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Serum total antioxidant capacity prior to liver transplantation for hepatocellular carcinoma is associated with 1-year liver transplantation survival

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Abstract

Objective: To determine whether there was an association between serum total antioxidant capacity (TAC) levels prior to in liver transplantation (LT) for hepatocellular carcinoma (HCC) and 1-year LT mortality.

Methods: This observational retrospective single-centre study of patients with LT for HCC measured serum levels of TAC and malondialdehyde (as a biomarker of lipid peroxidation) before LT. The study endpoint was 1-year LT mortality.

Results: This study included 142 patients who underwent LT for HCC. Patients who survived the first year ($n = 127$) had significantly lower aged liver donors, significantly higher serum TAC

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levels, and significantly lower serum malondialdehyde levels compared with the non-survivors ($n = 15$). Logistic regression analysis found that serum TAC levels (odds ratio [OR] 0.275; 95% confidence interval [CI] 0.135, 0.562) and the age of the LT donor (OR 1.050; 95% CI 1.009, 1.094) were associated with 1-year LT mortality. There was an inverse association between serum levels of TAC and malondialdehyde levels (rho = –0.22).

Conclusions: There was an association between low serum TAC levels prior to LT for HCC and mortality during the first year after LT. There was an inverse association between serum TAC levels and lipid peroxidation as measured by malondialdehyde levels.

Keywords

Serum total antioxidant capacity, hepatocellular carcinoma, liver transplantation, mortality, outcome

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Introduction

Hepatocellular carcinoma (HCC), the most common primary hepatic malignant tumour, is one of the most frequent causes of cancer-attributable death.[1–3] Liver transplantation (LT), which treats the hepatic insufficiency and eliminates the hepatic tumour, is the treatment of choice for some patients with HCC.[1–7]

The peroxidation of membrane lipids results in the production of different chemical entities.[8,9] Malondialdehyde is an aldehyde produced by the effects of free radicals on polyunsaturated fatty acids in the cellular membranes.[8,9] Malondialdehyde is released into the extracellular space and can reach the blood stream; therefore, it has been used as a circulating biomarker of lipid peroxidation.[8,9] Reactive oxygen species (ROS) are balanced by the action of antioxidant defences, and the determination of total antioxidant capacity (TAC) can provide information about the antioxidant status of patients.[10,11]

Lower circulating TAC levels have been found in LT patients compared with healthy controls.[12] LT patients also have lower circulating antioxidant vitamin levels than healthy controls;[13,14] and patients with HCC have lower circulating TAC levels than healthy controls.[15–18] Higher serum malondialdehyde levels have been demonstrated in LT patients compared with healthy controls,[12–14] and higher serum malondialdehyde levels have been found in HCC patients compared with healthy controls.[15,18–20] Higher circulating lipid peroxide levels have been found in patients that do not survive LT (i.e. non-survivor LT patients) compared with LT survivors.[21] Higher serum concentrations of reactive oxygen metabolites were found in HCC patients with a greater risk of disease recurrence after curative treatment.[22] The levels of circulating malondialdehyde were higher in non-survivor LT patients than survivors.[23] However, to the best of our knowledge, there are no published data describing circulating TAC levels in non-survivor and survivor patients with HCC who have undergone LT. Thus, the objective of this present study was to determine whether there was an association between serum TAC levels prior to LT and 1-year LT mortality in patients with HCC.

Patients and methods

Study design and patient population

This observational retrospective single-centre study included consecutive patients who underwent LT for HCC in the Hospital Universitario Nuestra Señora de Candelaria, Santa Cruz de Tenerife, Spain between January 1996 and January 2016. Livers for LT were collected from brain dead donors in all cases. The study was carried out with the approval of the Institutional Review Board of the Hospital Universitario Nuestra Señora de Candelaria and written informed consent was obtained from all patients or their family members.

The serum levels of malondialdehyde were determined previously in some of the patients who participated in this current study.[23] Serum TAC levels were analysed in the current study to determine their association with 1-year LT mortality.

Variables recorded

The following variables were recorded for each patient: degree of tumour differentiation; the Child-Pugh score;[24] presence of a multinodular tumour; met the Milan criteria before and after LT;[25] macrovascular invasion; microvascular invasion; model for end-stage liver disease (MELD) score[26] by hepatic function; age of liver recipient; infiltration of adjacent tissues; age of liver donor; serum alpha-fetoprotein (AFP) levels; portal hypertension (determined clinically or by hepatic venous pressure gradient); LT technique; treatment prior to LT; nodule size; ABO blood type; leukocyte count; serum albumin levels; serum protein levels; and sex. The 1-year LT survival was the study endpoint.

Determination of serum levels of TAC and malondialdehyde

A 5-ml sample of venous blood was collected approximately 2 h prior to LT from a peripheral intravenous catheter inserted in the middle third of the arm. The blood sample was transferred to a serum separator tube and centrifuged within 30 min at 1000 ***g*** for 15 min at 4°C. The serum was removed and frozen at −80°C until determination of serum TAC and malondialdehyde levels. TAC concentrations were determined in the Hospital Universitario de Canarias, Santa Cruz de Tenerife, Spain using the Antioxidant Assay Kit (Cayman Chemical, Ann Arbor, MI, USA), which is based on the ability of serum antioxidants to inhibit the oxidation of 2,2′-azino-di-(3-ethylbenzthiazoline sulphonate). The limit of detection of the assay was 0.04 nmol/ml, the inter-assay coefficient of variation was 3.0%, and the intra-assay coefficient of variation was 3.4%.

Malondialdehyde concentrations were determined in the Faculty of Medicine of La Laguna University, Santa Cruz de Tenerife, Spain using the thiobarbituric acid-reactive substance method as previously described.[27] The limit of detection of the assay was 0.079 nmol/ml, the inter-assay coefficient of variation was 4.01%, and the intra-assay coefficient of variation was 1.82%.

Statistical analyses

All statistical analyses were performed using the SPSS® statistical package, version 17.0 (SPSS Inc., Chicago, IL, USA) for Windows® and MedCalc® version 15.2.1 (MedCalc, Ostend, Belgium). Categorical variables are presented as frequency (percentage) and were compared using χ^2-test. Continuous variables are presented median (interquartile range) and were compared

using Mann–Whitney *U*-test. A receiver operating characteristic (ROC) curve was performed to estimate the prognostic capacity of serum TAC levels for 1-year LT mortality. Kaplan–Meier 1-year LT survival analysis of patients with serum TAC ≥2.98 nmol/ml and <2.98 nmol/ml was undertaken. The Youden J index was used to select this cut-off of serum TAC levels. Logistic regression analysis was used to determine the association between serum TAC levels prior to LT and 1-year LT mortality controlled for the age of LT donor. Odds ratio (OR) and 95% confidence intervals (CI) were used to estimate the clinical impact of each predictive variable. Spearman's rank correlation test was used to determine the association between serum levels of TAC and malondialdehyde. A *P*-value < 0.05 was considered statistically significant.

Results

Of the 142 patients that were included in the study, a total of 127 were alive at 1 year after LT and 15 died during the first year after LT. The demographic and clinical characteristics of the two groups of patients are presented in Table 1. Patients who survived the LT had a significantly lower age of liver donor ($P = 0.03$), significantly higher serum TAC levels ($P = 0.003$) and significantly lower serum malondialdehyde levels ($P = 0.02$) compared with non-survivor patients. There were no statistically significant differences between the two groups of patients (survivors versus non-survivors) in terms of sex, met the Milan criteria before and after LT, degree of tumour differentiation, presence of a multinodular tumour, portal hypertension, infiltration of adjacent tissues, macrovascular invasion, microvascular invasion, Child-Pugh score, ABO blood type, LT technique, treatment prior to LT, age of liver recipient, leukocyte count, serum albumin levels, serum protein levels, serum AFP levels, MELD score, and nodule size (Table 1).

The logistic regression analysis found that serum TAC levels (OR 0.275; 95% CI 0.135, 0.562; $P < 0.001$) and the age of the LT donor (OR 1.050; 95% CI 1.009, 1.094; $P = 0.02$) were associated with 1-year LT mortality (Table 2).

The ROC curve analysis found that the area under the curve of serum TAC levels for the prediction of 1-year LT survival was 74% (95% CI 66%, 81%; $P = 0.003$); and that serum TAC levels >2.98 nmol/ml had a sensitivity of 94% (95% CI 89%, 97%) and specificity of 53% (95% CI 26%, 78%) for the prediction of 1-year LT survival (Figure 1).

The Kaplan–Meier survival analysis found that patients with serum TAC levels ≥2.98 nmol/ml had a higher 1-year LT survival probability than patients with serum TAC levels <2.98 nmol/ml (94.5% [120/127] versus 46.7% [7/15]; hazard Ratio 13.4; 95% CI 2.04, 87.98; $P < 0.001$; Figure 2).

There was an inverse association between serum levels of TAC and malondialdehyde levels (as a biomarker of lipid peroxidation) (rho = –0.22; $P = 0.01$).

Discussion

The novel findings of this current study were: (i) that there was an association between low serum TAC levels (<2.98 nmol/ml) prior to LT for HCC and mortality during the first year after LT; and (ii) that there was an inverse association between serum TAC levels and lipid peroxidation as measured by malondialdehyde levels.

Previous studies have demonstrated higher serum malondialdehyde levels in LT patients[12–14] and in HCC patients[15,18–20] compared with healthy controls. In addition, lower circulating TAC levels in LT patients[12] and in HCC patients compared

Table 1. Demographic and clinical characteristics of patients ($n = 142$) with hepatocellular carcinoma who underwent liver transplantation stratified according to their 1-year survival.

	1-year survivors $n = 127$	1-year non-survivors $n = 15$	Statistical significance[a]
Sex, female	21 (16.5)	0 (0.0)	NS
Met the Milan criteria prior to LT	122 (96.1)	14 (93.3)	NS
Met the Milan criteria after LT	106 (83.5)	11 (73.3)	NS
Degree of tumour differentiation			NS
Well	95 (74.8)	12 (80.0)	
Moderate	29 (22.8)	2 (13.3)	
Poor	3 (2.4)	1 (6.7)	
Multinodular tumour	39 (30.7)	5 (33.3)	NS
Portal hypertension	87 (65.8)	11 (73.3)	NS
Infiltration	40 (31.5)	4 (26.7)	NS
Macrovascular invasion	7 (5.5)	0 (0.0)	NS
Microvascular invasion	27 (21.3)	3 (20.0)	NS
Child-Pugh score			NS
A	62 (48.8)	10 (66.7)	
B	36 (28.3)	3 (20.0)	
C	29 (22.8)	2 (13.3)	
ABO blood type			NS
A	59 (46.5)	6 (40.0)	
B	11 (8.7)	2 (13.3)	
O	51 (40.2)	6 (40.0)	
AB	6 (4.7)	1 (6.7)	
Transplantation technique			NS
By-pass	44 (34.6)	6 (40.0)	
Piggy back	83 (65.4)	9 (60.0)	
Treatment prior to LT	69 (54.3)	10 (66.7)	NS
Percutaneous ethanol injection	28 (22.0)	7 (46.7)	NS
Radiofrequency ablation	8 (6.3)	0 (0.0)	NS
Transarterial chemoembolization	27 (21.3)	3 (20.0)	NS
Liver resection	3 (2.4)	0 (0.0)	NS
Mixed treatment	3 (2.4)	0 (0.0)	NS
Age of LT recipient, years	59 (52–62)	56 (53–62)	NS
Serum alpha-fetoprotein, ng/dl	7.0 (4.0–42.0)	12.0 (4.8–164.9)	NS
Leukocyte count, $10^3/mm^3$	4.90 (3.60–6.25)	4.94 (3.49–7.92)	NS
Albumin, g/dl	3.33 (2.90–4.10)	3.31 (2.93–4.16)	NS
Protein, g/dl	6.70 (6.10–7.10)	6.70 (5.70–7.68)	NS
MELD score	15 (12–18)	15 (15–18)	NS
Nodule size, cm	3.0 (2.0–3.5)	3.2 (1.7–4.6)	NS
Age of liver donor, years	52 (35–63)	62 (49–72)	$P = 0.03$
Malondialdehyde, nmol/ml	2.96 (2.28–4.04)	3.66 (3.39–4.62)	$P = 0.02$
TAC, nmol/ml	4.00 (3.40–4.80)	2.98 (2.26–4.00)	$P = 0.003$

Data presented as *n* (%) or median (interquartile range).

[a]Categorical variables were compared using χ^2-test; and continuous variables were compared using Mann–Whitney *U*-test.

LT, liver transplantation; MELD, model for end-stage liver disease; TAC, total antioxidant capacity; NS, no significant between-group difference ($P \geq 0.05$).

Table 2. Logistic regression analysis of variables associated with 1-year mortality after liver transplantation in patients ($n = 142$) with hepatocellular carcinoma.

Variable	Odds ratio	95% confidence interval	Statistical significance
Serum total antioxidant capacity levels, nmol/ml	0.275	0.135, 0.562	$P < 0.001$
Age of liver donor, years	1.050	1.009, 1.094	$P = 0.02$

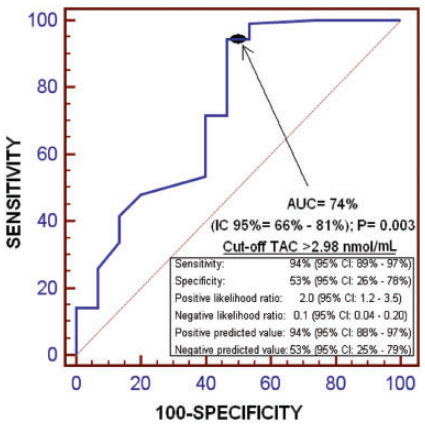

Figure 1. Receiver operating characteristic curve analysis to estimate the prognostic capacity of serum total antioxidant capacity (TAC) levels for 1-year liver transplantation survival. AUC, area under the curve; IC 95%, 95% confidence interval.

with healthy controls have been reported.[15–18] Thus, it was not unexpected to get similar data in patients with LT for HCC. However, to the best of our knowledge, this current study is the first to report an association between serum TAC levels $\geq$2.98 nmol/ml prior to LT for HCC and survival during the first year after LT, and an inverse association between serum levels of TAC and malondialdehyde.

These current findings suggest that an imbalance in the oxidative state might be of pathophysiological importance in HCC patients undergoing to LT. It is possible that LT patients who do not survive the first year after LT continue to have lower serum TAC levels and higher serum malondialdehyde levels (representing higher lipid peroxidation due to ROS overproduction that is not balanced by sufficient antioxidant compound production) than patients that survive during the first year after LT.

The use of different antioxidant agents reduced malondialdehyde levels and increased survival in animal models of sepsis[28–31] and of trauma-related brain injury.[32–34] In addition, lower circulating malondialdehyde levels and higher survival rates with the administration of different antioxidant agents were observed in clinical studies of asphyxiated newborn infants,[35] septic newborns,[36] adult burn patients,[37] and in patients with traumatic brain injury.[38] Thus, the administration of antioxidant agents could be a new class of treatment for patients with HCC undergoing LT.

This current study had a number of limitations. First, serum samples to determine serum levels of TAC and malondialdehyde levels during follow-up were not obtained. Secondly, data regarding other compounds associated with oxidant and antioxidant states were not available for analysis.

In conclusion, these current findings suggest that serum TAC levels could help in predicting mortality in patients with LT for HCC. In particular, patients with serum TAC levels <2.98 nmol/ml have a higher risk of death. However, serum TAC levels should not be used as the only prognostic biomarker, but should be used in combination with other factors such as age of LT donor, outside the Milan criteria, serum AFP levels, tumour size, tumour number, hepatic microinvasion, degree of

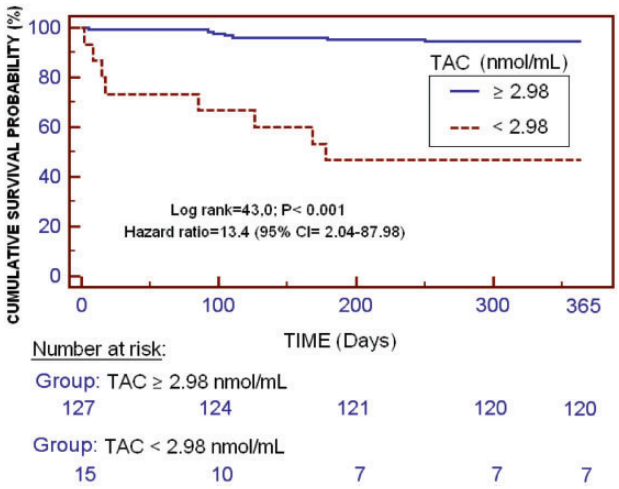

Figure 2. Kaplan–Meier 1-year survival analysis of patients with serum total antioxidant capacity (TAC) $\geq$ or $<$ 2.98 nmol/ml. 95% CI, 95% confidence interval.

differentiation, infiltration, and vascular invasion.[6,39] These current findings also suggest that further research should be undertaken to investigate the possible administration of antioxidant agents to patients with HCC undergoing LT.

Author contributions

Concept and study design: LL; acquisition of data: LL, STR, PS, APC, PAG, JP, DD, AG, MMM, PC, MAB; determination of serum total antioxidant capacity levels: APC; determination of serum malondialdehyde levels: PAG; analysis of data: LL, AJ; drafted the paper: LL. All authors revised the manuscript critically for important intellectual content and provided final approval of the version to be published.

Declaration of conflicting interests

The authors declare that there are no conflicts of interest.

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