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RESEARCH ARTICLES

Correlation between Total Antioxidant Capacity and Severity of Illness in Patients with Sepsis

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ABSTRACT | Severe sepsis is a challenging problem in the intensive care unit. It seems that reactive oxygen species play a role in causing or propagating the systemic inflammatory response syndrome in life-threatening conditions. Measurement of serum total antioxidant capacity (TAC) level is reported to provide an integrated index, as opposed to one based on simple summation of measurable antioxidants. This study aimed to evaluate the correlation between TAC and severity of illness in patients with sepsis and to correlate the various biomarkers with TAC and with the prognosis. Seventy five patients admitted to the medical emergency and intensive care unit with sepsis were enrolled and TAC levels with other routine investigations were done. Mean TAC levels of cases were found to be higher than those of controls, and the difference in TAC levels of cases and controls was found to be statistically significant. Mean serum albumin levels of controls were found to be higher than those of cases. Correlation of TAC with Glasgow Coma Scale (GCS) and serum albumin levels was found to be on the same direction while with Acute Physiology and Chronic Health Evaluation II (APACHE II) and Simplified Acute Physiology Score II (SAPS II) on the negative direction. Strength of correlation of TAC with APACHE II and SAPSII was found to be poor and statistically insignificant. In conclusion, serum TAC levels were elevated in severe sepsis cases as compared to healthy controls, but this study showed poor correlation of serum TAC levels with outcome (i.e., survivors and non-survivors), indicating that TAC levels were not positively correlated with the clinical severity of sepsis. Further studies are needed to confirm our observations and elucidate the underlying mechanisms.

KEYWORDS | Sepsis; Total antioxidant capacity

ABBREVIATIONS | APACHE II, Acute Physiology and Chronic Health Evaluation II; GCS, Glasgow Coma Scale; SAPS II, Simplified Acute Physiology Score II; TAC, total antioxidant capacity level; TRAP, total radical-trapping antioxidant parameter; UA, uric acid

CONTENTS

1. INTRODUCTION

Severe sepsis is a challenging problem in the emergency department or intensive care unit (ICU), and can lead to septic shock or multiple organ failure. The complex mechanisms underlying severe sepsis remain unclear. In sepsis, the overwhelming inflammatory response to the invading pathogen is the major pathophysiological challenge, rather than the pathogen itself [1, 2]. In a systemic inflammatory response, both endothelial cells and neutrophils are activated to release oxygen-derived free radicals. It seems that these oxygen free radicals play a role in causing or propagating the systemic inflammatory response syndrome (SIRS) in life-threatening conditions, such as severe sepsis, septic shock, multiple organ dysfunction syndrome, and that the imbalance in redox state reflects both oxidative stress and tissue damage [1–3].

Measurement of serum total antioxidant capacity (TAC) level was reported to provide an integrated index, as opposed to one based on simple summation of measurable antioxidants. It possibly could be used to assess the real change in antioxidant status in patients with severe sepsis and might lead to universally useful treatment [4–6]. Most believe that higher levels of oxyradicals and lower antioxidant levels in patients with SIRS or septic shock lead to multiple organ failure. However, serum TAC increases in critically surgical patients with septic shock. Moreover, endogenous peroxyl radical-scavenging ability in the plasma of SIRS patients was found to be elevated in nonsurvivors. The actual change in TAC in severe sepsis remains controversial [4–6]. Accordingly, this study aimed to evaluate the correlation between TAC and the severity of illness in patients with sepsis and to correlate the various biomarkers with TAC and with disease prognosis.

2. MATERIAL AND METHODS

2.1. Selection of Patients

Following ethical approval, consent was sought from either the patient, or assent from a near relative. Successive patients admitted to the medical emergency and Intensive Care Unit at King George's Medical University, UP, Lucknow, who fulfilled the following criteria for sepsis, given below, within a 24 h time window, were included. Inclusion criteria are patients admitted in Medical Emergency Trauma Centre or in Medical Intensive Care Unit with features of sepsis, severe sepsis or septic shock, clinical suspicion or evidence of acute infection with SIRS.

2.2. Measurements of Serum TAC and Other Serum Biomarkers

Serum TAC, uric acid (UA), albumin, and bilirubin were measured as indicators of antioxidative status. Serum levels of TAC and UA were determined within 6 h after a patient had arrived in the emergency department. Serum TAC was assessed using the total radical-trapping antioxidant parameter (TRAP) method and on a luminometer (AutoLumat LB 953; EG&G Berthold, Bad Wildbad, Germany) according to the procedure as previously described [7]. Briefly, a chemiluminescent reaction was generated in a collection tube by carefully mixing 800 µl of distilled water, 100 µl of signal reagent (luminol and 4-iodophenol in buffer solution), and 50 µl of 1:200 diluted horseradish peroxidase solution (Sigma-Aldrich, St. Louis, MO, USA). Then, 10 µl of the sample was added to inhibit the luminescence. The duration of quenching was measured and compared with that of water-soluble ascorbic acid. The precision of the assay (coefficient of variation) was 2.3%

for within-day variation and 5.1% for day-to-day variation. Serum concentrations of UA, albumin, and bilirubin were determined using commercial kits and an automated biochemical analyzer (Hitachi 747; Roche Diagnostics, Mannheim, Germany).

2.3. Evaluation of Clinical Severity and Primary Outcome

The Acute Physiology and Chronic Health Evaluation (APACHE) II [8] score was used to evaluate the severity of disease. APACHE II score was the first system to use a quantitative evaluation of disease severity in the ICU, and the score was calculated within 24 h of emergency department admission. The primary outcome was whether the serum TAC correlated with APACHE II score in patients with severe sepsis [7]. The secondary outcome was 28-day in-hospital mortality. In order to evaluate the relative contribution of serum TAC to patient outcome, patients were divided into "survivors" and "nonsurvivors". Survivors were those patients who were still alive 28 days after admission, including an ICU stay; nonsurvivors were patients who died within 28 days of emergency department admission.

2.4. Statistical Analysis

Continuous data were summarized as mean ± SD, while discrete (categorical) data were recorded as numbers and percentages. Two continuous independent groups were compared by parametric independent Student's t-test, and the significance of the parametric t-test was validated with the nonparametric alternative Mann Whitney U test, where appropriate. Discrete (categorical) groups were compared by chi-square test. Predictors of final outcome were evaluated using multivariate logistic regression analysis. A two-sided ($\alpha = 2$) p value < 0.05 was considered statistically significant. All analyses were performed on STATISTICA software version 17 (StatSoft Inc., Chicago, IL, USA).

3. RESULTS

3.1. Demographic Characteristics

Table 1 shows the demographic characteristics of the study population. A total of 75 patients admitted in the institution were recruited in the study as cases, along with 75 healthy age and gender matched controls. Out of the 75 cases included in the study, 39 (52%) were males and the rest 36 (48%) were females. Male: female ratio in overall cases was 1:0.92. The age of cases ranged from 14 to 92 years. Mean age of overall cases (n = 75) was 45.91 ± 19.00 years. Mean age of expired cases (49.37 ± 21.51 years) was higher than that of discharged cases (43.96 ± 17.37 years). The proportional differences in age groups of expired and discharged cases were not found to be statistically significant. Similarly, the proportional differences in gender of expired and discharged cases was not found to be statistically significant either (p = 0.985).

3.2. Outcome of the Study Population

Table 2 shows the various scoring systems based on the severity of illness. Glasgow Coma Scale (GCS) of discharged cases (12.63 ± 3.78) was found to be higher than that of expired cases (10.96 ± 3.99) but the difference was not found to be statistically significant (p = 0.077). APACHE II score of expired cases (17.00 ± 7.23) was found to be higher than that of discharged cases (9.23 ± 5.56), and the difference in mean APACHE score of expired and discharged cases was found to be statistically highly significant (p < 0.001). Simplified Acute Physiology Score II (SAPS II) of expired cases (49.11 ± 19.97) was found to be higher than that of discharged cases (27.88 ± 16.87), and the difference in mean SAPS II score of expired and discharged cases was found to be statistically highly significant (p < 0.001).

3.3. Duration of Hospital Stay

Table 3 shows comparison of duration of hospital stay in cases with different outcomes at discharge. Duration of hospital stay in overall cases ranged from 2 to 45 days (median: 13 days) and mean duration of hospital stay was 15.24 ± 9.32 days. Range of duration of hospital stay of expired cases and discharged cases was 2–33 days and 3–45 days, respectively. Mean duration of hospital stay of expired cases (14.96 ± 9.24 days) was shorter than that of discharged cases (15.40 ± 9.46 days) but the difference of hospital stay among discharged cases and expired cases was not found to be statistically significant (p = 0.848).

TABLE 1. Comparison of demographic variables of cases with different outcomes at discharge

Variables	Total (n = 75)		Expired (n = 27)		Discharged (n = 48)	
	No.	%	No.	%	No.	%
Age Group (years)						
Up to 20	7	9.33	3	11.11	4	8.33
21–30	13	17.33	2	7.41	11	22.92
31–40	12	16.00	5	18.52	7	14.58
41–50	16	21.33	7	25.93	9	18.75
51–60	10	13.33	2	7.41	8	16.67
61–70	11	14.67	3	11.11	8	16.67
71–80	4	5.33	3	11.11	1	2.08
>80	2	2.67	2	7.41	0	0.00
			$\chi^2 = 10.796$ (df = 7); p = 0.148			
Min–Max	14–92		14–92		14–77	
Mean ± SD	45.91 ± 19.00		49.37 ± 21.51		43.96 ± 17.37	
Gender						
Male	39	52.0	14	51.9	25	52.14
Female	36	48.0	13	48.1	23	47.9
			$\chi^2 = 0.000$ (df = 1); p = 0.985			

TABLE 2. Comparison of risk scores in cases with different outcomes at discharge

	Total (n = 75)		Expired (n = 27)		Discharged (n = 48)		Statistical Significance	
	Mean	SD	Mean	SD	Mean	SD	t	p
GCS	12.03	3.91	10.96	3.99	12.63	3.78	–1.793	0.077
APACHE II	12.03	7.22	17.00	7.23	9.23	5.56	5.206	< 0.001
SAPS	35.52	20.64	49.11	19.97	27.88	16.87	4.895	< 0.001

TABLE 3. Comparison of duration of hospital stay in cases with different outcomes at discharge

Outcome	No.	Min.	Max.	Median	Mean	SD
Expired	27	2	33	14	14.96	9.24
Discharged	48	3	45	13	15.40	9.46
Total	75	2	45	13	15.24	9.32

Note: t = 0.192; p = 0.848.

3.4. Comparison of TAC

Table 4 shows comparison of TAC levels in cases with different outcomes at discharge. Range of TAC among expired cases and discharged cases was 0.19–0.41 units and 0.20–0.42 units, respectively. Mean TAC levels of discharged cases (0.35 ± 0.05 units) was found to be higher than that of expired cases (0.33 ± 0.06 units) but the difference was not found to be statistically significant (p = 0.189). **Table 5** shows comparison of TAC between cases and controls. Range of TAC levels in cases was found to be 0.191–0.417 units while that in controls was found to be 0.022–0.245 units. The mean TAC level of cases (0.343 ± 0.055 units) was found to be higher than that of controls (0.151 ± 0.049) and the difference in TAC levels of cases and controls was found to be statistically significant (p = 0.001).

Table 6 shows serum albumin levels among cases and controls. The serum albumin levels among cases

TABLE 4. Comparison of TAC levels in cases with different outcomes at discharge

Outcome	No.	Min.	Max.	Median	Mean	SD
Expired	27	0.19	0.41	0.34	0.33	0.06
Discharged	48	0.20	0.42	0.35	0.35	0.05
Total	75	0.19	0.42	0.35	0.34	0.05

Note: z =1.314; p = 0.189 (Mann–Whitney U test).

TABLE 5. Comparison of TAC between cases and controls

Group	N	Mean	SD	Median	25th Percentile	75th Percentile	Min.	Max.
Cases	75	0.343	0.055	0.349	0.311	0.391	0.191	0.417
Controls	75	0.151	0.049	0.145	0.123	0.187	0.022	0.245
Total	150	0.247	0.109	0.221	0.145	0.349	0.022	0.417

Note: z = 10.356; p = 0.001.

TABLE 6. Comparison of serum albumin levels between cases and controls

Outcome	No.	Min.	Max.	Median	Mean	SD
Cases	62	1.27	4.50	3.00	2.94	0.76
Controls	58	2.07	5.60	3.80	3.73	0.76
Total	120	1.27	5.60	3.40	3.33	0.85

Note: t = 5.71; p < 0.001.

TABLE 7. Correlation of TAC with SAPS, APCHE, GCS and serum albumin (cases only) (Spearman's rank correlation)

SN	Correlation with	ρ	p	Strength and Significance
1	GCS	0.165	0.157	Poor and NS
2	APACHE II	–0.178	0.126	Poor and NS
3	SAPS II	–0.165	0.156	Poor and NS
4	Serum albumin	0.178	0.166	Poor and NS

Note: NS, not significant.

ranged from 1.27 to 4.50 units while that among controls from 2.07 to 5.60 units. The mean serum albumin levels of controls (3.73 ± 0.76 units) was found to be higher than that of cases (2.94 ± 0.76 units). The difference in serum albumin levels between cases and controls was found to be statistically highly significant ($p < 0.001$). **Table 7** shows the correlation of TAC with SAPS II, APACHE, GCS, and serum albumin (cases only). Correlation of TAC with GCS and serum albumin levels was found to be on the same direction while with APACHE II and SAPS II on the negative direction. Strength of the correlation of TAC with all of the above variables was found to be poor and statistically non-significant.

4. DISCUSSION

The present study was conducted in the department of medicine, at a tertiary care health center of Northern India. A total of 75 patients admitted in the institution were recruited in the study as cases, 75 healthy age and gender matched controls were also recruited in the study. In our study, 36% of cases expired and 64% were alive at conclusion.TAC levels were found significantly higher in cases as compared with controls ($p < 0.001$). The above results were also found in the studies by others [4, 9].

The range and mean levels of TAC among expired cases and discharged cases were not found to be statistically different ($p = 0.189$). TAC levels in patients with severe sepsis remain controversial. Although Ghiselli et al. [4] suggested that plasma rather than serum should be used to measure TAC levels, in one of our other experiments conducted in our own hospital there appeared to be no significant difference between the levels detected in the plasma and serum (data not shown). Pascual et al. [6] reported that plasma TAC levels were lower in patients with sepsis but higher in patients with septic shock as compared to control individuals. MacKinnon et al. [10] and colleagues, in a study conducted in 50 critically ill patients, reported that total antioxidant status and UA levels were higher in non-survivors than in survivors, and speculated that the higher total antioxidant status level might have reflected the higher UA levels caused by renal dysfunction. Tsai et al. [11] and co-workers reported that the plasma TAC levels were significantly higher in non-survivors than in survivors. However, most other studies found that the plasma antioxidant potentials of non-survivors were significantly lower than those of survivors among patients with severe sepsis. Additionally, mortality rates are much higher in patients of sepsis with adrenal involvement [12]. The discrepancy in the findings from these studies may mainly be due to differences in measurement methods. Instead of using a spectrophotometric method, we and two other groups used the TRAP method to measure the total antioxidant potential. The spectrophotometric method, proposed by Miller and coworkers, measures the reaction of plasma antioxidants and a mixture of different reactive species including 2,2′-azinobis(3-ethylbenzothiazoline 6-sulfonic acid; ABTS) radical cation, ferryl myoglobin, hydrogen peroxide, and other radicals. In the spectrophotometric method, albumin and UA account for 43% and 33%, respectively, of TAC. The marked decrease in serum albumin in patients with severe sepsis may lead to lower levels of TAC detected using the spectrophotometric method. On the other hand, the TRAP method measures the reaction of total plasma antioxidants and peroxyl radicals generated by 2,2′-azobis(2-amidinopropane hydrochloride; ABAP). The contribution of albumin to the TRAP reaction is very low (< 10%), whereas that of UA is higher (47–57%; could be up to 76.4%). Therefore, change in serum UA may influence serum TAC levels determined using the TRAP method.

Our results showed statistically significantly lower albumin levels in cases than in controls ($p < 0.001$). Chuang et al. [7] studied the correlation of serum albumin and sepsis, but found weak correlation between them. However, Qian et al. [13] in their study concluded that hypoalbuminemia was common with sepsis, severe sepsis, or septic shock, and serum albumin level was closely related to prognosis. They concluded that serum albumin level monitoring has important clinical significance in evaluating the prognosis of severe sepsis/septic shock patients. Similar correlation was shown by Sun et al. [14], who concluded that the severity markers and outcomes of patients with serum albumin level of ≤ 20 g/L were significantly worse than those with albumin levels 21–25 g/L and ≥ 26 g/L, whereas the latter two groups had similar prognosis. Every 1 g/L decrease of albumin level below the optimal cut-off (23 g/L) was associated with a 19.4% increase in hospital mortality and a 28.7% increase in the incidence of multiple organ dysfunction syndromes.

In the present study, correlation of TAC with GCS and serum albumin levels was found to on the same direction while with APACHE II and SAPS II on the negative direction. Strength of correlation of TAC with all of the above variables was found to be poor and statistically non-significant. Chuang et al. [7] in their study showed serum TAC levels in patients with severe sepsis correlated positively with APACHE II scores ($r = 0.426$, 95% confidence interval [CI] 0.2–0.6; $P < 0.001$).However, in our study APACHE II score was significantly higher in expired cases as compared to discharged cases, but the strength of correlation of TAC with APACHE and SAPS II was poor and statistically non-significant. One of the reasons for this may be the different sample size; others could be age group variability and

different population from which sample was taken. The discrepancy in the findings from these studies may also be due to differences in measurement methods. Instead of using a spectrophotometric method, we used the TRAP method to measure the total antioxidant potential.

In conclusion, our study demonstrated that serum TAC levels were significantly higher in patients with severe sepsis than in healthy control individuals. However, the mean TAC levels between discharged cases and expired cases were not found to be statistically different, indicating that TAC levels were not positively correlated with the clinical severity of sepsis in the patients. Due to the relatively small sample size of the present study, further studies are needed to confirm our observations and elucidate the underlying mechanisms.

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Following ethical approval (9318/ethics/R.Cell-16), consent was sought from either the patient, or assent from a near relative. The authors declare no conflicts of interest.

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