THE INFLUENCE OF ANTIBIOTIC TREATMENT ON THE DYNAMICS OF OXIDATIVE STRESS IN SPONTANEOUS BACTERIAL PERITONITIS

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THE INFLUENCE OF ANTIBIOTIC TREATMENT ON THE DYNAMICS OF OXIDATIVE STRESS IN SPONTANEOUS BACTERIAL PERITONITIS (Abstract): Bacterial infections play an important role in liver cirrhosis complications, being together with variceal bleeding and hepatic encephalopathy an important cause of morbidity and mortality in cirrhotic patients. Spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis, with a significant mortality. Recent studies have demonstrated the involvement of oxygen free radicals in the pathogenesis of liver cirrhosis, but the role of oxidative stress in the development of SBP is not very clear yet. Purpose: This study aims to evaluate the role of oxidative stress in the pathogenesis of spontaneous bacterial peritonitis and its changes after therapy. Material and Methods: The study is a prospective case-control, which included 33 patients divided into 3 groups: group I – 10 patients with decompensated cirrhosis and 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 staff, adapted to patients by age and sex. Malondialdehyde (MDA), a product of lipid peroxidation, was dosed in the blood and ascitic fluid of patients by assay thiobarbituric acid reactive substances (TBARS). Results: Serum MDA significantly increased in the group with decompensated cirrhosis and SBP compared with the control group. MDA levels in ascitic fluid showed a statistically significant increase in the SBP group compared with patients without SBP. There was a decrease of MDA after 6 months of antibiotic treatment compared with the initial stage, while MDA values increased in the absence of treatment. Conclusions: The study demonstrates the increased oxidative stress markers in the blood and ascitic fluid of cirrhotic patients with SBP, which can be considered a predictor of SBP and also a marker of treatment response. Keywords: SPONTANEOUS BACTERIAL PERITONITIS, OXIDATIVE STRESS, ANTIBIOTIC TREATMENT, PROPHYLAXIS.

Bacterial infections play an important role in liver cirrhosis complications, being together with variceal bleeding and hepatic encephalopathy an important cause of morbidity and mortality in cirrhotic patients (1). Compared with the general population, the incidence of infections such as peritonitis, pneumonia, urinary tract infections or meningitis is 10 times higher in cirrhotic patients and the mortality is 20 times high-
The influence of antibiotic treatment on the dynamics of oxidative stress

er, pointing out that liver cirrhosis is a status of acquired immune deficiency (2).

Spontaneous bacterial peritonitis (SBP) is a major complication of liver cirrhosis, with a significant mortality. The incidence of SBP in hospitalized patients with cirrhosis is between 7% and 23%. The probability for a patient with cirrhotic ascites to develop the first episode of SBP is 10% per year, but it increases in ascites with low protein concentration (below 1g / 100ml) and severe hepatic impairment (1, 2).

In most cases, SBP is a monobacterial infection of the ascitic fluid; its culture reveals a single microorganism, the number of neutrophils being over 250/mmc.

SBP probably develops as a consequence of ineffective defense mechanisms against infection, 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 infection is the bacterial gut, while the altered intestinal motility promotes the bacterial translocation, a key element in the pathogenesis of SBP (3).

Despite all protective measures, such as early diagnosis and prophylactic antibiotic therapy, as well as the introduction of new antibiotics, albumin infusions, the prognosis of these patients remains poor, with a mortality rate of 20-40% (4). The installation of an episode of spontaneous bacterial peritonitis reduces the survival rate to about 30% per year and 20% at 2 years.

The literature shows a mortality rate of 50% during hospitalization; 70% of SBP relapse, and 80% of patients die within the first year after the initial infectious episode (5). In this context, the identification of patients with increased risk of death is extremely important for improving their prognosis.

Early establishment of an appropriate antibiotic therapy is critical in most cases of SBP.

Knowing the risk factors for SBP is important not only in order to identify patients who could benefit from the preventive therapy, but also in understanding the pathogenesis of the disease.

Identifying risk factors for SBP is important in the development of a safe and cost-effective prophylactic strategy.

A new concept much-discussed lately is oxidative stress. It is defined as the imbalance between oxidants and antioxidants in the favor of oxidants, with destructive and pathogenic potential. The oxidants are normally formed during aerobic metabolism and their amount increases under certain conditions. In such circumstances, physiological antioxidant mechanisms that inactivate reactive oxygen species and repair several molecular lesions may prove insufficient. The imbalance between the oxidant and antioxidant capacity of the body is called oxidative stress and is the origin of various diseases with a high prevalence in modern medicine, such as proliferative diseases, atherosclerosis, glomerulonephritis, pulmonary fibrosis and cirrhosis (6).

Recent studies have demonstrated the involvement of oxygen free radicals in the pathogenesis of liver cirrhosis, but the role of oxidative stress in the development of SBP is not very clear yet. However, recent studies have shown 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. In the experimental conditions of lipid
peroxidation, infiltrations with neutrophils were observed in the intestinal mucosa, lesions which are correlated with bacterial translocation and portal hypertension, major factors in the development of SBP (7).

The free radicals, also called pro-oxidants, are compounds of circulating blood, very active, biochemically and biologically virulent, acting on membranes, nuclei and cell cytoplasm, producing and maintaining an intense oxidative stress (8). Antioxidant defense is achieved by the endogenous antioxidants and, where these systems are overcome, by means of the exogenous antioxidants. Antioxidants are designed to neutralize or remove reactive oxygen and nitrogen species and products of lipid peroxidation. It is always desirable that the level of pro-oxidants (free radicals) should be below the capacity of the antioxidants. When free radicals exceed the antioxidants (oxidative stress), phenomena of wear and early cellular aging appear (9).

Early establishment of an appropriate antibiotic therapy is critical in most cases of SBP. Empirical antibiotic treatment should be initiated prior to receiving the result of ascitic fluid cultures and blood cultures if the clinical picture is pathognomonic and the PMN number is above 250/mmc. The results of the cultures allow subsequent adaptation of the treatment regimen (10).

For over two decades, third generation cephalosporins were the antibiotics of choice in the treatment of SBP. Felisart et al. in 1985 have validated the use of cefotaxime as an empiric antibiotic choice in the treatment of SBP, further studies determining the optimal dose and duration of therapy. A recent study by Navas et al. demonstrated that oral therapy with ofloxacin is as effective as that with cefotaxime iv in patients with uncomplicated SBP (11).

Prophylactic antibiotic treatment aims to selective decontaminate the gastro-intestinal tract in order to reduce the risk of developing spontaneous bacterial peritonitis. Given the increased cost and risk of developing resistant organisms, antibiotic prophylaxis is indicated in three cirrhotic groups: those with a history of SBP, those hospitalized for upper gastrointestinal bleeding, those with low-protein ascitic fluid.

**MATERIAL AND METHODS**

The study is a prospective case-control, which included 33 patients divided into 3 groups: group I – 10 patients with decompensated cirrhosis and 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 staff, adapted to patients by age and sex. Malonildyaldehida (MDA), a product of lipid peroxidation, was dosed in the blood and ascitic fluid of patients by assay thiobarbituric acid reactive substances (TBARS). MDA levels were statistically analyzed using one-way analysis of variance (ANOVA). The results are expressed as mean ± SEM. The values of F for which p <0.05 were considered statistically significant.

**RESULTS**

Serum MDA levels, a marker of lipid peroxidation, have experienced significant growth both in the group 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) in comparison with the control group (fig. 1). Regarding MDA levels in ascitic fluid,
The influence of antibiotic treatment on the dynamics of oxidative stress

there was also a statistically significant MDA increase in the group with SBP compared with patients without SBP (F (1.20) = 5, p = 0.047) (fig. 2).

Fig. 1. MDA levels in serum in our research groups

Regarding the evolution of MDA in the context of prophylactic antibiotic treatment, there was a decrease in MDA after 6 months of antibiotic treatment compared to the initial stage, whereas MDA values increased in the absence of treatment (fig. 3).

Fig. 2. MDA levels in ascitic fluid in our research groups

Fig. 3. The evolution of MDA in patients with and without treatment
TABLE I

Average values of oxidative stress markers in patients with and without treatment, initially and after 6 months

<table>
<thead>
<tr>
<th>Oxidative stress marker</th>
<th>Without treatment (3 cases)</th>
<th>With treatment (6 cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>Ab. st.</td>
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<tr>
<td>MDA</td>
<td>101.86</td>
<td>18.383</td>
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<tr>
<td></td>
<td>110.87</td>
<td>19.351</td>
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**DISCUSSION**

The results obtained showed the presence of oxidative stress, increased markers of lipid peroxidation, suggesting the involvement of oxidative stress in SBP and its dynamics within the context of antibiotic therapy.

A recent study by S Bhandari et al. demonstrated the role of oxidative stress in worsening the severity of cirrhosis, assessed by Child-Pugh 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 (12).

Previous studies on rats with experimental model of liver cirrhosis induced by alcohol and iron found elevated levels of MDA in the liver. A significant increase in plasma MDA levels was also observed (13).

A recent study published by Shaden M et al., demonstrated the involvement of oxidative stress in SBP (14). Oxidative stress was evidenced by elevated MDA and by significantly reduced antioxidant components SOD, GPx. After antibiotic treatment, MDA, NO and TNF expression significantly decreased and there was significant increase in antioxidant elements. Also, in a study by Natarajan SK (15), a significant increase of ascitic MDA was observed in patients with SBP compared to controls. This study demonstrated the presence of oxidative stress in the ascitic fluid from patients with SBP and showed that the nitrate in the ascitic fluid may be a marker for diagnosis of SBP and an useful index to determine the therapeutic response to treatment with antibiotics.

Understanding this is important because the interaction between NO and superoxide may cause extremely damaging reactive species, such as Peroxynitrite.

Randomized trials have demonstrated the efficacy of intravenous cefotaxime, which is the antibiotic of choice in SBP (16).

In determining the therapeutic response in patients with SBP, Natarajan et al. measured the parameters of oxidative stress in relation to the number of neutrophils in the ascitic fluid. The study showed a decrease of NO in the ascitic fluid, in concordance with the number of neutrophils. Park et al demonstrated no significant change in nitrate from ascites of patients with SBP after treatment with antibiotics. However, the nitrate levels in ascites per se were altered in patients with SBP in this study, in contrast to data from other studies (17).

A Geetha et al. (12) demonstrated that in cirrhosis red cells undergo severe oxidative stress with significant changes in membrane properties. Oxyhemoglobin and methemoglobin levels were analyzed in blood red cells. Markers of oxidative stress, such as lipid peroxides, lipid hydroperoxides, as well as nitric oxide, were deter-
The influence of antibiotic treatment on the dynamics of oxidative stress

Methemoglobin level was significantly higher (p <0.001) in erythrocytes of patients with liver cirrhosis complicated with bleeding compared to patients without bleeding. Levels of oxidative stress markers, including nitric oxide has been shown to be higher in these patients.

Enzymatic antioxidants levels were low. Gines, in his study, showed that norfloxacin, a selective quinolone for gram-negative bacilli with low absorption in dose of 400mg/day reduces the risk of SBP recurrence in the first year from 68% to 20% (18).

Five randomized trials have shown the net benefit of using short-term antibiotic prophylaxis in cirrhotic patients admitted with variceal bleeding. A meta-analysis showed a significant decrease in the rate of infection in patients receiving antibiotic treatment on admission (19).

Grange et al. (20), in a randomized placebo-controlled trial evaluating norfloxacin as primary prophylaxis in cirrhotic patients with low ascitic fluid protein, demonstrated a decreased incidence of SBP in the group that received antibiotics (2%) compared with the placebo group (17%) and also decreased mortality rates at 6 months (19% vs. 36%).

CONCLUSIONS

Our results demonstrate the presence of increased oxidative stress in patients with decompensated cirrhosis and SBP, compared to those without SBP and those with compensated liver cirrhosis. This was shown by increased MDA, a marker of lipid peroxidation.

Thus, measurement of this parameter of oxidative stress may play an important role in the diagnosis and follow up of this important liver pathology

REFERENCES


**PHARMACOLOGICAL ACTIVITY OF MYRTUS NIVELLEI ESSENTIAL OIL**

Sahara myrtle is a medicinal plant traditionally used for dermatosis treatment. In order to justify the use of this plant, Bouzabata et al. aimed to elucidate the composition of Myrtus nivellei essential oil extracted by hydrodistillation of aerial parts, its antifungal properties and the cytotoxicity on keratinocytes. Chemical analysis using chromatographic and spectroscopic techniques elucidated the structure of two new compounds and showed a different composition of Myrtus nivellei oil from that of Myrtus communis. Broth macrodilution method was used to evaluate the activity against yeasts and filamentous fungi. Determination of MICs showed that the oil was more active against Cryptococcus neoformans (MIC = 0.16μL/mL), followed by dermatophytes (MICs of 0.64 and 1.25μL/mL). Evaluation of cell viability in HaCaT keratinocytes revealed no cytotoxicity at concentrations up to 1.25μL/mL. Thus, appropriate doses of Myrtus nivellei oil could be found, having antifungal activity and very low cytotoxicity on keratinocytes. (Bouzabata A, Bazzali O, Cabral C, Gonçalves MJ, Cruz MT, Bighelli A, et al. New compounds, chemical composition, antifungal activity and cytotoxicity of the essential oil from Myrtus nivellei Batt. & Trab., an endemic species of Central Sahara. *J Ethnopharmacol.* 2013 Jul 31. pii: S0378-8741(13)00487-X. doi: 10.1016/j.jep.2013.06.054.)