

Expressions of p53 and inducible nitric oxide synthase in congenital choledochal cysts

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BACKGROUND: Choledochal cyst, an isolated defect unrestricted to the bile duct, is more appropriately regarded as the sentinel feature of a constellation of anomalies affecting the pancreatobiliary system. This study was to assess the relationship between the expression of inducible nitric oxide synthase (iNOS) and the p53 gene as well as the pathogenesis of choledochal cysts.

METHODS: iNOS and p53 were detected by immunohistochemistry staining in 26 patients with congenital choledochal cysts. Histopathologically, hyperplasia of the mucosa of the cysts and the amylase level in the bile were also investigated.

RESULTS: Patients with a high level of amylase in the bile had higher expression of iNOS than those with a low level of amylase. p53 protein was expressed neither in fusiform type nor in cystic type. The incidence of mucosal hyperplasia was significantly higher in the fusiform type than that in the cystic type.

CONCLUSIONS: Higher expression of iNOS may participate in hyperplasia and carcinogenesis of the mucosa of choledochal cysts. The regurgitation of pancreatic juice into the biliary system might induce mucosal hyperplasia of the biliary tract and inflammatory reaction. In preventing regurgitation-caused hyperplasia and malignancy of the biliary tract, early surgery is important for children with congenital choledochal cysts.

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KEY WORDS: choledochal cyst, congenital; iNOS; p53

Introduction

Congenital choledochal cyst is a common disease in children. Its incidence is higher in Asia and occurs frequently in females, with a male to fe-

male ratio of 1:3 to 1:4.^[1] The etiology of extrahepatic cysts is unclear at present. Type I cysts are associated with an anomalous arrangement of the pancreatobiliary ducts (APBD), known as a "common channel", which is seen in up to 92% of patients with choledochal cysts.^[2] If choledochal cysts are not resected, the incidence (20% to 30%) of cholangiocarcinoma will be high after the second decade of life. This is the basis of resection as a state-of-the-art surgical treatment.^[3]

Inducible nitric oxide synthase (iNOS) can generate mutagenic concentrations of nitric oxide (NO) in mice.^[4] p53 is one of the most extensively studied genes. Identified as an oncogene, it has been confirmed to be an important tumor suppressor gene. In this study, we assessed the role of iNOS and p53 in the carcinogenesis of congenital choledochal cyst.

Methods

Patients

Between 1997 and 2002, 26 patients with type I choledochal cysts were treated at the Tianjin Children's Hospital, Tianjin, China. Their median age was 63 months (range 7 months to 156 months); the male to female ratio was 1:3. All the patients were subjected to resection of the dilated common and hepatic ducts, followed by Roux-en-Y hepaticojejunostomy. Among them, 8 patients received intraoperative cholangiography for the understanding of the anatomy of the common channel (Fig. 1). The resected samples were from either the level of the hepatic hilum or the level of the intrapancreatic region. All choledochal cysts were grouped clinically according to Todani's classification. Type I cysts were the most frequently encountered. Amylase levels in the bile of the choledochal cyst were measured in all 26 patients. Patients with amylase levels higher than 500 U/L were classified as high-amylase group and those with amylase levels lower than 500 U/L as low-amylase group.

Immunohistochemical staining of iNOS and p53

S-P immunohistochemistry method was used to stain iNOS and p53 protein. In each batch, known iNOS positive colorectal sample served as positive control. A

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sample without the primary antibody was used as negative control. p53 immunoreactivity was demonstrated by means of the same staining protocol. We used a monoclonal DO7 (DAKO) antibody, known to be specific for mutant forms of p53 protein at a working dilution of 1:1000. Breast carcinoma sample was used as positive control.

The staining intensity of iNOS in the epithelium and positive cells were assessed microscopically from the random fields. The staining intensity of iNOS in the epithelial cell was scored; 0 for negative staining, 1 for weak, and 2 for moderate and strong.

Results

Amylase levels in different subtype choledochal cysts

Twelve of 18 patients of the cystic type were classified into the high-amylase group (>500 U/L). The other 6 patients were included in the low-amylase group (<500 U/L). However, all of the 8 patients of the fusiform

Table 1. Choledochal dilatations and amylase levels in the cyst

SCC	n	Mean age (range, y)	AL (U/L)
Cystic type			
High-amylase group	12 *	4.5(1-14)	1187.18(901—>8600)
Low-amylase group	6 *	1.5(0.67-2)	151.50(26—466)
Fusiform type			
High-amylase group	8 *	3.0(1-6)	1317.66(936—>8000)

SCC; subtype of choledochal cyst; AL; amylase level; *: P<0.05.

type belonged to the high-amylase group. No significant difference was observed between the cystic type and the fusiform type of the high-amylase group (Table 1).

Mucosal hyperplasia of choledochal cysts

In the cystic type, 5 (41.6%) of 12 patients with high amylase levels and 2 (33.3%) of 6 with low amylase levels showed mucosal hyperplasia of choledochal cysts. In the fusiform type, however, 6 (75%) of 8 patients showed mucosal hyperplasia of choledochal cysts.

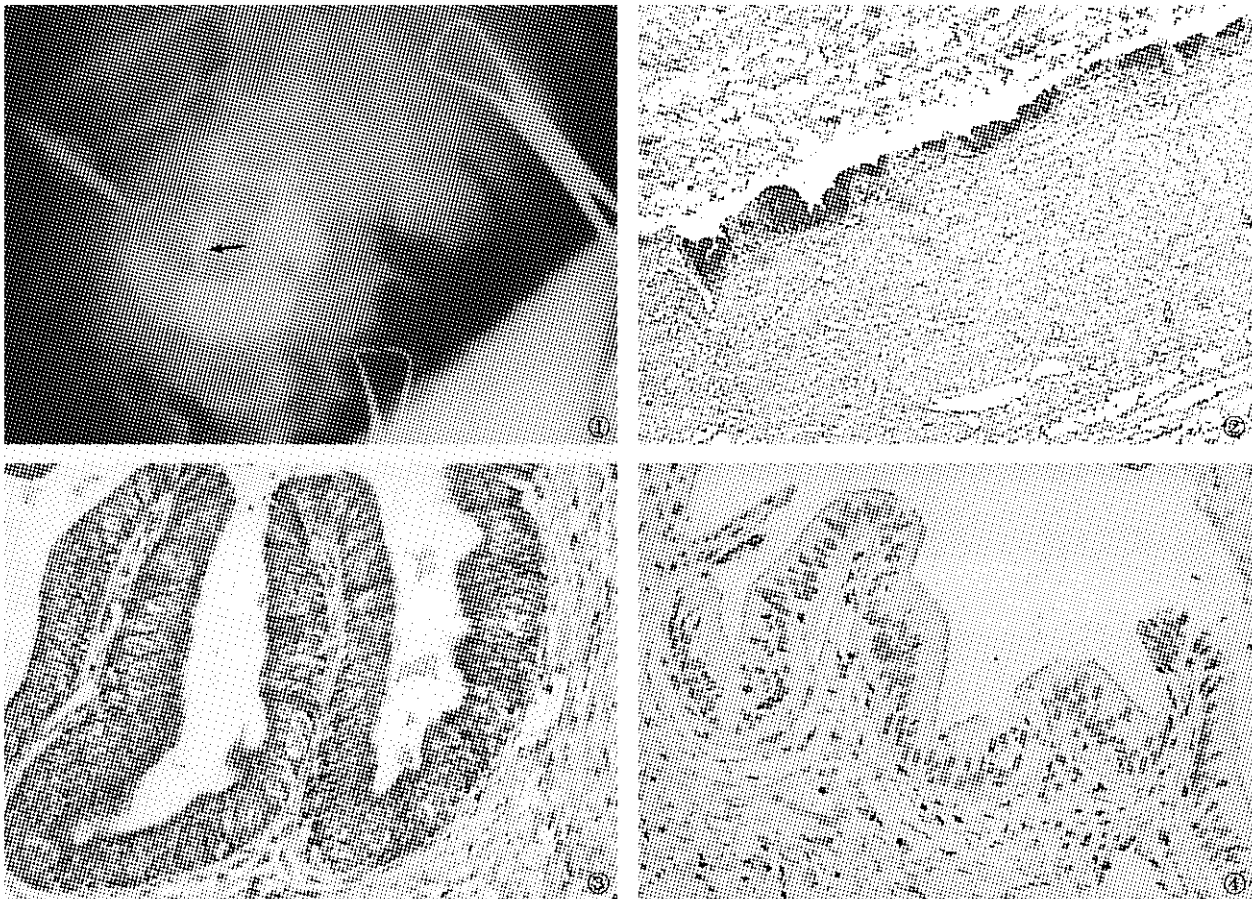


Fig. 1. A two-year-old boy with choledochal cyst. Cholangiogram showing the position of the common channel (arrow).
Fig. 2. iNOS immunohistochemical staining of epithelial hyperplastic choledochal cysts (original magnification×100).
Fig. 3. iNOS immunohistochemical strong staining of hyperplastic epithelial cells of the gallbladder (original magnification×400).
Fig. 4. p53 expression deficit in hyperplastic epithelial cells of the gallbladder (original magnification×400).

Table 2. Expression of iNOS and p53 in the epithelial cells of choledochal cysts

SCC	iNOS	p53
Cystic type		
High-amylase	+ - +++	-
Low-amylase	- - +	-
Fusiform type		
High-amylase	+ + - +++	-

SCC: subtype of choledochal cyst.

Expression of iNOS and p53 protein

The expression of iNOS (Fig. 2) was significantly higher in the high-amylase group of the cystic type than in the low-amylase group. The higher mucosal hyperplasia, the higher expression of iNOS. The expression of iNOS in the high-amylase group of the cystic type and fusiform type was higher than that in the low-amylase group ($P < 0.05$), and there was no significant difference between the values in the fusiform type and those in the high-amylase group of the cystic type. In the high-amylase group of the cystic type and fusiform type, the expression of iNOS was similar (Fig. 3). The expression of p53 protein in the mucosal hyperplasia cells of different groups was not detected (Fig. 4, Table 2).

Discussion

It has been shown that high amylase levels in the bile are commonly observed in choledochal cysts. Todani et al^[5] reported that amylase levels in the bile gradually increased with age, and reached high levels in children over the age of 13 months. In the present study, 22 of the 26 children were below 1 year old. Most of the children showed high amylase levels in choledochal cysts. In the other 4 children below 1 year of age, amylase levels in choledochal cysts were lower than 500 U/L (range 83–446).

The most widely used typing of choledochal cysts is Todani's classification, which is a modification of the Alonso-Lej classification. Like in most series, the majority of the children in this study had a type I cyst. We further subdivided type I cysts into cystic and fusiform cysts, and compared the difference of mucosal hyperplasia and amylase levels. The concept of treatment of extrahepatic choledochal cysts has changed in the past years because of a persistent high risk of malignancy after drainage procedure. In view of the high risk of cholangiocarcinoma, the state-of-the-art treatment of extrahepatic choledochal cysts is primary excision with a biliary digestive anastomosis. Since high-amylase and epithelial hyperplasia in children may be correlated with the risk of cholangiocarcinoma, early resection without internal drainage is the appropriate treatment for type I cysts and the extrahepatic part of IV biliary cysts.

The regurgitation of pancreatic juice into the biliary system may play an important role in the induction of inflammation or a premalignant condition of the biliary tract.^[6] Seven of 8 patients receiving intraoperative cholangiography in this study showed that there was a common channel of over 1 cm in length. The longer APBD may be the cause of pancreatic juice reflux into the biliary system, with resultant choledochal cysts. In this study, the incidence of mucosal hyperplasia of choledochal cysts was significantly higher in the fusiform type than in the cystic type. The isoenzymes of NOS are thought to play a role in maintaining intracellular physiology, and their loss could result in dysfunction of normal cellular homeostasis.

The role of iNOS in the epithelium of the biliary duct is not known at present.^[7] iNOS can produce high, persistent levels of NO upon induction with cytokines in many cell types and is expressed in the resting state in other cells, potentially resulting in cytotoxicity, tissue damage, or DNA damage. p53 is known to play an important role in safeguarding the genomic integrity of mammalian cells in response to DNA damage.^[8] Recent studies have shown that NO is able to stimulate p53 expression and apoptosis in rodent macrophages, pancreatic cell lines, and murine thymocytes, suggesting that NO-induced apoptosis results from DNA damage and subsequent accumulation of p53.^[9] To the present, we have not yet found the negative feedback loop in choledochal cysts. In this study, no p53 gene mutations were observed in the hyperplastic epithelial cells of choledochal cysts.

Cholangiocarcinoma in choledochal cysts has been reported in up to 26% of the patients. However, the overall incidence of cholangiocarcinoma is 14% (non-intrahepatic) comparable with that reported recently.^[10] In the present study, most of the children showed high amylase levels and high iNOS expression in choledochal cysts and the mucosa of the biliary tract, but none of a patient with cholangiocarcinoma was found. Albores-Saavedra et al's histological study^[11] suggested that epithelial hyperplasia might be a possible precursor of biliary tract carcinoma.

In conclusion, only mucosal hyperplasia of choledochal cysts can express high levels of iNOS, because of high-amylase level in choledochal cysts, which may be related to the regurgitation of pancreatic juice into the biliary system. However, the expression of p53 gene mutation was not detected in our patients, and further studies are necessary to ascertain whether there is a relationship between mucosal hyperplasia and carcinoma of the biliary tract.

Competing interest

No benefits in any form have been received or will be received

from a commercial party related directly or indirectly to the subject of this article.

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