Oxidative Stress and Antioxidant Defence in Cirrhotic Patients Associated with Spontaneous Bacterial Peritonitis.

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Abstract: Reactive species include reactive oxygen species and reactive nitrogen species. They are unstable chemicals generated in biologic systems under normal physiological as well as pathophysiological conditions in the liver of cirrhotic patients. Spontaneous bacterial peritonitis (SBP) is the most common complication of liver cirrhosis and accounts for significant mortality. SBP is associated with an important production of proinflammatory cytokines essential for an adequate host response against infection. The aim of the present study was to assess the state of oxidative stress, lipid peroxidation and nitric oxide levels as well as TNF-α mRNA expression level in sterile cirrhotic patients and others accompanied with spontaneous bacterial peritonitis in response to the treatment. Nitric oxide and Malondialdehyde were measured in ascitic fluid. Superoxide dismutase and glutathione peroxidase were measured in erythrocyte lysate and catalase activity in serum. RT-PCR was used to assess the expression of TNF-α mRNA in duodenal biopsy. These parameters were assayed in non-infected cirrhotic patients (n=10) and patients with SBP before and after treatment (n=20). In addition a group of healthy volunteers with matched age and sex (n=10) was involved in the study as a control group. Oxidative stress is demonstrated by increased MDA accumulation and reduced glutathione peroxidase and superoxide dismutase as well as serum catalase. A significant elevated base line of TNF-α mRNA expression level in SBP ascitic fluid (254.019±26.471, 2740±247.81, 9395±1242.779 respectively) (P<0.001). In addition to the increased concentration of NO level in patients with SBP but not in patients with sterile ascites (74.237±4.309, 40.537±3.625 respectively) (P<0.001). A notable positive significant correlation between TNF-α expression level and NO (P<0.001) among the three studied groups. After antibiotic treatment MDA, NO and TNF-α expression levels dropped back towards sterile values. In the mean time, antioxidant elements were also recovered. This study demonstrates that overproduction of NO, MDA and TNF-α expression levels may be used as markers for diagnosing SBP and a useful index in determining therapeutic response to antibiotic treatment. In addition to the down regulation of antioxidant defence elements in sterile cirrhotic patients predicting the development of complications such as SBP.

Key words: Oxidative Stress, Antioxidant Defence, Spontaneous Bacterial Peritonitis, TNF-α mRNA expression, Nitric oxide.

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is one of the most common and life-threatening complications of cirrhosis [1]. A bacterial overgrowth with translocation through the increased permeable small intestinal wall and impaired defense mechanisms is considered to be the main mechanism associated with its occurrence. The prompt diagnosis of SBP is the key factor for reduction observed in mortality rates in recent years. The clinical diagnosis of SBP is neither sensitive nor specific and the search for new practical and available tools for a rapid diagnosis of SBP is an important endpoint of current studies [2]. The prompt use of empirical antibiotics, mostly Cefotaxime, improves significantly the short-term prognosis of cirrhotic patients with SBP [23]. The recurrence rate of SBP is high and antibiotic prophylaxis has been recommended in high-risk settings. Unfortunately, the long-term prognosis remains poor [4].

The liver is well protected against free radicals. It is one of the best antioxidant supplied organs. An
important function of the liver is the detoxification of drugs, chemicals and toxic materials, with the subsequent release of free radicals and antioxidant tissue systems are reduced. In normal cells, a balance exists between oxidative damage and antioxidant defence. Although tumour necrosis factor (TNF-α) and highly potent oxidant molecules such as superoxide anion (O2•−), hydrogen peroxide (H2O2) and hydroxyl radical (OH•) are produced by the immune system, excessive production of oxygen-derived free radicals creates a condition known as oxidative stress, participating in various diseases and syndromes. The reactive oxygen species interacts with nucleic acids, proteins and lipids, thus enhancing TNF production, DNA mutations, cellular dysfunction, lipid peroxidation and death.

Nitric oxide (NO) is a messenger molecule with important biological functions as a powerful vasodilator agent present in ascitic fluid that also shows some bactericidal activity. Lipopolysaccharide (LPS) is one of the most powerful activators of the cytokine cascade, as well as of NO synthesis. Since NO is produced by stimulated macrophages has been implicated in the inhibition or killing of various microorganisms by inhibiting the respiratory cycle and the synthesis of adenosine triphosphate and DNA. An increased NO production by activated ascites macrophages would be expected to occur in the presence of infection and could be used as a diagnostic tool.

Other AF cytokine such as TNF-α is a cytokine that it is mainly produced by macrophages in response to inflammatory stimuli. An initial hypothesis suggested that bacterial products such as bacterial lipopolysaccharide would activate cytokine synthesis (TNF-α and others) and account for haemodynamic changes in advanced cirrhosis by stimulating expression of inducible NO synthase (iNOS).

Spontaneous bacterial peritonitis is associated with an important production of inflammatory mediators essential for an adequate host response against infection. However excessive production of TNF-α causes shock for the immune system and associated with high mortality rates. Therefore increased levels of AF cytokines and NO metabolites might be detected in patients who subsequently developed clinically apparent AF infection.

This study was designed to assess the state of oxidative stress, lipid peroxidation and nitric oxide levels as well as TNF-α mRNA expression level in sterile cirrhotic patients and others accompanied with spontaneous bacterial peritonitis in response to the treatment with antibiotic drug (Cefotaxime).

**Patients and Methods:**

**Characteristics of the Study Population:** Forty patients enrolled in this study were divided into four groups. Healthy controls (group I, n = 10, 6 males and 4 females, median age 55.5), cirrhotic patients with sterile ascites (group II, n = 10, 6 males and 4 females, median age 57), patients with SBP before treatment (group III, n = 20, 13 males and 7 females, median age 58.5), and patients with SBP after administrating a completed a 10-day course of treatment with antibiotic therapy (Cefotaxime, 2g every 8h) plus albumin (group IV, n = 20, 13 males and 7 females, median age 58.5). All patients were admitted to digestive disease unit, National Liver Institute, Menofiya University.

SBP was diagnosed on the basis of the following criteria; ascitic fluid polymorphonuclear cell (PMN) count > 250 cells/mm3 and a compatible clinical picture (abdominal pain, fever and unexplained encephalopathy). The possibility of secondary peritonitis was excluded in all patients at the time of paracentesis. Also in the absence of clinical or laboratory data suggesting hemorrhagic ascites, pancreatitis, mycobacterial, fungal peritonitis or carcinomatosis. The following data were collected prospectively; age, gender, etiology of cirrhosis and history of SBP. The severity of the disease was evaluated according to the classification of child Pugh.

Cirrhotic patients with sterile ascitic fluid were selected consecutively from those having a polymorphonuclear count in ascitic fluid lower than 250 cells/mm3 and the absence of clinical or laboratory signs of infection, as well as absence of microorganisms isolated in ascitic fluid were required. Serum, erythrocyte lysate, ascitic fluid and duodenal biopsy samples were obtained from the patients after an overnight fast in order to exclude diet-related nitrate intake. Ascites obtained at paracentesis was sent for total leukocyte count, PMN count and culture. And at the time of paracentesis blood was drawn for measurement of biochemical investigations (serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, albumin, total bilirubin and hemoglobin). All samples then stored until the time of analysis.

The study was approved by the Human Investigation Committees of each of the participating centers, and patients gave written informed consent to participate in the study.

**Estimation of Catalase Activity in Serum:** The catalase activity was estimated by the reaction of catalase with a known quantity of H2O2. The reaction is stopped after exactly one minute with catalase...
inhibitor. In the presence of peroxidase (HRP), remaining \( \text{H}_2\text{O}_2 \) reacts with 3, 5-Dichloro -2-hydroxybenzene sulfonic acid (DHBS) and 4-amino-phenazone (AAP) to form a chromophore with color intensity inversely proportional to the amount of catalase in the original sample that can be measured at 510 nm in a spectrophotometer\(^{[23]}\).

**Estimation of Superoxide Dismutase Activity in Erythrocyte Lysate:** Superoxide dismutase activity assay relies on the ability of the enzyme to inhibit the phernazine methosulphate-mediated reduction of nitroblue tetrazolium dye. Then measure the increase in absorbance at 560 nm for 5 min for control and for sample at 25°C\(^{[26]}\).

**Estimation of Glutathione Peroxidase Activity in Erythrocyte Lysate:** Glutathione peroxidase activity assay is an indirect measure of the activity of GPx. Oxidized glutathione (GSSG), produced upon reduction of organic peroxide by GPx is recycled to its reduced state by the enzyme glutathione reductase (GR). The oxidation of NADPH to NADP\(^+\) is accompanied by a decrease in absorbance at 340 nm providing a spectrophotometric means for monitoring GPx enzyme activity\(^{[23]}\).

**Estimation of Nitric Oxide in Ascitic Fluid:** The final products of NO in vivo are nitrite (\( \text{NO}_2^- \)) and nitrate (\( \text{NO}_3^- \)). The relative proportion of (\( \text{NO}_2^- \)) and (\( \text{NO}_3^- \)) produced from NO is variable. The exogenous source of \( \text{NO}_3^- \) ingested in the diet should be considered and can not be ignored (none-NO origin). Thus, one of the indexes of NO production is the (\( \text{NO}_3^- \)). The Biodiagnostic Nitrite Assay Kit provides an accurate and convenient method for measurement of endogenous nitrite concentration as indicator of nitric oxide production in biological fluids. It depend on the addition of Griess Reagents which convert nitrite into a deep purple azo compound, photometric measurement of the absorbance due to this azo chromophore accurately determines (\( \text{NO}_3^- \)) concentration . In acid medium and in the presence of nitrite the formed nitrous acid diazotise sulphanilamide and the product are coupled with \( \text{N}-(1-\text{naphthyl}) \) ethylenediamine. The resulting azo dye has a bright reddish – purple color which can be measured at 540 nm in a spectrophotometet\(^{[29]}\).

**Estimation of Malondialdehyde in Ascitic Fluid:** The lipid peroxidation products were estimated by the formation of thiobarbituric acid (TBA) and quantified in term of MDA where, thiobarbituric acid (TBA) reacts with MDA where thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm in a spectrophotometet\(^{[23]}\).

**Relative Quantitation of TNF-\(\alpha\) Using Real-time PCR Based on SYBR-green Fluorescence Technique:**

**RNA Isolation and CDNA Synthesis:** Isolation of total RNA from duodenal tissue biopsy was carried out with QIAamp RNA Blood Mini Kits (QIAGEN), Reverse transcription (RT)–PCR was carried out with High-Capacity cDNA Archive Kit (Applied Biosystems) that contains reagents for reverse transcription (RT) of total RNA to single-stranded cDNA.

**Primer Design:** The primer sequences used for human TNF-\(\alpha\) were the following: 5'-AGG CGC TCC CCA AGA AGA CA-3' (primer TNF-\(\alpha\) S2; nt 129 to 148 in mRNA for human TNF-\(\alpha\); DDBJ/EMBL/Gen-Bank accession no. M10988) and 5'-TCC TTG GCA AAACGT CAC CT-3'(primer TNF-\(\alpha\) R1; nt 164 to 183) (30). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (TaqMan GAPDH Control Reagents Kit) used as an endogenous control for relative quantitation w h e r e , F o r w a r d P r i m e r (GAAGGTGAAGGTCGGAGTC) and Reverse Primer (GAAGATGGTGATGGGA T TTC).

**SYBR Green Real Time PCR:** All reagents and equipment were purchased from PE Applied Biosystems. The SYBR Green PCR kit was used as 50 \( \mu \) l reactions in optical-grade PCR tubes. The volume for one 50 \( \mu \) l reaction for each PCR mixture contained 25 \( \mu \) l SYBR Green PCR master mix, 5 \( \mu \) l template DNA, and 1 \( \mu \) l of each primer and 18 \( \mu \) l sterile water. The final concentration of each PCR master mix mixture was 1×SYBR Green PCR master mix, 50ng template DNA, 100 nM of each primer. The cycling conditions used were as follows: initial incubation at 50°C for 2 minutes to activate Amp-Erase UNG and 95°C for 10 minutes to activate the AmpliTaq Gold polymerase. Thermal conditions followed 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. The no-template controls (NTC) were prepared by adding the appropriate volume of PCR-grade sterile water to SYBR Green PCR master mix.

**PCR Data Analysis:** At the end of the PCR, the SDS software saves the results, allowing instant manipulation or storage of the data for further analysis. Some initial cycles should be considered as a baseline (BL) or background in which no changes in fluorescence intensity occur, and the level above this, at which increments in fluorescence become detectable, is termed the threshold (Th). The software also determines the cycle number when a reaction reaches the Th. This value, termed the ‘cycle threshold’ (Ct),
always appears during the exponential phase of the PCR and is inversely proportional to the initial number of template molecules in the sample.

**Comparative Ct Method:** The amount of target, normalized to an endogenous reference and relative to a calibrator, is determined by the arithmetic formula $2^{-\Delta\Delta Ct}$, in which $\Delta\Delta Ct$ is the difference between the $\Delta Ct$ of any sample and the $\Delta Ct$ of the baseline sample.

**Melting Curve Analysis:** was performed to measure the specificity of quantitative PCR. The results were analyzed using the melting curve analysis software. The melting temperature (Tm) of the PCR products was calculated at the same time.

**Statistical Analysis:** All results were analyzed by SPSS software (version 10). Data were expressed as mean ± SEM. The student’s t test was used for statistical analysis of differences between each two groups. Comparison of mean values of studied variables among different groups was done using ANOVA test. Pearson’s correlation coefficient was used to quantify the relationship between the variables under study. P<0.05 was considered to be significant.

## RESULTS AND DISCUSSION

**Patients and Controls:** Ten healthy volunteers were considered as group I. Ten patients with sterile AF at the time of hospital admission who did not develop AF infection constituted group II. Twenty patients who develop AF infection during hospitalization admission constitute group III. Patients in group III subjected to a 10 day course of intravenous antibiotic therapy considered as group IV. There were no statistical differences noted in the mean age and sex distribution among the studied groups.

**Serum and Erythrocyte Lysate Concentrations of Some Biochemical and Antioxidant Parameters:** Biochemical characteristics and antioxidant elements concentrations of the four different studied groups were detailed in table I. Serum catalase activity showed a highly significant decrease in group II and III compared to group I (19.84±1.102, 18.066±0.839 vs. 23.477±0.761 respectively) (P=0.001). Group IV of patients after treatment showed a significant up-regulation of glutathione peroxidase activity (19.781±0.67) (P=0.001). As showed in table I.

Glutathione peroxidase enzyme activity showed a significant decrease in group II and III compared to group I (19.84±1.102, 18.066±0.839 vs. 23.477±0.761 respectively) (P=0.001). Group IV of patients after treatment showed a significant up-regulation of glutathione peroxidase activity (19.781±0.67) (P=0.001). As showed in table I.

**Ascitic Fluid Concentrations of Nitric Oxide and Malondialdehyde:** Table II summarizes the results of nitric oxide and malondialdehyde in AF among the three groups of patients. There was a highly significant increase in NO level in group III compared to group II (74.237±4.3.09 vs. 40.537±3.625 respectively) (P=0.000). NO level showed a significant decrease in group IV compared to group III (59.294±3.629 vs. 74.237±4.309 respectively) (P=0.012). As represented in figure 2.

Also there was a highly significant increase in MDA levels in group III compared to group II (16.06±0.916 vs. 8.406±0.751 respectively) (P=0.000). MDA level showed a high significant decrease in group IV compared to group III (11.369±0.838 vs. 16.06±0.916 respectively) (P=0.000).

**TNF-α mRNA Expression Level in Duodenal Biopsy:** There was a higher significant increase in TNF-α mRNA expression in duodenal biopsy from patients with SBP before treatment compared to cirrhotic patients with sterile AF (254.019±26.471 vs. 5.86±0.405 respectively) (P=0.000). Also there was a marked significant decrease in TNF-α mRNA expression level in SBP patients after treatment compared to SBP patients before treatment (47.99±3.813 vs. 254.019±26.471 respectively) (P=0.000). As represented in figure 3A.

In figure 3B, GAPDH and TNF-α mRNA amplification plots are shown in all studied groups and represents Ct values and threshold (Th). At the end of the PCR, the instrument performed a melting curve. As represented in Figure 3C.

**Correlations Between Different Studied Parameters among Different Groups:** In cirrhotic patients with sterile ascites group there was a negative significant correlation between serum catalase activity and both AF NO level and TNF-α mRNA expression level ($r=-0.885, p=0.001$; $r=-0.826, p=0.003$ respectively). In SBP after treatment group there was a negative highly significant correlation between GPx and both AF NO level and TNF-α mRNA expression level ($r=-0.889, p=0.000$; $r=-0.646, p=0.002$ respectively).
Table I: Statistical analysis of some biochemical parameters in serum and erythrocyte lysate in different studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (n=10)</th>
<th>Group II (n=10)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>sGOT (U/L)</td>
<td>24.8±2.568</td>
<td>43.3±3.253</td>
<td>56.45±5.982</td>
<td>55.35±6.232</td>
<td>0.004</td>
</tr>
<tr>
<td>sGP T (U/L)</td>
<td>25.4±2.864</td>
<td>38.2±4.647</td>
<td>46.35±4.582</td>
<td>45.4±4.786</td>
<td>0.025</td>
</tr>
<tr>
<td>Alb. (g/dl)</td>
<td>4.33±0.165</td>
<td>2.62±0.17</td>
<td>2.37±0.139</td>
<td>2.575±0.13</td>
<td>0.000</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>13.81±0.45</td>
<td>8.57±0.431</td>
<td>8.955±0.316</td>
<td>9.34±0.345</td>
<td>0.000</td>
</tr>
<tr>
<td>t.bil. (mg/dl)</td>
<td>0.56±0.091</td>
<td>2.79±0.301</td>
<td>3.656±0.205</td>
<td>3.593±0.202</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Cat. (U/L)</strong></td>
<td>509.337±39.869</td>
<td>398.343±23.477</td>
<td>328.614±15.231</td>
<td>414.608±22.294</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>Erythrocyte lysate</strong></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SOD (u/g Hb)</td>
<td>21.073±0.888</td>
<td>19.066±3.836</td>
<td>15.759±2.896</td>
<td>16.939±2.537</td>
<td>0.000</td>
</tr>
<tr>
<td>GPX (mu/g Hb)</td>
<td>23.477±0.761</td>
<td>19.84±1.102</td>
<td>18.066±0.839</td>
<td>19.781±0.67</td>
<td>0.001</td>
</tr>
</tbody>
</table>

sGOT: serum glutamate oxaloacetate transaminase, SGPT: serum glutamate pyruvate transaminase, Alb: Albumin, Hb: Hemoglobin, t.bil.: total bilirubin, Cat.: Catalase, SOD: Superoxide dismutase, GPX: Glutathione peroxidase. The values are expressed as mean±SEM. P<0.05 is considered significant.

Table II: Statistical analysis of some biochemical parameters in ascitic fluid in group II, III and IV

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group II (n=10)</th>
<th>Group III (n=20)</th>
<th>Group IV (n=20)</th>
<th>GP III vs. GP II</th>
<th>GP III vs. GP IV</th>
<th>GP III vs. GP II</th>
<th>GP III vs. GP IV</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO (nmol/l)</td>
<td>40.537±3.625</td>
<td>59.294±3.629</td>
<td>5.073</td>
<td>2.653</td>
<td>0.000</td>
<td>0.012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>8.406±0.751</td>
<td>11.369±0.838</td>
<td>5.442</td>
<td>3.78</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN (cells/mm³)</td>
<td>86.5±10.221</td>
<td>965±107.856</td>
<td>7.498</td>
<td>6.564</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLC (cells/mm³)</td>
<td>3580±892.442</td>
<td>3580±892.442</td>
<td>5.131</td>
<td>3.801</td>
<td>0.000</td>
<td>0.000</td>
<td></td>
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</tbody>
</table>

NO: nitric oxide, MDA: malondialdehyde, PMN: polymorphonuclear leukocytic, TLC: total leukocytic count. The values are expressed as mean±SEM. P<0.05 is considered significant.

Fig. 1: Serum catalase activity among different group
Fig. 2: Nitric oxide in AF among different groups of patients
Fig. 3: Determination of GAPDH and TNF-? mRNA expression. (A) Relative quantitation of mRNA expression level. (B) Amplification plot for GAPDH (•) and TNF-α (■) mRNA in cirrhotic patients with sterile AF, GAPDH (○) and TNF-α (▲) mRNA in SBP before treatment and GAPDH (×) and TNF-α (Δ) mRNA in SBP after treatment. (C) Melting curve of TNF-? mRNA product in all studied groups.

Fig. 4: Correlations between NO and both MDA and TNF-?.
The correlations showed in fig.4 revealed a positive highly significant correlations between AF NO level and both MDA level and TNF-α mRNA expression level in SBP before treatment group (r=0.963, p=0.000; r=0.963, p=0.000 respectively).

Discussion: The clinical course of patients with cirrhosis is often affected by a number of complications, including portal hypertension, ascites, and spontaneous bacterial peritonitis (SBP). Bacterial infection is responsible for up to one fourth of the deaths of patients with cirrhosis, and SBP is a major complication in cirrhosis that is common in patients with cirrhosis and ascites.

SBP probably develops as a consequence of impaired defense mechanisms against infection seen in cirrhotic patients. Although key steps in the pathogenesis of SBP are yet to be elucidated, it is evident that the gut is a major source of bacteria in SBP. Altered gut motility has been shown in cirrhosis, and this may facilitates bacterial overgrowth and produce bacterial peritonitis.

Cirrhosis can develop in a number of different conditions, including alcoholic liver disease, biliary disease, and viral infection. Oxidative stress plays an important role in the pathogenesis of toxic liver diseases and other hepatic alterations. Intestinal permeability is increased in patients with cirrhosis, Oxygen free radicals are known to play an important role in gut epithelial damage, which may alter the gut barrier function and facilitate bacterial translocation and release of endotoxin.

Macrophages are of central importance in the initiation and regulation of the nonspecific and specific immune responses. They take part as active phagocytic cells, serve as antigen-presenting cells for T lymphocytes, and are a major source of a wide range of inflammatory products including eicosanoids and cytokines. Monocytes or tissue macrophages undergo profound changes in morphology and function in the presence of proinflammatory substances or other mediators of the host defense response. Under these circumstances, they also secrete a large range of cytotoxic and bactericidal products, including NO. Rodent macrophages are extremely sensitive to agents promoting NOS II expression, and, in fact, this was the first cell type in which NOS II mRNA was cloned.

In this study oxidative stress is demonstrated by increased MDA accumulation and a significant reduction in antioxidant elements SOD, GPx as well as serum catalase. In addition to the increased concentration of NO level in cirrhotic patients with SBP but not in patients with sterile ascites. A significant elevated base line of TNF-α mRNA expression level in cirrhotic patients with SBP. After antibiotic treatment MDA, NO and TNF-α expression levels dropped back towards sterile values. In the mean time, antioxidant elements were also recovered. A notable positive significant correlation between TNF-α expression level and NO (P<0.001) among the three studied groups.

Nitric oxide (NO) is a powerful vasodilator agent present in AF that also shows some bactericidal activity. NO is elevated in peritoneal infections, and it has been suggested that this factor could play a protective role against bacterial infections in patients with cirrhosis. Our study was supported by a previous study in 2006, the presence of a significant increase in nitric oxide and MDA 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.

Bories et al. observed a long-lasting and increased intra-abdominal production of NOx in patients with SBP; Jimenez et al. demonstrated high local synthesis of NOx in patients with unresolved SBP, and finally Ugur Cos kun, et al reported that overproduction of nitric oxide in cirrhotic patients may be related to the severity of liver damage and spontaneous bacterial peritonitis. However, recent publications show conflicting results in this setting. Park YS, et al. showed that Ascites NO level was not different between ascites from SBP patients and ascites from cirrhotic patients with sterile ascites. There were no changes of ascites NO in SBP patients during treatment. Therefore ascites NO was not useful to predict the progress of SBP. However, the authors reported that mortality was significantly higher among patients whose NOx values were higher than 60 μmol/l.

TNF-α is a macrophage-derived cytokine produced in large amounts in response to endotoxin. High serum and AF levels of this cytokine have been observed in patients with cirrhosis and SBP. Since SBP is considered to be the consequence of repeated episodes of bacteremia following intestinal bacterial translocation with seeding of AF, it might be considered that the intermittent presence of bacteria in blood may be followed by an activation of the cytokine cascade. In our study there was a significant elevated base line of TNF-α mRNA expression level in cirrhotic patients with SBP. After treatment TNF-α expression level dropped back towards sterile values. A recent publication supports our results where, they found a significant increase in the production of nitric oxide and TNF-α in cirrhotic patients with SBP and reduction in these parameters after treatment.

Additional data from a previous study support our results where, Navasa M, et al. demonstrated high level
of TNH-alpha in SBP patients and said that the inflammatory response to the infection may be an important mechanism of renal impairment and the associated mortality in SBP (51). Also in 2001, Rodriguez-R.C., et al. showed that SBP was associated with significantly elevated ascitic fluid levels of every one of the proinflammatory cytokines (IL1β, TNF-α and IL6) compared to those in cirrhotic controls. And Ascitic fluid levels of proinflammatory cytokines decreased rapidly after resolution of the infection (52).

In conclusion, this study demonstrates the presence of significant oxidative stress in cirrhotic patients with SBP. Antibiotic treatment for SBP reverses the oxidative stress and decreases NO and MDA levels; also there is a significant decrease in TNF-α mRNA expression level. So the data suggests that measurement of these parameters may have a role in the diagnosis of SBP and follow up after antibiotic treatment.

REFERENCES


