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PLASMA REDOX STATUS IN CRITICALLY ILL PATIENTS WITH MULTIPLE ORGAN FAILURE

V. Hadjimitova[1], D. Tzoneva[2], Al. Todorov[2*], St. Hinev[2]

[1]Department of Medical Physics and Biophysics, Medical University – Sofia, Bulgaria
[2]University Hospital Alexandrovska – Sofia, Clinic of Anesthesiology and Intensive care; Department of Anesthesiology and Intensive Care, Medical University – Sofia, Bulgaria

ABSTRACT
PURPOSE: To examine the relation between disease outcome in critically ill patients and some markers of oxidative stress. METHODS: Nine critically ill patients with abdominal diseases and multiple organ failure (MOF) were included. The patients` oxidative status was studied by evaluating the level of TBA-RS in the blood plasma. The plasma antioxidant capacity was evaluated by ABTS method and results are presented in trolox equivalent. Blood samples were taken on day 1, 2, and 5 after patients` admission to the ICU. Vital signs (HR, BP, RR, RV, T, $SatO_2$) and laboratory tests (blood count, biochemistry, CRPr, BGA, ionic balance) were examined dynamically. RESULTS: We found decreasing in the TBA-RS levels and simultaneous increasing in antioxidant activity measured by trolox equivalent in survived patients. The group of patients with high TBA-RS levels and low trolox equivalent activity had worse course of the disease and some of them died. CONCLUSIONS: We found out that the changes in the levels of products of LP and total antioxidant activity correlate with the disease outcome in critically ill patients with MOF. These data suggest potential therapeutical strategies, including antioxidant supplementation.

Key words: oxidative stress • critically ill patients • multiple organ failure

INTRODUCTION

Multiple Organ Failure (MOF) is a severe, nonspecific stress-reaction of the human organism as a result of a systematic inflammatory response, with a universal effect on all organs and tissues by the aggressive mediators of the critical condition. The function of the damaged organs and systems is impaired and may consequently lead to irreversible changes in them. MOF is the main reason for lethal outcome in ICU (Intensive Care Unit) patients and comprises up to 80% of the total mortality (1-3). According to registered data critically ill patients, as for example those with ARDS (Adult Respiratory Distress Syndrome) (5, 6), with sepsis and septic shock (7-9), with burn injury (10), as well as with SIRS (Systemic Inflammatory Response Syndrome) (9, 10-13), develop oxidative stress (OxS) (3, 4). In all these cases certain changes of the markers of OxS are observed: increasing in the levels of TBA-RS (5, 6, 9, 12), decreasing in the total antioxidant capacity (TAOC) (5, 6, 9, 11-13) decreasing in the amount of certain molecules (such as glutathione) (10-16), as well as in some antioxidant enzyme systems (10, 17).

The purpose of this research is to find out the relation between severity of the condition of patients with MOF and the level of lipid peroxidation (LP), as well as to trace out the change of TAOC of blood plasma in the course of the disease.

MATERIALS AND METHODS

We studied nine critically ill patients, with both surgical diseases of abdominal organs and MOF. *Inclusion criteria*: critically ill patients, admitted to hospital as an emergency case, who have undergone surgical intervention upon their gastrointestinal tract with the developing of MOF in the early postoperative

***Correspondence to**: *Alexander Todorov, MD, University Hospital Alexandrovska – Sofia, Clinic of Anesthesiology and Intensive care, Department of Anesthesiology and Intensive Care, Medical University, Sofia, Bulgaria, Email: alexandrtodorov@gmail.com, Tel: +359885198350*

period; patient's agreement to participate in the study. *Exclusion criteria*: the presence of oncological disease. The severity of the clinical condition of the patients is evaluated by APACHE II (Acute Physiology and Chronic Health Evaluation) scale while the anesthesiological risk – by the ASA (American Society of Anesthesiology) classes. The surgical interventions are performed under standard general endotracheal anesthesia with Mechanical Ventilation (MV). In the postoperative period all studied patients develop acute respiratory failure, sepsis, which prompted the application of MV and combined antibiotic therapy. The vital signs (HR, BP, RR, RV, T, Sat O2) and laboratory tests (blood count, biochemistry, CRPr, BGA, ionic balance, microbiological cultures) are examined dynamically. The nine studied patients, including six women and three men, are of average age of 61 years (in the range of 46 to76 years). They have been operated on on emergency of diffuse peritonitis and /or of acute necrotic pancreatitis (6:3). All patients are at risk by ASA III E and severity of condition by APACHE II of averagely 29 (in the range of 21 to 34). Blood samples were taken from the patients on the1st, 3rd, and 5th postoperative day. The oxidative status of the patients is evaluated ex vivo by TBA-RS test and ABTS test, applied upon blood plasma. By TBA-RS we determined the level of the available oxidated lipids in the blood plasma while by ABTS method – TEAC (Trolox equivalent antioxidant capacity) of the same sample.

Registration of TBA-RS *(Thiobarbituric acid reactive substances)**:*** The TBA-RS of LP was measured by method of Asakawa and Matsushita (18) in two kinds of samples: either sample contained Fe^{2+}, or sample without Fe^{2+}. Each sample was prepared in 1 ml PBS, pH 7.4 containing 0.1 ml blood plasma, 0.1 ml $FeCl_2$ (with final concentration of 0.1 mmol/L) or none. Then the samples were incubated at 37°C for 30 min. The 0.5 ml of 2.8% trichloroacetic acid and 0.5 ml of 0.5 % TBA were added. The solution was heated at 100°C for 20 min. The absorption was measured at 532 nm. The results were calculated in MDA (malondialdehyde) equivalent.

ABTS radical cation scavenging assay: The $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) scavenging test was used to determine the antioxidant activity of blood plasma. $ABTS^{\bullet+}$ radical was obtained by reaction between ABTS and potassium persulfate (19). Blank sample was prepared from the daily solution by adding 1 ml PBS, which gives an absorbance of 0.7 ± 0.01. The radical scavenging activity was assessed by mixing 2 ml of $ABTS^{\bullet+}$ solution with 10 μl plasma. The reactive mixture was allowed to stay at room temperature for 20 min and the absorbance was recorded at 734 nm. The Trolox was used as a standard. The TEAC was expressed as mmol Trolox equivalent/liter plasma.

All chemicals were purchased from Sigma-Aldrich.

Statistical analysis All values were presented as the mean ± standard deviation. $P < 0.05$ was considered statistically significant.

The research follows the Declaration of Helsinki guidelines (1964) and we have applied for and obtained approval by the Ethics Committee as well as signed informed consent from participants.

RESULTS AND DISCUSSION

During their stay in ICU we examined dynamically the condition of each patient, using the former described vital signs and laboratory tests while the severity of condition was evaluated by the APACHE II scale. Meanwhile we took blood samples to evaluate the oxidative status of the patients by using two mutually supplementary methods. The purpose was to look for a relation between the course of the disease and the change in the examined OxS markers in time. The results we obtained were grouped in three figures. Each figure shows the results of three patients, as with I-1, I-3, I-5 the results of patient I are marked, which were accordingly recorded on the 1st, 2nd, and 5th day (Figure 1). The values of TBARS, obtained for each sample with or without adding iron (Fe^{2+}) are expressed by MDA (malondialdehyde) equivalent and are shown one beside the other. The difference between these values is a measure for the oxidative status of the patient and we called it oxidative difference. For patient I we found out that the value of the oxidative difference is the lowest on the 1st day and it goes up by the 5th day. This fact shows that the oxidative status marks certain improvement during the studied period.

Table 1 shows the results of the same samples for TEAC. For the same period of time the antioxidant capacity of blood plasma decreases, which is probably due to the fact that the patient was admitted to ICU in a severe impaired condition with the clinical manifestations of acute pancreatitis, and the illness grows worse and is accompanied by complications. As a result of the treatment the condition becomes stable and remains so during the period of the study. Analogical results were being observed with patient III **(Figure 1)**, patient IV **(Figure 2)**, patients VIII and IX **(Figure 3).**

Table 1. *Trolox equivalent antioxidant capacity (TEAC) for all patients on the 1st, 3rd and 5th day. The values are presented as mean ± SD (n = 3)*

Patient	I	II	III	IV	V	VI	VII	VIII	IX
1st day	8.373 ± 0.405	7.861 ± 0.136	8.823 ± 0.175	5.043 ± 0.278	6.166 ± 0.064	6.239 0.339	7.239 ± 0.075	5.178 ± 0.009	7.795 ± 0.009
3-rd day	7.843 ± 0.032	8.258 ± 0.132	7.728 ± 0.046	5.521 ± 0.591	5.911 ± 0.239	7.296 0.359	7.362 ± 0.123	5.872 ± 0.104	5.872 ± 0.104
5-th day	7.234 ± 0.516	- *	7.896 ± 0.033	5.258 ± 0.079	4.270 ± 0.079	7.012 0.113	8.446 ± 0.226	8.596 ± 0.456	8.596 ± 0.735

* the patient has died

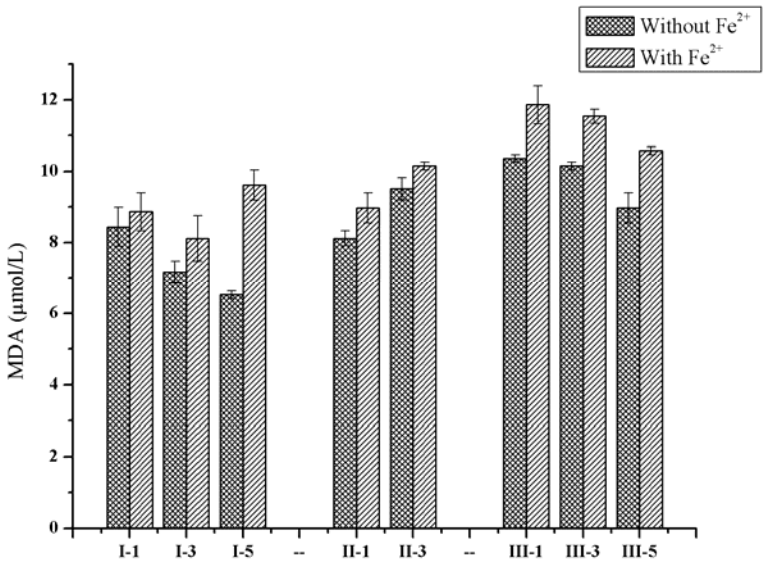

Figure 1. TBARS presented by MDA equivalent for patients I, II and III.

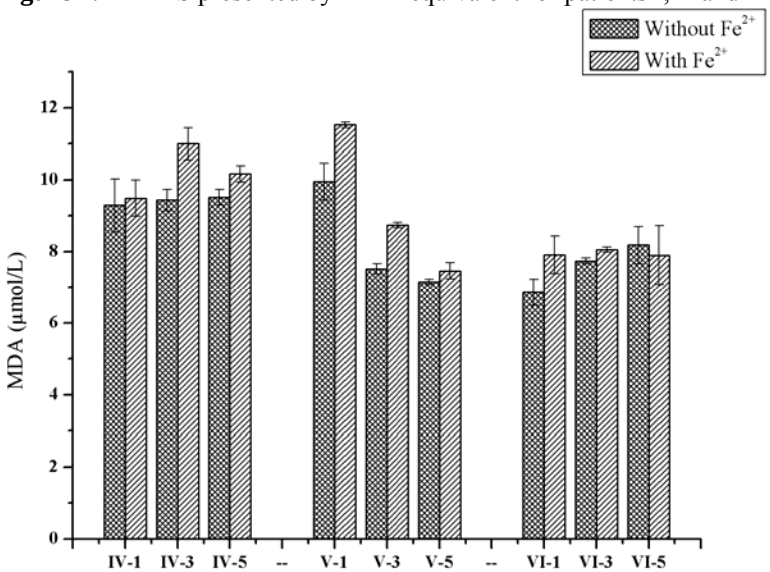

Figure 2. TBARS presented by MDA equivalent for patients IV, V and VI.

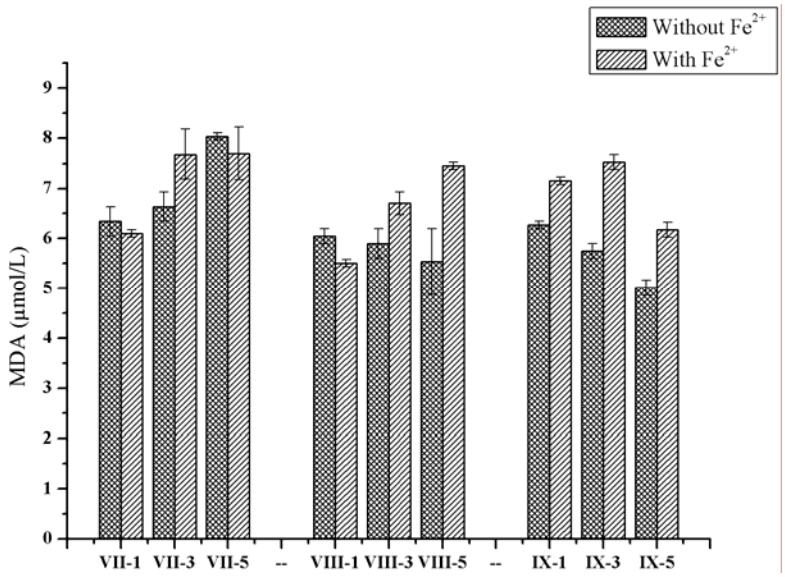

Figure 3. TBARS presented by MDA equivalent for patients VII, VIII and IX.

Of special interest is patient IX, who was admitted to ICU in an extremely severe impaired condition, high score by APACHE II and failure of two or three organ systems. As a result of the applied therapy his condition gradually became stable and he was discharged from hospital fully recovered. With this patient alanin-glutamine dipeptide was added to the standard treatment as supplementary to the parenteral nutrition. Unlike the rest of the patients in the group, what is observed with him is that together with the increasing in oxidative difference, there is also a sharp increasing in TEAC by the 5th day compared to the first **(Table 1)**. One possible explanation for the improved oxidative status is the fact that alanin-glutamine dipeptide can change favorably the proportion of oxidated to reduced glutathione (14, 15, 20, 21).

Figure 2 shows the results for patient V, whose course of illness quickly changes to the worse, develops all clinical manifestations of sepsis with MOF and has lethal outcome. Following the results of the oxidative status, we determined that it decreases from the 1st to the 5th day, which indicates that the oxidative status of the patient is getting worse and this corresponds to decreased TAEC **(Table 1)**. Analogical results were being observed with patient V **(Figure 1),** patient VI **(Figure 2)** and patient VII **(Figure 3)**. With some of these patients increasing in the values of TEAC was recorded, which corresponds neither to the clinical features nor to the worsen oxidative status. In scientific literature this result is explained with the increased values of urea in the blood plasma (22), what we already observed in the laboratory biochemical assays. We found out that when the general condition of the patient changes to the worse, a very small difference is observed between TBARS in the samples with or without iron (Fe^{2+}), as well as decreasing in TEAC. With patients in improved condition, it was determined that the oxidative difference increases, which means that the portion of oxidated lipids in the plasma decreases. Accordingly, TEAC goes up in comparison to the recorded data from the other days.

CONCLUSIONS

We found out that the increased levels of the products of LP correlate with the disease outcome in critically ill patients with MOF. TEAC should be evaluated in the context of the biochemical assays of the patients. Therapeutical strategies with antioxidant supplementation may influence the course of the disease of critically ill patients.

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REFERENCES

1. Зильбер, А.П., Полиорганная недостаточность как новый вид патологии: клиническая физиология, интенсивная терапия, профилактика.

Актуал. проблемы медицины критических состояний, 7:71-91, 2000.
2. Кижаева, Е.С., Закс, И.О., Полиорганная недостаточность в интенсивной терапии. *Вест. интенс. Терапии*, 1:12-24, 2004.
3. Goodyear-Bruch, C., Pierce, J.D., Oxidative stress in critically ill patients. *Am J Crit Care*, 11:543-551, 2002.
4. Winterbourn, C.C., Buss, I.H., Chan, T.P., Plank, L.D., Clark, M.A., Windsor, J.A., Protein carbonyl measurements show evidence of early oxidative stress in critically ill patients. *Crit Care Med*, 28:143-149, 2000.
5. Kumar, K.V., Rao, S.M., Gayani, R., Mohan, I.K., Naidu, M.U., Oxidant stress and essential fatty acids in patients with risk and established ARDS. *Clin. Chim. Acta*, 298:111-120, 2000.
6. Metnitz, P.G., Bartens, C., Fischer, M., Fridrich, P., Steltzer, H., Druml, W., Antioxidant status in patients with acute respiratory distress syndrome. *Intensive Care Med*, 25:180-185, 1999.
7. Tyml, K., Critical role for oxidative stress, platelets, and coagulation in capillary blood flow impairment in sepsis. *Microcirculation*, 18(2):152-162, 2011.
8. Cowley, H.C., Bacon, P.J., Goode, H.F., Webster, N.R., Jones, J.G., Menon, D.K., Plasma anti-oxidant potential in severe sepsis: a comparison of survivors and nonsurvivors. *Crit Care Med*, 24:1179-1183, 1996.
9. Goode, H.F., Cowley, H.C., Walker, B.E., Howdle, P.D., Webster, N.R., Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. *Crit Care Med*, 23:645-646, 1995.
10. Ritter, C.,Andrades, M., Guerreiro, M., Zavaschi, L., Gelain, D.P., Souza, L.F., Ribeiro, C.A., Clausell, N., Menna-Barreto, S., Moreira, J.C., Dal-Pizzol, F., Plasma oxidative parameters and mortality in patients with severe burn injury. *Intens Care Med*, 29:1380-1383, 2003.
11. Motoyama, T., Okamoto, K., Kukita, I., Hamaguchi, M., Kinoshita, Y., Ogawa, H., Possible role of increased oxidant stress in multiple organ failure after systemic inflammatory response syndrome. *Crit Care Med*, 31:1048-1052, 2003.
12. Alonso de Vega, J.M., Diaz, J., Serrano, E., Carbonell, L.F., Oxidative stress in critically ill patients with systemic inflammatory response syndrome. *Crit Care Med*, 30:1782-1786, 2002.
13. Tsai, K., Hsu, T., Kong, C., Lin, K., Lu, F., Is the endogenous peroxyl-radical scavenging capacity of plasma protective in systemic inflammatory disorders in humans? *Free Radic. Biol. Med*, 28:926-933, 2000.
14. Flaring, U.B., Rooyackers, O.E., Hebert, C., Bratel, T., Hammarqvist, F., Wernerman, J., Temporal changes in whole-blood and plasma glutathione in ICU patients with multiple organ failure. *Intensive Care Med*, 31:1072-1078, 2005.
15. Hammarqvist, F., Luo, J.L., Cotgreave, I.A., Andersson, K., Wernerman, J., Skeletal muscle glutathione is depleted in critically ill patients. *Crit Care Med*, 25:78-83, 1997.
16. Quinlan, G.J., Evans, T.W., Gutteridge, J.M.C., Oxidative damage to plasma proteins in adult respiratory distress syndrome. *Free Radic Res*, 20:289-298, 1994.
17. Halliwell B., Gutteridge, J.M.C., Free radicals in biology and medicine, 3rd ed. *Oxford*: Oxford University Press, 1999.
18. Asakawa, T., Matsushita, S., Thiobarbituric acid test for detecting lipid peroxides. *Lipid*;14: 401-406, 1980.
19. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med*, 26:1231-1237, 1999.
20. Estívariz, C.F., Griffith, D.P., Luo, M., Szeszycki, E.E., Bazargan, N., Dave, N., Daignault, N.M., Bergman, G.F., McNally, T., Battey, C.H., Furr, C.E., Hao, L., Ramsay, J.G., Accardi, C.R., Cotsonis, G.A., Jones, D.P., Galloway, J.R., Ziegler, T.R., Efficacy of parenteral nutrition supplemented with glutamine dipeptide to decrease hospital infections in critically ill surgical patients. *JPEN J Parenter Enteral Nutr*, 32(4):389-402, 2008.
21. Novak, F., Heyland, D.K., Avenell, A., Drover, J.W., Su, X., Glutamine supplementation in serious illness: a systematic review of the evidence. *Crit Care Med*, 30:2022-2029, 2002.
22. Rice-Evans, C. and Miller, N.J., Total antioxidant status in plasma and body fluids. *Methods Enzymol*, 234:279-293, 1994.