Evaluation of Oxidative Stress in the Late Postoperative Stage of Liver Transplantation

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ABSTRACT

Introduction. Liver transplant recipients are at an increased oxidative stress risk due to pre-existing hepatic impairment, ischemia-reperfusion injury, immunosuppression, and functional graft rejection. This study compared the oxidative status of healthy control subjects, patients with liver cirrhosis on the list for transplantation, and subjects already transplanted for at least 12 months.

Patients and Methods. Sixty adult male patients, aged between 27 and 67 years, were subdivided into 3 groups: a control group (15 healthy volunteers), a cirrhosis group (15 volunteers), and a transplant group (30 volunteers). Oxidative stress was evaluated by activity of reduced glutathione, malondialdehyde, and vitamin E.

Results. There was a significant difference ($P < .01$) in the plasma concentration of reduced glutathione in the 3 groups, with the lowest values observed in the transplanted group. The malondialdehyde values differed significantly ($P < .01$) among the 3 groups, with the transplanted group again having the lowest concentrations. The lowest concentrations of vitamin E were observed in patients with cirrhosis compared with control subjects, and there was a significant correlation ($P < .05$) among the 3 groups. No correlations were found between reduced glutathione and vitamin E or between vitamin E and malondialdehyde. However, there were strong correlations between plasma malondialdehyde and reduced glutathione in the 3 groups: control group, $r = 0.9972$ and $P < .0001$; cirrhotic group, $r = 0.9765$ and $P < .0001$; and transplanted group, $r = 0.8981$ and $P < .0001$.

Conclusions. In the late postoperative stage of liver transplantation, oxidative stress persists but in attenuated form.

STUDIES have established that increased oxidative stress accelerates the progression of liver fibrosis during chronic liver injury due to various etiologies. Theoretically, the increase in oxidative stress is essential for the development of portal hypertension and hyperdynamic circulation in cirrhotic patients [1].

Liver cirrhosis is a clinical condition that exhibits an increased and persistent oxidative imbalance, resulting in increased production of reactive oxygen species and a reduction in the bioavailability of antioxidants such as carotenoids, α-tocopherol, and glutathione (GSH) [2]. Liver transplant recipients have an increased risk for oxidative stress due to pre-existing hepatic impairment, ischemia-reperfusion injury, immunosuppression, and functional graft rejection [3].

Almost all clinical and experimental studies published on oxidative stress in liver transplantation limit their information to the intraoperative or immediate postoperative period. Studies involving postoperative biomarkers of oxidative stress are otherwise relatively scarce. The objective

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Table 1. Profile of the Cirrhotic Volunteers*

Cirrhosis Etiology	%	HPS (%)	Tabagism (%)
Alcohol	33.33	33.00	76.67 (No)
Alcohol/HCC	13.33	-	23.33 (Ex)
Cryptogenic	20.00	-	-
Virus C/alcohol	6.67	-	-
Virus C/HCC	13.33	-	-
Virus B/HCC	6.67	-	-
Virus B/Δ/alcohol	6.67	-	-

Abbreviations: HCC, hepatocellular carcinoma; HPS, hepatopulmonary syndrome.
*Model for end-stage liver disease varying between 6.66 and 33.33 (mean, 20.22 ± 6.48).

of the present study, therefore, was to compare the oxidative status of healthy control subjects, patients with liver cirrhosis who were on the list for liver transplantation, and subjects already transplanted for at least 12 months. The selected biomarkers to measure were plasma concentrations of reduced GSH, malondialdehyde (MDA), and vitamin E.

PATIENTS AND METHODS

This study was approved by the ethics committee of the Hospital of the Faculty of Medicine of Ribeirão Preto-FMRPUSP.

The study population included male subjects between 27 and 67 years of age. The first group comprised 15 healthy volunteers with a mean age of 37.13 ± 8.95 years (13.333% ex-smokers). The second group comprised 15 cirrhotic subjects (Table 1) with a mean age of 53.40 ± 9.75 years, and the third group (transplant group) comprised 30 subjects (Table 2) with a mean age of 54 ± 7.18 years.

Fifteen healthy subjects without clinical criteria for cirrhosis (ascites, jaundice, and encephalopathy) were selected for the control group. The volunteers enrolled in the study were not current smokers, and they were not taking medications that could interfere with measurements of biomarkers of oxidative stress. The cirrhotic group was composed of 15 individuals from the liver transplant list, with clinical, laboratory, and liver biopsy findings suggestive of cirrhosis, with or without hepatopulmonary syndrome. Liver disease severity in the cirrhotic group was assessed according to the model for end-stage liver disease scoring (United Network for Organ Sharing modification), varying between 6.66 and 33.33 (mean ± SD, 20.22 ± 6.48). The transplanted group consisted of 30 individuals operated on within at least 12 months at the Hospital of the Ribeirão Preto School of Medicine, University of São Paulo, São Paulo, Brazil. The model for end-stage liver disease score applied just before the liver transplant ranged from 8 to 33 (mean, 19 ± 5.9). The time of liver transplantation evolution (at the time of the blood sample collection) ranged from 1 to 7 years (mean, 2.5 ± 1.7 years), and no patient exhibited signs of rejection according to biochemical and ultrasonography criteria. The medications used at the time of blood sampling are those most frequently used for the treatment of the complications of portal hypertension such as propranolol, lactulose, pantoprazole, and treatment of viral hepatitis (5 patients). The following immunosuppressive drugs were added to the regimen of the transplanted patients: tacrolimus (11 patients), cyclosporine (2 patients), tacrolimus/mycophenolate (7 patients), cyclosporine/mycophenolate (3 patients), sirolimus/mycophenolate (6 patients), and mycophenolate (1 patient).

Blood samples were collected from a peripheral vein in the morning and transported on ice at −20°C. Samples of MDA, GSH, and vitamin E were stored in a tube containing gel separator with a clot activator. These samples were placed first in a bath set at 37°C and then centrifuged in a common centrifuge at 3000 rpm for 10 minutes to obtain serum. All samples were stored in a freezer at −70°C for later dosing.

Methods of Oxidative Stress Analysis

Oxidative stress was evaluated according to the levels of reduced GSH, and the final amount of lipid peroxidation was expressed by levels of MDA. The MDA content was determined according to the method proposed by Gerard-Monnier et al [4], with some adjustments. The reduced form of GSH was determined by using the method of Sedlack and Lindsay [5]. This method is based on the formation of the colored product (GSH−5-5'-dithiobis[2-nitrobenzoic acid]), which is determined by a change in absorbance at 412 nm and expressed as mole per liter of blood serum. Serum levels of vitamin E were measured by using high-performance liquid chromatography (model LC10A, Shimadzu Corporation, Kyoto, Japan). Vitamin E concentrations in the plasma were measured by using high-performance liquid chromatography with ultraviolet/visible detection after sample extraction with methanol and *n*-hexane, drying with nitrogen, and resuspension in the mobile phase (methanol/diclorometano/acetonitrile). The values were calculated by using a calibration curve for vitamin E [6].

Statistical Analysis

The data are presented as mean values ± SDs. To compare groups of data, dosages of GSH, vitamin E, and MDA, one-way analysis of variance (ANOVA) with Games-Howell posttest were used. Correlations between biomarkers of oxidative stress were calculated by using the Kendall Tau-b test. Differences were considered statistically significant when P values were $<.05$. All analyses were performed by using SPSS version 15.0 (IBM SPSS Statistics, IBM Corporation, Armonk, NY, United States).

RESULTS

Plasma concentrations of GSH in the 3 groups were significantly different ($P < .01$). The transplanted group had

Table 2. Profile of the Liver-Transplanted Volunteers

Cirrhosis Etiology	%	Immunosuppressive Agent	%	Hepatitis B or C Treatment (%)	Tabagism (%)
Alcohol	40.00	Tacrolimus	36.67	Hepatitis C, 13.33	63.33 (No)
Cryptogenic	6.67	Cyclosporine	6.67	Hepatitis B, 6.67	36.66 (Ex)
Virus C/alcohol	6.67	Tacrolimus/mycophenolate	23.33		
Virus C/ HCC	16.67	Cyclosporine/mycophenolate	10.00		
Virus B	6.67	Sirolimus/mycophenolate	20.00		
Virus C	26.67	Mycophenolate	3.33		

Abbreviation: HCC, hepatocellular carcinoma.