Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

Parisa Samadzadeh1*, Mohammad Rahmati2 and Homayun Dolatkhah3

1*Corresponding Author, MSc Student of Biochemistry, Dept. of Biology, College of Post Graduate, Ahar Islamic Azad University, Ahar, IRAN. Email: pari.saaaa@yahoo.com
2Associated Professor in Clinical Biochemistry, Dept. of Clinical Biochemistry and Laboratories Medicine, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, East-Azarbaijan, I. R. IRAN. Email:rahmati_bio@yahoo.com.
3Assistant in Clinical Biochemistry, Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, East-Azarbaijan, I. R. IRAN. Email: dolatkahhh@gmail.com.

ABSTRACT
Objective: Liver cirrhosis is the result of chronic inflammation of liver tissue which results for different reasons. One of the useful evaluation indexes for determining the reduction of liver biosynthesis capacity is checking the amount of oxidative stress in the liver cirrhosis patients which changes unnaturally in chronic liver diseases. Therefore, the main goal of this study is to check the amount of oxidative stress in blood and ascites fluid in liver cirrhosis patients.

Methods: This is a cross sectional research study in which the target population is liver cirrhosis patients with infectious or non-infectious ascites fluid. The participants are 120 people which are investigated in four different groups. Two samples of blood are taken from all the people and a sample of ascites is taken from cirrhosis patients. The activity of superoxide dismutase and glutathione peroxidase is measured in the samples of blood and ascites fluid.

Results: In the above mentioned groups, the activity of superoxide dismutase and glutathione peroxidase in the red blood cells and ascites fluid shows a significant decrease.

Conclusion: In the liver cirrhosis patients, the amount of oxidative stress has shown a significant increase. We can use this increase for monitoring the development of disease and also it shows serious damage to liver.

Key words: liver cirrhosis, infected and non-infected ascites fluid, oxidative stress

INTRODUCTION:
Liver cirrhosis is triggered by chronic inflammation of hepatic tissue which may be impaired for several reasons including any less or more inflammation (1). Accordingly, the impaired tissues are reconstituted as compensatory action (2). Chronically and frequently continued, this process causes disorders for the regular structure of liver, bringing about gradually fibrosis, liver stiffness and cirrhosis (3). The leading causes of cirrhosis in Iran include hepatitis B, hepatitis C and autoimmune hepatitis. However, drugs and toxic substances, fatty liver, Wilson disease, biliary strictures and occlusion of the hepatic veins are considered the main causes of the disease (4). Symptoms of liver cirrhosis depend on the stage of the disease, so that at the early stages the disease may not present any symptoms and can only be diagnosed with rigorous testing.
Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

Parisa Samadzadeh, et al.

radiology and histology. As the disease progresses, its symptoms and complications increase, as well (5). The very important symptoms of liver cirrhosis are ascites and accumulation of fluid in the abdomen which are caused due to decreasing serum albumin level and increased sodium and water in the body. This condition normally gives rise to swelling of the legs and abdominal distention in the body. Ascites is the very obvious mark of cirrhotic attack which implies a pathologic aggregation of fluid into peritoneal cavity. The term "ascites" is derived from the Greek word, "askos" meaning a wine bag and or sac. It seems not to make sense in this case. Clinical manifestations of ascites had been described/ investigated since the ancient era which was a logical and deductible overview in medical context in Egypt, Ebers Papyrus J. in 1550 BC. (6). Reasons given for the aggregation of cirrhotic ascites fluid are based on some widespread factors, the most important of which are regarded hormonal disorders, disorder in cytokines level and the volume linked with it in tuning venous blood pressure (7). Aggregated ascites stimulates the progress of cirrhosis. Ascites accumulation in patients with cirrhosis is considered the most prevalent cause rendering the patients being hospitalized (6). The results of epidemiological studies in different countries indicate the high death rate among patients with cirrhosis of the liver in recent years. As the fourteenth most common cause of death in most developed countries, it is the fourth leading cause of death in Central Europe. About a million and three hundred thousand people a year worldwide die from the disease (8).

Major complications of cirrhosis include high blood pressure (Hypertension), Esophagealvarices, hepatomegaly, ascites, spontaneous bacterial peritonitis, Hepaticencephalopathy, malnutrition, blood coagulation disorders, deficiency of fibrinolysis factor, thrombocytopenia, bone disorders, osteoporosis, rickets, blood disorders, anemia, hemolysis, neutropenia, diabetes, cancer, heart and kidney failure, pancreatitis and so on (2-4).

Patients with cirrhosis are required to undergo systematic continuous clinical and laboratory examination to keep their disease or its complications under control. However, the selection of appropriate treatment plan depends on the acuteness, type of damage to the liver and the possibility of its progress examination (1). Free radicals are now very toxic by themselves. The molecular oxygen in the form of reactive oxygen species (ROS) is counted as a natural part of aerobic life responsible for some of cellular functions, including signal transmission paths, defending against invasions by microorganism, and expression of genes, in order to enhance cell growth or death. (9). However, the excessive amount of the ROS is very toxic to cells. Oxidative stress causes damage to cell components such as to proteins, fat and DNA, being usually associated with pathogenesis of a variety of degenerative disease, such as diabetes, cancer, heart and vascular disorders, and nerve diseases (9-10). Exposure to high levels of ROS may lead to serious damage to the body, such as major disorders of the liver. Aside from these harmful effects, ROS, as the secondary messenger molecules, is produced in the response to growth, hormones, cytokine and extracellular ATP factors. Therefore, the role of oxidant factors is complicated in cells, depending on the balance between oxidants and antioxidant defense systems (11). The protection measures against ROS are taken by several important antioxidants enzymes such as: Superoxide dismutase (SOD) and Glutathione Peroxides (GPX), as well as the compounds, including Tocopherol, and vitamin E, beta-carotene, ascorbate and Glutathione(GSH) (12). When the capacity of this antioxidant structure is decreased, the amount of reactive oxygen species is increased. Finally, a dangerous condition of oxidative stress state is created and adverse impacts of oxidative factors may be made (13). With regard to the vital role of oxidative stress in liver diseases, antioxidant system level is
Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

Parisa Samadzadeh, et al.

studied for the treatment of liver disorders, which is considered to be a good therapeutic strategy (10). Unfortunately, the research conducted on the antioxidant defense level of the body in patients with liver disorders in general and with liver cirrhosis diseases in particular have not yielded results, remained controversial. Therefore, the main objective of this study was to assess oxidative stress in blood and as cites in patients with cirrhosis of the liver, which is evaluated by measuring the activity of superoxide dismutase and Glutathione peroxide enzymes in the blood and ascites fluid.

MATERIALS AND METHODS: The present study was a cross-sectional case-control one in which the cases were the patients who had liver cirrhosis. They had been diagnosed with liver cirrhosis by clinical, laboratory, endoscopy and ultrasound examination. The existence of ascites fluid in them was detected by clinical and ultrasound scans. The presence of SBP was revealed with ascites fluid PMN more than 250000/ ml, as well as positive cultures. The subjects in this study were studied in four groups: the control group (Group A) who were healthy individuals in terms of cirrhosis. They had referred to the Digestive Health Clinics because of digestive problems, and then, had been referred to the laboratory to undergo serological Anti-IgG test for the diagnosis of Helicobacter pylori infection. Being confirmed that their test result was negative and by explaining the study to them, the patients' blood samples were used for the purposes of this study with asking their permission. The other three groups of patients were: the cirrhotic patients without ascites fluid (group B), the cirrhotic patients with ascites and SBP infection (Group C), and the cirrhotic patients with ascites without SBP infection (Group D). Of groups C and D patients, a sample of blood was taken to use their red blood cell for measuring the activity of superoxide dismutase and glutathione peroxidase enzymes, along with an ascites fluid sample.

And from the control group and group B patients, a whole blood sample was obtained as mentioned above. It should be mentioned that blood samples were obtained in fasting. Microbial culture, the CBC and the measurement of superoxide dismutase and glutathione peroxidase activity were performed on the as cites fluid samples of the patients. Superoxide dismutase and glutathione peroxidase enzyme activity was carried out in whole blood.

Exclusion Criteria:
- Patients with renal impairment: To this end, of all blood samples collected urea and creatinine tests were taken and the samples with more than 40 mg/100 ml blood urea and more than 2.1 ml/ 100 ml creatinine were excluded from the study.
- The presence of diabetic signs with a history of high fasting blood glucose more than 120 mg/dl serum and glycosylated hemoglobin more than 6.7 percent.
- Having heart failure confirmed by lab results of LDH Serum over 500 IU/ L, CK-MB Serum over 25 IU /L and CTN-I more than 3.1 ng/ ml.

The activity of superoxide dismutase and glutathione peroxidase enzymes of whole blood and ascites fluid was measured by manual kit and colorimetric manufactured by Randox made in England.

Data analysis (statistical methods): First, since the groups were independent, the mean of obtained results was calculated by SPSS software in each group, and then, normal distribution of results was evaluated by Shapiro Wilks test. All results showed to have normal distribution. The results of ascites obtained from the two groups were compared between the groups by using Paired Sample t-test, and the results of whole blood as they were in four separate groups were compared by using one-way ANOVA. The tests were considered significant when p value was less than 0.05.
Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

RESULTS:
Comparison of demographic data of the groups:
In table 1, the demographic data of the patients was shown in four groups which were statistically compared and, as can be seen, the groups have been homogeneous in terms of age, gender, blood pressure, hemoglobin and hematocrit, urea and creatinine (in all cases p> 0.05).

Table 1. Demographic data of the studied groups

<table>
<thead>
<tr>
<th>Clinical factors</th>
<th>Groups</th>
<th>Control group (N = 30)</th>
<th>Case Group A (N = 30)</th>
<th>Case Group B (N = 30)</th>
<th>Case Group C (N = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td>60.70 ± 14.42</td>
<td>63.83 ± 15.25</td>
<td>63.66 ± 9.68</td>
<td>60.20 ± 15.28</td>
<td>0.132</td>
</tr>
<tr>
<td>Gender: Men (64)</td>
<td></td>
<td>14</td>
<td>16</td>
<td>17</td>
<td>17</td>
<td>0.854</td>
</tr>
<tr>
<td>Woman (56)</td>
<td></td>
<td>16</td>
<td>14</td>
<td>13</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td></td>
<td>113.66 ± 15.86</td>
<td>115.83 ± 16.71</td>
<td>115.00 ± 16.60</td>
<td>113.50 ± 16.77</td>
<td>0.938</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td></td>
<td>74.33 ± 11.50</td>
<td>75.16 ± 11.10</td>
<td>74.16 ± 12.04</td>
<td>75.00 ± 11.89</td>
<td>0.984</td>
</tr>
<tr>
<td>Urea (mg/ dl)</td>
<td></td>
<td>57.93 ± 37.5</td>
<td>64.30 ± 43.58</td>
<td>51.60 ± 38.46</td>
<td>55.73 ± 33.07</td>
<td>0.673</td>
</tr>
<tr>
<td>Creatinine (mg / dl)</td>
<td></td>
<td>1.13 ± 0.38</td>
<td>1.17 ± 0.63</td>
<td>1.06 ± 0.42</td>
<td>1.12 ± 0.56</td>
<td>0.299</td>
</tr>
<tr>
<td>Hemoglobin (g/ dl)</td>
<td></td>
<td>11.40 ± 1.84</td>
<td>11.51 ± 1.90</td>
<td>12.49 ± 1.63</td>
<td>11.79 ± 1.93</td>
<td>0.100</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td>35.13 ± 5.48</td>
<td>35.59 ± 5.63</td>
<td>36.54 ± 5.05</td>
<td>35.46 ± 6.01</td>
<td>0.786</td>
</tr>
</tbody>
</table>

Comparison of the mean of superoxide dismutase and glutathione peroxidase enzymes activity of blood among the four groups
As can be observed in table 2 and figure 1 and 2, there is a significant difference between the groups' mean. Note that using SPSS version 21 and one-way ANOVA test showed that the superoxide dismutase and glutathione peroxidase enzymes activity in the case groups was statistically significant in comparison with that of the control group. Results are shown as mean ± SD.

Table 2: Data on comparison of the mean of superoxide dismutase and glutathione peroxidase enzymes activity among the four groups

<table>
<thead>
<tr>
<th>Enzyme Activity</th>
<th>n</th>
<th>Mean ± SD (control group)</th>
<th>Mean ± SD (Case group A)</th>
<th>Mean ± SD (Case B group)</th>
<th>Mean ± SD (Case group C)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (IU/gHb)</td>
<td>30</td>
<td>136.57 ± 21.15</td>
<td>93.78 ± 9.58</td>
<td>50.14 ± 15.62</td>
<td>54.91 ± 16.18</td>
<td>0.002</td>
</tr>
<tr>
<td>Glutathione peroxidase (IU/gHb)</td>
<td>30</td>
<td>61.28 ± 15.75</td>
<td>26.98 ± 6.02</td>
<td>14.01 ± 5.10</td>
<td>19.12 ± 2.64</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Figure 1. The graph showing comparison of the mean of superoxide dismutase enzyme activity in the four groups
Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

Figure 2. The graph showing comparison of the mean of blood glutathione peroxidase activity in the four groups

Comparison of the mean of superoxide dismutase and glutathione peroxidase ascites enzymes activity in groups B and C:

As table 3 and Figure 3 and 4 depict, a significant difference can be observed between the mean of superoxide dismutase and glutathione peroxidase enzymes differed statistically. Results are shown as mean ± SD.

Table 3. Data on comparison of the mean of superoxide dismutase and glutathione peroxidase enzymes activity in groups B and C

<table>
<thead>
<tr>
<th>Enzymes Activity</th>
<th>n</th>
<th>Mean ± SD (CaseB)</th>
<th>Mean ± SD (GroupC)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (IU /mg Protein)</td>
<td>30</td>
<td>1.36 ± 0.49</td>
<td>3.02 ± 0.54</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Glutathione peroxidase (IU /mg Protein)</td>
<td>30</td>
<td>0.30 ± 0.16</td>
<td>0.71 ± 0.21</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

Figure 3. The graph showing comparison of the activity of superoxide dismutase enzyme in groups B and C
**DISCUSSION:**

In humans, cirrhosis of the liver may be caused at any ages and often trigger long-term effects (14). Cirrhosis is counted the eighth leading cause of death in the world and if enlarged liver is detected, ascites may be one of the most common major complications of cirrhosis. The other complications may be hepatic encephalopathy and variceal bleeding (15-16). Approximately 50% of patients are afflicted with (hidden) cirrhosis, i.e. ascites progresses without one of these complications within 10 years. Ascites is the most common complication of cirrhosis making the patient to be hospitalized. Fluid accumulation in cirrhosis environment causes the development of chronic liver disease (17). About 15% of patients with ascites die every year and 42% of them every 5 years. Many patients are referred to liver transplantation after the progress of ascites (17-18). Oxidative stress forms the very misty background for multiple disorders of liver (19). Oxygen free radicals and its increase are associated with the body's natural metabolic and proliferative diseases of the liver in duration of inflammation (20). Reactive oxygen species (ROS) are primarily produced in mitochondria and the endoplasmic reticulum of hepatocytes through cytochrome P450 enzymes. (21) In normal circumstances of body, cells control oxidative stress level with specific molecules strategies which are known as the antioxidant defenses, and maintain the balance between oxidants and antioxidants (22). Oxidative stress represents an imbalance between free radicals and antioxidant factors (23). In liver disorders, proteins, fats and DNA in the cell structures of the liver are primarily affected by reactive oxygen species and their natural structure are altered (24). This process leads to structural and functional abnormalities in the liver (25). So, in liver cirrhosis in general and in liver disorders, in particular, the phenomenon of oxidative stress and lipid profiles should be monitored for several reasons. First, oxidative stress and lipid profile may explain the reasons for the pathogenesis of various liver disorders. Secondly, markers of oxidative stress and lipid profile among liver cells may develop the required potential to detect liver damage and ultimately to monitor the response to drug therapy in patients with cirrhosis of liver. Accordingly, the present study aimed to investigate the oxidative stress and serum lipid profile and ascites in patients with liver cirrhosis.

The key factor playing a very important role in the progress of ascites in patients with liver inflammation is counted to be venous blood pressure which resulted from increased internal resistance of bloodstream and is combined by visceral vasodilation accounted for the produced
Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

Parisa Samadzadeh, et al.

vasodilators (26). Acute inflammation of liver (Cirrhosis) is brought about by deformation of liver caused by damage to chronic liver and sclerosis. As a result of inflammation of the liver and the coronary venous, bloodstream resistance is increased due to increased vasoconstrictor products, such as angiotensin, endothelin, cysteine, leukotrienes and thromboxane, leading to the gradual formation of the venous blood pressure, circulatory system and a change in the direction of blood circulation (27). Visceral vasodilation goes up constantly as venous blood pressure and thus, is involved in vasodilation factors, including nitric oxide, calcitonin, peptide, carbon monoxide and manabinoids (28-29).

Inflammation of liver is associated with splanchnic arterial carotid vasodilation. This ends with reducing blood flow volume and a powerful circulation. Decreasing the effective circulating volume makes the sodium of the kidneys and protective path active. Water and sodium gives rise to swelling in stomach which was rendered by sodium and water redundant precipitate of liver in the peritoneal cavity (30). During the stage of the disease, reduction in effective circulating volume results in high contraction of kidneys and a reduction in the degree of glomerular filtration (31). The onset of cirrhotic cardiomyopathy triggered by an increase in free radicals and abnormality in lipid profile doubles this problem. Circulation disorders along with organ failure end in death (32-33). Infectious poisoning is often accompanied by some stages. Elongation of endothelium and bacteria replacing are linked with excess producing of vasodilatation and other cytokines (34). The current information suggests that bacterial replacement is provided for mesenteric lymph nodes in inflamed liver, and stimulating the resultant of cytokines products plays an essential role in the arterial vasodilation stage (35). Visceral artopoly vasodilation and an effective accompaniment in blood circulation decrease effective arterial circulating volume and arterial blood pressure. In response to the change in effective arterial blood volume and visceral pressure receptor, the pressure receptors dependent on nervous system are activated. Renin-angiotensin system, aldosterone and antidiuretics hormones cause sodium retention and liver ascites (36). Restoring hemostasis in liver inflammation is also associated with increased sinus pressure and reduced plasma pressure. This enhances the development of liver infection, and when the liver lymph capacity goes back to hepatic lymph circulation, its speed improves. Excess lymph is shed into peritoneal cavity that creates ascites (37-38). The volume of cardiac output plasma rises at the early stages of liver inflammation that maintains the compensated circulation function. However, the cardiac output is decreased as a result of cirrhotic cardiomyopathy (39-40). At advanced stage, inflammation of the liver facilitates the volume of effective arterial blood volume, activating the mechanism of systematic vasoconstriction which impacts particularly on kidneys and increases glomerular and renal plasma flow. And at the intense state, it leads to kidney failure called liver-kidney syndrome (41-42).

In the study conducted by Galicia Moreno and colleagues (43) in 2014 on patients with alcoholic cirrhosis, it was reported that oxidative stress is significantly involved the development of liver lesions, and it was argued that extensive studies are required to be done on oxidative stress level in cirrhotic patients, so that the results of these studies could be used for the treatment of this disease by neutralizing free radicals. The results obtained from the present study showed that oxidative stress level (reviewed with superoxide dismutase and glutathione peroxidase enzymes activity) both in blood and in the ascites fluid was significantly increased with disease progression and also with infected peritoneal fluid, confirming the results of Galicia Moreno and colleagues’ research (43). Deshpande et al. (2013) study (44) reported that the reduced activity of superoxide dismutase and glutathione peroxidase enzymes in red blood cells on the one hand, and increased level of serum malondialdehyde in patients with
Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

Parisa Samadzadeh, et al.

liver cirrhosis on the other hand are caused by alcohol abuse and may result in the pathogenesis and the development of associated liver disease. The question arises whether in cirrhotic patients whose disease is not created as a result of alcohol consumption, has such a case been seen? We have answered the question in this research that in liver cirrhosis resulted from other than alcohol consumption, the activity of superoxide dismutase and glutathione peroxidase enzymes is also reduced inside red blood cells and the ascites fluid. On the other hand, LDL oxide level has demonstrated a significant increase in serum and ascites in patients with liver cirrhosis. These results and the results of Deshpande et al. (44) confirm and support each other.

CONCLUSION:
On the basis of the results of this study, it can be concluded that in patients with cirrhosis of liver, oxidative stress was increased significantly as the disease progressed that this increase can be used as a marker of easy for monitoring disease progression and also is the indicative of severe liver damage, rendering liver into more complicated problems and cancer.

REFERENCES:
Evaluation of blood and ascitic fluid oxidative stress in patients with liver cirrhosis

36. Mannucci PM, Triponi A. Hemostatic defects in liver and renal dysfunction. Hematology


