

## Effects of plastic stenting in common bile duct of rats. A quantitative reaction analysis using collagen and elastin morphometry<sup>1</sup>

Efeitos de prótese plástica em hepatocolédoco de ratos. Uma análise quantitativa utilizando morfometria do colágeno e elastina

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### ABSTRACT

**Purpose:** To evaluate the effects of biliary stenting in rats through analysis of collagen and elastin deposition in the bile ducts.

**Methods:** Twenty male rats underwent midline laparotomy, duodenotomy and transampullary stenting of the common bile duct with a 22Fr plastic stent. Animals were randomized in 4 groups, with 5 components in each: (I) control, (II) biliary stenting for 7 days, (III) biliary stenting for 14 days, and (IV) biliary stenting for 30 days. Sections of the common bile duct were stained using Hematoxylin Eosin; Sirius Red to quantitate the amount of collagen present; and Weigert's Resorcin-fuchsin to quantitate the amount of elastin present. **Results:** The percentage of area stained for collagen was 13.4; 21.5; 29.5 and 32.8, for groups I to IV, respectively. The percentage of area stained for elastin fibers was 7.0; 5.2; 4.0 and 2.9, for groups I to IV, respectively. Collagen/Elastin ratio was 2.4; 5.1; 11.0 and 14.4 for groups I to IV, respectively. **Conclusions:** The biliary stenting leads to collagen and elastin deposition in the bile ducts; and collagen deposition and collagen/elastin ratio are proportional to the period of stenting.

**Key words:** Prostheses and Implants. Collagen. Elastin. Common Bile Duct. Rats.

### RESUMO

**Objetivo:** Avaliar os efeitos da colocação de prótese biliar em ratos, analisando a deposição de colágeno e elastina no hepatocolédoco.

**Métodos:** Vinte ratos machos foram submetidos à laparotomia mediana, duodenotomia e colocação transpapilar de uma prótese plástica 22Fr no hepatocolédoco. Os animais foram randomizados em 4 grupos, com cinco componentes cada: (I) controle, (II) prótese biliar por 7 dias, (III) prótese biliar durante 14 dias e (IV) prótese biliar por 30 dias. Cortes do hepatocolédoco foram corados com Hematoxilina Eosina; Sirius Red para quantificar o colágeno; e Resorcina-fucsina de Weigert para quantificar a elastina. **Resultados:** A porcentagem de área corada para colágeno foi de 13,4; 21,5; 29,5 e 32,8 para os grupos I a IV, respectivamente. A porcentagem de área corada para fibras de elastina foi de 7,0; 5,2; 4,0 e 2,9, para os grupos I a IV, respectivamente. A razão colágeno / elastina foi de 2,4, 5,1, 11,0 e 14,4 para os grupos I a IV, respectivamente. **Conclusões:** O implante da prótese biliar leva à deposição de colágeno e elastina nos ductos biliares; e a deposição de colágeno e a relação colágeno / elastina são proporcionais ao tempo de permanência da prótese.

**Descritores:** Próteses e Implantes. Colágeno. Elastina. Ducto Colédoco. Ratos.

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### Introduction

Bile duct stenting is a common procedure for drainage of biliary obstructions due to malignant or benign causes. Surgically placed biliary drains predate the earliest report of endoscopically inserted biliary drains in 1989<sup>1</sup>. Little is known about the short and long term effects of such prosthesis on bile ducts and adjacent

organs. Changes related to such stents may be of relevance especially when biliary stents are placed for benign and potentially curable conditions. Karsten *et al.*<sup>2</sup> reported increased bacterial colonization, fibrosis and reactive hyperplasia in extrahepatic bile ducts of dogs in contact with the plastic stent system. In 2000, Dunkin *et al.*<sup>3</sup> performed histological analysis of liver and pancreatic tissue, and noted chronic inflammatory changes in the

bile ducts system, but none in the liver or pancreas. Waghlikar *et al.*<sup>4</sup> have previously reported significant morphological and fibroproliferative inflammatory changes at pancreaticoduodenectomy in bile ducts of 31 patients who had previously undergone bile duct stenting, making surgical excision something more difficult. Others studies have also reported that these stents lead to difficulty in surgical dissection due the local tissue reaction<sup>5,6</sup>.

This study aims to evaluate the effects of biliary stenting in rats through analysis of collagen and elastin deposition in the bile ducts, and the macroscopic and microscopic changes in the pancreatic and hepatic parenchyma.

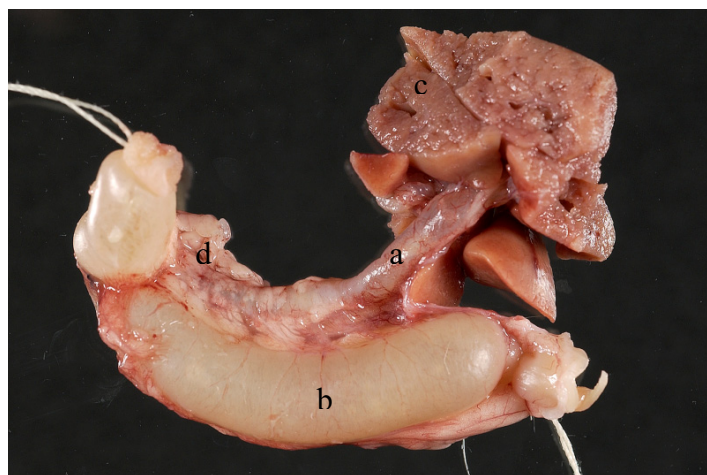
## Methods

Twenty Wistar male rats, weighing between 280 to 320g, were randomized in 4 groups: (I) control (n=5), (II) biliary stenting for 7 days (n=5), (III) biliary stenting for 14 days (n=5), and (IV) biliary stenting for 30 days (n=5).

Animals were anesthetized with intraperitoneal ketamine chloridrate 10% (100ml/Kg) and xylazine 2% (100ml/Kg). All groups underwent a midline laparotomy, duodenotomy and transampullary stenting of the common bile duct with a 22F plastic stent. Blood loss and trauma to the viscera were kept to a minimum. Principles of antisepsis were respected during the procedure.

In group I, the stent was immediately removed, acting as control. In groups II, III and IV, the stent was removed 7, 14 and 30 days after the procedure, respectively.

At the designated time, animals were euthanatized and en-bloc resection of the liver, bile ducts, pancreas and duodenum performed (Figure 1). The bloc was fixed in 10% formalin.



**FIGURE 1** - Image block formalized surgical specimen obtained where we observe the dilated bile duct and plastic stent inside (a), duodenum (b), liver (c) and pancreas (d).

### Macroscopic changes

Adhesions between surrounding viscera and the biliary system were recorded.

### Microscopic analysis

Sections of the common bile duct were properly processed for hematoxylin and eosin stain, and subsequently evaluated by an

experienced pathologist. Sirius red staining was performed to quantitate the amount of collagen present and Weigert's Resorcin-fuchsin staining was performed to quantitate the amount of elastin present. Computerized digital analysis was used to quantify the area of staining in each slide. Digital images (using Nikon DXM1200F a digital video camera attached to the Nikon Eclipse E600 microscope) were obtained of each histological section, at 20x magnification for collagen analysis and 40x for elastin analysis. Digitized images were processed using Nikon ACT-1 (version 2.62 2000) and Adobe Photoshop 7.0. Quantitative analysis was done by Image J Software, developed by National Institute of Health.

### Statistics

The data were arranged in the SPSS statistical system and values were expressed as percentage  $\pm$  standard deviation [95% confidence interval]. Tukey test was used for comparison among groups. A value of  $p < 0.05$  was considered significant.

### Ethics

The study was approved by the local ethical committee. All procedures followed the regulations of the Brazilian College for Animal Experimentation and the US National Institute of Health-Guide for care and use of laboratory animals guidelines.

## Results

### Macroscopic analysis

Macroscopic adhesions between surrounding viscera and the biliary system were present in 2 rats: 1 in group III and 1 in group IV and it was seen in the area of the duodenotomy to place the transpapillary plastic stent. It was not demonstrated adhesences between the adjacent visceras and common bile duct.

### Microscopic analysis

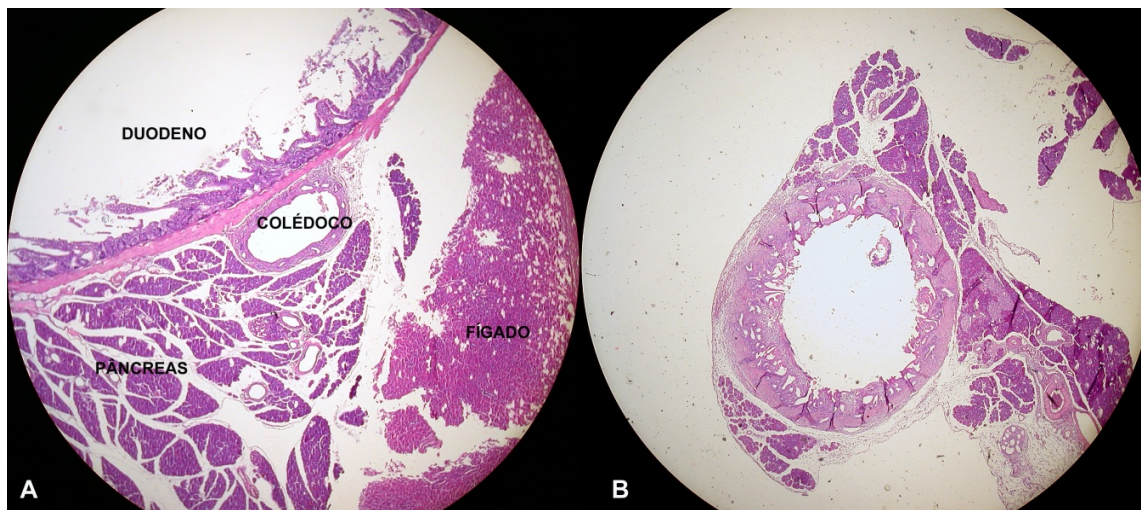
#### Histopathologic

In Group I there were solitary ulcers in the bile duct epithelium but no inflammatory cells. In Group II solitary superficial ulcerations were present mainly at the the middle portion of the bile duct. The submucosal layer showed slight thickening due vascular congestion and perivascular edema, presence of mast cells, eosinophils and elongated cells with round nucleus dispersed. In Group III there was extensive desquamation of the epithelium with re-epithelization in some areas and the bile duct wall was approximately 3 fold thickened. There was flattening of the epithelium with intense focal epithelial reactivity with increase in stratified layers. There was an intermediate increase in inflammatory cells with moderate number of fusiform appearing cells, intense mitotic activity with pyknotic nuclei. There was mild edema and formation of saculos de Beale. In Group IV, epithelium of difficult characterization, presenting areas of reactivity with purely cuboid cells and round nuclei. The bile duct wall was thickened around 3-4 times that normal. Large quantity of fusiform cells with small nucleus in the submucosa. Minimal inflammatory cells, with

predominance of lymphocytes and macrophages. Focal intestinal metaplasia in some saculos de Beale (presence of calciforms cells). Absence of submucosal edema.

Group I did not reveal any abnormalities in the liver. In the others groups there was the presence of inflammatory infiltrate

comprising of eosinophils, macrophages and lymphocytes, compatible with mild cholangitis along with focal dilation of intra-hepatic ducts. These results increased in mild and gradual in group II to IV. There was absence of any histopathological changes in pancreas (Figures 2 and 3).



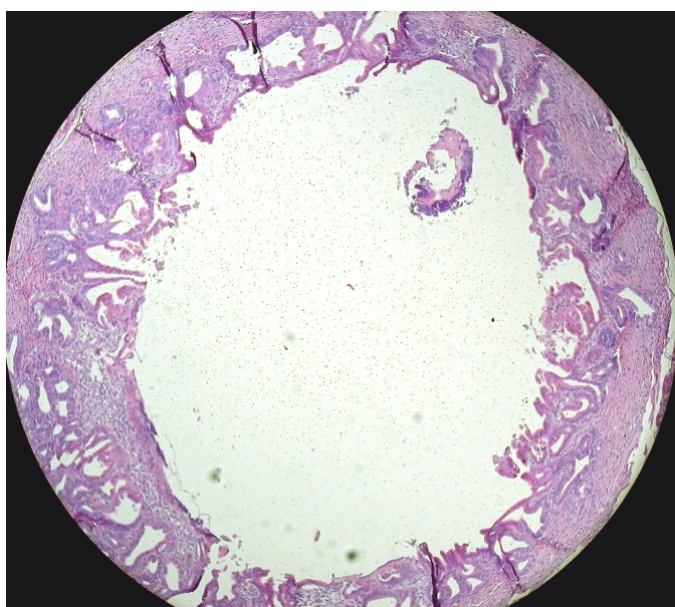
**FIGURE 2** – A. Microscopic large increase of common bile duct, pancreas and liver of control rat. B. Microscopic image with a small increase in common bile duct after the use of plastic biliary stent, highlighting the findings of wall thickening, presence of inflammatory cells and fibrocytes

*Collagen and elastic fiber the ratio of collagen fiber/ elastin deposition*

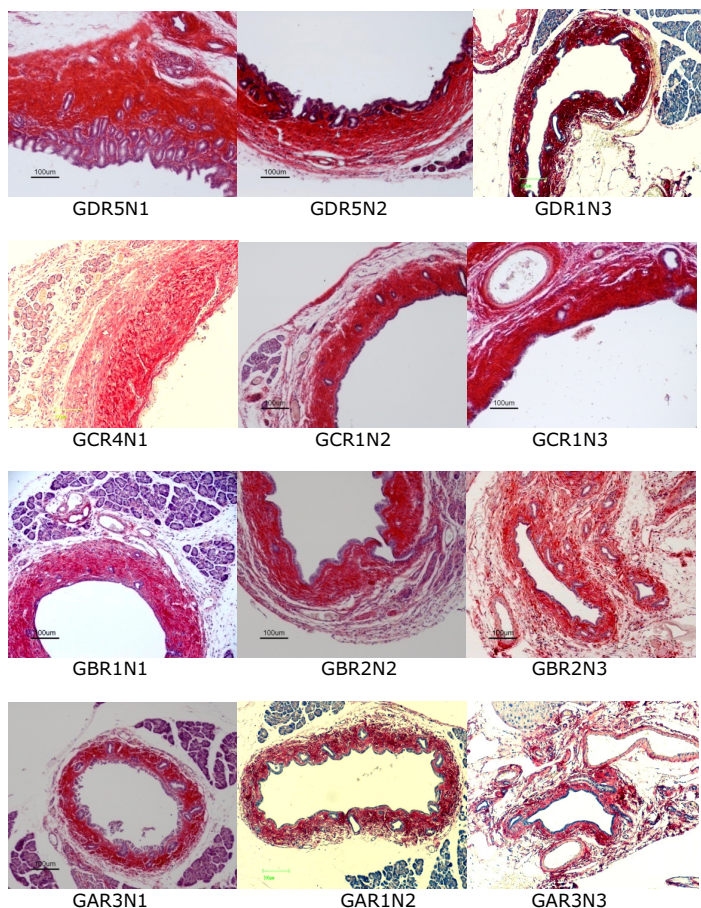
There was no difference in the amount of collagen deposition or elastic fiber in the 4 groups of rats. There was increasingly greater amount of collagen deposition in groups I, II and III, being similar in group III and IV. There was no significant difference in the elastin fiber in the 4 groups. The ratio of collagen/elastic fiber, between different groups showed values significantly higher in group IV when compared with control (group I) and group II.

The percentage of area stained for collagen was  $13.4 \pm 5.2$  [10.5-16.3];  $21.5 \pm 6.8$  [17.7-25.3];  $29.5 \pm 9.0$  [24.6-34.5] and  $32.8 \pm 6.1$  [29.4-36.2], for groups I to IV, respectively. Collagen staining was higher for group II compared to group I ( $p=0.014$ ) and higher for group III compared to group II ( $p=0.015$ ). There was no difference in collagen staining between group IV and III ( $p=0.585$ ) (Figure 4).

The percentage of area stained for elastin fibers was  $7.0 \pm 3.1$  [5.3-8.7];  $5.2 \pm 2.4$  [3.9-6.5];  $4.0 \pm 2.3$  [2.7-5.2]; and  $2.9 \pm 1.4$  [2.1-3.6], for groups I to IV, respectively. Elastin staining was higher for group III compared to group I ( $p=0.008$ ); IV compared to I ( $p<0,001$ ), and IV compared to III ( $p=0.05$ ). There was no difference in elastin staining between groups I and II ( $p=0.194$ ); II and III ( $p=0.537$ ); and III and IV ( $p=0.584$ ) (Figure 5). Collagen/Elastin ratio was 2.4; 5.1; 11.0; and 14.4 for groups I to IV, respectively.



**FIGURE 3** - Microscopic image with a large increase in common bile duct with evidence of inflammatory reaction after the use of biliary stent (Beale's outpouchings)



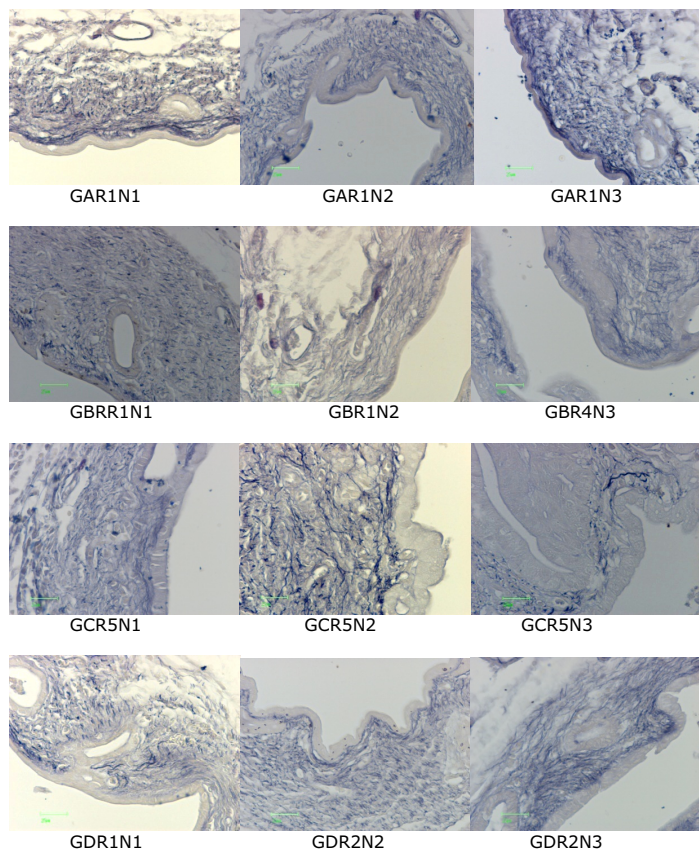
**FIGURE 4** - Sections of the common bile duct stained with the picosirius-hematoxylin (PSH) demonstrating the progressive increase in fibrillar collagen deposition in the bile duct observed in the groups A, B, C and D. Note the similar characteristics of deposition in different levels of the common bile duct of rats from the same group

**Discussion**

Clinical series of patients submitted to biliary stenting Karsten *et al.*<sup>2</sup> reported that patients with biliary obstruction, who underwent drainage by stenting had intense chronic inflammatory reaction and correlating histological changes in the bile duct in proximity to the lesion with irregular destruction of the mucosal surface and intense fibrotic reaction in the submucosa.

There are evidences indicating relationship between bile with bacteria and the frequency of postop complications<sup>7,8</sup>. Retrospective study has shown that patients with bacteria in the bile are epidemiologically similar to the patients with high risk for postop infections (15 to 20%)<sup>7</sup>. Although, the incidence of bacteria in the bile of the patients with jaundice due to pancreatic head Ca varies from 16 to 25% without endoscopic drainage and 1-3% with drainage by plastic biliary stent<sup>2,7,8</sup>. With this, can understand that the inflammatory thickening of the bile duct wall due to the infection represent one of the most important factors in postop complications of patients who underwent prior endoscopic drainage. Dunkin *et al.*<sup>3</sup> relates that the reactive inflammatory process due to biliary stent happens in a way to completely involve the extrahepatic biliary tree, including the cystic duct and the common bile duct. In our study it occurs mainly in the bile duct in contact with the stent.

Karsten *et al.*<sup>9</sup>, in study with dogs, showed that the



**FIGURE 5** - Sections of the common bile duct stained with resorcinfuchsin-oxidized (RFO) showing a progressive decrease in the deposition of elastic fibers in groups A, B, C and D. Note the similar characteristics of deposition in different levels of the common bile duct of rats from the same group

insertion of a stent in normal or obstructed bile duct induced extensive inflammation of the extra-hepatic biliary duct and the bile colonization, which persisted for two months after removal of the prosthesis. Furthermore, the occurrence of intra-abdominal adhesions and dehiscence of anastomosis surgery was greater in the group with biliary stent. There is evidence that linked the rise of microorganisms in biliary tract and postoperative complications, showing rates of infection from 15 to 20%<sup>9</sup>.

Carrasco *et al.*<sup>10</sup> found similar histological changes in the bile ducts of dogs drained with self expandable metal stent.

While the mechanisms underlying these inflammatory changes is not known, these may be a result of:<sup>11,12</sup> a) friction of the stent against the biliary epithelium permitting continuous solution and the immunoreactive elements diluted in the bile, provoke immunologic cascade; b) the pressure of the stent by direct contact with the epithelium resulting in local ischemia with formation of microulcers; c) reflux of unsterile duodenal contents from the duodenal lumen into the bile duct. Karsten *et al.*<sup>9</sup> did important study in dogs where it was verified that transpapillary stent caused bacterial contamination of bile and intense chronic inflammation causing thickening of the bile duct that was previously normal and also in the obstructed part. Other investigators have demonstrated that these inflammatory changes may persist even following removal of the biliary stent<sup>13-18</sup>. It should be considered that the persistence

of chronic inflammatory effect and of fibroblastic reaction in the bile duct in which the bile stent has already been removed occurred due to bacteria in the bile at the time of endoscopic drainage.

Regarding the type of material of the prostheses, both plastic, metal and, more recently, the biodegradable not differ in terms of histology, bile duct or serum determinations, and the findings of previous studies of the latter with low or no toxicity<sup>19</sup>.

In the present study was demonstrated that more strongly reaction of collagen and relationship between elastin and collagen occurred at the 15 days, meaning histologic events in the lumen and mainly in the extrahepatic biliary wall. Then, we should bear in mind that the difficulties problems related to the surgery can be avoided if the patient underwent to surgery procedure until 2 weeks after the stent had left in place. After this critical period, the wall become very tight and the inflammatory process could determine techniques problems during surgery procedure.

We verified in our study that the collagen deposition occurred in inverse proportion of the elastic fibers, and this probably resulted in more rigidity of the hepatobiliary duct that, due to the new reactive histologic structure, would permit the creation of the anastomosis with more risk of fistulae and dehiscence.

We conclude that: (a) biliary stenting leads to collagen and elastin deposition in the bile ducts, and (b) collagen deposition and collagen/elastin ratio are directly proportional to the period of stenting.

### Conclusion

Biliary stenting leads to collagen and elastin deposition in the bile ducts, and collagen deposition and collagen/elastin ratio are directly proportional to the period of stenting.

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