Hyaluronic Acid (HA) Level in Ascitic Fluid of Cirrhotic Patients with Spontaneous Bacterial Peritonitis (SBP)

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Abstract

Introduction: Spontaneous bacterial peritonitis (SBP) is a common problem that affects liver cirrhotic patients. It is also, a major contributor to the deterioration and aggravation of liver failure complications. Complement deficiency considered as a major complication of liver cirrhosis and bacterial overgrowth in the intestine is the major source of bacterial peritonitis. Hyaluronan or hyaluronic acid (HA) is a connective tissue polysaccharide, synthesized by many cell types, although mesenchymal cells are believed to be predominant. Serum level of HA is regulated by the influx from the tissues via lymphatic system and its receptor-mediated clearance by liver endothelial cells. So, marked increase in serum levels are noted in liver diseases, especially in patients with cirrhosis, when the clearance is impaired. Hyaluronic acids have an important role in controlling tissue permeation, bacterial invasiveness and macromolecular transport between cells. HA was observed to enhance cellular infiltration and migration by facilitating cell detachment. It also, increases the proinflammatory cytokines TNF-α and IL-8 production. It is interesting to note that HA not only can promote the inflammation, but can also moderate the inflammatory response. This may contribute to the stabilization of granulation tissue matrix. The innate immune system uses Toll Like Receptors (TLRs) to recognize microbes and initiate host defense. The repeating disaccharide structure of HA has features of pathogen-associated molecular patterns. Many pathogen-associated molecular patterns on pathogens utilize Toll-like receptors to initiate innate immune responses.

Aims and Methods: To measure the level of complement-3 (C3) and hyaluronic acid in ascitic fluid of liver cirrhosis patients with and without spontaneous bacterial peritonitis.

Results: In our study we found that there was a significant decrease in C3 level in ascitic fluid of cirrhotic patients in comparison to ascitic fluid of patients with other causes (i.e. Nephrotic syndrome) (p<0.05). Also, HA level was found to be highly significantly lower in ascitic fluid of cirrhotic patients in comparison to ascitic fluid of patient with other causes (i.e. Nephrotic syndrome) (p<0.001). HA level in serum of liver cirrhosis patients was significantly higher than the contro group (p<0.001). There was a highly significant decrease in HA level in ascitic fluid of cirrhotic patients with SBP in comparison to HA level in ascitic fluid of cirrhotic patients without SBP (p<0.001).

Conclusion: C3 and HA are significantly decreased in ascitic fluid of cirrhotic patients. HA significantly decreased in ascitic fluid of cirrhotic patients with SBP in comparison to patients without SBP.

Key Words: Hyaluronic acid (HA) – Spontaneous bacterial peritonitis (SBP).

Introduction

ACCORDING to some recent data [1], hepatic cirrhosis represents the tenth major cause of death world wide. Among the major complications of cirrhosis, ascites seems to be the most frequent one (85%), along with hepatic encephalopathy and the hemorrhage caused by the rupture of the esophageal varices. Patients with cirrhosis and ascites show a higher susceptibility to bacterial infections mainly because of the inadequate defence mechanisms. In these patients, the most frequent infectious complication that occurs (25% of the cases), and at the same time the most severe one is spontaneous bacterial peritonitis (SBP), followed by urinary infections (about 20%), pneumonia (about 15%) and bacteremia (12%) [2]. For SBP diagnosis, the number of polymorphonuclear leucocytes (PMN) from the ascetic fluid obtained by paracentesis must exceed 250 cells/mm³ and from bacteriological cultures only one germ must be isolated [3]. Spontaneous bacterial peritonitis is probably related to several impaired defense mechanisms, such as depressed reticuloendothelial system phagocytic activity, leukocyte dysfunction, reduced serum complement, and low bacterial activity of ascitic fluid. Infection of ascitic fluid is related to its antimicrobial activity. In cirrhotic patients, the

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bactericidal and opsonic activity of the ascitic fluid is lower than that observed in noncirrhotic ascites or in normal peritoneal exudate [4]. Patients with liver cirrhosis have a proposed three possible mechanisms for the development of SBP: Intestinal bacterial overgrowth, the alterations (structural and functional) of the intestinal mucosal barrier and the deficiencies of the local immune response [4]. In cirrhotic patient there is a local and systemic immune deficiencies, so bacteremia and ascitic fluid inoculation is a leading result. If the ascitic fluid complement level is low, this will determine a low bactericidal activity and thus a higher risk of SBP [5]. The hyaluronic acids (HA) are a class of macromolecular proteoglycans characterized by a highly polymerized chain of glucuronic acid and N-acetylglucosamine units bonded to protein. Due to its high water binding capacity hyaluronic acid is responsible for the hydration and osmotic balance of tissues. Hyaluronic acid network maintains matrix homeostasis by establishing spaces for fluid flow and diffusion barriers which regulate the distribution of proteins. It also influences cell proliferation and differentiation as well as the migration of cells and contributes to pericellular matrix formation by binding to the cell surface receptor CD44. Additionally, it is involved in tissue repair, so that large amounts of hyaluronic acid were observed in granulation tissue [6]. Occurring in intercellular ground substance of connecting tissue [7], they have an important role in controlling tissue permeation, bacterial invasiveness and macromolecular transport between cells [8]. HA acts as a promoter of early inflammation, HA was observed to enhance cellular infiltration [9][10]. Kobayashi and colleagues [9][11] showed a dose-dependent increase of the proinflammatory cytokines TNF-α and IL-8 production by human uterine fibroblasts at HA concentrations of 10 μg/mL to 1 mg/ml via a CD44 mediated mechanism. Endothelial cells, in response to inflammatory cytokines such as TNF-α, and bacterial lipopolysaccharide, also synthesize HA, which has been shown to facilitate primary adhesion of cytokine-activated lymphocytes expressing the HA-binding variants of CD44 under laminar and static flow conditions [12]. It is interesting to note that HA has contradictory dual functions in the inflammatory process. It not only can promote the inflammation, but also can moderate the inflammatory response, which may contribute to the stabilization of granulation tissue matrix. In chronic liver disease permanent inflammation might cause fibrosis or even cirrhosis of the liver at last. Liver fibrosis/cirrhosis is characterized by an enhanced extracellular matrix synthesis by hepatic stellate cells leading to progressive induration of the entire organ. The formation of scar tissue causes the progressive loss of liver function and also decreases the capacity of the liver sinusoidal endothelial cells for the clearance of hyaluronic acid. So, HA increases in liver cirrhosis both due to the loss of degradation capacity and an increased synthesis of hyaluronic acid [5].

**Material and Methods**

Thirty nine patients and 9 healthy subjects (group D) have been included in this study. Patients were divided into three groups: Group (A) included 16 patients with liver cirrhosis and ascites which develop spontaneous bacterial peritonitis (SBP) diagnosed according to the polymorphonuclear leukocytic count in ascitic fluid (>250/mm³) and +ve culture (with only one organism). Group (B) included 12 patients with liver cirrhosis and ascites without spontaneous bacterial peritonitis and group (C) included 11 patients with nephrotic syndrome and ascites.

Patients with pancreatitis, tuberculosis, peritoneal carcinomatosis and secondary peritoneal infection are excluded. Also patients with renal impairment are excluded from this study. All groups were subjected to the following investigations:

Complete blood picture, liver function (Bilirubin, total and direct, total proteins and albumin, AST, ALT, ALP, GGT), Serum Urea and creatinine, Ascitic fluid examination, Complement-3 level in ascitic fluid of group A, B and C and serum level in the four groups by ELISA technique (using Wkea Med Supplies Elisa kits, New York, USA).

Statistical analysis was performed using the SPSS 21.0 statistical software package. Continuous variables were expressed as means±SD. *p*>0.05 was considered statistically insignificant, *p*<0.05 was considered statistically significant while *p*<0.001 was considered statistically highly significant. Student *t*-tests were used to compare variables between patients groups.

**Results**

Table (1) demonstrates the sex distribution and mean±SD for age in all groups; while Table (2) illustrates the levels of AST, ALT, serum HA, ascetic HA and ascetic C3 for different groups.
The statistical analysis of these data shows that there was no significant statistical difference for age and sex in different groups. Also AST levels and ALT level showed no significant statistical difference between the four groups.

The ascitic fluid complement-3 (C3) level shows a significant decrease in group A in comparison to group C ($p<0.05$). Also, there was a significant decrease in C3 in group B in comparison to group C ($p<0.05$) Fig. (1).

There was a highly significant increase in serum HA in group A in comparison to group C and group D ($p<0.001$). Also, there was a highly significant increase in serum HA level in group B in comparison to group C and group D ($p<0.001$) Fig. (2).

Hyaluronic acid level in the ascitic fluid shows a highly significant decrease in group A (liver cirrhosis with ascites and SBP) in comparison to group C (nephrotic syndrome with ascites) ($p<0.001$). Also, there was a significant decrease in ascetic HA in group B (liver cirrhosis with ascites) in comparison to group C ($p<0.05$). Fig. (3).

There was a highly significant decrease in HA level in ascetic fluid of cirrhotic patients with SBP in comparison to HA level in ascetic fluid of cirrhotic patients without SBP ($p<0.001$).

Table (1): Demographic data of patients in all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>16</td>
<td>12</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>(Age in years) Mean±SD</td>
<td>43±9.13</td>
<td>45.75±3.5</td>
<td>34.27±5.34</td>
<td>45±11.0</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 males (75%)</td>
<td>7 males (58.3%)</td>
<td>7 males (63.6%)</td>
<td>6 males (66.6%)</td>
<td></td>
</tr>
<tr>
<td>4 females (25%)</td>
<td>5 females (41.7%)</td>
<td>4 females (36.4%)</td>
<td>3 females (33.4%)</td>
<td></td>
</tr>
</tbody>
</table>

Table (2): AST, ALT, serum HA, and Ascitic HA and C3 levels in all groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>Group D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST(U/L) Mean±SD</td>
<td>30±10</td>
<td>30.16±6</td>
<td>26±6.22</td>
<td>24.55±7.68</td>
</tr>
<tr>
<td>ALT (U/L) Mean±SD</td>
<td>39.5±5.80</td>
<td>38.33±0.5</td>
<td>36±3.54</td>
<td>39.88±5.89</td>
</tr>
<tr>
<td>Serum HA (ng/dl) Mean±SD</td>
<td>110.5±2.96</td>
<td>108.8±3.78</td>
<td>69.9±15</td>
<td>66.44±7.31</td>
</tr>
<tr>
<td>Ascitic HA (ng/dl) Mean±SD</td>
<td>16.94±1.32</td>
<td>38.05±0.98</td>
<td>41.73±0.79</td>
<td>–</td>
</tr>
<tr>
<td>Ascitic C3 (mg/dl) Mean±SD</td>
<td>25.36±6.40</td>
<td>37.36±3.67</td>
<td>52.02±1.08</td>
<td>–</td>
</tr>
</tbody>
</table>

Table (3): Significant difference between different groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>A Vs B</th>
<th>A Vs C</th>
<th>A Vs D</th>
<th>B Vs C</th>
<th>B Vs D</th>
<th>C Vs D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>ALT</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ascitic C3</td>
<td>NS</td>
<td>$p&lt;0.05$</td>
<td>–</td>
<td>$p&lt;0.05$</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Serum HA</td>
<td>NS</td>
<td>$p&lt;0.001$</td>
<td>NS</td>
<td>$p&lt;0.001$</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ascitic HA</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
<td>$p&lt;0.001$</td>
<td>NS</td>
<td>–</td>
</tr>
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</table>

– NS: Not significant.

Fig. (1): Complement 3 in ascitic fluid.

Fig. (2): Serum hyaluronic acid in different groups.

Fig. (3): Ascitic level of hyaluronic acid.
Discussion

Patients with severe acute or chronic liver disease are often deficient in complement and may also have malfunctioning of the neutrophilic and reticuloendothelial systems [18]. In these patients, the most frequent infectious complication that occurs (25% of the cases), and at the same time the most severe one is spontaneous bacterial peritonitis possibly due to three mechanisms; intestinal bacterial overgrowth, the alterations (structural and functional) of the intestinal mucosal barrier and the deficiencies of the local immune response [12]. In cirrhotic patient there is a local and systemic immune deficiencies, so bacteremia and ascitic fluid inoculation is a leading result. If the ascitic fluid complement level is low, this will determine a low bactericidal activity and thus a higher risk for SBP [14]. In our work we tested the complement-3 level in ascitic fluid as the first defense against the infiltrating gut organism (source of SBP). We found that the C3 level is significantly decreased in ascitic fluid of liver cirrhosis patient in comparison to ascites of other causes mainly Nephrotic syndrome patients. This may explain the high susceptibility of these patients to develop SBP. In patients with hepatic cirrhosis, because of intra- and extrahepatic shunts (due to portal hypertension), circulating bacteria do not come in contact with Kupffer cells (have a special role in preventing Infections), the result being bacteremia and high possibility of ascitic fluid inoculation of organism with development of SBP [14]. Hyaluronic acid is essential for the structure and organization of extracellular matrices since it forms a network interacting with proteins, receptors and cell surfaces. HA is synthesized at the plasma membrane and released directly into the extracellular environment [13]. This may contribute to the hydrated microenvironment at sites of synthesis, and is essential for cell migration by facilitating cell detachment. Most of HA are removed and terminally degraded by liver endothelial cells. A minor part (about 10%) is metabolized by the kidneys and the spleen. HA is closely associated with the cell migration process, and studies show that cell movement can be inhibited, at least partially, by HA degradation or blocking HA receptor occupancy [14]. In sepsis increased levels of hyaluronic acid were found to correlate with disease severity and prognosis with high values of hyaluronic acid associated with a poorer survival rate [12]. Our results show an increase in the mean value of serum hyaluronic acid in patients with ascites due to liver cirrhosis. The ascitic fluid level of hyaluronic acid shows a highly significant decrease in patients with ascites due to liver cirrhosis with or without spontaneous bacterial peritonitis (SBP) in comparison to ascitic patients due to nephrotic syndrome. From our results, and from the role of hyaluronic acid in inflammatory cell migration and activation and also its role in cytokine secretion, we conclude that the defense mechanisms are impaired in patients with liver cirrhosis even without SBP and patients with liver cirrhosis are more vulnerable to the dangerous attack of the gut organism (due to portosystemic shunt) and development of SBP with the bad prognostic results and rapid deterioration of cirrhotic patients. So, improvement of ascitic defense contents and even external supplementation of HA in those patients may impair the development of ascitic fluid infection and rapid deterioration of cirrhotic patients.

References

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