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Retrospective Study

DNA and RNA oxidative damage in hepatocellular carcinoma patients and mortality during the first year of liver transplantation

DNA damage and mortality in liver transplantation

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Abstract

BACKGROUND

Oxidative damage of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) has been associated with mortality of patients with different diseases. However, there is no published data on the potential use of DNA and RNA oxidative damage to predict the prognosis of patients with hepatocellular carcinoma (HCC) undergoing liver transplantation (LT).

AIM

Thus, the objective of this study was to determine whether patients with increased DNA and RNA oxidative damage prior to LT for HCC have a poor LT prognosis.

METHODS

Patients with HCC who underwent LT were included in this observational and retrospective study. Serum levels of all three oxidized guanine species (OGS) were measured prior to LT since guanine is the nucleobase that forms DNA and RNA most prone to oxidation. LT mortality at 1 year was the end-point study.

RESULTS

Surviving patients (n = 101) showed lower serum OGS levels (P = 0.01) and lower age of the liver donor (P = 0.03) than non-surviving patients (n = 13). An association between serum OGS levels prior to LT and one-year LT (Odds Ratio= 2.079; 95%CI= 1.356-3.189; P = 0.001) was found in the logistic regression analysis.

CONCLUSION

The main new finding was that high serum OGS concentration prior to LT was associated with the mortality one year after LT in HCC patients.

Key Words: DNA oxidative damage; hepatocellular carcinoma; liver transplantation; mortality.

Lorente L, Rodriguez ST, Sanz P, González-Rivero AF, Pérez-Cejas A, Padilla J, Díaz D, González A, Martín MM, Jiménez A, Cerro P, Portero J, Barrera MA. DNA and RNA oxidative damage in patients with hepatocellular carcinoma and mortality during the first year of liver transplantation. *World J Hepatol* 2022; In press

Core Tip: There is not published data about the potential use of DNA and RNA oxidative damage to predict prognosis of patients with hepatocellular carcinoma (HCC) underwent to liver transplantation (LT). In this retrospective study were measured serum levels of the three oxidized guanine species (OGS) before LT in 114 patients. One-year survivor patients showed lower serum OGS levels than non-survivor patients (P = 0.01). These preliminary results could induce studies to clarify the potential role of oxidative damage in prognosis of LT patients due to HCC and to explore the use of antioxidants agents to reduce oxidative stress in those patients.

INTRODUCTION

Liver transplantation (LT) could be the treatment of choice in some patients with hepatocellular carcinoma (HCC) [1-4], which is the most common liver malignant tumor and is responsible of many deaths, due to the fact that it treats liver failure and removes the liver tumor [5-8].

The possible contribution of oxidative state in the chronic liver diseases progression and in the hepatocarcinogenesis development has been suggested [9-12]. Ribonucleic acid (RNA), deoxyribonucleic acid (DNA), lipid and proteins could be damaged by reactive oxygen species (ROS) in the oxidative stress. The five types of nucleobases present in RNA and DNA are adenine, guanine, cytosine, uracil and thymine; but only four types of those nucleobases constitute RNA and DNA. In both, RNA and DNA, guanine,

adenine and cytosine are present. Besides, uracil is also present in RNA and thymine in DNA. Guanine is the nucleobase most prone to oxidation since it has the lowest redox potential [13-16]. The three species of oxidizied guanine species (OGS) are 8-hydroxyguanine (8-OHGua) from DNA or RNA, 8-hydroxyguanosine (8-OHG) from RNA, and 8-hydroxy-2'-deoxyguanosine (8-OHdG) from DNA.

An association between DNA and RNA oxidative damage and mortality has been found in patients with other diseases as sepsis [17]. Greater DNA oxidative damage (assessed by concentration of 8-OHdG in liver biopsy samples) has been found in patients with chronic hepatic disease with HCC than without it [18,19]. However, there is no published data about the potential use of DNA and RNA oxidative damage to predict the prognosis of patients with HCC and underwent to LT. Therefore, the aim in our study was to analyze the potential association between increased oxidative DNA and RNA damage before LT for HCC and poorer LT prognosis.

MATERIALS AND METHODS

Design and Patients

We included patients underwent to LT due to HCC between May 2001 to May 2017. LT were carried out in the Hospital Universitario Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain). This observational and retrospective study was performed after the approval by the Institutional Review Board. Patients were included after the written informed consent was obtained by the LT recipient or a family member. All LT donors were brain dead. We had serum samples obtained before LT and frozen at -80ºC and serum concentrations of 8-OhdG were determined in those samples.

Variables

Sex, age, nodules size, degree of tumor differentiation, Child-Pugh score [20], infiltration, serum alpha-fetoprotein (AFP) levels, macrovascular invasion, multinodular tumor, portal hypertension (determined either by clinical data or by hepatic venous pressure gradient), microvascular invasion, model for end-stage liver disease (MELD) score [21] by hepatic function, treatment before LT, LT technique, and inside Milan criteria [22] before and after LT were registered. In addition, age of LT donor was also registered. One-year LT survival was considered our end-point study.

Serum samples and determination of OGS concentrations

Serum samples were taken about two hours before LT. Afterwards samples were placed in a -80ºC freezer. We had previously determined serum caspase-3 Levels in some of these patients [23], and in this research we determined serum OGS levels. We use kits called DNA/RNA Oxidative Damage ELISA Kit® (by Cayman Chemical Corporation in Ann Arbor, USA) to determine serum OGS concentrations. The detection limit of these kits was of 0.45 ng/mL. All determinations were carried out in the same Laboratory Department blinded to clinical data.

Statistical Methods

Categorical variables, presented as frequency (percentage), were compared using the chi-square test. Continuous variables, presented as median (percentiles 25 and 75), were compared using the test of Mann-Whitney. The ability of serum OGS concentrations prior to LT to predict one-year LT mortality was analyzed using curve of receiver operating characteristic (ROC). The Kaplan-Meier one-year LT survival curves were constructed with a serum OGS concentrations cut-off (3.3 ng/mL) selected on the basis of Youden's J-index. The association between serum OGS levels and one-year LT controlling for serum caspase-3 levels and age of liver donor was analyzed using the

logistic regression analysis. MedCal 15.2.1 (Ostend, Belgium) and SPSS 17.0 (by SPSS Inc. in Chicago, IL, USA) were used to perform the statistical analyses.

RESULTS

We included 114 patients in the study, of which 101 remain alive after one year of LT and 13 died during the first year of LT. Surviving LT patients in comparison to non-surviving patients showed lower serum OGS concentrations prior to LT (P = 0.01) and lower liver donor age (P = 0.03) (Table 1). No significant differences showed surviving and non-surviving patients regarding sex, liver receptor age, nodule size, serum AFP levels, degree of tumor differentiation, microvascular invasion, multinodular tumor, infiltration, macrovascular invasion, Child-Pugh score, MELD score, portal hypertension, treatment prior to LT, LT technique and inside Milan criteria before and after LT (Table 1). Significant differences were no found (P = 0.20) in serum OGS concentrations according the cause of death: 8 (61.5%) sepsis, 3 (23.1%) multiple organ failure, one (7.7%) recurrence of hepatitis C virus infection and one (7.7%) recurrence of HCC.

In logistic analysis, an association was found between serum OGS and one-year LT mortality, controlling for serum caspase-3 and liver donor age (Odds Ratio= 2.079; 95%CI= 1.356-3.189; P = 0.001) (Table 2). On the ROC analysis, the area under the curve of pre-LT serum OGS concentrations for predicting 1-year LT mortality was found to be 71% (95%CI = 55%-88%; P = 0.009) (Figure 1).

Serum OGS levels with a cut-off point of 3.3 ng/mL showed a sensitivity of 69% (39%-91%), specificity of 66% (56%-74%), positive likelihood ratio of 2.1 (1.3-3.2), negative likelihood ratio of 0.5 (0.2-1.1), positive predictive value of 21% (14%-29%) and negative predictive value of 94% (88%-98%) for one-year LT mortality prediction. The Kaplan-Meier survival analysis showed a higher one-year LT mortality risk in patients with serum OGS levels prior to LT above 3.3 ng/mL (Hazard Ratio= 4.2; 95%CI= 1.36-13.11); P = 0.01) (Figure 2).

DISCUSSION

To our knowledge, our study is the first reporting data about the determination of DNA and RNA oxidative damage to predict prognosis of patients with HCC who underwent LT. The main finding was that high serum OGS prior to LT was associated with the mortality one year after LT. Greater oxidative DNA damage (assessed by 8-OHdG concentration in liver biopsy specimens) has been found in patients with chronic liver disease with HCC than without [18,19]. However, the association between serum OGS concentration and LT mortality is a new finding of our study.

These higher serum GOS found in non-surviving LT patients, are in line with those found in patients with other diseases as sepsis [17], and could be in relation with a higher oxidative status that could favorate multiple organ dysfunction and dead of patients.

Limitations of our study were: First, we have not determined serum 8-OH-dG change after LT to explore which is better serum marker for prognosis (before or after LT). Second, we have not determined serum 8-OH-dG in healthy control or chronic liver patients without HCC (however; the objective of our study was this study was to determine whether patients with increased oxidative DNA and RNA damage before undergoing LT for HCC have poorer LT prognosis). Third, we have not determined other markers of oxidative stress for nucleic acid, such as abasic sites (AP) or 8-nitroguanosine 3',5'-cyclic monophosphate (8-nitro-cGMP). Fourth, we have not determined 8-OH-dG in the liver to explore its correlation with serum levels. Fifth, the regression analysis do not allow to introduce more variables due to the low number of deceased patients. However, one strength of our study was that the association between mortality and serum OGS has been also previously found in patients with other diseases as sepsis [17].

The possible contribution of oxidative state in the chronic liver diseases progression and in the hepatocarcinogenesis development has been suggested; in addition, the potential use of antioxidant agents in patients with chronic liver diseases has also been suggested

[9-12]. Therefore, these preliminary results could induce studies to clarify the potential role of oxidative damage in prognosis of LT patients due to HCC and to explore the use of antioxidants agents to reduce oxidative stress in those patients.

CONCLUSION

The main new finding was that high serum OGS concentration prior to LT was associated with the mortality one year after LT in HCC patients.

ARTICLE HIGHLIGHTS

Research background

Oxidative damage of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) has been associated with mortality of patients with various diseases.

Research motivation

However, there is no published data on the potential use of DNA and RNA oxidative damage to predict the prognosis of patients with liver transplantation (LT) due to hepatocellular carcinoma (HCC).

Research objectives

The aim in our study was to analyze the potential association between increased oxidative DNA and RNA damage before LT for HCC and poorer LT prognosis.

Research methods

In this observational, retrospective study, patients with HCC who underwent LT were included. Serum levels of all three oxidized guanine species (OGS) were measured prior to LT due to that guanine is the nucleobase with higher risk of oxidation. LT mortality at 1 year was the end point of the study.

Research results

Surviving patients (n = 101) showed lower serum OGS levels (P = 0.01) and lower age of liver donor (P = 0.03) than non-surviving patients (n = 13). An association between serum OGS prior to LT and one-year LT (Odds Ratio= 2.079; 95%CI= 1.356-3.189; P = 0.001) was found in the logistic regression analysis.

Research conclusions

The main new finding was that high serum OGS concentration prior to LT was associated with one-year LT mortality.

Research perspectives

These preliminary results could induce studies to clarify the potential role of oxidative damage in prognosis of LT patients due to HCC and to explore the use of antioxidants agents to reduce oxidative stress in those patients.

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