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Serum thiamine concentration and oxidative stress as predictors of mortality in patients with septic shock

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ABSTRACT

Purpose: The purpose of the study is to determine the influence of serum thiamine, glutathione peroxidase (GPx) activity, and serum protein carbonyl concentrations in hospital mortality in patients with septic shock.
Materials and Methods: This prospective study included all patients with septic shock on admission or during intensive care unit (ICU) stay, older than 18 years, admitted to 1 of the 3 ICUs of the Botucatu Medical School, from January to August 2012. Demographic information, clinical evaluation, and blood sample were taken within the first 72 hours of the patient's admission or within 72 hours after septic shock diagnosis for serum thiamine, GPx activity, and protein carbonyl determination.
Results: One hundred eight consecutive patients were evaluated. The mean age was 57.5 ± 16.0 years, 63% were male, 54.6% died in the ICU, and 71.3% had thiamine deficiency. Thiamine was not associated with oxidative stress. Neither vitamin B1 levels nor the GPx activity was associated with outcomes in these patients. However, protein carbonyl concentration was associated with increased mortality.
Conclusions: In patients with septic shock, oxidative stress was associated with mortality. On the other hand, thiamine was not associated with oxidative stress or mortality in these patients.

1. Introduction

Thiamine (vitamin B1) is a water-soluble vitamin that is an essential component of cellular metabolism [1]. Thiamine acts as a cofactor in oxidative decarboxylation in 3 mitochondrial complexes (pyruvate, α-ketoglutarate, and α-ketoacids derived from branched-chain amino acids) and as a cofactor of transketolase, a cytosolic enzyme involved in the pentose cycle [2]. In addition, thiamine plays a role in maintaining cell redox status through the production of NADPH and glutathione [2]. Thiamine is essential for converting pyruvate from glucose into acetyl coenzyme A for entry to the tricarboxylic acid cycle, and a deficiency in thiamine is followed by changes in intermediate metabolism that culminate in lactic acidosis cxz [3].

Clinical syndromes of deficiency classically manifest as cardiovascular disease (wet beriberi), peripheral neuropathy (dry beriberi), and behavioral changes (Wernicke-Korsakoff syndrome), although subclinical manifestation has been described, especially in critically ill patients [3]. Thiamine deficiency is present in 20% of patients upon intensive care unit (ICU) admission and can develop over time in ICU patients [4,5]. Furthermore, thiamine deficiency is considered an uncommon source of lactic acidosis in severe sepsis and septic shock patients. However, the importance of thiamine deficiency in lactic acidosis induced by sepsis is likely underestimated.

In addition to its influence on cellular metabolism, thiamine plays a role in oxidative stress [2]. The disturbance between the oxidant and antioxidant organic balance, in favor of the former, leads to oxidative stress with subsequent oxidation of cellular components and activation of cytoplasmic and nuclear signal pathways. Thiamine is a precursor of thiamine pyrophosphate synthesis, which is a cofactor of various enzymes mainly involved in cellular metabolism and also protects against tissue oxidative damage by maintaining reduced $NADP^+$. Moreover, increased levels of thiamine increase the activity of glutathione peroxidase (GPx), a major component of the cellular

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antioxidant system [6]. Glutathione peroxidase is a selenoprotein enzyme that catalyzes the decrease of hydrogen peroxide and organic peroxides through glutathione metabolism. Alterations in selenium status may result in suboptimal amounts of GPx and have been associated with increased oxidative stress [7]. Sepsis is associated with increased oxidative stress; thus, thiamine deficiency could also interfere with septic shock outcomes through GPx reduction and consequently increase in protein damage, assessed by several markers, such as protein carbonyl. However, the relationship between thiamine levels, GPx activity, and serum protein carbonyl concentrations has not yet been evaluated in patients with septic shock.

Therefore, the objective of this study was to determine the influence of serum thiamine, GPx activity, and serum protein carbonyl concentrations in hospital mortality in patients with septic shock.

2. Methods

This study was approved by the Ethics Committee of Botucatu Medical School and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Written informed consent was obtained from all patients before their inclusion in the study. All patients older than 18 years with septic shock on admission or during their ICU stay who were admitted to 1 of the 3 ICUs at Botucatu Medical School, with 28 beds, between January and August 2012 were prospectively evaluated.

At the time of the patients' enrollment, demographic information, the Acute Physiology and Chronic Health Evaluation II (APACHE II) score, and the Sequential Organ Failure Assessment (SOFA) score were recorded. Blood sample was taken within the first 72 hours of the patient's admission or within 72 hours after septic shock diagnosis for determination of serum thiamine levels and GPx activity.

Septic shock was defined as an infection-induced systemic inflammatory response with systolic blood pressure less than 90 mm Hg or a mean arterial pressure less than 70 mm Hg requiring the introduction of vasopressor drugs [8]. Suspected infection was determined by the clinical team of the ICU. All patients were followed up during their ICU and hospital stay. The length of the hospital and ICU stay and hospital mortality were recorded.

2.1. Laboratory analysis

A hemogram was performed with a Coulter STKS hematological auto analyzer. Total serum levels of sodium, potassium, magnesium, total calcium, phosphorus, C-reactive protein (CRP), albumin, creatinine, and urea were measured using the dry chemistry method (Ortho-Clinical Diagnostics VITROS 950; Johnson & Johnson, New Brunswick, NJ), and lactate was measured using Roche OMNI S Blood Gas Analyzer (Holliston, MA).

An high performance liquid chromatography-based method (Immundiagnostik AG, Bensheim, Germany) was used to measure serum thiamine concentrations with the reference range of normality as 16 to 48 ng/mL [9]. In addition, we analyzed thiamine levels of 8 normal volunteers, which showed values of 23.0 (17.5-39.8) ng/mL. Data are expressed as the medians (including the lower quartile and upper quartile). Therefore, *deficiency* was defined as a serum thiamine concentration less than 16 ng/mL [10].

The GPx activity of red blood cell hemolysate was assessed with the method of Paglia and Valentine using the Ransel kit (Randox, Antrim, UK) [11]. Aliquots of erythrocytes were mixed into the diluting solution and incubated for 5 minutes, followed by the addition of 2× Drabkin's solution. The enzyme activity was evaluated at 37°C at a wavelength of 340 nm, and the results are expressed in units per gram of hemoglobin.

Protein carbonyl was analyzed through the reaction with dinitrophenylhydrazine and formation the of a Schiff base, according to method described by Reznick and Packer [12]. Serum samples were incubated in presence of the 10 mmol/L dinitrophenylhydrazine in the dark at room temperature for 1 hour, vortexing every 10 minutes. Proteins were precipitated with ice-cold 50% trichloroacetic acid (wt/vol) and centrifuged (10000*g* for 10 minutes). The pellets were washed 3 times with ethanol-ethyl acetate (1:1; vol/vol) mixture and resuspended in 6 mol/L guanidine hydrochloride at 37ºC for 10 minutes. The level of protein carbonyl was quantified spectrophotometrically at 360 nm using an extinction coefficient 22000 $M^{-1}.cm^{-1}$.

2.2. Statistical analysis

Data are expressed as the mean ± SD or the median (including the lower and upper quartiles). Comparisons between groups for continuous variables were performed using the Student *t* test (normal distribution) or the Mann-Whitney *U* test (nonnormal distribution). Fisher test or the χ^2 test was used for all categorical data. Spearman correlation was used to evaluate the association of continuous variables. Cox regression models were used to predict ICU mortality in patients with septic shock. Serum thiamine concentration, erythrocyte GPx activity, and serum protein carbonyl were tested as an independent variable and adjusted by age, sex, and APACHE II score. Data analysis was performed using SigmaPlot software for Windows version 12.0 (Systat Software, Inc, San Jose, CA). The significance level was set at 5%.

3. Results

One hundred eight consecutive patients were evaluated. The mean age was 57.5 ± 16.0 years, 63% were male, the median ICU and hospital stays were 7 (4-13) and 13 (5-24) days, respectively. Fifty-

Table 1
Demographical, clinical, and laboratorial data of 108 patients with septic shock

Variable	Hospital mortality		*P*
	Yes (n = 68)	No (n = 40)	
Age (y)	59.2 ± 15.6	54.5 ± 16.5	.141
Male, n (%)	39 (57.4)	29 (72.5)	.171
APACHE II score	21.6 ± 7.2	17.4 ± 7.0	.004
SOFA score	10 (7-12)	9 (7-10)	.035
Lactate (mmol/L)	2.2 (1.3-3.8)	1.4 (1.0-2.0)	<.001
Hemoglobin level (g/dL)	10.9 ± 2.3	11.3 ± 2.4	.382
Hematocrit (%)	32.7 ± 6.6	34.4 ± 7.4	.215
Leukocytes ($10^3/mm^3$)	15.3 (10.3-20.8)	13.0 (9.4-17.3)	.143
Sodium (mmol/L)	141 (137-148)	140 (137-145)	.238
Potassium (mmol/L)	4.2 (3.6-4.7)	4.2 (3.8-5.0)	.373
Phosphorus (mg/dL)	4.4 (3.3-5.5)	3.8 (2.8-4.6)	.169
Total calcium (mg/dL)	7.7 (7.0-8.2)	8.0 (7.5-8.4)	.087
Magnesium (mg/dL)	2.1 (1.8-2.3)	1.9 (1.7-2.2)	.086
Glycemia (mg/dL)	180 (111-250)	178 (139-250)	.674
CRP (mg/dL)	23.0 (8.0-31.5)	24.0 (6.6-37.5)	.677
Albumin (g/dL)	2.1 (1.7-2.6)	2.6 (2.2-3.0)	.002
Urea (mg/dL)	92.5 (50.5-138.5)	53.5 (42.3-91.8)	.006
Creatinine (mg/dL)	1.3 (0.7-2.5)	1.0 (0.7-2.8)	.687
MV, n (%)	64.0 (94.1%)	31.0 (77.5%)	.024
Thiamine (ng/mL)	7.8 (4.4-21.5)	8.6 (4.4-19.8)	.658
Protein carbonyl (nmol/mL)	5.3 (3.3-22.3)	13.8 (5.1-25.7)	.053
GPx (U/g Hb)	30.6 (23.5-37.2)	30.6 (24.9-39.1)	.698
Length of MV (d)	7 (4-13)	7 (2-14)	.838
Length of ICU stay (d)	6 (3-11)	10 (4-16)	.018
Length of hospital stay (d)	7 (3-15)	21 (15-34)	<.001
AH, n (%)	32 (47.1)	19 (47.5)	.915
DM, n (%)	24 (35.3)	9 (22.5)	.251
Cirrhosis, n (%)	9 (13.2)	0 (0)	.044

Data are expressed as the mean ± SD, median (including the lower and upper quartiles), or percentage. MV indicates mechanical ventilation; AH, arterial hypertension; DM, diabetes mellitus.