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THE RELEVANCE OF OXIDATIVE STRESS BALANCE (SUPEROXIDE DISMUTASE VERSUS MALONDIALDEHYDE) IN SPONTANEOUS BACTERIAL PERITONITIS

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Abstract - Among bacterial infections associated with hepatic cirrhosis, the most common is spontaneous bacterial peritonitis (SBP). Despite different protective measures, such as early diagnosis, therapy with albumin and the introduction of new antibiotics, the prognosis of these patients remains poor, with a mortality rate of 20-40%. In this context, the identification of patients with increased risk of death is extremely important for improving the prognosis. Thus, there is growing interest for studying the effects and mechanisms of oxidative stress, considering the requirements for identifying new substances with hepatoprotective functions and reducing various adverse effects. In this study, we assessed oxidative stress markers, the antioxidant enzyme superoxide dismutase (SOD) and the marker of lipid peroxidation, malondialdehyde (MDA), in the serum and ascitic fluid in patients with decompensated cirrhosis and SBP, in patients diagnosed with decompensated liver cirrhosis with ascites and in patients with compensated liver cirrhosis. Increased oxidative stress, demonstrated by a significant decrease of SOD and increase in MDA levels, was observed in patients with decompensated cirrhosis and SBP, compared with those without SBS, as well as those with compensated liver cirrhosis. Measuring these oxidative stress markers could have a fundamental importance in the diagnosis, treatment and follow-up of this liver pathology.

Key words: Spontaneous bacterial peritonitis, superoxide dismutase, malondialdehyde.

INTRODUCTION

Bacterial infections play an important role in the development of various complications in liver cirrhosis, such as variceal bleeding and hepatic encephalopathy, which are important causes of morbidity and mortality in cirrhotic patients (Runyon, 2004).

Among bacterial infections associated with hepatic cirrhosis, the most common are spontaneous bacterial peritonitis (SBP) (25%), urinary tract infections (20%) and pneumonia (15%) (Ghassemi and Garcia-Tsao, 2007). The incidence of SBP in hospitalized patients with ascitogenic cirrhosis is between 7% and 23%, while the probability of a patient with cirrhotic ascites developing the first episode of SBP is 10% per year. However, this increases when ascites are associated with low protein concentration (<1g/100 ml) and severe hepatic impairment (Stanciu et al., 2006).

The clinical course of patients with cirrhosis is often affected by a number of complications, such as portal hypertension, ascites and SBP. Bacterial infec-

tions are responsible for up to a quarter of the deaths in patients with cirrhosis, SBP being a major complication in patients with cirrhosis and ascites (Wyke et al., 2007).

SBP probably develops because of inefficient defense mechanisms against infection, as seen in patients with liver cirrhosis. Although the key steps in the pathogenesis of SBP are not fully elucidated, it is clear that the main source of bacterial gut and altered intestinal motility is bacterial translocation, a key element in the pathogenesis of SBP (Groszmann, 1994).

Despite different protective measures, such as early diagnosis, therapy with albumin and the introduction of new antibiotics, the prognosis of these patients remains poor, with a mortality rate of 20-40% (Nobre et al., 2008). In addition, an SBP episode reduces the survival rate at one year to about 30%, and 20% at two years. Moreover, mortality rates of 50% during hospitalization, 70% in SBP relapse and 80% within the first year after the initial infectious episode have been observed (Such and Runyon, 1998).

In this context, the identification of patients with increased risk of death is extremely important for improving the prognosis. Besides early appropriate antibiotic therapy, which is critical in most cases of SBP, knowing the risk factors for SBP is also important, not just to identify patients who could benefit from preventive therapy, but also to understand the pathogenesis of the disease. Additionally, identifying the risk factors for SBP is important in the development and optimal orientation of a cost-effective preventive strategy.

Oxidative stress, defined as an imbalance between pro-oxidant and antioxidant mechanisms in the body, is involved in liver pathology (Poli and Parola, 2007) and in the pathogenesis of liver cirrhosis (Irimia et al., 2013). The role of oxidative stress in the development of SBP is not clearly understood (Danulescu et al., 2013). In the present study, we examined oxidative stress markers, the antioxidant enzyme superoxide dismutase (SOD) and the marker of lipid peroxidation, malondialdehyde (MDA), in the serum and ascitic fluid of patients with decompensated cirrhosis and SBP, in patients diagnosed with decompensated liver cirrhosis with ascites, and in patients with compensated liver cirrhosis.

MATERIALS AND METHODS

The study is a prospective case control that included 33 patients divided into 3 groups: group I consisted of 10 patients with decompensated cirrhosis and spontaneous bacterial peritonitis (SBP); group II, 17 patients diagnosed with decompensated liver cirrhosis with ascites and group III, 6 patients with compensated liver cirrhosis. The control group consisted of 19 healthy subjects recruited from hospital personnel and matched to the patients by age and gender. SBP diagnosis was made based on clinical examination (fever, impaired general condition) and biological explorations (neutrophilic leukocytosis in blood and ascitic fluid). An essential criterion for the diagnosis of SBP was the presence of >250 neutrophils/mm^3. Compensated cirrhosis was defined as the absence of ascites in cirrhotic patients and presence of ascites marks the decompensated stage.

Biochemical estimations

Determination of SOD

Superoxide dismutase (SOD) activity was measured as the rate of inhibition rate of the enzyme with WST-1a, a water-soluble tetrazolium dye and xanthine oxidase using a SOD Assay Kit (FLUKA, 19160) according to the manufacturer's instructions. Percent inhibition was normalized by mg protein and presented as SOD activity units.

Determination of MDA

Malondialdehyde levels were determined by thiobarbituric acid reactive substances (TBARs) assay as described (Stefanescu et al., 2012).

Data analysis

The levels of SOD and MDA were statistically ana-

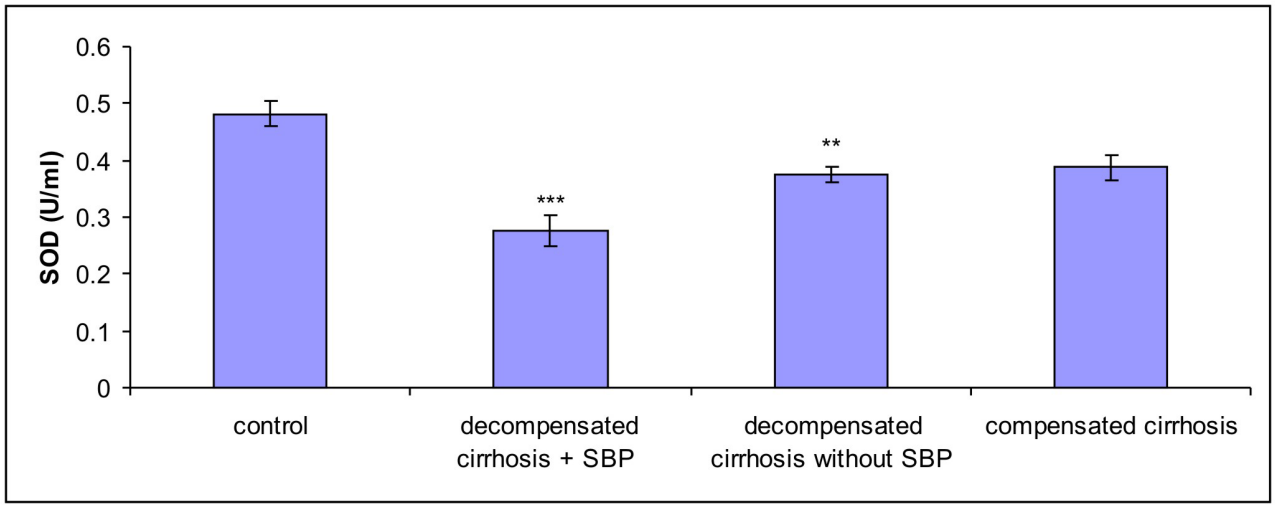

Fig. 1. SOD specific activity in the serum. The values are mean ± SEM. ***p = 0.00006 vs. control group, **p = 0.007 vs. control group.

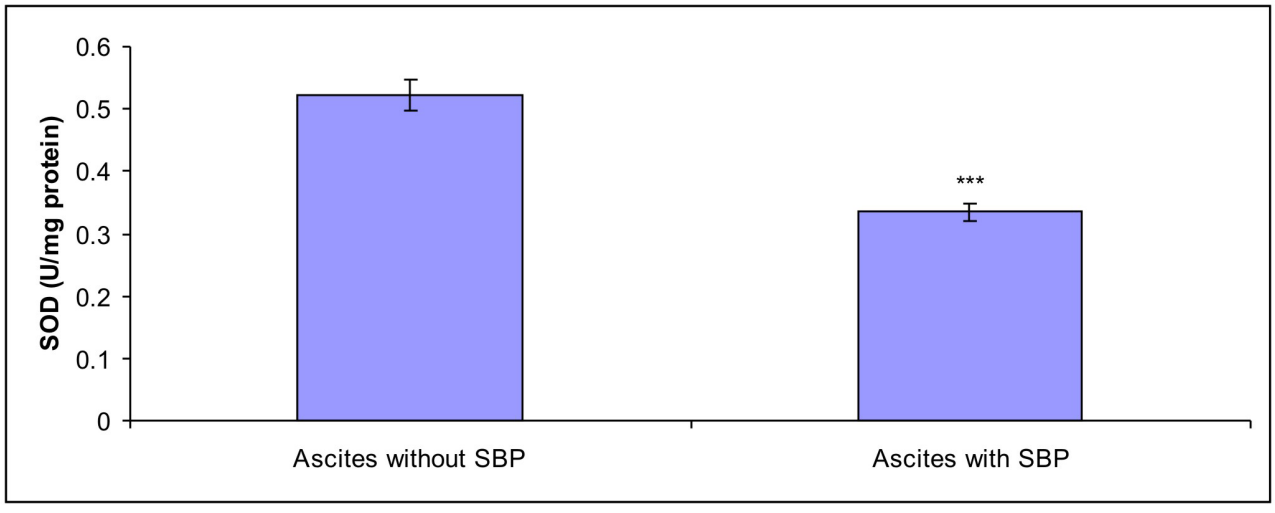

Fig. 2. SOD specific activity in the ascitic fluid. The values are mean ± SEM. ***p = 0.000002 vs. ascites without SBP.

lyzed by one-way analysis of variance (ANOVA). All results are presented as mean ± SEM. F values for which $p<0.05$ were regarded as statistically significant.

RESULTS

We observed a significant decrease in SOD activity in patients with decompensated cirrhosis and SBP ($F(1,28)=22$, $p=0.00006$), as compared to the control group (Fig. 1). A statistically significant decrease of SOD activity was also observed in the group with decompensated cirrhosis without SBP ($F(1,33)=8$, $p=0.007$). No significant differences were observed in patients with compensated liver cirrhosis ($F(1,24)=2$, $p=0.15$), as compared to controls (Fig. 1).

When we performed the *post-hoc* analysis, we observed statistically significant differences between the group with decompensated cirrhosis and SBP vs. decompensated cirrhosis without SBP-group ($p = 0.001$), as well as between the group with decompen-

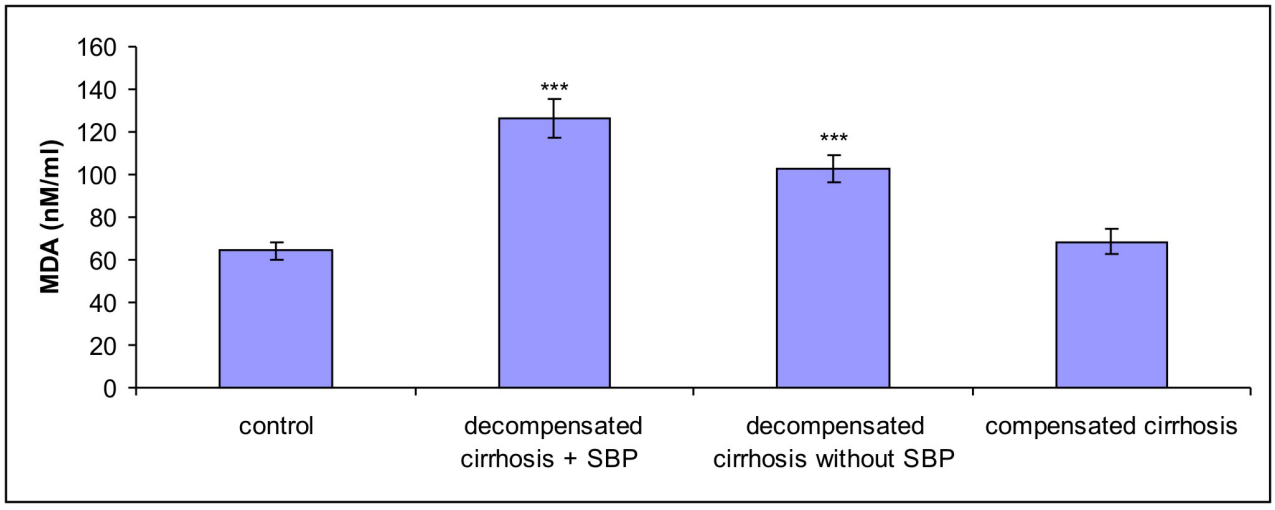

Fig. 3. MDA levels in the serum. The values are mean ± SEM. ***p < 0.0001 vs. control group.

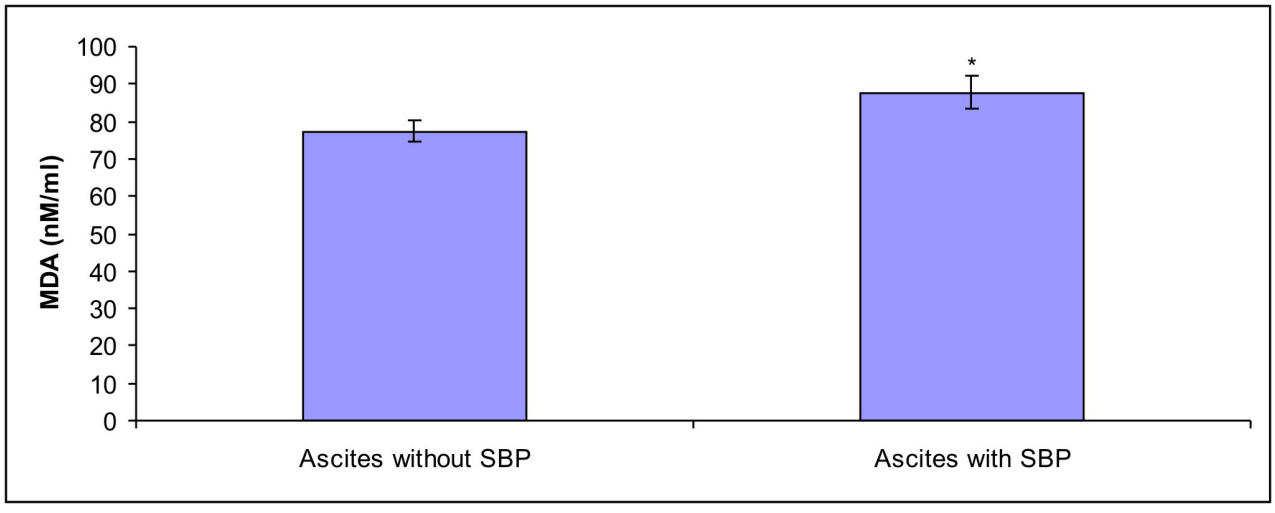

Fig. 4. MDA levels in the ascitic fluid of our research groups. The values are mean ± SEM. *p = 0.047 vs. ascites without SBP.

sated cirrhosis and SBP vs. the group of patients with compensated liver cirrhosis (p = 0.016). No significant differences were observed between the group of patients with decompensated cirrhosis without SBP-group vs. the patients with compensated liver cirrhosis (p = 0.591) (Fig. 1). A statistically significant decrease of ascitic fluid SOD was observed in the group with SBP, as compared to patients without SBP (F(1,20)=42, p=0.000002) (Fig. 2).

We report here a significant increase of serum MDA in both groups with decompensated cirrhosis and SBP (F(1,24)=53, p=0.0000001) and in the group with decompensated cirrhosis without SBP (F(1,31)=30, p=0.000005), when compared to control subjects (Fig. 3). Again, no significant differences were found for MDA levels in patients with compensated liver cirrhosis (F(1,22)=0.2, p=0.6) as compared to control group (Fig. 3).

When we performed the post-hoc analysis for serum MDA, we observed significant differences between the group with decompensated cirrhosis and SBP vs. the group with decompensated cirrho-

sis without SBP (p = 0.049), between the group with decompensated cirrhosis and SBP vs. the group of patients with compensated liver cirrhosis (p = 0.001), as well as between the group of patients with decompensated cirrhosis without SBP vs. the patients with compensated liver cirrhosis (p = 0.007) (Fig. 3). Regarding the levels of MDA from the ascitic fluid, we found a statistically significant increase of MDA in the group with SBP, as compared with the patients without SBP (F(1,20)=5, p=0.047) (Fig. 4).

DISCUSSION

Recent studies have demonstrated the important role of oxidative stress in increased intestinal permeability, an important element in the pathogenesis of SBP. In patients with liver cirrhosis, there is an intestinal motility disorder, which promotes intestinal bacterial overpopulation. Bacterial translocation is possible due to intestinal barrier alteration. Also, in experimental conditions, lipid peroxidation and the infiltration with neutrophils was observed in the intestinal mucosa lesions, correlated with bacterial translocation and portal hypertension (Chiva et al., 2003), which are important factors in the development of SBP.

Previous studies have shown a correlation between hepatic and plasma glutathione (a tripeptide synthesized in the liver, which acts as an antioxidant) levels (Shigesawa et al., 1992). It has also been shown that the antioxidant erythrocytic enzymes, such as SOD and catalase (CAT) are changed in liver diseases, in particular those that have progressed to cirrhosis. Lipoperoxides concentrations, which mean an increased production of free radicals, were also increased in cirrhotic patients compared to normal.

A recent study by Bhandari et al. demonstrated the role of oxidative stress in a worsening severity of cirrhosis, assessed by Child-Turcotte-Pugh (CTP) score, by determining the levels of pro-oxidant substances (serum levels of MDA) and of the antioxidant (superoxide dismutase and glutathione peroxidase) in cirrhotic patients (Bhandari et al., 2008).

Previous studies in rats with an experimental model of liver cirrhosis induced by alcohol and iron also found elevated levels of MDA in the liver. A significant increase in plasma MDA levels was also observed (Tsukamoto et al., 1995).

It was also shown that oxidative stress is associated with the development and progression of cirrhosis. This was done by measuring markers reflecting pro-oxidant (serum MDA) and antioxidant (CAT, SOD and blood reduced glutathione) factors. The level of oxidative stress was also assessed with the severity of liver disease, determined by Child-Turcotte-Pugh (CTP) scoring. A significant increase in serum MDA value in patients with cirrhosis was noted. On further assigning the cases with CTP scoring, Child C cirrhosis had a worsening level of oxidative stress. A significant decrease in antioxidant markers, RBC CAT, SOD and blood GSH was noted in cirrhotic patients compared to controls. Additionally, on grading the cases per CTP scoring, C had a significantly reduced level of antioxidant markers. The erythrocyte CAT was significantly lower in cases with liver cirrhosis in relation to controls. The decline in levels correlates well with decreased liver function, assessed by CTP classification (Gerli et al., 1992).

The idea that oxidative stress has a direct causative role in liver fibrogenesis was first mentioned by (Chojkier et al., 1999) who demonstrated *in vitro* the possible linkage between enhanced lipid peroxidation and induction of collagen gene expression (18). Bedossa et al. (1994) demonstrated the localization of increased collagen α-1 mRNA with carbon tetrachloride-induced lipid peroxidation using *in situ* hybridization and immunohistochemical studies in cultured rat hepatocytes. However, Gerli et al. (1992) in a study involving 73 cirrhotic patients (22 CTP A, 30 CTP B, and 21 CTP C) and 50 controls demonstrated no significant difference between erythrocyte SOD among the various groups. The disparities between these studies may be explained by the different biochemical methods applied and the acute condition of the patients, given the fact that CTP class may vary if there is any precipitating factor such as pharmacological incompliance.

A recent study published by Shaden M et al. (2009) demonstrated the involvement of oxidative stress in SBP. Oxidative stress was evidenced by elevated MDA and antioxidant components and by significantly reduced serum SOD, GPX and catalase. In addition, an increased concentration of NO in patients with liver cirrhosis with PBS was demonstrated, but not in patients with sterile ascites. Moreover, after antibiotic treatment, MDA, NO and TNF-α expression were significantly decreased, while antioxidant elements showed a significant increase (Shaden et al., 2009).

Rodríguez-Ramos et al., 2001 have shown that SBP is associated with increased levels of pro-inflammatory cytokines (IL-1, TNF-α and IL-6) in ascitic fluid, as compared with controls without liver cirrhosis. Levels of pro-inflammatory cytokines in the ascitic fluid decreased rapidly after eradication of the infection. These recent studies demonstrate the presence of oxidative stress in patients with liver cirrhosis and SBP; the measurement of these parameters may have an important role in the diagnosis and follow-up of SBP antibiotic efficacy.

In a study of Natarajan et al. (2006), a significant increase in MDA and protein carbonyl levels was seen in ascites from patients with SBP when compared to controls. This was accompanied by a decrease in total thiols and protein thiols. In addition, there was a significant increase in ascitic fluid nitrate in patients with SBP when compared to control patients. This study demonstrated the presence of oxidative stress in ascitic fluid from patients with SBP, and showed that ascitic fluid nitrate may be a marker for diagnosing SBP and a useful index in determining therapeutic response to antibiotic treatment.

Nitrate levels in ascitic fluid are elevated in SBP. This could be quite important, since an interaction between NO and superoxide can result in the production of highly damaging reactive species such as peroxynitrite (Bild et al 2013). Randomized trials have placed intravenous cefotaxime as the antibiotic of choice in SBP (Runyon et al., 1991). In determining the therapeutic response in patients with SBP, Natarajan et al. (2006) checked the oxidative stress parameters in correlation with neutrophil counts. This study demonstrated a parallel decrease of ascitic nitrate with neutrophil count. However, Park et al. (2004) did not demonstrate any significant change in ascitic nitrate in SBP patients after treatment with antibiotics. Ascitic nitrate levels *per se* were not altered in patients with SBP in that study, unlike data from other studies (Coskun et al., 2001). NO in ascitic fluid has also been suggested to be an independent predictor of the development of renal impairment in patients with cirrhosis and SBP (Such et al., 2004). Moreover, activated neutrophils and peritoneal macrophages are known to generate oxygen free radicals. Increased neutrophil count and oxidative stress parameters observed in the ascitic fluid from patients with SBP suggest that neutrophils and macrophages may be an important source of free radicals. In a study by Sakurai et al. (2013), the augmentation of oxidative stress of liver parenchymal cells can explain the close relationship between liver fibrosis and hepatocarcinogenesis.

CONCLUSIONS

The data presented here show the existence of an increased oxidative stress status in patients with decompensated cirrhosis and SBP, as compared with those without SBS and those with compensated liver cirrhosis; this is demonstrated through the significant decrease of SOD and by an increase in MDA levels.

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