Functional changes of intestinal mucosal barrier in surgically critical patients

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BACKGROUND: The gut is capable of inducing multiple organ dysfunction syndrome (MODS). In the diagnosis and treatment of critical ill patients, doctors should pay particular attention to the protection or recovery of intestinal barrier function. However, no reliable diagnostic criteria are available clinically. This study aimed to assess the changes of intestinal mucosal barrier function in surgically critical ill patients as well as their significance.

METHODS: Thirty-eight surgically critical ill patients were enrolled as a study group (APACHE II>8 scores), and 15 non-critical ill patients without intestinal dysfunction were selected as a control group (APACHE II<6). General information, symptoms, physical signs, and APACHE II scores of the patients were recorded. The patients in the study group were subdivided into an intestinal dysfunction group (n=26) and a non-intestinal dysfunction group (n=12). Three ml venous blood was collected from the control group on admission and the same volume of plasma was collected from the study group both on admission and in the period of recovery. The plasma concentrations of endotoxin, diamine oxidase (DAO), D-lactate, and intestinal fatty-acid binding protein (iFABP) were detected respectively. The data collected were analyzed by the SPSS 17.0 software for Windows.

RESULTS: The levels of variables were significantly higher in the study group than in the control group (P<0.01). They were higher in the intestinal dysfunction group than in the non-intestinal dysfunction group (DAO P<0.05, endotoxin, D-lactate, iFABP P<0.01). In the non-intestinal dysfunction group compared with the control group, the level of endotoxin was not significant (P>0.05), but the levels of DAO, D-lactate and iFABP were statistically significant (P<0.05). The levels of variables in acute stage were higher than those in recovery stage (P<0.01). The death group showed higher levels of variables than the survival group (endotoxin and D-lactate P<0.01, DAO and iFABP P<0.05).

CONCLUSION: The plasma concentrations of endotoxin, DAO, D-lactate, and intestinal fatty-acid binding protein (iFABP) could reflect a better function of the intestinal mucosa barrier in surgically critical ill patients.

KEY WORDS: Intestinal mucosal barrier; Endotoxin; Diamine oxidase; D-lactate; Intestinal fatty-acid binding protein

INTRODUCTION

Studies have focused on the function of intestinal barrier. The gut is thought to induce multiple organ dysfunction syndrome (MODS). In the diagnosis and treatment of critical patients, the protection or recovery of the intestinal barrier function is extremely important. However, no reliable diagnostic criteria are available clinically. This study was undertaken to observe the changes of endotoxin, diamine oxidase (DAO), D-lactate and intestinal fatty-acid binding protein (iFABP) in the plasma of critical patients, which accordingly could determine gastrointestinal dysfunction of patients.
METHODS

Patients

This study was a randomized, controlled trial. From February 2009 to August 2009, 38 surgically critical patients (APACHE II scores > 8) without gastrorrhexis, enterorrhexis or gastrointestinal operation were recruited at the First Affiliated Hospital of Bengbu Medical College, Bengbu. They were admitted to the hospital within 24 hours after onset of the disease. Among them, 24 were male and 14 female; their median age was 51 years (range 19-86 years). Eleven patients had severe multiple injuries, 5 had alimentary tract hemorrhage, 6 had severe acute pancreatitis, 10 had gastrointestinal operation (operation for cerebral hemorrhage in 5 patients, hysterectomy in 3 patients, bone fracture reestablishment in 1 patient and amputation in 1 patient), and 6 had shock. In this series, 25 patients had the condition improved and 13 patients died. The patients were divided into two groups: an intestinal dysfunction group (26 patients) and a nonintestinal dysfunction group (12 patients) according to the criteria set by the National Symposium on Intestinal Barrier Function in China in 2006. At the same time, 15 non-critical patients undergoing selective operation, 9 males and 6 females, aged on average 45 years (range 23-65 years), were enrolled in the control group. Age and sex distribution were not significantly different among the groups (P>0.05).

Collection and conservation of specimens

Three ml blood was collected from the control group on admission (acute stage), and the same volume of plasma was collected from the study group both on admission and in the period of recovery. The period of recovery was defined as the time when symptoms, physical signs and laboratory results were improved in addition to APACHE II score<6 and discharge from ICU. Strict aseptic conditions were subsequently ensured in plasma collection. Blood was kept in the desiccated anticoagulant test tube, and centrifuged for 10 minutes at 2000 r/min. The plasma sample was stored at -80 °C until further test.

Detection of laboratory parameters

The concentration of diamine oxidase (DAO) in the plasma was detected by absorption spectrometry introduced by Jun-you Li. D-lactate test was performed with O-dianisidine according to the Murray method. The intestinal fatty-acid binding protein (iFABP) test used enzyme linked immunosorbent assay (ELISA) in accordance with ELISA package's description. Endotoxin test was performed with dynamic turbidity assay. DAO standard, D-lactate standard, lactic dehydrogenase (LDH) and O-dianisidine were bought from Sigma, but ELISA package was purchased from R & D.

Statistical analysis

The data were expressed as mean±SD. Univariate analysis was made between groups where necessary, the chi-square test and Fisher's exact test were used. Statistical significance was defined as P<0.05. Data analysis was performed using SPSS 17.0 for Windows.

RESULTS

Analysis of parameters

The levels of endotoxin, D-lactate, DAO, and iFABP were higher in the intestinal dysfunction group than those in the nonintestinal dysfunction group (P<0.05). The levels of D-lactate, DAO, and iFABP were higher in the intestinal dysfunction group than in the control group, but there were no differences in endotoxin between the groups (Table 1).

Parameters on admission or in the period of recovery

The levels of endotoxin, D-lactate, DAO, and iFABP were higher on admission than in the period of recovery (P<0.01) (Table 2).

Parameters of the death group and survival group

The levels of endotoxin, D-lactate, DAO, and iFABP were higher in the death group and the survival group (P<0.05) (Table 3).

Table 1. Comparison of the parameters between the study group and control group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Endotoxin (pg/ml)</th>
<th>D-lactate (mg/l)</th>
<th>DAO (kU/L)</th>
<th>iFABP (μmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestinal dysfunction</td>
<td>26</td>
<td>9.94±8.92 &amp;</td>
<td>22.90±8.44 &amp;</td>
<td>18.40±11.46 &amp;</td>
<td>144.65±59.84 &amp;</td>
</tr>
<tr>
<td>Nonintestinal dysfunction</td>
<td>12</td>
<td>2.10±0.42</td>
<td>13.88±9.26</td>
<td>9.70±5.33</td>
<td>84.77±69.12</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>1.00±0.00</td>
<td>6.04±3.79</td>
<td>3.72±2.02</td>
<td>48.88±27.25</td>
</tr>
<tr>
<td>F</td>
<td></td>
<td>7.895</td>
<td>10.101</td>
<td>4.738</td>
<td>8.581</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Compared with the control group, *P<0.01; compared with the nonintestinal group, &P<0.01; *P<0.05; compared with the control group, *P<0.05.
DISCUSSION

The gut is easily damaged when the human body is in ischemic or anoxic conditions. The damage including trauma, hemorrhage, stress, operative injury, infection, shock, etc could make intestinal mucosa ischemia and hypoxia. With an increased intestinal permeability, bacterial translocation and even MODS may occur. With ischemia of the intestinal mucosa, dysbacteria are induced, and gram-negative bacteria in the gut grow. Then endotoxin is released increasingly, resulting in damage to the body and MODS. In this study, the levels of plasma endotoxin, D-lactate, DAO, iFABP were significantly higher in patients with intestinal dysfunction than in those without intestinal dysfunction and the controls. But the endotoxin level was not significantly higher in the patients without intestinal malfunction than in the controls. The accurate rate of the MB-80 microorganism detecting system was 1 pg/ml, and in the control group it was <1 pg/ml, showing that the rate of 1 pg/ml could influence the statistical results. D-lactate is a metabolic product of multiple bacteria in the intestine. Under normal conditions, D-lactate can not be absorbed or metabolized by the mammalian as an enzymatic system, but it can be released by the bacteria entering blood via the damaged mucosa. DAO as a high activity enzyme is seen in cells of the intestinal mucosa. When the intestine is ischemic with mucosal necrosis DAO enters cell spaces, lymphatic or blood vessels, resulting in an increased DAO level. If it is not released into blood vessels, Thus the increased levels of plasma endotoxin, D-lactate, DAO, and iFABP indicate the damage of the intestinal mucosa.

In this study the levels of plasma endotoxin, D-lactate, DAO, and iFABP were significantly lower in the recovery stage than in the acute stage (P<0.01), which were consistent with the results of other studies, for instance the changes of sepsis and endotoxin in hemorrhagic shock patients and the changes of iFABP and D-lactate in ischemia reperfusion rats. Moreover, the levels of variables were higher in the death group than in the survival group (endotoxin, D-lactate P<0.01; DAO, iFABP P<0.05), indicating that the damage of the intestinal mucosa barrier can reflect the prognosis of patients. Each patient in our study had damage of the intestinal mucosa barrier in different degrees and their levels of plasma endotoxin, DAO, D-lactate, iFABP were closely related among the patients, especially in those patients with intestinal dysfunction. Though the patients without intestinal malfunction did not show any clinical symptoms and signs, the intestinal damage was possibly insidious. The four variables can be used to detect functional changes of the intestinal mucosa barrier. At the same time, the levels of variables in the recovery stage decreased to normal with the improvement of intestinal ischemia. But inversely, the levels increased in the death group, indicating that the damage degree is closely correlated to the patients' prognosis.

In conclusion, monitoring of plasma endotoxin, DAO, D-lactate, and iFABP is helpful to evaluate the damage of the intestinal mucosa barrier, and possibly to make a judgement for patients' prognosis. Therefore, it is important for clinical doctors to early cure the surgically critical ill patients with intestinal dysfunction.

Table 2. The parameters on admission and recovery stage

<table>
<thead>
<tr>
<th>Time</th>
<th>Number of cases</th>
<th>Endotoxin (pg/ml)</th>
<th>D-lactate (mg/l)</th>
<th>DAO (mg/ml)</th>
<th>iFABP (μmol/ml)</th>
</tr>
</thead>
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<tr>
<td>At admission</td>
<td>25</td>
<td>7.52±4.42</td>
<td>25.22±5.39</td>
<td>16.54±10.30</td>
<td>154.10±56.60</td>
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<tr>
<td>Recovery period</td>
<td>25</td>
<td>3.93±2.98</td>
<td>12.66±6.23</td>
<td>12.52±5.07</td>
<td>77.60±59.63</td>
</tr>
<tr>
<td>( t )</td>
<td>3.025</td>
<td>5.061</td>
<td>4.429</td>
<td>6.163</td>
<td></td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. The parameters in the death group and survival group

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of cases</th>
<th>Endotoxin (pg/ml)</th>
<th>D-lactate (mg/l)</th>
<th>DAO (mg/ml)</th>
<th>iFABP (μmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death group</td>
<td>13</td>
<td>10.37±3.80</td>
<td>25.55±5.76</td>
<td>22.93±8.40</td>
<td>193.37±72.41</td>
</tr>
<tr>
<td>Survival group</td>
<td>25</td>
<td>4.50±2.58</td>
<td>18.55±6.38</td>
<td>15.63±5.29</td>
<td>140.36±43.68</td>
</tr>
<tr>
<td>( t )</td>
<td>10.854</td>
<td>10.214</td>
<td>4.651</td>
<td>6.921</td>
<td></td>
</tr>
<tr>
<td>( P )</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
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</table>

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