Idiopathic benign biliary strictures in surgically resected patients with presumed cholangiocarcinoma

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Abstract

Objective. Distinguishing between malignant and benign biliary strictures remains problematic. The aim of this study was to compare and contrast the clinical features of patients with benign and malignant biliary strictures.

Methods. Medical records of patients who underwent surgical resection for presumed cholangiocarcinoma were reviewed. Immunohistochemistry for hypoxia inducible factor-1-alpha (HIF-1-alpha) was performed on all bile ductule samples.

Results. Twelve patients with benign strictures (group I) were compared to 26 patients with cholangiocarcinoma (group II). Group I was predominantly female (ratio 2 : 1), (p < 0.01), whereas the gender ratio was 1 : 1 in patients in group II. Bismuth–Corlette type strictures in group I were more likely to be type I/II, whereas type III strictures predominated in group II. The CA 19-9 was ≤100 U/ml in 6 and >100 U/ml in 1 patient of group I and ≤100 in 13 and >100 in 11 patients in group II. Half of the patients in group I had positive immunoreactivity for HIF-1-alpha in bile ductules.

Conclusion. Benign biliary strictures masquerading as cholangiocarcinomas occur more often in women, are less often Bismuth–Corlette type III, have serum CA 19-9 values ≤100 U/ml, and hypoxia may play a role in a subset of these strictures.

Key Words: Bile duct strictures, cholangiocarcinoma, sarcoidosis, HIF-1-alpha

Introduction

Extrahepatic biliary strictures are often challenging diagnostic dilemmas. Patients may present with jaundice, cholangitis, isolated serum alkaline phosphatase elevations, or bile duct dilatation on imaging studies. These symptoms are diagnostically non-specific. Moreover, imaging modalities may also be non-specific. Cholangiograms, endoscopic and/or percutaneous, may display the site and extent of the stricture, but in the absence of stones, mass, adenopathy or the more diffuse biliary changes of primary sclerosing cholangitis (PSC), the underlying aetiology remains unclear. Cross-sectional imaging studies of the liver and bile ducts—computed tomography (CT) and magnetic resonance imaging (MRI)—can reveal bile duct dilation, atrophy of an obstructed lobe and hypertrophy of a contralateral lobe, masses, regional lymphadenopathy and loss of lobar blood flow. Masses and vascular encasement imply neoplastic obstruction. Lymphadenopathy, however, is non-specific. Unfortunately, tissue diagnosis is difficult given limited access to the bile duct, and the highly desmoplastic nature of biliary tract cancers. Negative biopsies and brushings, therefore, do not exclude bile duct cancers. Even with advanced cytological techniques such as digital image analysis and fluorescent in situ hybridization for aneuploidy, brushings and biopsies are frequently falsely negative [1].

The differential diagnosis of biliary strictures includes cholangiocarcinoma, gall bladder cancer, metastatic cancer to the perihilar region of the liver, primary and secondary sclerosing cholangitis, granulomatosis disease, biliary tract stone disease, surgical trauma and so-called “idiopathic strictures”. A major dilemma is distinguishing idiopathic strictures from early cholangiocarcinoma. Because current imaging and diagnostic modalities cannot exclude malignancy, all biliary strictures are approached from a management perspective as if they are malignant. In 8–13% of most surgical series on cholangiocarcinoma, the bile duct stricture is inflammatory and benign [2,3]. Accurate diagnosis of these idiopathic, inflammatory strictures may permit conservative stenting approaches without surgical extirpation. More information is necessary to characterize these strictures for accurate preoperative diagnosis.

The aetiopathogenesis of these idiopathic biliary strictures remains enigmatic. The biliary tree is quite susceptible to ischaemic injury. The tissue response to
chronic ischaemia is to adapt by expressing survival proteins. One adaptation to ischaemia is the expression of hypoxia inducible factor (HIF-1-alpha) [4]. Expression of this transcription factor can be identified by immunohistochemistry [4,5] and therefore, its expression may provide insight into the pathogenesis of idiopathic strictures.

Thus, the overall objective of this study was to further characterize idiopathic biliary strictures through a review of our clinical experience. These findings were compared to a group of patients who underwent surgical resection for proven cholangiocarcinoma. Our data suggest that idiopathic strictures occur more often in women and are more likely to include the mid-duct region than cholangiocarcinomas. A subset of these strictures may be ischaemic based on ductule expression of HIF-1-alpha.

Methods

Patient groups

This minimal risk retrospective study was approved by the Mayo Institution Review Board. We reviewed the medical records of patients who underwent surgical resection or exploration for presumed cholangiocarcinoma between 1990 and 2003. Clinical features, laboratory results, cholangiogram, imaging studies, surgical reports and histopathology were reviewed in the 12 patients with idiopathic benign strictures (group I) and compared with 26 patients with cholangiocarcinoma (group II) diagnosed over the same period. Patients with a diagnosis of PSC, inflammatory bowel disease, choledocholithiasis and previous cholecystectomy and gall bladder carcinoma were excluded from the study. Diagnosis of idiopathic benign stricture was confirmed after review by a hepatopathologist. The diagnosis of cholangiocarcinoma was also confirmed after histopathology review.

Review of cholangiograms and classification of biliary strictures

The cholangiograms were reviewed with particular regard to the site of stricture and biliary dilatation. The strictures were classified using the anatomical classification developed by Bismuth and Corlette [6]. Imaging modalities and surgical findings were reviewed to assess vascular involvement and the atrophy-hypertrophy complex.

Review of histopathology

The histopathology was reviewed systematically by a hepatobiliary pathologist. The specimens diagnosed as cholangiocarcinoma were graded into types I–IV according to UICC criteria [7]. All tissue underwent staining for acid-fast bacteria and fungi.

Immunohistochemistry for HIF-1-alpha

Unstained slides of tissue specimens from group I were deparaffinized and hydrated. Antigen retrieval was performed using EDTA (1 mM, pH 8.0); slides were placed in a vegetable steamer for 40 minutes at 97°C followed by a cooling off period of 20 minutes. Thereafter, the catalysed signal amplification system (DAKO, Carpinteria, CA, USA) was used for HIF-1-alpha staining according to the manufacturer's instructions [5]. The primary antibody utilized was anti-HIF-1-alpha mouse monoclonal antibody at a dilution of 1:1000 (Novus Biologicals, Littleton, CO, USA). Positive specimens required immunoreactivity of bile duct epithelial cells in >50% of ducts, staining above background and immunoreactive product in nuclei consistent with the biology of the transcription factor. Specimens with indeterminant immunostaining were considered negative.

Statistics

The results were expressed as mean values ± SE. Statistical significance in mean values was evaluated by the Student’s t test.

Results

Clinical characteristics

All patients presented with jaundice and proximal bile duct dilatation on ultrasound and CT imaging studies. There were 4 men and 8 women in group I compared with 14 men and 12 women in group II; the gender prevalence for women in group I was significant (p<0.01). The mean age of patients, serum bilirubin, AST, ALT and alkaline phosphotase levels in group I and group II were similar and not statistically different (Table I). The serum CA 19-9 value was >100 U/ml in 11 patients in group II, whereas it was elevated >100 U/ml in only 1 patient in group I. The patient in group I with elevated CA 19-9 (2888 U/ml) had active bacterial cholangitis, a known cause of elevated serum CA 19-9 values [3].

Diagnostic imaging was not standardized in the study because of the long duration of the study period. Ten patients in group I underwent CT; no masses were identified. Three patients in group I and nine patients in group II underwent endoscopic ultrasound; two of three in group I had ultrasonographic evidence for a mass and eight of nine in group II demonstrated a mass lesion, p = 0.08.

Cholangiography findings

Bismuth–Corlette type strictures were as follows. Group I: I = 6, II = 2, IIIa = 1, IIIb = 2 (Figure 1); group II: I = 5, II = 6, III = 1, IIIa = 4, IIIb = 10. Although group I had Bismuth type IIIa or IIIb strictures, no vascular encasement or lobar atrophy
and obstruction of secondary biliary radicals was observed in this group, but was observed in five patients in group II.

Surgical intervention

Eight of 12 patients in group I underwent resection of extrahepatic duct and Roux-en-Y hepaticojejunostomy. One patient underwent pylorus-preserving Whipple resection for a lesion of the distal bile duct involving the head of the pancreas. One patient underwent left hepatectomy and Roux-en-Y hepaticojejunostomy for a stricture of the left hepatic duct extending to the common hepatic duct with left lobar firmness. Two patients in group I had a mass noted at exploration. One patient had a 2-cm mass involving the common hepatic duct, cystic duct, Calot’s triangle and distal gall bladder that was subsequently diagnosed as sarcoidosis. The second patient had a 2-cm firm thick mass at the level of common hepatic duct and common bile duct whilst in remission from chronic lymphocytic leukaemia (CLL). This mass was fibrosed scar tissue, perhaps resulting from chemotherapy-induced necrosis of his CLL in this region. Both patients underwent resection of extrahepatic duct and Roux-en-Y hepaticojejunostomy. Two patients did not undergo resection, one underwent transduodenal sphincteroplasty and the other underwent t-tube

Table I. Clinical characteristics of patients

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I: benign stricture (n = 12)</th>
<th>Group II: cholangiocarcinoma (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) (median/range)</td>
<td>59.4 (20.8–79.8)</td>
<td>61.4 (41.8–78.9)</td>
</tr>
<tr>
<td>Age (mean)</td>
<td>54.7 ± 15.4</td>
<td>61.4 ± 10.3</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>4:8</td>
<td>14:12</td>
</tr>
<tr>
<td>Bismuth–Corlette type (I:II:III)</td>
<td>6:2:4</td>
<td>5:6:15</td>
</tr>
<tr>
<td>Total bilirubin mg/dl (median/range)</td>
<td>4.5 (0.3–21.6)</td>
<td>8.0 (0.4–37.9)</td>
</tr>
<tr>
<td>Mean</td>
<td>6.1</td>
<td>10.4</td>
</tr>
<tr>
<td>AST/ALT U/l (median)</td>
<td>60.5/83.5</td>
<td>70.5/82.5</td>
</tr>
<tr>
<td>Alkaline phosphatase U/l (median)</td>
<td>642.5 (91–2675)</td>
<td>809.5 (290–1722)</td>
</tr>
<tr>
<td>CA 19—9 U/ml (≤100: &gt;100)</td>
<td>6:1</td>
<td>13:11</td>
</tr>
</tbody>
</table>

Figure 1. Cholangiogram of a benign inflammatory bile duct stricture masquerading as cholangiocarcinoma. This representative cholangiogram epitomizes the difficulty in distinguishing between malignant and benign biliary strictures via ERC. There is lack of visualization of the right hepatic ductular system with left ductular dilatation.
choledochostomy; at the time of surgery, malignancy could not be confirmed and the stricture was assessed as inflammatory.

Twelve of 26 patients from group II underwent either left or right hepatectomy, extrahepatic bile duct resection and hepaticojejunostomy. Seven underwent extrahepatic bile duct resection and hepaticojejunostomy alone. Three patients had a Whipple resection for localized distal bile duct disease, two of which were pylorus-preserving procedures. Four patients had unresectable disease noted by laparotomy or laparoscopy, secondary to peritoneal carcinomatosis and portal vein invasion.

Clinical outcomes

Two patients in group I have died. Both patients underwent extrahepatic duct resection and Roux-en-Y hepaticojejunostomy. One patient died 8 years after resection from biliary cirrhosis and the other from transitional cell carcinoma of the bladder 3 years after biliary resection. The patient who developed biliary cirrhosis also had recurrent intrahepatic biliary strictures and hepaticolithiasis. The aetiology of this progressive stricturing syndrome was unclear. Eight patients remain healthy without recurrent disease 4–9 years after biliary resection. The patient who underwent t-tube choledochostomy experienced progressive intrahepatic strictureing from presumed PSC, and one patient was lost to follow-up. In contrast, none of the patients with cholangiocarcinoma, group II patients, had long-term survival (no 5-year survivors).

Immunohistochemistry for HIF-1-alpha in patients with benign strictures

HIF-1-alpha immunoreactivity was positive in 50% (6/12) of the patients in the benign biliary stricture group (Figure 2). Thus, a subset of these patients had biochemical and histological evidence for ongoing ischaemia or hypoxia within the resected biliary specimen. There was minimal to no immunoreactivity in the patient with sarcoidosis or the patient in remission from CLL as expected. As anticipated in an avascular cancer such as cholangiocarcinoma, all biliary tissue specimens from group II were positive for HIF-1-alpha immunoreactivity (data not shown).

Figure 2. Hypoxia inducible factor-1-alpha (HIF-1-alpha) is present in benign inflammatory bile duct strictures. Representative photomicrographs of biliary duct tissue specimens after immunohistochemical staining of HIF-1-alpha. (A) Positive staining of HIF-1-alpha in ductular epithelium (magnification ×20). (B) A higher magnification of (A) illustrating definite nuclear staining (arrows) (×40). (C) A biliary tissue specimen negative for HIF-1-alpha; note the lack of nuclear staining in ductular epithelium and clear nuclei.
Discussion

We examined a select group of patients with idiopathic biliary strictures by excluding patients with focal strictures secondary to primary sclerosing cholangitis (PSC), associated inflammatory bowel disease and choledocholithiasis. Our study demonstrates that patients with idiopathic biliary strictures are more likely to be women, demonstrate normal CA 19-9 levels, less frequently have strictures involving the bile duct confluence, and a subset of these strictures expressed HIF-1-alpha which is consistent with tissue hypoxia.

This study demonstrates that patients with benign strictures are more likely to have strictures of Bismuth–Corlette type I and II; in contrast patients with cholangiocarcinoma are more likely to have types II, III, IV. Our findings differ from those of the study by Verbeek et al. [8], who described predominantly type III strictures in more than half their 11 patients. However, our data are similar to those of Hadjis et al. [2], as all their patients exhibited type I or II strictures. Therefore, we suggest that strictures below the biliary confluence are more likely to be benign. However, most de novo strictures are malignant.

The location of these strictures is analogous to those occurring following cholecystectomy, which may be type III [9] or more than half the time type I–II because of compromise of axial and tenuous blood supply of the supraduodenal segment of the bile duct. [10]. These observations lead us to speculate that vascular compromise may contribute aetologically to idiopathic benign biliary strictures. To examine the hypothesis that vascular compromise and hypoxia may play a role in benign biliary strictures we performed immunohistochemical staining for HIF-1-alpha on excised biliary tissue from the benign stricture group. HIF-1-alpha is a transcription factor that is expressed in response to a decrease in the partial pressure of cellular oxygen and activates genes involved in glycolysis, modulation of vascular tone, angiogenesis and erythropoiesis [4,11].

We found that approximately half were positive for HIF-1-alpha protein in the nucleus. These data support hypoxic injury as a potential cause for benign biliary strictures. However, it is also possible that stricture formation itself may have rendered the tissue hypoxic. Nonetheless, hypoxia even as a secondary rather than an inciting event would likely contribute to stricture progression.

Two of six tissue specimens negative for HIF-1-alpha immunostaining had probable causes other than hypoxia. One stricture was most likely secondary to sarcoidosis. Although rare, cholestasis in sarcoidosis may be attributable to extrahepatic biliary ducal obstruction from sarcoid [12,13] or enlarged lymph nodes of the portahepatis [14]. The second patient likely had a stricture arising from chemotherapy for CLL. Rarely injury to the bile duct following radiation [15] and chemotherapy for lymphoma [16] has resulted in biliary stricture. Such a complication may have contributed to the stricture in this patient, although he was in remission from CLL. Recognizing these clinical scenarios as potential causes of benign strictures may help in their management.

Despite improvements in imaging techniques during the last decade, radically distinguishing benign from malignant strictures still remains a challenge [17,18]. ERC or MRC is helpful in identifying the site and extent of stricture but not the aetiology [19,20]. Endoscopic biopsies and brushings are only positive in 15–70% of patients with cholangiocarcinoma [21]. The difficulty of applying imaging techniques to clearly differentiate benign from a malignant process is highlighted by a non-contributory result from endoscopic ultrasound (EUS) in 3 of the 12 patients in the benign idiopathic group who underwent the procedure, 2 of whom had imaging criteria for a mass lesion. The presence of lymphadenopathy does not differentiate between malignancy and benign stricture; in fact, lymphadenopathy may be reactive and occur either as a part of the primary process or following placement of stents [1,22]. The results from newer techniques such as digitalized image analysis and fluorescent in situ hybridization [21,23] appear to be promising but need validation.

In conclusion, benign biliary strictures masquerading as cholangiocarcinomas occur more often in women and are less often Bismuth–Corlette type III than true malignant lesions. In the absence of cholangitis, serum CA 19-9 values are also seldom >100 U/ml in benign strictures. A subset of patients appears to have a hypoxic component, which may lead to the development of their benign biliary strictures.

Acknowledgements

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References