Association between Serum Malondialdehyde Levels and Mortality in Patients with Severe Brain Trauma Injury

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Abstract

There is a hyperoxidative state in patients with trauma brain injury (TBI). Malondialdehyde (MDA) is an end-product formed during oxidative stress, concretely lipid peroxidation. In small studies (highest sample size 50 patients), higher levels of MDA have been found in nonsurviving than surviving patients with TBI. An association between serum MDA levels and mortality in patients with TBI, however, has not been reported. Thus, the objective of this prospective, observational, multicenter study, performed in six Spanish intensive care units, was to determine whether MDA serum levels are associated with early mortality in a large series of patients with severe TBI. Serum MDA levels were measured in 100 patients with severe TBI on day 1 and in 75 healthy controls. The end-point of the study was 30-day mortality. We found higher serum MDA levels in patients with severe TBI than in healthy controls ($p < 0.001$). Nonsurviving patients with TBI ($n = 27$) showed higher serum MDA levels ($p < 0.001$) than survivors ($n = 73$). Logistic regression analysis showed that serum MDA levels were associated with 30-day mortality (odds ratio [OR] = 4.662; 95% confidence interval [CI] = 1.466–14.824; $p = 0.01$), controlling for Glasgow Coma Score, age, and computed tomography findings. Survival analysis showed that patients with serum MDA levels higher than 1.96 nmol/mL presented increased 30-day mortality than patients with lower levels (hazard ratio = 3.5; 95% CI = 1.43–8.47; $p < 0.001$). Thus, the most relevant new finding of our study, the largest to date on serum MDA levels in patients with severe TBI, was an association between serum MDA levels and early mortality.

Key words: brain trauma; injury; MDA; mortality; patients

Introduction

TRAUMATIC BRAIN INJURY (TBI) is an important cause of death, disability, and resource consumption.¹ Head trauma causes two kinds of injury in the neural tissue. One is the primary injury, which refers to the initial physical forces applied to the brain at the moment of impact. The other is secondary injury, which occurs over a period of hours or days after the initial trauma, resulting in a neuroinflammatory response and free radical generation.²

Reactive oxygen species (ROS) are produced after TBI, and they are involved in the secondary brain injury.^{3–6} Under physiologic conditions, ROS are carefully balanced by the action of pro-oxidant and antioxidant systems in cerebral tissue, and they are continu-

ously formed in small amounts as products of oxidative metabolism. TBI results in an increase of ROS and leads to lipid peroxidation, among other effects. Malondialdehyde (MDA) is an end-product formed during this lipid peroxidation, because of degradation of cellular membrane phospholipids.^{7,8} MDA is released into extracellular space and finally into the blood; it has been used as an effective biomarker of lipid oxidation.^{7,8}

Previous studies have reported higher levels of MDA in patients with TBI than in controls.^{9–13} In addition, in small studies (highest sample size 50 patients), higher levels of MDA were found in nonsurviving than surviving patients with TBI.^{12–14} To our knowledge, however, an association between serum MDA levels and mortality in patients with TBI has not been previously reported.

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Thus, the objective of this study was to determine whether MDA serum levels are associated with early mortality in a large series of patients with severe TBI.

Methods

Design and subjects

This prospective, observational, multicenter study was performed in six intensive care units of Spain. The study was approved by the Institutional Ethic Review Board of the six participating hospitals: Hospital Universitario de Canarias (La Laguna, Santa Cruz de Tenerife, Spain), Hospital Universitario Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain), Hospital General de La Palma (La Palma, Spain), Hospital Clínico Universitario de Valencia (Valencia, Spain), Hospital Insular (Las Palmas de Gran Canaria, Spain), Hospital Universitario Dr. Negrín (Las Palmas de Gran Canaria, Spain). Written informed consent from the patients or from their legal guardians was obtained.

We included 100 patients with severe TBI. Severity of brain trauma injury was classified according to Glasgow Coma Scale (GCS),¹⁵ and severe TBI was defined as GCS lower than 9 points.

Exclusion criteria were: age less than 18 years, pregnancy, inflammatory or malignant disease, and Injury Severity Score (ISS) in noncranial aspects higher than 9 points.¹⁶

Variables recorded

The following variables were recorded for each patient: sex, age, ISS, GCS, lactic acid, platelets, international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen, Acute Physiology and Chronic Health Evaluation II (APACHE II) score,¹⁷ and brain lesion according to the Marshall CT classification.¹⁸

End-point

The end-point of the study was 30-day mortality.

Blood sample collection

Blood samples were collected in tubes with separator gel to obtain serum from 100 patients with severe TBI on day 1 of TBI, and from 75 healthy controls to measure concentrations of MDA. After coagulation during 10 min at room temperature, serum was obtained by centrifugation at 1000 g for 15 min. The samples were aliquoted and frozen at -80°C until determination.

Serum MDA analysis

The assay of MDA levels was centralized in the Department of Physiology, Faculty of Medicine (University of the La Laguna, Santa Cruz de Tenerife, Spain). Serum MDA levels were measured using thiobarbituric acid (TBA)-reactive substance (TBARS) method as described by Kikugawa and associates,¹⁹ with some modifications. A volume sample of 0.2 mL was added to 0.2 mL of H_3PO_4 (0.2 M), and the color reaction was initiated by the addition of 25 μL of 0.11 M TBA solution. Samples were placed in a 90°C heating block for 50 min. After the samples were cooled, the TBARS (pink complex color) were extracted with 0.4 mL of *n*-

butanol. Butanolic phase was separated by centrifugation at $6000 \times g$ for 10 min.

Aliquots of the *n*-butanol phase were placed in a 96 well-plate and read at 535 nm in a microlate spectrophotometer reader (Benchmark Plus, Bio-Rad, Hercules, CA). The calibration curve was prepared with authentic MDA standards ranging from 0–20 nmol/mL. The detection limit of this assay was 0.079 nmol/mL; the intra- and interassay coefficient of variability were 1.82% and 4.01%, respectively. The serum concentration of MDA was expressed in nmol/mL. To avoid the possible dispersion of serum MDA level results, all the samples were processed at the same time, at the end of the recruitment process. MDA determination was performed by a laboratory technician blinded to all clinical data.

Statistical methods

Continuous variables are reported as medians and interquartile ranges. Categorical variables are reported as frequencies and percentages. Comparisons of continuous variables between groups were performed using Wilcoxon-Mann-Whitney test, and we reported the value of *U*. Comparisons between groups for categorical variables were performed with the chi-square test, and we reported the value of $\chi^2(\text{df})$.

Multiple binomial logistic regression analysis was applied to predict 30-day mortality. Because the number of events (death) was 27, we constructed three multiple binomial logistic regression models with only three predictor variables in each to avoid an overfitting effect that may lead to selecting a final model of slightly higher order than required.²⁰ We considered to include in regression analysis those significant variables in the bivariate analysis and were included in each model, avoiding the collinearity effect.²¹

In the first model, serum MDA levels, GCS, and age were included. In the second model, serum MDA levels, APACHE-II score, and CT classification were included. In the third model, serum MDA levels, APACHE-II score, and sex were included. Before including the variable CT classification in the regression analysis, it was recoded according to the risk of death observed in the bivariate analysis, as low (CT types 2 and 5) and high risk (CT types 3, 4, and 6), because we found a mortality rate of 3/24 (12.5%) in patients with CT classification type 2, 5/18 (27.8%) with type 3, 6/16 (37.5%) with type 4, 5/31 (16.1%) with type 5, and 8/11 (72.7%) with type 6. Thus, patients with CT classification types 2 and 5, with a combined mortality rate of 8/55 (14.5%), were considered to be at low risk; and patients with CT classification types 3, 4, and 6, with a combined mortality rate of 19/45 (42.2%), were considered to be at high risk. Odds ratio (OR) and 95% confidence intervals (CI) were calculated as measures of the clinical impact of the predictor variables.

Receiver operating characteristic analysis was performed to estimate the area under the curve (AUC), and we used the likelihood ratio between sensitivity/1-specificity as criteria to select the cutoff of the serum MDA level to predict 30-day mortality. Thirty-day Kaplan-Meier survival analysis and comparisons by log-rank test were performed using serum MDA levels lower/higher than 1.96 nmol/mL as the independent variable and survival at 30 days as the dependent variable. The association between continuous variables was assessed using the Spearman rank correlation coefficient.

TABLE 1. CHARACTERISTICS OF HEALTHY CONTROLS AND PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY

	Healthy controls (n=75)	Patients (n=100)	U or $\chi^2(\text{df})$ as appropriate	P value
Female sex–n (%)	22 (29.3)	23 (23.0)	5.31 (1)	0.51
Age–median years (p 25–75)	54 (43–63)	51 (31–67)	3699	0.38
MDA–median nmol/mL (p 25–75)	1.11 (0.79–1.51)	1.45 (1.12–1.98)	3039	<0.001

MDA, malondialdehyde.

FIG. 1. Serum malondialdehyde (MDA) levels in patients with traumatic brain injury and healthy controls.

A *P* value of less than 0.05 was considered statistically significant. Statistical analyses were performed with SPSS 17.0 (SPSS Inc., Chicago, IL) and NCSS 2000 (Kaysville, Utah) and LogXact 4.1, (Cytel Co., Cambridge, MA).

Results

Comparisons of age, sex, and serum MDA levels between patients with severe TBI (*n*=100) and healthy controls (*n*=75) are shown in Table 1. There were no significant differences between groups in terms of age and sex. We found higher MDA serum levels in patients with severe TBI than in healthy controls, however (*p*<0.001). These higher MDA levels (*p*<0.001) were also found in surviving and nonsurviving patients with severe TBI compared with healthy controls (Fig. 1).

Comparisons of demographic and clinical parameters between surviving (*n*=73) and nonsurviving (*n*=27) patients are shown in Table 2. Nonsurviving patients with TBI showed lower GCS, higher age and female rate, and APACHE-II score than survivors. We found statistically significant differences in CT classification between nonsurviving and surviving patients. In addition, nonsurviving patients showed higher serum MDA levels than surviving ones (*p*<0.001) (Fig. 1).

We found an association between serum MDA levels and APACHE-II ($\rho=0.232$; *p*=0.012) and GCS ($\rho=-0.212$; *p*=0.02) scores; however, no association was found with age ($\rho=0.104$; *p*=0.16).

TABLE 2. CLINICAL AND BIOCHEMICAL CHARACTERISTICS OF SURVIVOR AND NONSURVIVOR PATIENTS

	Nonsurvivors (n=27)	Survivors (n=73)	U or $\chi^2(df)$ as appropriate	p value
Gender female-n (%)	11 (40.7)	12 (16.4)	6.57 (1)	0.02
Age (years)-median (p 25-75)	66 (45-76)	47 (32-67)	380	<0.001
Temperature (°C)-median (p 25-75)	36.0 (35.0-37.0)	37. (35.6-37.3)	684	0.12
Sodium (mEq/L)-median (p 25-75)	141 (135-149)	139 (138-142)	730	0.19
Glycemia (g/dL)-median (p 25-75)	161 (142-189)	139 (120-163)	638	0.08
Leukocytes-median*10 ³ /mm ³ (p 25-75)	18.3 (10.7-23.9)	14.7 (10.2-19.3)	811	0.46
PaO ₂ (mm Hg)-median (p 25-75)	141 (104-186)	151 (116-217)	366	0.34
PaO ₂ /FIO ₂ ratio-median (p 25-75)	190 (154-316)	336 (242-407)	595	0.11
Bilirubin (mg/dL)-median (p 25-75)	0.75 (0.53-1.05)	0.50 (0.40-0.87)	528	0.045
Creatinine (mg/dl)-median (p 25-75)	0.95 (0.70-1.10)	0.80 (0.70-0.90)	808	0.44
Hemoglobin (g/dL) - median (p 25-75)	11.1 (9.4-12.3)	11.4 (10.4-13.0)	465	0.87
GCS score - median (p 25-75)	3 (3-6)	7 (6-8)	397	<0.001
Lactic acid (mmol/L)-median (p 25-75)	1.90 (1.15-4.55)	1.70 (1.23-2.50)	569	0.16
Platelets-median*10 ³ /mm ³ (p 25-75)	215 (139-264)	182 (143-252)	770	0.48
INR-median (p 25-75)	1.22 (1.01-1.67)	1.03 (0.92-1.15)	568	0.15
aPTT (sec)-median (p 25-75)	26 (25-31)	28 (25-32)	696	0.86
Fibrinogen (mg/dL)-median (p 25-75)	376 (246-560)	350 (282-444)	303	0.32
APACHE-II score-median (p 25-75)	26 (25-32)	19 (17-23)	212	<0.001
ISS-median (p 25-75)	25 (25-27)	25 (25-32)	876	0.24
ICP (mm Hg)-median (p 25-75)	20 (12-30)	15 (14-20)	112	0.27
CPP (mm Hg)-median (p 25-75)	60 (54-69)	68 (57-70)	103	0.46
CT classification-n (%)			16.99 (4)	0.002
· Type 1	0	0		
· Type 2	3 (11.1)	21 (28.8)		
· Type 3	5 (18.5)	13 (17.8)		
· Type 4	6 (22.2)	10 (13.7)		
· Type 5	5 (18.5)	26 (35.6)		
· Type 6	8 (29.6)	3 (4.1)		
MDA (nmol/mL) - median (p 25-75)	1.99 (1.31-2.76)	1.35 (1.02-1.79)	476	<0.001

p 25-75, percentile 25th-75th; PaO₂, pressure of arterial oxygen/fraction inspired oxygen; FIO₂, pressure of arterial oxygen/fraction inspired oxygen; GCS, Glasgow Coma Scale; INR, international normalized ratio; aPTT, activated partial thromboplastin time; APACHE II, Acute Physiology and Chronic Health Evaluation; ISS, Injury Severity Score; ICP, intracranial pressure; CPP, cerebral perfusion pressure; CT, computed tomography; MDA, malondialdehyde.

We found that females had higher serum MDA levels than males (1.70 [95% CI=1.23–2.57] vs. 1.40 [95% CI=0.80–1.86] nmol/mL; $U=644$; $p=0.048$); however, we found no significant differences in serum MDA levels according to CT findings grouping the patients as low (CT types 2 and 5) and high risk (CT types 3, 4 and 6) of death (1.41 [95% CI=1.00–1.80] nmol/mL vs. 1.54 [95% CI=1.23–2.40] nmol/mL; $U=983$; $p=0.08$).

Multiple binomial logistic regression analysis showed that serum MDA levels predicted 30-day mortality (OR=4.662; 95% CI=1.466–14.824; $p=0.01$), controlling for GCS and age (Table 3). In addition, serum MDA levels predicted 30-day mortality (OR=3.122; 95% CI=1.040–9.365; $p=0.04$), controlling for APACHE-II and CT classification (Table 3). In addition, serum MDA levels predicted 30-day mortality (OR=3.686; 95% CI=1.260–10.783; $p=0.02$), controlling for APACHE-II and sex (Table 3).

The AUC for serum MDA levels as predictor of 30-day mortality was 0.76 (95% CI=0.663–0.838; $p<0.001$) (Fig. 2).

Survival analysis showed that patients with serum MDA higher than 1.96 nmol/mL presented higher 30-day mortality than patients with lower levels (hazard ratio=3.5; 95% CI=1.43–8.47; $p<0.001$) (Fig. 3).

Discussion

To our knowledge, this study includes the largest series reporting data on MDA levels in patients with severe TBI. We found higher serum MDA levels in patients with severe TBI than in healthy controls, and in nonsurviving than in surviving patients. The most relevant new finding of our study was an association between serum MDA levels and early mortality in patients with TBI.

We found that patients with severe TBI had higher serum levels of MDA than healthy controls. These findings are consistent with the results of other smaller series.^{9–13} In these studies, higher MDA levels were found in serum,⁹ plasma,¹⁰ cerebrospinal fluid,¹¹ or erythrocytes^{12,13} of the patients with TBI than in control subjects.

In addition, nonsurviving patients with TBI showed higher serum levels of MDA than survivors. These findings are consistent

FIG. 2. Receiver operating characteristic analysis using serum malondialdehyde (MDA) levels as predictor of mortality at 30 days. AUC, area under the curve; CI, confidence interval.

with the results of other smaller series^{12–14} reporting higher MDA levels in erythrocytes^{12,13} or serum¹⁴ of nonsurviving patients than survivors. An association between MDA levels and mortality in patients with TBI has not been previously reported, however. The larger sample size of our study ($N=100$), compared with previous studies (highest sample size of 50 patients), allowed us to perform regression analysis to determine the independent contribution of MDA for the prediction of 30-day mortality. We report for the first time that serum MDA levels could be used to predict mortality in patients with severe TBI.

TABLE 3. MULTIPLE BINOMIAL LOGISTIC REGRESSION ANALYSIS TO PREDICT 30-DAY MORTALITY

Variable	Odds ratio	95% confidence interval	p
First model			
Serum MDA levels (nmol/mL)	4.662	1.466–14.824	0.01
GCS score (points)	0.574	0.409–0.804	0.001
Age (years)	1.083	1.035–1.134	0.001
Second model			
Serum MDA levels (nmol/mL)	3.122	1.040–9.365	0.04
APACHE-II score (points)	1.383	1.181–1.621	<0.001
CT classification (reference category: low risk of death)	5.311	1.253–22.517	0.02
Third model			
Serum MDA levels (nmol/mL)	3.686	1.260–10.783	0.02
APACHE-II score (points)	1.331	1.150–1.541	<0.001
Sex (reference category: female)	0.841	0.198–3.574	0.82

MDA, malondialdehyde; GCS, Glasgow Coma Scale; APACHE II, Acute Physiology and Chronic Health Evaluation; CT, computed tomography.

FIG. 3. Survival curve at 30 days using 1.96 nmol/mL of serum malondialdehyde (MDA) levels as cutoff. CI, confidence interval.

Taken together, these results indicate that alteration of the oxidative state may be of great pathophysiological significance in patients with TBI. The high serum MDA levels found in patients with TBI, especially so in nonsurvivors, represents an increase of lipid peroxidation from overproduction of ROS. The imbalance between antioxidant and pro-oxidant systems, produced by an increase of free radicals, favors a hyperoxidative state in patients with TBI. Increased ROS production in nonsurviving patients may contribute to different effects such as cellular dysfunction, loss of microvascular regulation, vasogenic edema, and progressive post-traumatic ischemia, finally leading to death.^{2,22}

From a therapeutic perspective, antioxidant agents (such as melatonin, resveratrol, lipoic acid, curcumin, or tempol) could be used as a new class of drugs for the treatment of patients with severe TBI.^{2,22} In animal models, the administration of different antioxidant agents such as melatonin^{23,24} or memantine²⁵ has been found to reduce MDA levels in brain tissues. In another animal study, melatonin has been shown to exert a beneficial effect on TBI.²⁶ In a small randomized clinical trial (18 patients with amantadine and 14 without amantadine), the administration of amantadine sulphate reduced MDA levels and mortality in patients with TBI (5.5% vs. 42.9%).²⁷

The strengths of our study are that it was a multicenter study (which increases the external applicability of results to other similar units) and it included a larger sample size in relation to previous studies (which allowed us to increase the accuracy of the parameters analyzed and report for the first time an association between serum MDA levels and mortality). Some limitations of our study should be recognized, however. First, we did not analyze serum MDA levels during follow-up to be able to describe their evolution in nonsurviving and surviving patients with TBI. Second, measurement of other compounds of oxidant and antioxidant states would be desirable to better evaluate this balance. Third, whereas low serum MDA levels have a high specificity, high levels of MDA have a low sensitivity; thus, the sole use of MDA levels to predict 30-day survival or nonsurvival in patients with TBI should be taken with caution. Additional studies are therefore needed to confirm the main finding of our study.

Conclusions

The most relevant new finding of our study, the largest to date on serum MDA levels in patients with severe TBI, was an association between serum MDA levels and early mortality.

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Author Disclosure Statement

No competing financial interests exist.

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