 0.526, p < 0.001$).

However, a certain increase in OSM values and a simultaneous decrease in OPP values were noticed, although the alterations were not significantly different from the controls (Table 1). Throughout the examined period OSM was higher than OPP, while OG levels gradually increased. A multiple increase in OG in the group of casualties as compared to the control group was noticed in the first terms, but the maximal values were observed on the 5th to 6th day after the injury ($p < 0.001$ vs control). At the end of the examined time after injury, the means of the total protein were below the control limits (Table 1). It occurred in both groups, but the decrease in the group II was greater, than in the group I (Figure 1B). A statistically significant inverse relationship between the total protein and urea levels were within the reference ranges, but the correlation with ISS ($r = 0.291, p < 0.05$) was observed.

Within seven days after the injury, the means of the total protein were below the control limits (Table 1). It occurred in both groups, but the decrease in the group II was greater, than in the group I (Figure 1B). A statistically significant inverse relationship between the total protein and urea levels were within the reference ranges, but the correlation with ISS ($r = 0.291, p < 0.05$) was observed.

The sodium level (Table 1) was within the control limits throughout the examined period, although in the term B (3rd and 4th day), it was significantly lower ($p < 0.05$) than the control values. No correlation between sodium and ISS was obtained ($r = -0.033, p = 0.811$).

### Table 1

<table>
<thead>
<tr>
<th>Time course alterations of biochemical parameters in casualties</th>
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<tr>
<td>MDA* (mmol/l)</td>
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<td>OG (mOsm/kg)</td>
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<td>Number of samples</td>
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*MDA – malondialdehyde; OSM – measured osmolality, OPP – calculated osmolality, OG – osmolar gap

Data presented as mean ± SEM

*p < 0.05 vs Control, **p < 0.01 vs Control, ***p < 0.001 vs Control

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**Fig. 1** – Time course alterations of serum water content (A) and total protein (B) in both less severely and severely injured patients

Legend: K – Control, A (1st and 2nd day), B (3rd and 4th day), C (5th and 6th day), D (7th to 10th day)

***p < 0.01 vs Control, **p < 0.001 vs Control, +++p < 0.01 vs Group II, Group I (ISS < 25), Group II (ISS ≥ 26), data presented as mean ± SEM

period, OG values started to decrease, but they were still by several times greater than the control limits (Table 1). A different OG trend in the examined groups was observed (Figure 2). Also, no significant correlations between ISS and OSM, OPP and OG were observed.

Simultaneously, MDA levels were almost doubled, in both groups throughout the examined period (Table 1, Figure 2). It should be pointed out that in the severely injured, the MDA reached a plateau level within the first terms, while in the group I the gradual increase occurred (Figure 2). Also, there was statistically significant correlation between MDA level and ISS \( (r = 0.414, p < 0.01) \). Although in both groups of casualties, the MDA and OG alterations showed the similar trends, no statistically significant linear correlation between these two parameters in the whole group of casualties was observed \( (r = -0.200, p = 0.155) \). Interestingly, considering the means of MDA and OG, a linear correlation in the group I was observed, but in the group II an exponential correlation between these parameters was obtained (Figure 2). In spite of some differences in their trends, at the end of the examined period in both groups these parameters reached similar high levels.

![Graph A](image1.png)  
**Group I**

![Graph B](image2.png)  
**Group II**

![Graph C](image3.png)

**C**

![Graph D](image4.png)

**D**

**Fig. 2** – Time course alterations of malondialdehyde and osmolal gap in less severely (A) and severely (B) injured patients. Correlation between means of malondialdehyde and osmolal gap in less severely (C) and severely (D) injured patients

Legend: K – Control, A (1st and 2nd day), B (3rd and 4th day), C (5th and 6th day), D (7th to 10th day)  
*\( p < 0.05 \) vs Control, **\( p < 0.01 \) vs Control, ***\( p < 0.001 \) vs Control,  
OG – osmolal gap, MDA – malondialdehyde, data presented as mean ± SEM  
Group I (ISS < 25), Group II (ISS ≥ 26)
Discussion

A prolonged, gradual, and increased OG levels, associated with the almost doubled MDA levels, persisting in the bombing casualties throughout the examined period, are the main observation of this study. It is interesting that in the present study there were similar trends of OG and MDA alterations in both less severely and severely injured casualties. Namely, the increased MDA levels were followed by the increase in OG levels. It has to be pointed out that at the end of the examined period, the increase in OG and MDA reached the similar levels in both patients’ groups. However, as compared to the control, OG was increased by several times, while MDA was doubled. Also, MDA alterations correlated with ISS, while the correlation between OG and ISS was not obtained.

Although in both groups of casualties a gradual increase of OG occurred, there was a difference in their time course of alterations, particularly during the very early posttraumatic period. Thus, within the first two days, the increase in OG was greater in the less severe than in the severely injured patients. Interestingly, in the less severe injured group of casualties, a linear correlation between the means of MDA and OG was obtained, while in the severely injured group an exponential correlation occurred. In our opinion, it might be partly a consequence of blood flow alterations, and/or infusions of different volumes of the applied solution in order to restore blood volume. Others have documented that the increased OG following trauma correlates well with the number of failed organs and the APACHE II score, particularly in the patients with multiple organs failure (MOF) 15. In addition, it has been shown that OG values in non-survivors were higher than in survivors with MOF 20, 21. They found that in survivors OG reached 20.0 mOsm/kg, while in non-survivors it was 34.4 ± 18.9 mOms/kg 20. In our studies, there were no lethal outcomes.

The results presented in this paper, also indicated a certain increase in OSM levels and decrease in OPP levels, but there were no significant differences between these values as compared to the controls. It should be pointed out that also a certain decrease in sodium level occurred, particularly 3–4 days after the injury, but it was within the reference limits. It could be the consequence of physiological solutions infusion during the early posttraumatic period. Also, the average sodium in the control group was close to the upper limit of the reference intervals ranging from 135 to 149 mmol/l. Moreover, due to the proper treatment, both glucose and urea levels were almost kept within the control limits. Thus, it is likely that the OG increase was not due to glucose, urea and sodium alterations (included in OPP calculation), but rather to the osmotically active solutes. On the other hand, a significant increase in serum water content has to be considered, too, particularly in regard to the total protein decrease. Others have confirmed that the nature of endogenous solutes which accounts for the increased osmolar gap, remains yet to be determined 14. Lactate, pyruvate, ketone body, nonesterified fatty acids, small peptides, amino acids and other metabolites could participate in the increase of OG 15,21,27. In addition, posttraumatic hormonal disturbances favouring cathobic processes, has to be considered, too 28, 29. There are opinions that about 75% of the increased OG could be accounted for by the increased amino acid concentrations during the postoperative state 21. On the other hand, others have not found any significant amino acid alterations in patients with the increased OG 14. This discrepancy was probably due to the difference in the examined patients groups and to the examined metabolic disorders. Amino acids are involved in the cell volume regulation 30–33 while their release from the cell after ischaemia or trauma has been documented 34. Also, our experimental investigation has demonstrated that blast injuries disturb the whole amino acid metabolism, particularly during a very early posttraumatic period 7. In addition, we demonstrated the relationship between plasma volume and amino acid metabolism after the occurrence of trauma 15. Recent investigations pointed out the inverse correlation between plasma taurine and sodium in the critically ill patients, particularly in trauma associated with sepsis 35. Moreover, the increase of cytokines (interleukin-1 and Tumor Necrosis Factor α – IL-1 and TNFα), eicosanoids, malondialdehyde, and other osmotically active components were observed following a blast injury, too 3–8, 36. All these substances could participate in the increased OG, which was observed in the present study.

It is well known that the increased formation of free oxygen radicals initiates the lipid peroxidation in the cell membrane. Lipid peroxidation is a chain reaction resulting in the MDA formation 37, characteristic particularly for an early posttraumatic period 31, 38. Recently, we reported a very early increase in nitric oxide formation, following blast injury 7, 9–11. In addition, we observed in the bombing casualties a very early increase of superoxide formation, associated with the decreased antioxidative defense 39. These findings suggested that in the injuries caused by the blast wave action, oxidative stress was intensified, and consequently participated in the oxidative damages of cell membranes. It is known that the lipid peroxidation could decrease the membrane fluidity, increase the “leakiness” of the membrane to substances that do not normally cross it (such as Ca2+ ions) and inactivate membrane-bound enzymes 12, 13, 37, 40. Also, oxidative disorders of the cell membrane associated with the intracellular metabolic disturbances could make osmotically active substances escape out of the cells in the extracellular space, resulting in the OG increase, reviewed in this paper. At the same time, a transient increase in intracellular enzymes activity occurred in the casualties, suggesting certain membrane damages 41. The presented results also demonstrated the relationship between plasma MDA and the severity of the initial injury. Interestingly, no correlation between ISS and OSM, OPP or OG was observed. However, it should be noted that at the end of the examined period OG values reached about 15 mOsm/kg, regardless the initial injury severity (at the same level in both group I and group II). It is likely that the observed metabolic disturbances could reduce the recovery following a blast injury. Others have also reported that in the posttraumatic stress disorder (PTSD), especially following a blast injury, persistent damages of some tissues, particularly those in central nervous system occur 36. The authors have found that the injured in explosions manifest the impaired vegetative functions more frequently than the gunshot injured ones 36, 42. It finally results as a psycho-organic

syndrome that might be specific for blast injuries. In our opinion, early oxidative cell damages participate in the overall metabolic disturbances following a blast injury.

Conclusion

A prolonged increase in OG and MDA levels and their similar alteration trends, suggesting the development of oxidative cell damages was related with general metabolic–osmotic disturbances in the bombing casualties. The initial oxidative damages of cellular membrane with the intracellular metabolic disturbances contributed to the gradual development of metabolic-osmotic damages of cells, which, consequently, caused the OG increase. The oxidative cell damages in the bombing casualties were dependent on the initial injury severity, while the metabolic-osmotic cell damages were not.

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The paper was received on January 18, 2006.