Analysis of natural history of the diaphragmatic injury on the right in mice

Análise da evolução natural dos ferimentos diafragmáticos à direita em ratos

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ABSTRACT

Objective: to evaluate natural evolution of right diaphragmatic injury after the surgical removal of a portion from hemi diaphragm. Methods: the animals were submitted to a surgical removal of portion from right hemi diaphragm by median laparotomy. The sample consists of 42 animals being 2 animals from pilot project and 40 operated animals. And the variables of the study were herniation, liver protection, healing, persistent diaphragm injury, evaluation of 16 channels tomography and the variables “heart rate” and “weight”. Results: we analyzed 40 mice, we had two post-operative deaths; we had 17 animals in this group suffered from herniation (42.5%) and 23 animals didn’t suffer from herniation (57.5%). Analyzing the tomography as image method in the evaluation of diaphragmatic hernia, we had as a method with good sensitivity (78.6%), good specificity (90.9%), and good accuracy (86.1%) when compared to necropsy. Conclusion: there was a predominance of healing of right hemi diaphragm, the size of initial injury didn’t have influence on occurrence of the liver protection or hernia in mice.


INTRODUCTION

Isolated diaphragmatic injuries are a major challenge for physicians working in the field of traumatology. They may go unnoticed both by the uncertainty of their evolution and by the acute or chronic clinical repercussions. The leading cause of diaphragmatic lesions are the penetrating injuries in the thoracoabdominal transition zone (TATZ), limited anteriorly by the fourth intercostal space, laterally by the sixth intercostal space, posteriorly by the tip of the scapula and inferiorly by the epigastric region and the costal margin. Such penetrating injuries to the TATZ can display up to 48% of diaphragmatic injuries. The most affected organs in diaphragmatic injuries are: lung, stomach, liver, spleen and colon, the left hemidiaphragm being the most affected both in penetrating and blunt trauma; stabbing wounds are the most frequent, and, in most cases, have less than two centimeters. Even with a substantial number of publications showing the high incidence of diaphragmatic injuries in TATZ trauma, this incidence is still unclear, given that a large numbers of these lesions still goes unnoticed.

Symptoms of patients with diaphragmatic injuries can range from referred pain in the shoulder, decreased chest expansion, bowel sounds on auscultation, dug abdomen or even no symptoms, and patients may progress from asymptomatic in and isolated small injuries to circulatory collapse, with severe respiratory failure, resembling tension pneumothorax. The most common symptoms are related to the digestive and respiratory systems, however, it is estimated that 25% of these patients may go asymptomatic.

For the diagnosis of a diaphragmatic injury, the main tools are the clinical examination and the imaging studies. A simple chest X-ray often shows nonspecific signs, such as hemothorax, pneumothorax, elevated hemidiaphragm and even a simple opacity in the costophrenic angle and, often, these changes may persist after thoracic drainage. Examinations with contrast media may render diagnoses when there is herniation of abdominal structures into the chest, but most of the injuries that affect the diaphragm are small, resulting in a low rate of herniations.

The use of computed tomography with three-dimensional reconstruction decreases the chances of a false negative. There are reports showing failure in diagnosing diaphragm lesions with the use of conventional tomography. Magnetic resonance imaging has shown promising results when evaluating diaphragmatic lesions in chest and abdomen, injuries but it is still a method with little use, is not yet available in most trauma services, is costly and requires a long time for its realization.

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Even when using all the above cited diagnostic tools along with a clinical exam, still we cannot achieve a good sensitivity and specificity of these tests for diaphragmatic injury, especially when faced with diaphragmatic injuries without hernia and with the patient stable. Healing is supposed to take place in parts of these injuries, many of whom, non-operated, showed good recovery without herniation.

Nowadays, there is still no consensus in the treatment of diaphragmatic injuries in stable patients, and in most emergency departments we did not find thoracoscopy as a test for routine use. We then decided to evaluate the natural evolution of diaphragmatic injuries after resection of a portion of the right diaphragm.

**METHODS**

We used 40 Rattus norvegicus albinus rats, known as the albino Wistar strain, with about 90 days of life (250g). They were kept in collective cages for ten days, with a population density of five animals per cage, with free and unrestricted access to water and food given to rodents, and light / dark cycle of 12 hours. The research project was submitted to the Ethics in Animal Experimentation Commission the of Dr. Paulo Prata Health Sciences School of Barretos (FACISB), which issued a favorable opinion under protocol 009/2013.

The identification of each animal was performed by numbered hidrocolor fountain on the tail and ear. After performing the surgical procedure, the animals were housed in individual cages. During the postoperative period of 30 days, we measured the heart rate and weight on days 0, 5, 10, 15, 20, 25 and 30. The heart rate was measured with the aid of a stethoscope. Weight was measured with a SF-400 CE precision balance. After this period, the animals underwent a CT scan of the chest and, soon after, euthanasia through a laparotomy associated with a double thoracotomy. During the autopsy we evaluated: 1) diaphragmatic hernia; 2) Liver protection; 3) diaphragmatic healing; and 4) persistent diaphragmatic injury without hernia or protection. Subsequently, we compared the occurrence of hernia or protection in the chest tomography with microscopy, checking whether the initial size of the lesion and the local inflammatory reaction (size of fibrosis and thickness of the membrane) influenced the occurrence of hernia or protection in the diaphragmatic injury.

Under general anesthesia, we performed a laparotomy and dissection of the right diaphragm in two Wistar rats of approximately 250g weight. The right diaphragm was withdrawn from one rat for the calculation of its total area, and from the other, to define the the portion of the right hemidiaphragm to be removed. The size of the lesion and the calculation of the right diaphragm area were measured using a computer graphics program (AutoCAD® by Autodesk®, version 2006), using integral calculus. We could not standardize the withdrawal of exactly 10% of the diaphragm due to difficulties of finding a surgical instrument able to extract a proper portion of the assessed hemidiaphragm and to anatomical and physiological conditions.

We performed the anesthesia to animals subjected to fasting ten hours before the procedure. The drugs chosen were ketamine hydrochloride at a dose of 50mg/kg intraperitoneally, and 2% xylazine 5mg/kg, intraperitoneally. The animals were ventilated with a continuous positive airway pressure (CPAP) mask fitted with a rubber tube at pressure 1.5Kgf/cm² and positive end-expiratory pressure (PEEP) 5mm of water, by an oxygen concentrator.

Once the anesthesia ensued and after abdominal trichotomy, they underwent laparotomy measuring 3cm in length, starting under the xiphoid process for viewing and incision of the right diaphragm with a scalpel (blade 11) to carry out a ipsilateral pneumothorax and cause pulmonary collapse, thus avoiding lung injury when performing the diaphragmatic injury. We performed resection of a portion of the right diaphragm on 40 animals, between the costal insertion and the central tendon of the diaphragm, without including it in the place where we had held the pneumothorax (Figure 1). There was a variation of 5.18% to 20% in the size of the resection portion withdrawn. Upon completion of the injury, we placed an open anatomical clamp next to the injury and photographed for later calculation of lesion size by the computer graphics software “AutoCAD”, since we already had knowledge of the size of the right hemidiaphragm. After the procedure and before the laparotomy closure, we held a lung hyperinflation followed by Valsalva maneuver to empty the pneumothorax and prevent atelectasis in the immediate postoperative period.

All animals were assisted till the end of anesthetic procedure and hydrated after surgery by applying 5ml of...
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We operated 40 rats. There were two deaths, with operative mortality of 5%. The deaths occurred on the tenth and 11th days postoperatively, the two animals had hernia and both were included in the study.

The result of the autopsy, according to the distribution of the evolution pattern of the lesion, showed liver protection in 50% of animals; there was herniation of abdominal structures, predominantly the liver followed by the omentum, large intestine, small intestine and spleen in 42.5% of animals and healing occurred in 7.5% (Table 1). When we compared the variables protection and hernia with the size of the initial lesion, which varied from 5.18% to 20% of right diaphragm removed, we found no statistically significant difference between them (Table 2).

When comparing the results of tomography and the microscopic analysis of the material in relation to what we consider the gold standard (necropsy), we noted that both microscopy and chest computed tomography showed good sensitivity (hernia), good specificity (protection) in evaluation of diaphragmatic hernia (Tables 3 and 4). Considering the reproducibility between the microscopy and tomography with the autopsy, we found acceptable Kappa coefficients. For microscopy versus autopsy it was 0.734 (p < 0.001), while for computerized tomography, 0.704 (p < 0.001).

This heart rate evaluation showed a statistical significance with respect to time, all animals displaying decreased heart rate over the days postoperatively (p < 0.001), but there was no statistical significance between protection and hernia groups (p = 0.250). Regarding the variable weight, we noted that there were significant differences over time (p < 0.001), all gaining weight during the postoperative period; and when comparing rats with protection and hernia, those with protection had more weight gain (p = 0.039) than those who had hernia.
In assessing the size of the initial lesion (macroscopic), the thickness of the diaphragm membrane at the injury site and the size of fibrosis at the site of injury (microscopic) in relation to the variables hernia and protection (microscopy), we noted no statistical significance that might explain the occurrence of hernia or protection (Table 5). When we evaluated the occurrence of persistent diaphragm injury without protection or hernia in both microscopy and in macroscopic, we found no cases of such injury in the animals studied.

**DISCUSSION**

When the diagnosis is made and the complications of diaphragmatic injury are repaired in the acute phase, there is a low morbidity and mortality. However, the results are different when it comes to a complication of diaphragmatic lesion with months or years of evolution. There are rates of approximately 48% mortality and 30% morbidity when treatment is started too late, reaching 80% of morbidity and mortality.

The reason that led some authors to reveal the possibility of spontaneous healing of diaphragmatic injuries was that, in clinical practice, only a relatively small number of patients with penetrating TATZ injuries developed hernia during clinical monitoring.

The pressure gradient between the pleural and peritoneal cavities, the constant movement of the diaphragm during the respiratory cycle and the radial tension between the center and its parietal tendon insertions are cited as factors that prevent diaphragmatic healing after trauma.

In a study with a porcine model using laparotomy, diaphragmatic injuries of 1.5cm to 2cm in length located at the central tendon of a hemidiaphragm and muscle of the contralateral hemidiaphragm, with pleural drainage for emptying of pneumothorax, using general anesthesia and sacrificing the animals after six weeks, they concluded that most stabbing injuries, regardless of the location, the central tendon or muscle part, heal spontaneously, not justifying the evaluation of diaphragm conditions in all patients with injuries in the thoracoabdominal transition area.

Evaluating existing studies in the literature, we found that healing is the rule for perforating injuries to the...
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In our experimental model, we sought to create a condition of healing difficulty when we removed a portion of the right hemidiaphragm, what none of the cited studies did, hampering the approach of the damaged edges, which would be conducive to wound healing. We were careful to specify the exact extent of the injury we carried out, which was located between the central tendon of the right hemidiaphragm and its insertion in the lateral thoracic wall, causing in this way an injury in one of the phrenic nerve branches, a fact which further corroborated the poor healing of the injury caused. We found, after reviewing the literature, that most works did not detail the site of injury, but one article which detailed and assessed the extent of the injury in the two main regions of the diaphragm (the central tendon and muscle part).

Regarding the anesthetic technique, the drugs chosen for the anesthesia were ketamine hydrochloride and xylazine to cause good analgesia, not having hypotensive action on the cardiovascular system and hardly causing respiratory depression. In this way, we did not establish a definitive airway and only held a ventilatory assistance by a rubber with intermittent pressure of 5 mm water, adapted as a CPAP, getting a good lung expansion during the procedure and a good evacuation of the pneumothorax, with no need of pleural drainage during the closure of the abdominal cavity.

We chose the median abdominal incision, approximately 3 cm in length from the xiphoid appendix, as a gateway to the right hemidiaphragm. Although there are reports of diaphragmatic dysfunction associated with laparotomy, we did not observe this fact, nor did other authors who used models similar to ours. This might be explained by the rapidity of the procedure and also by the use of CPAP, as we found no respiratory fatigue of the animals after the end of anesthesia.

The determination of 30 days for observation time was based on a work by Perlingeiro et al., showing no statistical significance between the dead animals in the period 11th to 60th day with the 120th to 150th days.

### Table 4 - Evaluation of sensitivity and specificity of Microscope and multidetector chest tomography in the evaluation of diaphragmatic Hernia.

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microscopy</td>
<td>100.0%</td>
<td>75.0%</td>
<td>86.5%</td>
</tr>
<tr>
<td>95% CI</td>
<td>[81.57%; 100.0%]</td>
<td>[53.1%; 88.8%]</td>
<td>[72.0%; 94.1%]</td>
</tr>
<tr>
<td>Tomography</td>
<td>78.6%</td>
<td>90.9%</td>
<td>86.10%</td>
</tr>
<tr>
<td>95% CI</td>
<td>[52.4%; 92.4%]</td>
<td>[72.2%; 97.5%]</td>
<td>[71.3%; 93.9%]</td>
</tr>
</tbody>
</table>

### Table 5 - Comparison of the initial injury size with the microscopic variables: 1) Thickness of the injury membrane; 2) Size of the fibrosis on the injury site.

<table>
<thead>
<tr>
<th>Variable Group</th>
<th>n</th>
<th>Average</th>
<th>Standard Deviation</th>
<th>Minimum</th>
<th>1st Quartile</th>
<th>Median</th>
<th>3rd Quartile</th>
<th>Maximum</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size of the initial diaphragmatic injury</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protection</td>
<td>15</td>
<td>10.11</td>
<td>3.73</td>
<td>5.76</td>
<td>6.76</td>
<td>9.41</td>
<td>12.62</td>
<td>19.37</td>
<td>0.866</td>
</tr>
<tr>
<td>Hernia</td>
<td>22</td>
<td>9.89</td>
<td>3.95</td>
<td>5.18</td>
<td>6.76</td>
<td>8.50</td>
<td>11.79</td>
<td>20.00</td>
<td></td>
</tr>
<tr>
<td>Membrane thickness in mm (Microscopy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protection</td>
<td>15</td>
<td>0.25</td>
<td>0.32</td>
<td>0.01</td>
<td>0.08</td>
<td>0.11</td>
<td>0.20</td>
<td>1.00</td>
<td>0.379</td>
</tr>
<tr>
<td>Hernia</td>
<td>22</td>
<td>0.16</td>
<td>0.32</td>
<td>0.00</td>
<td>0.03</td>
<td>0.05</td>
<td>0.10</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Fibrosis size in mm (Microscopy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protection</td>
<td>15</td>
<td>0.61</td>
<td>0.41</td>
<td>0.15</td>
<td>0.30</td>
<td>0.45</td>
<td>0.90</td>
<td>1.40</td>
<td>0.645</td>
</tr>
<tr>
<td>Hernia</td>
<td>22</td>
<td>0.70</td>
<td>0.68</td>
<td>0.20</td>
<td>0.30</td>
<td>0.48</td>
<td>0.82</td>
<td>3.30</td>
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</table>
progressive herniation of abdominal organs into the chest. In our study with 40 animals, there were two deaths with great herniation of abdominal organs into the chest cavity, there was protection of the injury in 50% of animals, complete healing of the diaphragm in 7.5%, and even a possible healing with muscles filling the wound. We found no cases of persistent injury without protection or herniated diaphragm. It was not possible in this study to determine why some animals evolve with hernia and others do not, since we showed that the lesion size was not statistically significant as for the occurrence of diaphragmatic hernia or protection. Many questions are still to be answered. It is still difficult to understand the natural evolution of perforating injuries to the thoracoabdominal transition zone.

In conclusion, there was a predominance of healing of the right hemidiaphragm injury, yet the size of the initial injury did not influence the occurrence of liver protection or hernia.

RESUMO

Objetivo: avaliar a evolução natural do ferimento diafragmático à direita após a retirada cirúrgica de uma porção do hemidiafragma. Métodos: os animais foram submetidos a ressecção de uma porção do hemidiafragma à direita através da laparotomia mediana. Foram operados 40 ratos. As variáveis analisadas foram: hemiação, proteção hepática, cicatrização, lesão persistente do diafragma, avaliação da tomografia computadorizada, frequência cardíaca e peso. Resultados: foram analisados 40 ratos. Houve duas mortes no pós-operatório. Dezessete animais tiveram hemiação (42,5%) e 23 (57,5%), não. Analisando emprego da tomografia computadorizada na avaliação da hérnia diafragmática, tivemos um método com boa sensibilidade (78,6%), boa especificidade (90,9%) e boa acurácia (86,1%) quando comparados com a necrópsia. Conclusão: houve predominio de cicatrização do hemidiafragma à direita e o tamanho da lesão inicial não influenciou na ocorrência de proteção hepática ou hérnia em ratos.


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