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Research paper

Oxidative stress influence on renal dysfunction in patients with obstructive jaundice: A case and control prospective study

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ABSTRACT

Background: Obstructive Jaundice (OJ) is associated with a significant risk of developing acute renal failure (ARF). The involvement of oxidative stress in the development of cholestasis has been demonstrated in different experimental models. However, its role in the morbidity of human cholestasis is far to be elucidated. The aim of the study was the evaluation of oxidative stress markers in blood from patients with OJ and its relation to complications and benign/malignant evolution of cholestasis. Methods: A prospective cross-sectional study of 105 patients with OJ and 34 control subjects were included. Several markers of liver function and oxidative stress, such as lipoperoxides (LPO), as well as reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were assessed. Results: The patients with OJ showed a marked increase in plasma levels of LPO, SOD and GSH, while GSH-Px levels were decreased. The increase in lipid peroxidation products and the depletion of SOD activity in blood were also related to renal dysfunction. The highest level of LPO was associated with malignant etiology of the disease. The logistic regression analysis showed that the age of the patient and the levels of LPO in blood were predictors of renal dysfunction in OJ patients. Conclusions: This study demonstrates a correlation between oxidative stress and renal dysfunction patients with OJ.

1. Introduction

The obstruction of extrahepatic biliary tract results in the dilatation of bile ducts with an accumulation of hydrophobic bile acids in hepatocytes. The toxic biliary products, such as glycochenodeoxicholate, as well as neutrophil migration promote the generation of oxygen-free radicals, unbalancing antioxidant status in the hepatic parenchyma [1]. Exogenous regulation of oxidative stress ameliorates liver injury induced by experimental cholestasis [2–4]. The pathogenesis of OJ involves a systemic alteration that affects different extrahepatic tissues. An increase in lipoperoxidation products is observed in extrahepatic tissues, including kidney and brain, in animals subjected to OJ [5,6].

Acute renal failure is a significant adverse outcome of OJ, observing rates of 6–18% [7], and is associated with a significant morbidity and postoperative mortality [8,9]. The administration of melatonin reduces lipid oxidation in kidney during experimental OJ [10]. Hypotension and sepsis, secondary to depletion of extracellular fluid and myocardial dysfunction has proven to be relevant in the development of kidney disease in cholestatic patients [11–19]. However, the role of oxidative stress during renal complications of OJ has not been studied in detail.

The main objective of the present study was to assess the correlation between the alteration of oxidative stress biomarkers in blood and the presence of renal failure in patients with OJ.

2. Methods

2.1. Design of study

The study was designed as a prospective cross-sectional observational study. 105 patients with OJ were enrolled and

Abbreviations: OJ, obstructive jaundice; ARF, acute renal failure; LPO, lipoperoxides; GSH, reduced glutathione; CAT, catalase; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDRD, modification of diet in renal disease; GFR, glomerular filtration rate; MDA, malondialdehyde; AP, alkaline phosphatase

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compared with 34 healthy subjects, matched by sex, age, weight, height, and body mass index. The patients were included over a period of 26 months. Inclusion criteria were OJ of benign or malign ethiology, levels of conjugated bilirrubin higher than 2 mg/dl or total bilirrubin higher than 3 mg/dl, biochemical cholestatic pattern, ultrasound evidence of extrahepatic bile duct dilatation higher than 12 mm and intrahepatic higher than 4 mm. Exclusion criteria were acute cholangitis, parenchymal liver disease, gastrointestinal hemorrhage, prior or concomitant intravenous fluid therapy, heart failure or chronic renal failure, undernourished patients, and treatment with diuretics, antihypertensives, cimetidine or trimethoprim. Informed consent was obtained from all participants.

The study was approved by the Ethical Committee for Clinical Research of the Institution.

2.2. Biochemical measurements

Biochemical and oxidative stress parameters were measured on patients peripheral blood samples while admission to hospital. Same determinations were performed in control subjects under similar conditions to baseline measurements in patients with obstructive jaundice. The levels of direct and total bilirubin, sodium, potassium, AST, ALT, alkaline phosphatase (AP), albumin and total protein were performed with Cobas Integra Roche-400 analyzer (Roche Diagnostics Ltd., Switzerland). Renal function was estimated by the modified formula known as MDRD (Modification of Diet in Renal Disease) [20]. Renal impairment was considered when the glomerular filtration rate (GFR) value was ≤ 70 ml/min (1.73 m^2). GFR was measured according to the MDRD using the following the formula:

$$\text{MDRD} = 186 \times [\text{creatinine, mg/dl}]^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ in women}) \times (1.212 \text{ if black population})$$

2.3. Oxidative stress markers

The markers of oxidative stress were assessed in plasma obtained after blood centrifugation at 3000 g for 5 min at 4 °C. Samples were stored at 80 °C until measurements.

LPO and GSH were determined using commercial assays (LPO-586 and GSH-400, Bioxytech SA, Oxis International, Portland, OR, USA). CAT and SOD were measured according to Aebi [21] and Sun [22] methods, respectively. GSH-Px was measured according to Flohe and Gunzler [23] method. The values were measured using a plate reader spectrophotometer Shimadzu UV-1603 (Shimadzu, Kyoto, Japan).

2.4. Statistical analysis

Results are expressed as mean ± standard deviation (SD). Comparisons between two groups were performed using Student's t test for unpaired groups or Mann–Whitney. Comparisons between three groups were performed using ANOVA analysis followed of post-hoc Kruskal–Wallis test. Comparisons between proportions were performed by chi-squared test. Correlations of variables were evaluated with Pearson coefficient or Spearman rho coefficient. Multivariate analysis was performed to identify factors associated with renal dysfunction. The main variables considered were: etiology of jaundice, age, duration of jaundice, bilirubin, sodium, potassium, AST, ALT, AP, albumin, total protein, LPO, GSH, GSH-Px, SOD, CAT. Linear and logistic regression were performed with the estimated renal function by MDRD. Stepwise method was used excluding variables with $P \geq 0.15$ (Student's t statistic for linear regression and Wald statistic for logistic regression).

3. Results

3.1. Oxidative stress in patients with OJ. Correlation with renal insufficiency

Table 1 shows general demographic and biochemical characteristics of the Control and OJ groups. OJ patients ($n=105$) were distributed in 67 men (64%) and 38 women (36%), with mean age of 69 ± 12.8 years old (15–93 years) (Table 1). The levels of peripheral LPO, GSH and SOD concentration were significantly higher in the group of patients with OJ compared to control subjects ($p=0.0001$) (Table 1). By contrast, levels of GSH-Px showed a significant decline ($p=0.0001$) (Table 1).

A strong positive correlation was found between levels of conjugated bilirubin and LPO in plasma ($r=0.744$, $p=0.0001$) (Fig. 1).

The creatinine clearance in control subjects (98 ± 31.1) was significantly higher than in OJ patients of benign ethiology (56 ± 25.9, $p=0.031$). There were no statistically significant differences with creatinine clearance in patients with malign ethiology (72 ± 35.2, $p=0.199$). The GFR of control subjects (104 ± 38.2 ml/min) was higher than in OJ patients of benign ethiology (84 ± 37.9 ml/min, $p=0.010$) with a moderate non statistically significant improvement in the malign ethiology (116 ± 93.3 ml/min, $p=0.184$). Renal failure was present in 32% of the patients, whereas it was only observed in 6% of controls. The presence of renal insufficiency in patients with OJ was associated with higher levels of total bilirubin ($p=0.002$), as well as an increase in serum levels of LPO ($p=0.017$) and GSH ($p=0.017$) (Table 2). By contrast, the levels of SOD showed a decrease in patients with renal impairment ($p=0.044$) (Table 2). There were no statistically significant differences in the levels of GSH-Px and CAT levels (Table 2).

A weak negative correlation was found between plasma levels of LPO and the GFR ($r=-0.303$, $p=0.001$) (Fig. 2).

3.2. Analysis of patients with OJ according to the benign/malign ethiology

There were not significant differences among the percentage of gender (men) in patients with benign (n=33, 31.5%) and malign ($n=72$, 68.5%) cholestasis (Table 3). The increased levels of total (20 ± 11.5 vs 12 ± 7.4, $p=0001$) and direct bilirubin (16 ± 9.6 vs 9 ± 6.0, $p=0001$), and AP (757 ± 554.3 vs 528 ± 577.3, $p=0.005$) in blood from patients with OJ of malign vs benign etiologies respectively, was probably due to the longer evolution period of the disease (mean duration for jaundice 18 vs 9 days) (Table 3). LPO levels were also higher in malignant etiology group. (603 ± 202.0 vs 473 ± 144.8 nmol/l) ($p=0.0001$) (Table 3).

3.3. Multiple linear regression analysis

Different variables such as age, duration of jaundice, bilirubin, sodium, potassium, AST, ALT, albumin, total proteins, GSH, SOD and CAT were eliminated from the model using the multiple F partial test ($F=-0.2764$, $p=0.735$; freedom degree=12.53). The multiple linear regression analysis for GFR estimated by the MDRD formula fulfilled the conditions of proper application such as linearity of independent variables, no co-linearity and independence among them, normality of residues and homoscedasticity of variances.

The linear adjusted equation for the GFR in OJ patients was: